STUDIES ON DIVERSITY OF *Sclerotium rolfsii* Sacc. AND INDUCED SYSTEMIC RESISTANCE IN GROUNDNUT (*Arachis hypogaea* L.) AGAINST STEM ROT PATHOGEN

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M.Sc. (Ag.)

DOCTOR OF PHILOSOPHY IN AGRICULTURE
(PLANT PATHOLOGY)

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BY

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M.Sc. (Ag.)

THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

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(PLANT PATHOLOGY)

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2017

DECLARATION

I, **NOORULLA HAVERI**, hereby declare that the thesis entitled **"STUDIES ON DIVERSITY OF *Sclerotium rolfsii* Sacc. AND INDUCED SYSTEMIC RESISTANCE IN GROUNDNUT (*Arachis hypogaea* L.) AGAINST STEM ROT PATHOGEN"** submitted to the **Professor Jayashankar Telangana State Agricultural University** for the degree of **Doctor of Philosophy in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

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Date: **I. D. No. : RAD/2012-20**

CERTIFICATE

Mr. **NOORULLA HAVERI** has satisfactorily prosecuted the course of research and that thesis entitled **"STUDIES ON DIVERSITY OF *Sclerotium rolfsii* Sacc. AND INDUCED SYSTEMIC RESISTANCE IN GROUNDNUT (*Arachis hypogaea* L.) AGAINST STEM ROT PATHOGEN"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any university.

Date:

(B. PUSHPAVATHI)
Chairperson

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON DIVERSITY OF *Sclerotium rolfsii* Sacc. AND INDUCED SYSTEMIC RESISTANCE IN GROUNDNUT (*Arachis hypogaea* L.) AGAINST STEM ROT PATHOGEN"** submitted in partial fulfilment of the requirements for the degree of "**DOCTOR OF PHILOSOPHY IN AGRICULTURE**" of the **Professor Jayashankar Telangana State Agricultural University**, Hyderabad is a record of the bonafide original research work carried out by **Mr. NOORULLA HAVERI** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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Hyderabad

13 October, 2017 **(NOORULLA HAVERI)**

LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
=	:	Is equal to
±	:	Plus or minus
^{0}C	:	Degree Celsius
a.i	:	Active ingredient
APS	:	Ammonium per sulphate
CD	:	Critical difference
cm	:	Centimetre
dNTP	:	Deoxynucleotide 5'-triphosphate
et al.	:	And others
etc.	:	And so on
EDTA	:	Ethylenediaminetetraacetic acid
Fig.	:	Figure
g	:	Gram
ha	:	Hectare
i.e.,	:	That is
lb *psi*	:	Pounds per square inch
mha	:	Million hectare
ml	:	Millilitre
mm	:	Millimetre
mt	:	Million tonne
mg	:	Miligram
M	:	Molarity
mM	:	Milimolar
N	:	Normality
ng	:	Nanogram
rpm	:	Rotations per minute
S.Em	:	Standard error mean
sp.	:	Species
SDS	:	Sodium dodecyl sulfate
t	:	Tonne
TEMED	:	Tetramethylethylenediamine
Tris	:	Tris-hydroxymethyl amino methane
viz.,	:	Namely
µm	:	Micrometre
µl	:	Microlitre
µg	:	Microgram

Student	: **NOORULLA HAVERI**
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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown in India. Of the various diseases inciting groundnut, the stem rot caused by *S. rolfsii* is a potential threat to groundnut production. The present investigations were carried out to assess the diversity of *S. rolfsii* causing stem rot of groundnut and to identify the potent fungal and bacterial biocontrol agents for effective management of disease.

The roving survey conducted during *kharif,* 2013 and *kharif,* 2014 in major groundnut growing areas of India recorded 11.23 to 55.40% and 10.11 to 59.33% incidence of stem rot respectively. Among various states surveyed, Gujarat recorded highest incidence whereas Telangana documented least incidence. Gujrat, parts in Maharashtra and Tamil Nadu recorded comparatively higher incidence where the crop was grown in black soils with susceptible cultivars continuously as sole crop. Totally 60 isolates of *S. rolfsii* were collected from varied geographical areas during survey. The medium Richards's broth was found to be best supporting medium for *in vitro* oxalic acid production by *S. rolfsii.* Further, the 60 of isolates exhibited wide variation in *in vitro* oxalic acid production. The 15g inoculum level of *S. rolfsii* per 7" pot was found most effective and suitable for glasshouse studies.

All the 60 isolates of *S. rolfsii* in the study were found pathogenic. Further, there was great variation among the isolates for virulence levels on three groundnut cultivars, hence the isolates were grouped into two categories *viz.*, highly virulent (*n*=56) and less virulent (*n*=4). Further, there was a positive correlation found between the amount of oxalic acid produced *in vitro* and virulence of the isolates. Thus, the highly virulent isolates produced significantly highest amount of oxalic acid (0.99-2.85 mg/ml) whereas less virulent isolates produced least amount (0.64-0.78 mg/ml).

Culturally the 60 isolates of *S. rolfsii* were found diverse. The growth rate, biomass production of isolates tested was ranged from 0.66 to 1.29 mm/hr and 6.82 to 14.62 mg/day respectively. All the isolates produced sclerotia on PDA medium. Most of the isolates produced the colonies which were raised at ends (*n*=27) followed by flat type (*n*=20) and raised type (*n*=13). As per mycelial growth type, most of the isolates were found highly profuse in growth (*n*=36) and few were profuse (*n*=24).

Likewise, the isolates exhibited considerable variation with respect to morphological characters. Wide variation in days to form (4 to 17 days) and days for maturation (7 to 23 days) of sclerotia was found among the isolates. Similar type of variation was found with respect to 100 sclerotial weight (0.12 to 1.19 g), number of sclerotia per plate (52 to 910), pattern of sclerotia produced in petri dish (scattered (*n*=38), peripheral (*n*=16) and central (*n*=6)), colour of sclerotia (brown (*n*=25), dark brown (*n*=20) and light brown (*n*=15)), size of sclerotia (0.15 mm to 2.81 mm). In mycelial compatibility study high rate of antagonistic reactions was found among paired *S. rolfsii* isolates indicating high extent of genetic diversity. Thus, based on mycelial compatibility, 15 mycelial compatibility groups (MCGs) were found among the 60 isolates of *S. rolfsii*. Further, the cultural and morphological variability of *S. rolfsii* isolates was not correlated with the virulence of isolates.

The molecular identity of 60 isolates of *S. rolfsii* was performed by amplification and sequencing of ITS-rDNA region of these isolates and confirmed as *S. rolfsii*. Further, the phylogenetic analysis of the ITS-rDNA sequences revealed relative uniformity in *S. rolfsii* population. The ITS-rDNA sequencing did not give detailed insight into the intraspecific diversity. Hence, to assess the same, the 15 isolates of *S. rolfsii* (one random isolate each from 15 MCGs) were subjected to diversity study using 30 RAPD primers which revealed wide genetic diversity among the isolates. Further, there was no link found between genetic diversity of isolates and their geographical origin.

The T1 isolate of *Trichoderma* sp. and B1 isolate of *Bacillus* sp. were found highly antagonistic to virulent isolate of *S. rolfsii* under *in vitro* conditions. Further, they were highly compatible with each other and with commonly used fungicides. The plants treated with bioformulation mixture of T1 and B1 fortified with chitin recorded higher level of resistance induction in the form of defense chemicals (Phenol), defence enzymes (PAL, PO, PPO and CAT) and PR proteins (*β*-1,3-glucanase-PR-2 and chitinase-PR-3) followed by bioformulation mixture of T1 and B1 without chitin against *S. rolfsii*. Further, the sharp increase in induction of resistance was noticed on 2nd dpi. Additionally, these treatments resulted in highest induction of isoform of defense enzymes (8 isoforms of PO, 13 isoforms of PPO and 3 isoforms of CAT). The chitin amendment of bioformulation had the positive effect on resistance induction.

The T1 and B1 isolates performed well in glasshouse and field evaluation (at two locations, Patancheru and Rajendranagar), wherein, the combined application of bioformulations of T1 and B1 with chitin through seed treatment and soil application was found most effective followed by combined application of bioformulations of T1 and B1 without chitin. Further, these treatments induced substantial amount of growth and yield attributing parameters and recorded highest pod yield and B:C ratio.

Overall, the study revealed the considerable variability among field isolates of *S. rolfsii*. Further, the T1 isolate of *Trichoderma* sp. and B1 isolate of *Bacillus* sp. were found more efficient in inducing of resistance against *S. rolfsii* and have the potential to be used in the field for effective management of stem rot of groundnut.

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INTRODUCTION

Chapter I

INTRODUCTION

Groundnut (Peanut; *Arachis hypogaea* L.) is an economically important legume crop of the world grown primarily for good quality edible oil and easily digestible proteins (Cobb and Johnson, 1973). The groundnut is also known as earthnut, monkey nut and goobers nut in various parts of the world, and is not a true nut but rather an annual legume crop. Groundnut is native of southern Bolivia/north west region of South America. It consists of 26% protein, 48% edible oil, 20% carbohydrates and 3% fiber and also rich in calcium, thiamine and niacin. Hence, it has all the potential to be used as a highly economical food supplement to fight malnutrition, thus groundnut is a nature gift to man in general and to the poor in particular.

Groundnut is a major crop in tropical and sub-tropical areas of the world. Globally the crop is cultivated in more than 100 countries in all six continents. It is currently grown on 24.70 mha worldwide with a total production of 40.32 Mt (USDA, 2016). On the global scale, India is a major producer of groundnut with a total production of 4.47 mt with an area of 4.56 mha (USDA, 2016). The states like Gujarat, Telangana, Andhra Pradesh, Karnataka, Tamil Nadu and Maharashtra are considered to be the major groundnut growing regions in India. Among these, Gujarat stands first in both area (1.40 mha) and production (2.22 mt), undivided Andhra Pradesh ranks second with 1.03 Mha area and production of 0.79 mt (DOES, 2015).

The production of groundnut is however threatened by various biotic and abiotic constraints such as pests and diseases, drought, low input use and socio economic infrastructure. Of the various diseases of groundnut, the soil borne fungal disease that adversely affects the plant health and productivity is stem rot caused by *Sclerotium rolfsii* Sacc. The disease was reported for the first time by Mcclintock (1917) in Virginia and is also known as white mold or southern blight. Depending on severity of field infestation, it has the potential to cause losses in pod yield of 10-25% and sometimes the loss can extend up to 80% in severe infestations (Mayee and Datar, 1988). Peg and pod rots caused by *S. rolfsii* are major consequences of the disease, resulting in serious pod losses at harvest. The warm and moist conditions favors stem rot development, while pod rots are favored by rather drier soil conditions. In heavy soils, *S. rolfsii* is most damaging to groundnut plants at or near the soil surface, but in lighter soils it can be active at greater depths, causing severe damage to pegs and pods

(Mehan and McDonald, 1990). In addition, oxalic acid produced by *S. rolfsii* causes blue discolouration and affects the seed quality and market value of the produce.

Owing to wide host range of *S. rolfsii* of over 500 plant species in over 100 plant families (Punja, 1985) the disease resistance in existing commercial cultivars is less effective and not long lasting. Further, it produces sclerotia on the infected portions of the plant near the soil line, which can survive in the soil from a few months to several years, depending on environmental conditions (Punja, 1985; Xu *et al.,* 2008), and are the primary inoculum source for disease development. Added to it, *S. rolfsii* is a necrotrophic pathogen and oxalic acid is principle metabolite of the pathogen which is known to play a significant role in pathogenesis (Kirtzman *et al.,* 1977). The oxalic acid sequesters the calcium in the host cell wall thereby favouring the pectic enzymes (polygalecturonase) secreted by the pathogen to hydrolyze the pectate in the middle lamella more rapidly leads to death of the tissue (Gawande *et al*., 2013). Further, the pathogen (*S. rolfsii*) on non-availability of host plants, survives as a saprophyte on plant debris, even on debris from non-host crops and produces the overwintering structures making it less vulnerable to control measures (Punja, 1985).

S. rolfsii has wide host range and certainly there is possibility of variations among the field isolates. Further, to device and for successful implementation of management practices the knowledge of distribution and diversity of the pathogen is essential. The diversity of *S. rolfsii* has been assessed for field populations in United States (Franke *et al*., 1998), Vietnam (Le *et al*., 2012) and Japan (Okabe and Matsumoto, 2000) but, for most other groundnut producing countries including India, the information on the distribution, severity and diversity is scarce or not available.

Although, stem rot disease can effectively be managed by applying the effective fungicides but, this strategy could not be considered as long term solution because of concerns about exposure risks, health and environmental hazards, residue persistence, emergence of fungicide resistance among the pathogens, pollution of ground water and food, development of oncogenic risks and more so greater production cost. Thus, the need for alternative approach to manage the stem rot of groundnut has become vital. Unfortunately there is no such viable, economical and eco-friendly practice currently available to serve this purpose. As a result, in recent years the focus is shifted towards biological control of diseases which could serve as eco-friendly, low cost and a potential component of integrated disease management. Therefore, considerable

attention has been paid to the beneficial rhizosphere antagonistic microflora (fungal and bacterial biocontrol agents) in the management of soilborne diseases (Weller, 1988).

Among the fungal biocontrol agents *Trichoderma* spp. has revolutionized the field of biological control of soilborne plant pathogens and proved to be valuable tool of integrated disease management (Radjacommare *et al*., 2010). Control of *S. rolfsii* infecting groundnut using *Trichoderma harzianum* was reported by several researchers (Muthamilan and Jeyarajan, 1996; Ganesan *et al.,* 2007). Additionally, *S. rolfsii* infecting other crops was also successfully managed by employing *T. harzianum* and *T. viride* (John *et al.*, 2015; Ekundayo *et al*., 2016; Sharma *et al*., 2016)

Rhizobacteria, saprophytic group of bacteria that live in the plant rhizosphere and colonize the root system have been studied as plant growth promoters for increasing agricultural production and as biocontrol agents against many plant diseases (Burris, 1998). Several strains of *Bacillus* spp. are known to suppress soilborne plant pathogens and improve plant health (Shrestha *et al*., 2016). Several workers reported the efficacy of *Bacillus* spp. which employs several mode of action in controlling the *S.rolfsii* infecting groundnut (Shifa *et al.* 2015; Ashok *et al*., 2014; Tonelli *et al*., 2011). Further, there are many reports suggesting the effective control of *S. rolfsii* infecting other crops using *Bacillus* spp. (Chakraborty *et al.*, 2016; Li *et al.*, 2016).

Understanding the mechanisms of biological control of plant diseases through the interactions between antagonists and pathogens may allow us to select and construct the more effective biocontrol agents and to manipulate the soil environment to create a conducive condition for successful biocontrol. The mechanisms of biocontrol may involve antibiosis, competition, mycoparasitism, production of cell wall degrading enzymes and volatile compounds and most important being induced resistance (Lo, 1998).

On the other hand, the induced systemic resistance (ISR) in which the plant's own defense mechanisms are induced by prior application of biotic and abiotic inducers has become a novel plant protection strategy to manage plant diseases (Tuzun, 2001; Kashyap and Dhiman, 2009) and this marked the greatest growth area in biocontrol in the last few years. Plants treated with biocontrol agents have latent defense mechanism against pathogens which can be systemically activated upon exposure of plants to stress or infection by pathogens (Bakker *et al.,* 1987). A wide variety of root-associated mutualists, fungi, including *Trichoderma* spp. (Pieterse *et al*., 2014; Solanki *et al*.,

2011; Gajera *et al*., 2015) and many strains of *Bacillus* spp. (Tonelli *et al*., 2011; Liang *et al*., 2011; Chakraborty *et al.*, 2015) sensitize the plant immune system for enhanced defense against *S. rolfsii* infecting groundnut and other crops without directly activating costly defenses. The mechanism operates through the activation of multiple defense compounds at sites distant from the point of pathogen attack (Dean and Kuc, 1985). These induced defense responses are regulated by a network of interconnecting signal transduction pathways in which salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play key roles (Dicke and van Poecke, 2002; Glazebrook, 2001; Thomma *et al.*, 2001). Recent investigations on ISR mediated resistance by plant growth promoting fungi and bacteria demonstrated that several strains protect the plants from soilborne pathogens attack by strengthening the epidermal and cortical cell walls with deposition of newly formed barriers beyond infection sites including callose, lignin and phenolics (Sudhagar *et al.*, 2000; Gajera *et al.*, 2014; Sarma and Singh, 2003) and by activating defense genes encoding peroxidase, polyphenol oxidase, catalase, chitinase, β-1,3-glucanase, and phenylalanine ammonia lyase (Muthukumar *et al*., 2011; Solanki *et al*., 2011; Tonelli *et al*., 2011; Gajera *et al*., 2015;).

On the other hand, some microorganisms as biological control agents have a relatively narrow spectrum of activity compared with commercial fungicides and often exhibit inconsistent performance in practical agriculture, resulting in limited commercial use of biocontrol agents to manage the plant pathogens. Most of the approaches for biocontrol of plant diseases have used single biocontrol agent as antagonists to a single plant pathogen, this partially accounts for the inconsistent performance by biocontrol formulation which is not likely to be active in all dynamic soil environments in which they are applied against all plant pathogens that attack the host plant (Guetsky *et al*., 2001). Thus, more emphasis is laid on the combined use of several biocontrol agents against soilborne pathogens with several mechanisms of control which turned out successful than either of them alone as reported by several workers (Senthilraja *et al.*, 2010: Thilagavathi *et al*., 2007). Further, development of suitable formulation and mixing of chitin with the *Bacillus* spp. (Ahmed *et al.*, 2003; Kishore *et al.*, 2005), or *Trichoderma* spp. (Solanki *et al.*, 2011; Loganathan *et al.*, 2010) has also been found to increase their biocontrol efficacy.

With the consideration of the above facts, the following objectives were formulated to obtain the substantial information on diversity of *S. rolfsii* and to evolve efficacious biocontrol agent bioformulations against stem rot of groundnut.

1. Survey and collection of isolates of *Sclerotium rolfsii* from major groundnut growing regions of India.
2. Morphological and molecular characterization of *S. rolfsii* isolates.
3. Efficacy of selected biocontrol agents against *S. rolfsii* under laboratory conditions
4. Characterization of induced systemic resistance (ISR) capacity of potential biocontrol agents in groundnut against *S. rolfsii* under glasshouse conditions.
5. Evaluation of potential biocontrol agents for effective management of stem rot of groundnut under glasshouse and field conditions.

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

Groundnut (*Arachis hypogaea* L.) is a major oilseed crop grown worldwide. In India Gujarat, Tamil Nadu, Andhra Pradesh, Telangana, Karnataka, Maharashtra, Rajasthan and Madhya Pradesh are major groundnut growing states which accounts for 4.68 million ha area under cultivation (USDA, 2016). Of the numerous diseases occurs on groundnut, stem rot caused by *Sclerotium rolfsii* Sacc. is an economically important disease. The disease causes yield loss of 30 per cent, under congenial environment the losses scales up to 80 per cent (Mehan *et al*., 1994). In the recent past the incidence of disease in groundnut was increasing year by year and has taken the heavy toll resulting in uneven stand of the crop, loss of plant population and subsequently the yields. The present studies were undertaken to investigate on the distribution, severity, diversity and to develop sustainable strategy to manage the *S. rolfsii* menace in groundnut. The literature reviewed on various aspects pertaining to objectives are given here under.

2.1. Distribution

Stem rot of groundnut is also known as root rot, southern blight, white mould, sclerotium rot and sclerotium wilt. The disease is widely distributed and rampant in the tropics and subtropics where temperatures are sufficiently high to permit the growth and survival of the fungus. The pathogen rarely occurs where winter temperature falls below 0°C average. Globally, this pathogen occurs in the tropical, subtropical and warm temperate regions of Central, North and South America, Australia, Southern Europe, Africa, Asia, Hawaii, Japan, China and Central Vietnam (Roberts, 2003). In India, it is wide spread in almost all the states especially in Gujarat, Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh. Irrigated groundnut crops grown in post rainy and summer seasons in India are often infected by the pathogen (Narendra Kumar *et al*., 2013). In India, stem rot of groundnut was first recorded by Butler and Bisby (1931).

2.1.1. Economic importance

The yield loss up to 75-80 per cent has been reported in New Mexico (Aycock, 1966). Yield losses were usually range from 10 to 25% in India, Thailand, Indonesia, Taiwan, and the Philippines but may reach 80% in severely infested fields (Mayee and Datar, 1988). About 20-60% of pod yield reduction was observed due to pod rot in

widely cultivated varieties JL 24, KRG 1, Dh 40, and TMV 2 in Karnataka and Andhra Pradesh (Anonymous, 1992).

2.1.2. Host range

The *S. rolfsii* has a very extensive host range including field (rice, maize, wheat, barley, groundnut, sunflower, pigeon pea, chickpea etc), vegetable (tomato, potato, chilli, capsicum, brinjal, cucurbits, onion, carrot etc), flower (chrysanthemum, crossandra, marigold etc), fruits and ornamental crops (Mordue, 1974; Singh and Allen, 1979; Wydra, 1996). Disease caused by this pathogen lead to heavy losses in vegetable crop especially during the wet season when weather conditions are favourable for both crop production and for the growth and dissemination of the sclerotia of the pathogen (Wokocha *et al*., 1986). These sclerotia are the primary inoculum of the pathogen and the sole organs by which the fungus survives adverse environmental conditions, awaiting susceptible host for infection when favourable conditions return (Wokocha *et al.*, 1986; Okabe *et al*., 1998).

2.1.3. Pathogen

The *S. rolfsii* Sacc. (telomorph: *Athelia rolfsii* Tu and Kimbrough) is a well-known polyphagous, ubiquitous, non-target, necrotrophic soil-borne fungal pathogen (Punja, 1985). This was first reported by Rolfs (1892) as a causal agent of southern blight of tomato blight in Florida. Later Saccardo (1911) named the fungus as *Sclerotium rolfsii*. In India, Shaw and Ajrekar (1915) isolated the fungus from rotten potatoes and identified as *Rhizoctonia detruens*. Later, Ramakrishnan (1930) confirmed that the fungus involved was *S. rolfsii*. Higgins (1927) worked in detail on physiology and parasitism of *S. rolfsii*. However, its perfect stage was first studied by Curzi (1931) and proposed generic name as *Corticium*. Further, in India Mundkur (1934) successfully isolated the perfect stage of *S. rolfsii*. The fungus produces white cottony mycelial growth on potato dextrose agar medium and the colony will be of was compact or fluffy. Initially, it produces white colored sclerotia later their color changes from white to off-white, light brown and dark brown as they attained maturity (Punja, 1985).

2.1.4. Disease Symptoms

The primary symptoms of stem rot of groundnut are browning and wilting of leaves and branches which are still attached with the plant. The fungus preferentially

infects stem by forming a whitish mycelial mat around the stem, but it can also infect any part of the plant including root, leaf and pod. In heavy soils, fungus damages groundnut plants near the soil surface but in light soils it can reach up to pod level, causing severe damage to pegs and pods (Mehan and McDonald, 1990). When the bark of infected pods is peeled off, the inner tissue shows a brown to yellow discoloration. Leaves of infected plants turn brown, dry and often remain attached to the dead stem. Drying or shrivelling of the affected branches ultimately lead to death of the complete plants after wilting (Narendra Kumar *et al.,* 2013).

2.1.5. Disease cycle and dissemination

The pathogen survives as a saprophyte on plant debris, even debris from non-host crops. Sclerotia survive well (3-4 years) at or near the soil surface but survive poorly when buried deep because the fungus has a high oxygen demand (Mehan *et al*., 1994). The hyphal growth resumes from infected tissues and germinating sclerotia in the presence of volatile compounds from decaying organic matter under warm and moist conditions. When hyphae come into contact with stem tissues, direct penetration occurs, but wounds facilitate infection (Punja, 1985). Hyphae may be intracellular or intercellular. Any part of the groundnut plant that comes in contact with the soil is infected with fungus. In warm and high moisture condition, the occurrence of stem rot usually coincides with early stages of peg and pod development. Stem rot develops at all the growth stages (10-90 days) but disease development is slows down as the plants grow older (Pande *et al*., 1994). The linear growth of hyphae towards healthy plants and spread of sclerotia through irrigation water and agricultural implements serves as Secondary source of inoculums.

2.1.6 Isolation and maintenance of the pathogen

The *S. rolfsii* can be isolated from different plant parts, *viz.,* collar (Rajalakshmi, 2002; Goud, 2011) and stem (Kumar and Sen, 2000) region of the affected portion of the plant tissue. The medium Potato dextrose agar (PDA) was found to be the best supporting medium for isolation of *S. rolfsii* (Naidu, 2000; Siddanagoudar, 2005; Raoof *et al.*, 2006; Shukla, 2008; Ozgonen, 2010; Rakh, 2011). Narain and Mishra (1979) opined that, the malt extract agar supported the production of numerous and bigger sized sclerotia by isolates of *S. rolfsii* collected from ragi. Further, *S. rolfsii* can also be maintained on potato sucrose agar medium (Ramarao and Usharaja, 1980).

2.1.7 Pathogenicity tests

To prove pathogenicity of *S. rolfsii* the pathogen was inoculated artificial through different inoculation methods. The soil infestation method was employed by Dange (2006), and Datur and Bindu (1974). Seedling root dip inoculation method was used by Chowdary (1997) to induce sclerotial wilt in bell pepper. Datur and Bindu (1974) prepared the inoculum of *S. rolfsii* by culturing the fungus on sterilized maize bran medium and mixed with the sterilized soil one week before sowing and they observed the typical symptoms of *S. rolfsii* on sunflower within a week of inoculation which was identical to those produced in the field. Further, Siddaramaiah and Chandrappa (1988) proved the pathogenicity of *S. rolfsii* on cardamom in pot culture studies by inoculating the sclerotial cultures which was grown on sand corn meal medium and observed the symptoms a week after inoculation. Similarly, Kulkarni *et al.* (1994) while studying the most susceptible growth stage of groundnut to *S. rolfsii* through artificial inoculation with inoculum prepared on sterilized maize bran medium and observed maximum mortality in 15 days old plants and the least mortality in 105 days old plants. Likewise, artificial inoculation of the groundnut plants with inoculum prepared on sorghum grain medium (SGM) to prove pathogenicity of *S. rolfsii* isolates was studied by Senthilraja *et al.* (2010) and Pande *et al.* (1994).

2.2. Survey to assess the incidence of stem rot of groundnut

In India, the stem rot pathogen occurs in all most all states, *viz.,* Karnataka, Andhra Pradesh, Telangana, Gujarat, Maharashtra and Tamil Nadu, Madhya Pradesh and Rajasthan (Narendra Kumar *et al*., 2013). Average of 7.80 and 11.30 per cent incidence of stem rot caused by *S. rolfsii* in different groundnut varieties was reported in Dharwad district (Karnataka) during *kharif*, 1975-76 and 1976-77 respectively (Siddaramaiah *et al.,* 1979). Further, Ramakrishna and Kolte (1988) reported the average incidence of pod rot of about 15-30 per cent in major groundnut growing areas of India. In Georgia, due to Southern stem rot (*S. rolfsii*) of groundnut losses to the extent of $ 30.6 and $ 37.5 million in 1987 and 1988 respectively have been reported (Brenneman *et al.,* 1990; Singh, 1987). Likewise, the pod yield loss of 10-25 per cent, but could reach over 80 per cent in heavily infected fields was noted by Porter *et al.* (1982), Mehan and McDonald (1990) and Bowen *et al.* (1992). Hanumanthegowda (1999) carried out survey on stem rot of groundnut during *kharif* 1998-99 and rabi/summer 1998-99 in

Dharwad, Belgaum and Haveri districts and reported a maximum disease incidence of 12.57 and 8.68 per cent in rainfed and irrigated fields, respectively.

A survey was conducted in farmers' fields during 1999 rainy season in major groundnut growing districts of Andhra Pradesh, Karnataka and Tamil Nadu revealed that, incidence of stem rot is low during seedling stage (0-4%), moderate to high during flowering and pod formation stage (9-16%) and very high incidence of >20 % was observed at maturity stage (Pande and Rao, 2000). Similar trend in incidence of stem rot in groundnut fields in Andhra Pradesh during *kharif* 2012 and 2013 was reported by Divya Rani *et al.* (2016). Field survey conducted in the Marathwada region of Maharastra, India, during 2003 revealed the 17.3 % average incidence of disease and the cultivar JL 24 recoded higher incidence compared to local cultivars (Kadam *et al*., 2011). Prevalence of stem and pod rot with 27% or more yield loss in all groundnut growing states of India, particularly very severe in Maharashtra, Saurashtra region of Gujarat and Raichur area of Karnataka was recorded by Ghewande *et al.* (2002). Further, Muthukumar and Venkatesh (2013) reported the 32.33 to 14.00 per cent incidence of Sclerotium wilt in major pepper mint growing regions of Tamil Nadu. Likewise, Mahato and Mondal (2014) recorded varied range of per cent incidence of stem rot caused by *S. rolfsii* from West Bengal in different crops during 2012-13 *viz.*, groundnut (0.88-1.55), tomato (0.45–3.60), brinjal (0.62–6.14), potato (0.12–4.52) and elephant foot yam (1.62–5.25).

2.3. Production of metabolites

Stem rot pathogen *S. rolfsii* was found to release oxalic acid in the host system as reported by Higgins (1927), Kirtzman *et al.* (1977), Mahadevan (1979), and Ansari and Agnihotri (2000). The pathogen was known to secrete oxalic acid *in vitro* (Higgins, 1927) and its toxicity to plants was also confirmed by Baudin (1956) and Maxwell and Bateman (1968). Further, Higgins (1927) found that, the oxalic acid was the principal toxic agent produced in the culture filtrates of *S. rolfsii* and it was responsible for the death of host cells. In addition, Mahadevan and Sridhar (1986) observed the production of oxalic acid by *S. rolfsii* enabled the pathogenesis by promoting the activity of poly-galacturononase and by creating an acidic environment in plant tissues, which inactivated the prohibitins and phytoalexins and thus suppresses the host resistance.

Degradative enzymes produced by *Sclerotium* spp. were thought to be important for infection but later oxalic acid production was found to play key role in their pathogenicity (Goday *et al.,* 1990). Further, Dickman and Chet (1998) identified oxalic acid as an organic acid required by *S. rolfsii*. Additionally, oxalic acid plays an important role in pathogenicity of *S. rolfsii* and virulence of different crop isolates was positively correlated with amount of oxalic acid produced by them under *in vitro* conditions (Gawande *et al*., 2013; Saraswathi and Madhuri, 2013).

2.4. Standardisation of inoculum levels for optimum infection under glasshouse conditions

Cultivars of groundnut with resistance or tolerance to stem and pod rot are needed, but screening for resistance in the field is complicated by the non-uniform spatial distribution of sclerotial inoculum (Shew and Beute, 1984). Further, consistent, reliable data to confirm the resistance of cultivars or breeding lines and to select the best treatment (bioagents) in management of disease are difficult to obtain in field with natural infestations of *S. rolfsii*. Thus, evaluation of different bioagents for management of stem rot and evaluation of genotypes for resistance would be more reliable if the information on level of inoculum needed for optimum infection is available.

Pande *et al*. (1994) evaluated the different inoculation techniques with different inoculum level (1 to 15g of inoculum per 15 cm plastic pot) spread on the soil and found the 15g inoculum spread on the soil surface and covered with leaf debris was most effective for screening groundnut germplasm against *S. rolfsii* under greenhouse conditions. Further, Shokes *et al*. (1996) observed the inoculum of *S. rolfsii* multiplied on sterilized oat seed and placed on the soil near the base of each central stem (20 g/8”pot) in the glasshouse and in the centre of rows (a full 150 ml beaker of inoculum per 2 m^2 row) in the field was found most effective level of inoculum for conducting glasshouse and field experiments.

Similarly, Sennoi *et al.* (2012) tested four inoculum densities of 1, 2, 3 and 4 of *S. rolfsii* infested sorghum seeds/plant using 10 Jerusalem artichoke genotypes and found that, the plants inoculated with four sorghum seeds had the highest disease incidence. Likewise, Muthukumar and Venkatesh (2013) observed that, the 5% inoculum load of *S. rolfsii* induced highest disease incidence in Peppermint.

2.5. Variability of *S. rolfsii*

2.5.1. Cultural and morphological variability

The fungus *S. rolfsii* has wide host range and certainly there is possibility of variations among the field isolates and was reported by various previous researchers. Jyothi (2006) reported the wide variation among the isolates of *S. rolfsii* collected from different crops species with respect to colony diameter (52.00 to 89.83 mm at 72 hours of incubation), colour (light to dark brown), size (1.3 to 3.40 mm), and shape (spherical to round) and dry mycelial weight (132.70 to 280.70 mg). Kokub *et al.* (2007) revealed the variation in the growth rate (0.86-1.35 mm/hr), sclerotial shape and diameter (0.5-2.0 mm) of eight isolates of *S. rolfsii*. Similarly, Hussain *et al.* (2010) reported the difference in morphological characters of *S. rolfsii* such as radial colony growth (fast growing, intermediate, and slow growing), size of sclerotia and number of sclerotia (higher producer, intermediate, and least producers). Similar kind of observations with respect to sclerotial characteristics among isolates of *S. rolfsii* collected from cowpea and tomato was reported by Okereke and Wokocha (2007), groundnut by Palaiah and Adiver (2004), and Colocasia by Tortoe and Clerk (2012).

Sulladmath *et al.* (1977) observed that, the weight of hundred sclerotia from groundnut isolate was five to eight times higher than other isolates collected from pigeonpea, sunflower and wheat; consequently, the number of sclerotia produced was less than remaining crop isolates. Similarly, Manjappa (1979) found variation among the different isolates of *S. rolfsii* collected from sunflower, groundnut, wheat, redgram, tomato, niger, lucerne and tamarind with respect to rate of growth, time taken form sclerotial initiation, size, number, weight of sclerotia and the virulence of pathogen. Likewise, variation in growth rate, sclerotial production and frequency of clamp formation among field isolates of *S. rolfsii* collected from 10 fields of California was reported by Punja and Grogan (1983).

Punja and Damiani (1996) recorded the differences in culture and morphological characteristics (colony type, sclerotial formation and size of sclerotia) in three different species of *Sclerotium, viz., S. coffeicola, S. rolfsii* and *S. delphini* collected from diverse geographical areas. Likewise, Ansari and Agnihotri (2000) reported the variation among 40 isolates of *S. rolfsii* collected from different soybean growing areas in India with respect to sclerotial arrangement, size and colour. Further, Sarma *et al.* (2002) recorded the wide variability among 26 isolates of *S. rolfsii* collected from various host and

localities in India with respect to colony morphology (fluffy/compact), mycelial growth rate, sclerotium formation (80-500 sclerotia/plate), teleomorph production and sclerotial size (1.0-2.2 mm) as well as colour (dark to reddish brown).

Prasad *et al.* (2010) noted the variation in cultural and morphological characters among the isolates of *S. rolfsii* collected from major groundnut growing areas of Andhra Pradesh with respect to colony type (fluffy/compact), growth rate (62.7-90 mm), days to form sclerotia (9-18 days) and size (0.90-2.2 mm). Similarly, Le *et al.* (2012) collected isolates of *S. rolfsi* from different crops like tomato, groundnut and taro, and reported the considerable variation among 103 isolates with respect to the days to form sclerotia, days to maturation, number (79-1080) and size (0.88-2.24 mm). Likewise, Rasu *et al.* (2013) reported the variability among *S. rolfsii* isolates from (different hosts) Tamil Nadu with regard to colony type (fluffy/compact), growth rate (slow growing, fast growing and intermediate), number of sclerotia, dry weight of 100 sclerotia and sclerotial colour (dark to light brown). Additionally, another report of Kumar *et al.* (2014) revealed the considerable morphological variations among isolates of *S. rolfsii* collected from groundnut growing areas of Rayalaseema with reference to sclerotial colour (light brown to dark brown), presence or absence of sclerotia, number, 100 sclerotial weight (2.4 to 17 mg) and pattern of sclerotia produced in petri dish (central/peripheral).

The variation in the dimensions of sclerotia of *S. rolfsii* from different hosts was reported by various investigators *viz.,*

References	Host	Sclerotial diameter (mm)
Wolf (1914)	Groundnut	0.50-0.85
Miller (1929)	Lippia	0.80-2.01
Palo (1933)	Mango	2.00-5.00
Dastur (1935)	Betel vine	1.00-1.50
Rayes (1937)	Groundnut	0.60-1.50
Singh and Srivastava (1953)	Tomato	0.20-2.40
Celino (1936)	Cotton	0.00-2.50
Foucart (1954)	Chrysanthemum	0.25-2.00

2.5.1.1. Mycelial compatibility

The mycelial compatibility indicates the extent of genetic diversity present among the field population of *S. rolfsii.* The studies on mycelial compatibility of *S. rolfsii* isolates were reported by many early researchers. Sarma *et al.* (2002) grouped the 26 isolates of different crop into 13 MCGs based on antagonistic reaction between the

isolates. Similarly, eight fungal strains of *S. rolfsii* isolated from diseased chickpea plants collected from different growing regions of Pakistan were subjected to mycelial compatibility reaction and were categorized into 2 MCGs by Kokub *et al*. (2007). Further, Punja and Sun (2001) conducted mycelial compatibility analysis of 132 isolates of *S. rolfsii* from 13 countries and differentiated into 71 mycelial compatibility groups (MCG). They further observed that, the isolates from close geographical locations were grouped into one MCG or *vice versa*. Likewise, Le *et al.* (2012) reported similar kind of variation among 103 isolates of *S. rolfsii* collected from major groundnut growing areas of Central Vietnam. They further noted that, the grouping of isolates into different MCGs was in part correlated with their geographical origin, whereas there was no correlation found between MCGs and virulence of the isolates.

2.5.2. Molecular variability

It is important to study the genetic variability of *S. rolfsii* isolates to understand their ecology and diversity in the field. The molecular variability among *S. rolfsii* populations from different geographical regions was demonstrated by Adandonon *et al.* (2005), Harlton *et al.* (1995), Okabe *et al.* (1998), Okabe and Matsumoto (2000), Kokub *et al.* (2007), Saude *et al.* (2004), Thilagavathi *et al.* (2013) and Sarma *et al.* (2002). Genetic variability of *S. rolfsii* in South Africa was studied by Cilliers *et al.* (2000) using ITS-AFLP and revealed the polymorphisms of 10 to 36 per cent. The RAPD analysis clearly showed genotypic differences of 22% among the *S. rolfsii* population. Further, they observed that, in the phylogenetic analysis the grouping of isolates were not in accordance with their geographical location and even with their virulence.

Similar kind of study was conducted by Punja and Sun (2001) who studied the genetic relationships among 132 isolates of *S. rolfsii* collected during 1967-97 from 36 different host species over a wide geographical range representing 13 countries using RAPD. They observed that, the most of the isolates exhibited least polymorphism in RAPD banding pattern and were assumed to clonally derived. Further, remaining isolates exhibited wide genetic variation with respect to genetic diversity. They finally concluded that, though the isolates of *S. rolfsii* were diverse at genetic level, the diversity cannot be correlated with the isolates aggressiveness and geographical origin.

Likewise, Almeida *et al.* (2001) confirmed the molecular identity of 30 isolates of *S. rolfsii* collected from different hosts and regions of Brazil using ITS-rDNA sequencing. The results showed that, the ‘ITS types’ within isolates were almost phylogenetically distinct but there was no clear correlation found between ITS based

phylogeny and isolate origin. They further studied the intra-specific genetic diversity among the *S. rolfsii* isolates using RAPD and revealed the higher extents of genetic diversity among isolates. Interestingly they found no defined correlation between the morphological variability with the molecular variability of isolates.

Further, Prasad *et al.* (2010) recorded the ITS-rDNA region amplified product of approximately 650 to 700 bp in all the isolates of *S. rolfsii* collected from Andhra Pradesh. In the genetic diversity study of isolates *S. rolfsii* using five RAPD primers they recorded the total of 221 reproducible and scorable polymorphic bands ranging approximately as low as 100 bp to as high as 2500 bp. Further, UPGMA cluster analysis of isolates revealed the wide genetic diversity among the isolates. In similar lines, Le *et al.* (2012) observed the ITS-rDNA region amplified product of approximately 680 bp in 103 randomly selected *S. rolfsii* field isolates collected from Central Vietnam and ITS-rDNA region sequencing and phylogeny revealed the three ITS groups in which majority of the isolates (*n*=90) grouped in one ITS group indicating genetic uniformity among the field isolates. They further did not find correlation between the morphological and molecular diversity of isolates.

Additionally, generation of 129 polymorphic bands in 17 different isolates of *S. rolfsii* using 11 RAPD primers was recorded by Gawande *et al*. (2013), they further observed relatively wide genetic diversity among the isolates as revealed by similarity matrix and UPGMA cluster analysis. Similarly, Rasu *et al.* (2013) studied genetic diversity of 10 isolates of *S. rolfsii* using five RAPD primers. They further observed that, the isolates of *S. rolfsii* had about 54% similarity coefficient indicating that they were genetically varied by their unique banding patterns. However, the most of the isolates shared more number of common bands and clustered together indicating their relative genetic uniformity. Alike, Parvin *et al.* (2016) studied the genetic diversity of eight isolates of *S. rolfsii* collected from Bangladesh using three RAPD primers (OPB 07, OPC-01, and OPF-15) and reported the co-efficient of gene differentiation (Gst) as 1.0 reflecting the existence of high level of genetic variations among the isolates. They further noted no linearity between cultural and morphological variability of isolates with their genetic diversity.

2.6. Sensitivity of *S. rolfsii* to commonly used fungicides

The studies on sensitivity of *S. rolfsii* isolates to commonly used fungicides gives an idea about the relative effectiveness of the fungicides against them. The fungicide sensitivity of more than 450 isolates of *S. rolfsii* from 11 different peanut fields in

Georgia to Tebuconazole, Flutolanil, and PCNB revealed that the most of the populations sampled were significantly more sensitive than the populations that had the longest exposure to the fungicides indicating effectiveness of fungicides (Franke *et al.*, 1998).

Similarly, Johnson and Subramanyam (2000) reported the sensitivity of isolates of *S. rolfsii* to Triazole group of fungicides whereas the same isolates were found comparatively tolerant to Carbendzim. Likewise, Mahato *et al.* (2014) reported the least efficacy of Carbendazim and Thiram in inhibiting the growth of *S. rolfsii* isolates. Further, Mohanty *et al.* (2016) also reported the similar results. Additionally, Bhagwan (2010b) revealed the effectiveness of PCNB, Tebuconazole, Hexaconazole and Propiconazole against the isolates of *S. rolfsii*. On the other hand the fungicides like Mancozeb, Thiram, Matalaxyl were found least effective against *S. rolfsii.*

2.7. Efficacy of rhizhosphere microorganisms against *S. rolfsii*

As seeds germinate and roots grow through the soil, the loss of organic material provides the driving force for the development of active microbial populations around the root, known as the rhizosphere effect (Whipps, 1992). Beneficial microorganism present in the rhizhosphere restrict the growth of soil borne pathogens, they produce antifungal substances, act as mycoparasite against the pathogenic fungi and secrete the lytic enzyme (Weller, 1988). Arun Arya and Mathiew (1993) study on rhizhosphere microflora of pigeon pea revealed that, out of 14 fungal species isolated from rhizosphere soils, three genera belonged to Zygomycetes, three of Ascomycetes and five of Deuteromycetes indicating the number and quality of fungi present in the rhizhosphere soil. Similarly, Pandey and Upadhyay (2000) reported the antagonistic interaction of *S. rolfsii* with different species of *Aspergillus*. The highest antagonistic activity of *Trichoderma* and *Aspergillus* sp. present in groundnut rhizhosphere against *S. rolfsii* and *Rhizoctonia batiticola* was observed by Thakare *et al*. (2002).

2.7.1. Fungal antagonists (*Trichoderma* spp.)

Trichoderma spp. has revolutionized the field of biological control of soilborne plant pathogens (Radjacommare *et al*., 2010). Biological control through induction of antagonists to the soil was attempted by Hartley in 1921 against damping off of coniferous seedling. Later, Millard and Taylor (1927) used this technique against common scab of potato. The potential use of fungal antagonists as biocontrol agents against plant diseases was suggested by Weindling (1932) who demonstrated the

parasitic activity of members of the genus *Trichoderma* for the first time against pathogen such as *Rhizoctonia solani*. Further, Sundheim (1977) reported that, application of *T. harzianum* to soil at sowing was better than other treatments including seed treatment. The use of antagonistic fungi, especially *Trichoderma* spp. and *Gliocladium* spp. has been more extensive than their bacterial counterparts as reported by Haran (1995), Babu and Seetharaman (2002), Ganesan (2004), Ganesan and Sekar (2004a), Ganesan and Sekar (2004b) and De Souza *et al.* (2008).

Control of *S. rolfsii* using *Trichoderma harzianum* was reported by Muthamilan and Jeyarajan (1996), Ganesan (2004), and Ganesan and Sekar (2004a). Likewise, Muthamilan and Jeyarajan (1996) observed the effective control of the root rot of groundnut by combined application of *T. harzianum*, *Rhizobium* with fungicide Carbendazim. In similar lines Ganesan *et al*. (2007) integrated *Rhizobium* and *Trichoderma harzianum* (ITCC 4572) in management of stem rot disease of groundnut caused by *S. rolfsii*. Further, the efficacy of several microorganisms, including bacteria, actinomycetes, a mycorrhizal fungus, and *Trichoderma* spp. against *S. rolfsii* under *in vitro* conditions was reported by Punja (1985). Additionally, the *Trichoderma* spp. have been found antagonistic to *S. rolfsii* (Elad *et al*., 1980) and were successfully used for the management of *S. rolfsii* in several crops under glasshouse and field conditions (Mathur and Sarbhoy, 1976; Elad and Chet, 1983).

The *Trichoderma* spp. are ubiquitous in the soil environment and are being successfully used and commercialized to combat a broad range of phytopathogenic fungi such as *Rhizoctonia solani*, *Pythium ultimum*, and *Botrytis cinerea* (Fravel, 2005; Hjeljord *et al*., 2000). In addition, the *Trichoderma* spp. can directly impact other fungi after sensing a suitable fungal host. Further, *Trichoderma* spp. respond with the production of antibiotic compounds, formation of specialized structures, and degradation of the host's cell wall, followed by the assimilation of its cellular content, a process known as mycoparasitism (Benitez *et al*., 2004; Chet and Chernin, 2002).

In similar lines, Srinivasulu *et al*. (2005) employed *Trichoderma* spp. in management of collar rot pathogen in elephant foot yam and Elad and Chet (1983) for control of *S. rolfsii* and *R. solani* in cowpea. Further, Anand Singh and Harikesh Bahadur Singh (2005) found different isolates of *Trichoderma* and two isolates of *Gliocladium virens* highly antagonist against *S. rolfsii* under *in vitro* conditions. They further found the effectiveness of these isolates in the management of collar rot of mint

(*Mentha arvensis*) caused by *S. rolfsii* under glasshouse and field conditions with significant increase in herb and oil yield. Additionally, Ekundayo *et al*. (2016) recorded the effective control of stem rot of tomato caused by *S. rolfsii* and enhanced growth promotion in the crop by employing *Trichoderma viride* under glasshouse and field. Likewise, Mukherjee *et al*. (2014) isolated *T. harzianum* strain CICR-G from tree-pathogenic *Ganoderma* sp. and found it as the highly effective strain against *S. delphinii* infecting cultivated cotton.

Similar study was reported by John *et al.* (2015) who recorded the effective suppression of collar rot of Amorphophallus caused by *S. rolfsii* by applying strains of *Trichoderma harzianum* (Tr9) and *Trichoderma asperellum* (Tr10). Similarly, Sharma *et al*. (2016) employed talc formulation of *T. harzianum* (Th3) through Seed treatment and soil application against stem and root rot complex disease of groundnut caused by *S. rolfsii* and *Macrophomina phaseolina* across various locations in India under field conditions and noted least average disease incidence of 21.6 per cent in all the field locations. They further observed the enhanced plant growth promoting attributes in groundnut by *Trichoderma* application. Likewise, effectiveness of three *Trichoderma* strains *viz., T. virens, T. viride,* and *T. harzianum* against *Aspergillus niger,* the collar rot disease causal organism in groundnut was recorded by Gajera and Vakharia (2010) and Gajera and Vakharia (2011).

2.7.2. Mode of action of fungal antagonists against *S. rolfsii*

Antagonism of *Trichoderma* spp. against the fungal pathogens may be accomplished by competition, parasitism and production of antibiotics or by a combination of these modes of action (Whipps, 1992) and the reviews pertaining to this are presented hereunder.

2.7.2.1. Lytic enzymes in disease suppression

. Parasitism involves the production of several hydrolytic enzymes that degrade cell walls of pathogenic fungi (Elad and baker, 1985). The importance of β-1,3-glucanase and chitinase as key enzymes responsible for fungal cell and sclerotial wall lysis and degradation has been reported (Cook and Baker, 1983). These enzymes have been shown to be produced by several antagonistic fungi and may be an important factor in biological control (Elad and Chet, 1983).

The direct mycoparasitic activity of *Trichoderma* species has been proposed as one of the major mechanisms for their antagonistic activity against phytopathogenic fungi (Baker, 1987; Chet, 1987; Chet, 1990). *Trichoderma* spp. attach to the host hyphae by coiling, hooks or apressorium like structures and penetrate the host cell walls by secreting hydrolytic enzymes such as a basic proteinase (Geremia *et al*., 1993), *β*-1,3-glucanase and chitinase (Elad and Chet, 1983). Elad *et al.* (1980) observed the interaction between *T. harzianum* and *S. rolfsii* by using the scanning electron microscope and fluorescent microscope, they further observed that the lysed sites and penetration holes appeared on the hyphae of the pathogenic fungi following removal of parasite hyphae.

Chitin, and *β*-1,3-glucan are the main structural components of most fungal cell walls, and are the basis for the suggestion that hydrolytic enzymes produced by some *Trichoderma* spp. play an important role in destruction of plant pathogens (Chet *et al*., 1981). Seyedi-Rashti (1994) reported on the xylanase production by *T. harzianum*. Elad and Kapat (1999) reported that proteases produced by *T. harzianum* may be directly toxic to germination of the pathogen and also may inactivate its enzymes.

Jackson *et al.* (1991) suggested that products of chitin degradation also regulate chitinase synthesis in *T. harzianum* and *Gliocladium virens* and were also found that the production of chitinase was markedly affected by pH, with the optimum at pH 6.0. Higher levels of chitinase and *β*-1,3-glucanase enzyme production by incubation with fresh or dried mycelium of *S. rolfsii* reflected the high content of chitin and glucan in the cell wall of that phytopathogenic fungus. Similarly, Lorito *et al*. (1998) reported a production of endochitinase from *T. harzianum* and has shown antifungal activity against *Sclerotium cepivorum*.

Qualhato *et al.* (2013) reported the production of cell wall degrading enzymes such as *β*-1,3-glucanase, NAGAse, chitinase, acidphosphatase, acid proteases and alginate lyase by *Trichoderma harzianum* and *T. asperellum* when grown in liquid cultures with cell walls of *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* Further these enzymes were found highly toxic against the above pathogens. Similarly, El-Katatny *et al.* (2001) recorded the production of extracellular chitinase and *β*-1,3-glucanase in culture filtrate of *T. harzianum* (T24) and found toxic to phytopathogenic basidiomycete *S. rolfsii*. Likewise, El-Komy *et al.* (2015) reported the production of extracellular chitinase and *β*-1,3-glucanase by *Trichoderma* isolates.

Further, these lytic enzymes were found responsible for the antagonistic capacity against *Fusarium oxysporum* f. sp. *lycopersici* in tomato.

2.7.2.2. Hyphal interaction in disease control

Coley-Smith and Cooke (1971) first reported the clamydospore production by *T. hamatum* invading sclerotia of *S. delphinii*, whereas Henis *et al.* (1982) first reported on clamydospore production by *T. harzianum* in sclerotia of *S. rolfsii*. Degraded sclerotia became dark in colour, soft, empty and disintegrated even under slight pressure. Saravanakumar (2002) observed the zone of interaction between *T. harzianum* and *S. rolfsii* which revealed the hyperparasitism of antagonist on the test pathogen. *T. harzianum* also produced several knob like structures and pegs, which entered into the hypae of *S. rolfsii*. This may result in disorganization or digestion of protoplasm contents or directly penetrated the hypae of *S. rolfsii*. Similarly, Elad and Chet, (1983) observed the formation of coiling hooks and appresoria by *T. harzianum* while attacking the hyphae of *S. rolfsii.* Further, Henis *et al.* (1982) recorded the penetration of *Trichoderma* into the rind and cortex of sclerotia and lead to its lysis. Likewise, Singh *et al*. (1999) noticed destructive parasitism *i.e.*, coiling around hypae with direct penetration leading to lysis of fungal hyphae by *T. harzianum* and *Gliocladium virens*.

2.7.2.3. Production of volatile compounds

The inhibition of soilborne plant pathogens by volatile compounds produced by the *Trichoderma* spp. was evidenced by Fravel (1988), Srinivasulu *et al.* (2005), Paramasivan (2006), and Kotasthane *et al.* (2014). The production of different volatile compounds likes alkyl pyrones, ethanol, isobutanol, isoamyl alcohol and isobutyric acid by *T. harzianum* having antagonistic activity against *Sclerotium cepivorum and S. rolfsii* was observed by Fravel (1988). He further noted, among the *Trichoderma* spp. the *T. harzianum* proved very effective in producing volatile antibiotics specific against *S. cepivorum* and *S. rolfsii* followed by *T. hamatum* and *T. viride*. Additionally, the volatile metabolites produced by these *Trichoderma* spp. were both fungicidal and fungistatic.

In similar lines Srinivasulu *et al.* (2005) reported the production volatile substances *T. viride, T. hamatum* and *T. harzianum* against *S. rolfsii* under *in vitro* conditions. Likewise, Kotasthane *et al.* (2014) found the highest antagonism by

Trichoderma viride (T14) against two soilborne plant pathogens *S. rolfsii* and *Rhizoctonia solani*. They further confirmed the antagonistic ability of *T. viride* and attributed that to the 6-Pentyl pyrone, one of the best studied secondary metabolites having both antifungal and plant growth-promoting activities). Similarly, Paramasivan (2006) reported the production of volatile and non-volatile compounds by *T. viride* and *T. harzianum* and were found antagonistic to *S. rolfsii.*

2.7.2.4. Antibiosis in disease suppression

Antibiotics, the low molecular weight (<1 KDa) secondary metabolites produced by antagonists directly inhibited the pathogen growth (Sabitha Doraisamy *et al.,* 2001). Antibiotics have long been suggested to be involved in biocontrol by *Trichoderma* (Weindling, 1932.). Sivasithamparam and Ghisalberti (1998) observed the production of 43 different substances by *Trichoderma* spp. having antibiotic property. Further, Howell (1998) observed the production of alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes, and steroids possessing antibiotic property by *Trichoderma* spp. associated with biocontrol activity against fungal plant pathogens.

Likewise, Jeyarajan and Nakkeeran (1998) characterized *Trichoderma* spp. for the production of antibiotics having antifungal activity. They found that, the *T. viride* produced the antibiotics *viz.*, trichodermin, dermadin, trichoviridin and sesquiterpene heptalic acid, and *T. harzianum* produced trichozianines whereas, *T. hamatum* produced trichoviridin 3 and isonitiin A. The strong antifungal activity of Peptaibol antibiotics, trichozianine A1 and trichozianine B1 produced by *Trichoderma* spp. against fungal pathogens was evidenced by Schirmbock *et al.* (1994) and also noted that, the Trichozianines depolarize the cell membrane by forming voltage gated ion channels and modify membrane permeability of pathogen cell. The production of other antibiotics like peptabiols and broad-spectrum antibiotics such as gliotoxin by *Trichoderma* spp. having antimicrobial activity was reported by Geremia *et al.* (1993), El-Hajji *et al.* (1989), Howell *et al*. (1993) and Pozo e*t al*. (2004).

2.7.2.5. Induced systemic resistance

Beneficial microbes in the microbiome of plant roots improve the plant health. Induced systemic resistance (ISR) emerged as an important mechanism by which selected plant growth–promoting fungi in the rhizosphere prime the whole plant body

for enhanced defense against a broad range of pathogens. A wide variety of root-associated mutualists, fungi, including *Trichoderma*, and mycorrhiza species sensitize the plant immune system for enhanced defense without directly activating costly defenses (Pieterse *et al*., 2014). Inducing the plant's own defense mechanisms by prior application of biotic and abiotic inducers is a novel plant protection strategy (Kashyap and Dhiman, 2009). Fungi belonging to *Trichoderma* genera are the well-known biological inducers and antagonistic towards a number of plant pathogenic fungi (Jyotsana *et al.*, 2008).

The *Trichoderma/Hypocrea* based formulations with chitin (1% v:v) in tomato resulted in enhanced activity of total phenols, peroxidase, polyphenoloxidase and phenylalanine ammonia lyase which lead to effective control of dry root rot of tomato caused by *Rhizoctonia solani* (Solanki *et al*., 2011). Similarly, Gajera *et al*. (2015) observed the enhanced activity of defense enzymes like polyphenol oxidase and phenylalanine ammonia lyase and higher accumulation of pathogenesis related proteins like *β*−1,3-glucanase and chitinase in *Trichoderma viride* (JAU60) treated groundnut plants upon collar rot pathogen inoculation (*Aspergillus niger*). Further, the strain JAU60 of *T. viride* induced increased synthesis of phenolics like gallic, ferulic and salicylic acids upon collar rot pathogen (*A. niger*) inoculation. These phenolics might be synthesized upon activation of PAL by *Trichoderma* in groundnut seedlings under pathogen infestation (Gajera *et al.*, 2014).

In similar lines Kishore *et al*. (2006) found significant elevation of activity of defense enzymes *viz.*, peroxidase and phenylalanine ammonia lyase (PAL) and PR protein activity *viz.*, chitinase and *β*-1,3-glucanase in *Pseudomonas aeruginosa* strain GSE 18 treated groundnut seedlings upon collar rot pathogen (*A. niger*) challenge. Likewise, Loganathan *et al.* (2010) observed that, the combined application of a chitin amended formulation of mixture of *Trichoderma* spp. resulted in increased activities of peroxidase and phenylalanine ammonia lyase, polyphenol oxidase and chitinase against *Sclerotinia sclerotiorum* and *Meloidogyne incognita* in cabbage.

The cumulative effect of combining the fungal and bacterial antagonists in inducing resistance against many fungal plant pathogens was evidenced by Alizadeh *et al*. (2013), Muthukumar *et al.* (2011) and Palani *et al.* (2016).

The significantly higher level of resistance in the form of increased activity of chitinases, *β*-1,3-glucanases, PAL and lipoxygenase in cucumber by combined application of *Trichoderma harzianum* (Tr6), and *Pseudomonas* sp. (Ps14) against *Fusarium oxysporum* f.sp. *radices-cucumerinum* was observed by Alizadeh *et al*. (2013). Similar study was reported by Muthukumar *et al.* (2011) who observed elevated level of activities of defense related enzymes such as PO, PPO, PAL, PR-protein like *β*-1,3-glucanase and accumulation of phenolics in chilli seedlings pre-treated with formulation mixture of *Trichoderma viride* (TVA) and endophytic *Pseudomonas fluorescens* (EBL 20-PF) against chilli damping-off caused by *Pythium aphanidermatum.* Likewise, Palani *at al.* (2016) executed the concept of bioconsortia (seri-bed waste+Pf1+Bs4+Th1+neem cake) against *Fusarium* wilt in mulberry. They further reported that, the reduced incidence of wilt in mulberry was mainly because of enhanced induction of defense enzymes such as PO, PPO, PAL, phenols, catalase and superoxide dismutase.

2.7.2.6. Compatibility of *Trichoderma* sp. with fungicides

In recent years, biocontrol of plant diseases is assuming importance in view of harmful effects of fungicidal control. Fungicides may have deleterious effects on the pathogen as well as the antagonist. Further, an understanding of the effect of fungicides on the pathogen and the antagonist would provide information on the selection of specific fungicides and fungicide resistant antagonists for compatibility studies (Malathi *et al*., 2002).

In these lines, the compatibility of bacterial and fungal biocontrol agent with Azoxystrobin 23 SC was observed by Archana *et al.* (2012), whereas with six commonly used fungicides by Mohiddin and Khan (2013) and with Carbendazim and Thiophenate methyl by Malathi *et al.* (2002). Likewise, Bhagwan (2010a) reported the compatibility of *Trichoderma* with fungicides which were commonly used in integrated management of soilborne diseases of soybean. Further, Dubey *et al.* (2015) observed the compatibility between fungal and bacterial bio-agents, together with fungicide and even with *Mesorhizobium* in integrated management of *Fusarium* wilt of chickpea.

2.7.3. Bacterial antagonists

The majority of work done on biocontrol of plant disease was related to soilborne diseases using either bacteria or fungal antagonists. Among bacteria, several

strains of *Bacillus* sp. are employed for management of various soilborne plant pathogens and was evidenced by the observations of Collins *et al.* (2003), Ganesan (2004), Ganesan and Sekar (2004a), McSpadden and Gardener (2004), Toure *et al*. (2004), Jayaraj *et al*. (2005), Szczech & Shoda (2006), Hu *et al*. (2014), Khabbaz and Abbasi (2014), Zhao *et al*. (2014), Shifa *et al*. (2015) and Shrestha *et al*. (2016).

The rapid increase in initial population densities of the bacterial inoculum in the immediate proximity of the germinating seed increases the probability of establishing the antagonist on individual roots. Further, the bacterial antagonists could also have an advantage over fungal antagonists in suppressing sclerotial fungi due to their rapid multiplication and higher populations in the rhizosphere (Suslow and Schroth, 1982).

The substantial reduction in foot rot of barley caused by *S. rolfsii* was noted by inoculation with strains of *Bacillus subtilis, B. licheniformis, Pseudomonas aeruginosa* and *Streptomyces diastaticus* (Singh and Dwivedi, 1987). Further, Dwivedi (1987) recorded the *in vitro* efficacy of *B. subtilis* and *Pseudomonas aeruginosa* against *S. rolfsii* with 58 and 36 per cent inhibition over control respectively in dual culture. Likewise, Chamswarng and Sangkaha (1988) recorded the enhanced efficacy of *Bacillus* spp. and *Pseudomonas* spp. in controlling tomato stem rot caused by *S. rolfsii* under field conditions.

Abeysinghe (2009) recorded satisfactory control of *S. rolfsii* incidence in chilli through seed bacterization and root bacterization by *B. subtilis* which resulted in maintaining higher numbers of bacteria at the collar region of chilli plants and may have shielded the most vulnerable area from the pathogen, resulting enhanced protection. In similar lines, Shifa *et al.* (2015) noted significant inhibition of *S. rolfsii* by *B. subtilis* (G-1) under *in vitro* conditions and the strain performed equally well in management of stem rot of groundnut in glasshouse and field conditions. They further observed that, the groundnut seedlings treated with the strain were exhibited significantly high root length, shoot length and seedling vigour and subsequently higher pod yield in field.

2.7.4. Mechanisms of *Bacillus* sp. as biocontrol agents

The antagonistic ability of *Bacillus* spp. against the fungal pathogens may be accomplished by competition, production of antibiotics, and inducing plant defense or by a combination of these modes of action (McSpadden and Gardener, 2004) and the reviews pertaining are presented hereunder.

2.7.4.1. Parasitism and production of extracellular enzymes

The production of extracellular lytic enzymes by bacterial antagonists against fungal plant pathogens was well documented by Collins *et al.* (2003), Toure *et al*. (2004), Jayaraj *et al*. (2005), Chernin *et al*. (1995), Chernin *et al*. (1997), Pleban *et al*. (1997), Helisto *et al.* (2001) and El-Gamal *et al*. (2016). The parasitism ability of bacterial antagonists against fungal plant pathogens was noted by Nagarajkumar *et al.* (2007), Rajendran and Samiyappan (2008), Solanki *et al.* (2012), Baysal *et al.* (2008) and Ongena and Jacques (2008).

The production of chitinolytic enzymes *viz.*, chitinases and *β*-1-3-gluconases by *Bacillus cereus* and *Pseudomonas fluorescens* and appear to be involved in biocontrol of *Rhizoctonia solani* Kuhn. in different crops was recorded by Thangavelu *et al.* (2001), Chernin *et al*. (1995), Chernin *et al*. (1997), and Pleban *et al*. (1997). Likewise, Helisto *et al.* (2001) observed the production of range of hydrolytic enzymes, composed of chitinase, chitosanase, laminarinase, lipase and protease by *Bacillus* sp. (strain X-b) in bio-control of range of fungal plant pathogens. Additionally, El-Gamal *et al*. (2016) recorded the presence of higher activities of chitinases, *β*-1,3-glucanases and *β*-1,4-glucanase in extracellular protein extracts of *Bacillus subtilis* and *Pseudomonas fluorescens.* Further, these strains were found promising in field and glasshouse against *Fusarium solani* and *Rhizoctonia solani* the pathogens of tomato.

2.7.4.2. Production of volatile metabolites

The production of volatile metabolites by *Bacillus* spp. was well studied by many early workers and their reports are listed hereunder.

The inhibitory effect of volatile metabolites produced by *P. fluorescens* and *B. subtilis* against *Sclerotium rolfsii* was well documented by Laha *et al.* (1996) and against *Phytophthora capsici* by Tehrani and Omati (1999). Further, the production of specific antifungal volatile compounds (AFV) against *S. rolfsii* by the *B. subtilis was observed by* Knox *et al.* (2000) and Ashok *et al*. (2014). They also noted that, the supplementation of chitin as a carbon source in culture medium was found most effective in inducing the production of bioactive compound by *B. subtilis*.

Giorgio *et al.* (2015) indentified array of volatile organic compounds (VOCs) responsible for inhibition of the growth range of soilborne pathogenic fungi in the

culture filtrate of eight strains of *Bacillus* spp. Furthermore, they found high efficacy of these VOCs against *Sclerotinia sclerotiorum.* Likewise, Li *et al.* (2016) evaluated VOCs produced by *Bacillus* strain against *Fusarium solani* in sealed petri dishes and found 56 to 82 per cent growth inhibition of pathogen. They later characterised in detail the chemical nature of these VOCs and found to be ketones, alcohols, aldehydes, pyrazines, acids, esters, pyridines and benzene compounds.

Additionally, Raza *et al.* (2015) characterised in detail the basis for antagonistic ability of VOCs produced by *Bacillus* spp. against *Ralstonia solanacearum* a pathogen of bacterial wilt of tomato. They later found that, these VOCs significantly inhibited the growth of pathogen on agar medium and in soil and in addition, the VOCs significantly inhibited the motility traits, production of antioxidant enzymes and exo-polysaccharides, biofilm formation and tomato root colonization by *R. solanacearum*.

2.7.4.3. Antibiosis

The production of antifungal metabolites (excluding metal chelators and enzymes) by bacterial antagonists under *in vitro* conditions that may also have activity under *in vivo* conditions is well known. Further, these antifungal metabolites includes ammonia, butyrol lactones, 2,4-diacetylphloroglucinol (DAPG), HCN, kanosamine, Oligomycin A, Oomycin A, phenazine-1-carboxylic acid (PCA), pyoluterin (Plt), pyrrolnitrin (Pln), viscosinamide, xanthobaccin, and zwittermycin A as well as several other uncharacterized moieties as reported by Milner *et al*. (1996), Keel and Defago (1997), Whipps (1992), Nielsen *et al*. (1998), Kang *et al*. (1998), Kim *et al*. (1999), Thrane *et al*. (1999), Nakayama *et al*. (1999), Raaijmakers *et al.* (2010), Mora *et al.* (2011), and Cazorla *et al.* (2007).

Li *et al.* (2016) observed that, the cell suspension of *Bacillus amyloliquefaciens* SYBC H47 and the cell-free supernatant of its culture showed significant antifungal activity against *Aspergillus niger*, *Mucor racemosus*, *Fusarium oxysporum*, *Penicillium citrinum*, and *Candida albicans* under in vitro conditions. The HPLC LC/ESI-MS/MS analysis of culture filtrate confirmed the presence of range of antibiotics *viz.*, bacillomycin L, fengycin, and surfactin. Similarly, Nagarajkumar *et al.* (2005) revealed the presence of several antifungal proteins in the culture filtrate of *Pseudomonas* sp. and *Bacillus* sp. which limited the mycelial growth and sclerotial production by *Rhizoctonia solani*

2.7.4.4. Induced resistance

The greatest growth area in biocontrol in the last few years has been concerned with induced resistance which is defined as the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (Kloepper *et al*., 1992). Most work has focused on the systemic resistance induced by non-pathogenic rhizosphere colonizing *Bacillus* and *Pseudomonas* species in systems where the inducing bacteria and the challenging pathogen remained spatially separate for the duration of the experiment and no direct interaction between the bacteria and pathogen was possible (Sticher *et al*., 1997; van Loon, 1997; van Loon *et al.,* 2006).

The full range of inducing moieties produced by bacteria is probably not yet known, but lipopolysaccharides (Leeman *et al*., 1995) and siderophores (Metraux *et al*., 1990; Leeman *et al*., 1996) are clearly indicated. Changes that have been observed in plant roots exhibiting ISR include strengthening of epidermal and cortical cell walls and deposition of newly formed barriers (Benhamou *et al*., 1996; Duijff *et al*., 1997), increased levels of enzymes such as chitinase, PO, PPO, and PAL (Chen *et al*., 2000), enhanced phytoalexin production (van Peer *et al*., 1991) and enhanced expression of stress-related genes (Timmusk and Wagner, 1999). Choudhary *et al.* (2007) elaborately described induced resistance and its mechanism of action in plants. Plants have the ability to acquire enhanced level of resistance to pathogens after exposure to biotic stimuli provided by many different PGPRs. These in association with plant roots elicit a steady state of defense or ISR in plants. This is often referred to as rhizobacteria mediated ISR. PGPR-elicited ISR was initially observed in carnation, common bean and in cucumber with reduced susceptibility to Fusarium wilt, halo blight, and *Colletotrichum orbiculare*, respectively. Several PGPR that colonize root systems with seed applications protect plant against foliar disease include *P. fluorescens, P. putida, B. pumilus,* and *Serratia marcescens* (Thomma *et al*., 2001).

The induction of systemic resistance by *Bacillus* spp. in different host crops against range of soilborne fungal pathogens was well documented by Conrath *et al.* (2006), Liang *et al*. (2011), Tonelli *et al*. (2011), Figueredo *et al.* (2014), Chakraborty *et al.* (2005), and Chakraborty *et al.* (2015).

Liang *et al*. (2011) observed that the *Bacillus megaterium* strain L8 applied through root dip in cucumber against seedling damping-off caused by *Pythium*

aphanidermatum induced the higher level denfense enzymes *viz.,* superoxide dismutase, peroxidise, polyphenol oxidase and phenylalanine ammonia-lyase and resulted in effective control of disease in glasshouse and field. Similarly, the induction of systemic resistance by strains of rhizobacteria *viz., Bacillus* sp. CHEP5 and *Pseudomonas* sp. BREN6 in groundnut against stem rot caused by *S. rolfsii* in the form of enhanced level of activities of defense enzymes *viz.,* polyphenol oxidase and phenylalanine ammonia-lyase upon pathogen attack was noted by Tonelli *et al*. (2011). Likewise, Chakraborty *et al.* (2015) recorded the significant increases in activity of phenolics along with increased induction of number of isoforms of catalase, peroxidase, chitinase, *β*-1,3-glucanase and PAL in plants previously treated with *Bacillus megaterium* and challenged with *S. rolfsii* a causal agent of sclerotial blight of tea. They further observed the growth promotion activity of *B. megaterium* in five varieties of tea, as evidenced by increase in plant height, emergence of new leaves, branches and increase in leaf numbers both in potted and field conditions.

The cumulative effect of combining the different bacterial antagonists in inducing resistance against many fungal plant pathogens was evidenced by Figueredo *et al.* (2014) and Chakraborty *et al.* (2016).

Figueredo *et al.* (2014) recorded enhanced PAL enzyme activity and higher levels of defence chemicals in groundnut plants co-inoculated with plant-growth-promoting bacteria *Bacillus* sp. CHEP5 and peanut nodulating strain *Bradyrhizobium* sp. SEMIA 6144 against *S. rolfsii*, the causal agent of stem rot. Similarly, Chakraborty *et al.* (2016) observed a sharp increase in polyphenolic accumulation and activities of defence enzymes *viz.,* PO, chitinase, *β*-1,3-glucanase and PAL in tea bushes dual inoculated with *Rhizophagus fasciculatus* and *Bacillus pumilus* upon *S. rolfsii* inoculation under glasshouse conditions. The elevated levels of defense in dual inoculated tea bushes lead to effective control of sclerotial blight under field conditions.

2.7.4.5. Compatibility of *Bacillus* sp with fungicides

Manifestation of biological control by *Bacillus* spp. against various soil borne plant pathogens has been observed from several years. Further, supplementation with specific compounds may provide a competitive advantage for the establishment of the introduced biocontrol agents and improve their biocontrol ability. In several disease management strategies, the addition of fungicide at reduced rates in combination with

biocontrol agents has significantly enhanced disease control, compared to treatments with biocontrol agent alone (Frances *et al.,* 2002). Integrated use of biocontrol agent at reduced dosage of fungicide was effective against *Fusarium* crown and root rot of tomato (Omar *et al*., 2006).

In these lines compatibility of *Bacillus* spp. with different fungicides were reported by many early researchers. Suneeta *et al.* (2016) found compatibility of *Bacillus* spp. with commonly used fungicides *viz.,* Carbendazim, Difenconazole, Azoxystrobin and Fosetyl Al. These strains were further found highly effective against *Fusarium oxysporum* f.sp. *gerberae* causal agent of *Fusarium* wilt of gerbera. Similarly, the compatibility of *Bacillus* spp. with Azoxystrobin was reported by Archana *et al.* (2012) and with six commonly used fungicides in controlling soil borne diseases by Mohiddin and Khan (2013). Further, Devi and Prakasam (2013) observed the compatibility of *Pseudomonas fluorescens* and *Bacillus subtilis* with Azoxystrobin. They further noted that, these strains were fitted well in integrated management of soilborne diseases.

2.7.5. Multiple microbial interactions

Use of different biocontrol agents with several mechanisms of control fits in well with the concepts of integrated disease management, in which several means of disease suppression are applied concurrently. When one or more means of mechanisms are not effective, the others may compensate for the former absence.

Guetsky *et al*. (2001) observed for the first time the additive contribution of several biocontrol mechanisms to total disease suppression. This work provides a theoretical explanation of increased disease control by mixing *Pichia guilermondii* and *Bacillus mycoides* against *Botrytis cineria*. This is a novel approach to biological control that may facilitate the more efficient use of this type of control on a larger commercial scale.

Combinations of fungi and bacteria have also been shown to provide enhanced biocontrol. For instance, *Trichoderma koningii* combined with either *Pseudomonas chlororaphis* or *P. fluorescens* provided greater suppression of take-all disease of wheat than *T. koningii* alone (Duffy *et al*., 1996), while *Trichoderma virens* GL-3 combined with *Burkholderia cepacia* provided greater protection than the antagonist used alone against four soil-borne fungal pathogens (Mao *et al*., 1998). A commercial product

consisting of a mixture of three *Bacillus subtilis* strains used to control fungal soil pathogens found very effective. These strains exhibited several biocontrol mechanisms, including production of antifungal compounds (including antibiotics and hydrogen cyanide), competition for ferric iron, competition for infection sites, and production of lytic enzymes (Pusey, 1989). A seed application of a combination of three PGPRs, *Bacillus pumilus*, *Bacillus subtilis* and *Curtobacterium flaccumfaciens* provided greater control of several pathogens on cucumber than when any were inoculated singly (Raupach and Kloepper, 1998).

The *T. viride* (Tv1) combined with *P. fluorescens* (Pf1), *B. subtilis* (Bs16), neem cake and zinc sulphate resulted in greater suppression of *Lasiodiplodia theobromae* causal organism of collar and root rot disease in physic nut under *in vitro* and *in vivo* conditions than their individual application (Latha *et al.*, 2011). Similarly, Thilagavathi *et al*. (2007) combined the application of *Trichoderma viride* (Tv13), *Pseudomonas fluorescens* (Py15) and *Bacillus subtilis* (Bs16) against *Macrophomina phaseolina* causal organism of dry root rot of greengram and found greater suppression of disease. They further observed the cumulative effect of three bioagents in inducing enhanced activity of defense enzymes (PAL, PO, PPO) in bioagent treated plants.

2.7.6. Chitin as inducer of systemic resistance in plants

Involvement of chitin or chitosan in inducing systemic resistance alone or in combination with biocontrol agents has been demonstrated in few crops. The interest in the utilization of chitinolytic antagonist bacteria and fungi has grown tremendously, as the chitin supplementations increased the attainable level of disease control. Chitin amendment of bioformulations has drastically reduced the populations of *Ph. personata* (Kishore *et al.,* 2005), incidence of stem rot of groundnut caused by *S. rolfsii* (Senthilraja *et al.,* 2010), root rot disease of pepper caused by *Rhizoctonia solani* and *Phytophthora infestans* (Ahmed *et al*., 2003).

The application of chitin alone or in combination with the biocontrol agents in managing plant diseases has been demonstrated in only few crops so far by Kishore *et al.* (2005), Nandakumar *et al.* (2001), Radjacommare *et al.* (2010), Rajkumar *et al.* (2008), Senthilraja *et al.* (2010) and Ahmed *et al*. (2003). The unique biological properties and antifungal potential of chitin oligomers against *Fusarium oxysporum* f. sp. *radices-lycopersici* and *Pythium aphanidermatum* in tomato was well documented by Manjula and Podile (2001).

The effective control of the disease complex incited by *Sclerotinia sclerotiorum* and *Meloidogyne incognita* in cabbage through application of chitin fortified formulation mixture of *Trichoderma* spp. was observed by Loganathan *et al.* (2010). They also noted the enhancement in activities of PAL, PO, PPO and chitinase by chitin fortification of bioformulations. Likewise, Kishore *et al.* (2005) reported that supplementation of *Bacillus circulans* GRS 243 and *Serratia marcescens* GPS5 with 1% colloidal chitin reduced lesion frequency of late leaf spot of groundnut caused by *Phaeoisariopsis personata* by 60%, when compared with application of bacterial cells alone under glasshouse conditions. Similarly, Ahmed *et al.* (2003) amended the formulation mixture of *Trichoderma* spp. and *Bacillus* spp. with 0.5% chitin and applied through seed treatment and root drench and observed the effective control of *Phytophthora* and *Rhizoctonia* root rot of pepper in glasshouse conditions compared to no chitin amended treatment. Additionally, Solanki *et al.* (2011) amended the talc formulation of *Trichoderma* spp. with 1% chitin and found significant control of root rot of tomato caused by *Rhizoctonia solani.* They further noted that, the chitin amendment has significantly increased the systemic resistance induction capacity of *Trichoderma* spp. In similar lines Senthilraja *et al.* (2010) amended the bioformulation of *Pseudomonas fluorescens* with 1% chitin and applied through seed treatment and soil application against stem rot of groundnut caused by *S. rolfsii* and observed effective control of disease in glasshouse and field conditions. Further, the chitin amendment has resulted in significant increase in activities of PAL, PO, PPO, CAT, *β*-1,3-glucanase and chitinase in groundnut plants upon challenge inoculation of *S. rolfsii* (Senthilraja *et al.,* 2013).

Moreover, chitin has wide applications against various plant pathogens (Kishore *et al.,* 2005; Yu *et al.,* 2008) as the monomer is the inducer for chitinase production by *Bacillus* spp. (Ahmed *et al.,* 2003), *Trichoderma* spp. (Solanki *et al.,* 2011) which, in turn, enhances the mycoparasitic potential of *Trichoderma* spp. or *Bacillus* spp. this perhaps justifies its use as an amendment in bioformulation mixtures.

2.7.7. Bioformulation of biocontrol agents

An important area of biological control is the development of formulations that would care for viable microbial activity for long period of time. Mass multiplication of PGPR in a suitable medium and development of a powder formulation was first carried out in 1980. A dried powder formulation of PGPR is especially important for seed treatment and soil application. The survival of PGPR in a dried formulation and the

effectiveness of methylcellulose in a powder formulation for coating sugar beet seed has been well documented by Suslow (1980). A talc-based powder formulation of PGPR has been developed for inoculation of potato seed pieces by Kloepper and Schroth (1981). They also tested the stability and efficacy of the product under field conditions and found that potato seed treatment with powder formulation produced a higher level of root colonization by PGPR than aqueous preparations. Similarly, the suitability of different carriers for the development of stable formulation has been tested by Vidhyasekaran and Muthamilan (1995) and found that in talc-based and peat-based formulations, the bacterial population was stable up to 240 days of storage period.

Recently, the carbon sources and minerals have been shown to have an important role in antifungal metabolite production by *Pseudomonas*, suggesting that nutrient amendments to formulations would be of useful strategy for improving biocontrol efficacy (Duffy and Defago, 1999). Also, PGPR formulation using chitin and neem as a carrier was formulated for application in the transplant soil-mix system for developing suppressive potting soil (Zehnder *et al*., 2001; Bharathi *et al*., 2004). Reddy *et al*. (1999) demonstrated, the use of LS213, a commercial formulation containing formulated spores of *B. subtilis* strain GB03, *B. amyloliquefaciens* strain IN 937a and chitosan has significantly increased the growth of tomato, cucumber, tobacco and pepper in addition to protection against bacterial spot and late blight of tomato, angular leaf spot of cucumber and blue mold of tobacco. Further, the same bio-formulation enhanced the pine seedling root and shoot growth (Enebak and Carey, 2000). Studies on talc formulation of *Trichoderma* spp. and *Bacillus* spp. has been reported by Solanki *et al.* (2011), Ahmed *et al.* (2003), Loganathan *et al.* (2010), Gajera and Vakharia (2010), Gajera and Vakharia (2011), Mukherjee *et al*. (2014) and Ekundayo *et al*. (2016).

2.7.8. Delivery of biocontrol agents

A variety of application methods has been evaluated in the field that should improve the integration of PGPR-mediated ISR into conventional agriculture and in some cases with improved efficacy. Delivery systems for formulated product include seed treatment (Mew and Rosales, 1986), root-dip (Maurhofer *et al*., 1994), sett treatment (Viswanathan and Samiyappan, 1999), sucker treatment (Saravanan *et al*., 2003), soil application (Vidhyasekaran *et al.,* 1997) and foliar application (Mew and Rosales, 1986; Chatterjee *et al.,* 1996) in various crops.

Vidhyasekaran and Muthamilan (1995) and Vidhyasekaran *et al.* (1997) observed the seed treatment followed by soil application of talc-based powder formulation of *Pseudomonas* spp. has effectively checked chickpea and pigeonpea wilt under field conditions and has also increased the yield. Likewise, Nandakumar *et al*. (2001) applied PGPR strains to rice plants through seed, root, soil or foliar application or in combinations of two, three and four application methods and found that combinations of three or four application methods were more effective than single methods in controlling sheath blight of rice in field trials. The more effectiveness of combination of different methods of application compared to single method of application was also noted by Vidhyasekaran *et al.* (1997), Vidhyasekaran and Muthamilan (1999), Meena *et al*. (2000), Nandakumar *et al*. (2001) and Saravanakumar (2006) in different crops.

MATERIAL AND METHODS

Chapter III

MATERIALS AND METHODS

The present research investigations on stem rot of groundnut caused by *Sclerotium rolfsii* Sacc. were carried out at the Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, and at Groundnut Pathology Unit, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India. The fine points of the methodology followed during the investigations are presented below.

3.1 General laboratory procedures

3.1.1 Glassware cleaning

For the complete laboratory experimental studies, borosil glassware was used. The glassware items were kept in cleaning solution containing 60.0 g of potassium dichromate ($K_2Cr_2O_2$) as an oxidizing agent overnight and washed with washing powder in tap water and then rinsed in distilled water before use.

3.1.2 Sterilization

Glassware used in the present investigation were kept in sterilization tins or wrapped in thick paper and were sterilized in hot air oven at 170 °C for 60 min. Surface of laminar air flow chamber was sterilized by wiping with tissue paper sprinkled with alcohol prior to research work. Inoculation loop, cork borer and scalpel were sterilized by dipping in alcohol and heated to red hot stage using a spirit lamp. The culture media and distilled water were sterilized in an autoclave at 1.5 kg/cm^2 (121.6^{0}C) for 15 minutes (Dhingra and Sinclair, 1995). Whereas the pot soil used in glasshouse studies was sterilized in an autoclave at 1.5 kg/cm^2 (121.6^{0}C) for 30 minutes (Williams and Ewel, 1984).

3.1.3 Culture media

The following nutrient media were used for isolation, culturing and maintenance of fungi and bacteria in the laboratory. The pH of all the media used was adjusted whenever necessary with 1 N NaOH or 1 N HC1 as the case may be with the pH meter.

The media *viz.*, Potato dextrose agar (PDA), Potato dextrose broth (PDB) and Bacillus differention agar (BDA) were obtained from Himedia laboratories, Mumbai (India). Further, the Nutrient agar (NA), Nutrient broth (NB), Czapek dox broth (CDB)

Richard's broth (RB) and *Trichoderma* selective medium (TSM) were prepared as described by Dhingra and Sinclair (1995) and Aneja (2003). The details of composition of culture media are hereunder;

Potato dextrose agar (PDA)

Ingredients	**Grams/Litre**
Potatoes, infusion from	200 g
Dextrose	20 g
Agar	15 g

PDA of Hi-media make was dissolved @ 39 g in 1000 ml of distilled water.

Potato dextrose broth (PDB)

Potatoes, infusion from	200 g
Dextrose	20 g

PDB of Hi-media make was dissolved @ 24 g in 1000 ml of distilled water.

Bacillus differentiation agar (BDA)

Yeast autolysate	0.2 g
Mannitol	5 g
Monohydrogen ammonium phosphate	1 g
Potassium chloride	0.2 g
Magnesium sulphate	0.2 g
Bromo cresol purple	0.0075 g
Agar	15 g

BDA of Hi-media make was dissolved @ 22 g in 1000 ml of distilled water.

Nutrient agar (NA)

Beef extract	3 g
Peptone	5 g
Glucose	2.5 g
Agar	15 g

Volume was made to 1000 ml with distilled water.

Nutrient broth (NB)

Beef extract	3 g
Peptone	5 g
Glucose	2.5 g

Volume was made to 1000 ml with distilled water.

Czapek dox broth (CDB)

Sucrose	30 g
Sodium nitrate	2 g
Dipotassium phosphate	1 g
Magnesium sulphate	0.5 g
Potassium chloride	0.5 g
Ferrous sulphate	0.01 g

Volume was made to 1000 ml with distilled water.

Richard's broth (RB)

KNO_3	10 g
KH_2PO_4	5 g
$MgSO_4.7H_2O$	2.5 g
Sucrose	35g

Volume was made to 1000 ml with distilled water.

***Trichoderma* selective medium (TSM)** (Elad *et al.,* 1980)

Dipotassium dihydrogen phosphate	0.9 g
Magnesium sulphate	0.2 g
Ammonium nitrate	1 g
Potassium chloride	0.15 g
Glucose	3 g
Agar	20 g
Metalaxyl	0.3 g
Rose bengal	0.15 g
Chloramphonicol	0.25 g

Volume was made to 1000 ml with distilled water and pH was adjusted to 7.0.

The sterilized medium was melted and distributed in petri dishes of 9 cm diameter at the rate of 20 ml per plate aseptically in a laminar air flow chamber and allowed to solidify. The plates containing the medium were used for culturing and maintenance of fungus in the laboratory.

3.1.4 Equipments

Compound microscope (OLYMPUS) was used for microscopic observations. Hot air oven and autoclave were used for sterilization of glassware and media respectively. BOD incubators (Sanyo) were used for incubating test materials at different temperatures. Cultures were stored in refrigerator (Kelvinator Scientific) for short term storage. Weighments were done on a single pan electronic balance (SDFCLEA3000) with a sensitivity of 0.001 g. Other tools that were used in the present investigation for various purposes include vortex (VORTEXGENIE-2, Scientific Industries), camel brush, inoculation needle and inoculation loops etc.

3.2. Survey and disease assessment

An intensive roving survey was conducted during *kharif*, 2013 and *kharif*, 2014 to assess the disease incidence in major groundnut growing states of India, *viz*., Karnataka, Telangana, Andhra Pradesh, Maharashtra, Tamil Nadu and Gujarat. Further, in each state intensive groundnut growing districts (Fig. 3.1), within each district major growing mandals/talukas and in each mandal/taluka major growing villages were selected for the study. The incidence of groundnut stem rot was assessed based on the external symptoms (presence of white mycelial growth, sclerotia, lesion on stem, wilting, drying or dead plants). In each field, five plots each with 5x5 m^2 area were selected. Among the five plots one plot was fixed at the centre of the field and the remaining four plots were fixed at random in different places in the field avoiding border rows. The stem rot incidence was assessed by counting the number of affected plants out of total number of plants in each plot (5x5 m^2). In each village, five fields were assessed and the mean disease incidence was calculated. Per cent disease incidence was calculated by using the formula:

Disease incidence (%) = (Number of plants infected / Total number of plants observed) x 100

Table 3.1. Survey format for taking observations

S. No.	State	District	Mandal	Village	Variety grown	No. of fields visited	Soil type	Planting date	Crop Stage	Crop density	Crop protection	Previous crop	Disease incidence (%)
1													
2													
3													
4													
5													

3.3. Isolation and maintenance of the pathogen

Groundnut plants showing typical symptoms of stem rot collected during survey from different states were used for isolation of *S. rolfsii* separately by tissue segment method (Rangaswami, 1993) using sterile Potato dextrose agar medium. The infected plants showing the presence of white mycelial mat with small round brown sclerotia near the collar region were pulled out and gently tapped to remove the soil and dirt particle. The infected portions of diseased plants collected from different area were cut into small pieces of 1 cm size using sterilized scalpel. These pieces were surface sterilized with 0.1 per cent sodium hypochloride for one minute and washed in sterile distilled water thrice and then placed at equidistance in a petri dish containing solidified Potato dextrose agar medium. These plates were incubated at 27±1°C in a BOD incubator for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogen was identified as *S. rolfsii* based on the morphological characters as described by Punja (1985).

3.4. *In-vitro* evaluation of different media for optimal production of oxalic acid by *S. rolfsii*

To know the best suited culture medium for optimal oxalic acid production, different media such as Richard's broth, Czapek dox broth and Potato dextrose broth were evaluated using the randomly selected isolate of *S. rolfsii* from each state. The isolates include SrKa-1, SrTs-1, SrAp-1, SrMh-1, SrTn-1 and SrGj-1.

3.4.1. Estimation of oxalic acid content in culture filtrate

Fifty ml of Richard's broth, Czapek dox broth and Potato dextrose broth were prepared in 250 ml Erlenmeyer flasks and autoclaved at 121°C (1.5 kg/cm^2) for 15 min. The flasks were inoculated with 6 mm mycelial disc from 5 days old actively growing culture of *S. rolfsii* isolates separately and incubated for 14 days at 27±1°C. The broth containing mycelial growth was filtered through Whatman No.1 filter paper to remove the mycelial mass. The filtrate was centrifuged at 10000 rpm for 10 min, to remove the mycelial fragment, if any. The supernatant obtained from each isolate was used for oxalic acid assay. To 5 ml aliquot of the cell-free culture filtrate in a centrifuge tube, 4 ml of calcium- chloride acetate buffer with pH 4.5 (solution A: 25 g of anhydrous calcium chloride was dissolved in 500 ml of 50% acetic acid; solution B: 330 g of sodium acetate tri-hydrate was dissolved in distilled water and made up the volume to

500 ml. The solutions A and B were mixed well and the pH was adjusted to 4.5 if necessary) was added and mixed thoroughly. The mixture was allowed to stand overnight at room temperature and centrifuged at 10000 rpm for 10 min. The supernatants were discarded and the sediments were dissolved in 5 ml of 5% acetic acid saturated with calcium oxalate and the suspension was centrifuged. Further, supernatants were discarded and sediments were dissolved in 5 ml of 4 N H_2SO_4. The solution was transferred to 100 ml conical flask and heated on a water bath to 80–90^oC and finally titrated while hot with 0.02 N potassium permanganate until faint pink colour persisted. One ml of 0.02 N $KMNO_4$ reacts with 1.2653 mg of oxalic acid. The oxalic acid present in the culture filtrate was calculated by multiplying the ml of 0.02 N $KMNO_4$ consumed with 1.2653 and expressed as mg/ml (Mahadevan and Sridhar 1986).

3.5. *In vitro* oxalic acid production by isolates of *S. rolfsii*

Richard's broth was used for production and quantification of oxalic acid. Fifty ml of Richard's broth was prepared in 250 ml Erlenmeyer flasks and autoclaved at 121°C (1.5 kg/cm^2) for 15 min. The flasks were inoculated separately with a 5 days old 6 mm culture disc of *S. rolfsii* isolates and incubated for 14 days at 27±1°C. Estimation of oxalic acid in the culture filtrate was performed as explained in 3.4.1.

3.6. Standardisation of inoculum level for optimum infection under glasshouse conditions

3.6.1. Raising of seedlings

Three susceptible groundnut cultivars *viz.*, TMV 2, JL 24 and J 11 were used for the study. The plastic pots of 7" diameter were filled with a 2:1:1 autoclaved mixture of alfisol soil, sand and vermicompost. The seeds were surface sterilized with 0.1 % sodium hypochloride and sown @ 8 seeds/pot. Finally four seedlings/pot was maintained.

3.6.2. Multiplication of inoculum of *S. rolfsii*

The most virulent isolate (Selected based on oxalic acid production assay) of *S. rolfsii* was multiplied on Sorghum grain medium (SGM) (Pande *et al.*, 1994). SGM was prepared by soaking 200 g sorghum grains for 16 h (mixture of several cultivars). The soaked grains were filled in one litre capacity Erlenmeyer flasks and autoclaved at 121°C (1.5 kg/cm^2) for 45 min. Each flask was seeded with a mycelial disc of 1 cm size

from a 5 day old actively growing culture of *S. rolfsii*, and incubated at 25±1°C for 20 days.

3.6.3. Inoculation and observations

The seedlings of 35 days old were inoculated with different levels of inoculum *viz.,* 5g, 10g, 15g, 20g, 25g, 30g and 35g per pot by spreading the inoculum on surface of soil. Suitable control without inoculation was maintained. Observations on incubation period (IP) and days to permanent wilting (DPW) were taken from next day of inoculation. Observations on disease severity (DS), disease incidence (DI) and mortality (M) were recorded at 8 days after inoculation and then on for every 8 days interval and continued up to 32 days after inoculation.

Disease severity assessments were made by using a 1-5 disease severity scale (Shokes *et al*., 1996). Where,

Severity scale	Description
1	A healthy plant
2	Lesions on stems only
3	Up to 25% of the plant symptomatic (wilted, dying, or dead)
4	26-50% of the plant symptomatic
5	>50% of the plant symptomatic

The per cent disease severity was calculated using the formula (Le *et al.,* 2012):

$$\text{Disease severity (\%)} = \frac{\Sigma ab}{AK} \times 100$$ Where,

a = Number of diseased plants having the same degree of infection

b = Degree of infection

A = Total number of plants examined

K = Highest degree of infection

3.7. Pathogenicity tests

3.7.1. Raising of seedlings

Three susceptible groundnut cultivars *viz.*, TMV 2, JL 24 and J 11 were used for the study. The plastic pots of 7” diameter were filled with a 2:1:1 autoclaved mixture of alfisol soil, sand and vermicompost. The pot accommodated three kilo grams of pot soil. The seeds were surface sterilized with 0.1 % sodium hypochloride and sown @ 8 seeds per pot. Finally, four seedlings per pot was maintained.

3.7.2. Multiplication of inoculum of isolates of *S. rolfsii*

The inoculum of all the isolates of *S. rolfsii* was prepared separately on SGM (Pande *et al.,* 1994). SGM was prepared by soaking 200 g sorghum grains for 16 h. The soaked grains were filled in one litre capacity Erlenmeyer flasks and autoclaved at 121°C (1.5 kg/cm^2) for 45 min. Each flask was seeded separately with a mycelial disc of 1 cm size from a 5 day old actively growing culture individual isolate of *S. rolfsii*, and incubated at 25±1°C for 20 days.

3.7.3. Inoculation and observations

Seedlings of 35 days old were inoculated with 15g inoculum per 7" pot by spreading the inoculum on the surface of soil. Suitable untreated control without inoculation was maintained. Observations on incubation period (IP) and days to permanent wilting (DPW) were taken from next day of inoculation. The disease severity (DS), disease incidence (DI) and mortality (M) observations were taken at 15 days after inoculation and then on for every 15 days interval and continued up to 45 days after inoculation. Re-isolation of pathogen was made from symptomatic plant tissues to compare with that of original isolate for conformity. A 1-5 disease severity scale was used for all disease severity assessments (Shokes *et al*., 1996) and per cent disease severity was calculated as per Le *et al* (2012).

3.8. Variability of *S. rolfsii* isolates

3.8.1. Cultural variability of *S. rolfsii* isolates

Cultural characters such as radial mycelial growth (growth rate), colony morphology and production of sclerotial bodies were studied by growing *S. rolfsii* isolates on Potato dextrose agar medium at 27±1°C.

3.8.1.1. Radial mycelial growth

The *S. rolfsii* isolates were cultured on Potato dextrose agar medium at 27±1°C. Petri dishes containing PDA were inoculated in the centre separately with a 6 mm mycelial disc of 5 days old actively growing cultures of *S. rolfsii* isolates. The radial mycelial growth of the fungal colony was determined at 24 and 48 h after incubation. Finally the radial mycelial growth was expressed as growth rate (mm/h).

3.8.1.2. Colony morphology and production of sclerotial bodies

Culturing of *S. rolfsii* isolates was carried out as explained in 3.8.1.1. When mycelial growth of *S. rolfsii* covered the full plate, observations on growth (highly profuse or profuse) and colony type (raised, flat, or raised at ends) were recorded. The production of sclerotial bodies was recorded at 21 days after incubation.

3.8.2. Morphological variability of *S. rolfsii* isolates

Morphological characters include sclerotial characters such as time required for sclerotial formation, sclerotial maturity, pattern of sclerotial production, color of sclerotial bodies, number of sclerotial bodies produced per plate, diameter of sclerotial bodies and weight of 100 sclerotial bodies.

3.8.2.1. Time required for sclerotial production

Culturing of *S. rolfsii* isolates was carried out as explained in 3.8.1.1. Observation on days to form sclerotial initials was recorded at two days after incubation and continued till all the isolates form sclerotial initials. Observation on days to maturity was noted when sclerotia turns to brown colour.

3.8.2.2. Colour, size, number and pattern of sclerotial production

Culturing of *S. rolfsii* isolates was carried out as explained in 3.8.1.1. Observation on pattern of sclerotial production (central, peripheral, or scattered), colour of sclerotial bodies, number of sclerotial bodies produced per plate, weight 100 sclerotia and sclerotial diameter were recorded at 21 days after incubation. The sclerotial diameter was determined by measuring the size of 30 randomly selected sclerotia from individual isolate using digital vernier caliper (Aerospace digimatic vernier caliper) and mean sclerotial diameter was calculated.

3.8.3. Mycelial compatibility groups (MCG)

Mycelial compatibility was assessed macroscopically by the method given by Punja and Grogan, 1983. For grouping isolates based on mycelial compatibility, fresh mycelial discs of 6 mm diameter were cut off from the edge of an actively growing colony (5 days old), and transferred to petri dishes containing solidified Potato dextrose agar medium, and incubated at 27±1°C. Three isolates per a plate spaced 2 to 2.5 cm apart were inoculated and visually examined after 5 to 8 days of incubation for the

presence of an aversion or a barrage zone. Pairings were marked either as incompatible when an antagonistic barrage zone was observed between two paired isolates and were put into different MCG groups or compatible when mycelia from two isolates intermingled without a barrage zone between them and in such case they were placed into the same MCG group.

3.8.4. Molecular identification of isolates of *S. rolfsii*

Molecular identification of all 60 isolates of *S. rolfsii* was carried out by ITS-rDNA amplification using ITS 1 and ITS 4 primers followed by sequencing and phylogeny.

3.8.4.1. DNA extraction

Mycelial discs of 6 mm diameter of all isolates of *S. rolfsii* were cut from the periphery of an actively growing 5 days old culture and inoculated on petri dish containing solidified Potato dextrose agar and incubated at 27±1°C till mature sclerotia were formed. Sclerotia (50-80 mg) were scraped from the surface of colonies and were used for genomic DNA isolation by the method described by Punja and Sun (2001) with minor modification.

The collected sclerotia (80 mg) were ground to fine powder using liquid nitrogen in pre-chilled mortar and pestle and transferred to a sterile 2 ml microcentrifuge tube containing 800 µl of Lysis buffer (200 mM Tris-HCl pH 8.0; 500 mM NaCl; 100 mM EDTA pH 8.0; 2% SDS; 1% 2-mercaptoethanol). The contents were mixed gently and incubated at 65°C in water bath for 30 min. Extraction buffer (phenol:chloroform:isoamyl alcohol, 25:24:1, v:v:v) was added (400 µl) stirred gently on a vortex until an emulsion had formed, and centrifuged at 6000 g for 5 min in a microcentrifuge at room temperature. The aliquot of the upper aqueous layer (600 µl) was collected, mixed with an equal volume of extraction buffer, and re-centrifuged at 6000 g for 5 min. The supernatant (500 µl) was carefully removed, 10 µl of RNase A (10 mg/ml) was added and the mixture was incubated at 37° for 30 min. Cold ethanol (1 ml) was added to the tubes and placed at -20°C for 2 h or overnight. The mixture was centrifuged (14000 g) for 30 min at 4°C, the pellet was collected and suspended in 70% ethanol, and re-centrifuged. Finally, the pellet was air-dried for 10-15 min, re-suspended in 100 µl of TE buffer (10 mM TrisHCl (pH 8.0); 1 mM EDTA).

3.8.4.2. Agarose gel electrophoresis

Agarose gel electrophoresis was performed to assess the quality of *S. rolfsii* genomic DNA.

Tools/materials required

a. Electrophoretic unit: Gel casting trey, gel casting stand, gel combs, power-pack, UV trans-illuminator

b. Agarose (1.2%)

c. Bromophenol blue (Loading dye)

d. Ethidium bromide (10 mg $m1^{-1}$)

e. 50 x TAE (stock) – (Tris base, 60.5 g; Glacial acetic acid, 14.25 ml;0.5 M EDTA, 25 ml; make up the volume to 250 ml; pH 8.0)

f. Working solution TAE (1x): 20 ml of 50x TAE was diluted to 1000 ml using distilled water

Procedure

The 1.2 g of agarose was taken into a 250 ml conical flask containing 100 ml of 1x TAE buffer and mixed well. The mixture was microwaved for 30 sec for three times and stirred well to ensure even mixing and complete dissolution of agarose. The solution was allowed to cool on the bench for 5 minutes down to about 60°C. Later, 3 µl of ethidium bromide (10 mg/ml) was added to the cooled solution, mixed well and poured into the gel casting platform after inserting the comb. While pouring sufficient care was taken for not allowing the air bubbles to trap in the gel. The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoretic apparatus containing sufficient buffer (1x TAE) so as to cover the wells completely. The amplified products (10 µl) to be analysed were mixed with loading dye (bromophenol blue) and carefully loaded into the sample wells with the help of micropipette. The Electrophoresis was carried out at 60 V, until the tracking dye migrated to the end of the gel. Finally, the gel was taken out of electrophoretic apparatus and DNA bands were viewed under UV-transilluminator and photographed in gel documentation unit (ChemiDoc, Bio-Rad).

3.8.4.3. Measurement of DNA Concentration

The quality and quantity of DNA was analyzed by running 2 µl of each sample mixed with 1 µl of 10x loading dye (Bromophenol blue) on 1.2% agarose gel. The gel

was visualized on a UV transilliminator and photographs were taken using gel documentation unit (ChemiDoc, Bio-Rad). Quantity and quality of the DNA was also measured by using spectrophotometer (Nanodrop ND 1000). All the 60 isolates of *S. rolfsii* had the concentration of DNA between 100-500 ng/µl and the ratio of 260/280 was 1.8 by quantification using spectrophotometer (Nanodrop ND 1000).

3.8.4.4. PCR amplification of ITS region

PCR amplification of Internal Transcribed Spacers (ITS) region of ITS-rDNA was performed using universal primers ITS-1 (5'-TCC GTA GGT GGA CCT GCG G - 3') as forward primer and ITS-4 (5' - TCC TCC GCT TAT TGA TAT GC - 3') as reverse primer (White *et al.*, 1990) in Eppendorf PCR master cycler. Amplification was carried out in 0.2 ml Eppendorf tubes with 25 µl reaction mixture.

PCR mixture (25µl) per reaction

a.	10x PCR reaction buffer	2.5 µl
b.	MgCl2 (25 mM)	4 µl
c.	Forward Primer (5 picomolar/µl)	1 µl
d.	Reverse primer (5 picomolar/µl)	1 µl
e.	dNTP mix (5 mM)	1 µl
f.	Taq polymerase enzyme (conc. 5 U µL-1)	0.5 µl
g.	Nuclease free water (Genei, Bangalore)	12 µl
h.	DNA (100-200 ng)	4 µl

The PCR amplification was carried out by 5 min of initial denaturation at 94°C followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min with final elongation at 72°C for 5 min (Le *et al.*, 2012).

3.8.4.5. Separation of amplified products by agarose gel electrophoresis

Agarose gel electrophoresis was performed to resolve the amplified products using 1.5 per cent agarose in 1x TAE buffer, 0.5µg/ml of ethidium bromide and loading dye (0.25% bromophenol blue in 40% sucrose). Four microliter of the loading dye was added to 20 µl of PCR product of all isolates of *S. rolfsii* and loaded on to the agarose gel. Electrophoresis was carried at 65 V for 1.5h. The gel was observed under UV light and documented using gel documentation unit. The size of the PCR product was

estimated by comparison with known DNA marker of 100 bp DNA ladder (Genei, Bangalore).

3.8.4.6. Sequencing of ITS region

The ITS-rDNA region amplified product of all isolates of *S. rolfsii* was sequenced to confirm the organism and to know the genetic variability present within them.

3.8.4.7. Sequencing and *in silico* analysis

The PCR product was sequenced using forward and reverse primers at Eurofins Genomics India Pvt. Ltd. Bengaluru (India). Homology search was done using BLAST algorithm available at the http://www.ncbi.nlm.nih.gov. Multiple alignments for homology search were performed using the Clustal W algorithm software and the phylogenetic tree was constructed using MEGA7 software (Patil, 2009).

3.8.5. Molecular diversity of *S. rolfsii* isolates

Molecular diversity of 15 isolates of *S. rolfsii* one each from 15 MCGs (Table 3.2) was studied by using 30 RAPD primers (Table 3.3).

3.8.5. 1. RAPD profiles through Polymerized Chain Reaction (PCR)

PCR mixture (25μl) per reaction

a.	10x PCR reaction buffer	2.5 µl
b.	MgCl2 (25 mM)	2 µl
c.	Primer (5 picomolar/µl)	1 µl
d.	dNTP mix (5 mM)	1 µl
e.	Taq polymerase enzyme (conc. 5 U µL-1)	0.5 µl
f.	Nuclease free water (Genei, Bangalore)	15.5 µl
g.	DNA (40-50 ng)	2.5 µl

PCR amplification was carried out by 5 min of initial denaturation at 94°C followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 37°C for 1 min and extension at 72°C for 2 min with final elongation at 72°C for 5 min. Amplified PCR products were subjected to 1.8 per cent agarose gel electrophoresis with 1x TAE as running buffer. The banding patterns were visualized under UV trans-illuminator with ethidium bromide (10 mg ml^{-1}) staining. The DNA banding profiles were documented

in the gel documentation system (ChemiDoc, Bio-Rad) and compared with 100 bp DNA ladder (Genei, Bangalore).

3.8.5.2. Scoring and data analysis

The DNA bands obtained for each isolate or group of isolates were scored based on their presence (1) or absence (0). Only reliable and repeatable bands were considered while scoring. Similarity index values were calculated based on genetic distance between isolates of *S. rolfsii* and cluster analysis was done by the unweighted paired group method using arithmetic averages (UPGMA). All calculations were performed with the aid of the program NTSYS pc version 2.02i (Exeter Software, New York).

3.9. Sensitivity of isolates of *S. rolfsii* to commonly used fungicides

The sensitivity of *S. rolfsii* isolates to four commonly used fungicides in groundnut cultivation *viz.,* Tebuconazole 2 DS (Raxil), Carbendazim 50 WP (Bavistin), Azoxystrobin 23.8 SC (Amistar) and Thiram 75 WP (Thiram) was studied at different concentrations using poison food technique. The fungicides measured as per the concentration to be tested and mixed with the Potato dextrose agar medium just before pouring into the petri dishes. Suitable controls were maintained without fungicide amendment. Tebuconazole and Azoxystrobin were tested at 100 to 1000 ppm, Carbendazim at 100 to 1500 ppm, and Thiram at 100 to 3500 ppm with an interval of 50 ppm between two concentrations. Mycelial discs of 6 mm diameter were cut from the margins of 5 day old actively growing cultures of each isolate and inverted in the centre of fungicide amended and un-amended PDA plates and incubated at 27±1°C. Colony diameters were measured when the full growth of isolates attained in control plates. The fungicide concentration which inhibits complete mycelial growth was considered as MIC (minimum inhibitory concentration), and the concentration which inhibits 50 per cent mycelial growth was considered ED_{50} (Effective dose).

3.10. Isolation of antagonists from the rhizosphere soil of groundnut

Antagonistic fungi and bacteria were isolated from the rhizosphere soil collected from different groundnut growing areas of India. The plants were pulled out gently with intact roots system and the soil adhering on roots was collected. Ten gram of this rhizosphere soil was transferred to 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water. After thorough shaking, the antagonists in the suspension were isolated

by serial dilution method. For isolation of fungal antagonists (*Trichoderma* sp.) one ml of aliquot from the final dilution of 10^{-3} was poured in sterilized petri dish containing *Trichoderma* specific medium. Similarly, for bacterial antagonists (*Bacillus* sp.) one ml of aliquot from the final dilution of $10^{-5}$and 10^{-6} was poured in sterilized petri dish containing Bacillus differentiation agar medium. The plates were gently rotated clockwise and anti-clockwise for uniform distribution and incubated at 27±1^{o}C for 24 hours. Colonies with characteristics of *Bacillus* sp. and *Trichoderma* sp. were isolated separately. The *Bacillus* sp. was purified by streak plate method (Rangaswami, 1993) on Nutrient agar medium, whereas *Trichoderma* sp. was subcultured on Potato dextrose agar. In total 100 isolates of *Trichoderma* sp. and 80 isolates of *Bacillus* sp. were collected from groundnut rhizosphere soils. Morphological identification was made through light microscopy and pure cultures were maintained on their respective agar slants at 4^{o}C.

Molecular identification of *Trichoderma* sp. was performed through ITS-rDNA amplification, sequencing, and phylogeny**.** ITS-rDNA amplification was carried out using ITS 1 and ITS 4 primers. The PCR product was sequenced using forward (ITS 1) and reverse (ITS 4) primers at Eurofins Genomics India Pvt. Ltd. Bengaluru (India). Homology search was done using BLAST algorithm available at the http://www.ncbi.nlm.nih.gov. Multiple alignments for homology search were performed using the Clustal W algorithm software and the phylogenetic tree was constructed using MEGA7 software.

Similarly, molecular identification of *Bacillus* sp. was performed by 16S rDNA amplification, sequencing, and phylogeny**.** ITS-rDNA amplification was carried out using 785 F and 907 R primers. The sequencing and phylogenetic studies were performed as explained for *Trichoderma* sp.

3.10.1. Preliminary screening of *Trichoderma* sp. against *S. rolfsii*

This was performed by dual culture assay (Dennis and Webster, 1971a) to assess the inhibitory effect of 100 isolates of *Trichoderma* sp. on radial growth and sclerotial production of virulent isolate of *S. rolfsii*. The 6 mm mycelial discs of actively growing colonies of *Trichoderma* isolates and the pathogen respectively were placed opposite to each other approximately 5 cm apart on petri dishes containing solidified Potato dextrose agar. Suitable control was maintained and the plates were incubated at 27±1°C

for 5 days. The efficacy of different isolates of *Trichoderma* sp. was assessed by calculating per cent inhibition of radial growth and sclerotilal production of pathogen over control using the formula:

I = (C-T/C) 100

Where,

I = Per cent inhibition over control

C = Radial growth and number of sclerotia of *S. rolfsii* in control plates

T = Radial growth and number of sclerotia of *S. rolfsii* in presence of isolates of *Trichoderma* sp.

In addition, the potentiality of 100 isolates of *Trichoderma* sp. was evaluated against *S. rolfsii* based on their ability to inhibit the oxalic acid production by the pathogen. In this study, 100 ml of Potato dextrose broth was distributed into 250 ml conical flasks and autoclaved. The flask were inoculated with a 6 mm culture disc of virulent *S. rolfsii* isolate into one side and on the other side of each flask was inoculated with 6 mm culture disc of each isolate of *Trichoderma* sp. PDB inoculated with *S. rolfsii* alone served as a control. These flasks were incubated at 27±1^{o}C for 14 days. The mycelial mat of the pathogen from each flask was harvested; filtrate was centrifuged separately at 2100 rpm for 30 min. The supernatant obtained from each treatment was used for assays of oxalic acid production (Paramasivan *et al.,* 2013). Estimation of oxalic acid in supernatant was carried out as explained in 3.4.1. Per cent inhibition of oxalic acid over control was calculated using the formula:

I = (C-T/C) 100

Where,

I = Per cent inhibition over control

C = Amount of oxalic acid produced by *S. rolfsii* in control flasks

T = Amount of oxalic acid produced by *S. rolfsii* in presence of isolates of *Trichoderma* sp.

From the preliminary screening, the best performing few isolates of *Trichoderma* sp. were selected for in detail characterisation of their bio-control traits against *S.rolfsii.*

3.10.2. Preliminary screening of *Bacillus* sp. against *S. rolfsii*

All the 80 isolates of *Bacillus* sp. were evaluated against virulent isolate of *S. rolfsii* by following the dual culture assay (Vidhyasekaran and Muthamilan, 1999). The

efficacy of these isolates was assessed based on their inhibitory effect on radial growth and sclerotial production of the pathogen. In the study, the isolates of *Bacillus* sp. were streaked separately on one side of petri dish containing Potato dextrose agar medium and on the opposite side a 6 mm mycelial disc of virulent isolate of *S. rolfsii* was placed. Suitable control was maintained and the plates were incubated at 27±1^{o}C for 5 days. The per cent inhibition of radial growth and sclerotilal production of pathogen over control was calculated as described in 3.10.1.

The potency of 80 isolates of *Bacillus* sp. was further evaluated based their ability to inhibit the oxalic acid production by the pathogen. This study was executed as explained in 3.10.1. However, except the isolates *Trichoderma* sp. a loop-full of cell suspension of each isolate of *Bacillus* sp. prepared from a 48-h old culture was used to inoculate the conical flasks.

From the preliminary screening, the better performing few isolates of *Bacillus* sp. were selected for in detail characterisation of their bio-control traits against *S.rolfsii.*

3.10.3. Characterization of potential isolates of *Trichoderma* sp. for bio-control traits against *S. rolfsii*

3.10.3.1. Dual culture assay

The inhibitory effect of potential isolates of *Trichoderma* sp. (selected based on preliminary data) on radial growth, number and size of sclerotia produced by pathogen was performed as explained in 3.10.1.

3.10.3.2. Metabolites assay

This method was designed to measure the effect of the volatile metabolites produced by the potential *Trichoderma* isolates against the pathogen. The PDA plates were inoculated centrally with 6 mm mycelial disc of each *Trichoderma* isolate and the lid of the plate was replaced by the bottom dish of another PDA plate inoculated centrally with 6 mm mycelial disc of *S. rolfsii*. The two plates were sealed using an adhesive tape (Dennis and Webster, 1971b) and incubated for 5 days at 27±1^{o}C. Both bottom lids with *S. rolfsii* disc was served as control. Observations on the inhibitory effect of *Trichoderma* sp. on radial growth, number and size of sclerotia of pathogen were recorded in control and *Trichoderma* inoculated plates. Per cent inhibition over control was calculated using the formula:

I = (C-T/C) 100

Where,

I = Per cent inhibition over control

C = Radial growth, number and size of sclerotia of *S. rolfsii* in control plates

T = Radial growth, number and size of sclerotia of *S. rolfsii* in *Trichoderma* sp. inoculated plates

3.10.3.3. Culture filtrate assay

To prepare the cell-free culture filtrate, the potential *Trichoderma* isolates were cultured in Potato dextrose broth and incubated for 8 days at 27±1°C on incubator shaker (150 rpm) and filtered through sterilized Whatman No. 1 filter paper. The culture filtrate was directly used to assess the inhibition of sclerotial germination. Culture filtrate of each isolate was placed in separate wells of greiner multi-well plates (Sigma) and well with sterile PDB served as control. Ten sclerotia of virulent *S. rolfsii* isolate were placed in each well and observation on sclerotial germination was recorded at 2 days after incubation. Per cent inhibition over control was calculated using the formula:

I = (C-T/C) 100

Where,

I = Per cent inhibition over control

C = Number of sclerotia germinated in control wells

T = Number of sclerotia germinated in wells containing culture filtrate

3.10.3.4. Inhibitory effect on oxalic acid production

This study was performed to assess the ability of potential *Trichoderma* isolates in inhibiting the oxalic acid production by virulent isolate of *S. rolfsii*. The experiment was executed as explained in 3.10.1.

From the above study, one potential best performing isolate of *Trichoderma* sp. was selected for further studies.

3.10.4. Characterization of potential isolates of *Bacillus* sp. for bio-control traits against *S. rolfsii*

3.10.4.1. Dual culture assay

The inhibitory effect of potential isolates of *Bacillus* sp. (selected based on preliminary data) on radial growth, number and size of sclerotia produced by pathogen was performed as explained in 3.10.2.

3.10.4.2. Metabolites assay

This study was performed to measure the effect of the volatile metabolites produced by potential isolates of *Bacillus* sp. against the *S. rolfsii* and was carried out as explained in 3.10.2.

3.10.4.3. Culture filtrate assay

To prepare the cell-free culture filtrate, the potential isolates of *Bacillus* sp. were cultured in nutrient broth and incubated for 8 days at 27±1°C on incubator shaker (150 rpm) and filtered through sterilized Whatman No. 1 filter paper. The assay was performed as explained in 3.10.3.3.

3.10.4.4. Inhibitory effect on oxalic acid production

This study was performed to assess the ability of potential *Bacillus* isolates in inhibiting the oxalic acid production by virulent isolate of *S. rolfsii*. The experiment was executed as explained in 3.10.2.

From the above study, one potential best performing isolate of *Bacillus* sp. was selected for further studies.

3.11. Compatibility of potential isolates *Trichoderma* sp. and *Bacillus* sp. with fungicides

Four fungicides *viz*., Thiram 75 WP, Carbendazim 50 WP, Tebuconazole 2 DS and Azoxystrobin 23.8 SC were tested for compatibility with potential isolates of *Trichoderma* sp. and *Bacillus* sp. at their recommended and half the recommended dose.

Compatibility study of potential isolates of *Trichoderma* sp. with fungicides was performed by employing the poisoned food technique. The recommended and half the recommended dose of each fungicide was amended in the PDA medium after autoclaving before dispensing into petri dishes. Suitable control was maintained without fungicide amendment. After solidification the plates were seeded with 6 mm mycelial discs of actively growing culture of potential isolates of *Trichoderma* sp. and incubated at 27±1ºC. After 5 days of incubation the radial growth of fungus was recorded. Per cent inhibition of over control was calculated using the formula:

$$I = (C-T/C)\ 100$$

Where,

I = Per cent inhibition over control

C = Radial growth of *Trichoderma* sp. in control plates

T = Radial growth of *Trichoderma* sp.in fungicide amended plates

Similarly, the compatibility of potential isolates of *Bacillus* sp. with fungicides was tested using turbidometric method (ISI, 1964). One ml of the actively growing culture of *Bacillus* isolates were transferred to a 250 ml conical flask containing 50 ml of NB amended with fungicides at recommended and half the recommended dose. Suitable control was maintained without amendment of fungicides. The flasks were incubated at 27±1°C in an incubator shaker. The optical density values of the inoculated and control broth were determined in Shimadzu UV-2600 spectrophotometer at 610 nm after 24h of incubation. Difference in absorption values were used to express the results as per cent inhibition of bacterial growth over the control.

3.12. Compatibility among potential isolates of *Trichoderma* sp. and *Bacillus* sp.

Compatibility among one each potential isolate of *Trichoderma* sp. and *Bacillus* sp. was tested by following dual culture technique. The one potential isolate of *Bacillus* sp. was streaked at one side of the petri dish containing PDA and 6 mm mycelial disc of 5 days old culture of one potential isolate of *Trichoderma* sp. was placed at the opposite side of the petri dish perpendicular to the bacterial streak. Inoculated plates were incubated at 27±1°C and observations on growth of *Trichoderma* in presence of *Bacillus* and vice-versa was recorded.

3.13. Preparation of chitin amended medium

To obtain the colloidal chitin, five gram of crab shell chitin (Sigma, USA) was slowly added into 100 ml of ice cold 0.25 N HCl with vigorous stirring and kept overnight at 4°C. The mixture was filtered through glass wool into 200 ml of ice cold ethanol at 4°C with rapid stirring. The resultant chitin suspension was centrifuged at 10,000 rpm for 20 min and the chitin pellets were washed repeatedly with distilled water till the pH become neutral. Chitin content of the suspension was determined by drying a sample in lyophilizer and the final concentration of 10 mg ml^{-1} (dry weight/volume) was adjusted with distilled water (Berger and Reynolds, 1958). The colloidal chitin (10 mg ml^{-1} w/v) was added to PDB and NB (1%, v/v) to obtain chitin amended medium.

3.14. Preparation of talc based formulation of biocontrol agents

3.14.1. Talc based formulation of potential isolate of *Trichoderma* sp.

Talc based formulation of one potential isolate of *Trichoderma* sp. was prepared as described by Mukherjee *et al.* (2014). A 6 mm mycelial disc of 5 days old actively growing culture of *Trichoderma* sp. was inoculated into 0.5x strength Potato dextrose broth (500 ml conical flask containing 100 ml of PDB) and incubated for 7 days at 27 ±1°C. After 7 days of incubation 200 ml of PDB medium with spore suspension, 1 kg of sterile talc powder, 15 g calcium carbonate (to adjust the pH to neutral) and 5 g CMC (adhesive) were mixed under sterile conditions (Plate 3.1). The final product was examined for spore load and adjusted to 10^8 cfu (colony forming units) per gram of formulation.

To prepare talc formulation of potential isolate of *Trichoderma* sp. with chitin amendment the 0.5x PDB medium was amended with 1% colloidal chitin.

3. 14.2. Talc based formulation of potential isolate of *Bacillus* sp.

A loopful culture of one potential isolate of *Bacillus* sp. was inoculated into the sterilized NB medium and incubated in an incubator shaker (150 rpm) for 72 h at 27±1°C (Plate 3.1). After 72 h, 400ml of bacterial broth suspension containing $9x10^8$cfu/ml, 1 kg of the sterile talc powder, 15 g calcium carbonate (to adjust the pH to neutral) and 5 g CMC (adhesive) were mixed under sterile conditions by following the method described by Nandakumar *et al.* (2001).

To prepare talc formulation of potential isolate of *Bacillus* sp. with chitin amendment the NB medium was amended with 1% colloidal chitin.

3.14.3. Mixtures of potential isolates of *Trichoderma* sp. and *Bacillus* sp.

Talc formulation of two bio-agents was mixed equally at the time of application. This procedure remains same even for chitin based talc formulation.

3.15. Plant growth promotion by potential isolates of *Trichoderma* sp. and *Bacillus* sp.

Plant growth promoting activity of bio-formulations of potential isolates of *Trichoderma* sp. and *Bacillus* sp. were assessed based on the seedling vigour index by the standard paper towel method (ISTA, 1993) using seeds of TMV 2 and J 11 groundnut cultivars. Ten seeds treated with talc formulation of potential isolates of

Trichoderma sp. and *Bacillus* sp. alone and in combination (with or without chitin) were kept over the pre-soaked germination paper. The seeds were held in position by placing another pre-soaked germination paper then rolled and incubated in growth chamber for ten days. Observations on germination, shoot length, root length, wet biomass and dry biomass were recorded to calculate vigour Index I, vigour Index II and germination percentage. The parameters like total root length, total root volume were assessed in root scanner instrument.

The vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973), where, Vigour Index I = (Mean root length + Mean shoot length) x Germination (%) and Vigour Index II = Dry weight (g) x Germination (%).

3.16. Evaluation of bio-formulations against stem rot under glasshouse conditions

Pot culture experiments were conducted to test the efficacy of potential isolates of *Trichoderma* sp. and *Bacillus* sp. bio-formulations (with and without chitin) through seed treatment (ST) and soil application (SA) in controlling stem rot of groundnut. Two set of pot experiments were executed by maintaining similar conditions. One to assess the disease control ability of bio-agents and another to check the defence enzymes induction capability of bio-agents in groundnut in response to *S. rolfsii* the causal organism of stem rot.

3.16.1.1. Raising of seedlings and treatment setup

Susceptible groundnut cultivar, TMV 2 was used during the study. The plastic pots of eight inch diameter were filled with a 2:1:1 autoclaved mixture of alfisol soil, sand and vermicompost. The experiment was conducted in a completely randomized block design with nine treatments having four replicates. The treatments include,

T1 – ST+SA of talc formulation of potential isolate of *Bacillus* sp.

T2 – ST+SA of talc formulation of potential isolate of *Bacillus* sp. (with chitin)

T3 – ST+SA of talc formulation of potential isolate of *Trichoderma* sp.

T4 – ST+SA of talc formulation of potential isolate of *Trichoderma* sp. (with chitin)

T5 – ST+SA of talc formulation of potential isolate of *Bacillus* sp. + potential isolate of *Trichoderma* sp.

T6 – ST+SA of talc formulation of potential isolate of *Bacillus* sp. + potential isolate of *Trichoderma* sp. (with chitin)

T7 – Chemical control

T8 – Inoculated control

T9 – Un-inoculated control

Where, ST is seed treatment and SA is soil application. The bio-formulations were applied with soil as basal application @ 5g/kg pot soil. Two days after soil application,

groundnut seeds treated with bio-formulations (10g/kg) were sown @ eight seeds/pot and finally four seedlings per pot were maintained. The treatments were compared with T7, T8 and T9. Where T7 was fungicide control in which the seeds were treated with tebuconazole 2 DS (@1g/kg seeds) at the time of sowing and azoxystrobin 23.8 SC @ 1 ml/l was applied as soil drench at 34th day after sowing. Whereas T8 was inoculated control (with pathogen inoculation) and T9 was un-inoculated control (without pathogen inoculation).

3.16.1.2. Multiplication of inoculum of virulent isolate of *S. rolfsii*

The inoculum of virulent isolate of *S. rolfsii* was prepared on SGM (Sorghum grain medium) as explained in 3.6.2.

3.16.1.3. Inoculation and observations

Seedlings of 35 days old were inoculated with 15g of inoculum per 7" pot except uninoculated control pots by spreading the inoculum on surface of soil (Plate 3.2). Germination percentage was recorded at 10 days after sowing. Observations on disease severity (DS) [A 1-5 scale was used for all disease severity assessments (Shokes *et al*., 1996) and per cent disease severity was calculated as per Le *et al* (2012)], disease incidence (DI) and mortality (M) were recorded at 15 days after inoculation and then on for every 15 days interval and continued up to 75 days after inoculation.

3.16.2. Assay of defense related enzymes (Induced systemic resistance)

The treatment setup and method of inoculation remain same as in 3.16.1.1 and 3.16.1.3 respectively.

3.16.2.1. Sample Collection

The stem samples were collected from individual treatments to study the induction of defense enzymes in response to pathogen attack in groundnut plants under glass house conditions. The samples were collected at 0, 1, 2, 3, 4, 5, 6, and 7 dpi (day post inoculation) at 1 day interval. The collected samples were immediately dipped in liquid N_2 and stored in -80^{o}C for further use.

3.16.2.2. Spectrophotometric assay of defense enzymes

Spectrophotometric assay of total protein, total phenols, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, catalase, chitinase and β-1,3 glucanase are explained hereunder.

3.16.2.2.1. Estimation of total proteins by Lowry's method

The aromatic amino acids present in a protein like tyrosine and tryptophan react with phosphomolybo-phosphotungstate (FCR) reagent to produce a blue coloured complex at 660 nm. The depth of the colour produced is proportional to the aromatic amino acids present in a given sample. A 0.5 g of sample was macerated using sterile pestle and mortar in 5 ml of 25mM Tris-HCl (pH 8.8) buffer at 4°C. The homogenate was centrifuged for 20 min (4^0C) at 15,000 rpm. Supernatant was used as enzyme extract for assay of total proteins (Lowry *et al.* 1995).

Reagent preparation:

Alkaline copper reagent:

Solution A – 2 % sodium carbonate in 0.1 N NaOH

Solution B – 1 % sodium potassium tartarate

Solution C – 0.5 % copper sulphate

Solutions A, B and C were mixed in the 100:1:1 proportion just before use.

Stock protein standard solution: A 50 mg of Bovine serum albumin (BSA) was dissolved in distilled water and made up to 50 ml with distilled water in a volumetric flask. This solution contained 1 mg of protein per ml.

Working standard solution: A 10 ml of stock standard solution was diluted to 50 ml distilled water in a volumetric flask. This solution contained 200 µg of protein per ml.

1 N Folin-ciocalteau reagent (FCR): Prepared as per instruction given by manufacturer (Fisher-scientific).

Assay: Working standard solution (40-200 µg) was pipetted out in to labelled test tubes and volume made to 2 ml with distilled water. A blank was maintained with 2 ml distilled water. A 0.2 ml of tissue extracts (all treatments) were taken in series of test tubes and volume made to 2 ml using distilled water. Later, 10 ml of alkaline copper reagent was added to all the test tubes and thoroughly mixed and allowed to stand for 10 minutes. Finally, 1 ml of 1 N FCR was added to all the tubes, mixed well and were kept in dark for 30 minutes (Blue colour will develop). The absorbance values of standards

and the samples were read against reagent blank which was set at 100% at 660 nm. Amount of protein in samples were calculated from standard graph and expressed as mg of protein per gram fresh weight of sample (mg g^{-1}FW).

3.16.2.2.2. Assay of Phenylalanine ammonia lyase (PAL)

The PAL activity (EC 4.3.1.5) was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm as per the method described by Ross and Sederoff (1992).

Reagents preparation:

0.1 M borate buffer (pH 8.8): The 6.183 g of boric acid and 1 g of NaOH was dissolved in 800 ml of distilled water and volume was made to 1000 ml. To this solution 0.1 g of polyvinyl pyrrolidine (PVP) was added.

12 mM L-phenylalanine (12 mM): 1.98 g of L- phenylalanine was dissolved in 1000 ml of distilled water. The solution was prepared a fresh.

Trans-cinnamic acid: 29.64 mg of trans-cinnamic acid was dissolved in 10 ml of acetone. 1 ml of this solution was diluted to 10 ml with borate buffer to obtain 2 moles trans-cinnamic acid/ml working standard solution. The buffer is stored under refrigerated condition.

1 M trichlro acetic acid (TCA): 16.339 g of TCA was dissolved in 100 ml of distilled water.

Preparation of enzyme extract: A 0.5 g of sample was homogenised with 5 ml of 0.1 M ice cold sodium borate buffer (pH 8.8). The homogenate was centrifuged at 15000 rpm at 4°C for 20 min. The supernatant was collected and used for estimation of PAL activity.

Assay: Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer (pH 8.8) and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The reaction was arrested by adding 0.5 ml of 1M TCA and incubated at 37 °C for 5 min. The blank contains 0.4 ml of crude enzyme extract and 2.7 ml of 0.1M borate buffer (pH 8.8) and absorbance was measured at 290 nm in Shimadzu UV-2700 spectrophotometer. Standard curve was drawn with graded amounts of cinnamic acid dissolved in acetone. The enzyme activity was expressed as

µmole of trans-cinnamic acid/min/g fresh weight of tissue/mg of protein (µmole trans-cinammic acid $min^{-1}g^{-1}$ FW mg^{-1} protein).

3.16.2.2.3. Estimation of total phenols

Phenols were estimated by Folin-ciocalteau (FCR) method (Malik and Singh, 1980). It is based on the reaction between phenols and an oxidizing agent, phospho-molybdate which results in the formation of a blue coloured complex with max at 650 nm.

Preparation of reagents:

Stock standard: 100mg of catechol was dissolved in 100ml distilled water.

Working standard: 5 ml of stock solution was diluted to 45 ml with distilled water. 1 ml of this solution contains 100 µg catechol/ml.

20% Sodium carbonate solution*:* 20 gm sodium carbonate was dissolved in 0.1 N NaOH and then the volume was made up to 100 ml with 0.1 N NaOH.

Folin-ciocalteau reagent (FCR): Just before use the FCR was prepared as per instruction given by manufacturer (Fisher-scientific).

Preparation of enzyme extract: 0.5 g of sample was homogenised with 10 times volume of 80% ethanol. The residue was re-extracted with 5 times volume of 80% ethanol, centrifuged and supernatant was pooled. Supernatant was evaporated to dryness (40^{o}C for 4-5 hrs in speed vacuum evaporator) and the residue was dissolved in known volume of distilled water (5 ml) and used for total phenol assay.

Assay: A 10 ml of FCR was added to 1 ml of the alcohol extract in a test tube followed by 2 ml of 20 per cent sodium carbonate solution and the mixture was heated in a boiling water bath for exactly 1 min. It was later cooled and made up to a known volume with distilled water. The blue coloured complex developed was read at 650 nm in spectrophotometer. The standards were prepared using catechol and concentration of phenols present in different samples was calculated from standard curve and expressed as mg/g fresh weight of tissue/ mg protein (mg g^{-1} FW mg^{-1} protein).

3.16.2.2.4. Assay of peroxidase (PO)

The spectrophotometric assay of peroxidase (EC 1.11.1.7) was carried out as per Hartee (1955).

Preparation of reagents:

1% hydrogen peroxide solution: 3.3 ml of H_2O_2 was mixed with 97.70 ml of distilled water to get 100 ml of 1% H_2O_2 solution. The solution was prepared fresh every time.

0.05 M Pyrogallol: 6.3005 g of pyrogallol was dissolved in 100 ml of distilled water. The solution was prepared every time freshly.

Preparation of enzyme extract: 0.5 g of sample was macerated using sterile pestle and mortar in 5 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min (4^0C) at 10,000 rpm. Supernatant was used as enzyme extract for assay of peroxidase defense enzyme.

Assay: The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol and 0.5 ml of the enzyme extract. The reaction was started by adding 0.5 ml of 1% H_2O_2. The change in absorbance was recorded at 470 nm for 3 min in spectrophotometer. The boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance at 420 nm min/g fresh weight of tissue/mg protein (ΔOD_{470nm} min^{-1} g^{-1} FW mg^{-1} protein) (Hammerschmidt *et al*., 1982).

3.16.2.2.5. Assay of polyphenol oxidase (PPO)

The polyphenol oxidase (EC 1.14.18.1) activity was determined as per the procedure given by Mayer *et al.* (1965) with minor modifications.

Preparation of reagents:

0.5 M catechol: 5.51 g of catechol was dissolved in small quantity of distilled water and final volume was made to 100 ml.

Preparation of enzyme extract: Performed as explained in 3.16.2.2.1.4.

Assay: The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer (pH 7.0) and 0.5 ml of the enzyme extracts. To start the reaction, 0.5 ml of 0.5 M catechol was added and change in absorbance was recorded at 420 nm for 3 min in spectrophotometer. The polyphenol oxidase activity was expressed as changes in

absorbance at 495 nm/min/g fresh weight of tissue /mg protein (ΔOD_{420nm} min^{-1} g^{-1} FW mg^{-1} protein).

3.16.2.2.6. Assay of catalase (CAT)

Preparation of enzyme extract: Performed as explained in 3.16.2.2.1.4.

Assay: Catalase activity (EC 1.11.1.6) was assayed spectrophotometrically as described by Chaparro-Giraldo *et al.* (2000) using 3 ml assay mixture containing 100 mM potassium phosphate buffer (pH 7.5) and 25 mM H_2O_2 prepared immediately before use and 100 µl enzyme extract. The activity was measured by monitoring the degradation of H_2O_2 using Spectrophotometer at 240 nm over 3 min, against a plant extract-free blank. The decrease in H_2O_2 was followed as the decline in optical density at 240 nm, activity was calculated using the extinction coefficient (ε_{240nm} = 40 M^{-1} cm^{-1}) for H_2O_2 and expressed in µmol of H_2O_2/ min/g fresh weight of tissue/mg protein (µmole $H_2O_2min^{-1}g^{-1}$ FW mg^{-1} protein).

3.16.2.2.7. Assay of Chitinase

The colorimetric assay of chitinase (EC 3.2.1.14) was carried out according to the procedure developed by Boller and Mauch (1988). 0.5 g of sample was extracted with 5 ml of 0.1 M sodium citrate buffer (pH 5.0). The homogenate was centrifuged for 10 min at 20,000 g at 4°C and the supernatant was used as enzyme source.

Preparation of reagents:

Colloidal chitin: It was prepared as described in 3.13.

Snail gut enzyme: 600 mg of the commercial lyophilized snail gut enzyme (Helicase, Sepracor, France) was dissolved in 10 ml of 20 mM potassium chloride (KCl) and chromatographed on a Sephadex G-25 column (38 x1.5 cm) using a 10 mM KCl solution, containing 1 mM EDTA and adjusted to pH 6.8 for equilibration and elution. The first 20 ml of enzyme was eluted and used for chitinase assay (Boller and Mauch, 1988).

***p*-dimethyl amino benzaldehyde (DMAB) reagent:** The DMAB reagent was prepared by the procedure described by Reissig *et al.* (1955). Stock solution of DMAB was prepared by mixing 8 g of DMAB in 70 ml of glacial acetic acid along with 10 ml of

concentrated HCl. One volume of stock solution was mixed with 9 volumes of glacial acetic acid immediately before use.

Assay: The reaction mixture consisted of 10 µl of 0.1 M sodium acetate buffer (pH 4.0), 0.4 ml enzyme solution and 0.1 ml colloidal chitin. After incubation for 2 h at 37°C, the reaction was stopped by centrifugation at 3,000 g for 3 min. An aliquot of the supernatant (0.3 ml) was pipetted into a glass reagent tube containing 30 µl of 1 M potassium phosphate buffer (pH 7.0) and incubated with 20 µl of 3% (w/v) snail gut enzyme for 1 h. After 1 h, the reaction mixture was brought to pH 8.9 by the addition of 70 µl of 0.1 M sodium borate buffer (pH 9.8). The mixture was incubated in a boiling water bath for 3 min and then rapidly cooled in an ice-water bath. After addition of 2 ml of DMAB, the mixture was incubated for 20 min at 37°C immediately thereafter; the absorbance was measured at 585 nm. *N*-acetyl-D-glucosamine (GlcNAc) was used as a standard and the enzyme activity was expressed as µmole N-acetyl-D-glucosamine/min/g fresh weight of tissue/mg protein (µmole N-acetyl-D-glucosaminemin^{-1}g^{-1} FW mg^{-1} protein).

3.16.2.2.8. Assay of *β*-1,3 glucanase

β-1,3 glucanase activity (E.C. 3.2.1.39) was assayed by the laminarin-dinitrosalicylic acid method (Pan *et al.*, 1991).

Preparation of reagents:

Dinitro-salicylic acid (DNS) reagent: 1.0g of 3,5-dinitrosalicylic acid and 30g sodium potassium tartrate tetrahydrate was dissolved in 50 ml of distilled water. Slowly 20 ml 2N NaOH solution was added to it and final volume was made to 100 ml.

Preparation of enzyme extract: 0.5 g of sample was extracted in 5 ml of 0.05 M sodium acetate buffer (pH 5.0). The homogenate was centrifuged at 20,000 g for 10 min at 4°C and the supernatant was used as enzyme source.

Assay: The reaction mixture consisted of 62.5 µl of 4% laminarin (Sigma) and 62.5 µl of enzyme extract. The reaction was carried out at 40°C for 10 min. The reaction was stopped by adding 375µl of dinitrosalicylic acid and heating for 5 min in boiling water, vortexed and its absorbance was measured at 500 nm. D-glucose was used as a standard. The enzyme activity was expressed as µmole glucose released/min/g fresh weight of tissue/mg protein (µmole glucosemin^{-1}g^{-1} FW mg^{-1} protein).

3.16.2.2.2. Activity gel electrophoresis

Native PAGE assay was carried out to study the effect bio-formulations in induction of isoforms of peroxidase, polyphenol oxidase and catalase defense enzymes in groundnut against *S. rolfsii.*

Stock chemicals required for native PAGE analysis

Stock acrylamide solution: 29.22 g of acrylamide 30% and 0.78 g of bis-acrylamide 0.8% was dissolved in 60 ml distilled water. The volume was made to 100 ml and stored at 4°C.

1.5 M Tris-HCl: 47.28 g of Tris-HCl was dissolved in 200 ml of distilled water; pH was adjusted to 8.8 and stored at 4^{o}C.

0.5 M Tris-HCl: 15.76 g of Tris-HCl was dissolved in 200 ml of distilled water, pH was adjusted to 6.8 and stored at 4^{o}C.

10% APS: 0.1 g of APS was dissolved in 1.0 ml of distilled water. Prepared a fresh just before use.

5X Loading/Sample buffer (8 ml): 1.0 ml of 0.5 M Tris-HCl (pH 6.8), 0.8 ml of β – mercaptaethanol, 1.6 ml of Glycerol, 0.4 ml of 0.5% bromophenol blue and 4.2 ml of distilled water were mixed well and stored at room temperature.

Electrode/gel running buffer (pH 8.3): 4.32 g of Glycine, 0.9 g of Tris base was dissolved in distilled water and volume made to 300 ml. The pH was adjusted to 8.3.

Resolving/separating gel (12%) (10 ml)

a. Stock acrylamide solution : 4.18 ml
b. 1.5 M Tris-HCl (pH- 8.8) : 2.5 ml
c. Distilled water : 3.3 ml
d. 10% APS : 150 µl
e. TEMED : 10 µl

Stacking gel (4%) (10 ml)

a. Stock acrylamide solution : 1.35 ml
b. 1.5 M Tris-HCl (pH- 8.8) : 1.25 ml
c. Distilled water : 7.5 ml
d. 10% APS : 50 µl
e. TEMED : 10 µl

Preparation of enzyme extract: 0.5 g of sample was macerated using sterile pestle and mortar in 5 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min (4^0C) at 10,000 rpm. Supernatant was used as enzyme extract to study the activity gel electrophoresis of peroxidase, polyphenol oxidase and catalase. Enzyme extracts of all the samples were diluted with sterile distilled water to adjust equal concentration of protein in all the samples. 40 µl of enzyme extract was mixed with 20 µl of loading buffer before loading the samples to wells on gel.

3.16.2.2.2.1. Peroxidase (PO)

To study the expression pattern of different isoforms of peroxidases in different treatments, activity gel electrophoresis was carried out. For native polyacrylamide gel electrophoresis, resolving gel of 12% and stacking gel of 4% were prepared. Native electrophoresis was carried out in mini Protean (Bio-Rad) PAGE setup at 80 V (4^0C) for 7 hr. After electrophoresis the gels were incubated in 0.2 M acetate buffer (pH 5.0) containing 1.3 mM benzidine (dissolve benzidine in acetate buffer by heat and constant stirring) and fresh 1.3 mM H_2O_2 added to the benzidine solution just prior to placing gels in incubation medium. Peroxidase isozymes will appear after 30 min of incubation. After staining, the gel was washed with distilled water and photographed (Sindhu *et al.,* 1984).

3.16.2.2.2.2. Polyphenol oxidase (PPO)

To study the expression pattern of different isoforms of poly phenoloxidase in different treatments, activity gel electrophoresis was carried out. For native polyacrylamide gel electrophoresis, resolving gel of 12% and stacking gel of 4% were prepared. After native electrophoresis, the gels were equilibrated in 50mM sodium phosphate buffer (pH 7.0) containing 0.1 % *p*-phenylenediamine (coupling reagent) in dark for 20 min, followed by addition of 0.5 M catechol. The addition of catechol was followed by a gentle shaking which resulted in appearance of dark brown discrete bands (Gayatridevi *et al.,* 2014).

3.16.2.2.2.3. Catalase

Electrophoresis was carried out under native condition in 8% polyacrylamide mini-gels for CAT activity staining. Electrophoresis running conditions were as described by Vitoria *et al.* (2001) and buffers and gels were prepared as described by Laemmli (1970) lacking SDS. The activity was assayed as described by Woodbury *et*

al. (1971). After native electrophoresis, the gels were washed 3 times in distilled water and incubated in 0.003% H_2O_2 for 10 min. Later, rinsed twice with distilled water to remove traces of H_2O_2. In two separate conical flasks 1% ferric chloride (w/v) and 1% potassium ferricyanide (w/v) prepared separately and poured directly on the top of the gel. When achromatic bands begin to form, the stain was poured off and the gels were rinsed extensively with distilled water and photographed.

3.16.2.2.2.4. Construction of zymogram of PO, PPO and CAT

In zymogram the presence of isoforms were indicated with + sign and absence with – sign. Further the intensity of band was highlighted with ++ sign. The isoforms of PO, PPO and CAT were designated as PO1, PO2, PO3; PPO1, PPO2, PPO3; and CAT1, CAT2, CAT3 respectively.

3.17. Evaluation of bio-formulations against stem rot under field conditions

A field trial was conducted at two locations during *kharif,* 2016 to test the efficacy of potential isolate each of *Trichoderma* sp. and *Bacillus* sp. against stem rot of groundnut.

3.17.1. Location I

The trial was conducted at ICRISAT, Patancheru (India) during *kharif* 2016. The experiment was carried out with beds of 1.5 × 4.0 m^2 size in a randomized complete block design (RCBD) with 9 treatments and 3 replications with spacing of 30 x10 cm. The groundnut cultivarTMV-2 which is susceptible to stem rot was used. Production practices were followed as recommended by ICRISAT, Patancheru, India. The treatments of the experiment were as follows;

T1 – ST+SA of talc formulation of potential isolate of *Bacillus* sp.

T2 – ST+SA of talc formulation of potential isolate of *Bacillus* sp. (with chitin)

T3 – ST+SA of talc formulation of potential isolate of *Trichoderma* sp.

T4 – ST+SA of talc formulation of potential isolate of *Trichoderma* sp. (with chitin)

T5 – ST+SA of talc formulation of potential isolate of *Bacillus* sp. + potential isolate of *Trichoderma* sp.

T6 – ST+SA of talc formulation of potential isolate of *Bacillus* sp. + potential isolate of *Trichoderma* sp. (with chitin)

T7 – Chemical control

T8 – Inoculated control

T9 – Un-inoculated control

Where, ST is seed treatment and SA is soil application. Fifteen days before sowing, the bioformulations were mixed with well decomposed farm yard manure

(FYM) and applied at 2.5 kg/ha as basal. The seeds were treated with bioformulations @ 10g/kg of seeds and sowing was taken up. The treatments were compared with T7, T8 and T9. Where T7 was fungicide control in which the seeds were treated with tebuconazole 2 DS (@1g/kg seeds) at the time of sowing and azoxystrobin 23.8 SC @ 1 ml/l was applied as soil drench at 44th day after sowing. Whereas T8 was inoculated control (with pathogen inoculation) and T9 was un-inoculated control (without pathogen inoculation). The inoculum of virulent isolate was prepared on SGM as explained in 3.16.1.2. On 45th day after sowing artificial inoculation was taken up in field in which, 400 g of inoculum of virulent isolate was applied per 4 meter row. The inoculum was placed near the collar region of plants (Plate 3.2).

A 1-5 severity scale was used for all disease severity assessments (Shokes *et al*., 1996) and per cent disease severity was calculated as per Le *et al* (2012). For disease severity (DS) observations, about 20% of plant population was considered and the data was recorded at regular intervals. Other observations like disease incidence (DI) and mortality (M) were also recoded. All the observations were taken at 15 days after inoculation and then on for every 15 days interval till harvesting. At harvest plants were uprooted and observed for stem discoloration, lesions on pod and pod rot. After harvest, observations on yield related attributes *viz.*, shoot length (cm), root length (cm), nodulation (no./plant), number of pods/plant, pod yield/plot, biomass yield/plot, 100 kernel weight, shelling (%), Oil content (%) and protein content (%) were recorded.

3.17.2. Location II

The same field trial was conducted at college farm, PJTSAU, Rajendranagar (India) during *kharif* 2016. All experimental setup was as similar as location I.

3.18. Statistical analysis

All the *in-vitro*/laboratory experiments were carried out in a completely randomized block design (CRBD) with 5 replications. The glasshouse experiments were also conducted in a completely randomized design block design (CRBD) with 4 replications. Field experiments were conducted in a randomized complete block design (RCBD) with 9 treatments and 3 replications. The data were statistically analysed using the Genstat 14th edition developed by the Rothamsted research station, England. Prior to statistical analysis of variance (ANOVA) the percentage values were arc sine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were separated using critical difference (CD).

RESULTS AND DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

The results of the investigations conducted on morphological, cultural and molecular diversity of *Sclerotium rolfsii* Sacc. and induced systemic resistance in groundnut against stem rot pathogen are presented and discussed in this chapter.

4.1. Survey and disease assessment

The roving survey conducted during *kharif,* 2013 and *kharif,* 2014 in major groundnut growing areas of India indicated the incidence of stem rot in all the groundnut fields surveyed. During *kharif,* 2013 the incidence of stem rot in major growing areas of India was ranged from 11.23 to 55.40% (Table 4.1). Further, the Gujarat state recorded the highest per cent mean stem rot incidence of 28.86, followed by Maharashtra (27.02), Tamil Nadu (24.47), Karnataka (22.81), and Andhra Pradesh (22.55). The lowest mean per cent incidence was recoded in Telangana (20.65). Among the various districts surveyed, highest per cent mean incidence was observed in Porbander (32.31) followed by Amreli (31.80) and Rajkot (30.31) of Gujarat and the lowest per cent mean incidence was found in Raichur (19.06) of Karnataka followed by Warangal (19.56) of Telangana (Table 4.2).

During *kharif,* 2014 the similar pattern in incidence of stem rot was followed wherein the overall per cent incidence of stem rot in major groundnut growing areas of India was ranged from 10.11 to 59.33. Among the different states, Gujarat recorded highest per cent mean incidence of 27.62 which was followed by Maharashtra (26.80), Tamil Nadu (24.13), Andhra Pradesh (24.11) and Karnataka (22.92) (Table 4.1). Further, lowest per cent mean incidence was recorded in Telangana (22.39). Of the different districts surveyed Amreli recorded highest per cent mean incidence of 32.62 followed by Porbander (28.09) of Gujarat and Nanded (26.86) of Maharashtra. Further, the lowest per cent mean incidence was found in Raichur (20.94) of Karnataka followed by Warangal (21.35) of Telangana (Table 4.2).

In the present study, the varied incidence of stem rot of groundnut from one locality to another was might be due to cultivation of different groundnut varieties (K 2, K 6, TG 1, TG 17, VRI 2, TMV 7, TMV 10, GG 20, GG 2, GG 11, TMV-2, JL-24 and local cultivars), prevalence of different soil conditions (temperature and soil moisture content) and adoption of different cropping patterns such as pigeonpea-groundnut,

chickpea-groundnut, chilli-groundnut, ragi-groundnut, cotton-groundnut, wheat-groundnut, cumin-groundnut, castor-groundnut, and groundnut-groundnut. Even, it could also be attributed to the existence of pathogenic variability in the fungus. The results are in agreement with Kulkarni (2007) who reported the variable incidence of stem rot of potato at different localities of Belagavi, Dharwad, and Haveri districts of Karnataka and Siddaramaiah *et al.* (1979) who observed the varied levels of stem rot incidence in various groundnut growing villages in Dharwad district of Karnataka. Similarly, Ramakrishna and Kolte (1988) reported the different levels of stem rot incidence in major crop growing areas of India which was ranged from 15-30 per cent.

Divya Rani *et al.* (2016) noticed the varied levels of incidence of stem rot of groundnut in different villages of Andhra Pradesh during *kharif,* 2012 and *kharif,* 2013. Further, Kadam *et al.* (2011) recorded higher incidence of stem rot in cultivar JL 24 (17.3%) among various cultivars grown in marathwada region of Maharastra. Additionally, Ghewande *et al.* (2002) reported the average incidence of 27 per cent in major groundnut growing areas of India. They also noticed the higher incidence of stem rot in Maharashtra, Saurashtra region of Gujarat compared to other areas surveyed. Our findings were also in agreement with Okabe and Matsumoto (2000) who reported 10 to 40 per cent incidence of stem rot in different groundnut growing areas of Japan.

In a study, among different districts surveyed in Karnataka the higher incidence of stem rot was recorded in Chitradurga and Tumkur (Table 4.1). This is in line with findings of Karunakaran *et al.* (2013) who observed the higher incidence of groundnut in major groundnut growing areas of Chitradurga district where the susceptible groundnut varieties such as TMV 2 and K 6 were grown in large area as a sole crop. Thus, the continuous cultivation of any crop over the seasons and years will build up inoculum level to such an extent that the epidemic will become a common phenomenon (Chaube and Singh, 2001).

During the survey it was observed that the incidence of stem rot was comparatively higher in crop which was at pod stage (Table 4.1). The findings are in agreement with Pande and Rao (2000) who opined that though stem rot occurred in the seedling stage, its incidence increased as the crop grew older. The disease reached maximum and even lead to death of plants and rotting of pods at maturity.

Similarly, during survey, higher incidence of stem rot was observed in all the districts of Gujrat and parts in Maharashtra and Tamil Nadu where the crop was grown in black soils with susceptible groundnut cultivars (TMV 2, JL 24, GG 20, and GG 11) continuously in a sole cropping pattern (Table 4.1). The findings are in agreement with Le *et al.* (2012) who reported that, the continuous cultivation of groundnut in Central Vietnam lead to multiplication and build-up of pathogen inoculum in soil which resulted in higher incidence of disease. Further, the black soils contains higher organic matter content which is known to support the germination of sclerotia and subsequent hyphal growth towards the host plant and leads to higher disease incidence (Punja, 1985).

The least incidence of stem rot was noted in Telangana, parts of Karnataka and Tamil Nadu where the crop is cultivated in red soils and diverse cropping pattern such as pigeopea-groundnut, chickpea-groundnut, chilli-groundnut, ragi-groundnut was practiced (Table 4.1). The findings are in line with Anahosur (2001), who reported that, the cereal crops are less preferred hosts of *S. rolfsii* as the root exudates of these crops have detrimental effect on sclerotial germination. Further, lesser organic content of the red soil do not favour the germination of sclerotia and subsequent hyphal growth toward the host plant and hence leads to lesser disease incidence (Punja, 1985).

4.2. Symptoms of the disease

The primary symptoms of stem rot of groundnut were browning and wilting of leaves and branches which were still attached with the plant. The fungus preferentially infected stem by forming a whitish mycelial mat around the stem which was later spread over the soil and around the basal canopy of the plant. In advanced stage the fungus produced sclerotia which were like mustard seeds in size and colour, appeared on the infected area. In later stage the entire plant was killed or only few branches were affected. Infected pods were completely covered with white mycelial growth and in severe cases rotting of pods were observed (Plate 4.1). The similar kind of symptoms of stem rot of groundnut was observed by previous workers Mehrotra and Aneja (1990), Aken and Dashiell (1991), Le *et al.* (2012) and Narendra Kumar *et al.* (2013).

4.2.1. Isolation and identification of pathogen

The fungus was successfully isolated on Potato dextrose agar medium by following tissue isolation method from stem samples of diseased groundnut plants

collected from major growing regions of India. The *Sclerotium rolfsii* produced white cottony mycelial growth on potato dextrose agar medium and the colony morphology was compact or fluffy. Initially, the white colored sclerotia were formed. Then their color was changed from white to off-white, light brown and dark brown as they attained maturity. The sclerotia were mostly spherical and sometimes sub-spherical in shape. The surface of sclerotia was mostly smooth and sometimes with fine wrinkles. Looking to the cultural and morphological characters exhibited by the fungus it was identified as *Sclerotium rolfsii* based on description given by Punja (1985). Additionally, the description of pathogen was in conformity with the report of early workers Kokub *et al.* (2007) and Prasad *et al.* (2010). Further the molecular identity of fungus was performed by ITS-rDNA amplification, sequencing and phylogeny. The fungus at molecular level was identified as *Sclerotium rolfsii*. The detailed results of molecular identity are presented in 4.6.2.

Totally 60 isolates of *Sclerotium rolfsii* (Plate 4.2) were collected from major groundnut growing areas of India. Among them, 20 isolates were from Karnataka, 10 each from Telangana and Andhra Pradesh, 6 each from Maharashtra and Tamil Nadu and 8 from Gujarat (Table 4.3). Similar type of studies was conducted by Kumar and Sen (2000), Rajalakshmi (2002) and Goud (2011) who isolated the fungus, *S. rolfsii* from stem portion of diseased groundnut plants and mentioned the similar kind of observations.

4.3. *In-vitro* evaluation of different culture media for optimal production of oxalic acid by *S. rolfsii*

To know the best suited culture medium for optimal production of oxalic acid (OA) three different media *viz.,* Richard's broth (RB), Czapek dox broth (CDB) and Potato dextrose broth (PDB) were evaluated using one each randomly selected isolate of *S. rolfsii* from each state. The isolates selected includes SrKa-1, SrTs-1, SrAp-1, SrMh-1, SrTn-1 and SrGj-3 (Plate 4.3).

In the study significantly highest amount of OA production was noticed in RB (1.86 mg/ml of culture filtrate) followed by CDB (1.40 mg/ml) and PDB (1.19 mg/ml). Hence, the RB was found to be best supporting medium for *in vitro* evaluation of oxalic acid production by *S. rolfsii* (Table 4.4).

Our results are in agreement with the findings of Gawande *et al.* (2013) who reported the highest amount of OA production by different crop isolates of *S. rolfsii* in

RB medium. Further, Saraswathi and Madhuri (2013) opined that CDB supported well for OA production by *S. rolfsii* compared to PDB. The *in vitro* OA production by *S. rolfsii* was greatly influenced by medium composition. Hence, the media rich in carbohydrate and devoid of minerals induces the biomass and sclerotial production whereas the medium rich in minerals favoured the OA production (Punja and Jenkins, 1984). Further, abundant mycelial growth does not always insure high OA production (Gawande *et al.*, 2013).

4.3.1. *In vitro* OA production by isolates of *S. rolfsii*

The *in vitro* OA production by isolates of *S. rolfsii* was studied using Richards's broth and results are presented in table 4.5. The 60 of isolates of *S. rolfsii* exhibited wide variation in the amount of oxalic acid production under *in vitro* conditions (Plate 4.4) and was ranged from 0.64 to 2.85 mg/ml of culture filtrate.

The significantly highest amount of oxalic acid was produced by the isolate SrGj-3 (2.85 mg/ml) followed by SrGj-2 (2.51 mg/ml), SrKa-7 (2.28 mg/ml), SrKa-3 (2.26 mg/ml), SrKa-5 (2.24 mg/ml), and SrTs-10 (2.16 mg/ml). Further, the isolates SrTn-3 and SrTn-4 were produced significantly lowest (0.64 mg/ml) amount OA followed by SrKa-12 (0.76 mg/ml) and SrAp-2 (0.78 mg/ml) (Table 4.5).

These results are in conformity with Punja and Jenkins (1984) who reported the variation in OA production by isolates of *S. rolfsii* in culture filtrate. Similarly, Ansari and Agnihotri (2000) characterized 44 isolates of *S. rolfsii* from soybean and classified them into 4 groups on the basis of quantity of oxalic acid produced. Likewise, Sarma *et al.* (2002) also demonstrated marked variation in HPLC profiles of culture filtrates of *S. rolfsii* isolates and observed a distinct difference in the production of oxalic acid. Further, Gawande *et al.* (2013) reported the varying amount of oxalic acid production by different crop isolates *S. rolfsii*.

The *S. rolfsii* is a necrotrophic pathogen and oxalic acid is a principle metabolite produced by *S. rolfsii* which is known to play a significant role in pathogenesis of this fungus (Kirtzman *et al.*, 1977). The oxalic acid sequesters the calcium in the host cell wall thereby favouring the pectic enzymes (polygalecturonase) secreted by the pathogen to hydrolyze the pectate in the middle lamella more rapidly. The process leads to rapid disintegration and death of the tissue which leads to the expression of symptoms like stem/collar rot (Gawande *et al.*, 2013). Once the pathogenesis is established the oxalic

acid acts as virulence factor and leads to the rapid wilting and death of the plants (Kirtzman *et al.*, 1977). Further, production of oxalic acid by *S. rolfsii* in host tissue enables the pathogenesis by promoting the activity of poly-galacturnonase and by creating an acidic environment, which in turn inactivates the prohibitins and phytoalexins and leads to suppression of host defense mechanism (Ferrar and Walker, 1993; Mahadevan and Sridhar, 1986).

4.4. Standardisation of inoculum level for optimum infection under glasshouse conditions

The study was conducted to determine the optimum level of inoculum of *S. rolfsii* which produces optimum infection and provides the reliable and effective results for conducting glasshouse studies. The virulent isolate of *S. rofsii*, SrGj-3 (which had produced highest amount of OA in culture filtrate under *in vitro* conditions) was multiplied on sorghum grain medium (SGM). The 35 days old groundnut seedlings of TMV 2, JL 24 and J 11 cultivars were inoculated with different levels of inoculum *viz.*, 5g, 10g, 15g, 20g, 25g, 30g and 35g per 7" pot (Plate 4.5).

The results indicated that, all the inoculum levels produced disease incidence of 100 per cent in all the cultivars. However, they differed with respect to incubation period (IP), days to permanent wilting (DPW) (Table 4.6), disease severity (Table 4.7) and mortality (Table 4.8).

The inoculum level 5g exhibited significantly highest mean IP (7.25 days) followed by 10g (6.33 days). Further, the inoculum levels from 15g to 35g produced significantly lowest mean IP (5.50 to 5.83 days) and were at par with each other. In addition the inoculum level 5g and 10g did not induced complete wilting and all these plants were survived with partial wilting till the end of the experiment (32 dpi). Whereas the inoculum levels from 15g to 35g produced significantly lowest mean DPW (7.11 to 7.42 days) and were at par with each other (Table 4.6).

The inoculum levels of 5g and 10g exhibited significantly least mean per cent disease severity of 46.67 to 50.00, 53.33 to 67.22, 62.78 to 75.55 and 63.89 to 77.22 at 8, 16, 24 and 32 dpi respectively. Whereas, the inoculum levels from 15g to 35g produced significantly highest mean per cent disease severity of 77.78 to 84.44 at 8 dpi and was at par with each other. Additionally, at 16, 24 and 32 dpi the 100 per cent mean disease severity was noted in these inoculum levels (Table 4.7). Further, at 8 dpi the inoculum levels of 5g and 10g did not induced mortality. Whereas at 16, 24 and 32 dpi

these inoculum levels exhibited 13.89 to 25.00, 30.56 to 58.34 and 33.33 to 63.89 mean per cent disease severity respectively which was significantly lowest compared to other inoculum levels in the study. Intrestinally, except at 8 dpi, the inoculum levels from 15g to 35g recorded 100% mean mortality at all observation intervals (Table 4.8). Hence, looking to the above results the inoculum level of 15g per 7" pot was found most effective and suitable for glasshouse studies.

Similar type of studies were reported by Sennoi *et al.* (2012), who observed the inoculum level of 5g was most effective to prove pathogenicity of *S. rolfsii* in Jerusalem artichoke. They further observed no difference in disease incidence, severity and DPW at higher inoculum levels. Similarly, Yaqub and Shahzad (2005) reported that, the one sclerotia/g of soil was found to be the effective inoculum level to prove pathogenicity of *S. rolfsii*. They further found no statically significant difference in root colonisation index in treatments containing one, five and ten sclerotia/g of soil.

Likewise, Muthukumar and Venkatesh (2013) studied the correlation between inoculum densities of *S. rolfsii* Sacc. and incidence of collar rot of peppermint. They found that, the 5% inoculum load registered the maximum incidence collar rot which was followed by the 4% inoculum load. Further, they noted increase in disease incidence with the increase in inoculum load up to 5% beyond which the disease incidence was found to remain static.

4.5. Pathogenicity of isolates of *S. rolfsii*

A pot culture experiment was conducted to test the pathogenicity of 60 isolates of *S. rolfsii* collected from major groundnut growing areas of India using three susceptible groundnut cultivars *viz.,* TMV 2, JL 24 and J 11 (Plate 4.6; Plate 4.7; Plate 4.8). The results indicated that all 60 isolates tested were pathogenic on all three cultivars and exhibited 100 per cent disease incidence. However, the isolates differed with respect to incubation period (IP) (Table 4.9), days to permanent wilting (DPW) (Table 4.9), disease severity (Table 4.10) and mortality (Table 4.11). The individual isolates were re-isolated from the inoculated plants and were exhibited the characteristic features of the original culture.

The mean IP of 60 isolates of *S. rolfsii* tested on three groundnut cultivars was ranged from 5.06 days in SrGj-3 to 8.97 days in SrAp-2 and SrTn-3. Significantly lowest mean IP was recorded in isolate SrGj-3 (5.06 days). The isolates SrKa-13 (5.58

days) followed by SrGj-7 (5.61 days), and SrKa-7 (5.64 days) recorded the lowest mean IP which were at par with each other. Further, the isolates SrKa-12, SrAp-2, SrTn-3 and SrTn-4 recorded the significantly highest mean IP of 8.95, 8.97, 8.97 and 8.95 days respectively and were found at par with each other (Table 4.9). In similar way, the mean DPW of 60 isolates was ranged from 6.75 days in SrGj-3 to 15.14 days in SrKa-11. The isolates SrKa-7 (7.81 days) followed by SrKa-2 (7.89 days), and SrGj-7 (8.00 days) recorded the significantly lowest mean DPW and were at par with each other. Further, the isolates SrKa-12, SrAp-2, SrTn-3 and SrTn-4 did not induced complete wilting and all these plants were survived with partial wilting till the end of the experiment (45 dpi) (Table 4.9).

Further, significantly lowest mean disease severity was recorded in plants inoculated with isolates SrKa-12 (48.33%), SrAp-2 (43.89%), SrTn-3 (42.78%) and SrTn-4 (46.67%) and were at par with each other at 15 dpi (days post inoculation). The similar trend was observed at 30 and 45 dpi. Further, the mean disease severity induced by remaining 56 isolates was significantly high and were at par with each other at all observation intervals (Table 4.10). Likewise, significantly lowest mean mortality was recorded in plants inoculated with isolates SrKa-12 (13.89%), SrAp-2 (5.55%), SrTn-3 (2.78%) and SrTn-4 (8.33%) and were at par with each other at 15 dpi. The similar trend was observed at 30 and 45 dpi. Further, the mean mortality induced by remaining 56 isolates was significantly highest and were at par with each other at all observation intervals (Table 4.11).

From the above results it was interesting to note that, there was great variation among the isolates for virulence levels on their host plants. The isolates *viz.,* SrKa-12, SrAp-2, SrTn-3 and SrTn-4 exhibited the mean IP of more than 8 days, induced no permanent wilting, mean disease severity of less than 60% and mean mortality of less than 25%, hence were categorized into less virulent. Similarly, the remaining 56 isolates exhibited the mean IP of less than 8 days, DPW of less than 16 days, mean disease severity of more than 60% and mean mortality of more than 25%, hence were categorized into highly virulent.

Further, there was a positive correlation found between the amount of oxalic acid produced under *in vitro* conditions and virulence of the isolates. In the study highly virulent isolates produced significantly highest amount of oxalic acid which was ranged from 0.99 mg/ml in SrTn-2 to 2.85 mg/ml in SrGj-3, whereas the less virulent isolates

produced least amount of oxalic acid which was ranged from 0.64 mg/ml in SrTn-3 and SrTn-4, to 0.78 mg/ml in SrAp-2 (Table 4.5).

Among all highly virulent isolates, the SrGj-3 exhibited lowest IP (5.06 days), lowest DPW (6.75 days), highest per cent mean disease severity (100) and highest per cent mean mortality (100). Hence, the isolate SrGj-3 was used in all experiments in the subsequent study.

The results are in line with the report of Eslami *et al.* (2015) who tested the virulence of 78 isolates of *S. rolfsii* on groundnut genotypes. They observed the positive correlation between virulence of isolates and per cent disease incidence and per cent stem area affected. Further, they categorised the 78 *S. rolfsii* isolates in to most virulent and less virulent based on the reaction with groundnut genotypes.

Similarly, Sennoi *et al.* (2012) proved the pathogenicity of ten isolates of *S. rolfsii* on three varieties of Jerusalem artichoke. They observed the variation in virulence of isolates and grouped them in to most aggressive and least aggressive. Likewise, Flores-Moctezuma *et al.* (2006) proved pathogenicity of 20 *S. rolfsii* isolates on 12 plant species and observed the variation in virulence of isolates. Further, Le *et al.* (2012) proved the pathogenicity of *S. rolfsii* isolates obtained from groundnut (n = 8), tomato (n = 5), and taro (n =5) on groundnut and reported the variation in their virulence level.

4.6. Variability of *S. rolfsii* isolates

The *S. rolfsii* Sacc. is a major soilborne pathogen of groundnut. Fundamental knowledge of the diversity of *S. rolfsii* populations in groundnut fields may help to adopt and develop effective and sustainable control measures (Le *et al.*, 2012). Further, the study of pathogenic variability is essential for breeding disease resistance in crop improvement programs. A potential pathogen is often blessed with biodiversity within its population. Basically, the variation in pathogen is desirable trait for its existence in nature. The variability among the pathogens underlies their diverse nature and ability to withstand the host environment. Hence the present study was conducted to assess the cultural, morphological and molecular variability present in 60 isolates of *S. rolfsii* collected from major groundnut growing areas of India.

4.6.1. Cultural variability of isolates of *S. rolfsii*

Cultural diversity of 60 isolates of *S. rolfsii* was studied with respect to growth rate, colony type, growth type, presence of sclerotia and biomass production. The growth rate of 60 isolates tested exhibited wide range (0.66 to 1.29 mm/hr). Significantly the lowest growth was found in isolate SrTs-1 (0.66 mm/hr) followed by SrMh-2 (0.74 mm/hr) and SrTs-5 (0.78 mm/hr). The growth rate of SrMh-2 and SrTs-5 was at par with each other. Further, significantly highest growth of 1.29 mm/hr was recorded in three isolates SrAp-7, SrMh-6 and SrTn-2 (Table 4.12). There was no significant correlation found between the growth rate and virulence of isolates.

All the 60 isolates of *S. rolfsii* under study produced sclerotia on PDA medium. The isolates found diverse with respect to colony type. Most of the isolates produced the colonies which were raised at ends (*n*=27) followed by flat type (*n*=20) and raised type (*n*=13) (Plate 4.9). Here too there was no relationship between colony type and virulence of isolates was observed. The most virulent isolate SrGj-3 produced flat type of colony, while the less virulent isolates *viz.*, SrKa-12, SrAp-2, SrTn-3 and SrTn-4 exhibited all the three types of colonies. As per the mycelial growth type, most of the isolates were highly profuse in growth (*n*=36) and few were profuse in growth (*n*=24). However, there was no linkage between virulence and growth type of isolates was noticed (Table 4.12).

With regard to biomass production the isolates exhibited wide variation ranging from 6.82 mg/day to 14.62 mg/day (Plate 4.10). Further, significantly lowest biomass production was found in isolates SrTs-10 (6.82 mg/day) followed by SrTn-1 (8.27 mg/day), SrMh-5 (8.62 mg/day), SrAp-2 (8.82 mg/day). Whereas, significantly highest biomass production was recorded in isolate SrTs-9 (14.62 mg/day) followed by SrTs-1 (12.87 mg/day), SrKa-16 (12.84 mg/day), SrAp-6 (12.80 mg/day) and SrAP-7 (12.78 mg/day). However, majority of isolates under study were found at par with each other for growth rate indicating the vigorous growth characteristic feature of *S. rolfsii* in nature (Table 4.12).

The results are in line with Sarma *et al.* (2002) who reported the wide variability among 26 isolates of *S. rolfsii* collected from different localities in India with respect to colony morphology (fluffy/compact), mycelial growth rate, sclerotial formation (80-500 sclerotia/plate) and biomass production. Similarly, Jyothi (2006) observed the wide variation among the isolates of *S. rolfsii* collected from different crops with respect to

growth rate (52.00 to 89.83 mm at 72 hours of incubation). Further, she opined that groundnut isolates recorded the highest dry mycelial weight and was not correlated with their virulence on different crops tested.

Likewise, Hussain *et al.* (2010) classified the *S. rolfsii* isolates in to very fast, intermediate and slow growing based on their growth rate. Similar type of studies on cultural variability of *S. rolfsii* in different crops such as cowpea, tomato, colocasia and groundnut was reported by many researchers (Okereke and Wokocha, 2007; Tortoe and Clerk, 2012; Palaiah and Adiver, 2004).

4.6.2. Morphological variability of isolates of *S. rolfsii*

The 60 isolates of *S. rolfsii* tested, exhibited considerable variation with respect to days to form sclerotia, days to mature, pattern of sclerotial production in petri dish, color of sclerotia, 100 sclerotial weight, number of sclerotia per plate, and size of sclerotia.

The time required to form sclerotia by 60 isolates of *S. rolfsii* was ranged from 4 to 17 days. Most of the isolates (*n*=41) were taken less than 10 days to form sclerotia and few (*n*=19) were taken more than 10 days. Similarly, time required for sclerotial maturation by the isolates was ranged from 7 to 23 days. Most of the isolates (*n*=48) were required less than 20 days for sclerotial maturation and few (*n*=12) were taken more than 20 days (Table 4.13).

As per the pattern of sclerotia produced in petri dish, most of the isolates were fell into scattered category (*n*=38) followed by peripheral (*n*=16) and central (*n*=6) (Plate 4.11). The colour of sclerotia was ranged from brown to dark brown, most of isolates produced brown colour sclerotia (*n*=25) followed by dark brown (*n*=20) and light brown (*n*=15). The 100 sclerotial weight of the isolates was ranged from 0.12 to 1.19 g. Wherein, the significantly least sclerotial weight was found with isolate SrAp-8 (0.12 g) followed by SrTs-1 (0.13 g), SrTs-4 (0.15 g) and SrKa-3 (0.16 g). Whereas, the isolates SrGj-5 and SrGj-2 produced significantly highest sclerotial weight of 1.19 g followed by SrKa-13 and SrMh-3 (1.09 g) (Table 4.13).

Likewise, the number of sclerotia produced per petri dish by 60 isolates of *S. rolfsii* was ranged from 52 in SrKa-13 to 910 in SrAp-1. The significantly least number of sclerotia was produced by isolate SrTs-5 (55) followed by SrKa-20 (73), SrGj-6 (75) and SrMh-4 (97) which were at par with each other. Whereas, significantly highest

number of sclerotia was produced by isolate SrAp-1 (910) followed by SrKa-8 (837) and SrKa-11 (823) which were at par with each other. There was wide variation in size of sclerotia produced by isolates tested and was ranged from 0.15 mm to 2.81 mm. Additionally, most of the isolates (*n*=32) produced the sclerotia of more than 1 mm in size and few (*n*=28) produced sclerotia of less than 1 mm in size. Further, as the size of sclerotia increased, the number of sclerotia per plate was decreased (Table 4.13). It was interesting to note that, there was no relation found between the morphological variability and virulence of isolates.

The results are in conformity with Le *et al.* (2012) who reported considerable morphological variations among 103 *S. rolfsii* isolates with respect to the days to produce sclerotia, days for maturation, number (79-1080) and size (0.88-2.24 mm) of sclerotia. Further, they stated that the morphological variability had no correlation with virulence of isolates. Similarly, Rasu *et al.* (2013) reported the wide variability among *S. rolfsii* isolates collected from Tamil Nadu state with respect to number of sclerotia, dry weight of 100 sclerotia and sclerotial colour (dark to light brown).

Likewise, Kumar *et al.* (2014) reported wide morphological variations among isolates of *S. rolfsii* collected from groundnut growing areas of Andhra Pradesh with respect to presence or absence of sclerotia, sclerotial colour (light brown to dark brown), number, 10 sclerotial weight (2.4 to 17 mg) and pattern of sclerotia produced (central/peripheral). In addition, Prasad *et al.* (2010) observed the considerable morphological variation among the isolates of *S. rolfsii* isolated from groundnut collected from Andhra Pradesh with respect to days to form sclerotia (9-18 days) and size (0.90-2.2 mm) of sclerotia. Further, Kokub *et al.* (2007) also revealed the variation in sclerotial size (0.5-2.0 mm) of 8 fungal strains of *S. rolfsii*.

4.6.3. Mycelial compatibility study

The study was conducted to know the extent of genetic diversity present among the 60 isolates of *S. rolfsii.* The investigation revealed the wide genetic diversity among 60 isolates of *S. rolfsii.*

In the study there were 1800 pairings of the 60 isolates. The development of aversion zones between the paired isolates of *S. rolfsii* on PDA was apparent within 8 to 14 days. Of the total pairings only 104 combinations showed a compatible reaction (5.78%) where the mycelia of the paired isolates intermingled at the zone of interaction.

The remainder of the combinations (94.22%) showed antagonistic reactions with each other in which the clear lytic zone was observed between paired isolates (Table 4.14; Plate 4.12). Based on mycelial compatibility, 15 mycelial compatibility groups (MCGs) were found among the 60 isolates of *S. rolfsii* under study (Table 4.15).

In all the antagonistic reactions, initial intermingling of hyphae of incompatible isolates was followed by lysis and a clear lytic zone was rapidly developed in the region of interaction**.** In some cases, sclerotia were produced at the retreating edge of the mycelium by leaving clear zone between two incompatible isolates and was observed in most of the combinations. Few incompatible combinations failed to produce sclerotia at retreating edge of the lytic zone, but sclerotial production was observed away from lytic zone at the centre of each isolate. On prolonged incubation, the lytic zone, in some combinations, was broadened parallel to both sides of incompatible isolates (Plate 4.12).

The high rate of antagonistic reaction in the study shows the extent of genetic diversity among the isolates. Further, the 15 MCGs found in our study revealed in part the relationship with the geographical origin of the isolates of *S. rolfsii* with few exceptions. Thus, the isolates collected from Karnataka state were grouped into 4 MCGs (MCG 1, MCG 2, MCG 3 and MCG 6) with few exceptions. Herein, the MCG 2 and MCG 6 contained one isolate each from Telangana and Andhra Pradesh and were collected from areas which are in close proximity of geographical area of Karnataka. Similarly, the isolates from Telangana were grouped in to 2 MCGs (MCG 4 and MCG 5) wherein MCG 4 contained one isolate from Karnataka which was collected from area which was in close proximity of geographical area of Telangana. Likewise, isolates from Andhra Pradesh were grouped into 2 MCGs (MCG 7 and MCG 8), Maharashtra into 2 MCGs (MCG 9 and MCG 10), Tamil Nadu into 2 MCGs (MCG 11 and MCG 12) and Gujarat into 3 MCGs (MCG 13, MCG 14 and MCG 15) (Table 4.15).

From the above observations it was found that, majority of MCGs contained the isolates from same state. However, few MCGs contained one isolate from geographically adjoining state. Further, the isolates collected from same state did not fall under single MCG, instead they were distributed into 2 to 5 MCGs.

The results are in agreement with Sarma *et al.* (2002) who noted high rate of antagonistic reactions in the mycelial compatibility test among 26 isolates of *S rolfsii* and grouped them into 13 MCGs. They further stated that, the identified MCGs in the

study revealed the genetic relatedness of isolates or intraspecific variation within field populations of *S. rolfsii* collected from different geographical location of India.

In similar lines, Le *et al.* (2012) reported the high level of genetic divergence among the 103 isolates of *S. rofsii* and grouped them into 17 MCGs. Likewise, Punja and Sun (2001) reported the total number of 71 MCGs among the world-wide collection of 132 isolates of *S. rolfsii.* Further, they revealed no clear relationship between hosts of origin of isolates and their MCGs which reflects the extremely wide host range of this pathogen. They also stated that, the characterization of MCG within a fungal species, in particular plant pathogenic fungi is a useful method to monitor distribution and spread of isolates over time as well as to determine the population structure in a specific region.

Further, Cilliers *et al.* (2000) revealed 13 MCGs within 121 field isolates of *S. rolfsii* collected from 15 localities and from seven plant species throughout South Africa. They further observed that, the few MCGs containing isolates from the same host plant or geographic area, suggesting a possible relationship between MCG and host plant or locality but, most MCGs, however, contained isolates from a variety of hosts from various localities hence they found no clear association between MCG, host and geographical distribution. Similarly, Remesala *et al.* (2012) identified 12 MCGs from the 459 *S. rolfsii* isolates collected from autumn-sown sugar beet crops grown in many countries and observed that, the isolates collected from different countries were grouped into same MCG or *vice-versa.* Hence they found no solid correlation between geographical origin, virulence of isolates and MCGs identified.

4.6.4. Molecular variability of isolates of *S. rolfsii*

4.6.4.1. Molecular identification of isolates of *S. rolfsii*

The study was conducted to confirm the molecular identity of 60 isolates of *S. rolfsii* collected from different groundnut growing areas of India. The molecular identification of isolates of *S. rolfsii* was performed by ITS-rDNA amplification, sequencing, and phylogeny. The amplification of ITS-rDNA region of 60 isolates of *S. rolfsii* was performed by using ITS-1 and ITS-4 primers. The amplification yielded an amplicon of 700 bp in all 60 isolates (Plate 4.13).

The high quality forward and reverse sequences of ITS-rDNA fragment of 60 isolates of *S. rolfsii* were performed by the nucleotide blast in NCBI, Genebank, (USA) and confirmed as *S. rolfsii* (Table 4.16).

For the phylogenetic analysis, the sequences were trimmed to 550 bp and aligned to reference sequences of *S. rolfsii* available in NCBI databases. The aligned sequences were used to construct the phylogenetic tree with MEGA7 software using the ITS-rDNA sequence of *S. delphini* as an out-group. In the phylogenetic tree the 60 isolates were categorised into four main groups designated as ITS groups 1 to 4 (Fig. 4.1). Further, most of the isolates were clustered into ITS group 1 (n=30), followed by ITS group 2 (n=15), ITS group 4 (n=12) and ITS group 3 (n=2) in phylogenetic tree.

Looking to the grouping of isolates in phylogenetic tree it was found that the *S. rolfsii* population in groundnut fields in major growing areas of India was relatively uniform. However, the ITS-rDNA sequencing does not give detailed insight into the intraspecific diversity. Hence, to study the intraspecific diversity among *S. rolfsii* isolates random amplified polymorphic DNA (RAPD) was used and results are presented in 4.6.4.2. Further, there was no correlation drawn between the MCGs and ITS groups, as *S. rolfsii* isolates belongs to different MCGs were clustered into single ITS group or *vice-versa*.

Our results are in accordance with the report of Adandonon *et al.* (2005) who recorded the amplicon of 700 bp from amplification of ITS-rDNA region of *S. rolfsii*. Similarly, Prasad *et al.* (2010) also reported a amplicon of about 650-700 bp while studying the molecular variability of *S. rolfsii* isolates collected from groundnut growing areas of Andhra Pradesh. Likewise, Harlton *et al.* (1995) screened a world-wide collection of *S. rolfsii*, using universal primer pairs ITS1-ITS4, ITS1-ITS2 and ITS3-ITS4, and revealed variation in ITS regions with 12 sub-groups. They further reported that, the ITS-rDNA region amplification of *S. rolfsii* and *S. delphinii* yielded a unique band of about 700 and 720 bp respectively.

In addition, Okabe and Matsumoto (2000) reported the molecular diversity of isolates of *S. rolfsii* collected from different countries based on ITS-rDNA sequence phylogeny. They further reported the 3 ITS groups in phylogenetic tree each group containing isolates from different countries or *vice-versa*. Further, Le *et al.* (2012) reported the similar pattern of grouping of 103 isolates of *S. rolfsii* in phylogenetic tree.

The results of the above study and available literature indicates that molecular diversity study by ITS-rDNA sequencing does not give detailed insight into intraspecific genetic diversity. Hence, to study the same other molecular markers have to be used.

4.6.4.2. Molecular diversity of isolates of *S. rolfsii* by RAPD

The study was conducted to reveal the molecular diversity among isolates of *S. rolfsii.* The genomic DNA of 15 isolates of *S. rolfsii* (one random isolate each from 15 MCGs) amplified using 30 random decamer primers demonstrated the extent of intraspecific variation among them. Collectively the 30 RAPD primers generated 207 polymorphic bands (Plate 4.14). The binary data, in the form of one (1) or zero (0), based on the presence or absence of a particular band, was used for the estimation of similarity matrix to calculate genetic divergence and relatedness among *S. rolfsii* isolates tested using NTSYS pc version 2.02i. The similarity matrix revealed that, the isolates SrMh-1, SrMh-6 and SrAp-10 are genetically most similar followed by SrKa-1 and SrKa-5. On other hand, the isolates SrGj-6 and SrAp-2 are genetically most distant followed by SrGj-1 and SrTn-1 (Table 4.17).

Further, the cluster analysis (UPGMA analysis) grouped the isolates into two clusters (Fig. 4.2). The cluster I comprised of 13 isolates *viz.*, SrKa-1, SrKa-5, SrKa-20, SrTs-1, SrTs-10, SrKa-12, SrAp-2, SrAp-10, SrMh-1, SrMh-6, SrTn-1, SrTn-5 and SrGj-3 while the cluster II contained isolates SrGj-6 and SrGj-1. The grouping of isolates was not correlated with geographic origin of the isolates however, few isolates were grouped together in a cluster based on their geographical origin *viz.,* SrKa-1 and SrKa-5 from Karnataka, SrMh-1 and SrMh-6 from Maharashtra were grouped in a close proximity in cluster I, whereas SrGj-6 and SrGj-1 from Gujarat were grouped into in cluster II. Further, SrGj-3 isolate from Gujarat was found in cluster I. Hence, looking to the style of grouping of isolates it was concluded that, there was no defined correlation found between the genetic diversity of isolates and their geographical origin.

Our results are in conformity with Prasad *et al.* (2010) who reported the high level of intraspecific genetic diversity among eight *S. rolfsii* isolates using five random amplified polymorphic (RAPD) primers and found 2 main clusters in UPGMA analysis which did not revealed any correlation between the genetic diversity and pathogenic virulence of isolates of *S. rolfsii*. Likewise, Punja and Sun (2001) reported the extent of genetic diversity among MCG of *S. rolfsii* and *S. delphinii* by their unique banding

patterns using six primers and found no discernible relationships among the various MCG using UPGMA analysis.

Similar kind of genetic diversity was reported by Kokub *et al.* (2007) among 8 fungal strains of *S. rolfsii* collected from Pakistan, Gawande *et al.* (2013) and Saude *et al.* (2004) among the *Sclerotium* species (*S. rolfsii* and *S. delphinii*) and Thilagavathi *et al.* (2013) among the 10 isolates of *S. rolfsii* collected from different host plants and geographic locations in Tamil Nadu.

Hence, looking to the above results and available literature it was concluded that the intraspecific diversity of *S. rolfsii* isolates may not always have direct correlation with virulence or geographical origin of isolates.

In total, the knowledge of cultural, morphological and molecular variability of *S. rolfsii* will help to understand the present status of pathogen and accordingly help to design effective management practice for the disease.

4.7. Sensitivity of isolates of *S. rolfsii* to commonly used fungicides

The studies on sensitivity of *S. rolfsii* to commonly used fungicides gives an idea about the relative effectiveness of the fungicides against them. The findings in present investigation indicated that there was a significant variability among the isolates of *S. rolfsii* with regard to sensitivity to fungicides tested. Across the isolates tested, significantly lowest mean minimum inhibitory concentration (MIC) was recorded with Tebuconazole (832 ppm) followed by Azoxystrobin (949 ppm) and that of highest was recorded with Thiram (3012 ppm) followed by Carbendazim (1105 ppm) (Table 4.18).

The MIC of Thiram and Carbendazim to the 60 isolates of *S. rolfsii* tested was ranged from 2700 to 3200 ppm and 900 to 1300 ppm respectively. On the other hand, the MIC of Azoxystrobin and Tebuconazole to different isolates was ranged from 800 to 1000 ppm and 800 to 900 ppm respectively. Further, among the four fungicides tested, Tebuconazole and Azoxystrobin were found to be highly effective in inhibiting the growth of *S. rolfsii* under laboratory conditions (Table 4.18).

To assess the resistance development in isolates of *S. rolfsii* to commonly used fungicides, the ED_{50} values were determined. The ED_{50} values were further used to calculate the resistance factor. In our findings there was a wide variation found in ED_{50} values of fungicides to *S. rolfsii* isolates. The significantly lowest mean ED_{50} values was recorded with Tebuconazole (416 ppm) followed by Azoxystrobin (474 ppm) and that

of the highest ED_{50} values was recorded with Thiram (1506 ppm) followed by Carbendazim (553 ppm). The ED_{50} values of Thiram, Carbendazim, Azoxystrobin, and Tebuconazole were ranged from 1350 to 1600 ppm, 450 to 650 ppm, 400 to 500 ppm and 400 to 450 ppm respectively (Table 4.19).

It was interesting to note that, Carbendazim had recorded higher resistance factor (1.12) among all fungicides tested. Further, the higher resistance factor of Carbendazim was noted in isolates collected from all states except for Gujarat isolates (1.03). Additionally, the higher resistance factor of Tebuconazole was recorded in isolates collected only from Gujarat (1.11) than the other state isolates. Furthermore, the higher resistance factors of fungicides Carbendazim and Tebuconazole were region specific and were probably due to their routine usage in groundnut cultivation at the respective locations (Table 4.20). However, to state clearly the resistance development in *S. rolfsii* isolates against commonly used fungicides in groundnut cultivation, a detailed investigation need to be conducted.

Our findings are in agreement with Bhagwan (2010b) who reported the higher efficacy of tebuconazole and least efficacy of thiram against *S. rolfsii* in groundnut under *in vitro* conditions. Similarly, Johnson and Subramanyam (2000) observed complete inhibition of radial growth of *S. rolfsii* by tebuconazole and least inhibition with carbendazim. Likewise, Franke *et al.* (1998) reported the higher efficacy of tebuconazole fungicide in controlling the stem rot of groundnut in Georgia under field conditions and *S .rolfsii* isolates under *in vitro* conditions.

In the study, tebuconazole was found to be most effective. The effectiveness was probably due to mode of action of tebuconazole (Bhagwan, 2010b), which exhibited directional selection process in pathogen, indicating the resistance mechanism may be under the influence of many genes, or at least more than one (Franke *et al.*, 1998).

4.8. Isolation of antagonists from rhizosphere soil

Totally 100 isolates of antagonistic fungi and 80 isolates of antagonistic bacteria were isolated from the groundnut rhizosphere soil collected from different groundnut growing areas of India. Both antagonistic fungal and bacterial isolates were subjected to preliminary screening against SrGj-3, the virulent isolate of *S. rolfsii* to test their biocontrol ability. The isolates of fungal antagonists exhibited varied level of biocontrol traits against the virulent isolates of *S. rolfsii* (Fig. 4.3). Further, among the 100 isolates of antagonistic fungi 8 most promising isolates were selected for further studies and

were designated serially from T1 to T8. The details of these isolates with their geographical origin and designations are given in Table 4.21. Molecular identification of these promising antagonistic fungal isolates was performed by ITS-rDNA amplification, sequencing and phylogeny. The ITS-rDNA amplification was carried out using ITS 1 and ITS 4 primers and the amplification yielded an amplicon of 600 bp size (Plate 4.15). The quality forward and reverse sequence data of amplified fragment of 8 isolates was subjected to nucleotide blast in NCBI data base and all the isolates were confirmed as *Trichoderma* sp. (Table 4.22). For the phylogenetic analysis, the sequences were trimmed to 500 bp and aligned to reference sequences of *Trichoderma* sp. available in NCBI databases. The phylogenetic tree was obtained with MEGA7 software.

In the phytogenic tree the isolate T1 was clustered with *Trichoderma harzianum,* T2 with *Trichoderma viride,* T3, T4, T5, T6, T7, and T8 isolates with *Trichoderma asperellum* reference strains (Fig. 4.4).

The *Trichoderma* spp. has revolutionized the field of biological control of soil-borne plant pathogens (Radjacommare *et al.*, 2010). The use of antagonistic fungi, especially *Trichoderma* and *Gliocladium* spp. has been more extensive than their bacterial counterparts (Ganesan *et al.*, 2003; Ganesan, 2004; Ganesan and Sekar, 2004a, 2004b). Control of *Sclerotium rolfsii* using *Trichoderma harzianum* was reported by Ganesan *et al*. (2003), Ganesan (2004) and Ganesan and Sekar (2004a). Further, Muthamilan and Jeyarajan (1996) reported the enhanced efficacy of combined application of *T. harzianum, Rhizobium* and Carbendazim in controlling the root rot of groundnut. Similarly Ganesan *et al.* (2007) reported the integrated management of stem rot disease of groundnut using a combined application of *Rhizobium* and *Trichoderma harzianum*. Likewise, Ekundayo *et al.* (2016) reported the efficacy of *Trichoderma viride* in reducing the severity of southern blight of tomato caused by *S. rolfsii* under pot culture studies. Further, efficacy of *Trichoderma* strains (*T. virens, T. viride, T. harzianum*) against the collar rot disease causing fungus *A. niger,* under pot culture studies was reported by Gajera and Vakharia (2010), and Gajera and Vakharia (2011).

From the above study 8 most promising isolates of *Trichoderma* sp. (Table 4.21) were selected and were evaluated for their biocontrol traits against virulent isolate of *S. rolfsii* (SrGj-3).

Similarly, in the preliminary screening, the isolates of bacterial antagonists exhibited varied level of biocontrol traits against the virulent isolate of *S. rolfsii* (Fig. 4.5). Further, among the 80 isolates of bacterial antagonist 5 most promising isolates were selected for further studies and were designated serially from B1 to B5. The details of these bacterial isolates with their geographical origin and designations are given in table 4.23. Molecular identification of these 5 promising antagonistic bacterial isolates was performed by 16S rDNA amplification, sequencing, and phylogeny. The 16S rDNA amplification was carried out using 785 F and 907 R primers and the quality forward and reverse sequence data of 5 isolates was subjected to nucleotide blast in NCBI data base and confirmed as *Bacilus* sp. (Table 4.24). For the phylogenetic analysis, the sequences were aligned to reference sequences of *Bacillus* sp. available in NCBI database. The phylogenetic tree was obtained with MEGA7 software.

In the phylogenetic tree the isolates B1 was clustered with *Bacillus megaterium*, whereas B2, B3, B4 & B5 isolates were clustered with *Bacillus pumilus* reference strain (Fig. 4.6).

Among bacterial antagonists, several strains of *Bacillus* sp. are known to suppress the soil borne plant pathogens and improve plant growth (Hu *et al.*, 2014, Khabbaz and Abbasi, 2014, Zhao *et al.*, 2014; Shifa *et al.*, 2015; Shrestha *et al.*, 2016). The results are in conformity with Suslow and Schroth (1982) who reported the effective suppression of *S. rolfsii* infection in groundnut, chickpea and beans by *Bacillus* sp. They further opined that, the *Bacillus* sp. have the rapid multiplication ability in the immediate proximity of germinating seeds thus increased probability of establishment of antagonist on individual roots. *Bacillus* sp. could also have an advantage over fungal antagonists in suppression of sclerotial fungi due to their rapid multiplication in the rhizosphere.

Further, Singh and Dwivedi (1987) reported the substantial reduction in foot rot of barley caused by *S. rolfsii* by the strains of *Bacillus subtilis, B. licheniformis, Pseudomonas aeroginosa* and *Streptomyces diastaticus*. Likewise Dwivedi (1987) observed the efficacy of *B. subtilis* and *Pseudomonas aeruginosa* in reducing the growth of *S. rolfsii* under *in vitro* conditions. Similarly, Chamswarng and Sangkaha (1988) reported the efficacy of *Bacillus* spp. and *Pseudomonas* spp. in management of stem rot of tomato caused by *S. rolfsii*. In addition, Abeysinghe (2009) noted the enhanced ability of *B. subtilis* in reducing the *S. rolfsii* incidence in chilli through Seed

bacterization and Root bacterization which resulted in maintaining higher numbers of bacteria at the collar region of chilli plants and leads to shielding of the most vulnerable area from the pathogen, resulting in enhanced protection. Further, Shifa *et al.* (2015) observed the effective control of stem rot of groundnut caused by *S. rolifsii* by *Bacillus subtilis* (G1 strain).

From the above study 5 most promising isolates of *Bacillus* sp. (Table 4.23) were selected and were evaluated for their biocontrol traits against virulent isolate of *S. rolfsii* (SrGj-3).

4.8.1. Evaluation of potential isolates of *Trichoderma* sp. for biocontrol traits against *S. rolfsii*

4.8.1.1. Dual culture assay

The 8 potential isolates of *Trichoderma* sp. were evaluated against the virulent isolate of *S.rolfsii* (SrGj-3) in dual culture assay. The results indicated that, the T1 isolate induced significantly highest per cent inhibition of radial growth (77.44), number of sclerotia produced (94.13) and reduction in sclerotial size (54.74) of pathogen over control. The isolates T2, T3, T4 and T5 were found next best antagonists and were at par with each other in respect to efficacy against the pathogen. Whereas, the T7 and T8 isolates were found to be significantly least effective against the pathogen (Table 4.25; Plate 4.16).

The results are in conformity with Paramasivan (2006) who reported that, the *T. viride* and *T. harzianum* were highly effective in reducing the radial growth of *S. rolfsii* in dual culture. The *in vitro* inhibition of radial mycelial growth of *S. rolfsii* was also reported by Srinivasulu *et al.* (2005) and Kotasthane *et al.* (2014).

4.8.1.2. Metabolite assay

The 8 potential isolates of *Trichoderma* sp. were evaluated against the virulent isolate of *S. rofsii* (SrGj-3) in metabolite assay. Among them, the T1 isolate produced significantly highest per cent inhibition of radial growth (59.22), number of sclerotia produced (68.78) and reduction in sclerotial size (52.50) of pathogen over control. Further, the T8 isolate was recorded significantly least per cent inhibition of radial growth (48.22), number of sclerotia produced (44.50) and reduction in sclerotial size (25.26) of pathogen over control (Table 4.25; Plate 4.17).

The results are in agreement with the report of Fravel (1988) & Kotasthane *et al.* (2014) who observed the effect of volatile metabolites of *Trichoderma* sp. against *S. rolfsii.* Fravel (1988) identified the alkyl pyrones as volatile compounds produced by *T. harzianum* suppressive to *S. cepivorum*. He further recorded the production of volatile organic metabolites *viz.*, ethanaol, isobutanol, isoamyl alcohol and isobutyric acid by *T. harzianum* which were found very effective against *S. rolfsii.* Similarly Kotasthane *et al.* (2014) found the highest antagonism by *Trichoderma viride* isolate against two soil borne plant pathogens *Scelrotium rolfsii* and *Rhizoctonia solani*. Further, they opined that, the antagonistic ability of the isolate was due to the 6-Pentyl pyrone which is one of the best studied secondary metabolites having both antifungal and plant growth-promoting activities.

4.8.1.3. Culture filtrate assay

The effect of culture filtrates of 8 potential isolates of *Trichoderma* sp. were tested for their inhibitory effect on sclerotial germination of virulent isolate *S. rolfsii* (SrGj-3). Among them, the significantly highest per cent reduction of sclerotial germination (96.00) of pathogen over control was recorded with culture filtrate of T1 isolate and that of least was observed with T8 isolate with 47.00 per cent reduction of sclerotial germination of pathogen over control. Further, the effectiveness of culture filtrate of T1 isolate in the study was might be due to presence of antifungal compounds (Table 4.25).

The inhibitory effect of culture filtrate of *Trichoderma harzianum* on germination of sclerotia of *S. rolfsii* was reported by Jeyarajan & Nakkeerun (1988). They further opined that, the inhibiting effect of culture filtrate was due to presence of antibiotics such as trichodermin, dermadin, trichoviridin and sesquiterpene heptalic acid. Antibiotics have long been suggested to be involved in biocontrol by *Trichoderma* (Weindling, 1932). Sivasithamparam and Ghisalberti (1998) listed 43 substances produced by *Trichoderma* spp. that have antibiotic nature. Of these, alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes and steroids have frequently been associated with biocontrol activity of strains of *Trichoderma* (Howell, 1998).

4.8.1.4. Inhibitory effect on oxalic acid production

Among the 8 isolates of *Trichoderma* sp. tested for their inhibitory effect on oxalic acid production by virulent isolate of *S. rolfsii* (SrGj-3), the T1 and T2 isolates were found most effective with significantly highest reduction of oxalic acid (92.74 and 89.73% reduction over control) produced by the pathogen and least reduction was observed with T7 and T8 isolates (79.82 and 78.41% reduction over control) (Table 4.25).

The results are in agreement with Komathi (2002) who demonstrated that, the *P. fluorescens, T. viride* and *T. harzianum* were capable of inhibiting oxalic acid production by *S. rolfsii*. Likewise, Paramasivan *et al.* (2013) also reported the degradation of oxalic acid produced by *S. rolfsii* by *T. viride* and *T. harzianum*. Similarly, Maheswari *et al.* (2002) reported the efficacy of *T. viride* in inhibiting the oxalic acid production by *S. rolfsii*. Oxalic acid is a major virulence factor of *S. rolfsii*, thus biological degradation of oxalic acid by bioagents will be the promising approach in controlling stem rot of groundnut (Paramasivan *et al.,* 2013).

4.8.2. Evaluation of potential isolates of *Bacillus* sp. for biocontrol traits against *S. rolfsii*

4.8.2.1. Dual culture assay

The results of evaluation of 5 potential isolates of *Bacillus* sp. against *S. rolfsii* in dual culture assay indicated that, the isolates of *Bacillus* sp. were equally effective as *Trichoderma* sp. against *S. rolfsii.* Among the 5 potential isolates tested, the B1 isolate was found to be most potent against test pathogen with significantly highest reduction of radial growth (70.33%), inhibition of number of sclerotia produced (83.37%) and reduction in sclerotial size (46.58%) over control followed by B2. Further, the least performance was noted with B5 isolate which produced 58.11%, 76.32% and 27.11% of reduction of radial growth, inhibition of number of sclerotia produced and reduction in sclerotial size over control respectively which was at par with B3 and B4 (Table 4.26; Plate 4.18).

The findings are in agreement with Solanki *et al.* (2012) who reported the highest inhibition of radial growth of *R. solani* by strain MB101of *Bacillus* spp. Similarly, Baysal *et al.* (2008) revealed the highest growth reduction of *Fusarium oxysporum* f. sp. *radicis-lycopersici* causal organism of fusarium wilt of tomato by

Bacillus subtilis strain (EU07) under *in vitro* conditions. Likewise, Ongena and jacques (2008) reported the antagonistic ability of *Bacillus lipopeptides* against various soil borne pathogens including *S. rolfsii*.

4.8.2.2. Metabolites assay

The similar trend was observed while testing efficacy of 5 potential isolates of *Bacillus* sp. against *S. rolfsii* in metabolic assay. The B1 isolate was found most effective with significantly highest reduction of radial growth (74.22%), inhibition of number of sclerotia produced (84.32%) and reduction in sclerotia size (49.08%) of pathogen over control. The B2, B3 and B4 were nest best potent isolates which were at par with each other. Further, the significantly least performance was recorded with B5 isolate with 51.56% reduction of radial growth, 58.53% inhibition of number of sclerotia produced and 33.42% reduction in sclerotia size of pathogen (Table 4.26; Plate 4.19).

The results are in line with Knox *et al.* (2000) who reported the strong inhibition of several plant pathogenic fungi on agar plate by two strains of *B. subtilis* and opined that, the effectiveness was due to antifungal volatile compounds (AFV) produced by them. Similarly, Ashok *et al.* (2014) noted the production of bioactive compound by *Bacillus subtilis* which has antagonistic ability against *S. rolfsii*. Likewise, Giorgio *et al.* (2015) revealed production of volatile organic compounds (VOCs) by eight *Bacillus* strains responsible for inhibition of the growth of *Sclerotinia sclerotiorum*. Further, Li *et al.* (2015) reported the production of VOCs from *Bacillus* strain which significantly inhibited the mycelial growth of *Fusarium solani* under *in vitro* conditions.

4.8.2.3. Culture filtrate assay

Among the 5 potential isolates of *Bacillus* sp. the significantly highest inhibition of germination of sclerotia of virulent isolate of *S. rolfsii* (SrGj-3) over control was recorded with culture filtrate of B1 (94.00%) followed by B2 isolate (87.00%) and significantly least inhibition was observed with B5 isolate (59.00%) (Table 4.26).

The *Bacillus* spp. which are capable of inhibiting the numerous pathogens under *in vitro* conditions produces the extracellular cell wall degrading enzymes, such as chitinase and *β*-1, 3-glucanase (Solanki *et al.*, 2012) and antifungal compounds

(Raaijmakers *et al.,* 2010; Mora *et al.,* 2011; Cazorla *et al.,* 2007). Thus, the results of present investigation are in conformity with Solanki *et al.* (2012) who reported the maximum reduction of radial growth of *R. solani* by culture filtrate of MB101 strain of *Bacillus* spp. Similarly, Nagarajkumar *et al.* (2005) revealed the presence of several antifungal proteins in the culture filtrate of *Pseudomonas* sp. and *Bacillus* sp. which limited the mycelial growth and sclerotial production by *Rhizoctonia solani.*

4.8.2.4. Inhibitory effect on oxalic acid production

Among the 5 potential isolates of *Bacillus* sp. evaluated for their inhibitory effect on oxalic acid production by virulent isolate of *S. rolfsii* (SrGj-3) the B1, B2 and B3 isolates were found most effective with significantly highest reduction of oxalic acid (95.93 92.39 92.74% reduction over control respectively) produced by pathogen. Further, the B4 and B5 isolates were found significantly least effective (Table 4.26).

The results are in agreement with Paramasivan *et al.* (2013) who identified potential *B. subtilis* strains which significantly reduced the oxalic acid production by *S. rolfsii*. Similarly, Nagarajkumar *et al.* (2005) revealed the detoxification of oxalic acid by potential *Pseudomonas* sp and *Bacillus* sp. isolates. As the oxalic acid is a principal pathogenicity factor of *S. rolfsii,* it would be ideal to identify potential rhizosphere strains capable of degrading oxalic acid produced by *S. rolfsii.*

4.8.3. Compatibility of potential isolates *Trichoderma* sp. and *Bacillus* sp. with fungicides

The results of compatibility study of potential isolates of *Trichoderma* sp. with fungicide revealed that, all the isolates were grown profusely and highly compatible with the fungicides tested at their half the recommended dose (Plate 4.20). However, by increasing the fungicidal concentrations to the recommended dose the growth of *Trichoderma* isolates was reduced slightly (Plate 4.21). The significantly least reduction in radial growth over control of 20.56%, 21.48%, 31.67% and 65.74% to recommended dose of Thiram, Carbendazim, Azoxystrobin and Tebuconazole respectively was recorded in T1 isolate. Further, significantly highest reduction in radial growth was noticed in T8 isolate (30.07, 30.63, 37.46 and 75.39 per cent reduction in radial growth over control to recommended dose of Thiram, Carbendazim, Azoxystrobin and Tebuconazole respectively) (Table 4.27).

Our results are in conformity with Bhagwan (2010a) who reported the compatibility of *Trichoderma viride* and *Trichoderma harizianum* with thiram, copper oxychloride and Mancozeb. Further, Tapwal *et al.* (2012) reported compatibility of *Trichoderma* species with dithane, tebuconazole, ridomil and carbendazim. The efficiency of the biological control agent could further be improved when it was applied with the recommended fungicide at lower concentration. The ability of *Trichoderma* sp. to withstand and proliferate in the presence of fungicides at reduced dose makes them the most potential antagonist. Further, this type of strains can be used as one of the components of integrated disease management modules (Bhagwan, 2010a).

In the present study, the T1 isolate of *Trichoderma* sp. recorded the significantly highest biocontrol traits against virulent isolate of *S. rolfsii* (SrGj-3) under *in vitro* condition. Further, this isolate was compatible with commonly used fungicides in groundnut cultivation. Hence, its efficacy was further evaluated under glasshouse and field conditions.

The results of compatibility study of potential isolates of *Bacillus* sp. with fungicide revealed that, the growth of all the isolates were slightly inhibited in presence of fungicides at their half the recommended dose. Significantly least inhibition was observed in B1 isolate with 6.28, 5.23, 6.28 and 26.91 per cent inhibition over control at half the recommended dose of Thiram, Carbendazim, Azoxystrobin and Tebuconazole respectively. However, by increasing the fungicidal concentrations to the recommended dose the growth of bacterial antagonists reduced slightly. The significantly least per cent reduction in growth over control of 18.83, 37.97, 42.45 and 49.78 to recommended dose of Thiram, Carbendazim, Azoxystrobin and Tebuconazole respectively was observed in B1 isolate. Further, significantly highest reduction of growth was found in B5 isolate (31.69, 41.11, 49.93 and 51.12 per cent reduction in growth over control to recommended dose of Thiram, Carbendazim, Azoxystrobin and Tebuconazole respectively) (Table 4.28).

Our findings are in line with Suneeta *et al.* (2016) who reported the compatibility of *Bacillus* spp. with carbendazim, azoxystrobin, tebuconazole, and tebuconazole+trifloxystrobin. Similarly, Archana *et al.* (2012) reported the compatibility of *Bacillus subtilis* with azoxystrobin. Likewise, Mohiddin and Khan (2013) revealed the compatibility of *Bacillus* spp. with six commonly used fungicides which were used in controlling soil borne diseases.

The manifestation of biological control by *Bacillus* spp. against various soilborne plant pathogens has been observed from several years. Further, supplementation with specific compounds may provide a competitive advantage for the establishment of the introduced biocontrol agents and improve the biocontrol of plant diseases. In several disease management strategies, the addition of fungicide at reduced rates in combination with biocontrol agents has significantly enhanced disease control, compared to treatments with biocontrol agent alone (Frances *et al.*, 2002).

Looking to the results, the T1 isolate of *Trichoderma* sp. and B1 isolate of *Bacillus* sp. were recorded the significantly highest biocontrol traits against virulent isolate of *S. rolfsii* (SrGj-3) under *in vitro* conditions and found compatible with commonly used fungicides in groundnut cultivation. Hence, their efficacy was further evaluated under glasshouse and field conditions.

4.8.4. Compatibility among *Trichoderma* sp. (T1) and *Bacillus* sp. (B1)

The study was conducted to know the compatibility between T1 isolate of *Trichoderma* sp. and B1 isolate of *Bacillus* sp. The result indicated that, the mycelial growth of T1 was not inhibited by growth of B1 on potato dextrose agar medium and *vice versa.* Further, the mycelium of T1 was overgrown on B1 and there was no inhibition zone observed (Plate 4.22). Similar type of study was reported by Latha *et al.* (2011) who observed the compatibility between strains of *P. fluorescens, B. subtilis* and *T. viride*. Likewise, Thilagavathi *et al.* (2007) found compatibility between *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis.*

4.8.5. Plant growth promotion by *Trichoderma* sp. (T1) and *Bacillus* sp. (B1)

The study was conducted to know the plant growth promotion ability of talc formulation (with or without chitin) of T1 and B1 in TMV 2 groundnut cultivar using standard paper towel method.

The results indicated that, the talc formulation of T1 and B1 with or without chitin, alone and in combination exhibited significantly higher amounts of plant growth promotion parameters in TMV 2 compared to control (Plate 4.23). Among the treatments, the combined application of bioformulation of T1 and B1 with chitin exhibited significantly higher levels of growth attributes like germination (100%), shoot length (27.67 cm), root length (26.56 cm), biomass (31.20 g), total root length (852.65 cm), root volume (0.91 cm^3), vigour index-I (5423.33) and vigour index-II (283.33)

followed by combined application of T1 and B1 without chitin when compared to other treatments (Table 4.29). The higher growth promotion attributes in these treatments was might be due to the cumulative action of T1 and B1.

The results are in conformity with Prashanth and Mathivanan (2010) who reported the enhanced growth promotion in groundnut by IAA producing rhizobacteria, *Bacillus licheniformis.* Similarly, Dey *et al.* (2004) reported growth promotion and yield enhancement in groundnut by application of plant growth-promoting rhizobacteria. Further, Navya *et al.* (2015) reported the cumulative action of *Pseudomonas fluorescens*, *Bacillus* sp. and *Trichoderma atroviride* in growth promotion and enhanced yields of groundnut.

Several studies revealed the production of phytohormone IAA by *Trichoderma* sp. involved in growth promotion in several crops (Harman *et al.*, 2004; Morteza *et al.*, 2011; Zhang *et al.*, 2013; Badawi *et al.*, 2011). Recently, Solanki *et al.* (2011) reported IAA production and phosphate solubilisation by plant growth promoting fungi (PGPF) included different *Trichoderma* spp.

4.9. Evaluation of bioformulations against stem rot of groundnut under glasshouse conditions

The study was conducted to test the efficacy of talc based bioformulations of T1 isolate of *Trichoderma* sp. and B1 isolate of *Bacillus* sp. with or without chitin, individually and in combination through seed treatment and soil application in controlling the stem rot of groundnut under glasshouse conditions (Plate 4.24). The efficacy of these bioformulations were evaluated based on their effect on stem rot severity, incidence and mortality induced by virulent isolate of *S. rolfsii* (SrGj-3).

Results of efficacy of bioformulation on severity of stem rot revealed that, the combined application of bioformulation of T1 and B1 fortified with chitin recorded significantly least disease severity (13.67%) followed by chemical control (17.58%) at 15 dpi (days post pathogen inoculation). The similar trend was observed at 30, 45, 60 and 75 dpi. Additionally, the progress in disease severity in above treatments from 15 to 75 dpi was very slow compared to other treatments. In total, the significantly lowest mean disease severity was recorded with combined application of T1 and B1 fortified with chitin (33.20%) which was on par with chemical control (35.70%) followed by combined application of T1 and B1 fortified without chitin (38.67%) (Table 4.30).

Further, the similar trend was observed in efficacy of bioformulation on stem rot incidence. Significantly least stem rot incidence of 38.06% was noted with the combined application of bioformulation of T1 and B1 fortified with chitin followed by chemical control (45.31%) and combined application of bioformulation of T1 and B1 without chitin (48.44%) at 15 dpi and similar trend was observed at 30, 45, 60 and 75 dpi. Additionally, the progress of disease incidence in the above treatments from 15 to 75 dpi was very slow compared to other treatments. Overall, significantly least mean disease incidence of 60.73% was recorded in combined application of bioformulation of T1 and B1 fortified with chitin followed by chemical control (64.86%) and combined application of bioformulation of T1 and B1 fortified without chitin (67.81%) (Table 4.31).

Similarly, the combined application of bioformulation of T1 and B1 fortified with chitin exhibited significantly lowest stem discoloration (7.81%) and pod rot (14.06%) and was at par with the chemical control (9.38% and 15.63% stem discoloration and pod rot respectively) followed by combined application of bioformulation of T1 and B1 without chitin (28.09% and 14.30 % stem discoloration and pod rot respectively) (Table 4.32).

Likewise, the bioformulation differed in controlling the mortality in groundnut plants induced by *S. rolfsii* under glasshouse conditions. At 15 dpi no mortality was observed in all the bioformulation treatments except inoculated control which recorded 20.31% mortality. Further, at 30 dpi the combined application of bioformulation of T1 and B1 fortified with chitin recorded significantly least mortality (9.38%) which was at par with chemical control (7.81%) followed by the combined application of bioformulation of T1 and B1 without chitin (10.94%) and same trend was evidenced at 45, 60 and 75 dpi. Here too, the progress of mortality in the above treatments from 30 to 75 dpi was very slow compared to other treatments. In total, the combined application of bioformulation of T1 and B1 fortified with chitin recorded significantly least mean mortality (13.33%) which was on par with chemical control (14.06%) followed by combined application of bioformulation of T1 and B1 without chitin (19.69%) (Table 4.33).

Looking to the above results it was found that, the combined application of bioformulation of T1 and B1 fortified with chitin through seed treatment and soil application was found significantly most effective in controlling stem rot of groundnut

under glasshouse conditions and was on par with chemical control followed by combined application of T1 and B1 bioformulation without chitin.

Similar reports of the use of biocontrol agents in combination for better disease control have been reported by Saravanan (2006), Jadhav and Ambadkar (2007), Muthukumar *et al.* (2011). Improved suppression of damping-off caused by *Pythium ultimum* by combined application of *T. virens* and *Burkholderia ambifaria* was observed by Roberts *et al.* (2005).

Similarly, Janisiewicz (1996) reported that combining antagonists which occupy different nutritional niches and coexist in the infection court are more effective biological control treatments than individual antagonists. Further, the combined application of a formulation mixture of *Trichoderma* spp. with fortified chitin significantly reduced the incidence of the complex of diseases incited by the combined action of *Sclerotinia sclerotiorum* and *Meloidogyne incognita* in cabbage was reported by Loganathan *et al.* (2010). Likewise, Ahmed *et al.* (2003) recoreded the enhanced efficacy of formulation mixture of *Trichoderma* spp. and *Bacillus* spp. along with 0.5% chitin through seed treatment and root drenching against *Phytophthora* and *Rhizoctonia* root rot of pepper in glasshouse studies.

In the present investigation the chitin amendment enhanced the disease suppression ability of bioformulations. This is in agreement with Kishore *et al.* (2005) who reported enhanced antibiotic ability of bacterial antagonists against *Phaeoisariopsis personata* upon chitin amendment. The enhanced efficacy of biocontrol agents upon chitin amendment in managing plant diseases has been demonstrated by Kishore *et al.*(2005), Nandakumar *et al.* (2001), Radjacommare *et al.* (2010), Rajkumar *et al.* (2008) and Solanki *et al.* (2011).

4.10. Induced systemic resistance

The study was conducted under glasshouse conditions to know the resistance inducing ability of talc based bioformulation of T1 isolate of *Trichoderma* sp. and B1 isolate of *Bacillus* sp. individually and in combination (with or without chitin) against stem rot pathogen.

The biochemical and molecular analysis of groundnut plants applied with bioformulations through seed treatment and soil application revealed that the higher activity of defense enzymes (PAL, peroxidases, polyphenol oxidase and catalase), PR proteins (chitinase and β-1,3 glucanase) and defense chemicals (total phenol).

The greatest growth area in biocontrol in the last few years has been concerned with induced resistance which is defined as the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (Kloepper *et al.*, 1992). Most work has focused on the systemic resistance induced by non-pathogenic rhizosphere colonizing *Bacillus* and *Pseudomonas* species against range of soil borne plant pathogens (Sticher *et al.*, 1997; van Loon, 1997; van Loon, 2006). Further, a wide variety of root-associated mutualists, fungi, including *Trichoderma*, and mycorrhiza species were known to sensitize the plant immune system for enhanced defense without directly activating costly defenses (Pieterse *et al.*, 2014).

4.10.1. Total proteins

The total protein content analysis in groundnut plants applied with bioformulations through seed treatment and soil application and challenge inoculated with SrGj-3, the virulent isolate of *S. rolfsii* indicated that, significantly highest mean protein content was recorded in combined application of T1 and B1 with chitin (43.14 mg g^{-1} FW) followed by combined application of T1 and B1 without chitin (37.19 mg g^{-1} FW). Further, among the sampling intervals, significantly highest amount of total protein was expressed in bioformulation applied plants on 2^{nd} day post inoculation of pathogen (dpi) followed by 3^{rd}, 4^{th} and 5^{th} dpi (Table 4.34).

The results are in line with Doley *et al.* (2015) who reported the highest total protein level in *Trichoderma* and mycorrhiza inoculated groundnut plants followed by only mycorrhiza treatment. The elicitation of host protein synthesis is considered to be brought about by pathogen penetration in host plants which in turn restrict the further growth of pathogen in host (Adrienne and Barbara, 2006).

4.10.2. Total phenols

The similar trend was observed with respect to total phenol content. The combined application of T1 and B1 fortified with chitin rerecorded significantly highest mean total phenol content expression (2.30 mg g^{-1} FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (1.94 mg g^{-1} FW mg^{-1} protein). Further, there was a significantly sharp increase in total phenol content on 2^{nd} dpi and persisted up to 7^{th} dpi with slightly decrease in T1 and B1 bioformulation mixture amended with chitin treatment followed by T1 and B1 without chitin. In other treatments the sharp increase in phenol content was persisted till 4^{th} dpi then on the sharp decrease was noticed (Table 4.35).

These findings are in agreement with the findings of Khaleifa *et al.* (2006) in groundnut against damping off and root-rot diseases, and of Sudhagar *et al.* (2000) in groundnuts against the rust pathogen *Puccinia arachidis*. Similarly, Gajera *et al.* (2014) reported the higher content of phenolic and ferulic acid in response to *A. niger* infection in *T. viride* treated groundnut plants. Likewise, Sarma & Singh (2003) identified highest amount of three major phenolic acids (gallic, vanillic and ferulic acids) in *S. rolfsii* infected chickpea plants.

In addition, Singh *et al.* (2003) observed that, the plant growth promoting rhizobacteria (PGPR) strains induced the synthesis of specific phenolic acids, with varied amounts at different growth stages of chickpea seedlings against *S. rolfsii* infection. The maximum induction of phenolics (gallic, protocatechuic, chlorogenic, caffeic, ferulic, and ellagic acids) in PGPR treated betelvine plants infected with *Phytophthora nicotianae* was recorded by Lavania *et al.* (2006).

Thus, the total phenols are another group of compounds associated with biocontrol which offer a practical way of immunizing plants against the pathogen ingress. The present investigation also revealed that application of the T1 and B1 bioformulations (with or without chitin) in groundnut modified the composition of host-defense molecules, especially total phenols in response to *S. rolfsii* infection.

4.10.3. Phenylalanine Ammonia Lyase (PAL)

The bioformulation were most effective in inducing higher activity of PAL in groundnut against *S. rolfsii*. In the study, significantly highest mean PAL activity was observed with combined application of T1 and B1 with chitin (9.31 µmole trans-cinammic acid $min^{-1}g^{-1}$ FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (7.97 µmole trans-cinammic acid $min^{-1}g^{-1}$ FW mg^{-1} protein). Further, significantly least mean PAL activity was noticed in chemical, inoculated and uninoculated controls. Among the sampling intervals significantly sharp increase in PAL activity was noticed on 2^{nd} dpi and persisted up to 6^{th} dpi in all bioformualtions treatments (Table 4.36).

The results are in agreement with Muthukumar *et al.* (2011) who reported fourfold increase in PAL activity in *Trichoderma viride* and endophytic *Pseudomonas fluorescens* treated chilli seeds. Generally, induction of PAL enzyme is correlated with increased resistance to pathogenic infection (Bell *et al.,* 1984). Induction of PAL by

fluorescent *Pseudomonas* and *Bacillus* sp. was reported in tomato against *Fusarium oxysporum* f.sp. *lycopersici* (Ramamoorthy *et al.,* 2002).

Early and increased synthesis of PAL in the *T. viride*, *P. fluorescens* and *B. subtilis* pre-treated peppermint plants challenged with *R. solani* was observed by Kamalakannan *et al.* (2003). Similarly, Sangeetha *et al.* (2010) reported that, the banana fruits treated with bacterial antagonists (individual and in combination) and challenge inoculated with crown rot pathogens recorded up to four fold increase in PAL activity. Likewise, Solanki *et al.* (2011) demonstrated the improved control of tomato root rot by chitin supplemented applications of *Trichoderma/Hypocrea* spp. which was reflected in the enhanced activities of defense related enzymes (PO, PPO and PAL) in tomato. Strains, *Bacillus* sp. CHEP5 and *Pseudomonas* sp BREN6 inoculation in groundnut plants induced the higher expression of phenylalanine ammonia-lyase (*S. rolfsii*) challenge (Tonelli *et al.,* 2011).

The early induction of PAL is more important, since it is the first enzyme in the phenylpropanoid pathway that leads to production of phytoalexin and phenolic substances leading to the formation of lignin with peroxidases (Solanki *et al.*, 2011).

4.10.4. Peroxidase (PO)

The bioformulations of T1 and B1 differed in their ability to induce the activity peroxidase in groundnut against *S. rolfsii.* The significantly highest mean peroxidase activity (2.53 $\Delta OD_{470nm} min^{-1} g^{-1}$ FW mg^{-1} protein) was recorded in combined application of T1 and B1 with chitin followed by combined application of T1 and B1 without chitin (1.99 $\Delta OD_{470nm} min^{-1} g^{-1}$ FW mg^{-1} protein) compared to other treatments. Further, significantly least peroxidase activity was noticed in chemical, inoculated and uninoculated controls.

Among the sampling intervals significantly sharp increase in peroxidase activity was noticed on 2^{nd} dpi and persisted up to 5^{th} dpi in bioformualtions treatments. Whereas, in chemical, inoculated and uninoculated controls no such increase in peroxidase activity was observed (Table 4.37).

4.10.4.1. Isoform pattern of PO

The bioformulations were found effective in inducing the expression of isoforms of PO in response to stem rot pathogen in groundnut. The bioformulations induced the expression of nine isoforms *viz.,* PO 1, PO 2, PO 3, PO 4, PO 5, PO 6, PO 7, PO 8 and

PO 9 in groundnut upon *S. rolfsii* challenge (Plate 4.25; 4.26; 4.27; 4.28; 4.29; 4.30; 4.31; 4.32).

On 2nd and 3rd dpi the combined application of T1 and B1 bioformulation with chitin induced the expression of eight isoforms *viz.*, PO 1, PO 3, PO 4, PO 5, PO 6, PO 7, PO 8, PO 9 and PO 1, PO 2, PO 3, PO 4, PO 5, PO 6, PO 7, PO 8 respectively. Followed by, the combined application T1 and B1 without chitin which recorded the induction of five isoforms with high intensity. In uninoculated, inoculated and chemical control least number of isoform with less intensity was observed (Table 4.38).

In the study, groundnut plants treated with the bioformulation T1+B1 (amended with or without chitin) mixture and challenged with pathogen showed higher induction of peroxidases. Our results are in conformity with Muthukumar *et al.* (2011) who reported high level of peroxidase activity in *Trichoderma viride* and endophytic *Pseudomonas fluorescens* amended with chitin against *Pythium aphanidermatum* in chilli. Similarly, Thilagavathi *et al.* (2007) revealed the higher activity of PO in greengram plants treated with the bioformulations containing *Trichoderma, Pseudomomas* and *Bacillus* against *M. phaseolina*.

Suppression in the wilt incidence of cucumber and higher level of defense enzymes peroxidase and catalase in plants applied with *T. viride* indicating that the production of phytoalexin or lignin which might be involved in disease suppression was observed by Zehnder *et al.* (2001). Further, Liang *et al.* (2011) stated that, the cucumber seeds treated with *Brevibacillus brevis* showed a higher activity of peroxidase which may contribute to cross linking of hydroxyl proline-rich glycoproteins (HRGPs), and lignifications that act as barriers against pathogen entry. Likewise, Solanki *et al.* (2011) reported high level of PO activity in tomato plants treated with chitin-fortified *Trichoderma/Hypocrea* formulation against *Rhizoctonia solani* the causal organism of root rot of tomato. Increased activity of peroxidase in groundnut plants treated with strains of *Bacillus* sp. and *Pseudomonas* sp. upon *S. rolfsii* challenge was recorded by Tonelli *et al.* (2011).

Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Further, peroxidases have been implicated in a number of physiological functions that may contribute to resistance including exudation of hydroxy cinnamyl alcohol into free radical intermediates (Gross, 1980), phenol oxidation (Schmidt and Feucht, 1980), polysaccharide cross linking (Fry, 1986), cross linking of extensin

monomers (Everdeen *et al.*, 1988) and lignification (Walter, 1992) and are also associated with deposition of phenolic compounds into plant cell walls during resistance interactions (Graham and Graham, 1991).

4.10.5. Polyphenol oxidase (PPO)

In the study the bioformulations were found most effective in inducing the higher activity of polyphenol oxidase (PPO) in groundnut against *S. rolfsii*. In these lines, the significantly highest mean PPO activity was observed in combined application of T1 and B1 with chitin amendment (2.21 $\Delta OD_{420nm} min^{-1} g^{-1}$ FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (1.58 $\Delta OD_{420nm} min^{-1} g^{-1}$ FW mg^{-1} protein). Whereas the chemical, inoculated and uninoculated controls notes the significantly least mean PPO activity.

Further, on 2nd dpi significantly sharp increase in PPO activity was observed in bioformulation treatments and was persisted up to 5th dpi. Significantly least PPO activity was noted in uninoculated control (Table 4.39).

4.10.5.1. Isoform pattern of PPO

Induction of isoforms of PPO by bioformualtions in groundnut against *S. rolfsii* was studied through Native PAGE analysis. The results indicated that, the maximum activity PPO was found during the study. Induction of about 13 isoforms *viz.*, PPO 1, PPO 2, PPO 3, PPO 4, PPO 5, PPO 6, PPO 7, PPO 8, PPO 9, PPO 10, PPO 11, PPO 12, and PPO 13 was noted in different bioformulation treatment (Plate 4.25; 4.26; 4.27; 4.28; 4.29; 4.30; 4.31; 4.32). Further, **t**he isoforms PPO1, PPO3, PPO5 and PPO7 were found to be constitutive as they were expressed in all treatments at all sample intervals and the isoforms PPO 2, PPO 4, PPO 6, PPO 8, PPO 9, PPO 10, PPO 11, PPO 12, and PPO 13 were specific and induced as a result of pretreatment with bioformulations.

In the study, highest expression of PPO isoforms *i.e.*, 11 (PPO 1, PPO 2, PPO 3, PPO 5, PPO 6, PPO 7, PPO 9, PPO 10, PPO 11, PPO 12, and PPO 13), 9 (PPO 1, PPO 2, PPO 3, PPO 5, PPO 6, PPO 7, PPO 9, PPO 10 and PPO 11) and 11 (PPO 1, PPO 2, PPO 3, PPO 4, PPO 5, PPO 7, PPO 9, PPO 10, PPO 11, PPO 12, and PPO 13) were noted on 2nd, 3rd and 4th dpi respectively in combined application of T1 and B1 with chitin treatment. Further, in inoculated and chemical control less number of specific PPO isoforms were induced. Further, in uninoculated control no specific PPO isoforms were induced (Table 4.40).

The results are in conformity with Loganathan *et al.* (2010) who reported the combined application of a formulation mixture of *Trichoderma* spp. along with chitin in cabbage resulted in enhanced activity of polyphenoloxidase (PPO) against *Sclerotinia sclerotiorum* and *Meloidogyne incognita.* Similarly, Muthukumar *et al.* (2011) revealed the high level of polyphenol oxidase activity in co-inoculation of *Trichoderma viride* and endophytic *Pseudomonas fluorescens* amended with chitin against *Pythium aphanidermatum* in chilli. Likewise, Vivekanandhan *et al.* (2004) noted the elevated level of polyphenol oxidase activity in mango treated with *Pseudomonas fluorescens* bioformulation (amended with chitin) upon challenge inoculation of anthracnose pathogen. Further, Gajera *et al.* (2015) stated the enhanced activity of polyphenol oxidase in collar rot pathogen (*Aspergillus niger* Van Tieghem) challenged groundnut seedlings in response to *Trichoderma viride.*

Polyphenol oxidase is known to catalyze the oxidation of phenolics to free radicals of quinone which can react with biological entities, thus creating unfavourable environment for pathogen development (Mayer and Harel, 1979). The inactivation of pathogen pectolytic enzymes by the oxidized substrate of PPO is reported as a part of host resistance mechanism (Sarwar *et al.,* 2003). The higher induction of PPO in plants in response to pathogen might represent a broad defensive role of PPO in protection of plants from subsequent attack by broad spectrum of pathogen (Thipyapong and Steffens, 1995).

4.10.6. Catalase (CAT)

The bioformulation of T1 and B1 with or without chitin individually and in combination differed in their ability to induce the catalase activity in groundnut plants against *S. rolfsii*. Wherein the combined application of T1 and B1 with chitin recorded significantly highest mean catalase activity (2.16 µmole H_2O_2 $min^{-1}g^{-1}$ FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (1.72 µmole H_2O_2 $min^{-1}g^{-1}$ FW mg^{-1} protein). Significantly least catalase activity was observed in uninoculated control. Further, significantly sharp increase in catalase activity was found in 2^{nd} dpi and persisted up to 4^{th} in bioformulation treatments. Further, in chemical, inoculated and uninoculated control the sharp increase in catalase activity was observed only at 2^{nd} dpi then on there was sharp decline in catalase activity was evidenced (Table 4.41).

4.10.6.1. Isoform pattern of CAT

The boformulations differed in their ability to induce catalase isoforms in groundnut against stem rot. In the study, the isoform CAT 1 was found to be constitutive as it was expressed in all treatments and at all sample intervals and the isoforms CAT 2 and CAT 3 were found to be specific and induced as a result of bioformulation pre-treatment (Plate 4.25; 4.26; 4.27; 4.28; 4.29; 4.30; 4.31; 4.32). Further, on 2^{nd}, 3^{rd}, 4^{th} and 5^{th} dpi all three catalase isoforms CAT 1, CAT2 and CAT 3 were expressed in groundnut plants pretreated with combined application of T1 and B1 bioformulation with or without chitin through seed treatment and soil application (Table 4.42).

The findings are in conformity with Saravanakumar (2006) who reported the higher activities of catalase in rice plants treated with fluorescent pseudomonad bioformulation mixture (amended with chitin) and challenged with sheath rot pathogen. The enhanced expression of catalase in groundnut plants against *S. rolfsii* by plant growth promoting rhizobacterial bioformulations amended with chitin was observed by Senthilraja *et al.* (2013).

Plants produce active oxygen species (AOS) such as superoxide anion ($O2^{-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) as one of the earliest responses attempted to infection by pathogens (Grant and Loake, 2000). Further, production of reactive oxygen species (ROS), particularly H_2O_2 has repeatedly been associated with diverse plant pathogen and plant insect interactions (Vera-Estrell *et al.*, 1993; Orozco-Cardenas *et al.*, 2001). Hence, scavengers of active oxygen species like catalase (catalyzes the decomposition of H_2O_2) suppress the oxidative burst and inhibit the tissue necrotization.

4.10.7. *β*-1,3 glucanase

The induction of *β*-1,3 glucanase by bioformulations of T1 and B1 in groundnut against *S. rolfsii* showed the ability of *Trichoderma* and *Bacillus* bioformulations in inducing the PR-2 protein. The T1 and B1 bioformulation treated plants challenged with stem rot pathogen synthesized higher levels of *β*-1,3-glucanase than the untreated plants. Significantly highest mean activity of *β*-1,3 glucanase was recorded with combined application of T1 and B1 with chitin (16.87 µmole glucose $min^{-1}g^{-1}$ FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (13.86 µmole glucose $min^{-1}g^{-1}$ FW mg^{-1} protein). Further significantly sharp increase in *β*-1,3

glucanase activity was found on 2nd dpi and persisted upto 5th dpi in bioformulation treatments (Table 4.43).

4.10.8. Chitinase

The induction of chitinase by bioformulations of T1 and B1 in groundnut challenged with *S. rolfsii* showed the ability of *Trichoderma* and *Bacillus* bioformulations in inducing PR-3 protein. The efficacy of *Trichoderma* and *Bacillus* bioformulations on induction of chitinase activity varied in groundnut plants against stem rot pathogen. Further, the combined application of T1 and B1 with chitin recorded significantly highest mean chitinase activity (3.52 µmole N-acetyl-D-glucosaminemin^{-1}g^{-1} FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (2.98 µmole N-acetyl-D-glucosaminemin^{-1}g^{-1} FW mg^{-1} protein). Significantly least activity was found in untreated control. Among the sample intervals on 2nd dpi significantly sharp increase in chitinase activity in bioformulation treatments was recorded and was persisted up to 6th dpi (Table 4.44).

Similar kind of studies was reported by many researchers. Higher level of the activities of *β*-1, 3 glucanase and chitinase in *B. subtilis* and *P. chlororaphis* pre-treated chilli plants challenged with *P. aphanidermatum* was observed by Kavitha *et al.* (2005). Similarly, maximum induction of *β*-1,3 glucanase and chitinase in rice plants treated with antagonistic bacteria against *R. solani* was recorded by Nagarajkumar *et al.* (2005). Likewise, Chakraborty *et al.* (2016) reported that, the joint application of *Rhizobium fasciculatus* and *B. pumilus* in tea bushes lead to sharp increase in *β*-1,3 glucanase and phenylalanine ammonia-lyase which played a key role in disease suppression.

Further, Gajera *et al.* (2015) revealed the highest accumulation of pathogenesis related proteins (*β*-1,3 glucanase and chitinase) in *Aspergillus niger* challenged groundnut seedlings in response to *Trichoderma viride.* In addition, Loganathan *et al.* (2010) reported the cumulative action of combined application of different strains of *Trichoderma* sp. amended with chitin in induction of *β*-1,3 glucanase in cabbage against *Sclerotinia sclerotium.*

Production of lytic enzymes like chitinase and *β*-1,3 glucanase by antagonists forms the basis for control of plant-pathogenic fungi in the rhizosphere (Mauch and Staehelin, 1989). Synthesis and accumulation of PR-proteins have been reported to play an important role in plant defense against the attack of pathogens. Further, chitinases

and β-1,3 glucanase (which are classified under PR-3 and PR-2 groups of PR-proteins, respectively) have been reported to be associated with plant disease resistance against the various soilborne fungal pathogens (Maurhofer *et al.*, 1994; van Loon, 1997).

Looking to above results, the combined application of bioformulations T1 and B1 fortified with chitin was found to be most effective in induction of resistance against stem rot of groundnut under glasshouse conditions followed by combined application of T1 and B1 bioformulations without chitin.

4.11. Evaluation of bioformulations against stem rot of groundnut under field conditions

The study was conducted to test the efficacy of talc formulations of T1 isolate of *Trichoderma* sp. and B1 isolate of *Bacillus* sp. with or without chitin individually and in combination through seed treatment and soil application in controlling stem rot of groundnut under field conditions at two locations one at ICRISAT, Patancheru (Location I) (Plate 4.33) and another at PJTSAU, Rajendranagar (Location II) (Plate 4.34) during *kharif*, 2016.

The efficacy of these bioformulations were evaluated based on their effect on stem rot severity, incidence, stem discolouration, pod rot and mortality induced by virulent isolate of *S. rolfsii* (SrGj-3) in groundnut under field conditions.

4.11.1. Disease severity

Results of efficacy of bioformulation on severity of stem rot in groundnut induced by *S. rolfsii* revealed that, at location-I, the combined application of bioformulation of T1 and B1 fortified with chitin recorded significantly least disease severity (16.67%) followed by chemical control (17.08%). Further, the combined application of bioformulation of T1 and B1 without chitin was found to be the next best treatment (20.42%) at 15 dpi (days post pathogen inoculation). The similar trend was observed at 30, 45, 60 and 75 dpi. Additionally, the progress in disease severity in above treatments from 15 to 75 dpi was very slow compared to other treatments. In total, the significantly lowest mean disease severity was recorded with combined application of T1 and B1 fortified with chitin (38.42%) followed by chemical control (45.83%) and combined application of T1 and B1 without chitin (48.92%).

Likewise, the similar tendency was recorded even at location-II wherein the significantly lowest mean disease severity was observed with combined application of T1 and B1 bioformulation with chitin (41.92%) followed by chemical control (48.25%) and combined application of T1 and B1 bioformulation without chitin (54.17%). Interestingly the similar trend was noted in pooled data, where the combined application of T1 and B1 bioformulation with chitin was found most effective followed by chemical control and the combined application of T1 and B1 bioformulation without chitin (Table 4.45).

4.11.2. Disease incidence

The similar trend was observed in efficacy of bioformulation on stem rot incidence in groundnut induced by *S. rolfsii* under field conditions. At location-I, the significantly least disease incidence of 25.37% was noted with the combined application of bioformulation of T1 and B1 fortified with chitin. Further, the combined application of bioformulation of T1 and B1 without chitin was found to be next best treatment (31.39%) which was at par with chemical control (30.49%) at 15 dpi. The similar type of observations was recorded at 30, 45, 60 and 75 dpi. Additionally, the progress of stem rot incidence in the above treatments from 15 to 75 dpi was very slow compared to other treatments. Overall, significantly least mean disease incidence of 35.40% was recorded in combined application of bioformulation of T1 and B1 fortified with chitin. The combined application of bioformulation of T1 and B1 without chitin exhibited the disease incidence of 43.07% which was at par with chemical control (41.93%).

Likewise, the similar tendency was recorded even at location-II wherein the significantly lowest mean disease incidence was noted with combined application of T1 and B1 bioformulation with chitin (36.22%) followed by chemical control (41.84%) and combined application of T1 and B1 bioformulation without chitin (42.23%). Further, the similar trend was noted in pooled data, where the combined application of T1 and B1 bioformulation with chitin was found most effective. Additionally, the combined application of T1 and B1 bioformulation without chitin was found to be next best treatment which was at par with chemical control (Table 4.46).

4.11.3. Stem discoloration and pod rot

The bioformulations varied in their efficacy in controlling the stem discolouration and pod rot induced by *S. rolfsii* under field conditions. Significantly least stem discoloration (3.67%) and pod rot (1.32%) was observed in the combined

application of bioformulation of T1 and B1 fortified with chitin followed by chemical control (with 4.67% of stem discoloration and 2.72% of pod rot) and combined application of bioformulation of T1 and B1 without chitin (with 5.26% of stem discoloration and 2.01% of pod rot) which were at par with each other at location-I. Further, the similar trend was recorded at location-II and in pooled data (Table 4.47).

4.11.4. Mortality

Likewise, the bioformulation differed in controlling the mortality in groundnut plants induced by *S. rolfsii* under field conditions. At location-I, no mortality was observed at 15 dpi in all the bioformulation treatments. Further, at 30 dpi the combined application of bioformulation of T1 and B1 fortified with chitin recorded significantly least mortality (9.04%). Whereas the combined application of bioformulation of T1 and B1 without chitin noted 13.61% mortality which was at par with chemical control (12.44%) and the same trend was evidenced at 45, 60 and 75 dpi. Here too, the progress of mortality in the above treatments from 30 to 75 dpi was very slow compared to other treatments. In total, the combined application of bioformulation of T1 and B1 fortified with chitin recorded significantly least mean mortality of 12.57%. The combined application of bioformulation of T1 and B1 without chitin (18.49%) was found to be next best treatment and was at par with chemical control (17.61%). Interestingly, the similar trend was recorded at location-II and in pooled data wherein the combined application of bioformulation of T1 and B1 fortified with chitin was found to be most effective in controlling the mortality induced by *S. rolfsii* under field conditions. Further, the combined application of bioformulation of T1 and B1 without chitin was found to be next best treatment and was at par with chemical control (Table 4.48).

4.11.5. Growth promoting traits

The bioformulations recorded significant growth promoting traits in groundnut under field conditions. At location-I, the combined application of T1 and B1 bioformulation with chitin induced significantly higher germination (78.33%). Further, the combined application of T1 and B1 bioformulation without chitin was found to be next best treatment with 72.92% germination and was at par with chemical control (75.42%). Similar observations were noted with respect to plant height, wherein the combined application of T1 and B1 bioformulation with chitin was significantly superior (55.77 cm). The combined application of T1 and B1 bioformulation without chitin was found to be next best treatment with 51.55 cm plant height and was at par

with chemical control (51.65 cm). With respect to nodules per plant, the combined application of T1 and B1 bioformulation with chitin performed significantly superior (251.77) followed by the combined application of T1 and B1 bioformulation without chitin (285.13) which were at par with each other. Further the chemical, inoculated and uninoculated controls recorded least nodules per plant compared to other bioformulation treatments. Likewise, the different treatments in the study did not differed significantly with respect to the oil and protein content and was ranged from 46.22 to 48.90% and 22.76 to 24.78% respectively, indicating no deleterious effect of these bioformulations on oil and protein content of groundnut under field conditions. Interestingly, the similar trend was observed at location-II and in the pooled data (Table 4.49).

4.11.6. Yield and yield related traits

The bioformulation application in groundnut had positive effect on yield and yield related traits under field conditions. At location-I, the combined application of T1 and B1 bioformulation with chitin recorded significantly highest number of pods per plant (16.88) followed by the combined application of T1 and B1 bioformulation without chitin (15.58) which was at par with each other. In regard to 100 kernel weight, the combined application of T1 and B1 bioformulation with chitin (38.37 g) performed significantly superior followed by the combined application of T1 and B1 bioformulation without chitin (36.00 g). Likewise, significantly highest shelling percentage was noted in combined application of T1 and B1 bioformulation with chitin (74.30) followed by combined application of T1 and B1 bioformulation without chitin (71.25). Similar observations were recorded with respect to pod yield, wherein the combined application of T1 and B1 bioformulation with chitin was significantly superior (1888.26 kg/ha) followed by the combined application of T1 and B1 bioformulation without chitin (1780.49 kg/ha). Additionally, the significantly highest biomass yield was noted with the combined application of T1 and B1 bioformulation with chitin (2798.61 kg/ha) followed by combined application of T1 and B1 bioformulation without chitin (2638.89 kg/ha) and chemical control (2500.00 kg/ha) which were at par with each other. Interestingly, the combined application of T1 and B1 bioformulation with chitin recorded the highest B:C ratio of 3.11 followed by the combined application of T1 and B1 bioformulation without chitin (2.94). Further, the similar trend was noted at location-II and in pooled data (Table 4.50).

Hence, looking to the above results it was found that, the combined application of bioformulation of T1 and B1 fortified with chitin through seed treatment and soil application was found significantly most effective in controlling stem rot of groundnut under field conditions and was at par with chemical control followed by combined application of T1 and B1 bioformulation without chitin.

The results are in consistency with the findings of Thilagavathi *et al.* (2007), Karthiba *et al.* (2011) and Senthilraja *et al.* (2010) who demonstrated the combined use of antagonistic microorganisms against various soil borne fungal pathogens. Similarly, Latha *et al.* (2011) reported the greatest reduction in collar and root rot incidence in physic nut in plots treated with the mixture of bioagents (*T. viride, P. fluorescens* and *B. subtilis*). Further, growth promotion in physic nut by combined application of *T. viride, P. fluorescens* and *B. subtilis* was observed by Latha *et al.* (2011). Similarly, growth promotion in chilli by combined application of *T. viride* and *P. fluorescens* was recorded by Manoranjitham *et al.* (2000).

In the present investigation the chitin amendment in bioformulations has enhanced the efficacy of bioagents in disease suppression ability and induced the defense response in treated plants against the pathogen. Chitin is a copious renewable natural resource obtained from marine invertebrates, insects, fungi and algae. More than 80,000 Mt of chitin is obtained per year from marine waste (Loganathan *et al.,* 2010). Role of chitin or chitosan in inducing systemic resistance alone or in combination with biocontrol agents has been demonstrated in few crops. The interest in the utilization of chitinolytic antagonist bacteria and fungi has grown tremendously, as the chitin supplements increased the attainable level of disease control. However, the application of chitin alone or in combination with the biocontrol agents in managing plant diseases has been demonstrated in only a few crops by Kishore *et al.* (2005), Nandakumar *et al.* (2001), Radjacommare *et al.* (2010), Rajkumar *et al.* (2008) and Senthilraja *et al.*(2010).

In similar lines Ahmed *et al.* (2003) reported the efficacy of a 0.5% chitin amended formulation mixture of *Trichoderma* spp. and *Bacillus* spp. over their non-chitin amended bioformulations against *Phytophthora* and *Rhizoctonia* root rot of pepper. Similarly, significant control of *Rhizoctonia solani* by application of *Trichoderma/Hypocrea*-based formulations with chitin (1% v:v) was observed by Solanki *et al.* (2011).

In the present study, the application of mixture of bioformulation through seed treatment followed by soil application had effectively checked the disease in glasshouse and field conditions. The results are in conformity with Vidhyasekaran *et al.* (1997) who reported the effective control of chickpea and pigeonpea wilt by application of talc formulation of bioagent mixtures through seed treatment and soil application. Further, efficacy of combination of different methods of application bioagents was reported by Vidhyasekaran *et al.* (1997), Vidhyasekaran and Muthamilan (1999), Meena *et al.* (2000), Nandakumar *et al.* (2001) and Saravanakumar (2006) in control of various soil borne fungal pathogens.

Table 4.1. Prevalence of stem rot in major groundnut growing areas of India

State	District	Mandal/Taluka	Village	Variety	Soil type	Planting date	Crop Stage	Crop density	Crop protection	Previous crop	Disease incidence (%)	
											Kharif 2013	*Kharif* 2014
Karnataka	Gadag	Gadag	Asundi	TMV 2	Red	June	Pod	High	-	Groundnut	33.10	25.50
			Harti	TMV 2	Red	June	Pod	Medium	-	Groundnut	21.55	31.60
			Hirekoppa	TMV 2	Red	June	Pod	Medium	Thiram ST	Pigeonpea	19.50	20.11
			Hulkoti	Local	Red	June	Pod	Medium	-	Groundnut	15.40	15.20
			Kanavi	Local	Black	June	Pod	Medium	-	Chickpea	20.10	23.45
			Mulgund	Local	Red	June	Pod	High	Carbendazim ST	Groundnut	31.50	20.15
			Shirol	Local	Red	June	Pod	Medium	-	Groundnut	24.50	23.50
		Ron	Asuti	TMV 2	Red	June	Pod	Medium	-	Groundnut	21.40	15.66
			Budihal	TMV 2	Red	June	Pod	High	-	Groundnut	29.60	34.55
			Honnapur	TMV 2	Red	June	Pod	High	Thiram ST	Groundnut	28.70	25.50
			Itagi	Local	Black	June	Pod	Medium	-	Chickpea	19.80	21.30
		Mundargi	Dindur	TMV 2	Red	June	Pod	Medium	-	Groundnut	14.50	10.50
			Hallikeri	TMV 2	Red	June	Pod	Medium	-	Groundnut	14.66	10.44
		Shirahatti	Balehosur	Local	Red	June	Pod	Medium	-	Pigeonpea	19.66	23.56
			Bellatti	Local	Red	June	Pod	Medium	-	Groundnut	15.24	21.55
			Chabbi	TMV 2	Red	June	Pod	High	-	Groundnut	21.10	15.23
	Raichur	Raichur	Yapaldinni	Local	Red	May	Pod	Medium	-	Pigeonpea	15.45	10.25
			Yermarus	Local	Red	June	Pod	Medium	Carbendazim ST	Groundnut	12.10	23.50
			Mirzapur	Local	Red	June	Pod	Medium	-	Groundnut	19.50	21.50
			Mallapur	Local	Red	July	Peg	High	-	Groundnut	24.70	20.14
		Devadurga	Bagur	Local	Red	July	Peg	Medium	-	Groundnut	12.30	21.14
			Devatgal	Local	Red	May	Pod	High	-	Groundnut	31.40	29.33
			Gabbur	TMV 2	Red	June	Pod	Medium	-	Groundnut	16.40	23.55
		Lingasugur	Anahosur	TMV 2	Red	June	Pod	Medium	-	Pigeonpea	16.80	15.00
			Chittapur	Local	Red	June	Pod	Medium	Thiram ST	Groundnut	18.40	19.50
			Hatti	Local	Red	June	Pod	High	-	Groundnut	23.50	25.50
	Bellary	Bellary	Bevinahalli	TMV 2	Black	July	Peg	Medium	-	Chilli	24.60	27.80
			Honnahalli	TMV 2	Red	July	Peg	High	-	Groundnut	14.90	20.40
			Jalihal	Local	Red	July	Peg	High	-	Groundnut	26.50	31.50
			Janikunte	Local	Red	July	Peg	High	-	Groundnut	31.80	36.00
			Kolur	Local	Red	May	Pod	Medium	-	Groundnut	16.50	20.15
		Sirguppa	Basarahalli	TMV 2	Red	July	Peg	Medium	-	Groundnut	21.60	23.40
			Budaguppa	TMV 2	Red	May	Pod	Medium	-	Groundnut	21.50	15.40
			Sirigeri	Local	Red	July	Peg	Medium	-	Groundnut	15.40	10.50
		Kudilgi	Bellikatti	Local	Black	July	Peg	Medium	Thiram ST	Chilli	21.50	20.45
			Gudekota	Local	Red	August	Peg	Medium	-	Groundnut	23.40	31.50
			Kodihalli	Local	Red	August	Peg	Medium	-	Groundnut	22.40	20.15

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State	District	Mandal/Taluka	Village	Variety	Soil type	Planting date	Crop Stage	Crop density	Crop protection	Previous crop	Disease incidence (%)	
											Kharif 2013	*Kharif* 2014
Karnataka	Chitradurga	Challakere	Balenahalli	TMV 2	Red	July	Peg	High	-	Ragi	41.55	30.65
			Belegere	Local	Red	July	Peg	High	-	Groundnut	31.50	30.55
			Chikkahalli	TMV 2	Red	July	Peg	High	-	Groundnut	26.70	24.55
			Donehalli	TMV 2	Red	August	Peg	Medium	-	Ragi	21.50	20.45
			Gollahalli	Local	Red	July	Peg	Medium	Thiram ST	Groundnut	31.22	30.15
			Heggere	TMV 2	Red	August	Peg	Medium	-	Groundnut	14.66	10.55
			Hirehalli	Local	Red	July	Peg	Medium	-	Groundnut	12.35	15.50
		Hiriyur	Berenahalli	Local	Red	July	Peg	Medium	Thiram ST	Pigeonpea	11.45	10.22
			Devarakotta	TMV 2	Red	August	Peg	Medium	-	Groundnut	16.33	20.65
			Gollahalli	TMV 2	Red	July	Peg	Medium	-	Groundnut	21.44	24.11
			Hartikote	Local	Red	July	Peg	Medium	Carbendazim ST	Groundnut	25.33	20.11
			Hosahalli	TMV 2	Red	July	Peg	Medium	-	Ragi	21.33	23.12
	Tumkur	Pavagada	Chikkahalli	K 6	Red	July	Peg	Medium	Carbendazim ST	Groundnut	25.66	31.22
			Chikkanayakanahalli	Local	Red	July	Peg	Medium	Carbendazim ST	Groundnut	21.30	15.44
			Hosahalli	TMV 2	Red	July	Peg	Medium	-	Pigeonpea	25.40	30.22
			Dodahalli	K 2	Red	June	Pod	Medium	Carbendazim ST	Groundnut	21.60	15.11
			Hanumanbetta	Local	Red	July	Peg	High	Carbendazim ST	Groundnut	21.40	20.11
			Kodigehalli	TMV 2	Red	August	Peg	High	-	Groundnut	35.60	40.12
			Lingadahalli	Local	Red	July	Peg	High	-	Ragi	41.50	38.55
			Yattinahalli	TMV 2	Red	July	Peg	High	Thiram ST	Groundnut	45.80	39.44
		Sira	Karehalli	K 2	Red	August	Peg	High	-	Groundnut	45.60	40.11
			Koratakere	TMV 2	Red	July	Peg	High	Carbendazim ST	Groundnut	21.50	20.11
			Archalli	K 6	Red	July	Peg	Medium	Carbendazim ST	Groundnut	14.50	15.45
			Bettanahalli	Local	Red	July	Peg	Medium	Thiram ST	Groundnut	20.15	21.33
			Devarahalli	TMV 2	Red	August	Peg	Medium	Carbendazim ST	Ragi	25.66	26.88
			Gopikunte	TMV 2	Red	July	Peg	High	-	Groundnut	31.22	30.12
Telangana	Mahabubnagar	Balmoor	Balmoor	Local	Red	June	Pod	Medium	Thiram ST	Groundnut	21.44	15.22
			Jinkunta	K 2	Red	June	Pod	Medium	Thiram ST	Castor	16.44	10.44
			Polepalli	K 6	Red	July	Peg	Medium	-	Groundnut	21.40	31.22
			Ramagiri	K 2	Red	June	Pod	High	Carbendazim ST	Groundnut	26.00	31.22
		Lingal	Bakaram	K 2	Red	June	Pod	High	-	Groundnut	31.20	34.11
			Lingal	Local	Red	June	Pod	Medium	-	Castor	14.50	21.44
			Madapur	K 6	Red	July	Peg	Medium	-	Groundnut	21.50	20.14
			Rampur	K 6	Red	June	Pod	Medium	Thiram ST	Groundnut	21.44	23.55
	Warangal	Zaffergadh	Aliyabad	Local	Red	June	Pod	Medium	Thiram ST	Groundnut	14.55	20.14
			Kunoor	Local	Red	June	Pod	Medium	-	Groundnut	14.21	10.11
			Sagaram	Local	Red	July	Peg	Medium	Carbendazim ST	Castor	13.56	15.22
		Paravathagiri	Enugal	K 2	Red	June	Pod	High	-	Groundnut	26.55	30.12
			Kalleda	Local	Red	June	Pod	High	-	Groundnut	31.24	32.33
			Ravoor	K 6	Red	June	Pod	Medium	-	Groundnut	15.45	16.22
			Vadlakonda	K 6	Red	July	peg	Medium	Carbendazim ST	Groundnut	21.33	25.33

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State	District	Mandal/Taluka	Village	Variety	Soil type	Planting date	Crop Stage	Crop density	Crop protection	Previous crop	Disease incidence (%)	
											Kharif 2013	*Kharif* 2014
Andhra Pradesh	Anantapur	Kadiri	Kadiri	K 6	Red	July	Pod	High	-	Groundnut	34.65	30.66
			Yerododdi	K 6	Red	August	Peg	Medium	-	Groundnut	24.51	20.14
			Kadirikuntapalle	K 2	Red	July	Peg	Medium	Thiram ST	Groundnut	14.56	15.22
			Pandulakunta	K 2	Red	July	Peg	Medium	-	Groundnut	21.45	20.13
		Nallacheruvu	Allugundu	K 6	Red	July	Peg	High	-	Groundnut	14.33	15.22
			Kadiripulakunta	Local	Red	July	Peg	High	-	Groundnut	41.23	40.15
		Nallamada	Gopepalli	K 2	Red	June	Pod	High	Carbendazim ST	Groundnut	21.55	20.13
			Vellamaddi	Local	Red	July	Peg	Medium	-	Groundnut	19.65	15.23
		Singanamala	Budepalli	Local	Red	July	Peg	Medium	-	Groundnut	34.52	39.11
			Kallumadi	Local	Red	June	Pod	High	-	Groundnut	14.52	16.33
	Chittoor	Chittoor	Anathapuram	K 2	Red	June	Pod	High	Thiram ST	Groundnut	12.35	15.22
			Arathala	K 6	Red	July	Peg	Medium	-	Groundnut	21.44	30.11
			Bandapalle	K 6	Red	July	Peg	Medium	Carbendazim ST	Groundnut	25.60	30.11
			Krishnapuram	K 6	Red	July	Peg	Medium	Carbendazim ST	Groundnut	25.80	25.66
		B Kothakota	Gollapalle	K 6	Red	August	Peg	Medium	-	Groundnut	30.11	31.45
			B Kothakota	K 6	Red	July	Peg	High	-	Groundnut	25.11	28.77
		Ramachandrapuram	Nadavaluru	K 6	Red	July	Peg	Medium	Thiram ST	Groundnut	15.22	23.11
			Netha Kuppam	Local	Red	July	Peg	Medium	-	Groundnut	12.33	15.45
Maharashtra	Latur	Latur	Akoli	JL 24	Red	June	Pod	Medium	Carbendazim ST	Pigeonpea	34.25	36.44
			Bhadgaon	TG 1	Red	May	Pod	High	-	Groundnut	31.52	35.12
			Chikurda	TMV 2	Red	June	Pod	Medium	-	Groundnut	19.68	20.14
			Borwati	TMV 2	Black	June	Pod	Medium	Thiram ST	Cotton	31.24	23.99
			Gangapur	JL 24	Red	July	Peg	High	-	Groundnut	21.54	23.55
			Gategaon	JL 24	Red	May	Pod	High	-	Groundnut	23.55	21.14
	Solapur	Solapur North	Bhogaon	JL 24	Red	June	Pod	Medium	Thiram ST	Pigeonpea	14.55	15.24
			Dongaon	TG 17	Red	June	Pod	High	-	Groundnut	24.68	25.66
			Karamba	JL 24	Red	June	Pod	Medium	-	Pigeonpea	21.85	23.11
			Khed	TG 17	Black	June	Pod	Medium	Carbendazim ST	Groundnut	35.61	38.12
		Solapur South	Aurad	JL 24	Black	July	Peg	Medium	-	Groundnut	41.42	45.10
			Doddi	JL 24	Red	May	Pod	Medium	-	Groundnut	24.66	25.33
			Hatur	JL 24	Red	June	Pod	Medium	Thiram ST	Pigeonpea	21.33	15.12
	Nanded	Nanded	Bhalki	JL 24	Red	June	Pod	Medium	-	Groundnut	26.78	19.56
			Daryapur	JL 24	Black	July	Peg	Medium	-	Groundnut	41.51	45.23
			Jaitapur	TG 17	Red	June	Pod	High	Carbendazim ST	Groundnut	24.68	31.22
			Kalhal	JL 24	Red	June	Pod	High	-	Groundnut	24.70	15.99
			Naleshwar	TG 17	Red	July	Peg	Medium	-	Pigeonpea	21.30	22.30

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State	District	Mandal/Taluka	Village	Variety	Soil type	Planting date	Crop Stage	Crop density	Crop protection	Previous crop	Disease incidence (%)	
											Kharif 2013	*Kharif* 2014
Tamil Nadu	Coimbatore	Coimbatore North	Kallipalayam	VRI 2	Red	June	Pod	High	-	Ragi	31.22	29.77
			Panchapalayam	TMV 2	Black	June	Pod	High	-	Groundnut	41.56	40.00
			Aliarmagar	TMV 7	Red	June	Pod	Medium	Carbendazim ST	Groundnut	21.44	25.47
			Pannimadai	TMV 2	Red	July	Peg	Medium	-	Pigeonpea	11.23	12.30
		Coimbatore South	Myleripalayam	TMV 10	Red	June	Pod	Medium	-	Groundnut	14.33	16.30
			Malumichampatti	TMV 2	Red	June	Pod	High	-	Pigeonpea	21.45	25.80
		Pollachi	Achipatti	VRI 2	Red	June	Pod	High	Carbendazim ST	Groundnut	24.50	23.66
			Kallipatti	TMV 7	Red	June	Pod	Medium	-	Ragi	21.50	20.11
	Erode	Erode	Avalpoondurai	VRI 3	Red	July	Peg	Medium	-	Groundnut	14.50	15.45
			Ellapalayam	TMV 10	Red	June	Pod	High	-	Groundnut	22.80	16.33
			Erode	VRI 2	Red	June	Pod	High	-	Pigeonpea	24.50	24.55
			Gangapuram	TMV 7	Red	June	Pod	High	-	Ragi	21.33	15.23
			Koorapalayam	TMV 2	Red	July	Peg	Medium	-	Groundnut	21.47	15.21
		Bhavani	Bhavani	TMV 7	Red	June	Pod	Medium	Carbendazim ST	Pigeonpea	15.23	15.11
			Andlikulam	TMV 2	Black	June	Pod	High	-	Groundnut	50.14	40.12
			Kesarimangalam	TMV 10	Red	June	Pod		-	Groundnut	24.15	26.33
	Selam	Selam	Adikarapatti	VRI 2	Red	July	Peg	Medium	-	Groundnut	24.71	25.44
			Basavanathampatti	TMV 2	Red	June	Pod	Medium	Thiram ST	Pigeonpea	19.78	20.14
			Chettichavadi	VRI 3	Red	July	Peg	Medium	-	Groundnut	21.33	22.14
			Thippampatti	TMV 12	Red	June	Pod	Medium	-	Groundnut	24.55	26.30
		Gangavalli	Belur	TMV 2	Red	June	Pod	Medium	Carbendazim ST	Ragi	14.52	15.44
			Goodamalai	TMV 10	Black	June	Pod	Medium	-	Groundnut	41.12	45.66
			Panchamalai	VRI 2	Red	June	Pod	High	-	Groundnut	34.11	35.11
Gujarat	Junagadh	Junagadh	Mandanpara	GG 20	Black	July	Peg	Medium	Tebuconazole ST	Groundnut	31.45	25.33
			Navagam	GG 20	Black	June	Pod	High	Tebuconazole ST	Groundnut	26.45	29.56
			Khalipur	GG 20	Black	June	pod	High	Tebuconazole ST	Groundnut	21.33	20.11
			Makhiyala	GG 2	Black	July	Peg	Medium	Tebuconazole ST	Wheat	24.33	25.33
			Surajkund	GG 2	Black	June	Pod	High	-	Cumin	25.45	25.00
			Chokli	GG 20	Black	June	Pod	Medium	-	Cumin	18.50	15.22
			Khadiya	GG 20	Black	June	Pod	Medium	Tebuconazole ST	Groundnut	34.50	23.66
			Ramnath	GG 20	Black	June	Pod	High	Tebuconazole ST	Groundnut	50.45	50.11
			Patapur	GG 2	Red	June	Pod	High	Tebuconazole ST	Groundnut	24.60	20.40
			Bhalgam	GG 2	Red	June	Pod	Medium	Tebuconazole ST	Groundnut	21.40	20.55
			Jamka	GG 20	Black	June	Pod	Medium	Tebuconazole ST	Groundnut	34.80	35.60
		Bhesan	Barwala	GG 20	Black	June	Pod	Medium	-	Groundnut	31.20	30.10
			Dholwa	GG 20	Black	June	Pod	High	-	Wheat	25.00	20.45
			Mandva	GG 20	Red	June	Pod	Medium	Tebuconazole ST	Groundnut	24.50	30.14

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State	District	Mandal/Taluka	Village	Variety	Soil type	Planting date	Crop Stage	Crop density	Crop protection	Previous crop	Disease incidence (%)	
											Kharif 2013	*Kharif* 2014
Gujarat	Rajkot	Rajkot	Golida	GG 2	Black	June	Pod	Medium	-	Cotton	16.50	10.12
			Dhamalpur	GG 20	Black	June	Pod	High	Tebuconazole ST	Groundnut	31.40	26.88
			Kalipat	GG 20	Black	June	Pod	High	Tebuconazole ST	Groundnut	51.60	45.23
			Bedla	GG 20	Black	June	Pod	High	Tebuconazole ST	Cotton	24.50	20.34
			Umrali	GG 20	Black	June	Pod	Medium	-	Cotton	21.45	20.11
			Nakaravadi	GG 11	Black	June	Pod	High	Tebuconazole ST	Groundnut	45.33	40.15
			Jamgadh	GG 20	Black	July	Peg	Medium	-	Cotton	21.40	20.12
	Porbandar	Porbandar	Bhetkadi	GG 20	Black	June	Pod	High	Tebuconazole ST	Cotton	24.50	26.50
			Chikasa	GG 20	Black	June	Pod	High	Tebuconazole ST	Cumin	21.45	20.11
			Rajhivada	GG 20	Black	June	Pod	High	-	Groundnut	31.55	29.56
			Kantela	GG 20	Black	June	Pod	Medium	Tebuconazole ST	Cotton	25.45	15.23
			Khistri	GG 20	Black	June	Pod	Medium	-	Cotton	26.14	20.14
			Madhavpur	GG 20	Black	July	Peg	High	-	Groundnut	51.40	45.00
			Modhavada	GG 2	Black	July	Peg	High	-	Groundnut	45.70	40.12
	Amreli	Amreli	Devarajiya	GG 11	Black	June	Pod	Medium	-	Groundnut	26.40	25.33
			Vadera	GG 20	Red	June	Pod	High	-	Groundnut	21.50	22.30
			Malvan	GG 20	Black	June	Pod	High	Tebuconazole ST	Groundnut	36.50	40.11
			Haripura	GG 20	Black	June	Pod	High	Tebuconazole ST	Groundnut	55.40	59.33
			Chandgadh	GG 20	Black	June	Pod	High	-	Cotton	26.50	23.00
			Devaliya	GG 20	Black	June	Pod	High	-	Cotton	24.50	25.66
	Gir Somnath	Somnath	Bhojde	GG 2	Black	July	Peg	High	-	Groundnut	19.90	20.13
			Borvav	GG 20	Black	June	Pod	High	-	Cotton	21.30	25.12
			Khushiagir	GG 11	Black	July	Peg	Medium	-	Cotton	21.30	23.55
			Semarvav	GG 20	Black	June	Pod	Medium	-	Groundnut	24.50	30.11

Table 4.2. District-wise mean per cent incidence of stem rot of groundnut in major growing areas of India

State	District	Disease incidence (%)	
		***Kharif* 2013**	***Kharif* 2014**
Karnataka	Gadag	21.89	21.11
	Raichur	19.06	20.94
	Bellary	21.83	23.39
	Chitradurga	22.95	21.72
	Tumkur	28.35	27.44
	Mean	**22.81**	**22.92**
Telangana	Mahabubnagar	21.74	23.42
	Warangal	19.56	21.35
	Mean	**20.65**	**22.39**
Andhra Pradesh	Anantapur	24.10	23.23
	Chittoor	21.00	24.99
	Mean	**22.55**	**24.11**
Maharashtra	Latur	26.96	26.73
	Solapur	26.30	26.81
	Nanded	27.79	26.86
	Mean	**27.02**	**26.80**
Tamil Nadu	Coimbatore	23.40	24.18
	Erode	24.27	21.04
	Selam	25.73	27.18
	Mean	**24.47**	**24.13**
Gujarat	Junagadh	28.14	26.54
	Rajkot	30.31	26.14
	Porbandar	32.31	28.09
	Amreli	31.80	32.62
	Gir Somnath	21.75	24.73
	Mean	**28.86**	**27.62**

Table 4.3. List of isolates of *Sclerotium rolfsii* collected from major groundnut growing areas of India

S. No.	Isolate Designation	Geographical origin			Date of collection
		State	District	Village	
1	SrKa-1	Karnataka	Gadag	Asundi	16-09-2013
2	SrKa-2	Karnataka	Gadag	Harti	16-09-2013
3	SrKa-3	Karnataka	Gadag	Bellatti	16-09-2013
4	SrKa-4	Karnataka	Gadag	Hulkoti	18-09-2013
5	SrKa-5	Karnataka	Bellary	Bevinahalli	18-09-2013
6	SrKa-6	Karnataka	Bellary	Sirigeri	19-09-2013
7	SrKa-7	Karnataka	Bellary	Janikunte	19-09-2013
8	SrKa-8	Karnataka	Raichur	Yapaldinni	19-09-2013
9	SrKa-9	Karnataka	Raichur	Hatti	22-09-2013
10	SrKa-10	Karnataka	Raichur	Anahosur	22-09-2013
11	SrKa-11	Karnataka	Chitradurga	Belegere	22-09-2013
12	SrKa-12	Karnataka	Chitradurga	Hirehalli	25-09-2013
13	SrKa-13	Karnataka	Chitradurga	Hartikote	25-09-2013
14	SrKa-14	Karnataka	Chitradurga	Hosahalli	25-09-2013
15	SrKa-15	Karnataka	Tumkur	Hanumanbetta	25-09-2013
16	SrKa-16	Karnataka	Tumkur	Yattinahalli	25-09-2013
17	SrKa-17	Karnataka	Tumkur	Dodahalli	27-09-2013
18	SrKa-18	Karnataka	Tumkur	Chikkahalli	27-09-2013
19	SrKa-19	Karnataka	Tumkur	Karehalli	27-09-2013
20	SrKa-20	Karnataka	Tumkur	Gopikunte	27-09-2013
21	SrTs-1	Telangana	Mahabubnagar	Balmoor	07-10-2013
22	SrTs-2	Telangana	Mahabubnagar	Polepalli	07-10-2013
23	SrTs-3	Telangana	Mahabubnagar	Bakaram	07-10-2013
24	SrTs-4	Telangana	Mahabubnagar	Lingal	07-10-2013
25	SrTs-5	Telangana	Mahabubnagar	Rampur	07-10-2013
26	SrTs-6	Telangana	Warangal	Kunoor	07-10-2013
27	SrTs-7	Telangana	Warangal	Sagaram	09-10-2013
28	SrTs-8	Telangana	Warangal	Kalleda	09-10-2013
29	SrTs-9	Telangana	Warangal	Ravoor	09-10-2013
30	SrTs-10	Telangana	Warangal	Vadlakonda	09-10-2013
31	SrAp-1	Andhra Pradesh	Anantapur	Kadiri	14-10-2013
32	SrAp-2	Andhra Pradesh	Anantapur	Yerododdi	14-10-2013
33	SrAp-3	Andhra Pradesh	Anantapur	Allugundu	14-10-2013
34	SrAp-4	Andhra Pradesh	Anantapur	Gopepalli	14-10-2013
35	SrAp-5	Andhra Pradesh	Anantapur	Kallumadi	14-10-2013
36	SrAp-6	Andhra Pradesh	Chittoor	Arathala	14-10-2013
37	SrAp-7	Andhra Pradesh	Chittoor	Bandapalle	14-10-2013

Cont.

S. No.	Isolate Designation	Geographical origin			Date of collection
		State	District	Village	
38	SrAp-8	Andhra Pradesh	Chittoor	Gollapalle	16-10-2013
39	SrAp-9	Andhra Pradesh	Chittoor	B Kothakota	16-10-2013
40	SrAp-10	Andhra Pradesh	Chittoor	Nadavaluru	16-10-2013
41	SrMh-1	Maharashtra	Latur	Akoli	21-10-2013
42	SrMh-2	Maharashtra	Latur	Chikurda	21-10-2013
43	SrMh-3	Maharashtra	Solapur	Bhogaon	21-10-2013
44	SrMh-4	Maharashtra	Solapur	Khed	21-10-2013
45	SrMh-5	Maharashtra	Nanded	Jaitapur	21-10-2013
46	SrMh-6	Maharashtra	Nanded	Kalhal	21-10-2013
47	SrTn-1	Tamil Nadu	Coimbatore	Kallipalayam	29-10-2013
48	SrTn-2	Tamil Nadu	Coimbatore	Aliarmagar	29-10-2013
49	SrTn-3	Tamil Nadu	Coimbatore	Achipatti	29-10-2013
50	SrTn-4	Tamil Nadu	Erode	Ellapalayam	29-10-2013
51	SrTn-5	Tamil Nadu	Erode	Bhavani	29-10-2013
52	SrTn-6	Tamil Nadu	Selam	Belur	29-10-2013
53	SrGj-1	Gujarat	Junagadh	Khalipur	01-11-2013
54	SrGj-2	Gujarat	Junagadh	Surajkund	01-11-2013
55	SrGj-3	Gujarat	Rajkot	Umrali	01-11-2013
56	SrGj-4	Gujarat	Porbandar	Bhetkadi	01-11-2013
57	SrGj-5	Gujarat	Porbandar	Khistri	01-11-2013
58	SrGj-6	Gujarat	Amreli	Malvan	01-11-2013
59	SrGj-7	Gujarat	Gir Somnath	Bhojde	01-11-2013
60	SrGj-8	Gujarat	Gir Somnath	Semarvav	01-11-2013

Table 4.4. *In vitro* evaluation of different culture media for optimal production of oxalic acid by *S. rolfsii*

Isolates	Oxalic acid (mg/ml)			
	Richards broth	**Czapek dox broth**	**Potato dextrose broth**	**Mean**
SrKa-1	2.01	1.45	1.22	1.56
SrTs-1	1.99	1.31	1.14	1.48
SrAp-1	2.24	1.73	1.47	1.81
SrMh-1	1.97	1.42	1.13	1.51
SrTn-1	1.97	1.53	1.27	1.59
SrGj-3	2.85	2.36	2.12	2.44
Control	0.00	0.00	0.00	0.00
Mean	1.86	1.40	1.19	-

Factors	CD (0.01)	S.Em.±	CV (%)
Isolates	0.231	0.081	7.53
Media	0.151	0.053	
Interaction	0.400	0.140	

Table 4.5. *In vitro* oxalic acid production by isolates of *S. rolfsii*

S. No.	Isolates	Oxalic acid (mg/ml)	S. No.	Isolates	Oxalic acid (mg/ml)
1	SrKa-1	2.00	31	SrAp-1	2.12
2	SrKa-2	2.08	32	SrAp-2	0.78
3	SrKa-3	2.26	33	SrAp-3	1.18
4	SrKa-4	2.08	34	SrAp-4	1.28
5	SrKa-5	2.24	35	SrAp-5	1.18
6	SrKa-6	1.90	36	SrAp-6	1.20
7	SrKa-7	2.28	37	SrAp-7	1.78
8	SrKa-8	1.04	38	SrAp-8	1.98
9	SrKa-9	1.12	39	SrAp-9	1.80
10	SrKa-10	1.58	40	SrAp-10	1.82
11	SrKa-11	1.58	41	SrMh-1	1.94
12	SrKa-12	0.76	42	SrMh-2	1.36
13	SrKa-13	1.18	43	SrMh-3	1.12
14	SrKa-14	1.39	44	SrMh-4	1.42
15	SrKa-15	1.72	45	SrMh-5	1.38
16	SrKa-16	1.84	46	SrMh-6	1.83
17	SrKa-17	1.59	47	SrTn-1	1.10
18	SrKa-18	1.26	48	SrTn-2	0.99
19	SrKa-19	1.21	49	SrTn-3	0.64
20	SrKa-20	1.54	50	SrTn-4	0.64
21	SrTs-1	1.98	51	SrTn-5	1.03
22	SrTs-2	2.14	52	SrTn-6	1.09
23	SrTs-3	1.47	53	SrGj-1	1.16
24	SrTs-4	1.20	54	SrGj-2	2.51
25	SrTs-5	1.88	55	SrGj-3	2.85
26	SrTs-6	1.63	56	SrGj-4	1.80
27	SrTs-7	1.18	57	SrGj-5	2.14
28	SrTs-8	1.84	58	SrGj-6	2.12
29	SrTs-9	2.11	59	SrGj-7	1.44
30	SrTs-10	2.16	60	SrGj-8	1.32

CD (0.01)	0.019
S.Em.±	0.070
CV (%)	7.80

Table 4.6. Effect of different inoculum levels on incubation period (IP) and days to permanent wilting (DPW) of *S. rolfsii* in groundnut

Inoculum levels (g/pot)	IP (days)				DPW (days)			
	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean
5g	7.25	7.25	7.25	7.25	>32*	>32*	>32*	>32*
10g	6.33	6.33	6.33	6.33	>32*	>32*	>32*	>32*
15g	5.83	5.83	5.83	5.83	6.92	6.83	8.50	7.42
20g	5.67	5.67	5.67	5.67	6.58	6.75	8.92	7.42
25g	5.58	5.58	5.58	5.58	6.67	6.50	8.17	7.11
30g	5.50	5.50	5.50	5.50	6.50	6.75	8.42	7.22
35g	5.58	5.58	5.58	5.58	6.83	6.92	8.08	7.28
Mean	5.22	5.22	5.22	-	6.70	6.75	8.42	-

Factors	IP			DPW		
	CD (0.01)	S.Em.±	CV (%)	CD (0.01)	S.Em.±	CV (%)
Inoculum levels	0.38	0.12	10.81	1.22	0.43	13.50
Cultivars	0.21	0.07		0.75	0.26	
Interaction	0.59	0.21		2.12	0.74	

*Permanent wilting in inoculated plants was not observed even at 32 days post pathogen inoculation (dpi) and were excluded from statistical analysis

Table 4.7. Effect of different inoculum levels of *S. rolfsii* on severity of stem rot of groundnut

Inoculum level	Disease severity (%)															
	8 dpi*				16 dpi				24 dpi				32 dpi			
	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean
5g	50.00 (45.00)**	43.33 (41.17)	46.67 (43.09)	46.67 (42.13)	60.00 (50.77)	50.00 (45.00)	50.00 (45.00)	53.33 (46.92)	70.00 (56.79)	58.33 (49.80)	60.00 (50.77)	62.78 (52.45)	70.00 (56.79)	60.00 (50.77)	61.67 (51.75)	63.89 (53.10)
10g	53.33 (46.91)	48.33 (44.04)	48.33 (44.04)	50.00 (44.04)	65.00 (53.73)	66.67 (54.74)	70.00 (56.79)	67.22 (55.09)	80.00 (63.43)	73.33 (58.91)	73.33 (58.91)	75.55 (60.42)	80.00 (63.43)	78.33 (62.26)	73.33 (58.91)	77.22 (61.53)
15g	88.33 (70.03)	71.67 (57.84)	73.33 (58.91)	77.78 (62.26)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
20g	91.67 (73.22)	75.00 (60.00)	73.33 (58.91)	80.00 (64.04)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
25g	91.67 (73.22)	78.33 (62.26)	78.33 (62.26)	82.78 (65.91)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
30g	90.00 (71.57)	85.00 (67.21)	78.33 (62.26)	84.44 (67.01)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
35g	91.67 (73.22)	80.00 (63.43)	76.67 (61.12)	82.78 (65.92)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Mean	79.52 (72.25)	68.81 (56.56)	67.86 (55.80)	-	89.29 (79.21)	88.10 (78.53)	88.57 (78.83)	-	92.86 (81.46)	90.24 (79.82)	90.48 (79.95)	-	92.86 (81.46)	91.19 (80.43)	90.71 (80.09)	-

Factors	8 dpi			16 dpi			24 dpi			32 dpi		
	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)
Inoc. levels	6.33	2.23	14.6	4.53	1.53	11.3	6.55	1.23	9.7	2.73	0.90	8.9
Cultivars	3.39	1.13		3.62	1.24		2.25	0.75		1.83	0.62	
Interaction	12.6	4.21		8.14	2.69		6.31	2.10		3.97	1.27	

*__dpi__ – days post inoculation **Figures in the parenthesis are arc sine transformed values

Table 4.8. Effect of different inoculum levels of *S. rolfsii* on mortality of groundnut

Inoculum level	Mortality (%)															
	8 dpi*				16 dpi				24 dpi				32 dpi			
	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean
5g	0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	25.00 (30.02)	0.00 (0.00)	16.67 (20.01)	13.89 (16.68)	50.00 (45.02)	25.00 (30.02)	16.67 (20.01)	30.56 (31.68)	50.00 (45.02)	33.33 (35.02)	16.67 (20.01)	33.33 (33.35)
10g	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	33.33 (35.02)	25.00 (25.01)	16.67 (20.01)	25.00 26.68	66.67 (55.03)	41.67 (40.02)	66.67 (55.03)	58.34 50.03	66.67 (55.03)	58.33 (50.03)	66.67 (55.03)	63.89 (53.36)
15g	41.67 (40.02)	8.33 (10.01)	8.33 (10.01)	19.44 (20.01)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
20g	58.33 (50.03)	25.00 (30.02)	16.67 (20.01)	33.33 (33.35)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
25g	58.33 (50.03)	33.33 (35.02)	16.67 (20.01)	36.11 (35.02)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
30g	50.00 (45.02)	41.67 (40.02)	16.67 (20.01)	36.11 (35.02)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
35g	58.33 (50.03)	25.00 (30.02)	25.00 (25.01)	36.11 (35.02)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Mean	38.09 (33.59)	19.05 (20.73)	11.91 (13.58)	-	79.76 (73.61)	75.00 (67.89)	76.19 (70.04)	-	88.10 (78.61)	80.95 (74.33)	83.33 (75.04)	-	88.10 (78.61)	84.52 (76.47)	83.33 (75.04)	-

Factors	8 dpi			16 dpi			24 dpi			32 dpi		
	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)
Inoc. levels	10.52	3.69	15.10	6.61	2.32	12.30	5.27	1.85	10.40	5.65	1.98	8.90
Cultivars	6.45	2.26		4.04	1.43		3.23	1.14		3.46	1.22	
Interaction	18.24	6.41		11.43	4.01		9.14	3.21		9.77	3.44	

***dpi** – days post inoculation **Figures in the parenthesis are arc sine transformed values

Table 4.9. Incubation period (IP) and days to permanent wilting (DPW) of isolates of *S. rolfsii* on groundnut

Isolates	IP (days)				DPW (days)			
	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean
SrKa-1	6.58	5.50	6.25	6.11	8.25	8.33	9.00	8.53
SrKa-2	6.08	5.75	6.17	6.00	7.33	7.50	8.83	7.89
SrKa-3	6.33	5.83	6.25	6.14	7.58	7.58	9.50	8.22
SrKa-4	5.67	5.50	6.08	5.75	7.08	7.17	10.42	8.22
SrKa-5	6.58	5.25	6.17	6.00	10.00	7.58	11.50	9.69
SrKa-6	6.92	5.67	6.17	6.25	10.42	8.50	11.25	10.06
SrKa-7	5.42	5.25	6.25	5.64	6.50	6.92	10.00	7.81
SrKa-8	6.58	5.75	6.33	6.22	8.25	10.25	9.75	9.42
SrKa-9	6.58	6.08	6.25	6.30	9.75	11.17	9.25	10.06
SrKa-10	7.42	6.00	6.17	6.53	11.25	7.50	12.75	10.50
SrKa-11	8.25	5.92	6.42	6.86	14.58	17.50	13.33	15.14
SrKa-12	9.00	8.92	8.92	8.95	>45*	>45*	>45*	>45*
SrKa-13	5.42	5.25	6.08	5.58	7.17	9.17	10.83	9.06
SrKa-14	8.42	6.67	6.08	7.06	11.67	8.08	12.08	10.61
SrKa-15	6.00	5.67	6.08	5.92	8.58	7.42	11.00	9.00
SrKa-16	5.92	5.42	6.17	5.84	10.25	7.00	12.33	9.86
SrKa-17	8.08	6.92	6.08	7.03	10.75	7.92	12.50	10.39
SrKa-18	7.83	6.50	6.33	6.89	14.50	9.25	13.83	12.53
SrKa-19	6.42	6.42	6.17	6.34	8.50	8.33	11.17	9.33
SrKa-20	6.42	5.50	6.42	6.11	8.25	8.92	14.58	10.58
SrTs-1	7.92	6.67	6.50	7.03	12.50	10.75	12.58	11.94
SrTs-2	8.08	6.08	6.00	6.72	12.92	9.83	12.83	11.86
SrTs-3	7.67	6.67	6.25	6.86	12.92	9.17	12.58	11.56
SrTs-4	6.92	6.17	6.17	6.42	11.83	11.33	13.17	12.11
SrTs-5	8.67	6.33	6.33	7.11	12.58	11.08	13.92	12.53
SrTs-6	8.42	6.58	6.33	7.11	14.00	12.08	13.50	13.19
SrTs-7	8.33	6.50	6.33	7.05	13.92	11.50	12.08	12.50
SrTs-8	8.50	6.33	6.25	7.03	13.33	12.00	14.67	13.33
SrTs-9	7.92	6.08	6.25	6.75	14.33	9.83	13.67	12.61
SrTs-10	7.83	5.58	6.42	6.61	13.67	11.17	16.83	13.89
SrAp-1	7.92	5.75	6.50	6.72	13.58	13.92	17.17	14.89
SrAp-2	9.00	9.00	8.92	8.97	>45*	>45*	>45*	>45*
SrAp-3	6.75	5.83	6.25	6.28	10.67	11.08	13.17	11.64
SrAp-4	7.00	5.92	6.25	6.39	11.33	10.92	13.50	11.92
SrAp-5	7.33	5.50	6.25	6.36	10.67	10.25	10.58	10.50
SrAp-6	8.67	5.42	6.17	6.75	14.42	10.83	13.25	12.83
SrAp-7	6.92	5.33	6.17	6.14	12.58	9.00	13.67	11.75
SrAp-8	8.67	5.33	6.33	6.78	15.17	9.75	13.75	12.89

Cont.

Isolates	IP (days)				DPW (days)			
	TMV 2	**JL 24**	**J 11**	**Mean**	**TMV 2**	**JL 24**	**J 11**	**Mean**
SrAp-9	8.00	5.83	6.25	6.69	13.75	11.08	14.58	13.14
SrAp-10	8.50	5.83	6.42	6.92	12.92	11.08	13.00	12.33
SrMh-1	7.92	6.08	6.58	6.86	16.58	13.33	12.83	14.25
SrMh-2	8.50	6.00	6.42	6.97	13.83	13.92	12.50	13.42
SrMh-3	8.50	6.25	6.75	7.17	14.25	13.83	13.33	13.80
SrMh-4	7.08	5.17	6.42	6.22	12.17	11.58	12.00	11.92
SrMh-5	7.25	6.00	6.42	6.56	11.00	12.75	12.50	12.08
SrMh-6	6.67	5.50	6.25	6.14	12.08	11.08	12.25	11.80
SrTn-1	7.58	5.83	6.25	6.55	10.42	12.58	12.00	11.67
SrTn-2	6.58	6.08	6.25	6.30	8.58	14.33	16.92	13.28
SrTn-3	9.00	8.92	9.00	8.97	>45*	>45*	>45*	>45*
SrTn-4	8.92	9.00	8.92	8.95	>45*	>45*	>45*	>45*
SrTn-5	7.58	5.50	6.17	6.42	11.83	11.42	11.67	11.64
SrTn-6	7.17	6.42	6.42	6.67	9.42	10.17	13.83	11.14
SrGj-1	7.83	6.08	6.25	6.72	10.08	12.33	11.75	11.39
SrGj-2	7.92	5.50	6.25	6.56	11.50	10.25	12.67	11.47
SrGj-3	4.75	4.67	5.75	5.06	6.00	6.08	8.17	6.75
SrGj-4	6.92	5.33	6.00	6.08	8.08	9.67	11.17	9.64
SrGj-5	7.08	5.58	6.17	6.28	8.08	7.08	9.67	8.28
SrGj-6	6.92	5.17	6.33	6.14	7.92	9.33	10.00	9.08
SrGj-7	5.33	5.33	6.17	5.61	6.42	7.83	9.75	8.00
SrGj-8	8.08	5.42	6.42	6.64	12.17	15.33	15.17	14.22
Mean	7.38	6.04	6.44	-	11.04	10.92	12.33	-

Factors	IP			DPW		
	CD (0.01)	S.Em.±	CV (%)	CD (0.01)	S.Em.±	CV (%)
Isolates	0.52	0.19	10.4	2.67	0.97	13.6
Cultivars	0.12	0.05		0.61	0.22	
Interaction	0.89	0.33		4.66	1.68	

*Permanent wilting of inoculated plant was not observed even at 45 days post pathogen inoculation (dpi) and were excluded from statistical analysis

Table 4.10. Variation in severity of stem rot of groundnut by isolates of *S. rolfsii*

Isolates	Disease severity (%)											
	15 dpi*				30 dpi				45 dpi			
	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean
SrKa-1	100.00 (90.05)**	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-2	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-3	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-4	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-5	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-6	98.33 (82.58)	100.00 (90.05)	100.00 (90.05)	99.44 (87.57)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-7	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.00)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-8	100.00 (90.05)	90.00 (71.57)	100.00 (90.05)	96.67 (83.90)	100.00 (90.05)	96.67 (79.48)	100.00 (90.05)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-9	100.00 (90.05)	90.00 (71.57)	100.00 (90.05)	96.67 (83.90)	100.00 (90.05)	96.67 (79.48)	100.00 (90.05)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-10	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-11	90.00 (71.57)	78.33 (62.26)	85.00 (67.21)	84.44 (67.05)	100.00 (90.05)	91.67 (73.22)	96.67 (79.48)	96.11 (80.94)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-12	50.00 (45.00)	50.00 (45.00)	45.00 (42.13)	48.33 (44.07)	55.00 (47.87)	50.00 (45.00)	48.33 (44.04)	51.11 (45.66)	55.00 (47.87)	50.00 (45.00)	50.00 (45.00)	51.67 (45.98)
SrKa-13	100.00 (90.05)	96.67 (79.48)	100.00 (90.05)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-14	100.00 (90.05)	98.33 (82.58)	100.00 (90.05)	99.44 (87.57)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-15	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-16	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-17	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-18	90.00 (71.57)	96.67 (79.48)	95.00 (77.08)	93.89 (76.08)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-19	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-20	100.00 (90.05)	96.67 (79.48)	95.00 (77.08)	97.22 (82.23)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-1	100.00 (90.05)	100.00 (90.05)	90.00 (71.57)	96.67 (83.90)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-2	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-3	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-4	100.00 (90.05)	91.67 (73.22)	95.00 (77.08)	95.56 (80.14)	100.00 (90.05)	96.67 (79.48)	100.00 (90.05)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-5	85.00 (67.21)	95.00 (77.08)	93.33 (75.04)	91.11 (73.15)	100.00 (90.05)	96.67 (79.48)	100.00 (90.05)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-6	88.33 (70.03)	95.00 (77.08)	100.00 (90.05)	94.44 (79.08)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)

Cont.

Isolates	Disease severity (%)											
	15 dpi				30 dpi				45 dpi			
	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean
SrTs-7	100.00 (90.05)	96.67 (79.48)	100.00 (90.05)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-8	95.00 (77.08)	93.33 (75.04)	93.33 (75.04)	93.89 (75.76)	100.00 (90.05)	96.67 (79.48)	100.00 (90.05)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-9	95.00 (77.08)	100.00 (90.05)	100.00 (90.05)	98.33 (85.74)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-10	100.00 (90.05)	95.00 (77.08)	90.00 (71.57)	95.00 (79.59)	100.00 (90.05)	100.00 (90.05)	96.67 (79.48)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-1	100.00 (90.05)	90.00 (71.57)	85.00 (67.21)	91.67 (76.30)	100.00 (90.05)	96.67 (79.48)	96.67 (79.48)	97.78 (83.03)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-2	45.00 (42.13)	40.00 (39.23)	46.67 (43.09)	43.89 (41.50)	56.67 (48.83)	43.33 (41.17)	50.00 (45.00)	50.00 (45.02)	65.00 (53.73)	50.00 (45.00)	58.33 (49.80)	57.78 (49.53)
SrAp-3	100.00 (90.05)	98.33 (82.58)	100.00 (90.05)	99.44 (87.57)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-4	100.00 (90.05)	96.67 (79.48)	95.00 (77.08)	97.22 (82.23)	100.00 (90.05)	100.00 (90.05)	98.33 (82.58)	99.44 (87.57)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-5	100.00 (90.05)	98.33 (82.58)	100.00 (90.05)	99.44 (87.57)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-6	93.33 (75.04)	98.33 (82.58)	100.00 (90.05)	97.22 (82.58)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-7	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-8	93.33 (75.04)	98.33 (82.58)	95.00 (77.08)	95.56 (78.27)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-9	100.00 (90.05)	98.33 (82.58)	100.00 (90.05)	99.44 (87.57)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-10	100.00 (90.05)	100.00 (90.05)	90.00 (71.57)	96.67 (83.90)	100.00 (90.05)	100.00 (90.00)	95.00 (77.08)	98.33 (85.74)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-1	55.00 (47.87)	100.00 (90.05)	100.00 (90.05)	85.00 (76.00)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-2	100.00 (90.05)	96.67 (79.48)	100.00 (90.05)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-3	90.00 (71.57)	91.67 (73.22)	85.00 (67.21)	88.89 (70.70)	100.00 (90.05)	96.67 (79.48)	93.33 (75.04)	96.67 (81.55)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-4	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-5	100.00 (90.05)	93.33 (75.04)	100.00 (90.05)	97.78 (85.06)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-6	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTn-1	100.00 (90.05)	95.00 (77.08)	90.00 (71.57)	95.00 (79.59)	100.00 (90.05)	100.00 (90.05)	96.67 (79.48)	98.89 (86.54)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTn-2	100.00 (90.05)	93.33 (75.04)	85.00 (67.21)	92.78 (77.46)	100.00 (90.05)	96.67 (79.48)	96.67 (79.48)	97.78 (83.03)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTn-3	40.00 (39.23)	45.00 (42.13)	43.33 (41.17)	42.78 (40.86)	50.00 (45.00)	48.33 (44.04)	48.33 (44.04)	48.89 (44.39)	55.00 (47.87)	55.00 (47.87)	43.33 (41.17)	51.11 (45.66)
SrTn-4	40.00 (39.23)	50.00 (45.00)	50.00 (45.00)	46.67 (43.10)	50.00 (45.00)	51.67 (45.96)	53.33 (46.91)	51.67 (45.98)	53.33 (46.91)	55.00 (47.87)	55.00 (47.87)	54.44 (47.57)
SrTn-5	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTn-6	100.00 (90.05)	100.00 (90.00)	88.33 (70.03)	96.11 (83.38)	100.00 (90.05)	100.00 (90.05)	93.33 (75.04)	97.78 (85.06)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-1	100.00 (90.05)	95.00 (77.08)	96.67 (79.48)	97.22 (82.23)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)

Cont.

Isolates	Disease severity (%)											
	15 dpi				30 dpi				45 dpi			
	TMV 2	**JL 24**	**J 11**	**Mean**	**TMV 2**	**JL 24**	**J 11**	**Mean**	**TMV 2**	**JL 24**	**J 11**	**Mean**
SrGj-2	88.33 (70.03)	100.00 (90.05)	95.00 (77.08)	94.44 (79.08)	85.00 (67.21)	100.00 (90.05)	100.00 (90.05)	95.00 (82.45)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-3	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-4	100.00 (90.05)	98.33 (82.58)	96.67 (79.48)	98.33 (84.06)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-5	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-6	100.00 (90.05)	100.00 (90.05)	95.00 (77.08)	98.33 (85.74)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-7	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-8	86.67 (68.58)	90.00 (71.57)	90.00 (71.57)	88.89 (70.61)	100.00 (90.05)	95.00 (77.08)	95.00 (77.08)	96.67 (81.43)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Mean	93.72 (82.72	93.89 (81.08)	93.47 (81.15)	-	96.61 (86.78	95.83 (84.91)	95.97 (85.11)	-	97.14 (87.32	96.83 (87.14)	96.78 (87.11)	-

Factors	15 dpi			30 dpi			45 dpi		
	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)
Isolates	5.45	1.83	11.80	3.61	1.21	13.80	2.61	0.87	12.60
Cultivars	1.23	0.41		0.66	0.22		0.61	0.19	
Interaction	9.36	3.12		7.26	2.43		4.19	1.43	

*__dpi__ – days post inoculation **Figures in the parenthesis are arc sine transformed values

Table 4.11. Variation in mortality due to stem rot of groundnut by isolates of *S. rolfsii*

Isolates	Mortality (%)											
	15 dpi*				30 dpi				45 dpi			
	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean
SrKa-1	100.00 (90.05)**	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-2	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-3	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-4	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-5	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-6	91.67 (80.04)	100.00 (90.05)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-7	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-8	100.00 (90.05)	66.67 (60.03)	100.00 (90.05)	88.89 (80.04)	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-9	100.00 (90.05)	66.67 (60.03)	100.00 (90.05)	88.89 (80.04)	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-10	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-11	83.33 (75.04)	58.33 (50.03)	75.00 (65.03)	72.22 (63.37)	83.33 (75.04)	66.67 (55.03)	83.33 (70.04)	77.78 (66.70)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-12	16.67 (20.01)	16.67 (20.01)	8.33 (10.01)	13.89 (16.68)	21.67 (25.02)	16.67 (20.01)	8.33 (10.01)	15.56 (18.35)	25.00 (25.01)	16.67 (20.01)	16.67 (20.01)	19.45 (21.68)
SrKa-13	100.00 (90.05)	83.33 (70.04)	100.00 (90.05)	94.44 (83.38)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-14	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-15	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-16	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-17	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-18	83.33 (75.04)	83.33 (70.04)	91.67 (80.04)	86.11 (75.04)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-19	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrKa-20	100.00 (90.05)	83.33 (75.04)	83.33 (75.04)	88.89 80.04)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-1	100.00 (90.05)	100.00 (90.05)	83.33 (70.04)	94.44 (83.38)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-2	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-3	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-4	100.00 (90.05)	75.00 (65.03)	83.33 (70.04)	86.11 (75.04)	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-5	75.00 (60.03)	91.67 (80.04)	83.33 (70.04)	83.33 (70.04)	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-6	75.00 (65.03)	75.00 (70.04)	100.00 (90.05)	83.33 (75.04)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)

Cont.

Isolates	Mortality (%)											
	15 dpi				30 dpi				45 dpi			
	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean	TMV 2	JL 24	J 11	Mean
SrTs-7	100.00 (90.05)	83.33 (75.04)	100.00 (90.05)	94.44 (85.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-8	91.67 (80.04)	83.33 (70.04)	83.33 (70.04)	86.11 (73.37)	100.00 (90.05)	91.67 (80.04	100.00 (90.05)	97.22 (89.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-9	91.67 (80.04)	100.00 (90.05)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTs-10	100.00 (90.05)	83.33 (75.04)	83.33 (70.04)	88.89 (78.38)	100.00 (90.05)	100.00 (90.05)	83.33 (70.04)	94.44 (83.38)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-1	100.00 (90.05)	75.00 (65.03)	75.00 (65.03)	83.33 (73.37)	75.00 (70.04)	91.67 (80.04)	83.33 (75.04)	83.33 (75.04)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-2	8.33 (10.01)	0.00 (0.00)	8.33 (10.01)	5.55 (6.67)	15.00 (17.03)	0.00 (0.00)	16.67 (20.01)	10.56 (12.35)	21.67 (23.02)	16.67 (20.01)	25.00 (25.01)	21.11 (22.68)
SrAp-3	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	91.67 (80.04)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-4	100.00 (90.05)	83.33 (70.04)	91.67 (80.04)	91.67 (80.04)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-5	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-6	83.33 (75.04)	91.67 (80.04)	100.00 (90.05)	91.67 (81.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-7	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-8	66.67 (55.03)	91.67 (80.04)	75.00 (65.03)	77.78 (66.70)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-9	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrAp-10	100.00 (90.05)	100.00 (90.05)	83.33 (75.04)	94.44 (85.05)	100.00 (90.05)	100.00 (90.05)	83.33 (75.04)	94.44 (85.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-1	8.33 (10.01)	100.00 (90.05)	100.00 (90.05)	69.44 (63.37)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-2	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	91.67 (80.04)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-3	66.67 (55.03)	83.33 (70.04)	75.00 (65.03)	75.00 (63.37)	100.00 (90.05)	91.67 (80.04)	83.33 (75.04)	91.67 (81.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-4	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-5	100.00 (90.05)	83.33 (70.04)	100.00 (90.05)	94.44 (83.38)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrMh-6	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTn-1	100.00 (90.05)	83.33 (70.04)	83.33 (70.04)	88.89 (76.71)	100.00 (90.05)	100.00 (90.05)	91.67 (80.04)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTn-2	100.00 (90.05)	83.33 (70.04)	75.00 (65.03)	86.11 (75.04)	75.00 (70.04)	91.67 (80.04)	91.67 (80.04)	86.11 (76.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTn-3	0.00 (0.00)	8.33 (10.01)	0.00 (0.00)	2.78 (3.34)	8.33 (10.01)	8.33 (10.01)	0.00 (0.00)	5.55 (6.67)	25.00 (25.01)	25.00 (30.02)	0.00 (0.00)	16.67 (18.34)
SrTn-4	0.00 (0.00)	16.67 (20.01)	8.33 (10.01)	8.33 (10.01)	100.00 (90.05)	16.67 (20.01)	16.67 (20.01)	14.45 (17.35)	23.33 (27.02)	25.00 (30.02)	25.00 (25.01)	24.44 (27.35)
SrTn-5	91.67 (80.04)	100.00 (90.05)	100.00 (90.05)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrTn-6	100.00 (90.05)	100.00 (90.05)	75.00 (60.03)	91.67 (80.04)	100.00 (90.05)	100.00 (90.05)	83.33 (70.04)	94.44 (83.38)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-1	100.00 (90.05)	75.00 (65.03)	83.33 (70.04)	86.11 (75.04)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)

Cont.

Isolates	Mortality (%)											
	15 dpi				30 dpi				45 dpi			
	TMV 2	**JL 24**	**J 11**	**Mean**	**TMV 2**	**JL 24**	**J 11**	**Mean**	**TMV 2**	**JL 24**	**J 11**	**Mean**
SrGj-2	75.00 (65.03)	100.00 (90.05)	91.67 (80.04)	88.89 (78.37)	75.00 (70.04)	100.00 (90.05)	100.00 (90.05)	91.67 (83.38)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-3	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-4	100.00 (90.05)	91.67 (80.04)	83.33 (70.04)	91.67 (80.04)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-5	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-6	100.00 (90.05)	100.00 (90.05)	91.67 (80.04)	97.22 (86.71)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-7	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
SrGj-8	75.00 (60.03)	83.33 (75.04)	83.33 (70.04)	80.55 (68.37)	100.00 (90.05)	83.33 (75.04)	83.33 (70.04)	88.89 (78.38)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Mean	88.06 (78.79)	85.97 (76.46)	87.64 (77.79)	-	92.72 (83.86)	91.95 (82.59)	91.67 (82.30)	-	94.92 (85.71)	94.72 85.71)	94.44 85.21)	-

Factors	15 dpi			30 dpi			45 dpi		
	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)
Isolates	11.11	3.99	15.40	9.34	3.38	12.10	3.89	1.39	14.9
Cultivars	2.49	0.89		2.09	0.76		0.87	0.32	
Interaction	19.25	6.92		16.26	5.90		6.37	2.42	

*__dpi__ – days post inoculation **Figures in the parenthesis are arc sine transformed values

Table 4.12. Variation in cultural characteristics of *S. rolfsii* isolates

S. No.	Isolate	Growth rate (mm/h)	Colony type	Growth type	Biomass (mg/day)
1	SrKa-1	0.83	Raised	Profuse	10.13
2	SrKa-2	0.96	Raised	Profuse	10.07
3	SrKa-3	0.93	Flat	Profuse	9.11
4	SrKa-4	1.14	Flat	Highly Profuse	11.93
5	SrKa-5	1.12	Flat	Highly Profuse	12.60
6	SrKa-6	1.21	Flat	Profuse	10.58
7	SrKa-7	0.88	Raised	Profuse	12.67
8	SrKa-8	0.98	Raised at ends	Profuse	11.27
9	SrKa-9	1.14	Flat	Highly Profuse	10.56
10	SrKa-10	0.98	Raised at ends	Highly Profuse	10.44
11	SrKa-11	1.01	Flat	Profuse	10.27
12	SrKa-12	1.01	Raised at ends	Highly Profuse	9.33
13	SrKa-13	0.79	Raised at ends	Profuse	9.78
14	SrKa-14	0.93	Raised at ends	Profuse	11.16
15	SrKa-15	1.18	Flat	Highly Profuse	9.53
16	SrKa-16	1.22	Flat	Highly Profuse	12.84
17	SrKa-17	0.88	Raised at ends	Profuse	12.13
18	SrKa-18	0.87	Raised	Profuse	10.84
19	SrKa-19	1.19	Flat	Highly Profuse	12.29
20	SrKa-20	0.95	Raised at ends	Profuse	10.33
21	SrTs-1	0.66	Raised at ends	Profuse	12.87
22	SrTs-2	0.80	Raised at ends	Profuse	8.09
23	SrTs-3	0.79	Raised at ends	Profuse	11.04
24	SrTs-4	0.94	Raised	Profuse	11.00
25	SrTs-5	0.78	Raised at ends	Profuse	11.69
26	SrTs-6	0.84	Raised	Profuse	10.27
27	SrTs-7	0.85	Raised	Profuse	11.47
28	SrTs-8	0.89	Raised at ends	Profuse	10.93
29	SrTs-9	0.98	Flat	Highly Profuse	14.62
30	SrTs-10	0.93	Raised	Profuse	6.82
31	SrAp-1	0.98	Raised	Highly Profuse	10.07
32	SrAp-2	0.78	Raised at ends	Profuse	8.82
33	SrAp-3	0.86	Raised at ends	Profuse	12.36
34	SrAp-4	0.86	Raised at ends	Profuse	11.73
35	SrAp-5	1.20	Raised	Highly Profuse	12.04
36	SrAp-6	1.14	Flat	Highly Profuse	12.80
37	SrAp-7	1.29	Flat	Highly Profuse	12.78
38	SrAp-8	1.17	Flat	Highly Profuse	10.04

Cont.

S. No.	Isolate	Growth rate (mm/h)	Colony type	Growth type	Biomass (mg/day)
39	SrAp-9	1.07	Flat	Highly Profuse	11.00
40	SrAp-10	1.20	Flat	Highly Profuse	12.22
41	SrMh-1	1.09	Raised	Highly Profuse	11.71
42	SrMh-2	0.74	Raised	Highly Profuse	10.84
43	SrMh-3	1.20	Raised at ends	Highly Profuse	12.00
44	SrMh-4	1.13	Raised at ends	Highly Profuse	11.33
45	SrMh-5	1.13	Raised at ends	Highly Profuse	8.62
46	SrMh-6	1.29	Flat	Highly Profuse	10.87
47	SrTn-1	1.11	Raised at ends	Highly Profuse	8.27
48	SrTn-2	1.29	Flat	Highly Profuse	11.02
49	SrTn-3	1.31	Flat	Highly Profuse	12.24
50	SrTn-4	1.20	Raised at ends	Highly Profuse	12.47
51	SrTn-5	1.08	Raised at ends	Highly Profuse	10.36
52	SrTn-6	1.08	Raised at ends	Highly Profuse	11.42
53	SrGj-1	1.10	Raised at ends	Highly Profuse	10.91
54	SrGj-2	1.20	Flat	Highly Profuse	11.62
55	SrGj-3	1.21	Flat	Highly Profuse	9.47
56	SrGj-4	1.17	Raised at ends	Highly Profuse	11.36
57	SrGj-5	1.12	Raised at ends	Highly Profuse	12.07
58	SrGj-6	0.84	Raised	Highly Profuse	10.64
59	SrGj-7	1.03	Raised at ends	Highly Profuse	11.16
60	SrGj-8	1.17	Raised at ends	Highly Profuse	12.87
CD (0.01)		0.119	-		2.115
S.Em.±		0.042	-		0.755
CV (%)		7.20	-		11.90

Table 4.13. Variation in sclerotial characteristics of *S. rolfsii* isolates

S. No.	Isolates	Time required to (days)		Pattern	Color	Weight (g/100 Sclerotia)	Number/ Plate	Size (mm)
		Produce	Mature					
1	SrKa-1	5	9	Peripheral	Light brown	0.51	310	1.35
2	SrKa-2	6	11	Scattered	Dark brown	0.32	730	0.87
3	SrKa-3	13	19	Scattered	Brown	0.16	150	0.53
4	SrKa-4	15	21	Scattered	Brown	0.23	470	0.33
5	SrKa-5	17	23	Scattered	Dark brown	0.21	705	0.73
6	SrKa-6	16	22	Scattered	Brown	0.69	391	0.84
7	SrKa-7	16	23	Scattered	Light brown	0.58	433	1.51
8	SrKa-8	14	21	Peripheral	Dark brown	0.31	837	0.53
9	SrKa-9	12	19	Scattered	Dark brown	0.61	553	1.30
10	SrKa-10	16	23	Peripheral	Brown	0.27	760	0.43
11	SrKa-11	4	7	Scattered	Dark brown	0.19	823	0.44
12	SrKa-12	5	9	Scattered	Dark brown	0.33	590	0.77
13	SrKa-13	4	7	Central	Dark brown	1.09	52	0.92
14	SrKa-14	9	13	Peripheral	Dark brown	0.51	505	1.31
15	SrKa-15	8	13	Peripheral	Brown	0.57	493	1.35
16	SrKa-16	16	19	Scattered	Brown	0.63	230	0.94
17	SrKa-17	16	21	Peripheral	Light brown	0.63	290	1.30
18	SrKa-18	15	21	Peripheral	Brown	0.91	174	1.86
19	SrKa-19	16	23	Scattered	Brown	0.15	570	0.20
20	SrKa-20	9	12	Peripheral	Dark brown	0.64	73	0.99
21	SrTs-1	15	21	Scattered	Light brown	0.13	435	0.55
22	SrTs-2	13	20	Peripheral	Brown	0.83	153	1.98
23	SrTs-3	16	22	Peripheral	Brown	0.93	175	1.57
24	SrTs-4	5	9	Scattered	Brown	0.15	430	1.83
25	SrTs-5	4	11	Scattered	Dark brown	0.17	55	0.82
26	SrTs-6	8	11	Scattered	Light brown	0.52	95	0.87
27	SrTs-7	9	11	Central	Brown	0.31	130	0.86
28	SrTs-8	5	9	Scattered	Brown	0.22	195	0.53
29	SrTs-9	4	7	Central	Dark brown	0.18	870	0.35
30	SrTs-10	13	14	Peripheral	Dark brown	0.89	115	1.75
31	SrAp-1	4	7	Scattered	Brown	0.19	910	0.27
32	SrAp-2	4	7	Scattered	Dark brown	0.43	210	0.73
33	SrAp-3	5	9	Scattered	Dark brown	0.39	195	0.82
34	SrAp-4	4	9	Scattered	Dark brown	0.69	345	0.88
35	SrAp-5	4	11	Scattered	Dark brown	0.53	510	0.61
36	SrAp-6	5	9	Scattered	Dark brown	0.41	530	0.50
37	SrAp-7	5	13	Peripheral	Light brown	0.66	290	1.06

Cont.

S. No.	Isolates	Time required to (days)		Pattern	Color	Weight (g/100 Sclerotia)	Number/ Plate	Size (mm)
		Produce	Mature					
38	SrAp-8	15	21	Scattered	Light brown	0.12	437	0.15
39	SrAp-9	5	9	Scattered	Dark brown	0.33	310	0.43
40	SrAp-10	4	9	Scattered	Dark brown	0.29	590	0.46
41	SrMh-1	4	7	Scattered	Light brown	0.95	410	1.77
42	SrMh-2	6	13	Central	Light brown	0.53	310	1.24
43	SrMh-3	5	11	Central	Brown	1.09	395	1.69
44	SrMh-4	6	12	Central	Light brown	0.96	97	1.86
45	SrMh-5	7	9	Peripheral	Brown	0.63	360	1.66
46	SrMh-6	5	9	Scattered	Brown	0.59	390	1.56
47	SrTn-1	6	9	Scattered	Brown	0.81	290	1.76
48	SrTn-2	5	8	Scattered	Brown	0.70	210	2.00
49	SrTn-3	6	8	Scattered	Brown	1.07	290	2.02
50	SrTn-4	9	15	Peripheral	Light brown	0.94	312	1.59
51	SrTn-5	7	10	Scattered	Brown	0.91	513	1.67
52	SrTn-6	9	13	Scattered	Light brown	0.76	357	1.05
53	SrGj-1	6	11	Peripheral	Light brown	1.03	120	1.51
54	SrGj-2	4	8	Scattered	Brown	1.19	220	1.63
55	SrGj-3	7	15	Scattered	Brown	1.01	392	1.55
56	SrGj-4	10	13	Scattered	Light brown	0.92	273	1.23
57	SrGj-5	14	20	Scattered	Light brown	1.19	190	2.81
58	SrGj-6	5	8	Scattered	Light brown	0.89	75	1.16
59	SrGj-7	6	11	Scattered	Brown	0.83	310	1.45
60	SrGj-8	5	13	Peripheral	Brown	0.95	260	1.24
CD (0.01)		1.39	1.652	-	-	0.142	67.088	0.023
S.Em.±		0.49	0.590	-	-	0.051	23.961	0.008
CV (%)		10.10	10.70	-	-	13.10	15.80	7.20

Table 4.14. Mycelial compatibility of 60 isolates *S. rolfsii*

Is/Is	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	-	C	C	C	I	I	I	I	I	I	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
2	-	-	C	C	I	I	I	I	I	I	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
3	-	-	-	C	I	I	I	I	I	I	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
4	-	-	-	-	I	I	I	I	I	I	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
5	-	-	-	-	-	C	C	C	I	C	I	I	I	I	I	I	I	I	I	I	I	I	C	I	I	I	I	I	I	I
6	-	-	-	-	-	-	C	C	I	C	I	I	I	I	I	I	I	I	I	I	I	I	C	I	I	I	I	I	I	I
7	-	-	-	-	-	-	-	C	I	C	I	I	I	I	I	I	I	I	I	I	I	I	C	I	I	I	I	I	I	I
8	-	-	-	-	-	-	-	-	I	C	I	I	I	I	I	I	I	I	I	I	I	I	C	I	I	I	I	I	I	I
9	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I	I	C	C	I	C	C	I	I	I	I	I
10	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I	I	I	C	I	I	I	I	I	I	I
11	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
12	-	-	-	-	-	-	-	-	-	-	-	-	C	C	I	I	I	C	C	I	I	I	I	I	I	I	I	I	I	I
13	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I	C	C	I	I	I	I	I	I	I	I	I	I	I
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	C	C	I	I	I	I	I	I	I	I	I	I	I
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	I	I	C	I	I	I	I	I	I	I	I	I	I
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	C	I	I	I	I	I	I	I	I	I	I
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	C	I	I	I	I	I	I	I	I	I	I
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I	I	I	I	I	I	I	I	I
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I	I
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	C	C	I	I	I	I	I
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	C	C	I	I	I	I	I
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I	I	I
25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	C	C
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	C
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Cont.

Is/Is	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
1	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
2	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
3	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
4	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
5	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
6	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
7	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
8	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
9	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
10	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
11	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
12	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
13	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
14	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
15	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
16	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
17	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
18	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
19	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
20	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
21	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
22	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
23	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
24	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
25	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
26	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
27	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
28	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
29	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
30	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I

Cont.

Is/Is	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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Cont.

Is/Is	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
31	-	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
32	-	-	C	C	I	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
33	-	-	-	C	I	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
34	-	-	-	-	I	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
35	-	-	-	-	-	I	C	C	C	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
36	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
37	-	-	-	-	-	-	-	C	C	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
38	-	-	-	-	-	-	-	-	C	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
39	-	-	-	-	-	-	-	-	-	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
40	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
41	-	-	-	-	-	-	-	-	-	-	-	C	C	I	I	I	I	I	I	I	I	I	C	I	I	I	I	I	I	I
42	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I	I	I	I	I	I	I	C	I	I	I	I	I	I	I
43	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I	I	I	I	I	I	I	I	I	I	I	I
46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I	I	I	I	I
47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	C	I	I	I	I	I	I	I	I	I	I
48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	I	I	I	I	I	I	I	I	I	I
49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I	I	I	I	I	I	I	I
50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I	I	I
51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I	I	I	I	I	I
52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	I	I	I
53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I	I	I	C
54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I	I	I	C
55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	C	I	I	I
56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I	I	I
57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	I
58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	I
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

I – Incompatible reaction **C – Compatible reaction** **Is = Isolates of *S. rolfsii***

Table 4.15. Summary of mycelial compatibility groups among the 60 isolates of *S. rolfsii*

Isolates	Mycelial compatibility groups (MCGs)														
	MCG 1	**MCG 2**	**MCG 3**	**MCG 4**	**MCG 5**	**MCG 6**	**MCG 7**	**MCG 8**	**MCG 9**	**MCG 10**	**MCG 11**	**MCG 12**	**MCG 13**	**MCG 14**	**MCG 15**
	SrKa-1	SrKa-5	SrKa-15	SrKa-9	SrTs-6	SrKa-12	SrAp-2	SrAp-5	SrMh-1	SrMh-4	SrTn-1	SrTn-5	SrGj-1	SrGj-3	SrGj-6
	SrKa-2	SrKa-6	SrKa-16	SrTs-1	SrTs-7	SrKa-13	SrAp-3	SrAp-7	SrMh-2	SrMh-5	SrTn-2	SrTn-6	SrGj-2	SrGj-4	SrGj-7
	SrKa-3	SrKa-7	SrKa-17	SrTs-2	SrTs-8	SrKa-14	SrAp-4	SrAp-8	SrMh-3	SrMh-6	SrTn-3		SrGj-8	SrGj-5	
	SrKa-4	SrKa-8	SrKa-20	SrTs-4	SrTs-9	SrKa-18	SrAp-6	SrAp-9			SrTn-4				
	SrKa-11	SrKa-10		SrTs-5	SrTs-10	SrKa-19		SrAp-10							
		SrTs-3				SrAp-1									

Table 4.16. Molecular identity of 60 isolates of *S. rolfsii* sequenced and deposited in Genebank, NCBI, USA

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
1	SrKa-1	*S. rolfsii* (AB075318)*	95	>ATGCTTGCTCGAGTAGCAATGGAGTGATGCTGTGATGATATTGCATGTACATGCTCTGTAGTGCGCATGATTTTCTCAGGTGTGCATACTGCTAGAGGAGACTTCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATTTCATA TTGAAGCTTTGTTTTCTGACACGTTTATCTTTATTTAGAATATATCACTTTCTACAACGGATTGACTTGTATCTTGTTTTGTTGAAGAAAGAATCGAAATGCTCTCAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAGGCTTTTTAAGAACCTCGCT GCCTCTGTGGTTTCTAGGAGCCAGCTTGTTTGAGAGTCTTCAGTGTCTTCTCCATACAAAAAATTGTAGATATCTCGGCTGGTGAGTGGTATTTGTTTTTTAAAGAAAAAATCTTGGCGGAAAACTTTATTACTATTATAAAGACATCTAGGAAACCCCA CAGGTGGTGTGATAAGATGAATACCCCTACGGATCTCGGCAGAATCTTGTGTGTATGAGCTATTAAAAAATATATGCCGATAGAGTGTGCACATGAGTATTATTACGACCACCACCTCCTCCGCATATGTGGGTCCACCCGCGGCTCTCCCGCAGTCAC CCGGGGGAAAAAAAAAAAACTATGGAATCATATGGCGAGGAGGTGTGCTGCTAAATGAAATATTGCAGTGTGCCCCCT >GGTAGCAATTTGCTACAGTGTAATGAGTGAGGCAGTGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTAGGGGCGGAGATATATTCTCACACATTGCGTGGGTTTTTCTAC GTGTTCCTATTGGAGTTTTGGAGAGAGCCAGTCATATTATACTCTAACAAATATCACTTTCATCCAAGCCTTGACATATATATTTTTTTGTTGGGGAAAGAATTGAATGACTCTCAGACATGCGCGCCCCAGAATATACCAGAGGGCGCGAGGTGTTTTA AGACACTCGATGACTCTGTGGTTTCTGCGAGTCAGCTTACTTGAGAGTTTTCGCTGTCTTCTCCATACAAGAAAGAGCAGATATCTCGGGTGGTGAGAGGTGTTTGTTTTTTAGAAAAAAAAACTTGGCAGAAAACTTTGTTCTATTAGAAGGCGTTCT AGGAAACACCACAGGAGGTGTGATGAGGTGTATCCTCCTATGGATTTCGGCTGAATATGGTTGGTTCAAGCTGTTTATAATATAATGCTAGAGAGTGGGGACAAGAGTAATTTATTACCACCACCACCTCCTCCGCATATGTGAATTCACCCAGCGACC CTTCCGCAGGTACCCCCGGGGGGAAAAAAGATTTTTTTGTATTTTTATTTGCGAGGGGGTTGTGCTGGTAATAAATA
2	SrKa-2	*S. rolfsii* (DQ484060)	93	>GCCTGTGACTCTATTAGCGATGGAGTGGTGCTGGGATGATATTGCATGTGTATGCTCTGTAGTGCGCATGATATTATAATGTGTACATACTGCGAGAGGACATTCCGGACTATGCGTACACTATATTCTCTTATTGGATGTTACATAGAACGATCTCAT ATTGAATTTTTGTTTTCTGACACGTTTCTCTTTATTAAAAAAAATCTACTTTCAACAACGGTTGACTTGGCTATTGTTTCGATGGTGAAGGATTCGAAGTGCCACAAGTAGTGTTGATTGTAGAAACACCAGAGATCATCGAATCTTTTAAAAAACCCGG TGCCCCTTGGTATTCCGAGGGTCATGTCTCTTTGAGAGTTTTGCTGTTCTCTTCCTTACTACAATTTGTAGTAGTCCCGGCGTGGAGGGTAGAATTGTTGTTTATAAAAAAATCTTGACGGGCTACCTTTTTTAATTATTAGAGGATGTGTTGAAATACCC ACGAGGGGTGATATGATGTGTTCACCCCTATGATTTCTGGGGACTACTGTTGGTTCGTACTTGTTTATAATATCATGCTCCATAGTGTGCAGATGAAATATTTTTTTTTGACCACCACTCCCCTCGCTGATGTGGGACTCACCCGCTGTCCTTCCGCATGT CCCCCGCCGGGAAAAAAAAACTTATGGAATTCATATAGCGAAGGAGTTGTGCTGGTAATAAAAATTGCGGTCCCCCCCCGGGATC >GGAAGTGCTGGCATATAGTAATGAGTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTATAGGCGTACACTATTATCTACCAACGGAAGTTATTTCTAACGGT CCCATAATGAATTTTAAATATAGCCAGTCAGAATTTTTTAATAAGAAAATACTACTTTCTACAACCGTTGAAAATGACAAATTTTTGGATGGAGAAGAATTTGAAGAGTCAAAAACAGTGTTGCTTGTAGAAATACCAGGGGGCATCAGGTCTTTTAAA AGATCCGATGCCCCTCGGTATTCTGCGAGGCATACTACTTTTAGAATTTTTCTGTTTTCTTCCTCACTACAATTTCTAATAGTTCCGGTGTGGAAGGTAGAATATTTTTTTTAAAAAAAAATCTTGTCAAAAAACATTTTTTAATTATAAGAGCATTCTATG AAACACACAACGGGAGTGATATAAGATAATCACCCCTAGGATTTCTCCGGACTACAGTTGGTTCACAGGTGTTTATATATTATGGCTCCAGAGGGTGCACATGCAATATTTATTTTGACCACCACTCCTCCGCATATGTGGGATCACCCGCGATCCTTCC GCAGATCTCCCCCCGGGAAAAAAAAAAACTTATTGAATTCATATATGCGAAGGAGTTGTGCTGGTAATGAATATTGCATGTC
3	SrKa-3	*S. rolfsii* (AB075318)	95	>3GGCATGGCTGTAGTAGCGATGGAGTGATGCTGTGGTGATTTTGCACGTGTATGCTCTGTAGTGCGCATGATTTTATAATTAGTACATACTAGCTAGAATCCCCTTCCGGACTATGCGTACACTATTAACTCTTATTGGATGTTACATAGAACGATCTCA TATTGAATTTTTGTTTTCTGACACGTTTTTCTTTAATAAGAATATACTACTTTCTACAACGGTTGACTTGACTATTGTTTCGATGGAGAACGATTCGAAGTGCCACAAGTAATGTTGCTTGTAGAATCCACTGAATCATCGAATCTTTTAAAAAACCTGGT GCCCCTCGGTATTCTGAGGGGCATGCTTGTTTGAGAGTTATGCAGTTCTCCTCCTCACTAAAAGTTGTAGTAGATCCGGTGTGGAGGGTAGAATATTTGTTTATAAAAAAATCTTGGAAGGCTCCCTTTTATCATTATAAGAAGATGTGTTGAAACACCC ACCAGGGGTGATATAATGTAATCACACCTATGATTTCTGGGGACTCCTGGTTGTTCGTAGGTGTTTATAATATAATGCCTCCATAGTGTGCAGATGCAATAATTTTTTTGACCACTACTCCTCTCGCTGATGTGGGACTACCCGCGGTCCTTCCGCAGTCC CCCCGGGAAAAAAAAAAAATTTTTGGATTCATATATGCGAAGGAGTTGTGCTGGTAATGAATATTGCATGTGCACCCTCTGGAAGCTATTATA >GAGCAATGCTGCATTCGTACTGATGTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTATGGGCGTACACTATAATCTACCAACGGAAGTTATTTCTAACGGTC CCACAATGAATTTTAAAGATAGCCGGTGAGAATATACTCAAACCAGCTACTACAACATCCCCGCCTTGACAATGACAATTTTTTGGATGGTGAAGAATTTGAAGACCCTAAAGCAGGGTGGCCCGTAGAAACACCAGGGGGCGCGAGGTCTTTTAAAA ACTCCGGTGCCCCTCGGTATTCTGCGGTGCATATTACTTTGAGAATTTTGCTGTTCTCTTCCTCACTACAAGTTCTAATAGTTCCGGTGTGGAAGGTAGAATATTTTTTTATAAAAAAAACTTGGCAAAAAACAATGTTTAATTATGAGAGCATTTTTTGA AACACCCAACGGGAGTGATATAATGTAATCACCCCTAGGATTTCTCCGGACTACTGTTGGTTCACAGGTGTTTATATTATAATGGCTCCAGATGGGGCACATGCAATATTTTTTTTGACCACCACTCCTCTCGCATATGTGAGATCACTAATGATCCTTC CGCAGATACCCCCCGAAAAAAAAAAACTTATTGAATTCATATCTGCGAAGGAGTTGTGCTGGTGATGAATATTGCATGTGCACA
4	SrKa-4	*S. rolfsii* (DQ484060)	83	>TGCTTTGACTGGTATTAGCGATGGAGTGGTGCTGTGATGATATTGCATGTGCACGCTCTGTAGCGCGCATGATATTCTCAGGTGTGCATACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATT TCATATTGAAGCTTTGTTTTCTTACAAGTTTATCTTTATTTAAAATATACCACTTTTAACAACGGAGCTCTTGTATCTTGTTTCGTTGAAGAAAGAAGCGACATGCTATCAGTAATGTGAATTGCAGAATCCTGTGAATCATCGAATCTTTGTACGAACCT CGAGGCCTCTGTTGTTTCTAGGAGCATGCTTGTTTGATAGTAATCAATTTCTTCTCCATACAAATAATTGTAGATATCAACGCTGGTGAGTGATAGTTGTTTTTTAAAAAAAATTCTGGGGGGCTCTCTTTTAAACTATTAGTAAGACTTGTAGAAAACC CCACAGGTGGTGTGATAATGTGATTACCCCTATTACAGAGGGGGGAATCTGGTGGGTTCGACGGTTTATAAAAAATAATGCGGATAGAGTGGGCGCATGAGTATTTTTTGCACCACCACCCCTCCGCATAGGTGGGACCACCGATCATCCTTCCGCAG GTGCCCAGGGGAAAAAAAGACTTGTGGGATTCTTTTGCCGAGGGGTTTGTGCTGGAAATAAATATGGTGGGTGCACCCCGGGAGCTA >GGGCTTTGCTACAGACGTAATGATTGAGGCAGTGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTGGGGTATAGGCGTAGACATATTATCACTCATTGCGTGGGATTTTCTACGTG TTCCTATTAAAGTTTTGGAGAGAGCCAGTGATATTTTTATCTAGAATATATCACATTTTCCAAGGATGACATATATATATTTTTTTGTTGGGGAAAGTATTGAAACTCTCTCAGACATGCGCGCCGTAGAAACTACCGGAGGGCGCGAGGTGTTTAAAGAC ACTCGATGACTCTGTGGATTCTGCGAGTCAGCTTATTTGAGAGTTTTCGCTGCCTTCTCCATCCAAGAAATAGTAGATATCTCCGTTGGAGAGAGGTGTTTGTTTTTTATTAAAAAAAAACTTTGGGAGAAAACTTTTATTCTATTATAAGTCGCTGTAG GAAACACCCCAGGGGGTGTGTTTAGGTGACTCCTCCCTACGGATTTCGGCGGCATCTGGTTGGTTCACGGGTGTATTAAAAAAAAATGCCCCCAGAGGGGGCACGTGCAGTTTTTTTTTACCCCCCCCCCCCCTCCGCATATGTGAGTCCACCATCGCCCT TCCGCAGTGACCCGGGGAAAAAAAAAAATTTTTTTGAATTCTTTTTTGCGAGGGGTTTGTGGTGGTAATGAAAAAGGTAGGTGCACTC
5	SrKa-5	*S. rolfsii* (GQ358518)	93	>GGCTATGGACTCTATTAGCGATGGAGTGATGCTGTGGTCGATTTATGCATGTGTATGCTCTGTATGTGCGCATGATTTTCTAATGTGTACATACTGCTAGAATCACCTTCCGGACTATCGTACACTATAAACTCTTATTGGATGTTATTTAGAACGATCT CATATTGAATCTTTGTTTTCTGACATGTTTCTCTTAATTTACCATATACTACTTTCAACAACGGATCTATTGACTATTGTTTCGATGGAGAACAATTCGAAGTGCCATAAGTAATGTTGCTTGTAGAAACAAGTGAATCATCGAATCTTTTAAAAGACCTG GTGCCCCTCGGTATTCTGAGGGTCATGCTTCTTTGAGAGTTTTGCTGTTCTCTTCCTTACTACAATTTGTATTAGTCCCGGTGTGGATGGTAGAATTGTTGTTTATAAAAAAATCTTGACAGAAAACCTTTTATAATTATAAGAGGATCCTTTGTAATACC CAACGGGGGTGATATAATGTAATCACCCCTATGATTTCTGGGGACTACTGTTGGTTTTGTAGGTGTTTATATTATAATGCGCCCATATTGTGCAGATGCAGTGCTTAATTTTGACCACATCTCCTCCGCTGATGTGGGATCACCCGCGGTCCTTCCGCAGT CCCCCCCGGGAAAAAAAAAAAATTTTTTGAATTCATATATGCGAAGGAGTTGTGCTGGTAATAAATAATTGCATGTGCACCCCCCTGGAGCTATATATAA >GGGCATTTGCTAGATACGTAATGAGTGAGGCAGTGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTCTGGCGTACAATATAATCTCCCATCGGAAGTTATTTCTAACGGTCC CATAATGAATTTTTGTTTTAGCCGGTCAGAATCTCTCTAACCAGCTACTACTACATCCCCGCCTTGACAAAGACAATTGTTTGGATGGAGAAGGATTTGAAGACTCTCAAGCAGTGTTGCCCGTAGAAATACCAGGGGGCACCAGGTCTTTTAAAAGAT CCGGTGCCCCTCGGTATTCTGAGGTGCATATTACTTTGAGAGTTTTGCTGTTCTCTTCCTCACTAAAATATCTAAGAGATCCGGTGTGGATGGTAGAATTTTTTTTTATAAGAAAAACTTGGCAAAAAACATTTTATCATTATAAGAGGATTCTTTGAAAC ACACAAGAAGGGTGATATAATGTAATCACACCTAGGATTTCTGGGGACTACAGTTGGTTCACAGGTGTATATAATATCATGCCTCCAGAGTGTGGCACATGCAATATTTATTATGAGCACAACTCCTCCGCATATGTGGGTTCACCCGCGGTCCTTCCG CATGTCTCCCCCCGGGAGAAAAAAAAAACTTTTTGAATTCTATATGCGAAGGAGTTGTGCTGGTAATGAATATTG
6	SrKa-6	*S. rolfsii* (AB075318)	94	>GGGCCTTCTGACTCTATTAGCCAGGAGTTGTGCTGTGATGATTTTGCATGTGCACGCTCTGTAAGCTATATAATATTTTCTCATGTTAAATAACTGTAGTAGGAGATTCCTAACTATGATTACACTATATAACTCTTATTGTATGTTACATAGAACGATC TCATATTGAATCTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACTACTTTCAACAACGGATCTCTTGGCTCTTGTTTCGATGAAGAACACAGCGAAATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACC TTGTGCCCCTTGGTATTCTGAGGGGCATGCTTGTTTGAGAGTCATTATATTCTCATCCTTACTAATTTTTGTATTAGTCAAGGCGTGGATGGTAGAGTTGTTGGTTATAAAAAATTCTGACGGGCTCCTTTTTTTAATATTAATAGGACGTGTTGAAATGC CCACGGAGAGTGATATAATATGTTCACCCCTATGACGGCGGGGGACTACAGCTGGTTTGTACTACTTATAAAATCATGCGCCATATTGAGCAGATAAGTGCATATTTGACCATTTCACCTCTCATCAGGTAGGACTACCCGCGGTCCTTCCGCAGGCAC CGGCGGGAAAAAAGAACTTTTTGAATTCTTTTGCGAAGGAGTTGTGCTGGTAATGAATATGCAGGTGCCCCTCTGGAGCAT >AGGCTAATGCTGCATTAGTAATGATGTGAGGTCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTATAGGCGTACACATATTATCACACCAACGGAAGGCATTTCTAA AGGTCCCACAATGAATTTTAAAGAGAGCCGGTCAGAATATACTCTAACCAGCTACTACCACATCCCCGCCTTGACAAATACAATTTTTTGGATGGTGAAGAATTTGATGACTCTCAAACAGGGTTGCCCCTAGAAACACCAAAGGGATAAGGGGCGTT AAAAGATTCGATGATTCTCGGTATTCTGCGGTTCATATTTCTTATAGAATTTTGCTGCTTTCTTCATCGCTACAAGATCTAAGAGACCCGTTGTGGATGGTAGAATATTTTTTTATAAAAAAATCTTGGCAAAAAACAAATTATCATTATGAGAGCATTCT TTGAAACACCCAATAGGAGTGATATGAGGTAATCACACCTAGGATTTCTGGGGACTACAGTTGGTTCACAGGTGTTTATATAATAATGCCCCCAGAGTGTGCACATGCAATATTTATTACCACCACAACTCCTCTCGCATATGTGAATTCAATAATGAT CCTTCCGCAGATACCCCCCGAAAAAAAAAAATTTTTTTGAATTCATATATGCGAAGGAGGTGTGCTGGTAATGAATATTGCAGGTGCCCACT

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
7	SrKa-7	*S. rolfsii* (GQ358518)	89	>GCCATGACTCTATTGCGATGAGTGGTGCTGGGGTGAAATGCATGTGTACGCTCTGTAGTGCGCATGATTTTCTAAGTTGTGCTACTGTAGTCAGGAGAAATCCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATAT TGAAACTTTGTTTTCTGACAAGTGTCTCTTTATTAAGAAATATACACCTCTTAACAACGGAGCTATTGGCTCTTTTTCGAGGTAGAAACAGCGCGATGCGAAAGTGTTGTGCATTGCGGAATACCGAGGGCACCGGATCTTTGAAAGCACCTCGCGCCC CCTGGTATCCGAGGGGCATGCCCGTATGAGAGTCTCAAATCTCTCCATCACACATCTTCGAGTATCTCAGGGGTGGAAGTGGGAGTTTTTGTTTTAAGAAAATCTGGCAGAATCTCTTTATACTATAGGGCGCTCGTAGAAACCCCCCCGGGGGTGTGT AAGAGTCTCGCTAGACTGAGGGGATCCTGGTGGTTCGCGTATATAAAAAAGCCCCCTAGTGCCATAGTGCTCTTCCGCGTACCCCTCGTGGGGGGATCCCGGGACTCGGGTCACGGGGAAAAAG >GGCAGTGCTGCATTGTACTGAGTGATGCAGTGGTCATATATGCACGTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAAGCTAGAATCCCCTTCCGGACTATGATCACACTATATAACTCTTATCGGATGTTATTTAGAACGATCTC ATATTGAATTTTTGGTTTAGCCAGCGAGTCTCTTTATTAAGAAACATTCACCTTTCAACAACGTTGAAAAATGCAAAATTTTTGGATGAGGAAAATTTAGAGATGCTAAAAGAGGTGTGCCCTGCGGAATACAGGGAGGCATCGGGGGCTTTGAAAGA CCCGATGGCCCCTTGGTATCCGCAGGTGCAATTACTTTTGAGAGTTCGCAGATTTCCTCCACTACACAAATTCGAATATATCCGGGGTTGGATGTGAGATTTTTTTTTTAGAGAAAATTCTTGACTGGCTCTCTTTTTTCCTTTTAGGAGCACTTCTTTGA AACGCCCACGAGGGGTGTTATAAGGTGTCCACGCCTAGACTTTCGGGGGACTCCTGTTGGTTTACAGTTTTTTATAAAAATAAGCCCCCCCAAAGGGGGGCCCATGGATTTTTTTTTACCAGAACATGACTTTCGCTAAGGTAGAATCTCACACAGTGG TCCTTCCGCGTATACCCGGGGGGAGAGAAAAAATCTTGAACCTAACCAGGGGGGGCGGAAAAATG
8	SrKa-8	*S. rolfsii* (GQ358518)	95	>GGCTTGCTCTAGTGCGATGAGTGATGCTGTGGTGATATTGCACGTGTATGCTCTGTAGTGCGCATGATTTTATAATTAGTAATACTGCTAGAATCCCCTTCCGGACTATGCGTACACTATAAACTCTTATTGGATGTTACATAGAACGATCTCATATTGA ATTTTTGTTTTCTGACATGTTTCTCTTAATTTACCATATACTACTTTCTACAACGGATCTATTGGCTATTGTTTCGATGGAGAAGAATTCGAAGTGCCACAAGTAATGTGAATTGTAGAAACCAGTGAATCATCGAATCTTTTAAAAAACCTGGTGCCCCT CGGTATTCCGAGGGGCATGCTTGTTTGAGAGTTACTCAGTTCTCTACCTCACTACAATTTGTAGTAGTCCCGGCGTGGATGGTAGAATTGCTGTTTATAGAAAAAATCTTGCAGGAAAACATTTTTTCATTATAAGAGCATCCTTTGAAACACCCACCGG TGGTGATATAATGTATTCACCCCTATGATTTCTGGGGACTCCTGCTTGTTCACACGTGTTTATAATATCATGCGCCCATATTGTGGCAAATGCAATATTTTTTTTGACCACTACTCCTCCGCTGATGTGGGACTCCCCGCGGTCCTTCCGCAGTCCCCCCC GGGGAAAAAAAAAAATTTTTGAATTCTTATCTGCGAAGGAGTTGTGCTGGGAATGAATATTGCATGTGCCCC >GGAGGCATCTTGAGCATTCGTAATGAGTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGAAAATAAGTAGTACATACAAGCTAGAATCACCTTCCGGTATATGCGTACACTATTATCTACCATCGGAAGTTATTTCTACAGGT CCCATAATGAATTTTTGAGAGAGCCAGTGAGTATCTTTAATAACAAACTACTCCTTCTTCCAACCGTTGACAATGACAATTGTTTGGATGGAGAAGAATTTGAAGTCTCACAAGGAGTGTTGCTTGTAGAAACACCAGAGGGCGCCAGGTCTTTTAAAA GATCCGGTGCCCCTCGGTATTCCGCGGTGCATATCTCTTTGAGAGTTTTGCTGTTCTTCTCCTCACTACAATTTCTAGTAGTCCCGGTGTGGATGTTAGAATATTTTTTTATAAAAAATACTTGGCAGAAAACATTTTATCAATATAAGAGCATCCTATGA AACACCCACCAGGGGTGATATAGTGTAATCACCGCTAGGATTTCTGCGGACTACTGTTGGTTCACAGGGACTTATAAAAACATCGCCCCCATAGTGTGCACATGAAATATTTATTACGACCACCTACTCCTCTCGCATATGTGGGACTCACCCGCGGTC CTTCCGCAGGTCCCCCCCGGGGAAAAAAAAAATTTTGATTCTATTTAAAGAGTGTGCGGGAAAAATAGGGT
9	SrKa-9	*S. rolfsii* (DQ484060)	96	>GGGATGTTGATGATAGTGCACTGGAGTGGTGCTGGGGTGATATTGCATGTGTATGCTCTGTAGTGCGCAAGATTTTCTCCTTTGTACTACTGTAGTCAGGACCTTCCGGACTATGATTACACTATATAACTCTTATTGTATGTTACATAGAACGATCTCA TATTGAATCTTTGTTTTCTGACACGTTTCTCTTAATTTAAAATATACTACTTTCAACAACGGATCTCTTGACTCTTGTTTCGATGAAGAACAATGCGAAGTGCCACAAGTAATGTTGCTTGCAGAAACACCTGAATCATCGAATCTTTGAACGCCCCTGGT GCCCCTTGGTATTCTGCGGGGCATGCTTCTTTGAGAGTTATGCTATTCTCATCCTTACTAAAATTTGTATTTGTCCCGGCGTGGATGGTAGAATTGCTGGTTATAAAAAAATCTGACGGGCTCTTTTTTATAATTATAAGAGCATTCTTTGAAATACCCAC GAGGGGTGATATAATGTGATCACACCTATGACTTCTGGGGACTCTAGCTTGTTTGTACTACTTATAATATCATGCGCCCATATGGAGCAGATGCAATATTTATTTTGACCACTTCTCCTCCGCAGATGTGGGACTACCCGCGGTCCTTCCGCATATTCTTC CGCGGGGAGAGAAAGACCTGTGTCTATTCGAGGTGGTGAAAAAATGA >GGGAACTGGCTGGTATACGTACTGAGTGAGGCAGTGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGACTAGGCGTACACTATAATCTCCCATCGGAAGGCATTTCTAACGG TCCCATAATGAATTTTTGTTTTAGCCAGTCAGAATCTTTTCTAAGAATATACTACTTCCTCCAACCGTTGAAAATGACTATTGTTTGGATGGTGAAGAATTTGAAGTGTCACAAGCAGTGTTGCTTGTAGAAACACCAGAGGGCATCAGGTCTTTTAAAA GACCTGGTGCCCCTCGGTATTCTGCGGTGCATGTTACTTTGAGAATTTTGCTGTTTTCTTCCTCACTACAAGTTGTAGTAGACCCGGTGTGGATGGTAGAATATTTTTTAATAAAAAAATCTTGGAAAAAAACATTTTTTCATTATAAGAGCATTCTATGT AACACCCACCAGGGGTGATATAGTGTAATCACCGCTAGGATTTCTGGGGACTACAGTTGGGTCACAGGTGTTTATAATATCATACCTCCATAGTGTGCACATGCAATATTTATTACCACCACAACTCCTCTCGCATATGTGAGATCACCCGTGGTACTTC CGCAGGTCTCCCGCCGGGAAAAAAAAAATTTTTTTGTTTCTTTCTCCGAGGGAGTTGTGGGGGTGAAAATTGGTCGCCCAC
10	SrKa-10	*S. rolfsii* (AB075318)	93	>GGGCATGTGGCTGAGAGTAGCAATGGAGTGGTGCTGGGAATCGATATATGCACGTGCACGCTCTGTATATGCGCATGATTTTCTCAGGTGTGCATACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAG AACGATTTCATATTGAAGCTTTGTTTTCTGATACGTATATCTTTATTAAGAATATACCACTTTTTCCACGGAGTGACTTGTATCTTGTTTTGTTGAAGAAAGAATCGAAATGCTCTCAGTAATGTGAATTGTAGAATCCAGTGGATCATCGAAGCTTCTTA CGACACTCGCTGCCTCTGTTGTTCCTAGGAGCCAGCTTATTTGAGAGTAATCAGTGTCTTCTCCATACAAAAAATTGTAGATATCACGGCTGGTAAGTGATATTTGTTTTTTAATAAAAAAAATCTTGGGGAGAAAACTATTTTACTATTATAAAGCTTT CTAGAAAACACCACAGGTGGTGTAAAAATATGTATACTCCTATGCATCTGGGGGGCATCTGGTGTGTTGGAGCTTTTTTATAATATAACGCTCCAGAGTGTGCACGCGAGTATTTATTACCACCACCACCCCTCCATATATGAGGATACACTCATCCGT CCTCCCGCAGATCCCCCGGGGAGAAAAAAAAAATTTTTTAATTTTATTTCGAGGGGTTGTGGGGAAAAAAATG >GGGCAAATGATGGAGTAGTCTGATTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAGCTAGAATCCCCCTTCCGGTATAGGCGTAGACATATTATCACTCACCGCGTGGGATTTTCTACGTGT TCCTATTAAAGTTTTGGAGAGAGCCAGTGATATTATACTCTAGAATATATCACTTCCATCCAAGGGAGTGACATATACATTTTTTTGTTGGGGAAAGTATTGAAACTCTCTCAGACATGCGCGCCCGCAGAATCCACCAGAGGGCGCGAGGCGTTTAAA GACACTCGATGACTCTGTGGATTCTACGAGACAGATTATTTAAGAGTTTTCGCTGCCTTCTCCATCCAAAAAAGAGTAGATATATCCGTTGGAGAGAGATGTTTGTTTTTAAAAGAAAAAAAGTGTGGGAGAAAACTATTTTTCTATTATAAGGCGTCG TTGGAAACACACCAGGGGGTGTAAAAAGGAGATCCCCCGTACGGATTTCGCGGAAAGACGGTTGGGTCAGAGGTGTATATAAATATAATCCCCCCAGAGGGGGCGCGTGCAGTTTTTTTTTATGACGAATATTTCTTCGCATATGAGGAGATCACGAT CGACCCTCCCCGCAGTCCCCCCGGGGGAAAAAAAAAAAATTTTTGGAATTTCTTATTTCCGAAGGAGTGGGCGGGGAAAAAAAAATTGGGCGGCC
11	SrKa-11	*S. rolfsii* (DQ484060)	94	>GTGCCATCTGATCATATACGCGATGGAGTGGTGCTGGGATGATATTGCATGTGTATGCTCTGTAGCTATATAATATTTTCTCCTGTGTACATACTGCGTCAGGACATTCCGGACTATGATTACACTATATAACTCTTATTGTATGTTACATAGAACGATC TCATATTGAATCTTTGTTTTCTGACAAGTTTCTCTTTATTAAAAATATACTACTTTCTACAACGGATGACTTGACTCTTGTTTCGATGGAGAACGATTCGAAGTGCCATAAGTAATGTGAATTGTAGAATCAAGTGAATCATCGAATCTTTTAAAAGACCT TGTGCCCCTTGGTATTCCGAGGGTCATGCTTCTTTGAGAATTTTTCAATTCTCATCCTTACAACAATTTGTATTTGTCCCGGCGTGGATGGTAGAATTGTTGGTTAAAAAAAATTCTTGACTGGCTATATATTATCATATTAAGAGGATTCGTTGAAATAC CCACCAGTGGTGATATAATGTAATCACCCCTATGATTTCTGGGGACTACAGCTTGTTTGTACTACTTTATAATATCATGCTCCATATTGTGCACATAAATACTTATTTTGACCACTACTCCTCCGCTGATGTGGGACTACCCGCGGTCCTTCCGCATCTCT TCCGCGCAGAGAGAGAAAAACGTATGAGTCTATTCGAGGGTGTGGGAAAAATACGGGCC >GGGCAATTTTGGATGATATACGTACTGAGTGATGCTGTGGTCGATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTCTGGCGTACACTATTATCTCCCATCGGAAGTTATTTCTAAC TGTCCCATAATGAATTTTTGTTTTAGCCAGTGAGAATTTTCTCTAACCATATACTACTACTTCCCAACGGTTGACAATGACTATTGTTTGGATGGTGAAGAATTTGAAGAGCCTCAAGCAGTGTTGCCCGTAGAAACACCAGGGGGCACCAGGTCTTTTA AAAGACCCGGTGCCCCTCGGTATTCTGCGGTGCATGTTACTTATAGAATTTTGCTGTTTTCTTCCTCACTACAATTTGTAGTAGTCCCGGTGTGGATGGTAGAATATTTTTTAATAAAAAAATCTTGTCAAAAAACATATTTTCATTATAAGAGCATTCTT TGTAACACCCACCAGGAGTGATATAATGTAATCACACCTAGGATTTCTGGGGACTACTAGTTTGTTCATAGGTGTATATAATATCATGCCCCCATATTGTGCACATGCAATATTTATTTTGAGCACTACTCCTCCGCATATGTGAAATCACCAATGATCC TTCCGCATATCCCCCCCCGGAAAAAAAAAATTTTTTTGTTTTCATATCTGCGGAGGTGGTTGTGCGGGAAAAAAAAATGTGGGGGCCCCCCCCG
12	SrKa-12	*S. rolfsii* (DQ484060)	91	>TGGGCATGTGACTGATATAAGCACTGAGTGGTGCTGGGATCGATATTGCACGTGTATGCTCTGTAGCTGCGCATGATATTATAATGTGTGCATACTAGCGTCAGGAGAAATCCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAAC GATTTCATATTGAAGCTTTGTTTTTTTTTACGGATATCTCTAATTTGAATATCTCACTTCTTGAACTGAGCTCCTGTAGATTTTTGCGTTGAAGAATGTAGCGACACTCTATCAGACATGCGCATTGTAGAATCCAATGAGCGCTCAACGAATTTTTCGAG GCTCGTTGACTCTGTTGGTTCTCCGAGGCAGATGATTTGATAGGACTCAGTGTATTATCAATACAAAAAATTGTAGATATCTTCGTGGGTGTGGTGTATTTGTTGCTTATAAAAAATTCTTGGGGGCTCCTCTTTTTACTTATATGAGGGTTTCTTGGAAA CCCCACCAGGGGTGATATAAAGTGATCACCGCCACTACTCCAGGGGACAGCAGGTTGTTTCCACTAATTATAAAATAAAAGCCCGCGTATGTGGGCCAGAGATTTTTTTTTAGCCCCCCCCCCCCCCCCCAAGTATGACACCACCGATCCTCCCTCCCC CCAGATATCCCCGGGAAGAAGAAAAATTTTGATCAATCGAGGTGGGGGAAAA >GGGCAAATTGATCGTAGTGTACTGAGTGAGGCAGTGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAAGCTAGAATCCCCTTTGGGGTAGGCGTTACTCTTTATCTCACTCACCGCATGTTATTTTATACG TTCTCCTATTAGAGTTTTGGAGAGCGCCACAGATATTCTTCTCTAGAAAATCTCACATCTTTCCCTGAGTGATATATAGATTTTTTTGTTGGAGAATATATTGACTCTCTCACAGGCATGCGCCTTGTAGAATCAAAGGGAGCATCAGGGTGTTTTTAGAC GCTCGTTGCCTCTGTTGTTCCTGCGAGACAGCTGATTTGATGTTGCTGCGTTCTTTCTCCATCCAAGAAATTGTAGATATCTTCGTGGGAGTGGTGTATTTGTTGCTTAAAAAAAATTTTTGGGGGAAACTCATTTTTATAATATGAGCGTTTGTTGGAAA CACCACCAGGGGTGATATAAAGTCATCACCACCAGGACTTCTGCGGACAGCTGGTTGGTTTGCAGGGTTTTTTAATATAAAATGCCCCGTGTGTGGGCACGTGAGTATTTTTTTAGCGGAGATATTTCTTCAATAAGTAGGGTACCACCATCACCCTCC CGCGTCCCCCCCGGGAAAAAAAAAATTTTTTTTTTTATTCTACGGAGGGTTGGGGGGGAAAAAAAAATTGGGGGCCCCCCCCAGA
13	SrKa-13	*S. rolfsii* (GQ358518)	93	>GATTGGATCTACTGGAGTAGCAAGGAGTGGTGCTGTGATGATATTGCACGTATATGCTCTGTAGCTGCGCATGATAATACAGGGAACTACTGGCGAGAGTCCCCATCCTGACTATGATTACCCTATATAACTCTTATTGGATGTTATTAGAATGATCTC ATATTGAATTTTTAGTTTGTTTTACGATCTTCTTATAAAAAAAATACTACCTTCTAGCCCTGAGGAATATGATTATTTCTTGGGTGGAGAAATTATCGGAGTCTCTAAAGGCATGTGAGTTGAAATAACAAACGGGGCGGGGGGGGGTATCTAAGACC CCTGCTCGCTCTGTTCTTCCGACCGACATGCTTGTTGGAGTTTGATTCATTTCTCTTCCATACAAAAAAATGTATGTGCTTTGGTGGGGATGGGAGAATTTTTAGTTATTAAAAACTGGCGGAAAAAAAAACTATTTTTAATTATAAGGCGGTTGTGAAAA CACCCCGCGGGGGGGTATAAAGTCTTTATCCCCACGGATTTCCGGGGGAAGACTGGTTGGTTGAAGTATTTATAAAAAAAGGCGCCCCAAAGGGGGGACAAAAAAATAATATTTTGCCCCAAACCCCCCCCTACAAGGGGGGAATAACGAAAGGTTTT TGGGAGGATCAACCGGGGGAAGAAAATTTTTTGTAAAAAAAAAAAAAAAAAAAAAATGTG >GGGAAACTGATGGCAGTAGTAATGATTGAGGCAATGGTCATGTATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAGCTAGAATCCCCTTCGGGATAAGGGTTAAACTATTAACCCACCCACCGGAAGGCTTTCTTCATG GTCCCATAAAAAATTTTAAGAAAGCCCGGCCGAAATTCTTAACAAGCAGCTCTTACCTCCTAGCCTTGACAAAAACTACAATTTTTTGGGTGGAGAAATTAATGACTCTCATACAGGCGTGCCCCTTGGAAAAACAAAGGGGGCAGGGGGGGGTTTAA AAAAACCCGATGCGCCGGGGTCTGCAGTTCATCATACTTGTTTGAGTTTGATGCGTTCTTCTTCCATGCTAAAACATCTAGATCTTTTGTTGTGGATGGTAGATTTTTTTTTTAAAGAAAAATTTCTGAAAAAAAACTATCTTTCATTATTGGAGGGTTGT ATGAAACAACACACGGGGTGATGTGGGAATCATTGCTCGTATGGATTCCGCCGACAGACTGGTTGGTTCGAGGGTGTTTTATATAACATGCCCCCTGAGGGGGCGCATGCATTATTTATTTGCCCCCACCCCCCCCTCCGTGGAGGAGGAATCACCATG GTCCTCCCGCGGTTCACCGGAAGGAAAGAAAAA

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
14	SrKa-14	*S. rolfsii* (DQ484060)	94	>TGCATGTGATCTATAAGCGATGGAGTGGTGCTGGGATGATATTGCATGTGCATGCTCTGTAGCTCGCATGATATTCTCATGTGTGCATACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATTT CATATTGAAACTTTGTTTTCTGACACGTTTATCTTAATCTAGAATATATCACTTTAACAACGGATGATTGTATCTTGCTTTGTTGAAGAAAGAATCGAAATGCTATCAGTAATGTGAATTGCAGAAACCAGTGAATCATCGAAGCTTTTAACGAGACTCG ATGCCTCTGGTGTTCCTAGGAGCCAGCCTATTTGAGAGTCTTCAATGTCTTCTCCATACAAACAAGTGTAGATATCACCGTGTGTGAGTGATATTTGTTTTTTAAAGAAAAAATCTTTGGGAGCTCTCTTTTTTTCTATTAGAAAGCCTTGTAGAAAACAC CACAGGTGGTGTGATAATGTGATTCCCCCTATGGATTTCGGGGGGAATACGGTTGGGTACAGAACGATTATATAATACATGCCCCCAGAGTGGGCGCATGCAGTTATTTATTTACGACCACTACTTCTCCGCATATGAGAATATCACTCATCGATACTT CCGCAGTCACCCAAAAAAAAAAAAAAAAAAAATTATTGATTCATATTTCGAGGAGTTGGTGGG >GGGCAAAATGATGGTAGTCGTACTGATTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAGCTAGAATCCCCCTTCCGGTATAGGCGTAGACATATTATCACTCACTGCGTGTGATTTTCTACG TGTTCCAATTAAAGTTTTAAAGAGAGCCAGTCATATTATACTCTAACAAGTATCTCTTACATCCAAGCCTTGACATGTACATTTGTTTGTTGGGGAAAGAATTGAATGACTCTCAGACATGCGCGCCCCTGAAAATACCGGAGGGCGCAAGGTGCTTAC AAAGACTCGATGACTCTGTGGATTCTGCGAGTCAGCTTACTTATCGCATTTCGCTGTCTTCTCCATCCATGCAAGAGCAGATATATCGGTTGGTGAGAGGTATTTGTTTTTAAATAAAAAAAAACTTGTCAGAAAACAATGTTTCTATAAGAAATCGTTC TATGAAACACCACAAGAGGTGTAATTAGGTGAATCATAGTATGGATTTCTCCTGAAGACGGTTGGGTTCACAGGTGTTTTTATATATGGCTCCAGAGTGGGGCGCGTGCAATATTTTTTCCACCACCACTCCTCCGCATATGTGGATTCACTAACGATC CTCCCGCAGTCCCCCCCGAAAAAAAAAAAAAACTTTTTTGAATTTCTATTTCCGAAGGATTGGTGCTGGTAAAAAAAATGGCGCGC
15	SrKa-15	*S. rolfsii* (GQ358518)	93	>TGGCATGTGGCTGTAGTAGCGATGGAGTGGTGCTGGGATGAAATTGCATGTGCACACTCTGGTGCTATATAATATTTTCTCCTGTGTACTACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGAT TTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACCACTTTCTCCACGGATTGACTTGGCTCTTGTTTTGATGGAGAAAGAATTGAAATGCTCTAAGTAATGTGAATTGCAGAAACCACCTGAATCATCGAATCTTTTAAAAAA ACTCGAGGCCTTTGGGATTCCGAGGGGCCAGCTTATTTGAGAATTTTTAAATTCTTCTCCATACAAAATTTTGTAATTATACAGGCTTGGAAGAGATAATTGTTTTTTAATAAAAAAAACTGGCTGGAAAACTTTTATACTATTAGAAAGACTTGTAGGA AAGCCCACAGGTGGTGTGAAAATGTGAATACCCCTATAATTTCAAGGGGGACTACTGGTTGTGTTCCAAGGTGTTTATAAAATAAGCCCCCAGAGTGTGCCCTAGAAATATTAATTACGAGCATTAGTCTTCGCATAGGTGAGATCACCAACGACCCTTC CCGCAGTCACCCCGGGGGAAGAAAAAAAACTTTGATTCTTTTAAGGGGTGTGCGG >GGGGCATTTTGCTGGGAGTCGTACTGATTTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATATGCGTACCCTATTTATCACTCATTGCGTGGGATTTTCA ACGTTTTCATATTGAAGTTTTAAAGAGAGCCAGGTTTAATATTCTCTAACAAGTACCTCTTTTTCCAACCCTTGACATGGACAATGTTTTGTAGGGGAAAGAATTTAAAGACTCTCAAACAGGCTTGCCCCTGGAAACACCAAAGGGCGCAGGGTGTTT TAAAAGATTCGAGGATTCTGGGGATTCGGCGAGTCAGATTATTTAAGGAATTTCGCTGCTTTCTTCATACAAAAAAATAGCAAATAGATCGGGTTGGTGAGAGTTGTATGTTTTTTATTAAAAAAAACTTGGCAGAAAACAATGTTTCTATTATAAAG TCTACGTGTTCCAATTAAAGTTTTAAAGAGAGCCAGTCATAATATACTCTAACCTATATCTCTTACATCCCCGGAGTGACATATACATTGTTTTGTTGGGGAAAGAATTGAGTGGCTCTCAGACAGGCGGCGCCCCTGAATATACCGGAGGGCGCAGGGT GCTTACAAAGACTCGATGACTCTGTGGATTCTGCGAGTCAGCTTATTTATCGCATTTCGCTGTCTTCTCCATCCAAGAAAGAGCAGATATATCCGTTGGTGAGAGGTGTTTGTTTTTTAATAAAAAAATATTTGGCAGAAAACAATGTTTCTATTATAAG GCGTTCTATGAAACACCACAGGAGGTGTATTGAGGTGAATCCCCCGATGGATTTCGGCGGACTACTGGTTGGTTCACGGGTGTATATAATATAATGGCTCCAGAGTGGGCACGTGCAATATTTTTTACCACCACCACTCCTCCGCATATGTGAAGATCA CTAACGATCCTCCCGCAGATCACCCCACGGAAAAAAAAAAAACTTATTGAATTCATATTTCCGAAGGAGTTGGTGCTGGTAAAAAAATAGGCGGGTG
16	SrKa-16	*S. rolfsii* (DQ484060)	91	>GGGGAAATGGACTGGCATGTCGTACTGATTGAGGCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATAGGCGTAGACATATTATCACACACAGCGTGGGATTT TCTACGTGTTCCAATTAAAGTTTTAAAGAGAGCCAGTCATAATATACTCTAACCTATATCTCTTACATCCCCGGAGTGACATATACATTGTTTTGTTGGGGAAAGAATTGAGTGGCTCTCAGACAGGCGGCGCCCCTGAATATACCGGAGGGCGCAGGGT GCTTACAAAGACTCGATGACTCTGTGGATTCTGCGAGTCAGCTTATTTATCGCATTTCGCTGTCTTCTCCATCCAAGAAAGAGCAGATATATCCGTTGGTGAGAGGTGTTTGTTTTTTAATAAAAAAATATTTGGCAGAAAACAATGTTTCTATTATAAG GCGTTCTATGAAACACCACAGGAGGTGTATTGAGGTGAATCCCCCGATGGATTTCGGCGGACTACTGGTTGGTTCACGGGTGTATATAATATAATGGCTCCAGAGTGGGCACGTGCAATATTTTTTACCACCACCACTCCTCCGCATATGTGAAGATCA CTAACGATCCTCCCGCAGATCACCCCACGGAAAAAAAAAAAACTTATTGAATTCATATTTCCGAAGGAGTTGGTGCTGGTAAAAAAATAGGCGGGTG
17	SrKa-17	*S. rolfsii* (DQ484060)	94	>GGCATCTGGCTGATAGTAGCAATGGAGTGGTGCTGGGAATCGATATTGCATGTGCATGCTCTGTAGCTATGCAAGATTTTATCATGTGTGCATACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAAC GATTTCATATTGAAGCTTTGTTTTCTGACAAGTTTATCTTAATTTAAAAATATACATTCTACAACGGAGTGACTTGTATCTTGCTTTGATGAAGAAAGAATTGAAATGCTCTAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAAGCTTTTAAC GAACCTCGATGCCTCTGTTGTTCCTAGGAGTCAGCTTGTTTGAGAGTTTTCAATGTCTTCTCCATACAAAATATTGTAGATATCTCCGCTGGTGAGAGGTATTTGTTTTTTAATAAAAAAAAACTGTGGGAGAAAACTTTTTTTCTATTATAAGGACTTCT ATAAAACACCCACAGGGGGTGTAATGAGGTGTATCCCCCCTATAGATCTCGGCAGGATACTGTTGGGTCACGGGTATTATATAAATAAAATCCCCCCAGAGTGGGGACAAAAAATATTTTTTTACCCCCACCACCCCCCCCGTATATGAGAATACACTC AGCGGCCCTCCCGCGGTCCCCCCGGGGGGGGGGGGGTACTTTTTTGAATTCTTATTTCCGAGGGAGTTGTGCGGGTAATAAAAATGGTGC >GGGAATTTGCTGTGAGTAGTACTGATTTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATAGGCGTAGATATATTATCACACACCGCGTGGGATTTTCTAC GTGTTCCAATTAAAGTTTTAAAGAGAGCCAGTCATATTATACTCTAACCAGTATCACTTACATCCAAGCCTTGACATATACATTTTTTTGTTGGGGAAAGAATTGAATGACTCTCAGACATGCGCGCCCCAGAAACCACCAGAGGGCGCGAGGGCGCTTA CAGAGACTCGATGACTCTGTGGATTCTACGAGTCAGCTTATTTATCGCATTTCGCTGTCTTCTCCATCCAAGAAAGAGCAGATATATCGGTTGGTGAGAGGTATTTTTTTTTTAATAAAAAAATATTTGGGAGAAAACAATGTTTCTATATGAAGTCGTT GTAGGAAACACCACAGGAGGTGTATTAAGGTGATTACTCGTACGGATTTCGCCTGAATATGGTTGGTTCACGGGTGTTTTTTATATATCCCTCCAGAGGGGGGACGTGCAGTATTTTTTCCACCACCACTTCTCCGTATATGTGAATTCACTGATCGATC CTCCCGCAGTCCCCCCGGGGGGGGGGGGGATCTTTTTGAATTATATTCCGAAGGA
18	SrKa-18	*S. rolfsii* (GQ358518)	97	>GGCAATGATCTATACGCGAGGAGTTATGCTGTGGTGAAATTGCATGTGCACGCTCTGGAGCTATATAATATATACACCTGTGAACCAACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACTTAGAACGATCTC ATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAAATATCCACCTTTCCCAACGGATCTCTTGCCTATAGTTTGAAGGAAGAAAACTTCAAGATGCTAAAAGTAGTGTGAATGGCGGAATCCAGTGGGCCCCCGGATCTTTGAAAGAACCT GGGGCCCTTTGGAATTCGGAGGTTCTTGCACGTTTGGATTTTTCTGCTTTTCTCCTTTACAAATTTTCAATTTATCCGGCTTGGAGGTGGATTTTTTTTTTAAGAAAAACTGACCGGCTCTCTTGATTACTATTGAAGGACTTGTGAATGCCAAAGGTGGT GTGATAGGTGACACGCTTAGCCGTAACGGAATTCAGTTGGTTACAGATTTTATACATGGCCCATATTGCCATGCTGCTTATTTCCCCCTAGTCCTCCCCCAGTTGATTACCGTGAACTCGCGTCTCACGGAAAGAAACTTATGATTCTATCGAGATGTGC GGTATGAAATGGAGTGCC >GGGAATTTGGATGATATATCGTACTGAATGAGGCTGTGGTCGATATATGCACTTATATGCTAGATATATGCGCATGATTTTATCAGTAGTAATACAAGCTAGAATCCCCTTTCGGGCTATCATACACTATTTTCATCTCATCGGATGGCATTTCTAACG GTCCCATATAGAAATTTAAGAGAGCCCGTGTTAATCTTAATTAACCAACAACTCCCTTTCCCAGCCTTGGCAAATGCCAAAATTTTGAAGGGTGAAAATTTTAAGACCCTTAAACAAGGGTGCCCCTCGGAATCCCAAGGGGCGCCAGGTGCTTTCCAA GAATCCATGGCTCCCTGGATTCTGCAATTCACTGTAAGTATCAGAGTTTGGTGCTTTTCTCCCTATGCATGAGCCAATAGTCCAGGTGTTGAAGGTGGATTTTTTGTTTTAAAAGAAACTTGCCGAAAACCTGATTCAATTGAAGCCTTGTAGGAAGCCA AAGAAGGGGTTTATAGGTGATTACGTTAGCCGTCGCGGATACAGTTGGTTCAACACTTATATATATAGCCCAGATTGCATTGCATATTATTTCCCCACTTCTCCTCGTAAGTGAATTACACTGACTCGCATATCACGCGAAGAGATTATGATTCATTCAA GATTGTGTGTAAGATTGCAGGCCCCT
19	SrKa-19	*S. rolfsii* (AB075318)	93	>GGCAAGTGACTGATATAAGCGAGGAGTGGTGCTGGGATGATATTGCATGTGATCTCTGAGTGTATATTATATAAACAGGTGTGCATACTGTAGTCAGGAGAAATTCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATTTC ATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTATGAATATATCACTTTCTTCAACGGATGACTTGTATCTTGTTTTGATGAAGAAAGAATCGAAATGCTCTAAGTAATGTGAATTGCAAAACTCAGTGAATCATCAAATCTTTTAACGCGACTCG CTGACTTTGTGATTTCTAGGAGTCAGCTTATTTGAGAGTCTTCAGTTTCTTCACCATACAAATTTTTGTAGATGTCACGGCTTGTAAGTGATATTTGCTGGTTAAAGAAATTCTGACTGGATCTCTTTTAATCTATTAGGAAGACATGTAGAAAACCCCCC GGGGGGTGTGAATATATGATCCCCCCTATGCATTTCGGGGGGAATATTGTGTGTTCACCGGTATTTATAAAATAACGCCCCCAGAGGGGGGCACAAGAAATATTTTTTTCCCCGCACCCCCCCCCCCGCATATGTGAATATCACCCATGACCCTCCCCG CATGTTCCCCCGAGGGAAAGAAAAAAACAGAGGGCCTCACCAGGGGGGGGGAAAGAGGGGGG >GGGCATTTGGCTGGATACGTACTGATTGAGGCAGTGGTCATATATGCACGTATATGCTAGATATATGCGCATGATTTTATAAGTTGTGCATACAAGCTAGAATCCCCCTTCCGGTATATGCGTAGACATTTTATCACTCATTGCGTGTGACTTTCTACG TGTTCATATTAAAGTTTTAAAGAGAGCGTGTCATAATATACTCTAACCTATATCACTTTATTCACTGATGACATGTACATTGTTTTGTTGGGGAAAGAATTGAAAGGCTCTCAGACATGCGGATTGCAAAATATACCTGAAGGCGCAAAGCGTTTTACG AGACTCGATGACTCTGTGGATCCTAGGAGTCAGCTTACTTGAGAGTCTTCACTGTCTTCTCCATACAAGAAATAGTAGATATATCCGCTGGTGAGAGATGTTTGTTTTTTAAAAAAAAAATACTTGCGGGAAAACTATGTTTCTATTAGAAAGCGTTCTA TGAAACACCACAGGAGGTGTAATAAGGTGAATACGCCTATGGATTTCGGCTGAATACGGTTGGTTCACAGGTGTTTAATATAATGCCTCCAGAGTGGGCACATGAGTATTATACCACCACCACCTCCTCCGCATATGTGAGTTCACTCAGCGATC CTTCCCGCATGTACCCCCACAGAAAAGAAAAAACCTGATCTTGCCGAGGTGGGGGGAAAAATTGGGGCCC
20	SrKa-20	*S. rolfsii* (AB075318)	89	>GGCATTGACTGGTATTAGCGATGGAGTGGTGCTGGGATCGATATTGCATGTGCACGCTCTGGTGTGCGCAATATTTTCTCCTGTGTACCACTGTAGTCAGGACAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACTTAGAACGATTT CATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATAACACTTTTTCCACGGATGACTTGGCCCTTGTTTTGATGGAGAAAGAATTTAAATGCTCTAAGTAATGTGAATTGCAGAAACCACCAGGATCATCGAGGCTTTTAAAAAGACT CGAGGCCTCTGGGATTTCGAGGGGTAAGATTATTTGAGAGTTTTTAAAGTTTTCTCCATACAAAATTTTGCAATTATACAGGTTTGGAAGATATTTGTTTTTTATTAAAAAATATTTGGCGGAAAACTTTTTTTCTATTAGAAAGCCTTGTAGAAAACC CCACAGGTGGTGTGATAAGGTGTTTACCCCTAAGGATTTCTGCGGGAATCCGGTTGGTCAAGCTGTTTATAATATAATGCCCCCAGATTGTGGCCAAGAGTATTTTTTTATGAGCATTAGTTCTTCACATATGATGAGATCACTAATGATACTTCCGCAGT CACCCGGCGGAAAAAAAAAACTTTTGAATTCATATTTGCGAAGGAGTTTGTGCTGGTTAATAAATATTGCGTGTGCACTT >GGCATTTGGCTGGGAGTCGTACTGATTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCGGGTATATGCGTACCCTTTTTATCACTCATCGCGTGGGATTTTCAACG TTTTCAAATAGAAGTTTTAAAGAGACCCAGTCATAACTCCTCAAACCGGCACCTCTTTTTCCAGCCTTGACATGGACAAAATTTTGTAGGGGAAAGAATTTAAAGACTCTCAAACAGGCGTGCCCCTGGAAACACCAAAGGGCGCAGGGTGTTTTAAA AGATTCGATGACTCTGGGGATTCGGCGAGTCAGACTATTTAAGGAATTTCGCTGTTTTCTTCATACAAAAAATAGCAAATAGATCGGTTTGGGAAAGTTGTTTTTTTTTATTAAAAAAAATTTGGCAGAAAACATTTTTCTATTAGAAGGCGTTCTTG GTAACCCCCAAGAGGTGTGATAAGGGGAATCCCCCTAAGGCTGCCGGGGGACTACGGTTGGGTCACAGGTGTATATAATATAATGCCCCTGAGTGGGCCCATGCAATATTTTTTTCGAGCATACTCCTTCGCATATGTGAATTCAACAATGATCCTTC CGCAGTCTCCCCAGGGAAAAAAAAAACTTTTGAATTCATATTTGCGAAGGAGTTGGGCTGGTAAAGAAATATGGCATGTGCACAC

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
21	SrTs-1	*S. rolfsii* (DQ484060)	87	>TGGGCTCTGATCTATTAGCGAGGAGTTGTGCTGTGGTGAATATTGCATGTGCACGCTCTGGAGCTATATAATATATACACCTGTGAACTACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGAACGATC TCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAAATAACCACTTTTCCCAACGGACCTTTTGCCAATAGTTTGAAGGAAGAAAACTTCAAAATCCTAAAAGTAGTGTGAATTGCGGAATCCCAAAGGGCCACCGGATCTTTGAAAGAA CCTGGGGCCCTTTGGAATCCGGAGGTCCATGCACTTTTTGGAGTTTCAACTTTCCACCTTTACACATTTTTCAATAGGTCAGGTCTTGAAGGTGGATTTTTGTTTTAAGAAAAATACTGGCCGGGAACTCAATGTTACAATTAAAGCCTTGTGAAAGCCC CGGTGAGTGTGATATGTGATCACGCTTAGCCTGCAGGGGATTCAGCTGGTTCAAAGGCTTATAAAATCAGGCCCCAATTGTGCACAGGCTTCTATTTCCCCACAGCTCCTCCACCAAGTTGGATTACCACTGAACCTCCGCCAGTCTCACCGGGAAGAA AAATTTTTTGAATTCTATTGCGAAGGAGTGTGCGGTAAGAATATGCAG >GGCAATTGACTGGCAGACGTACTGGTTTGATGCTGTGGTCGATATATGCACGTATATGCTAGATATATGCGCATGATTTTATCAGTAGTAATACAAGCTAGAATCCCCTTTCGGTCTATCATCACCCTATATAACTCTCATCGGATGGTATTTAGAACG GTCCCATATAGAAATTTTGTTTTCTGAGTGTTTCTCTTAATTAAAAAATAACTCCTTTTCCCAGCCTTGGCAAATGCCAAAAGTTTGAAGGGTGAAAAATTAAAGACCCTTAAACAGGGGTGCCCCTCGGAATACCAAAGGGCGCAAGGGGCTTTCAAA GAATCGATGGCCCATTGGAATCCGCAGTTCAAATCACTTTTAGATTTCGCTGCTTTTCTCCTTGATGCATGTTCCATTAGTTCGGCTTGAAGGTGGATTTTTTTTTTAAGAAAAACTGACGAAACCAGATTCAATTAGAAGGGTTGTAGAAAGCCCCGGT GGGTGTGAAAGATGATCAACTAAGATGTAACGGATTCAGTTGGTTCAAGAGCTAATATAAAAGCCCCATAGTGTGCCTTGCATACTATTTCAGCACAGCTCCTCGCAGTTGGATTACCACGACTTCGCATCTCTGCGGGAAGAGACTATGAATCAATGC CGAGAGTGGCTGTATGAATATGCAGTCCCCTCGCA
22	SrTs-2	*S. rolfsii* (GQ358518)	91	>GTGCATTGGCTCTAGTAGCGTGGAGTGATGCTGGGATGATATTGCATGTGCACGCTCTGTAGTGCGCAAGATTTTCTCATGTGTGCATACTGCTAGAGGACACTTCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACTTAGAACGATTTCAT ATTGAAGCTTTGTTTTGTGACACGTTTATCTTAATTTAAAATATACCACTTTCTCCACGGATGACTTGTATCTTGCTTTGTTGAAGAAAGAATCGAAATGCTCTCAGTAATGCGGATTGCAGAATCCACCTGAATCATCGAAGCGTCTAACGACACTCGA TGACTCTGTTGTTCCTAGGAGTCAGCTTGTTTGATAGTTTTCAATTTCTTCTCCATACAAACTATTGTAGATATCACGGCTGGTGAGTGGTATTTGTTTTTTAAAAAAAAATCTTGGGGGCTCTCTTTTTTTCTATTATAAAGCTTTCTATAAAACACCCCA GGTGGTGTAATTAAGTGAACACCACTATGCCTCTCGAGGGAATACGGTTGTGTTCACCGGTGTTTATTATATAATCCCTCCAGAGTGTGCACGTGAGTATTTTTTACCACCACCACCTCCTCCGCATATGTGAGTTCACTAATCGATCCTCCCGCACTTCC CCCGGGGGGGGGAAGATCTTTTTGAATTCATATTTGCGAAGGATTTGTGCTGGAAAAAAAATGGCAC >GGGGCAATTTCCTGGCAGTAGTACATGATTTGAGGCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAAGCTAGAATCCCCCTTGCGGTATAGGCGTAGACATATTATCACACACAGCGTGGGCTTT TCTACGTGTTCCAATTAGAGTTTTAAAGAGAGCCAGTCATAATATACTCTAACCAGTATCACTTACATCCAAGCCTTGACATATACATTTTTTTGTTGGGGAAAGAATTTAATGACTCTCAGACATGCGCGCCCCTAGAAAATACCAGAGGGCGCAGGG TGCTTTAAAAGACTCGATGACTCAGTGGATTCTGCGAGTCAGCTTATTTATCGCATTTCGCTGTCTTCTCCATCCAAGAAAGAGTAGATATATCCGTTGGTGAGAGGTGTTTGTTTTTTAAAGAAAAATATTTGGCAGAAAACAATGTTTCTATATGAAA TCGTTGTATGAAACACCACAAGAGATGTATAAAGGTGAATCATAGTAGGGATTTCGGCTGACGACGGTTGGGTTCACGGGTGTATATTATATATGCCTCCAGAGTGTGGCGCGTGCAGTATTTATTTACCACCCACCCCTCCTTCGCATATGTAAAATC ACTGATCGATCCTCCCGCAGTCCCCCCGCGGGAAAAAAAGAAATTTTTGAATTCATATTTCCGAGGGAGGTTGGGCGGGTAAATAAAGATGGCGGGCCCCCCCCCCG
23	SrTs-3	*S. rolfsii* (DQ484060)	90	>GGCCATCTGACTCTATATGCGAGGAGTGGTGCTGGGATGATATTGCATGTGCACACTCTGGTGCTATATAATATTTACTCCTGTGTAATACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATT TCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATATCACATTCTACAACGGATCTCTTGGCTCTTGCTTCGTTGAAGAAAGCAGCGAAATGCTATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTTAACGCACC TCGCGGCCTCTGTTATTCCTAGGAGCAAGCTTATTTGAGAGTAATTAATTTCTTCACCATACAAATTTTTGTATTTATCACGGCTTGTATGTGATATTTGTTGGTTAATAGAAAAAAACTGACTGGCTCTCTTTTATACTATTAGGAAGACTTGTAGGAAA CCCCACAGGTGGTGTGAAAATGTGTCTACCCCTACGCAAGACGGGGGACTCTGGCGTGTTCAGAAGTATTTATAAAAACATGCTATATAGTGTGACGAGAGTACTTATTTACGACCAACCTCACCTCCGCATATGTGAGACCACCCACTATCCTCCCGC AGTTAACCCACAGAAAAGAAAAAATTTTCGGGACGCAGATAGGCAAC >GGGCATTGGATTGATATACGCCATGAGTGATGCTGTGGTCGATATATGCACGTATATGCTCGATATATGCGCATGATTTTATCAGTTGTGCATACAGCTAGAATCCCCCTCCCGACTATGATTACCCTATATAACTCTCATTGTATGTGACTTAGTACG ATTTCATATTGAAGCTTTGTTTTCTGACACGTATATCTTAATTAAGAATATATCACTTTCTCCAAGGATGACTTGTATCTTGCTTTGTTGAAGAAAGAATCGAAATGCTATAAGACATGCGAATTGCAGAATCCACCTGAATCATCGAGGCGTTTAACGA GACTCGAGGACTCTGTGATTCCTAGGAGCAAGCTTATTTGAGAGTTATTGATGTCTTCTCCATACAAACAATTGTAGATATCACGGCTTGTGAGTGATATTTGCTTGGTTAATAGAAAAAAACTTGGGGCTCTCTTTTTCACTATTATAAAGCCTTGTAG GAAACACCACAGGTGGTGTAATAATGTGAATACCCCTACGCATTTCGCCGGAATACGGTGTGTTCACAAGTATATATAAAAACATGCCCCCAGAGTGTGGACATGAATATTATTTACCCGCACCACTCCTCCGCATATGTAGTACACCCATGACCCTTC CCGCACATCCCCCCCCGGGGGGGGGGATTTGTGATTCATGGGCGGGGGGTGGGTGCG
24	SrTs-4	*S. rolfsii* (GQ358518)	94	>TGCTCGTAGCTCTATTGCAATGGAGTGGTGCTGGGATGAATATTGCATGTGCACACTCTGGAGCTATATAATATTTACACCTGTGACCAACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGAACGATC TCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAAATAAACAACTTTCAACAACGGATCTCTTGCCTCTAGTTTGGATGAAGAAAACTTCAAAATGCTAAAAGTAAGGTGAATTGCGGAATCCAGTGAATCATCGAATCTTTGAAAGCA CCTGGCGCCCTTTGGAATTCGGAGGTCAATGCATTTTTGGGATTTATTAAATTCTCACCCTTACAAATTTTTGAATTTGTCAGGTCTTGAAAGTTAGATTTGTTTTAATTAGAAAATCCTTGACTGGCTCTCTTTATACCTATTGGAAGGACTTGTAGAAA TGCCTACGATGGGTGAGATAGTATGTCTACGCTTATACGGGAGGCGGATTCCAGCTGGTTTGTACGACTTAAAAAAATATGCGCCCAGATTTTGCAAATCGCAGCCTATTTTCGACCACCTGAAACTACCCACTGTACTTCCGC ATATCCTCAACCCGGAAGAAGTCTTTGATTCTTTCAGATGGGGAA >GGATATGAGGGATTAGTATGAGTGATGCTGTGGTGATAATGCACGTATATGCTAGGGTATGCGCATGATTTTCTCAGGTGTAATACTGCTAGAATCCCCTTCCGGGCTATCATCACCCTATATTTATCTCATTGGATGGTACTTAGAACTGTCCCATAT AGAAACTTTGAGTTCGGACAAGTTTCTCTTAATTAAAAAATAACTCTCTTTCCCAACCTTGGCAAATGCCAAAATTTTGAAGGATGAAAATTTAAAGACCCTTAAACAAGGGTGCCCCTCGGAATCCCAAAGGGCGCAAGGTGCTTTCAAAGATTCCAT GGCTCTTTGGAATCCGGAGGTCCAAGTACTTTTGGGAGTTTGCTGCTTTCCTCCTTGACGCATGTTGCTAGAGGTCCGGTGTTGGAAGTTGGATTTGTTTTAATTAAGAAAAACTGGCCGGGCTACCTTGTTTCCTATTGAGGGGTTCGTGGAAATGCCA AAGGAGGGGTGAAAAAGATGGTCTAGCTTAGCCTGCACCGGATTCAGCTGGTTTAGAAGAATAAAAAATATGGGCCCATAGTGTGCAATTCAATGCTCATTTCACCCTTTGTCCTTGTCAAGTTGAATTCACTCATGAAACTTCGGCAGTCATCAGACGG AAAAACTTTTGTTCTTCCAGTTGGGGCAAAAGGGC
25	SrTs-5	*S. rolfsii* (DQ484060)	93	>GGAATAAGAACCGTGGTTGGATCTATATGCGAGGTTTGCCGGGAAAATTTGGCGTGCACCACCCGGGATCTTAAAGGAAGCCATCAAACGGGGTGGGGGAATACCGAAATGAAAAAAGGAAAAACTCCTATGGGGGGCCTAAAAAAGCCCTTATTT AGAATTTTTGGGGGGCGGGCAGGTTTTTCCTAATAAAAAAAAATCCCCCTTTTCCCCCGCGGGGTGTTTCGCTTTTTTTTTTGGGGAAAAAAACTTCGAGACCCCCCAAAAGGGGGCGCCGCGGAAACACCCAAGAGCGTCGGAGGGTTTTTTTAAAAA CAACCCGCCCCCCCTTTGGTTTTCCGGGGGGGGCTCCCTTTTTGGGGGGGTTAGGTTCTCTTCCCCCCCCCACAGAATATTTTATATAATCCGGGGTGGGGGGGGGGGGCTGCCTTATAAAAAAAAAACCCGGCCCCCACCCCCTTTTCTTCTATTTTTT AGGGGGCGAACACCCCCCTCCGGGGGGGGGGTAGATATATTGTCTTT >GGATAAAAGTCTAGAATATATGTAGAGTATGATTGGGCAATGACATATTGCCTTCATGCTAAAATATCGCCACTTTAAGGGGGGGAACAACAGTGAGAAGCCACCTCCGACCATGTTTACACTATTTACACCAACGCGGGTGATTATAACCCCCCCCCT AAAAAAGTTTTTTTGTAAGGAGAGTGTTTATTATTTATAAAGATTCTCTTCCCCCCCCCGGCGATGGGTGATTTTTGTGGGAGGAAGATAATAAAATAAGAATGGTGTGTTGATAAAAAAAAAAGAAGGGGGGTTTTTTTTCATCTATTCTCTTCTTTATG GCTTCTTAGATACTGGAAAGAAGGTGGTGGGGCGGCCCCCTCCCTAAAGCGGGAAGAGCCGGTCCGGGGAGGAGTTTTTTTTTTTTTATAGGGGAAGCAGCCCCC
26	SrTs-6	*S. rolfsii* (DQ484060)	91	>TGCTTCTGGCTGGAGTAGCGTGGAGTTGTGCAGGTAATGATATTGCATGTGCACACTCTGAAGCTATACAAGATATTCTCCTGTGACCACTAGTAGTCAGGAGATTCCGGACTATGATTACACTATATAACTCTTATTGTATGTTACATAGAACGATCT CATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACTACTTTCATCAACGGATCTCTTGGCTCTTGTTTCGATGAAGAACACTTCGAAGTGCCATAAGTAATGTGAATTGCAGAATCAAGTGAATCATCGAATCTTTTAAAAGACCTT GTGCCCCTTGGTATTCTGAGGGGCATGCTTGTTTGAGAGTTTTTCTGTTCTCATCCTTACTAAAATTTGTATTAGTCCCGGTGTGGATGTTAGAATTGTTTTTTATAAAAAAATCTTGGCAAAAAACTTTTTATAATTATAATAGGATTCTTTGAAACACC CACCGAGGGTGATATAATGTGATCACCCCTATGATTTCTCCGGACTACAGTTTGTTTACAGGTGTTTATATTATAATAGCCCCAGAGGGGGCAGATAAAATATTTATTACCACCACCACTCCTCTCTCATATGTGAAATCACCAGTGATCCTTCCGCAGC TTCTTCCGCCAGGAAAAAAAAAACTTTTTTGAATTCATATATGCGAAGGAGTTGTGCTGGTAATGAATATTGCATGTGCACAC >GCATATAAGCTTCGATATCGTCTTGATTGCGCAGTGGTCAATATATGCACTTATATGCTATATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGACTGGCGTACACTTATTATCACCCCAACGGAAGGCATTTCTAACG GTCCCATAATGAATTTTAAAGATAGCCGGTCAGAATATACTCTAACCAGCTACTACCACATCCCCGCCTTGAAAATGACAATTGTTTGGATGGTGAAGAATTTGATGACTCTCAAACAGGGCTGCCCCTAGAAACACCAAAGGGCGGAAGGGGCGTTC AAAGATTCTATGATCCTCGGTATTCTGAGGTTCATATTTCTTATCGAATTTCGCTGTTTTCTTCCTCGCTACAATATCCAAAAGATCCGTTGTGGAAGGTTGAATTTTTTTTAATAAAAAAAACTTGGCAAAAAACAATGTTTCATTATGAGATCACTCTT TGTAACACACAATAAGAGTGATATGAGGTAATCATAGTTAGGATTTCTCCTGACTACTGTTGGGTCACAGGTGTTTTTATTATAAAGCCCCCAGAGTGTGCTCATGCAATATTTTATTACCACCACAACTCCTCCGCATATATGAATATACTAATGATCC TTCCGCATGATCACCCGGCAGAAAGAAA
27	SrTs-7	*S. rolfsii* (DQ484060)	93	>GGGCATGTGGATCTATATGCGAGGAGTGGTGCTGGGAATGATATTGCATGTGCACACTCTGGTGCTATATAATATTTTCTCCTGTGTAACCACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGA TCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAAATTACCACTTTTTACCAACGGTTCTCTTGCCTTTTGTTTGGATGAAAAAAGCTTCAAAATCCGATCCTTTAAAAGCA CCTGGGGCCCTTTGGATTTCGCAGGGGAAGGCTCTTTTGGGAGTTTTTAAATTTTCACCCTTACACATTTTTGAATTTGTCAGGGCTTGAATGTGAGATTTTTTTTTTAAGAAAATACTGCCGGGCTCTCTTTATAACTATAAGAGGGACTTGTAGAAACG CCAACGGTGGGTGTGATAAGATGTCTCCGCTTATACTTTAGGGGGATTCAGTTGGTTGAGGATTTTTATATAATGCCCCAAAGGTGGCCAATAAATCTTTTTTTCCCCACTTGACCTCCGCCAGGTGGATTAACACTCGACCTCCGCAGTATCACCCGGAA AAAAATTTTTTGATTTCTTATGCGAGGGTTTGCGCGGATACAATACGCGCCCCCCTGGG >GGGGAAATTGATGATATATGTACTGAGTGATGCTGTGGTCGATATATGCACTTATATGCTAGATATATGCGCATGATTTTATCAGTTGTACATACTGCTAGAATCCCCTTTCGGACTATCATTACCCTATATTACTCTCATTGTATGTTATTTAGAACGA TCCCATATTGAAATTTTGTTTTCTGACATGTTTCTCTCAATTAAAAAATAACCACCTTTTACCAGCCTTGGAAAATGCCAAAAGTTTGGAGGAGAAAAATTTCAAGACCTTAAAAGCGGGGGGCCTGGCGGAATCCCGAGGGGCAGCAGGTCTTTAAAA GAACCTGGTGCCCATGGTATTCCGCGGGGCAAGTAATTTTGAGAGTTTGCAGTTTTCTCCTTGATACTGATTCAGTAGTTAGGCTTGGATGTTGTAATTGTTTTTTTTAAAAAAACTGGCTGAATCTATTTATACTATTGGGGGGCTTGTAGAACACCCCA AGAGTGTATGATATGATCACCGCTATGATTTAGGCGAATACGGTGGTACAAGTATTTTAAAAAAGGCCCTGAGTGTGCCATTGAGTATTATACACCACACTCCTCGATAGTAGATCACTATGACTCGCTGTCTCTGCGAAAAATTTTGTTTATCCAAG GGTGCGGAAAATG

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
28	SrTs-8	*S. rolfsii* (GQ358518)	94	>GGCATCTGGATGATATAAGCGAGGAGTGGTGCTGGGATGATATTGCATGTGCACACTCTGTAGCTATATAATATTTTCTCATGTGTACTACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATT TCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATATCACTTTTTACAACGGATCTCTTGTATCTTGCTTCGTTGAAGAAAGCAGCGAAATGCTATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTTAACGAACCT CGATGCCTCTGGTGTTTCTGCGAGCAAGCTTATTTGAGAGTAATTAATTTCTTCACCATACAAATTTTTGTATATATCACGGCTGGTGTGTGATATTTGCTTTTTAAAGAAAATACTGACGGGCTCTCTTTAAATCTATTAGAAAGACATGTAGAAAACC CCACAGGTGGTGTGATAATGTGACTACCCCTATGGATTTCGGGGGGGAGATAGTGTGTTCAAGCTATATATAAAAACATGCTCCAGAGTGTGTACATGCAATACTATTACCCCCACCACCCCTCCGCATATGTGAGATCACCCGCGATCCTTCCGCAGA TAACCCAGGGAAAAAAAAAAAATTTTTGGATTTTATTTGTGCAAGGGAGTTGTCGTGGATAATAAATAGGCGGCCCCCCCCCGGCCA >GGGCAATTTGGAGGGTATATCGTACCTGATTGAGGCAGTGGTCATATATGCACGTATATGCTAGATATATGCGCATGATTTTATAAGTTGTACATACAGCTAGAATCCCCCTTCCGGTATATGCGTAGACATTTTATCACTCATTGCGTGTGACTTTCT ACGTGTTCCTATTAAAGTTTTAAAGAGAGCCAGGGATATTATACTCTAACAATATATCACTTACATCCAAGGATGACATGTACATTGTTTTGTTGGGGAAAGAATTGAATGACTCTCAGACATGCGCGCCCCAGAATCCACCAGAGGGCGCGAGGCGTT TAAAAAGACTCGATGACTCTGTGGATTCTAGGAGTCAGATTATTTAAGAGTTTTCGCTGTCTTCTCCATACAAAAAATAGTAGATATATCGGCTGGTGAGAGATGTATGTTTTTTAAAGAAAAATATTTGGGAGAAAACTATGTTACTATAAGAAAGCG TTGTAGGAAACACCACAGGAGGTGTATATAGGTGAATCATAGTAAGGATTTCTCCTGAATCTGGTTGGTTCACAAGTGTTTATAATATAATGCTCCAGAGTGTGCACGTGCAATATTTATTACGCCCACCACTCCTCCGCATATGTGAGATCACTGAGC GATCCTTCCCGCAGTCACCCCAACAAAAGAAAAAAACTAGGATCATACACGAGGGGGGGGGGAAAAAAAGGGGG
29	SrTs-9	*S. rolfsii* (DQ484060)	94	>GGGATCTGGACTCGTATATAGCGATGGAGTGGTGCTGGGAATCGATATTGCATGTGCACGCTCTGTATGTGCGCAAGATTTTCTCATGTGTACATACTGTAGTCAGGACAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACTTAGA ACGATTTCATATTGAAACTTTGTTTTCTGACACGTTTATCTTAATTTAAAATATATCACTTTCTACAACGGATGACTTGTATCTTGCTTTGTTGAAGAAAGAATTGAAATGCTCTCAGTAATGTGAATTGCAGAAACTACCTGAATCATCGAGGCTTTTAA AGAGACTCGATGACTCTGTGGTTCCTAGGAGTCAGCTTATTTGAGAGTCTTCAATGTCTTCTTCATACAAAAAATTGTAGATATATCCGCTGGTATGTGATATTTGTTTTTTAATAAAAAAAAACTTGGGAGAAAACTATTTTTCTATTATAAAGCTTTGT ATGAAACACCACAGGTGGTGTGATAATATGACTACCCCTATGGATTTCGGGGGACTCTGGTGTGTTCACAGGTATTTATAAAATAATGCTATAGAGTGTGGTACATGAGTATTTATTACGACCACCACTCCTCCGCATATGTGAGTTCACTAACGATCC TCCCGCAGTCACCCCAGAAAAAAAAAAAAACTTTTTGAATTTCATATTTGCGAGGGGATTTGTGCTGGTAAAAAAATATGGCGTGTCGACCCTCAG >GGGGGAATTGGATGGGAGTCGTACCTGATTTGAGGTCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATAGGCGTAGACATATTATCACTCACTGCGTGTGATTT TCTACGTGTTCCAATTAAAGTTTTAAAGAGAGCCAGTCATATTATACTCTAACATATATCTCTTACATCCAAGCCTTGACATATACATTGGTTTGTTGGGGAAAGAATTGAATGACTCTCAGACATGCGCGCCCCAGAATATACCAGAGGGCGCAGGGC GCTTTCAAAGACTCGATGACTCTGTGGATTCTGCGAGTCAGCTTATTTATCGCATTTCGCTGTCTTCTCCATCCAAGCAAGAGCAGATATATCGGCTGGTGAGAGGTGTATGTTTTTAAATAAAAAAAAATTTGGGAGAAAACAATGTTTCTATATGAAGT CGTTCTATGAAACACCACAAGAGGTGTGATGAGGTGAATCATCGTAGGGATTTCGCCTGAATACTGGTGGGTTCACAGGTGTATAATAATATATGCTCCAGAGTGTGCACATGAGTATTTATTACGACCACCACTCCTCCGCATATGTGATATCACTAA TGATCCTTCCGCAGTCACCCAAAAAAAAAAAAAAAACTTTTGAATTCATATACCGAGGGAGTGGTGCTGTAAAAAAATATGGCGGGCGCA
30	SrTs-10	*S. rolfsii* (GQ358518)	91	>GGCAATTGACTCTATATGCGAGGAGTGGTGCTGGGATGATATTGCATGTGCACACTCTGTAGTATATAATATATTCTCATGTGTGCATACTGTAGTCAGGAGAAATCCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATCT CATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATATACAACATTCAACAACGGATCTCTTGGGTCTTGTTTCGATGAAGAAAACTTCGAAATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAAAGCACC TTGCGCCCCTTGGTATTCCCAGGGGCATGCCTTTTTGAGAGTCAGTTAATTTTCTCCCTCACACAAATTTGTATTTGTCAAGGGTTGAATGTGAGAGTTGTTTTTTAGAGAAAATTCTTGAGTGGCTCTCTTTAAAACTATTAGGAGCACATGTAGAAATG CCCACGGGGGGTGTGATAATGTGTCTACGCCTATACTTCAGGGGGATTCCAGTTGGTTTCAAGTATTAAATAAAATAAGGCCCCAATATTGGGGCAAATAAATTATTTTTTTGCCGGATTTGACTTTCATAAGGTAGAACTACCCGCGGACCTTCCGCA GTCACCCGGGGGGAGAGAAAAATTTTTTTTTTTTCTATCTCCGAGGGGTGTGTGGGAAAAAAAAAGGGGGGGCCCCCCC >GGGCCAATTGATGATATACGTACTGAGTGATGCTGTGGTCGATATATGCACGTATATGCTAGATATATGCGCATGATTTTATAAGTTGTGCATACAAGCTAGAATCCCCTTCCGGACTATGATCACACTATAAACTCTTATCGGATGTTATTTAGAACG ATCTCATATTGAATTTTTGTTTTCTGACACGAGTCTCTTTATTAAGAAACATACACCATTCAACAACGTTGAAAATTGCAACTTGTTTCGATGAAAAAAATTTCGCAATGCTAAAAGTGGTGTGCCTTGCAGAATACAGTGAGGCATCGGGGGCTTTGAA AGCACCTGATGGCCCCCTGGATTCCGCAGGGGCATGCCCTTATGAGAGTCAGCTGATTTTCTCCCCTACACAAATTTGAATATATCCGGTGTTGGATGTGAGAGATTTTTTAAGAGAAAATACTTGACTGGCTCTCTTTTTTACTTTTAGGAGCACATG TTGAAATGCACACGAGGGGTGTGATAATGTGTCTACGCCTATACTTTCTGCGGATTCCAGTTGGGTTACACTATTATATAATATAATAGCCCCTGAGTGGGCCCATGAAATATTTTTTTACAAGAACATTTCTTTGATAAGGAGAACTCCCAGATGATCC TCCCCGCAGTTCCCCCGCGGGAAAAA
31	SrAp-1	*S. rolfsii* (GQ358518)	96	>TTGCAACTAGCTCGAGTGTACTGAGTGGTGCAGTGATCGATATTGCATGTGTATGCTCTGTATATGCGCATGATTTTCTCCTGTGTACTACTGCTAGAATCACCTTCCGGACTATGCGTACACTATAAACTCTTATCGGATGTTATTTAGAACGATCTCA TATTGAATCTTTGTTTTCTGAGTGTTTCTCTTAATTTACCATATACTACTTTTCCAACGGATCTATTGACTATTGTTTCGATGGAGAAGAATTTGAAGTGCCATAAGTAATGTTGCTTGTAGAAACACCGTGAATCATCGAATCTTTTAAAAGACCTGGTG CCCCTTGGTATTCTGCGGGGCATGCTTCTTTGAGAATTTTGCTGTTCTCATCCTCACAAAAATTTGTAATAGACCCGGTGTGGATGGTAGAATTGTTTTTAATAAAAAAATCTTGACAAAAAACATATTTTAATTATAAGAGCATTCTTTGAAATACCCA CGAAGGGTGATATAATGTAATCATACCTATGATTTCTGGGGACTACAGTTGGTTCACAGGTGTATATATAATCATGCCCCCATATTGTGGCAGATGCAATATTTATTACCACCACTACTCCTCCGCATATGTAGAATTCACCAATGATCCTTCCGCAGGT TCATCAGCCGAAAAAAAAAAAACTTTTTTGAATTCATATATGCGAAGGAGTTGTGCTGGTAATGAAATATGGCGTGTGCC >GTGCAAATGCGCAATATCGTAATGATTGAGGCAGATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTATAGGCGTACACATATTATCACACCAACGGAAGGCATTTCTA CAGGTCCCACAAAGAATTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCTACTCTCACATCCCAGCCTTGACAAAGACAATTGTTTGGAAGGTGAAGAATTTAATGACTCTCAAACAGGGTTGCCCCTCGAAACACCAGAGGGCGCAAGGTGCG TTAAAAGATTCGATGATCCACTGTATTCTGCAATTCATATTACTTATCGAATTTCGCTGCTTTCTTCATCGATACAAGATCCAAGAGATCCGTTGTGGATGGTAGAATTTTTTTTAATAAAAAAATCTTGGCAGAAAACAAAGTTTCATTATGAGATCAT TCTATGTAACACACAATAAGAGTGATATAGGGTAATCATAGTTAGGATTTCTCCTGACTACAGTTGGTTCACAGGTGTATATATTATATAGCTCCAGAGTGTGCACATGCAATATTTATTACCAGCACAACTCCTCCGCATATGTGAATTCAATAATGAT CCTTCCGCAGATTCACCCCCCGGAGAAGAAAAAACTTTTTGAATTCTTATATGCGAAGGAGTTGTGCTGGTAAAAAATATGGGGGGTC
32	SrAp-2	*S. rolfsii* (DQ484060)	89	>TGGCATGTGACTGGATGTAGCAATGGAGTGGTGCTGGGATCGATATTGCATGTGCACACTCTGGAGCGCACAAGATTTTCTCCTGTGTGCCAACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACG ATTTCATATTGAAGCTTTGTTTTCTGACAAGTTTATCTTAATTAAAAATATACCACTTTCAACAACGGAGCTCTTGTCTCTTGCTTCGTTGAAGAAAGCAGCGAAATGCTATCAGTAATGTGAATTGCAGAATCCACCTGAATCATCGAATCTTTTTACGC ACCTCGCGGCCTCTGTTGTTCCTACGAGCCTGCTTGTTTGAGAGTCATCAATTTCTTCTCCATACAAAATATTGTAGATATCTCCGCTGGTATGTGATATTTGTTTTTAAAAAAAATTCTGTGGGAGCTCTCTATTTTACTATTAGTAAGACTTGTAGAA AACCCCACAGGTGGTGTGATTATGTGAATACCCCTACGGATGTCGGGGGAATCTGGTTTGTTCGAGGATTTTATAATATAATGCCTCCAGAGTGTGCACATGAGTAATTTTTTACCACCACCACCTCCTCCGCATATGTGAAATCACTCAGCGATCCTTC CGCAGGTTCACCCGGAAGAGAGAGAAAAAAACTTTTGAATTCATATTTCCGAAGGGGTTTGTGCGGGTAAATAAAATATGG >TGGGGATATTGCTGCGATGTCGTAATGATTTGAGGTCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAAGCTAGAATCCCCCTTGCGGTATAGGCGTAGACATATTATCACACACAGCGTGGGTTTT TCTACGTGTTCCAATTAAAGTTTTAAAGAGAGCCAGTCATAATATACTCTAACCAGTCTCTCTTACATCCCCGCCTTGACATATACATTTGTTTGTTGGGGAGAGAATTGACTGACTCTCAGACATGCGTGCCCCTGAAAATACCGGAGGGCGCAGGGT GCGTTCAAAGATTCGATGACTCAGTGGATTCTGCGAGTCAGCTTACTTATCGCATTTCGCTGCGTTCTCCATCCATGAAAGAGCAGAGATATCGGTTGGTGAGAGGTGTATATTTTTAAAAAAAAAAAATGTGGCAGAAAACAATGTTTCTATTAGAAG TCGTTCTATGAAACACCACAAGAGATGTATATAGGGGAATCATAGTAAGGATTTCGCCTAACGACGGTTGGTTCACGGGTGTATATTATATATGGCTCCGAGAGTGGGGACATGAGTATTTTTTACCACCACCACTCCTCCGTATATGTGAGTTCACTA ATGATCCCTCCCGCAGTGCCCCCCCGGGAGAAAAAAAAAAACTTTTGAATTCATATTCGAGGGGTTGTGCGGGTAAAAAAA
33	SrAp-3	*S. rolfsii* (GQ358518)	83	>GGGCATTTGACTCGTATATGCGAGGAGTTGTGCTGGGAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAACCAACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGAA CGATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAAATAACCACCTTTCCCAACGGACCTCTTGCCTCTAGTTTGAAGGAAGAAAACTTCAAAATGCTAAAAGTAGGGTGAATTGCGGAATCCCCAAGAATCATCGAATCTTTGAA AGCCCCTGGGGCCCTTTGGAATCCGGAGGTCCATGTTTTTTTGGGATTTTTTAACTTTCCTCCTTTACAAATTTTTCAATTGGTCCGGTCTTGGAAGTTGGATTTGTTTTATTTAGAAAATCCTTGACGGGATCTCTTTATTCCTATTGAAAGCATTTGTGG AAATGCCCACGGTGGGTTAGATAGGATGTCTACACTTATACTTTCTGCGGACTCCAGCTGGTTCGTACGACTTAAAATAATATGGCCCCAGATTTTGCACATGCAATGCTTCATTACCCGCACTTCACCTCCCATCAGGTAGGACTACCCGCTGATCCTT CCGCAGTATCACCCGGGAGAAAAAACTTTTTGAATTCATATCTGCGAAGGAGTTGTGCTGTAATGAATATGCATGGTGCAC >GGGCATTGATGGGAGTCGTACCTGATTTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATCAGTAGTACATACAAGCTAGAATCCCCTTCCGGGCTGGCGGTGAATATTTTTACCCCAACGGATGGCATTTCTAACGG TCCCATATAGAAATTTTAAGAAAGCCCATGTTAAAAAACTCCAACCCGCAACCCTTTTTCCCAGCCTTGAAAAATGCCAAAATTTGGAAGGGTGAAAAATTAAAGACCCTTAAACAGGGGTGCCCCTCGGAATACCCAAGGGCGCAAGGGGCTTTCAA AGAATCCATGAATCATTGGATTTCGGAATTCAAATTACTTTTCGGATTTTGCTGCTTTCCTCCTTGATGCAAGAACCAATAGATCCGGTGTTGAAAGTTGGATATTTTTTTATTAAAAAAAACTTGGCGGAAAACAAAGTTTCCATATGAAAGCGTTCTA GGAAACACACAAGAAGAGTTAAATAGGGTGATCACACTTAGGATTTCTCCGGACTACAGTTGGTTCACAGGTGTTTATATAATATGCCTCCAGAGTGTGCACATGCATTATTCATTACCACCACAACTCCCTCGCATATGTGGATCAACAACTGATCCT TCCGCAGGCACCCCCCCGGGAAAAATTCTTGATCAATTCAGATG
34	SrAp-4	*S. rolfsii* (DQ484061)	93	>TGGGCAATGACTCTATAAGCGAGGAGTGGTGCTGTGATCGATATTGCATGTGTACACTCTGTAGTATACAATATTTTCTCCTGTGTACATACTAGTAGTCAGGACATTCCGGACTATGATTACCCTATAAACTCTTATTGTATGTTACATAGAACGATC TCATATTGAATCTTTGTTTTCTGACAAGTTTCTCTTAATTTTAAATATACTACTTTCAACAACGGATCTATTGACTCTTGTTTCGATGAAGAACACTTCGAAGTGCGATAAGTAATGTGAATTGTAGAAACAAGTGAATCATCGAATCTTTTAACGCACCT GGTGCCCCTTGGTATTCCGAGGGGCATGCTTGTTTGAGAGTTTTTCTATTCTCATCCTTACTACAATTTGTATTAGATCCGGTGTGGATGTTAGAATTGTTGGTTAAAAAAAAATCTTGACGGGATCTTTTTTATAATATTAATAAGCATCTGTTGAAACA CCCACCGGGGGTGATATGATGTATTCACCCCTATGATTTCTGGGGACTACTGTTTGTTTGTACTACTTTATAAAATCATGGCCCCATATTGTGGCACAAGAAATATTTATTTCGAGCACTACTCCTCTCGCATATGTGGGATTACTCGCGGTCCTTCCGCA TTCCCCCCCGGGAAAAAAAAAACTTTTTGAATTCATATTTCCAAGGGAGTTGTGCTGGTAAAAAATATTGGAGGTCCCCCCCCCGGGA >GGGGATTTGACTGGGAGTCGTACTGAGTGAGGCAGTGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAGCTAGAATCCCCTTCCGGTATAGGCGTACACTATAATCTACCATCGGAAGGCATTTCTAAAGG TCCCACAATGAATTTTTGTTTTAGCCAGTGAGAATATACTCTAACCAGCTACTACTACATCCAGGGTTGACAAAGACAATTGTTTGGATGGAGAAGGATTTGAAGACTCTCAAGCAGTGTGGCCCGTAGAAACACCAGGGGGCGCCAGGTCTTTTAAA AGACCCGGTGCCCCTCGGTATTCTGCGGTGCATACTACTTAGAGAATTTTGCTGTTCTCTTCCTCACTACAAGATCTAAGAGACCCGGTGTGGATGGTAGAATTTTTTTTATAAGAAAATCTTGGCAAAAAACAATTTATCATTATAAGAGCATTCTTT GTAATACACAAGAGGAGTGATATAGTGTAATCACAGCTATGATTTCTCCTGACTACAGTTTGTTCACAGGTGTTTATATTATAAAGCTCCATAGTGTGGTCATGCAATATTTATTACCACCACAACTCCTCCGCATATGTGAATTCAATAATGATACTTC CGCAGCTTATCCCGCCGGGAGAGAAAAAACTATGAGTCTATTCGAGGAGTGTGGGGAAAAAAG

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
35	SrAp-5	*S. rolfsii* (DQ484061)	93	>GTGCATTTGATGATATAAGCGAGGAGTGGTGCTGGGATCGATGTTGCATGTGCACACTCTGTAGTGCGCATGATTTTCTCCTGTGTACATACTGCTAGAGGACCTTCCGGACTATGATTACACTATAAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAATCTTTGTTTTCTGACACGTTTCTCTTAATTAAGAATATACTACTTTCTACAACGGATGAAATTGACTCTTGTTTCGATGGAGAACACAGCGAAGTGCGATAAGTAATGTGAATTGTAGAATCACAGTGAATCATCGAATCTTTTAACGCACCTGGTGCCCCTTGGTATTCTGCGGGGCATGCTTCTTTGAGAGTTATGCTATTCTCATCCTTACTAAAATATGTATTTGTTCCGGCGTGGATGGTAGAATTGTTGGTTAAAAAAAATTCTGGGGGGATCTATTTTATCATTATAAGAGCATGCTTTGAAATACCCACCAGGGGTGATATAATGTAATCACCCCTATTATTTCTGCGGACTACAGCTTGTTTGTACGTGTTTATAATATCATGCTCCATAGGGTGCAGATACAATACTTATTTTGACCACAACACCTCCGCTGATGTAGGACTACTCGCGATCCTTCCGCAGCTCTTCCGGCGGGAAAAAAAAAATTTTTTTTTCTATTTCGAAGGAGTTGTGCTGATAAAAAAAATGGCGTGGCGCCCCCCGCGGGATCATTAA >TGGGCATTTGGATTGTATACGTACTGAGTGATGCTGTGGTCATATATGCACGTATATGCTAGATATATGCGCATGATTTTATAAGTTGTACATACAGCTAGAATCCCCTTCCGGACTAGGCGTACACTATAATCTCCCATCGGAAGTTATTTCTAACGATCCCATAATGAATTTTTGTTTTAGCCAGTGAGAATTTTCTCTTAGAATATACTACTTCTTCCAACCGTTGAAAATGACTATTGTTTGGATGGAGAAGAATTCGAAGACCCACAAGCAGTGTGGCTTGCAGAAACACCAGAGGGCATCGGGTCTTTTAAAAGACCTGGTGCCCCTCGGTATTCTGCGGTGCATATTTCTTTTAGAGTTTTGCTGTTTTCTTCCTCACTACAAGATGTAGTAGTTCCGGTGTGGATGGTAGAATTGTTGTTAATAAAAAAAATCTTGTCAAAAAACATTGTATAATTATAAGAGCATTCTATGTAACACACAAGAGGAGTGATATGATGTAATCACACCTAGGATTTCTGGGGACTACTGTTTGTTCACAGGTGTTTATTATATCATGGCTCCAGAGTGTGGCAGATGCAATATTTATTACGACCACAACTCCTCCGCATATGTGAGATCACCCACGATCCTTCCGCATGTTCTCCCCCCGGGAGAAAAAAAACGGTGACTCTATTCGGAGAGGGTGGGGAAAAATA
36	SrAp-6	*S. rolfsii* (GQ358518)	94	>TTGCCTCTGACTGGTAGTAGCAATGGAGTTGTGCTGGGAATCGATATTGCATGTGCACACTCTGTAGCGCGCATGATTTTCTCAGTTGTGCATACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATTTCATATTGAAGCTTTGTTTTCTGATACGTTTCTCTTAATTATGAATATACCACTTTTTTCAACGGATGACTTGTATCTTGCTTTGTTGAAGAAAGAATCGACATGCTATCAGTAATGTGAATTGCAGAATCCCCTGAATCATCAAAGCTTTTTCGAGACTCGATGACTCTGTTGATTCTAGGAGTCAGCTTATTTGATAGTCTTCAATTTCTTCTCCATACAAAATATTGTAGATATCTCCGCTGGTGAGTGGTAGTTGTTTTTAAAGAAAAAAACTGGGGAGCTCTCTATTTTTCTATTATAAGGCGTTCTTGAAAACCCCCCAGGGGGTGTGATAATGTGATCACCCCTAAGGATTTCGGGTGAATCTGGTTGGTTCACGGGTTTTTAAAAAAAAATGCCCCGAGAGGGGGGACAAGAGTAATTTTTTATGAGGATTATTTCTTCGTATATGAGAGTACACTAGCGGTCCTCCCCCCTGTCACCCGGGGGAAAAAAAAAAACTTTTTTGATTTCATATTTTCCAAGGGATTTGTGCTGGTAAAAAAAAATGGTGGGCCCCCCCCCGGAACCTTTAAA >GGGGAATAGTGATTGGAGTAGCATGAGTGAGGCAGTGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTAGGCGCGGCCCTTATTCTCACTCACCGCGTGTGTTTTTCTACGTGTTCCTATTAAAGTTTTGGAGAGAGCGTGTGATATTATACTCTAAAAATATATCACATTTTCCAAGCCTTGACATATACATTTTTTGTTGGGGAAAGTATTGAAACGCTCTCAGACATGCGCGCCCCAGAAACCACCAGAGGGCGCGAGGTGTTTAAAAACACTCGATGACTCTGTGGTTTCTGCGAGTCAGCTTATTTAAGAGTTTTCGGTGTCTTCTCCATCCAAGAAAGAGTAGATATCTCGGTTGGAGAGAGGTATATGTTTTTTAAAAAAAAAAAAGTTGGGAGAAAACTTTTTTTCTATTTTAGAGCGCTGTATAAAACACCCCACAGGGGTGTGATGAAGTGTATCCCCCTATGGCTCTCGCCGGGAGACTGTTGGTTCAGAGGTATTATAAAAAAAAAAACCCCCCAGAGGGGGGGGCAAGAAATATTTTTTATGAGCAATATTCTCCGTATATGAGAAACAACGATCGACCCTCCCCGCAGATCCCCCCGAGAGAGAGAAAGAAACTTTTGAATTCAATACCGAGGGATTGGTGGGGTAAAAAATAGGA
37	SrAp-7	*S. rolfsii* (GQ358518)	95	>GTGCAGTGATGATATACGCGAGGAGTGATGCTGGGGTCGATTTGCATGTGTATGCTCTGTAGTGCGCATGATTTTCTCCTGTGTACATACTGCTAGAGGACCTTCCGGACTATGATTACACTATAAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAATCTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACTACTTTCTACAACGGATGACTTGACTCTTGTTTCGATGGAGAACACAGCGAAGTGCCATAAGTAATGTGAATTGCAGAAACACCGTGAATCATCGAATCTTTTAAAAGACCTTGTGCCCCTTGGTATTCTGCGGGGCATGCTTCTTTGAGAGTTTTTCTATTCTCATCCTTACTAAAATATGTATTTGTTCCGGCGTGGATGTTAGAATTGTTGGTTATAAAAAAATCTTGACGGGCTACTTTTTATCATTATAAGAGGATTCTATGAAATACCCAAGGGGGGTGATATAATGTATTCACACCTATGATTTCTGGGGACTACAGTTTGTTCATAGGTACTTATAATATCATGCTCCATATTGTGCAGATGCAGTACTTATTACGACCACTTCTCCTCCGCTGATGTGGGATCACCCGCTGTCCTTCCGCAGATCTCCGCCGGAAAAAAAAAAACTTTTTGAATTATATCTGCGAAGGAGTTGTGCTGGTAAAAAATATTGCCTGTCCCCCCCCGCATAA >GGGGGAAATGGCTGGATATCGTACTGAGTGAGGCTGTGGTCGATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGACTAGGCGTACACTATAATCTACCATCGGAAGGCATTTCTAACGGTCCCATAATGAATTTTTGTTTTAGCCAGTGAGTATCTTTTCTAACCAGCTACTACTACTTCCAACGGTTGACAAAGACAATTGTTTGGATGGTGAAGAATTTGAAGACTCTCAAGCAGTGTTGCCCGTAGAAACACCAGAGGGCGCCGGGTCTTTTAAAAGACCCGATGATCCTCGGTATTCTGCGGGTCATATTACTTATAGAATTTTGCTGTTTTCTTCCTCACTACAATATCTAAGAGATCCGTTGTGGATGGTAGAATTGTTTTTAATAAAAAAATCTTGACAAAAAACATAGTTTAATTATAAGAGCATTCTATGAAACACACACGAGGAGTGATATAGTGTATTCACACCTAGGATTTCTCCTGACTACAGTTGGTTCACAGGTGTATATATTATCATGGCTCCAGAGTGTGCACATGCAATATATATTACCACCACAACTCCTCCGCATATGTGGGTTCACTAATGGTCCTTCCGCAGGTGCATCCGGCCAGGAGGAAAGAACTTGTGACTCTATCTGCGAAGGAGTGGTGCGGAAAAAATGGGGGGC
38	SrAp-8	*S. rolfsii* (DQ484061)	95	>GGCATTGATCTATATGCGAGGAGTGGTGCTGGGATAATATTGCATGTGCACACTCTGTAGCTATACAAGATATTCTCATGTGTGCATACTGTAGTCAGGAGAAATCCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAGCTTTGTTTTTGTTACGAATTTCTCTAATTTGAAAATATCACATTTTCAACGGAGGACTTGTTGCTTTTTTCGTTGAAGAAAGAAGCGACACGCTATCAGTAATGATGATTGTAGAAGAACCCGGAGCATCGACGAATCTTACGACGCTCGTTGCCCCCGTTGTTCCTACGAGGGTGCTGATTTGATAGTAATCAGTTTCTTCTCCATCTAACAAATTGTAGATATCATCGCGGGTAGGATGTAATTGTTGCTTAAAAAAAATATTTGGGGGCTCCTCATTATAATTATAAGAAGGTTTGTTAGAAACCCCAGCGGTGGTGATATTAAGTCATCACCGCCACTACTGCAGGGGGAAGCTGGTTGGTTCGACTAATTATAAAATAAAAGCCCTGCGTATGTGGGCCAGAGAATATTTTTTAGGGGGATATTTGTTGATTAAGGAGGACACCCCCACCCACCCTCCCCCCATTCACCCGCGGGAAAAAAAAGA >GGGGCTTATTGGATCTAGTAGTACTGAGTGAGGCAGTGGTCATATATGCACGTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACTAGCTAGAATCCCCTTTGTGACTATCGTTACTCATTATCTCACTTATTGCATGTTACATAGTACGATCTCCTATTGAAGCTTTGGAGAGTGAGACGGATATTCTTAACTAGAAAATCTCACATCTTTCAACTGAGGATATGTAGATTTTTGCGTTGGAGAAAGTATTGACTCTCTAACAGGCATGCGCCTTGTAGAATCAAAGGGAGCATCAACGTGTCTAGAGACACTCGATGACTCTGTTGTTCCTACGAGACAGCTTATTGGATATGGCTGCGTGCCTTCTCCATCCAAGAGATTGTAGATATCTTGGTGGGAAGGATGTATTTGTTGCTTAAAAAAAATTTTTGGGGAGAAACTCATTTTTATAATATGAAGGTTCGTTGGAAACCCCACCAGGGGTGAGATAAAGTCATCACCGCTAGGACTTCTGCGGACAGCTGGTGGGTTCCACGGTTTTTTATAATAAAAGGCCGCTGAAGGGGGCCAGTGCATTTTTTTTAGAACCAATATTTTTTTGGCATGTATGAATTCACCGATGCTCCCTCCCCCCAGTCACCCGGCGGGAGAAAAAGAGTTTTTTTTTCCCGAGGGGGGGGGGGAAAATT
39	SrAp-9	*S. rolfsii* (GQ358518)	94	>GGCCACTGATGATATATGCAAGGAGTGGTGCTGGGATGATATTGCATGTGCACACTCTGTAGCTATATATGCTTTTACCTGAGAACCTACTGTCGTCAGGAGCATCCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAGCTTTGTTTTTTTTTACAATTTTCTCTTAATTAAAATATAACCTACTTTAAAGGACGGATTGACTGTTTCTTGGATTGAGAAAGAACGGAACTGAATACAGTAATGTGTGTTGAAAAAACAATCGAATCAATCAACGAATCAATGAACGTAGCGCGCCTCGGTAGGCAGTCCGGGGGACATGTCTGAGTGAGAGTCATTACTCTCCCATCCTAACGAATGTATGTATTTGGGCGGGGATGGTAGAGTTGTTTGTTAGAAAAAAATCTCTCAGTCTCTCTCTTTTTCATTATAAGAGGGACGTATAAAACAACCCGCGGGGTGTGTGTATATATATCTCCGCACGTATTTCTGGGGAATACTGGTTGGTTCGACCAATTAAAAAAAAAAATGCCCCCTATGGTGGCAAAAAAAATATTTTTTAGGACCAAAATTCTCTATTAGGAGAAATCACCACCGATCCTCCCCCAGTTCTCCGACGGAAAAAAAAAA >TGGGCAATTTTGGACTATATACGCCATGAGTGATGCTGTGGTCATATATGCACGTATATGCTCTATATATGCGCATGATTTTATAAGTAGTACATACTATCGAGAGTCCCCTTCCGGACTATGATTACACTATATAACTCTTATTGTATGTTATTAGAATGATCTCATATTGAATCTTTGTTTTTTTTTACAATTTCTTTTTAATGAAATATAACCTCCTACCACCGACGAATTGACTGTTTCTTGGATTGAGAAAGAAATGACCCTCATACAGGAATGCCCCTTGCAAAAACAAAAGGGGCAAGGGGGGGTATCAAAGAACGCGATTCGCCTCGGTATGCAGTTCAAGATGCTTGTCTGAGTGAGATGCATTTTTCTTCCATGCTAAAGCATGTATGTCTTTTGTTGTGGATGGTAGATTTTTTTGTTAGAAAAAAATTTTTGAAAAAAAAACTATTCTTCATTATAAGAGGGACGTATGAAAAAACCCGCAGGGTGTGTGAGAATATATCTACGTATGTATTCTGAGGACTACTGGTTGGTTCGTCGGATTAATAAAACCATGCCTCCTAAGTGGGCGCAAGAATAATTTTTTGGGGGAATACATCTTCGTTAAGGAGGAAATAACAGACCAACCTCCCGCAGTTACCCGCCGGAAAAGAAAAACTTTTGGATTTCTTGCAGCGAAGGAGTGGTGGGGGCAAAATTGGGGGGGCCCCCCCCCCGCCTCTT
40	SrAp-10	*S. rolfsii* (DQ484061)	94	>GGCATGTGACTCATATATAGCACATGGAGTTATGCTGGGGTCGATAATTGCATGTGCACGCTCTGGTGTGCGCAAGATTTTCTCCTGTGTACATACTGTAGTCAGGACAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATTTCATATTGAAACTTTGTTTTCTGACACGTTTATCTTAATTTAAAATATATCACATTCTACAACGGATGACTTGTATCTTGCTTCGTTGAAGAAAGAATCGAAATGCTCTCAGTAATGCGAATTGTAGAAACCACCTGAATCATCGAATCTTTTAAAAAACCTCGATGCCTCTGTTGTTCCTACGAGTCAGCTTATTTGAGAGTCTTCAATTTCTTCTCCATCCAAACAATTGTAGATATCTCGGCTGGTAAGTGATATTTGTTTTTTAAAAAAAAATTCTTACGGGCTCTCTATTTTTCTATTAGAAAGACTTCTAGAAAACACCACAGGTGGTGTGATAATGTGATTACCCCTATGGATTTCGGGGAACTATGGTTTGTTCATAGGTGTATATAAAAACATACCTCCAGAGTGTGTACGTGAGTATTTATTTACCACCACCACTCCTCCGCATATGTGAGATCACCAGCGATCCTTCCGCAGGTCCCCCGCAAGAAAAGAAAAAAACTTTTTGAATTCATATTTCCGAGGGATTTGTGCTGGGTAATGAAAATGGCAGGC >GGGGAACTGGACTGGATGTCGTACCTGAGTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAGCTAGAATCCCCCTTGCGGTAGGCGCGGCCACATATTCTCACTCACTGCGTGTGTTTTTCTACGTGTTCCAATTAAAGTTTTAAAGAGAGCCTCTCATAATATACTCTAACCTATAACTCTTACATCCCCTGCTTGACATATACATTGTTTTGTTGGGGAAAGAATTGACTGACTCTCAGACATGCGCGCCCCTCAAAATACCGAAGGCGGAGGCGTTTTCAGAGATTCGATGACTCTGTGGATTCTGCGAGTCAGACTATTTATCGCATTTCGCTGTCTTCTCCATCCAAGCAAGAGCAGATATATCCGTTGGTGAGAGATATATGTTTTTTAATAAAAAAAAATTTGGCAGAAAACAATGTTTCTATATGAAATCGTTCTATGAAACACCCCAGGAGATGTATTTAGGTGAATCATAGTATGGATTTCTCCGGACTACGGTTTGTTCACAGGTGTATATTATATAATGCTCCAGAGTGTGCACATGAGTATTTTTTACCACCACCACTCCTCCGCATATGTAAGTTCACTAACGATCCTTCCGCAGGTCCCCCCGCGAGAAAAAAAAAATCTTATTGAATTCATATTTCCGAGGGATTTGGGCGGG
41	SrMh-1	*S. rolfsii* (AB075318)	96	>TGCAATCTGGCTCTAGTGCGATGGAGTGTGCTGTGATGATATTGCATGTGCACACTCTGGAGCTATATAAGATATTCTCCTGTGACCACTGTAGTCAGGAGATTCCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAACTTTGTTTTCTGACACGTTTCTCTTAATTAAAAATATACTACTTTCAACAACGGATCTCTTGGCTCTTGTTTCGATGAAGAACAATTCGAAGTGTCATAAGTAATGTGAATTGCAGAAACAACTGAATCATCGAATCTTTTAACGCACCTTGTGCCCCTTGGTATTCTGAGGGGCATGCTTGTTTGAGAATTTTTATATTCTCCTCCTTACTAAAATTTGTATTAGTTCCGGCGTGGATGGTAGAATTGTTGGTTATAAAAAAACTTGGCGGGCTCTCTTTTATAATTATAATAGCATGTCTTTGAAACACCCACCAGGGGTGATATAATATGATCACACCTACGATTTCTGCAGGACTACTAGTTGGTGTGTAGTGTTTATTATATATAATAGCTCCATAGTGTGCACATGAAATATTTTTACCACCACAACTCCTCCGCATATGTGGTATTACCACCGATCCTTCCGCAGCTCTCACGGCGAAAAAAAGAACTTATTGTATTCATATATGCGAAGGAGTTGTGCTGCTAATGAATATGCATGTGCACAC >GGGCAATATGCTGGAGTCGTATTGATGTGAGGTCAGATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTATAGGCGTAAACTTATTATCACCCCAACGGAAGGCATTTCTACAGGTCCAACAAAGAATTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCTACTCTCACATCCCAGCCTTGACAAATACAAATTTTTGGAAGGTGAAGAATTTGATGACTCTCAAACAGGGCTGCCCCTCGAAACACCAAAGGGCGGGAGGGGCGTTCAAAGATTCGATGATTCACTGGATTCTGCAATTCATATTACTTATCGAATTTCGCTGCTTTCTTCATCGATACAAGATCCAAGAGATCCGTTGTTGAAAGTAGAATTTTTTTTTATAAAAAAAACTTGGCAAAAAACAAAGTTTCATTATGAGATCATTCTATGTAACACACAATAAGAGTTATATAGGGTAATCATAGTTAGGATTTCTCCTGACTACTGTTGGTTCACAGGTGTATATTATTATATAGCTCCAGAGTGGGCACATGCAATATTTATTACCAGCACAACTCCTCCGCATATGTGAATTCAATAATGATCCTTCCGCATGTTCTCCTGCGAGAAAGAAAGAACTTATTGAATTCATATATGCGAAGGAG

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
42	SrMh-2	*S. rolfsii* (DQ484061)	93	>TGCACTGACTGATATTAGCAATGGAGTGGTGCTGGGATGATATTGCATGTGCACACTCTGAAGCTATATATTATATTCTCAGGTGAACCAACTGTAGTCAGGAGAAATCCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAATCTTTGTTTTTTTTTACGAGTTTCTTATATATGAAATATACACCTTTTAACAACGCAGCTCTTGTGACTTGTGTCGATGAGAAAAACATAGCGATGCGACAAGTATTGTGAATTGCAGAATCCAGGGGGAAGCGAATCTTTGAACGCCCCCCTGGCCCCCTGTGTTCCCCAGAGGCAAATGCTTTTGAGAGTCAGCTTATATTCTCACCTATACAAATTTGTAAATGTCTCGGGTTGGATGTGAGAGTTTTTCTTTAGAGAAAATTGTGAGTGGCTCTCTTTTTAACTATTAGGAGGACATATAGAAATGCCCACGGGGGGTGTGATAAGATGTCTACGCCTATACCTCAGGGGGATTCCGGTTGGTTACAAGTTTTTTAAAAAAAATCCCCCCATTATGTGGCCAATAGAATATTTTTTTGGCCATTATGTCTCTTATGAAGGGGGGAACCCACCCCGGGACCTCCCCCGTTCACCCCGGGGGAGAGAAAA >GGGATTTTGCTGGATACGTACTGAGTGAGGCGTGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAAGCTAGAATCCCCTTCCGGACTAGGGTTACCCTATATCCTCCCACCGGAAGTTATTCCAAATGATCTCATAATGAATTTTTGGTTTGGCAGTCAAATATTTTTAATAAGAAACATTCACAATTCAACAGCGTTAAAAAATAAGATTTTTGAAGATGAGAAAATTATAGCGATTCTACAAGTATGGTGCCCTGCAGAATACAGGGGGGCAGGGGGGGCTTTGAAAGCTCATGATTCCCCCTGTATGCTGCAGTTGAAATTACTTATGTGAGTCTGCTGACTTTCTCAACGATACAAATTTGTGAATCTCTGTTGTTGAATGTGTGAGATTTTATTAAGAGAAAATTCTTGAGAGACTCTCTATTTCAATATTAGGAGCACATGTAGAAATGCACACAGAGGGGTGTGATAAGGTGTCTACGCCTATATCTCCTCAGGATACCGGGGGTCACAGTCTTTATATATAATCGCCCCTATGTGTGCACTGAGATATTTTTACCCCCACCTCACCCCTGTACATGTGGTATCCACAGTGCTCCCTCCGCGGTTCTCTCGGGGGAAAAAAAAATTTCGTTCTTCCAAGGGGG
43	SrMh-3	*S. rolfsii* (DQ484062)	89	>CCGGGGGGTTCGGCGCACTGATCTATGAGCAAAGAGTTGTGCTGGTAATGATTTGCTGTCCACCCCGGTCCTTAAGTTCTAAAAGGGGCCACCCGGGGTAGGACATTTCGAAATCCCAATCGATAATACTCTCATGGGGGGCCATAGAAGGTTTTCTAATTAAAGTTTTGGTGGTTGCCGGAGTTTTCTCTTTTAAAAAAAAATCACCTTCTTCCCCCCGCCGGTTCTTTGCTTTTTGTGTGGAGGGAAAAAAGTACAACCCCCCAAAAAGGGGGGCTCCCCGAAAAAAACCAGGGGGAGGGGGGGGTTTTTAAAAACCCCCCTCGCCCCCGTGTTTTCTCCCGGGGGGCCCCTCTTTGTGAGGAGTCATTTTTTCCCCCCCCCCCAAAAATTATAATAATTCCGGCCGGGGGGGGGGGGAGTGTTGGCTTTAAAAAAATTTGGGGGGGGCCCCCACAACCTTATTAAAGGGGGGGGGGGGGACTCCCCCCCCGGGGGAGGAAGGGAGTTTAAGCCACAGTTCTCCGGGAGCCCGGGGGTGTGGGGTTC >GGGGGTGGCTGAGGGGAAATGCTAATACGTCAATGGATTGGGGCAGGGATCAATTTTGCACGTACACGCTGGGAACGGCACACCCCCCTTTAAAAAGGGAAAACATAGGGGCACAACCCCCCCATTCCGCCCCTGGAAAACCTTTTTTTCCCCCCCCCGGGGGGGTTTCTTCCCCCCGCCCCACATAAATATTTTTAGGGGGGGCAAAAAATTATACTCAAAAAACATCCCCCCCCTACCACCGAGGGGTGAAAATATATCCCTCCCCCCCCCAAAACAATTTAACTTCTACAAGAGGGGCCCCCTTTAAATGGCTTTAAAAAAATTTGGGGGGGGCCCCCACAACCTTATTAAAGGGGGGGGGGGGGACTCCCCCCCCGGGGGAGGAAGGGAGTTTAAGCCACAGTTCTCCGGGAGCCCGGGGGTGTGGGGTTCvTGGCTTTAAAAAAATTTGGGGGGGGCCCCCACAACCTTATTAAAGGGGGGGGGGGGGACTCCCCCCCCGGGGGAGGAAGGGAGTTTAAGCCACAGTTCTCCGGGAGCCCGGGGGTGTGGGGTTC
44	SrMh-4	*S. rolfsii* (GQ358518)	89	>TGCATGTGGCTGATAGCAGCACTGGAGTGGTGCTGGGATCGATATTGCACGTGCACACTCTGTAGCTATATATCACATTACATGAGAACATACTGGCGACAGGAGCATCCTGACTATGATTACACTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAATCTTTGTTTTTTTTTTACAATTTTCTCATTAATAAAAAATATCCTTCTACAACCGACGGATCTACTGTTTTTTGGATGGATGAAGAAACGAAATGTCATCAGTAATGTGCCTTGAAGAAACAAAAGGGGGCAAGGGGGGATTCATAGAACCCAGCGCGCCCCGGTTGTCAGTCCGAAGGACATGTTTGAGTGAGAGTCATTTCTTATCCATACTAAAAAATGTATGTATTTTGTCGTGGATGGTAGAGATGTTTTTTATTAAAAAAATTTCTGAGTCTCTCTCTTAATATATATAAGAGGGGCGTAGGAAAAAGCCCGCAGGGTGTTTAAAAATATATATTCATACATATTCGGGCGAAAGCTGGTTGGGTGGAGGGATTTATTAAAAAAAATCCCCCCCAAGGGGGGGGAAAAAAATAATTTTTTTGGAGGCAATATTTCTTTAATAAGGAGGAGAACACCGACGGTCCCTCCCCGCGGGTCATCCCGCGAAAAAAAAAAA >GGGGGAATATTTGGTTGGCATTCGTAATGAGTGAGGCAATGGTCATGTATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGATTAGGGTTAAACTATTAACCCACCCACCGGAAGGCTTTCTTCATGATCTAACAATAAATTTTAAGATTGGCCGTCCGATATTCTTTACTAGCAGCTCTTACCTCCCAGCCTTGACGAAAACTACAATTTTTTGGGTGGAGAAAATAATGACGCTCAAACAGGCATGCCCCTCGGAAAAACACAAGGGGGCGGGGGGGGAATCAGAAAAGGCAATTCACCGCTGTCTTCAGTTCATCATACTTATCGGAGTTTGATGCGGTCTTCTTCCATGCTACAGCATCTATGACCTTTGTTGTGGATGGTAGATTTTTTTTTTATGAAAAACTTTTGGAAAAAAAACTATCTTTAATTATAAGAGGGTCGTTGGAAACAACCAACAGGGGTTGTGGGGGTGTATGTCCGTACGTATTCTGCCGACAGCTGGTGGGTCGAGGGTTTTTTTATATAAAATCCCCCCTAAGGGGGCAAAAGAAATATTTTTTTGCCGCCCCACCTCCCCCCCCTAGGAAAAATCAACAATCCATCCTCCCGCCGGTTCACCGGCGGAAAAAAAAAATGTTTTTTGGGTAAAAGTTTCTATAATGGCTTTAAAAAAATTTGGGGGGGGCCCCCACAACCTTATTAAAGGGGGGGGGGGGGACTCCCCCCCCGGGGGAGGAAGGGAGTTTAAGCCACAGTTCTCCGGGAGCCCGGGGGTGTGGGGTTC
45	SrMh-5	*S. rolfsii* (DQ484062)	92	>TTGCACTGCTGGTAGTGTACTGGTGTTGTGCAGTGAATCGATATTGCATGTGCACACTCTGTATATGCGCAAGATTTTCTCCTGTGTACATACTGTAGTCAGGACCTTCCGGACTATGATTACCCTATATTACTCTTATTGGATGTTACATAGAACGATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTATAAATATACTACTTTCAACAACGGATCTATTGACTCTTGTTTCGATGGAGAACAATTCGAAGTGCCATAAGTAATGTAATTGTAGAAACAAGTGAATCATCAAATCTTTTAAAAGATCTTGTGCCCCTTGGTATTCTGAGGGGCATGTCTGTTTGAGAATTTTTCTGTTCTCATCCTCACTAAAATTTGTATTAGTCCCGGTGTGGATGGTAGAATTGTTTTTAATAAAAAAATCTTGACGGGCTCCCTTTTTTCATTATAATAGCATCCGTTGAAACACCCACGAGGAGTGATATAATGTGATCACACCTATGATTTCTGGGGACTACAGTTGGTTCACAGGTGTATATAATATAATAGCTCCATAGGGGGCAGATGAAATATTCTTTACCAGCACTACTCCTCCGCATATGTGAGTACACTAATGATCCTTCCGCATGTCTTCCGCGAGAAAAAAGAACTTTTTGAATTCATATTGCGAAGGAGTTGTGCTGGTAAAGAATATGCATGCCGCCCCCGCCAGCACTA >TGGGAATTTGAGGAAGTCGTCTTGATTGAGGTCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTATAGGCGTAGACTTATTATCACACCAACGGAAGGCATTTCTACAGGTCCTACAAAGAGTTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCTACTCTCACATCCAAGCCTTGACAAATACAAATATTTGTAAGGTTGAGAATTTAATGACTCTCAAACAGGGTTGCCCCTAGAAATACCAAAGGGCGCAAGGTGTGTTCAAAGATTCGATGATTCACTGTATTCTGCAATTCATATTACTTATCGCATTTCGCTGCGTTCTTCATCGATACAAGATCCAAGAGATCCGTTGTGGAAAGTTGAATTTTTTTTTATAAAAAAAACTTGGCAGAAAACAATGTTTCATTATGAGATCGTTCTATGTAACATACAATAAGAGTGATATAGGGTAATCATAGTTAGGATTTCTCCTGACTACAGTTGGTTCACAGGTGTATATTATTATATAGCTCCAGAGTGTGCACATGCAATATTTATTACCACCACAACTCCTCCGCATATATGAATTCAATAATGATCCTTCCGCAGGTTCTCCCGGGGGGAAAAAAAAACATTATTGAATTCATATCTGCGAAGGAGTTGTGCT
46	SrMh-6	*S. rolfsii* (GQ358518)	92	>GGGCTTGGACTCATATAAGCGAGGAGTGGTGCTGGGAATGATATTGCATGTGCACACTCTGTAGCTATATAATATTTTCTCATGTGTGCATACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAAATTTACAACTTTTAACAACGGATCTCTTGGCTCTTGTTTCGATGAAGAAAGCATCGAAATGCGATAAGTAATGTGAATTGCAAAATCCAGTGAATCATCGAATCTTTGAAAGCACCTTGCGCCCCTTGGTATTCCCAGGGGCATGCCTTTTTGAGAGTCATTAAATTTTCTCCCCTACAAAAATTTGTATATGTCAGGGGTTGGATGTGAGAGTTGTTGTTTATAGAAAATACTGACTGGCTCTCTTTAAAACTATTAGTAGGACATGTAGAAATGCCCACGGGGGGTGTGATAATGTGTCTACGCCTATACCGCAGGGGGATACCAGTTGTTTTCAACTAATTAATAAAAAAAATGGCGCCTAATGTGGCAAATACAATATTTATTACCCAATATGACTTCCGATAAGGTGGGACTACCGCGGTCCTCCGGATGTATTCGGCGGAAAAAAAAATTTTTTTTTTTTTTTTTTCAAAGTTTATTGGGAAAAAAAAATCGAGGGCCCCCCCCCGACT >GGGGGATTTGGCTGGGATACGTACTGAGTGAGGCTGTGGTCATATATGCACGTATATGCTAGATATATGCGCATGATTTTATAAGTTGTGCATACAAGCTAGAATCCCCTTCCGGACTATGATCACACTATAACCTCTTATCGGATGTTATTTAGAACGATCTCATATTGAATTTTTGTTTTAGCCGGCGAGTATCTTTATTAAAAAACATACACCTTTCAACAGCGGTGAAAAATGGAAATTGTTTCGAGGAGGAAAATTTAGCAATGCGAAAAGTGGGGTGCCTTGCAGAATCCAGGGAGGCATCGGGTCTTTGAAAGACCCGATGGCCCCCTGGATTCTGCAGGGGAAATTACTTTTGAGAGTTCGCTGATTTTCTCCCCTACACAAATTTGTATATATCCGGTGTTGAATGTGAGATATTCTTTTAAGAGAAAATTCTGAGAGGCTCTCTTTATCACTATTAGGAGCACATGTTGAAACACACACGAGGGGTGTGATAATGTGTCTACGCCTATATTTTCTGCGGACTCCAGTTGGTTTCCACGAATTTAAAAAAATAATAGCCCCAGTGTGGTGGCACATAGAAGCTTTATATAGAGAATATGTTCTTTGGCAATGGAGAATCTCACTAGTGGATCCTCCCGCAGATCTCCGGGGGAAAGAAAAATTTGGACTAGGTAAGGTTGCGA
47	SrTn-1	*S. rolfsii* (DQ484062)	93	>GGGCATGTGACTCGATTAGCAATGGAGTGATGCTGGGGTGATATTGCATGTGTATGCTCTGTAGCTCGCATGATTTTCTAAGGTGTGCATACTGCGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATTTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATATCACTTTTTCAACGGAGCTCTTGTATCTTGCTTTGATGAAGAAAGCAGCGAAATGCTATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCAAATCTTTTTACGAGACTCGATGACTCTGTGGTTCCTAGGAGTAAGATTATTTGAGAGTCTTCAATTTCTTAACCATACAAATTTTTGTATTTATCACGGCTGGTGAGTGATATTTGTTTTTTATAAAAAATTCTGGGGGGCTCTCTTTATTACTATTAGTAGGACATGTATGTAACACCACAGGTGGTGTGATAATATGATTACCCCTATGCATGTCGGGGAAATCTGGTGTGTTTGAGGTTTATATAAATATAATGCCCCTAGAGTGCGCACAAGAGTATTTTTTTACCCCCACCCCTCCCCCGCATAGGTGAGACACCAGCGGTCCTCCCGCAGTCCCCCGGGGAAAAGAAAGAGTTAGGGATTCATATGGCGAGGGGGTGGTTGGGGGAAAAAAAATAGGGCGGCCCCCCCCAGGA >TGGGGATTTGATTGCAGTCGTACTGATTTGAGGCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATAGGCGTAGACATATTCTCACACATAGCGTGGGCTTTTCTACGTGTTCCAATTAGAGTTTTAAAGAGAGCCAGTCAGATTATACTCTAACAAGTATCTCTTACATCCAAGCCTTGACATATACATTTATTTGTTGGGGAAAGAATTTAATGACTCTCAGACATGCGTGCCCCAGAAAATACCAGAGGGCGCAAGGTGCTTACAAAGACTCGATGACTCAGTGGATTCTGCGAGTCAGCTTATTTATCGCATTTCGCTGTCTTCTCCATCCAAGCAAGAGTAGATATATCCGCTGGTGAGAGGTATTTGTTTTTAAATAAAAAAAAATTTGGCAGAAAACAATGTTTCTATATGAAGTCGTTGTATGAAACACCACAGGAGATGTATATAGGTGAATCATAGTATGGATTTCTGCTGAATACTGTTGGCTCACAGGTGTATATAATATATAGCTACAGAGTGGGTACATGAGTATTTATTTCCACCACCACCCCTCCGCATATGTGAATTCACTAATGATCCTTCCGCAGGTCCCCCAGGGGAAAAAAAAAAATGCGATTACTTCTGGGGG
48	SrTn-2	*S. rolfsii* (AB075318)	93	>GGCATGTAGCTTCGATGAGTAATGAGTGGTGCAGGGAATCGATATTGCATGTGCACACTCTGTATATGCACATGATATTATAAGGTGTGCATACTAGCTAGAGGAGAAATCCTGACTATGATTACTCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAGCTTTGTTTTCTTTTACGAATATCCCTAATTTGAATATCTCACTTCTTGAACTGAGCTCTTGTAGATTTTTTTGTTGAAGAATTTATTGACACGCTATCAGGCATGCGCATTGTAGAATCCTCCAATGGCTCAAAGAATTTTTCAAGACTCGTTGACTCTGTTGTTTCTACGAGAGTGCTTATTTGATAGTACTCAGTTTCTTCTCCATCCAAGAAATTGTAGATATCGTCGCGGGTAGGGTGTATTTGTTGCTTAAAAAAAACTTTTGGGGGCTCCTCATTTTAATTTTATGAAGGTTTGTTGTAAACCCCACCGGGGGTGTTAATAAGATATCACCCCCACTACTCCAGGGGACAGCTGGTTGGTTCCACGGATTATAAAATAAAATCCCCCGAAGTGGGGCAGAAAATTAATTTTTTAGAGCAAATATCTCTTGGTAAGGAGGAAATCCACCGCGCGGCCTCCCCCGCCAGTCACCCCGGGGAGAGGAAAAAAGTATTATTTTATTCAAGGATTTGGGGAAAAAAATGC >GGGGCAATTTTGATGCATATCGTATTGATTTGAGGTCAGATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAAGCTAGAATCCCCCTTCCGGTAGGCGTAGACATATTATCTCACCCACCGCGGGGGTTTCTCTACGTGCTCCAATTAGAGTTTTAGAGAGCGCGTCAGATATTCTTAACTAACCACTCTCACATCCAAGCCTTGAGTGATACATAAATTTGTGTGGTGGGGAAATTATTGACTCTCTCTCAGACATGCGCCTCGCAAAAAATAAGGGCGCGCGGTGCGTTTTTGATAGACTCGATGACTCAGTGGAGCTGCGACACACCTTATCGCATTTCGCTCCGTGCTTTATCGATCCAAGAGAGAGGAGATATATTGGTTGGAGAGGTGTATTTTTTTTTTAAAGAAAAAATGTTGGCAAAAAACAATCTTTCTTATATGATCGCTCGTTGTAAACAACACAAGAGGTGTTTTAAAGTGATCCCTACCAGGACTTTCCGCCGACAGACTGGTGTGTTGCCGGGTTTATTATATAATATGCCCCGAGGGGGGGGCGCAAGATTTTTTTTACCGCCCCCCCCCCCTCCCTAAGTATGAATTAACGATGGTTCCCCCCCCCGTTCCCCCGCGGGAGAAGAAAAAATTTTGAATTCTTTCCT

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
49	SrTn-3	*S. rolfsii* (AB075318)	94	>TGGCATTGATGATATAAGCAAGGAGTGGTGCTGGGATAATATTGCATGTGCACACTCTGTAGCTATACATGATTTTCTCATTTGAGCATACTGTAGTCAGGAGAAATCCTGACTATGATTACTCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAGCTTTGTTTTTGACACAAGTTTCTTTAATAAGAAAATATCACATTTTTCAACGGCGGACTTGTTGCTTGCTTCGTTGATGAAAGTAGCGACATGCTAACAGGAATGATGATTGTAGAATCAAGTGGAGAATCAACGATTCTTTGAACCCTCGTTGCCCCTGTTGTTCCTACGAGAGAGCTGACTTGATAGTAATCAATTTCTTCTCCATCCAAAAAATTGTAGTTATCGTCGCGGGTAGGATGTATTTGTTGCTTAAAAAAAAATTGTTGGGGGCTCCCTTTTTTAATTATATGAGGGCTTTTGGAAACCCCCCCGGGGGTGATAATAAGTCATCTCCCCCAATACACAAGGGGATATCAGGTGGGTTCCCCGGTTTTTTTTTTTTCCGGCCCCTTATGTGGGCAGACAATTTTTTTTTTGAGGAAATATTTCTTCATTAAGGAGGACACCACCATCACCCTCCCCCCGTTCCCCCCCGGAGAGAAAAAAATTCACGATCTTTTCGGGGGGTGGGAAAAATTGGGC >TGGCATTTGCTGGATATCGTAATGAGTGAGGCAGATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAAGCTAGAATCCCCCTTCCGATAGGCGTAGACATATAATCACACTCATTGCGTGTTTTTCTCTACGTGCTCCTATTAGAGTTTTGGAGAGCGAGACAGATATTCTTAACTAGAAAATCTCACATCCAAGCCCTGAGAAATACATAGATTTTTTTGTTGGAGAAAGTATTGACTCTCTCTCAGACATGCGCCTTGTAGTATACAAGGGCGCAGCAACGTTTCTAGAAACGCTCGATGACTCTGTGGTTCCTGCGAGACACATTATTTGATGTTGCTCCGTGCTTCCTCCATCCAAGAAAGTGTAGATATCTTGGTGGGAGAGATGTATTTGTTGCTTAAAAAAAAATTTTGGGGGCAAACTCTTTTTTCTTTTATGGGGGGTCGTTGGAAACACCACCAGGGGTGTTGAGAAGTGATCTCCCCCAGTACTCCTGCGGACAGCAGGGGGGGGTTGCAGGGTTTTTTATAAAAAATCCCCCGAGTGTGGGCGCAAGCATTTTTTATTAGCAGGAATAAGTCGTTGGGTAGGGATAATACACCGAGGTCCCTCCCGGCAGTCCCCCCGGGGAAAAAAAATGTCACGACCGTG
50	SrTn-4	*S. rolfsii* (DQ484062)	95	>TGCATGTGACTGATAGGTCGCACTGGAGTTGTGCAGGGAATCGATAATTGCATGTGCACACTCTGGAGCTATACAAGATATTCTCCTGTGTACCACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATTTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAAAATATCACTTTCTTCAACGGATCTCTTGTATCTTGCTTCGTTGAAGAAAGAAGCGAAATGCTATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTTAACGCACCTCGCTGCCTCTGTTGTTCCTACGAGCCTGCCTGTTTGAGAGTCATCAATTTCTTCACCATACAAATTATTGTATTTATCACCGCTGGTGAGTGATATTTGCTTTTTAATAAAAAATTCTGGGGGGCTCTCTTTTTTACTATTAGTAGGACTTCTAGAAAACACCACAGGAGGTGTGATTATGTGTATCCCCCTATAGATGTGGGGGGAATCTTGTTGGTTCGAGCTTTTTAATAATATAATGCGGCGAGAGTGTGGTACGTGAGTATTATTTACCACCACCACTCCTCCATATATGTGAAATCACTCACGACCCTCCCGCAGGTCCCCCCACAAAAAAAAAAAAACTTTTTTGAATTCATATTTTCGAGGGAGTTGTGGCTGGTAATAATAATGGCAGGTGCCCCCCGG >GGGCACTGCTCGAGTCGTCATGATTTGAGGTCAGATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATAGGCGTACACATATTATCACACCCAGCGTGGGCTTTTCTACGTGTTCCAATTAGAGTTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCAACTCTCACATCCCCGCCTTGACATATACATTTTTTTGGTGGGGAGAGAATTGAATGACTCTCAGACATGCGTGCCCCTCAAAATACCAAAGGGCGCAAGGCGCGTTCAAAGATTCGATGACTCTGTGGATTCTGCGAGTCACATTACTTATCGCATTTCGCTGCGTTCTCCATCCAAGAAAGAGCAGAGATATCCGTTGGAGAGAGGTGTATTTTTTTTAATAAAAAAAAATTTGGGAGAAAACAATGTTTCTATATGAAATCGTTCTATGAAACACCCCACAAGGTGTGTTTAGGGGAATCATCGTAAGGATGTCTCCTGAATACTGTTGGTTCACAGGTATATATATATATATAATGCCCCCAGAGTGGGCACATGCTATATTTTTTACCCCCACCCCCTCCCCCGCATAAAAGGAAATCAACAATGACCCTTCCGCAGGTTCCCCCGGGGGAGAAGAAAAAAATTTATTGAATTCAATTCG
51	SrTn-5	*S. rolfsii* (GQ358518)	95	>GGGCATTTGATGATATATGCGAGGAGTGGTGCTGGGGTCGATATTGCACGTGCACACTCTGTAGCTGCGCATGATTTTACATTTGTACATACTGTCGACAGGACCATCCTGACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAGCTTTGTTTTTTTTTAGAATTTTCTCATTAATAAAATATAACTTTCTTCAACCGACGAAATTGACTGTTTCTTGGATGGAGAAAGAATTGAACTGCATACAGTAATGTGAGTTGCTAAAACAAAGGAATCATCCAACCTTTCAAAGAACCCTGCGCGCCTCGGTATTCCTTCCGGGAGACTTGTTTGATTGTGATTCATTTCTCTTCCATACTAAAAAATGTATATGTTTTGTTGGGGATGGTAGAGTTGTTTGTTAAAAAAAAAATTTCTGGGGCTCTCTATTTTTATTTATATGAGAGGTGTATGAAAAAACCCGGGGGGTGTGAGTATATATATCCCCCTACGTATTCCGGGGAATACTGGTTGGTTGGACCTATTAATAAAAAAATGCCCACTTTTGGGGCCAAGAATAATTTATTTGCACCCCCCCCCCCCCCGCTAAGGAGGAACCACCACCGTTCCTCCCCCTGTCTCCCCCGGGAAAAAAAAAACTTTTGTACTTCATTCGCCGAGGGAGTGCTGGCTGGCAATAAAAATGGCAGTCCCCCCCCCCCAACTTA >GGGGGAAATTGGCTGGATATCGTAATGAGTGAGGCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTTGGGATAAGGGTTAAACTATTAACCCACCCACCGGAAGCCTTTCTACATGATCCCCCAATGAATTTTTAGATTGCCCGTCCGAAATTCTTAACTAGCAGCTCTTACATCCTAGCCTTGACGAATACGAAAATTTTTTGGGTGGAGAAAATATTGACGCTCATACAGGCGTGCGCCCCGGAAAAACAAAGGGGGCAAGGGGGGGTCTAAAAAAACATGATTCACCTCGGTATTCCGTTCAGCATACTTGTTTGAGTTTGATGCGTTCTTCTTCCATACTAAAACATGTAGATCTCTTGTTGTGGATGGTAGATTTTTTTTTAAAAAAAAAATATTTGGAAAAAAAACTATTTTCCTTTATAAGACGGTTGTAGGAAACAACCAAGAGGGTGATTTGAAATATTTCTCCGTAGGTATTCTGAGGAATACTGGTTGGTTCCAAGGGGTTTTTATAAAAAACCCCCCGAGGGGGGCCCAAGCATTATTTTTTTGCCCCCCCCCCCCCCCGCTAGGGTGGATTAACCAAGGTTCCTCCCCCGGGTACCCGCAGGAAAAAAAAAGTATGGCGCGAGTCGGTGGCTTTAAAAAAATTTGGGGGGGGCCCCCACAACCTTATTAAAGGGGGGGGGGGGGGACTCCCCCCCGGGGGAGGAAGGGAGTTTAAGCCACAGTTCTCCGGGAGCCCGGGGGTGTGGGGTTC
52	SrTn-6	*S. rolfsii* (GQ358518)	93	>GGGCTATTGATCTATATGCGAGGAGTTGTGCTGGGAATGATATTGCATGTGCACACTCTGGAGCTATATAATATTTTCTCCTGTGTAAATACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATTTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACCACTTTTTCAACGGATCTTTTGGCTCTTGCTTTGATGAAGAAAGAATTGAAATGCGATAAGTAATGTGAATTGCAGAAACCACCTGAATCATCGAATCTTTTAAAACACCTCGAGGCCTTTGGGATTCCGAGGGGCAAGCTTGTTTGAGAGTTTTTAAATTCTTCACCTTACAAATTTTTGTATTTATACACGCTGTGAAGGTAGAATTGTTTTTTAAAGAAAAAATCCGGGTGGCTCTCTTTTATACTATTAGAAAGACTTGTAGAAAAGCCCACAGGTGGTGTGATAATATGATTACCCCTAAGGATTTCGGGGGATTCCGGTTTGTTTGAAGGATTTTAAAAAAACATGCCCCCCATAGTGGGCCAAATAAGTGCTTTTTTTGACCATTTGATCTCCAATAATGTGAAATCACCCCCGAACCTTCCGCAATACCCCGCGGGAAAAAAAAAATTTTTTTGGATTCATATTTGCAAAGGATTTGTGCTGGTAAAAAAAATGGGGGGGCCCCCCCGGGATGGCTTTAAAAAAATTTGGGGGGGGCCCCCACAACCTTATTAAAGGGGGGGGGGGGGGACTCCCCCCCCGGGGGAGGAAGGGAGTTTAAGCCACAGTTCTCCGGGAGCCCGGGGGTGTGGGGTTC >GGGGGAATTGGCTGGGATATCGTACCTGATTTGAGGTCAATGGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATGGGCGTAGATTTTTATCACCCAACGCGTGGGATTTTCAACGTGTCCAAATAAAAGTTTTAAAGAGAGCCAGTCATAAATATACTCTAACCAGTAACTCTTACATCCACGCCTTGACAAATACAAAATTTTGTAGGGGAAAGAATTTAATGACTCTCAAACAGGCGTGCCCCTGGAAATACCAAAGGGCGCAAGGTGCTTTCAAAGATTCGATGATTCAGGGGATTCTGCGATTCAGATTATTTATGGCATTTCGCTGCGTTCTCCATCCAAACAAGAGCAAATAGATCCGTTGTGGAAAGTTGTTTATTTTTTATTAAAAAAAACTTGGCAGAAAACAATGTTTCTATTTGAAAGCGTTCTATGTAACACAACAGAAGAGGTGATAAGGGGAATCATAGTTAGGATTTCTCCGGACTACGGTTGGTTCACAGGTGTATATAATATATATAGCTCCAGAGTGTGCACATGCAATATTTATTACCAGCACAACTCCTTCGCATATATGGATTCACTAATGATCCTTCCGCAGGTCACCCTAGGGAGAAAAAAAAACTTTGATTAATTCTG
53	SrGj-1	*S. rolfsii* (GQ358518)	91	>GCCTTGGACTGGTATTGTCAATGGAGTTGTGTCTGTGTATCGAGATTGCATGTGCACGCTCTGTAGCTATATATGATATTCTCCTGTGTACCACTGTAGTCAGGACATTCGTAACTATGATTACACTATATTACTCTTATTGTATGTTACATAGAACGATCTCATATTGAAACTTTGTTTTGCGACAAGTTTCTCTTTTCTTAAGAATATACTACTTTTTTACAACGGAGAACTTGGCGCTTGTTTCGATGGATGAACAATGCGAAATGTCTATAAGTAGTGTGGATTGTAGAAATACAGTGGAGCATCGGATCTTTTAAAAAACCCCGTGGCCCACGGTATTTCTGCGGGGCAGACTGTTTGGAGAGTCATGAGATTCTTCTCCTCACTAAAATTTGTAGATGATACGCGTGAAGGTGGAATTTTTGCTATAAAAAAAATCTGCGGACACCTTTTTACTATTAGAGGGCTCTTGAAAACACCACGAGGGTGTGAAGAATTATCGCTAGACTCTGCGGATCTGGTGGTCACGGCTATATATATAGCTCACATGGCCGTGAGTATTATTGCACCTCATCTCTACAGAGAGAGATTACAGAGGTCTCCGGTCTCGGGGAATGGCTTTAAAAAAATTTGGGGGGGGCCCCCACAACCTTATTAAAGGGGGGGGGGGGGACTCCCCCCCCGGGGGAGGAAGGGAGTTTAAGCCACAGTTCTCCGGGAGCCCGGGGGTGTGGGGTTC >GGGGGATGTGGTGCAGTCGTCTGATTTGAGGTCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTATAGGCGTAGACTTATTATCACACCAACGGTAGGCATTTCTACAGGTCCAACAAATAGTTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCAACTCTCACATCCCAGCCTTGACAAATACAAATTTTTGTAAGGTTGAGAATTTAATGACTCTCAAACAGGGATGCCCCTAGAAATACCAAAGGGAGCGGGGGGTGTTAAAAGATTCGATGATTCACTGGATTCTGCAATTCATATTACTTATAGAATTTCGCTGCGTTCTTCATCGATACAAGATCCAGGAGATCCGGTGTTGAAAGTTGAATTTTTTTAATTAAAAAAAACTTGGCAAAAAACAAAGTTTCATTATGAGATCATTCTTTGTAACAAACAATAAGAGTTATATAGGGTAATCATAGTTAGGATTTCTCCTGACTACAGTTGGTTCACAGGTGTATATTATTATATAGCTCCAGAGTGTGCACATGCAATATTTATTACCAGCACAACTCCTCCGCATATATGAATTCAATAATGATCCTTCCGCAGGTTCTCCCGCCGGAAGAAAAGAACTTGTGAATCTATCT
54	SrGj-2	*S. rolfsii* (GQ358518)	93	>TGCTTCTGGCTCGAGTGTAATGGAGTTGTGCAGGGATCGATATTGCATGTGCACGCTCTGTATATGCGCATGATTTTCTCCTGTGTACATACTAGCTAGAGGACCTTCCGGACTATGATTACACTATAAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAATCTTTGTTTTCTGACACGTTTCTCTTAATTTAAAATATACTACTTTCAACAACGGATCTATTGACTATTGTTTCGATGGAGAACAATTCGAAGTGCCACAAGTAATGTGAATTGTAGAAACAAGTGAATCATCAAATCTTTTAAAAGACCTTGTGCCCCTTGGTATTCTGCGGGGCATGCTTGTTTGAGAGTTTTTATGTTCTCCTCCTTACTACAATTTGTAATAGTCCCGGTGTGGATGGTAGAATTTTTTTTATAAAAAATTCTTGACGGGCTCCATTTTATAATTATAAGAACATTCTTTGAAACACCCACTAAGAGTGATATAATGTAATCACACCTATGATTTCTCCGGACTACAGTTGTGTCACACTACTTTATATATCATAGCTCCAGAGTGTGCAGATAAGTATTTATTACCACCACCACTCCTCCGCTGATGTGAGATCACTAGTGGTCCTTCCGCATGTCTCCGGCAGGAAAAAAGAACTTATTGAATTCATATATGCGAAGGAGTTGTGCTGGTAAATGAATATGCATGTGCCACCCGCGGCGATAT >GGGGAATTTGGCTTGAGTCGTCTGATTTGAGGTCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGAATAGGCGTAAACATATTATCACACCAACGGAAGGCATTTCTACAGGTCCAACAAAGAATTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCTACTCTCACATCCCAGCCTTGAAAAATACAAATTTTGGAAGGTGAAGAATTTGATGACTCTCAAACAGGGCTGCCCCTAGAAACACCAAAGGGCGGAAGGGGCGTTCAAAGATTCGATGATTCACTGGATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATACAAGATCCAAGAGATCCGTTGTTGAAAGTTGAATTTTTTTTATAAGAAAAACTTGGAAGAAAACAATGTTTCATTATGAGATCGTTCTATGTAACACACAATAAGAGTTATATAGTGTAATCATAGTTAGGATTTCTCCTGACTACAGTTGGTTCACAGGTGTATTTATTATATAGCTCCAGAGTGTGCACATGCAATATTTATTACCAGCACAACTCCTCCGCATATGTGAGTTCAATAATGATCCTTCCGCAGGTTCATCCGCCGAGAAAAAGAACTTATTGATCTATATCGAAGA
55	SrGj-3	*S. rolfsii* (GQ358518)	94	>TGGCATGGTAGGCTGGAGTGTAATGATTTGTGCAGTGAATGATATTGCATGTGCATGCTCTGTATGTGCGCAAGATTTTCTCCTGTGTACCACTGTAGTCAGGACCTTCCGGACTATCGTACACTATAAACTCTTATTGGATGTTACATAGAACGATCTCATATTGAATCTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACTACTTTCTACAACGGTTGACTTGACTCTTGTTTCGATGGAGAACACTTCGAAGTGTCATAAGTAATGTGAATTGTAGAAACCAGTGAATCATCGAATCTTTTAAAAGACCTTGCGCCCCTTGGTATTCTGAGGGGCATGCCTGTTTGAGAGTTTTCAGTTCTCCTCCTCACAAAAATTTGTAATAGTCCCGGTGTGGATGGTAGAATATTTTTTAATAAAAAAATCCTGGCAAAAAACCTTTTTCATTATAATAACATCCTTTGAAACACCCACTAGTGGTGATATAATGTATTCACCCCTATGATTTCTGGGGACTACAGTTTGTTTACACTACTAATTAATATAAACCTCCATATTGTGGCAATGAAATATTTATTACCACCACAACTCCTCTCGCATATGTGAGAATCACCCGTGGTCCTTCCGCAGATTCACCAGCGAGAGAAGAAAAAACTTATTGAATTCTTATATGCGAAGGAGTTGTGCTGGTAAAAAATGTGGCAGGT >GGGGGATTTTGAATGCAGTCGTAATGATTGAGGTCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGTATAGGCGTAGACATATTATCACACCAACGGTAGGCATTTCTACATGTCCAACAAATAATTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCAACTCTCACATCCAAGCCTTGAAAAATACAAAAATTTGGAAGGTTGAGAATTTAATGACTCTCAAACAGGGATGCCCCTAAAAATACCAAAGGGCGGAAGGGGCGTTCAAAGATTCGATGATTCACTGGATTCTGCAATTCATATTACTTATCGAATTTCGCTGCGTTCTTCATCGATGAAAGAGCCAAGAGACCCGTTGTGGAAAGTTGAATTTTTTTATATAAGAAAAACTTGGCAAAAAACAAAGTTTCATTATGAGATCGTTCTTTGAAACACACAATAAGAGTTATATAGGGTAATCATAGTTAGGATTTCTCCTGACTACAGTTGGTTCACAGGTGTTAATATTATATAGCTCCAGAGTGGGGCACATGCAATATTTATTACCACCACAACTCCTCCGCATATGTGATATTCAATAATGATCCTTCCGCAGGTTCACCCGGCGGGAAGAAAAAAACTTATTGAATCTATATCGAG

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *S. rolfsii*
56	SrGj-4	*S. rolfsii* (GQ358518)	93	>TGGACTGATGCGATCAGTCATGGTTGGTGCAGTGAATCAATAATTGCACGTAGCACGCTCTGTATGTGCGCATGATATTCTCAGGTGTGCATACTGCTAGAAGCAGAAATCGTGACTATGATTACTCTATATAACTCTTATTGTATGTTACTTAGAACG ATCTCATATTGAAGCTTTGTTTTTTTTTGAGATAACTCCTCTTGAATATCTCACATTTTTCAACTGATGATATGTAGATTTGTGCGGTGAAGAATTTAGCGACACTCGAACAGGCATGCCCCTTGGAAAAGAACCCAAAGGCTCAACGCATTTTTAAAGA CTCCTTGACTCTGTTGTTTCTACGAGGGTGCTTATTGGATAGGACTCAGTTCTTTCTCCATCTAAGAAATTGTAGATATCTTGGTGGGTGTGATGTGTTTGTTGCTTAAAAAAAAATTTTTGGGGAAAAACTCTTTTTTATATTTAGAGGGCCTCTTGTAAA CCCCCCCAGGGGTGGTGTAAAGTCATCACCGCCATTACTTCTGGGGACAGCTGGTTGGTTTGCACGGGTTATATAAAAAATGCCCCCTAAGTGGGCACGAGAATATTTTTTTTGGGGATTATTCGTTCATTAGGTATAGATTCACCGATGGACCCTCCCG CAGTTCCCCGCCGGAGAGAGAAAAAATTTTTGAATCTTTTCAGAGTGGGGAAAAA >TGCATGTGCTAGACATCATGATTTGAGGTCAGTGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTGCATACAAGCTAGAATCCCCTTTGGGGTATACGCGTACACATTATAACACCAACCGCATGCGTTTTTATACGT CCTCCTATTAGTTTTTTAGAGAGACCGTGTGATAATATACTCTAGCGACTCTCTCACATTTGCCTTGTTTGATATATAAATTTGTTTGGTGGAGAATTTATTGACACTCTCACAGGCATGCCCGCCGGAAAACAAAACAAAGCGCGCAACGCGCTTAGAG ACACTCATTGACTCTGTTCTTTCTGCGAGACTACTTATTTCATGTTGCTGCGTTCTTTCTCCATCCAAGAGAGAGGAGATATCTTGTTGGGAGAGGTGTGTTTTTTTGCTTAATAAAAAATATTTTGGCAAAAAACCATCTTTATTATATGAGGGTCTGTT GTAAACAACACAAGGGGTGTTGAGAAGTCATCGCCGCGAGGACTTCTGCCGACAGACGGTTGGTTCCACGGTGTTTTTTATATTAGCCCGCCTGTGGGGGCGCTCGATTTTTTTTTATAGGGCTACTTCTTCATATGTATGAATTCATGATGCTCCCTCC CGCAGACCCCCCAAGAGAGAAAAAAGAAACTTTTTAATTTATTTTTTCGAGGGGGTGGGGGGGGAAAAAAAAAGG
57	SrGj-5	*S. rolfsii* (GQ358518)	94	>TGCTGTGCTGCTATAAGCGAGGAGTTATGCTGTGATAATATTGCATGTATATGCTCTGATGTGCGCATGATTTATAAGGTGTACTACTGTAAGAAGGAGAAATTGTGACTATGATTACCCTATATAACTCTTATTGTATGTTACTTAGAACGATTTCAT ATTGAAACTTTGTTTTCTTACAAGTTTCTCTTATCTATGAAGTACAACTTTTTTCAACGGATGACTTGTATCTTGCTTTGTTGAAGAACGCATTGAAATGCTCTCAGTAATGTGAATTGTAGAATCCACCAGAATCATCGAAGCTTTTAACGAACCTCGAT GCCTCTGTGATTCCGAGGAGCCAGCTTATTTGAGAGTTTTTAAATTCTTCACCATACAAGTTATTGTATATATCACGGCTTGTGAGAGGTATTTTTTTGGTTATTAAAAAATTCTTGGGGAGATCTCTTTTTTACTATTAGAAAGCTTTGTAGAAAACACC CCAGGGGGTGTGAAAATATGTCTCCCCCTATGGATTTCGGGGGAATCTAGTTGGTTCACAGCTATAAAATAAATATAATGCCCCCAGAGGGGGGCCAAGAAATATTTTTTTTGACCACCACCCCCTCGCATAGGGGGGGATCACCCATGATCCTCCCGC ATGTCCACCGGGGGGAAAAAAAAATACTGGTTCACTCGGGTGGGA >GAGGTAATGATGCATACGTCGTGAGTGAGGCTATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATATGCGTAGACATATTATCACACATTGCGTGTGACTTTCTACG TGTTCCAATTAGAGTTTTAAAGAGACCGTGTCAGAATATACTCTATCCCGCCTCTCACATATCCAAGCCTTGACATATAAAAAAATTTGATGAAGAGAGAATTGTGACGCTCTCAGACATGCATGCTTGCAGAATATACCAAAGGGCGCAAAGCGTTTT ACGAGACTCGATGACTCTGTGAATTCTGCGAGTCAGATTATTTGACGGTGCTCGATGCTTCCTCCATACAAAAAAAAGAGTAGATATCTCCGTTGGAGAGAGAGATGTTTGTTTTTTAATAAAAAAAAAACTTTGGGAGAAAACTTTGTTTCTATAAGAAGGC GTTCTAGAAAACACCCCAGGGGGGTGTGTAAAAGGTGAACACCCCTAAGGATTTTCGCCGGACTACTGGTGTGTTCACCGCTATTTAATAAAAAAATGCCCCGAGAGTGGGGCCCAGAAATATTTTTTTACCCCGCACCCCCCCCCTCCACATAGTGTG AGTTCACTAATGATCCTCCCGCAGTCACCCAAGAGAAAAAAAAAATTTTTTTGTTTTTTTTTTCCGGGGGGTTGGGGGGGAAAAAAAAGGGGGGCC
58	SrGj-6	*S. rolfsii* (AB075318)	95	>TGCTTAGTGACTGCTATCAGCGTGGAGTTGTGCTGTGTAATCGATTATTGCATGTGCACACTCTGGTGCTCTATAATATATTCTCCTGTGTAAATACTGCAGTCAGGAGAAATCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAAC GATTTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACCTCTTTTTCCAACGGATGACATGGATCTTGTTTTGAAGGAGAAAGAATTGAAATGCTCTAAGTAATGTGGATTGCAGAAACCCGTGAGGCCTCGAATCTTTTTAAA AAACTCGATGCCTTTGGGATTTCGAGGAGCCAGCTTATTTGAGAGTTTTTAAAGTCTTCACCATACAAAATTTTGCAAATATAAAGGCTTGGAAGAGATATATGTTTTTTAATAAAAAATCTTGCCGGAAAACTTTTTTTCTATTAGAAAGCCCTCTAGG AAACCCCACAGGTGGTGTGATAGGTGATTACCCCTATAGATTTCGGGGGAATACTAGTTGGTCACAGGTGTTTATAAAAAAATGCCCCCAGAGTGTGGCCATGAATATTTATTTTGAGCATTACTCTTCACATATGTGAGATCAACAAGCGATCCCTCC GCAGGTCACCCCGGCGGGGGGGGAGGATCTTTTTTGATTTCTATATGCGAAGGGGTTGTGCGGG >GGGGGAAAGTGCTTCCATTCATACTGATTTGAGGTCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATAGGCGTACACATTTTAACACCCAACGCGTGGGATTTT CAACGTGTCCAAATAAAAGTTTTAAAGAGAGCCAGTCAGAATATTCTCTAACCAGCAACTCTCACTTCCAAGCCTTGACAAATACAAAATTTTGTAGGGGAAAGAATTTAATGACTCTCAAACAGGCGTGCCCCTGGAAATACCAAAGGGCGCAGGGT GTGTTAAAAGATTCGATGACTCAGGGGATTCGGCAATTCAGATTACTTATCGAATTTCGCTGCGTTCTTCATCGAAGAAAGAGCAAATAGATCGGTTGTTGAGAGTTGTATATTTTTAATTAAAAAAAAAATTTGGCAGAAAACAATGTTTCTATAAGAA AGCCTTCTAGGAAACACCCAAAAAGGGGGTTTATAGGGGATCATACTAGGATTCCCCCGGAATACAGGTTGGTTCACAGGGTGTATATAATATAAAGCCTCCCAGAGTGTGGCACATGCAATATTATTACCACCACCACCTCCCTCCGGATAGATGAA ATCAACGAGTGGTACCTCCCGCAGTCACCCGAGCGGAAAAGAAAATTTTATGTATTCCTATTTCGCAGGGGGTGTGCGCGGGAAAGAAAATGGCAGGCGAACCGGGCTCTTTAAAATATC
59	SrGj-7	*S. rolfsii* (GQ358518)	95	>GGCATTATGGACTGGTATTAGCCTGGAGTTGTGCTGTGGTCGAATTTGCACGTATATGCTCTGTATGTGCGCATGATTTTATAAGTAGTACATACTGCTAGAATCACCCTTGTGACTATGATTACCCTATATAACTCTTATTGCATGTTACTTAGAACGA TTTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTATCTAAAAATATATCACATTCTCCAACGGATGACATGTATCTTGTTTTGTTGAAGAAAGAATTGAAACGCTCTCAGTAATGCGCGCTGCAGAAATATACCAGAGGGAGCGAGGCGTTTAAAGA GACTCGATGACTCTGTGGTTTCTGCGAGTCAGCTTATTTGAGAGTCCTCAGTGCCTCATCCATACAAGAAATAGTAGATATCTCGGCTGGTGAGAGGTGTTTGTTTTTAAATAAAAAAAACTTTGGGAGAACACTATGATACTATTAGAAAGCGCTCTA GGAAACACCCCAGAGTGGTGTGATAAGATGAATCATCGTACGTATTTCTGGGGGCCTACTAGTGGGGTCAGCGGGTGTATATAATATATGCCGCTAGAGTGCGCGCATGAAGTATTTTTTTACGCGCACCACCTCCTCCGCATATGATGAGATCACTGA GCGATACTTCCGCAGTCACCCGGGGGAAAAAAAGAACTGTTTGAATTTATTATGTCGAGGGTGTGCGCTGCTAAATAAATAATGGTGTGTGCAC >GAGGATGTTGATGGATTATCCTGATTTGAGGTCAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTGCGGTATATGCGTACACATTATAACACACACAGCGTGGGCCTTTCTA CGTGTTCCTATTAGAGTTTTAGAGAGAGCCAGTCAGAATATACTCTAACCAGTCTCTCTCACCTCCAAGCCTTGACATATACATTTTTTTGTTGGGGAGAGAATTGAATGACTCTCAGACAGGCGCGCCCCTAGAAACTACCAGAGGGCGCGAGGCGTT TAAAAAGACTCGATGACTCTGTGGATTCTGCGAGTCAGCTTACTTATCGCATTTCGCTGCTTTCTCCATACAAGAAAGAGCAGATATATCCGCTGGTGAGAGATGTTTGTTTTTAAATAAAAAAAAATTTGGCAGAAAACATTGTTTCTATTTGAAGTCG TTCTAGGAAACACCCCACAGAGGTGTGATGAGGTGAATCATCGTAGGGATTTCGGCGGCCTACTGGTGGGTTCACGGGTGTATTATTATAATGGCTCCAGAGTGGGCCCGTGCAGTATTTATTTACCACGCACCACCTCCCCCGCATAGGATGAGAT CACTAAGCGATCCTCCCCGCACGTCCCCCGCGAGAGAGAGAAAAAAATTTTTTGATTTTATTCGAGGGTGGG
60	SrGj-8	*S. rolfsii* (GQ358518)	93	>GTGGCACTGATGCTATAAGCGAGGAGTTGTGCTGTGGTGATTATTGCATGTGCACGCTCTGGAGCTATATAATATTCACCTGTTAACCAACTGCAGTCCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACTTAGAACGA TCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAAATATCTCCCTTTCCCCAACCTTGGCCATTGCCAAAAGTTTGAAGGAAGAAAAATTCGAAATCCTTAAACAAGGGTGCCCCTCGGAATCCAAAGGGCCACAGGATCTTTGAAA GAACCTTGGGCCCTTTGGAATCCGCAGGTCCATGCACTTTTAGATTTTTCAGCTTTTCTCCCTAAAAATTTTCAATAGATCAGGCTTGAAGGTAGATTTTTTTTTTAAGAAATCTTACGGCAATCATTTTCAATTAGAAGCCTTGTAGGAAGCCCCGATGG TTTATAGGTGATCACGCTAAGCCGGAACGGATACAGTTGGTTAAAGGATAATATATAAAGCCCCAAGTGTGGCCATGCGTGATTATTTTCCACCACAGTCCTCCACCAAGTTGGATTCACCAGTGAACTTCGCCAGTCTCTACCGAAAGAAGACTATGA TCTATTCAAGAT >GGGAAATGATGCTAGACGTACTGAATGAGGCTGTGGTCGATATATGCACGTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATCCCCTTCCGGGACTATCATCACCCTATATAACTCCCATCGGATGGCATTTAGAAC GGTCCCATATAGAAATTTTGTTTTCTGAGTGTTTCTCTTAATTACCCGCAACTCTCTTTCCCAGCCTTGGCAAATGCCAAAATTTTGAAGGGTGAAAATTTAAAGACCCTTAAACAAGGGTGGCCCTCGGAATCCAAAGGGCGCCAGGTGCTTTGAAAG AACCCGTGGCTCATTGGATTCCGCAGGTCAAGTAAGTATCGGATTTTTCTGCTTTTCTCCTTGATGCATTTTCAATGGTCCGGTGTTGAAAGTTGGATTTGTTTTAATTAAGAGATACTGGCCGGAAATCAGTGTTCCAATTAGAAGGGTTGTAGAAATG CAAAAGATAGGTGTGATAGGGTGACCACGCTAAGGCTGTCAGCGGATTCAGTTGGTTCGAGGAATTTATATTATCTAGCCCCATATTGTGCCCTGCAATCTTCATTTCCACCATTGTCCCCGCAAGTGGATTCACACTGACTTCGCATACTCACTCGGAA GAAAATTTTGATCTTTCAAGATG

*Strains in the parentheses are reference strains of *S. rolfsii* present in NCBI genebank

Table 4.17. Similarity index values of isolates of *S. rolfsii* based on RAPD analysis

S. No.	Isolate	SrKa-1	SrKa-5	SrKa-20	SrTs-1	SrTs-10	SrKa-12	SrAp-2	SrAp-10	SrMh-1	SrMh-6	SrTn-1	SrTn-5	SrGj-1	SrGj-3	SrGj-6
1	SrKa-1	1.000														
2	SrKa-5	0.640	1.000													
3	SrKa-20	0.523	0.463	1.000												
4	SrTs-1	0.604	0.468	0.368	1.000											
5	SrTs-10	0.375	0.395	0.425	0.477	1.000										
6	SrKa-12	0.544	0.535	0.598	0.515	0.516	1.000									
7	SrAp-2	0.291	0.387	0.558	0.322	0.450	0.522	1.000								
8	SrAp-10	0.464	0.462	0.567	0.443	0.582	0.519	0.546	1.000							
9	SrMh-1	0.497	0.485	0.610	0.451	0.514	0.564	0.497	0.660	1.000						
10	SrMh-6	0.462	0.400	0.545	0.463	0.480	0.482	0.405	0.632	0.723	1.000					
11	SrTn-1	0.237	0.331	0.336	0.381	0.482	0.382	0.400	0.503	0.387	0.427	1.000				
12	SrTn-5	0.410	0.432	0.541	0.395	0.456	0.618	0.462	0.510	0.538	0.503	0.526	1.000			
13	SrGj-1	0.439	0.283	0.375	0.305	0.259	0.338	0.263	0.307	0.388	0.326	0.132	0.411	1.000		
14	SrGj-3	0.305	0.364	0.288	0.330	0.400	0.357	0.220	0.429	0.316	0.361	0.484	0.379	0.189	1.000	
15	SrGj-6	0.365	0.299	0.357	0.264	0.256	0.380	0.122	0.291	0.262	0.246	0.130	0.354	0.451	0.267	1.000

Table 4.18. Minimum inhibitory concentration (MIC) of different fungicides against growth rate of *S. rolfsii* isolates

S. No.	Isolates	MIC (ppm)				
		Thiram	Carbendazim	Azoxystrobin	Tebuconazole	Mean
1	SrKa-1	3200	1200	1000	900	1575
2	SrKa-2	3000	1200	1000	900	1525
3	SrKa-3	3200	1300	1000	800	1575
4	SrKa-4	3100	1300	900	800	1525
5	SrKa-5	3100	1000	900	800	1450
6	SrKa-6	3000	1100	1000	800	1475
7	SrKa-7	3000	1300	1000	800	1525
8	SrKa-8	3100	1300	1000	800	1550
9	SrKa-9	3100	1300	900	800	1525
10	SrKa-10	3100	1200	1000	800	1525
11	SrKa-11	2800	1000	900	800	1375
12	SrKa-12	2800	1000	900	800	1375
13	SrKa-13	2800	1000	900	800	1375
14	SrKa-14	2700	1000	900	800	1350
15	SrKa-15	2900	1200	900	900	1475
16	SrKa-16	3100	1100	900	900	1500
17	SrKa-17	3000	1100	1000	900	1500
18	SrKa-18	3100	1000	1000	900	1500
19	SrKa-19	3000	1100	1000	800	1475
20	SrKa-20	3100	1200	1000	800	1525
21	SrTs-1	3000	1300	1000	800	1525
22	SrTs-2	3100	1300	900	800	1525
23	SrTs-3	3200	1300	800	900	1550
24	SrTs-4	2800	1200	900	800	1425
25	SrTs-5	2900	1100	1000	800	1450
26	SrTs-6	3000	1100	900	800	1450
27	SrTs-7	3100	1000	1000	900	1500
28	SrTs-8	3000	1000	1000	900	1475
29	SrTs-9	3100	1100	1000	900	1525
30	SrTs-10	3000	1000	900	900	1450
31	SrAp-1	2700	1000	900	800	1350
32	SrAp-2	2700	900	900	800	1325
33	SrAp-3	2900	1000	900	800	1400
34	SrAp-4	3000	1200	1000	800	1500
35	SrAp-5	3100	1200	900	800	1500
36	SrAp-6	3200	1000	900	800	1475
37	SrAp-7	3200	1000	1000	900	1525
38	SrAp-8	3100	1000	1000	900	1500
39	SrAp-9	3000	1100	900	900	1475
40	SrAp-10	3200	1100	900	800	1500
41	SrMh-1	3100	1200	1000	900	1550

Cont.

S. No.	Isolates	MIC (ppm)				
		Thiram	**Carbendazim**	**Azoxystrobin**	**Tebuconazole**	**Mean**
42	SrMh-2	3000	1100	900	900	1475
43	SrMh-3	3000	1100	900	800	1450
44	SrMh-4	2900	1100	900	800	1425
45	SrMh-5	2900	1000	1000	800	1425
46	SrMh-6	2800	1000	900	900	1400
47	SrTn-1	3000	1200	900	900	1500
48	SrTn-2	3100	1100	900	800	1475
49	SrTn-3	2800	900	800	800	1325
50	SrTn-4	2800	900	900	800	1350
51	SrTn-5	3000	1000	1000	800	1450
52	SrTn-6	3000	1000	1000	800	1450
53	SrGj-1	3100	1000	1000	900	1500
54	SrGj-2	3100	1200	1000	800	1525
55	SrGj-3	3200	1100	1000	800	1525
56	SrGj-4	3200	1100	1000	800	1525
57	SrGj-5	3100	1100	1000	800	1500
58	SrGj-6	3100	1000	1000	800	1475
59	SrGj-7	3100	1200	1000	800	1525
60	SrGj-8	2900	1200	1000	800	1475
Mean		3012	1105	949	832	-

Factors	CD (0.01)	S.Em.±	CV (%)
Fungicides	35.26	12.69	-
Isolates	136.57	49.15	-
Interaction	237.15	98.29	11.50

Table 4.19. ED_{50} of different fungicides against growth rate of isolates *S. rolfsii*

S. No.	Isolates	ED_{50} (ppm)				
		Thiram	Carbendazim	Azoxystrobin	Tebuconazole	Mean
1	SrKa-1	1600	600	500	450	788
2	SrKa-2	1500	600	500	450	763
3	SrKa-3	1600	650	500	400	788
4	SrKa-4	1550	650	450	400	763
5	SrKa-5	1550	500	450	400	725
6	SrKa-6	1500	550	500	400	738
7	SrKa-7	1500	650	500	400	763
8	SrKa-8	1550	650	500	400	775
9	SrKa-9	1550	650	450	400	763
10	SrKa-10	1550	600	500	400	763
11	SrKa-11	1400	500	450	400	688
12	SrKa-12	1400	500	450	400	688
13	SrKa-13	1400	500	450	400	688
14	SrKa-14	1350	500	450	400	675
15	SrKa-15	1450	600	450	450	738
16	SrKa-16	1550	550	450	450	750
17	SrKa-17	1500	550	500	450	750
18	SrKa-18	1550	500	500	450	750
19	SrKa-19	1500	550	500	400	738
20	SrKa-20	1550	600	500	400	763
21	SrTs-1	1500	650	500	400	763
22	SrTs-2	1550	650	450	400	763
23	SrTs-3	1600	650	400	450	775
24	SrTs-4	1400	600	450	400	713
25	SrTs-5	1450	550	500	400	725
26	SrTs-6	1500	550	450	400	725
27	SrTs-7	1550	500	500	450	750
28	SrTs-8	1500	500	500	450	738
29	SrTs-9	1550	550	500	450	763
30	SrTs-10	1500	500	450	450	725
31	SrAp-1	1350	500	450	400	675
32	SrAp-2	1350	450	450	400	663
33	SrAp-3	1450	500	450	400	700
34	SrAp-4	1500	600	500	400	750
35	SrAp-5	1550	600	450	400	750
36	SrAp-6	1600	500	450	400	738
37	SrAp-7	1600	500	500	450	763
38	SrAp-8	1550	500	500	450	750
39	SrAp-9	1500	550	450	450	738
40	SrAp-10	1600	550	450	400	750

Cont.

S. No.	Isolates	ED_{50} (ppm)				
		Thiram	**Carbendazim**	**Azoxystrobin**	**Tebuconazole**	**Mean**
41	SrMh-1	1550	600	500	450	775
42	SrMh-2	1500	550	450	450	738
43	SrMh-3	1500	550	450	400	725
44	SrMh-4	1450	550	450	400	713
45	SrMh-5	1450	500	500	400	713
46	SrMh-6	1400	500	450	450	700
47	SrTn-1	1500	600	450	450	750
48	SrTn-2	1550	550	450	400	738
49	SrTn-3	1400	450	400	400	663
50	SrTn-4	1400	450	450	400	675
51	SrTn-5	1500	500	500	400	725
52	SrTn-6	1500	500	500	400	725
53	SrGj-1	1550	500	500	450	750
54	SrGj-2	1550	600	500	400	763
55	SrGj-3	1600	550	500	400	763
56	SrGj-4	1600	550	500	400	763
57	SrGj-5	1550	550	500	400	750
58	SrGj-6	1550	500	500	400	738
59	SrGj-7	1550	600	500	400	763
60	SrGj-8	1450	600	500	400	738
Mean		1506	553	474	416	-

Factors	CD (0.01)	S.Em.±	CV (%)
Fungicides	12.85	4.62	-
Isolates	49.77	17.91	-
Interaction	99.54	35.82	8.40

Table 4.20. Sensitivity distribution of isolates of *S. rolfsii* to different fungicides

Location	Isolates	Thiram				Carbendazim				Azoxystrobin				Tebuconazole			
		ED_{50} (ppm)			Resistance factor*	ED_{50} (ppm)			Resistance factor*	ED_{50} (ppm)			Resistance factor*	ED_{50} (ppm)			Resistance factor*
		Range		Mean		Range		Mean		Range		Mean		Range		Mean	
Karnataka	20	1350	1600	1505.0	1.06	500	650	572.5	1.14	450	500	477.5	1.05	400	450	415.0	1.08
Telangana	10	1400	1600	1510.0	1.06	500	650	570.0	1.14	400	500	470.0	1.06	400	450	425.0	1.06
Andhra Pradesh	10	1350	1600	1505.0	1.06	450	600	525.0	1.14	450	500	465.0	1.08	400	450	415.0	1.08
Maharashtra	6	1400	1550	1475.0	1.05	500	600	541.7	1.11	450	500	466.7	1.07	400	450	425.0	1.06
Tamil Nadu	6	1400	1550	1475.0	1.05	450	600	508.3	1.18	400	500	458.3	1.09	400	450	408.3	1.10
Gujarat	8	1450	1600	1550.0	1.03	500	600	556.3	1.08	500	500	500.0	1.00	400	450	406.3	1.11

*Resistance factor = Maximum ED_{50} /Mean ED_{50}

ED_{50} values were taken from table 4.19

Table 4.21. List of potential isolates of *Trichoderma* sp. isolated from groundnut rhizosphere soils

S. No.	*Trichoderma* isolate	Soil sample location	18S rDNA sequence identification
1	*Trichoderma* sp. (T1)	ICRISAT (Telangana)	*Trichoderma harzianum* (KR232487.1)
2	*Trichoderma* sp. (T2)	Kadiri (Andhra Pradesh)	*Trichoderma viride* (JF304319.1)
3	*Trichoderma* sp. (T3)	Enugal (Telangana)	*Trichoderma asperellum* (KU987251.1)
4	*Trichoderma* sp. (T4)	Hartikote (Karnataka)	*Trichoderma asperellum* (LN846677.1)
5	*Trichoderma* sp. (T5)	Sirigeri (Karnataka)	*Trichoderma asperellum* (KU987247.1)
6	*Trichoderma* sp. (T6)	Kanavi (Karnataka)	*Trichoderma asperellum* (JX422014.1)
7	*Trichoderma* sp. (T7)	Bedla (Gujarat)	*Trichoderma asperellum* (KT876619.1)
8	*Trichoderma* sp. (T8)	Umrali (Gujarat)	*Trichoderma asperellum* (KC113288.1)

Table 4.22. Molecular identity of potential isolates of *Trichoderma* sp. sequenced and deposited in Genebank, NCBI, USA

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *Trichoderma* sp.
1	T1	*T.harzianum* (KR232487.1)*	97	>GGCGTCCGTAGTCGATTGCAGCGCTTACTGCGCGGCGAAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAA CACAATTTAATTATTTTTACAGTTAGTCAAATTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGC AGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGT CGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTGGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGGATATTTCTGGTATTGTTGG CCCGGCCTTACAACAACCAAACAAGTTTGACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATTATCAACCGGAGGAATCATTACAGTATTCTTTTGCAGCGTTTACTCGCGGCGA AAAACATACACACAGTGTTTTTTTGATTCAAAATTATGCTTTTGTTTGCTAGAGAAGGTGGGCGAAGGTTTAC >GGAAAAGGTGGGATGCGTACTGATTGAGGTCAACTTGTTTGGTTGTTGTAAGGCCGGGCCAACAATACCAGAAATATCCCGCCACACCATTCAACGAGTTGGATAAACCTAATACATTGAGA GGTCGACAGCACTATCCAGTACTACCCATGCCAATACTTTTCAAGCAAACGCCTAGTCCGACTAAGAGTATCACTCAATACCAAACCCGGGGGTTTGAGAGAGAAATGACGCTCAAACAGGCA TGCCCTCTGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACGAAAATCTGCAATTCATATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAACCAAGAGATCC GTTGTTGAAAGTTTTGAAGATTAATTCAAAATTTGACTAACTGTAAAAATAATTAAATTGTGTTTTGTTAAACCTCTGGCCCATCCTATCTCTAGGCCAAAGCAAAGCAAGAGTTCTGTATCAA AAAGACACTGTGTGTTAGGTTTTTCGCCGCGCAGTTAAGCGCTGGCAAAAGAATACTGTAATGATCCTTCCGCAGGCACCCAACCGGAAAAAACTTACAGTTTTCCTTTTGCAAGCCCTTAATC CCCGGGAAAAAAAAAAAAAAAACGCCTTTTTTATAAAAACAATCCCTTGGTTGGCCTAGGCAAGGGGGGGCGGGGCTTAAAATCAAAAA
2	T2	*T. viride* (JF304319.1)	96	>GGGCTCTGACGTCACTCCAACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAACTCTT TCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAG TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGATCGGC GTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCTAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGCCCACGTCCGTA AAACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCTCGCTGAACTTAAGCATATCAGAAACCCCGGAAAGGAAAATACCGAGTTTAAAATCCCAACCCAATGTGAACGTT ACCAACTGTTGCAGCGGGGGCCCCCGCGGCGTCAGCCCCCGACAGGGCCCCCGGAAGAACACAACACCTTTTCGTGAGCCCCCGCGGGAATTTTTCTAAG >GGATAGGTGGCTTCATACATGATCGAGGTCAACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGGCGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCG AGACCGCCACTGTATTTAGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAGAATACTGG CGGGCACATGGTGCGTTCAAAGATTCGATGATTCACTGATTTCTGCAATTCACATTACTTATCGCATTTCGCTGGGTTCTTCATCGATGAGCGAACCTTAAAATCCGGTGTTGAAAGTTTGGATT CGTTTTGAGTTTTGGGTCCCATCTGTCGGAAATCCCTCCCCCAAGGGGACTGCAGATAGAGCTTGGTTGGCTCCTCCGCCGGGGGCTGGGTTCCTGGGGCTGCGACGCACCCCGGGGCGTCATC CCGCAAACGCACCAGTTTGTTAATGTGTTACCTTGGGTTTGGGGAGTAGTAACCTGGGGAATGATCCCTCGTCAGCACCCCGGAAAAAAAAATTTCCTGTTTTTCATTTCCAAACCCATGGGCC GTTCCAAACTGTCCCCCGGGGCCCCCGGGGGGCCCCCCCCCGACCAGCCCCCCCGAGGGAACCCCAAGGTGTTTGAGGCCCCCGCGGAAATTTTTTTG
3	T3	*T. asperellum* (KU987251.1)	99	>CCAGGGTAGTTGTCTAGCGTCAGCACCATACCATGTGACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCA AACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGC GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGG ATCGGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCTAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGCCCACG TCCGTAAAACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCCGCTGAACTTAAGCATATCATAGCCGGAGGAAAATATTATTTGTTCAGTAACCCCGAGAACCTAAGCA GGTGGACACGCTCGCCACCTTATACTATCAAGAG >GATAGCTGCAATACATAATGATCATGGTCACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGGCGCGCCGCTTTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAG ACCGCCACTGTATTTAGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGTTCCGAGGGTTGAAATGACGCTCGAAAAGGAATGCCCGCAAAAATACGGGCG GGCGCATGGAGTGTTTAAAGATTCGATGATTCACTGAATTCTGCAATTTACATTACTTATCGAATTTCGCTGCGTTTTTCATCGATGCCAGAAACCAAGAGATCCGTTGTTGAAAAGTTTTGATT CTTTTTGAATTTTTGCACAGAGCTGAAAAAAATACGTCCGCGAGGGGGACAACAGAAAGGTTTTGGTTGTCTCCTCGGGGGGGGGCTCGGGTTCCGGGGGATGCGACGCACCCCGGGCCGTGA CCCCGCAGAGACAAAATTTTGGAAACCGTTCACATTGGGTTTGGGAGTTGTAACCCCGGGAAATGATCCCTCCGCAGGTCACCCAACAGAAAGGAATGTCCCTTTTTTACCCCCCCAAAACCC TTGGTAAAATGACAAAAAGGGAAGGGGCGGCCCCAACGTCAGTGAAAACAAAAAAGAGGCCCCCCGGGGAAAACCACAACTTTTTTTTGACCAAAGCACAGTT
4	T4	*T. asperellum* (LN846677.1)	98	>TGGCAACTAGCGGCAGCTCCATACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAAC TCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGATC GGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCTAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGCCCACGTCC GTAAAACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAGTAGCCGGAAGAAAA >GGATAGTAGGTCATAATGATCGAGGTCACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGGCGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAGAC CGCCACTGTATTTAGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGGTTCGGAGGGTTGAAATGCCGCTCGGACAGGCATGCCCGCCAAAATACTGGCGG GCACAATGTGCGCTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCTTTCTCCATCGATGCGAGAAACCAAAAGATCCGTTGTTGAAAGTTTTGATTCA TTTTGAATTTTTGCTCAGAGCTGTAAGAAATACGTCCCCAAAGAGACTACAGAAAGAGTTTGGTTGTCTCCTGCGGCGGGCGCTGGGAACTGGGACTGCGAGCACCCGGGGCGTGACCCCGCA AACACACAGTTTTGTAACGTTCCATTGGGTTTGGGAGTTAAAACTCGGGAACGACCCTCGCAGTTCCCCTGGAAAA
5	T5	*T. asperellum* (KU987247.1)	99	>TGGCCAAACTGAGCGTTCAGCTCCAACCCATGTGACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAAC TCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGATC GGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCTAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGCCCACGTCC GTAAAACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTTAAGCATATCATAGCCGGAAGGAAAAA >GGGATATGGGTTCATCATACATGATCGAGGTCAACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGGCGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGG CGAGACCGCCACTGTATTTAGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGGTTCGGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAAAATAC TGGCGGGCGCGATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCTGGTGCGTTCTTCATCGATGCCAGAACCTAGAGATCCGTTGTTGAAAGTTTT GATTCATTTTGAATTTTGGCTCCGAGCTGAAAGAAATACGCCCCCGAGGGGACTACAGAAAGAGTTGGGTTGTTTCCTCCGGCGGGGGGCTGGGGTTCCGGGGCTGCGAGCACCCCGGGCCGT GACCCCTCAAAGACACCAGTTTGTTAACGTTCACATTGGGGTTTGGGAGTTGTAACCTCGGGGAATGATCCCTCCGCAGTCCCCCCCAAAAAAAAAGTTGTTTCGGGGTTTCCCCCCCCGAAAC GGGGGGAGGCCGTTTCCCATTTGTGGGGGGGGGGGCTCCGCCGGTGGGGGGCACGAAAAAAAGGGCGGAGGGGGGAGAAAAAACGTTTTATGTTTGTTCCCAGGG

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of ITS-rDNA region of isolates of *Trichoderma* sp.
6	T6	*T. asperellum* (JX422014.1)	97	>TGGCCTACGGACGTTCAGCTCCAACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAAC TCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGATC GGCGTTGGAGATCGGGACCCATCACACGGGTGCCGGCCCCTAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGCCCACGCCC GTAAAACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGATACCCGCTGAACTAAAGCATATCATAACCCGGAAGAAAA >GGGATAGATGGGATCATACATGATCGAGGTCAACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGGCGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGC GAGACCGCCACTGTATTTAGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATGCGCGCCAGAATACTG GCGGGCACGATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAACCAAGAGATCTGTAGTTGAAAGTGTTGA TTCATTTTGAATATTTGCTCAGAGCTGTAAGAAACACGTCCGCGAGGGGACTACAGAAAGAGTTTGATTGGCTCCTCCGCCGGGCGCTTGGTTCCGGCACTGCGACGCACGCGGGCGTGACCC CGCCGAGACAACAGTTTGTTAACGTTCACATTGGGTTTGGGAGTTGTAACCTCGGGAATGATCCCTCCGCAGACCCCCGTAACAAAA
7	T7	*T. asperellum* (KT876619.1)	98	>TTGGGCTCCTGAGTTGCAGCACCATACCCATGTCACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGCTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGACAACCAACCAAACT CTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGAATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCGGGGGGATCGG CGTTGGAGATCGGGACCCCTCACACGGATGCCGGCCCCGAAATACAGTGGCGGTCTCGCTGCAGCCTCTCCTGCGCAGTAGTTTGCACAACTGGCACCGGGAGCGCGGCGCGTCCACGTCCGT AAAAGACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCCGCTGAACTTACCGCACATCAGTAGGCGGGAAGAAAAA >GGCAAGCTGCACGTAATGATCCGAGGTCACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGACCCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAGA CCGCCACTGTATTTCGGGGCCGGCACCCGTGTGATGTGTCCCGATCCCCAACGCCGATCACCCGGAGGGGTTCGAAGCTTGAAATGACGCTTGAAAGGGAATGCCCGCCAAAATACGGGCGG GCGCTGGGTGCGTACAAAGATTCGATGATTCACTGAATTCTGCAATTCAAATTACTTATCGCATTTCGCTGCGTTCTTCATCTATGCCGGAACCAAGAGATCTGTTGTTGAAAGTTTAGATCTCT TTTTGAATTTTTGCACACAGCTGTAAGATATACGTCCGCGAGGAGACTACAGAAATGTTTTGGTTGATTCCTCCGGGGGGGGTCTGGTTCCGGGACTGCGACGCCCCCGGGGGCGTTACCCCTA AAAGCCCACAGTTTTCGAAACGTTCACATTGGGTTCGGGGAGTTGTAACCTCGGTAATTATTCCCTCCGCAGTCCCCCAACCAAAAGAAGAATCCCGTAGGTGACACTCCAAAACCTTTTAGTC CCTCGAAAAATGTGGGCACAGGGGTGTTGTTTGTTAAGTGACTCGGT
8	T8	*T. asperellum* (KC113288.1)	96	>TGGGCCTGCTGAGCGTGAAGCACCATACCCATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCA AACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGC GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGG ATCGGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCCGAAATACAGTGGCGGTCTCGCCGCAGCCTTCTCCCTGCGCAGTAGTTTTGCACAACTCGCACCGGGGAGCGCGGCGGCG CCCCACGTTCCGTAAAAACACCACAACTTTTCTGAAATGTTGACCTCGGTATCAGGGAAGGATATACCCCGCTGAACTTAAGGCATATCAGAAGCCGGGAAAGGAAAAATTAGGATCCCCTAA AGGAAACCCTTGGGAGACTTTTACAAACTGTTGAAAAAACTGAAAACACCCGGTGTTGTTAATCCCCAAAAACAAGCCCCGGTGGAGACCCACTAATTTTTTCTGTGAACCCGG >GGGAACGCTAGCATCATACATGATCCGAGGTCACATTTCAGAAAGTTGGGTGTTTTACGGACGTGGACGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGGC GAGACCGCCACTGTATTTCGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGCCGATCCCCCGGAGGGGTCCGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAGAATACTG GCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTCCGCTGCGTTCTTCATCGATGCCAGAACCAAGAAGATCCGTTGTTGAAAGTTTTG ATTCTTTTTGAATTTTTGCCCACAGCTGTAAGAAAATACGTCCCCCGAGGGGACTACAGGAAATAGTTTGGGTCGTTTCCTCCGGGCGGGGCCTCGGGTTCCGGGGGCTGCGACCCCCCCGGGG CGTGACCCCCGCAAAGGCACCAGTTTGGTAACGTTCAACATTGGGTTTGGGAGTGGTAAACTCGGTAATGATCCCTCCGCAGGTCCCCCCTAACGAAAAGAAAGTATCCCCTACCGAAAACTC CAAAAACAGTTTGAAAGGGGAAAATTGTTCCCCGGGGGGGTCCGGCGGGAAGGGGCCAGGAGGAGCCCCCGGGTATCCTAACCCCCGGTG

*Strains in the parentheses are reference strains of *Trichoderma* sp. present in NCBI genebank

Table 4.23. List of potential isolates of *Bacillus* sp. isolated from groundnut rhizosphere soils

S. No.	*Bacillus* isolate	Soil sample location	Gram stain	Cell shape	16S rRNA sequence identification
1	*Bacillus* sp. (B1)	ICRISAT (Telangana)	+	Rod	*Bacillus megaterium* (KJ721214.1)
2	*Bacillus* sp. (B1)	Kadiri (Andhra Pradesh)	+	Rod	*Bacillus pumilus* (EU855197.1)
3	*Bacillus* sp. (B1)	Hosahalli (Karnataka)	+	Rod	*Bacillus cereus* (KX242264.1)
4	*Bacillus* sp. (B1)	Chokli (Gujarat)	+	Rod	*Bacillus pumilus* (KJ767390.1)
5	*Bacillus* sp. (B1)	Khadiya (Gujarat)	+	Rod	*Bacillus pumilus* (KF475865.1)

Table 4.24. Molecular identity of potential isolates of *Bacillus* sp. sequenced and deposited in Genebank, NCBI, USA

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of 16S-rRNA region of isolates *Bacillus* sp.
1	B1	*B. megaterium* (KJ721214.1)*	99	>AGGCGTGATGAGTGCTAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCA GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA AATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCT ACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTGTG GAGCCAGCCGCCTAATGTGGGACAGATGATTGGGTGAAGTCGTAACAGCAAACCGTAACGTGTACTTTTCGGGCCGGTAACGGCACCTGAGGGGCGATAACACGACGGAAAAGACGCGATCACATAT GGTGGTCATCACTATCCTGTAACGAAGTCAGCGCAACGGTGAGAAGGCCTCCTCCCTTTGCGGCAAGATTCCCCTATTAACGACTGGCCCTGGAGGAGACAGCCGGGAGGCTTTACACTCAAAGTAT >CGTCGCGTCTCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCT TTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAAT GACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCC GTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCAT TGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAAT GCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCGCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACG TGTTACTCACCCGTCCGCCGCTAACTTCATACGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTACTCACGCACGCCGCCAGCGTTCATCCTGAACAGAAAAAAAAAAATTGTACGGGCGGCGGAAT AGTTGTACGCCACTAGTTTTTTTTATTTACG
2	B2	*B. pumilus* (EU855197.1)	96	>ATAGGGGTGATGAGTGCTAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCAC AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGT CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT CAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCTGCAAGACCGCAAGGTTTAGCCAATCCCATAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGA CTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCTTTA TGGAGCCAGCCGCCGAAGGTGGGGCAGATGATTGGGTGAAGTCATAACAAGTAACCGTAACGCTGCTCTGGTGTGTCACTGACCCTCAGAGCGTAAGCGTGGGGAAGAGAACGGCATTAGATCCCCT GGTGCTCCTCCACGTCACTACGTATTTGTCGCTGCAGGGGAATTTCACCTCTCTTCTGTGCACTCAAGTTCTACGAGTTTACCATGAACCCTCACCTGTTGCGCGAGGGGTGCTTCACATGAGTAATT >ACGTTGCGTCTCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACG CTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCA ATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG CCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCA TTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTA ATGCGCCGCGGGTCCATCTGTAAGTGACAGACGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCA CGTGTTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCGCTCGACTTGCATGTATTAGGCACGCCGGCAGCGTTCGTCCTGAGCAAGATTCATATCTACTAGGGCCTCCCAGAGAA ACCGGGTTGACGCGGTTATATTCTCGTAA
3	B3	*B. cereus* (KX242264.1)	97	>GTAGGGGAGATGAGTGCTAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCAC AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGT CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA CGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACT CGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCG TAAGGAGCTAGCCGCCTAAAGTGGGACAGATGATTGGGTGAGTCGTACAGTTAACCGTAACGTTTTTTTCCGCCTGTTATTGCACCTCTAAGCGCGGATAACGAGGCAAAAAACCCGCTATCCACATCGC TGTGTCCCCCCCACTCTTAACCCATTGACCGCTATACTGTGGAGAGTCCGCTTCTCTTCTTCGCACACAATATCCCAATATCACACGACCCTGCCGATGTAGGCCCGGAGGACTTACAACTATCA >GGGTGCGTCTCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACG CTTTCGCGCCTCAGCGTCAGTTACAGACCAAAAAGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCGCTTTTCTCTTCTGCACTCAAGTTCCCCAGTTTCCA ATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG CCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACAAGCAGTTACTCTCGTACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCA TTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAA TGCACCGCGGGCCCATCTGTAAGTGATAGCCGAAACCATCTTTCAATCATCTCCCATGAAGGAGAAGATCCTATCCGGTATTAGCTTCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCCCACG TGTTACTCACCCGTCCGCCGCTAACGTCATAGAAGCAAGCTTCTAATCAGTTCGCTCGACTTGCATGTATTAGCACGCCGCCAGCGTTCATGCTGAGCA

Cont.

S. No.	Isolate	Identified as	% homology	Forward and reverse nucleotide sequences of 16S-rRNA region of isolates *Bacillus* sp.
4	B4	*B. pumilus* (KJ767390.1)	97	>CTGGGGGTGAAGAGTGCTAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCAC AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGT CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT CAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCTGCAAGACCGCAAGGTTTAGCCAATCCCATAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGA CTGCGTGAAGCTGGAATCACTAGTAATCGCGGATCAGCATGCGGCGGTGAATACGTTCCCGGGCCTTGCACACACCGCCCCTCACACCACGAGAGTTTGAAACACCCGAAGTCGGTGACCCTAACCTTT ATGGAGACATCCGCTGAATCGTGGGCCCGATGATGTAGTGAAGTCATAACCAGGGTAACCGTAAGCGTTCTCTGCGCCCGTCACTGCGCCTGAAGAGCGAAGTGTGGGGAACCGAA >GCGGTCATCTCCCAGGCGGAGTGCTTATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGC TTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCAA TGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGC CGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCAT TGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCTCCTTGGTGAGCCATTACCCCACAATCTAGCTGAT GCGCCGCGGGTCCATCTGAAAGTGAGAAACAAAACCGTCATACATCCTTGACCGTGCGGATTCTGGACCTATCCAGTATTAGCTGCGGTATCCCGGAGGTTTCCCACCCTGACTGGCCCCCTAGTACCTG ATGACTGAACCGTACGCAGGGGGAAATCCGGCAGCAATACTCCCTCCTAACAGCTGAAGTAGTATGAATAGGGAGTCGGCAG
5	B5	*B. pumilus* (KF475865.1)	99	>ACTTTCCTGGCGAGATGAGTGCTAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCC CCCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTT GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT GACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCTGCAAGACCGCAAGGTTTAGCCAATCCCATAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAA CTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAAC CTTTATGGAGCCAGCCGCCGAACGTGGGGCATATGATTGGGGTGAAGTCGTAACAAGGTAACCGTAAGGTGTG >GGGGCTTACTTTCTCCCAGGCGGACTGCTTATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTACCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCA CGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTTCCCAGTTTC CAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTT AGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGT CCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAG CTAATGCGCCGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCAGTCTTACACGCGGTTACCCA CGTGTTACTCACCCGTCCGCCGCTACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGATCCAAGATCAAAGCTCTCGGGCGGGCCGGAC

*Strains in the parentheses are reference strains of *Bacillus* sp. present in NCBI genebank

Table 4.25. Characterization of potential isolates of *Trichoderma* sp. for biocontrol traits against *S. rolfsii*

Isolates	Dual culture assay			Metabolite assay			Culture filtrate assay	Inhibitory effect on oxalic acid production
	Radial growth[1]	Number of sclerotia[2]	Size of sclerotia[3]	Radial growth[1]	Number of sclerotia[2]	Size of sclerotia[3]	Sclerotial germination[4]	Oxalic acid[5]
T1	77.44 (61.70)*	94.13 (76.05)	54.74 (47.74)	59.22 (50.34)	68.78 (56.08)	52.50 (46.46)	96.00 (79.70)	92.74 (74.43)
T2	73.00 (58.74)	91.53 (73.14)	46.84 (43.21)	55.67 (48.28)	66.06 (54.40)	42.50 (40.69)	86.00 (68.23)	89.73 (71.39)
T3	69.56 (56.56)	90.51 (72.11)	41.58 (40.17)	51.22 (45.73)	60.89 (51.32)	33.82 (35.54)	73.00 (58.90)	84.42 (66.80)
T4	66.22 (54.52)	89.95 (71.57)	38.16 (38.16)	50.78 (45.47)	57.83 (49.53)	33.16 (35.11)	64.00 (53.18)	84.96 (67.23)
T5	67.33 (55.18)	86.94 (68.86)	36.84 (37.38)	50.22 (45.15)	53.44 (47.00)	34.87 (36.16)	66.00 (54.45)	84.42 (66.81)
T6	64.56 (53.50)	86.17 (68.21)	26.84 (31.12)	50.11 (45.09)	48.28 (44.03)	33.68 (35.42)	65.00 (53.83)	85.13 (67.39)
T7	60.11 (50.89)	77.40 (61.66)	28.42 (32.15)	47.22 (43.43)	46.61 (43.08)	26.32 (30.80)	59.00 (50.23)	79.82 (63.36)
T8	58.78 (50.08)	75.87 (60.62)	24.74 (29.66)	48.22 (44.00)	44.50 (41.86)	25.26 (30.10)	47.00 (43.29)	78.41 (62.35)
CD (0.01)	4.107	2.338	1.047	3.256	1.489	1.713	1.658	3.234
S.Em.±	1.623	0.807	0.361	1.124	0.514	0.591	0.572	1.117
CV (%)	6.80	7.30	8.21	6.70	7.70	8.90	7.60	6.90

*Figures in the parenthesis are arc sine transformed values

1 – Per cent inhibition of radial growth over control, **2** – Per cent reduction of number of sclerotia over control, **3** – Per cent reduction of size of sclerotia over control, **4** – Per cent inhibition of sclerotial germination over control, **5** – Per cent reduction of oxalic acid content over control

Table 4.26. Characterization of potential isolates of *Bacillus* sp. for biocontrol traits against *S. rolfsii*

Isolates	Dual culture assay			Metabolite assay			Culture filtrate assay	Inhibitory effect on oxalic acid production
	Mycelial growth[1]	Number of sclerotia[2]	Size of sclerotia[3]	Mycelial growth[1]	Number of sclerotia[2]	Size of sclerotia[3]	Sclerotial germination[4]	Oxalic acid[5]
B1	70.33 (57.03)*	83.37 (65.97)	46.58 (43.04)	74.22 (59.53)	84.32 (66.71)	49.08 (44.48)	94.00 (77.50)	95.93 (78.42)
B2	65.22 (53.90)	80.68 (63.98)	37.76 (37.93)	68.00 (55.58)	71.53 (57.80)	39.08 (38.70)	87.00 (69.10)	92.39 (74.05)
B3	61.56 (51.73)	78.89 (62.71)	36.45 (37.15)	67.11 (55.05)	66.58 (54.72)	41.18 (39.94)	74.00 (59.43)	92.74 (74.43)
B4	62.44 (52.25)	77.16 (61.51)	25.00 (29.96)	56.11 (48.54)	62.68 (52.38)	26.32 (30.87)	66.00 (54.45)	85.66 (67.81)
B5	58.11 (49.73)	76.32 (60.93)	27.11 (31.31)	51.56 (45.92)	58.53 (49.94)	33.42 (35.33)	59.00 (50.23)	85.49 (67.67)
CD (0.01)	4.909	3.081	1.130	3.773	1.520	1.455	1.861	4.127
S.Em.±	1.637	1.028	0.377	1.258	0.507	0.485	0.621	1.377
CV (%)	5.90	8.31	7.33	7.80	8.63	9.13	7.50	8.10

*Figures in the parenthesis are arc sine transformed values

1 – Per cent inhibition of radial growth over control, **2** – Per cent reduction of number of sclerotia over control, **3** – Per cent reduction of size of sclerotia over control, **4** – Per cent inhibition of sclerotial germination over control, **5** – Per cent reduction of oxalic acid content over control

Table 4.27. Compatibility of potential isolates of *Trichoderma* sp. with fungicides under *in vitro* conditions

Isolates	Thiram			Carbendazim			Azoxystrobin			Tebuconazole		
	3000 ppm[a]	1500 ppm[b]	Mean	1000 ppm[a]	500 ppm[b]	Mean	1000 ppm[a]	500 ppm[b]	Mean	1000 ppm[a]	500 ppm[b]	Mean
T1	20.56* (26.92)**	0.00 (0.00)	10.28 (13.46)	21.48 (27.62)	0.00 (0.00)	10.74 (13.81)	31.67 (34.24)	0.00 (0.00)	15.84 (17.12)	65.74 (54.20)	0.00 (0.00)	32.87 (27.10)
T2	25.56 (30.35)	0.00 (0.00)	12.78 (15.18)	27.04 (31.34)	0.00 (0.00)	13.52 (15.67)	34.98 (36.26)	0.00 (0.00)	17.49 (18.13)	68.70 (56.01)	0.00 (0.00)	34.35 (28.01)
T3	28.44 (32.22)	0.00 (0.00)	14.22 (16.11)	28.50 (32.28)	0.00 (0.00)	14.25 (16.14)	36.22 (37.01)	0.00 (0.00)	18.11 (18.51)	68.89 (56.13)	0.00 (0.00)	34.45 (28.07)
T4	27.56 (31.65)	0.00 (0.00)	13.78 (15.83)	29.89 (33.15)	0.00 (0.00)	14.95 (16.58)	37.58 (37.81)	0.00 (0.00)	18.79 (18.91)	71.73 (57.92)	0.00 (0.00)	35.87 (28.96)
T5	29.79 (33.07)	0.00 (0.00)	14.90 (16.54)	30.16 (33.33)	0.00 (0.00)	15.08 (16.67)	37.59 (37.82)	0.00 (0.00)	18.80 (18.91)	72.73 (58.56)	0.00 (0.00)	36.37 (29.28)
T6	29.80 (33.08)	0.00 (0.00)	14.90 (16.54)	31.08 (33.90)	0.00 (0.00)	15.54 (16.95)	38.68 (38.46)	0.00 (0.00)	19.34 (19.23)	73.40 (58.99)	0.00 (0.00)	36.70 (29.50)
T7	29.57 (32.93)	0.00 (0.00)	14.79 (16.47)	30.63 (33.62)	0.00 (0.00)	15.32 (16.81)	38.79 (38.53)	0.00 (0.00)	19.40 (19.27)	75.41 (60.32)	0.00 (0.00)	37.71 (30.16)
T8	30.07 (33.25)	0.00 (0.00)	15.04 (16.63)	30.63 (33.62)	0.00 (0.00)	15.32 (16.81)	37.46 (37.74)	0.00 (0.00)	18.73 (18.87)	75.39 (60.29)	0.00 (0.00)	37.70 (30.15)
Mean	27.67 (31.68)	0.00 (0.00)	-	28.68 (32.36)	0.00 (0.00)	-	36.62 (37.23)	0.00 (0.00)	-	71.50 (57.80)	0.00 (0.00)	-

Factors	Thiram			Carbendazim			Azoxystrobin			Tebuconazole		
	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)
Isolates	1.344	0.456	7.2	0.330	0.114	11.7	1.523	0.527	6.9	0.679	0.235	12.10
Fungicide dose	0.672	0.233		0.165	0.057		0.761	0.264		0.339	0.117	
Interaction	1.901	0.658		0.467	0.162		2.154	0.746		0.960	0.332	

*Values represent the per cent inhibition over control **Figures in the parenthesis are arc sine transformed values

a – recommended dose of fungicides, **b –** half the recommended dose of fungicides

Table 4.28. Compatibility of potential isolates of *Bacillus* sp. with fungicides under *in vitro* conditions

Isolates	Thiram			Carbendazim			Azoxystrobin			Tebuconazole		
	3000 ppm[a]	**1500 ppm[b]**	**Mean**	**1000 ppm[a]**	**500 ppm[b]**	**Mean**	**1000 ppm[a]**	**500 ppm[b]**	**Mean**	**1000 ppm[a]**	**500 ppm[b]**	**Mean**
B1	18.83* (25.73)**	6.28 (14.50)	12.55 (20.12)	37.97 (38.06)	5.23 (13.17)	21.60 (25.62)	42.45 (40.68)	6.28 (14.50)	24.37 (27.59)	49.78 (44.89)	26.91 (31.26)	38.35 (38.08)
B2	27.80 (31.84)	8.22 (16.58)	18.01 (24.21)	42.90 (40.94)	7.77 (16.11)	25.34 (28.53)	44.99 (42.15)	8.82 (17.27)	26.91 (29.71)	52.02 (46.18)	31.99 (34.46)	42.01 (40.32)
B3	27.65 (31.57)	10.61 (18.94)	19.13 (25.26)	44.84 (42.06)	9.27 (17.57)	27.06 (29.82)	46.34 (42.92)	6.58 (14.82)	26.46 (28.87)	50.82 (45.49)	33.48 (35.37)	42.15 (40.43)
B4	37.07 (37.53)	13.30 (21.35)	25.19 (29.44)	43.80 (41.45)	9.72 (18.13)	26.76 (29.79)	44.99 (42.15)	9.87 (18.32)	27.43 (30.24)	53.06 (46.78)	34.98 (36.28)	44.02 (41.53)
B5	31.69 (34.24)	11.51 (19.79)	21.60 (27.02)	41.11 (39.90)	6.43 (14.66)	23.77 (27.28)	49.93 (44.98)	7.92 (16.35)	28.93 (30.67)	51.12 (45.67)	33.33 (35.28)	42.23 (40.48)
Mean	28.61 (32.18)	9.98 (18.23)	-	42.12 (40.48)	7.68 (15.93)	-	45.74 (42.58)	7.89 (16.25)	-	51.36 (45.80)	32.14 (34.53)	-

Factors	Thiram			Carbendazim			Azoxystrobin			Tebuconazole		
	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)	CD (0.01)	S.Em ±	CV (%)
Isolates	2.854	0.960	9.3	2.140	0.720	6.3	0.879	0.269	10.5	0.774	0.261	9.6
Fungicide dose	1.805	0.607		1.353	0.455		0.556	0.187		0.490	0.165	
Interaction	4.036	1.358		3.026	1.019		1.243	0.419		1.095	0.369	

*Values represent the per cent inhibition over control **Figures in the parenthesis are arc sine transformed values

a – recommended dose of fungicides, **b –** half the recommended dose of fungicides

Table 4.29. *In vitro* plant growth promotion by bioformulations of *Trichoderma* sp. (T1) and *Bacillus* sp. (B1) in groundnut

Treatments	Germination %	Shoot length (cm)	Root length (cm)	Biomass (g)	Total root length (cm)	Root Volume (cm^3)	Vigour index-I	Vigour index-II
T1	83.33	17.11	22.57	21.00	479.43	0.48	3301.97	127.23
T2	90.00	21.49	25.30	22.20	557.80	0.61	4211.70	139.80
T3	90.00	25.80	27.23	23.37	694.58	0.68	4773.00	175.50
T4	96.67	24.01	25.93	25.87	770.81	0.78	4821.63	175.00
T5	96.67	25.55	26.73	27.93	766.63	0.81	5057.00	261.33
T6	100.00	27.67	26.56	31.20	852.65	0.91	5423.33	283.33
T7	90.00	18.35	26.45	17.67	367.66	0.42	4032.30	104.40
CD (0.01)	6.75	3.38	2.17	1.86	94.75	0.05	407.20	29.46
S.Em.±	2.21	1.11	0.71	0.61	31.01	0.02	133.30	9.62
CV (%)	4.10	8.40	4.80	4.40	8.40	3.90	5.10	9.20

T1 – ST+SA of talc formulation of *Bacillus* sp. (B1), **T2** – ST+SA of talc formulation of *Bacillus* sp. (B1) (with chitin), **T3** – ST+SA of talc formulation of *Trichoderma* sp. (T1), **T4** – ST+SA of talc formulation of *Trichoderma* sp. (T1) (with chitin), **T5** – ST+SA of talc formulation of *Bacillus* sp. + *Trichoderma* sp. (T1+B1), **T6** – ST+SA of talc formulation of *Bacillus* sp. + *Trichoderma* sp. (T1+B1) (with chitin), **T7** – Control

Table 4.30. Effect of bioformulations on stem rot severity in groundnut under glasshouse conditions

Treatment	Disease severity (%)					
	15 dpi*	**30 dpi**	**45 dpi**	**60 dpi**	**75 dpi**	**Mean**
T1	31.25 (33.99)**	62.50 (52.24)	70.31 (56.98)	74.61 (59.74)	76.95 (61.31)	63.12 (52.85)
T2	31.25 (33.99)	60.94 (51.32)	68.75 (56.01)	73.83 (59.23)	77.73 (61.84)	62.50 (52.48)
T3	26.17 (30.77)	44.92 (42.09)	52.34 (46.34)	56.25 (48.59)	57.81 (49.49)	47.50 (43.46)
T4	23.44 (28.96)	43.36 (41.18)	50.00 (45.00)	57.42 (49.27)	56.25 (48.59)	46.09 (42.60)
T5	17.19 (24.49)	35.55 (36.60)	44.92 (42.09)	46.09 (42.76)	49.61 (44.78)	38.67 (38.14)
T6	13.67 (21.70)	29.69 (33.02)	37.50 (37.76)	42.19 (40.51)	42.97 (40.96)	33.20 (34.79)
T7	17.58 (24.79)	35.16 (36.36)	39.06 (38.68)	41.80 (40.28)	44.92 (42.09)	35.70 (36.44)
T8	48.44 (44.10)	74.61 (59.74)	99.61 (86.42)	100.00 (90.00)	100.00 (90.00)	84.53 (74.05)
T9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	23.22 (26.98)	42.97 (39.17)	51.39 (45.48)	54.69 (47.82)	56.25 (48.78)	-

Factors	CD (0.01)	S.Em.±	CV (%)
Treatments	3.197	1.142	13.30
dpi	2.097	0.749	
Interaction	8.534	3.048	

***dpi** – days post pathogen inoculation **Figures in the parenthesis are arc sine transformed values

T1 – ST+SA of talc formulation of *Bacillus* sp. (B1), **T2** – ST+SA of talc formulation of *Bacillus* sp. (B1) (with chitin), **T3** – ST+SA of talc formulation of *Trichoderma* sp. (T1), **T4** – ST+SA of talc formulation of *Trichoderma* sp. (T1) (with chitin), **T5** – ST+SA of talc formulation of *Bacillus* sp. + *Trichoderma* sp. (T1+B1), **T6** – ST+SA of talc formulation of *Bacillus* sp. + *Trichoderma* sp. (T1+B1) (with chitin), **T7** – Chemical control, **T8** – Inoculated control, **T9** – Un-inoculated control

Table 4.31. Effect of bioformulations on stem rot incidence in groundnut under glasshouse conditions

Treatment	Disease incidence (%)					
	15 dpi*	30 dpi	45 dpi	60 dpi	75 dpi	Mean
T1	84.38 (66.85)**	96.88 (82.80)	98.44 (86.42)	100.00 (90.05)	100.00 (90.05)	95.94 (83.24)
T2	76.56 (61.35)	93.75 (79.69)	98.44 (86.42)	100.00 (90.05)	100.00 (90.05)	93.75 (81.52)
T3	60.94 (51.40)	76.56 (61.92)	78.13 (62.92)	78.13 (62.92)	78.13 (62.92)	74.37 (60.41)
T4	57.81 (49.59)	71.88 (58.04)	79.69 (63.29)	81.25 (64.53)	81.25 (64.53)	75.31 (60.61)
T5	48.44 (44.11)	76.56 (61.12)	71.88 (58.04)	73.44 (59.12)	73.44 (59.12)	67.81 (55.68)
T6	38.06 (36.69)	58.5 (52.44)	64.88 (51.18)	70.11 (62.20)	72.13 (64.20)	60.73 (53.34)
T7	45.31 (42.31)	67.19 (55.62)	71.75 (59.53)	74.75 (67.53)	75.31 (69.48)	64.86 (51.36)
T8	87.50 (69.65)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	97.50 (85.97)
T9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	55.44 (46.88)	71.26 (60.19)	73.69 (61.98)	75.30 (65.16)	75.58 (65.60)	-

Factors	CD (0.01)	S.Em.±	CV (%)
Treatments	3.310	1.183	9.80
dpi	2.467	0.882	
Interaction	7.402	2.646	

*__dpi__ – days post pathogen inoculation **Figures in the parenthesis are arc sine transformed values

Treatment details are given below table 4.30

Table 4.32. Effect of bioformulations on stem discolouration and pod rot in groundnut under glasshouse conditions

Treatment	Stem discolouration at harvest (%)	Pod rot at harvest (%)
T1	65.08 (53.86)*	32.94 (34.90)
T2	51.39 (45.82)	28.17 (32.02)
T3	46.11 (42.74)	16.39 (23.55)
T4	37.63 (37.83)	18.81 (25.69)
T5	28.09 (31.75)	14.30 (21.76)
T6	7.81 (11.6)	14.06 (21.16)
T7	9.38 (17.6)	15.63 (23.19)
T8	100.00 (90.05)	100.00 (90.05)
T9	0.00 (0.00)	0.00 (0.00)
Mean	41.41 (39.62)	25.98 (29.12)
CD (0.01)	5.690	7.190
S.Em.±	1.951	2.471
CV (%)	9.801	14.90

*Figures in the parenthesis are arc sine transformed values

Treatment details are given below table 4.30

Table 4.33. Effect of bioformulations on mortality due to stem rot of groundnut under glasshouse conditions

Treatment	Mortality (%)					
	15 dpi*	**30 dpi**	**45 dpi**	**60 dpi**	**75 dpi**	**Mean**
T1	4.69 (8.80)**	40.63 (39.47)	45.31 (42.31)	46.88 (43.21)	51.56 (45.94)	37.81 (35.95)
T2	0.00 (0.00)	37.50 (37.62)	39.06 (38.62)	45.31 (42.31)	54.69 (47.73)	35.31 (33.26)
T3	0.00 (0.00)	25.00 (29.93)	35.94 (36.84)	40.63 (39.61)	42.19 (40.52)	28.75 (29.38)
T4	0.00 (0.00)	21.88 (27.60)	28.13 (31.87)	32.81 (34.86)	32.81 (34.86)	23.13 (25.84)
T5	0.00 (0.00)	10.94 (18.84)	26.56 (30.54)	29.69 (32.78)	31.25 (33.78)	19.69 (23.19)
T6	0.00 (0.00)	9.38 (17.60)	15.63 (23.19)	20.31 (26.76)	20.31 (26.76)	13.13 (18.86)
T7	0.00 (0.00)	7.81 (11.60)	14.06 (21.16)	21.88 (27.60)	26.56 (30.78)	14.06 (18.23)
T8	20.31 (26.76)	42.19 (40.50)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	72.50 (67.48)
T9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	3.13 (4.45)	24.42 (27.90)	38.09 (39.32)	42.19 (42.15)	44.92 (43.80)	-

Factors	CD (0.01)	S.Em.±	CV (%)
Treatments	2.884	1.031	16.50
dpi	2.150	0.768	
Interaction	6.449	2.305	

***dpi** – days post pathogen inoculation **Figures in the parenthesis are arc sine transformed values

Treatment details are given below table 4.30

Table 4.34. Effect of bioformulations on total protein content of groundnut plants

Treatments	Sampling intervals (U)								
	0 dpi*	**1 dpi**	**2 dpi**	**3 dpi**	**4 dpi**	**5 dpi**	**6 dpi**	**7 dpi**	**Mean**
T1	8.26	10.42	38.70	24.75	33.80	21.62	10.88	10.04	19.81
T2	9.44	14.42	51.09	38.92	29.90	23.31	14.90	13.02	24.38
T3	10.65	17.16	52.60	43.63	51.02	40.33	14.89	15.11	30.67
T4	11.61	18.41	50.05	59.00	49.33	26.98	17.53	15.69	31.08
T5	12.82	21.42	61.68	60.01	55.39	41.77	25.72	18.70	37.19
T6	18.84	24.92	72.46	65.71	60.36	49.64	28.43	24.73	43.14
T7	9.52	10.39	21.73	24.53	24.76	15.89	10.79	12.54	16.27
T8	8.89	8.59	15.37	19.62	20.94	12.80	10.05	9.74	13.25
T9	9.11	10.18	13.97	14.83	17.32	11.10	9.93	9.15	11.95
Mean	11.02	15.10	41.96	39.00	38.09	27.05	15.90	14.30	-

Factors	CD (0.01)	S.Em ±	CV (%)
Treatments	0.351	0.126	5.70
Sampling intervals	0.370	0.133	
Interaction	1.111	0.398	

***dpi –** days post inoculation **U –** mg g^{-1} FW

T1 – ST+SA of talc formulation of *Bacillus* sp. (B1), **T2** – ST+SA of talc formulation of *Bacillus* sp. (B1) (with chitin), **T3** – ST+SA of talc formulation of *Trichoderma* sp. (T1), **T4** – ST+SA of talc formulation of *Trichoderma* sp. (T1) (with chitin), **T5** – ST+SA of talc formulation of *Bacillus* sp. + *Trichoderma* sp. (T1+B1), **T6** – ST+SA of talc formulation of *Bacillus* sp. + *Trichoderma* sp. (T1+B1) (with chitin), **T7** – Chemical control, **T8** – Inoculated control, **T9** – Un-inoculated control

Table 4.35. Effect of bioformulations on total phenol content of groundnut plants

Treatments	Sampling intervals (U)								
	0 dpi*	**1 dpi**	**2 dpi**	**3 dpi**	**4 dpi**	**5 dpi**	**6 dpi**	**7 dpi**	**Mean**
T1	1.01	1.16	1.56	1.97	1.62	1.41	1.61	1.26	1.45
T2	1.02	1.17	1.92	1.96	1.53	1.48	1.53	1.23	1.48
T3	1.17	1.29	2.05	2.00	1.87	1.58	1.70	1.40	1.63
T4	1.29	1.40	2.02	2.13	2.01	1.56	1.81	1.50	1.72
T5	1.42	1.57	2.22	2.35	2.05	2.01	2.01	1.88	1.94
T6	1.35	1.74	3.01	3.00	2.46	2.29	2.29	2.27	2.30
T7	1.10	1.24	1.18	1.72	1.62	1.27	1.24	1.37	1.34
T8	1.00	1.11	1.61	1.82	1.01	0.92	1.18	0.81	1.18
T9	0.93	1.09	1.45	1.63	1.06	0.87	1.05	0.79	1.11
Mean	1.14	1.31	1.89	2.06	1.69	1.49	1.60	1.39	-

Factors	CD (0.01)	S.Em ±	CV (%)
Treatments	0.044	0.016	5.90
Sampling intervals	0.047	0.017	
Interaction	0.140	0.050	

***dpi –** days post inoculation **U –** mg g^{-1} FW mg^{-1} protein

Treatment details are given below table 4.34

Table 4.36. Effect of bioformulations on phenylalanine ammonia-lyase (PAL) activity in groundnut plants

Treatments	Sampling intervals (U)								
	0 dpi*	**1 dpi**	**2 dpi**	**3 dpi**	**4 dpi**	**5 dpi**	**6 dpi**	**7 dpi**	**Mean**
T1	2.69	3.91	11.02	6.50	5.75	5.34	4.33	2.93	5.31
T2	2.80	4.93	11.29	7.86	7.73	5.31	5.35	2.80	6.01
T3	2.40	5.35	10.35	7.09	8.60	7.27	4.47	4.19	6.22
T4	2.38	5.05	12.60	10.16	8.71	7.40	5.45	3.78	6.94
T5	2.88	6.24	15.06	11.48	9.28	8.50	5.69	4.59	7.97
T6	2.97	6.64	18.61	13.82	10.31	9.68	7.32	5.12	9.31
T7	2.70	2.97	4.28	4.89	3.18	2.68	4.25	0.94	3.24
T8	2.30	3.24	3.39	3.62	1.95	1.07	3.95	0.08	2.45
T9	1.62	2.61	2.37	2.60	2.15	1.66	3.46	0.61	2.14
Mean	2.53	4.55	9.89	7.56	6.41	5.43	4.92	2.78	-

Factors	CD (0.01)	S.Em ±	CV (%)
Treatments	0.013	0.005	5.98
Sampling intervals	0.014	0.005	
Interaction	0.043	0.015	

***dpi –** days post inoculation **U –** µmole trans-cinammic acid $min^{-1}g^{-1}$ FW mg^{-1} protein

Treatment details are given below table 4.34

Table 4.37. Effect of bioformulations on peroxidase (PO) activity in groundnut plants

Treatments	Sampling intervals (U)								
	0 dpi*	**1 dpi**	**2 dpi**	**3 dpi**	**4 dpi**	**5 dpi**	**6 dpi**	**7 dpi**	**Mean**
T1	0.85	0.94	1.87	1.31	1.41	1.26	0.45	0.05	1.02
T2	1.24	1.32	2.48	1.79	1.34	1.32	1.27	0.87	1.45
T3	1.04	1.05	2.55	1.40	1.39	1.60	1.35	0.93	1.41
T4	1.33	1.15	2.46	2.11	1.75	1.89	1.55	1.14	1.67
T5	1.37	1.43	3.00	2.75	2.68	2.25	1.42	1.04	1.99
T6	1.39	1.77	3.95	3.29	3.13	2.95	2.08	1.69	2.53
T7	0.76	0.82	1.81	1.28	1.14	1.23	1.06	0.64	1.09
T8	0.56	0.81	1.70	1.01	0.93	0.36	0.19	0.07	0.70
T9	0.40	0.44	1.28	1.12	0.80	0.83	0.74	0.33	0.74
Mean	0.99	1.08	2.34	1.78	1.62	1.52	1.12	0.75	-

Factors	CD (0.01)	S.Em ±	CV (%)
Treatments	0.029	0.011	6.30
Sampling intervals	0.031	0.011	
Interaction	0.093	0.033	

***dpi –** days post inoculation

U – $\Delta OD_{470nm} min^{-1} g^{-1}$ FW mg^{-1} protein

Treatment details are given below table 4.34

Table 4.38. Zymogram showing banding pattern of peroxidase isozymes in bioformulation treated groundnut plants challenged with *S. rolfsii*

dpi*	Band no.	Isoform	Rf**	Treatments								
				T1	T2	T3	T4	T5	T6	T7	T8	T9
0	1	PO 1	0.04	+++	++	++	+++	+++	+++	++	+	++
	2	PO 3	0.09	++	+	+	++	++	++	+	-	+
	3	PO 4	0.19	++	+	+	++	++	++	+	+	+
1	1	PO 1	0.04	+++	+++	+++	+++	+++	+++	++	++	++
	2	PO 3	0.09	++	++	++	++	++	++	+	+	+
	3	PO 4	0.19	+	+	+	+	+	+	+	+	-
2	1	PO 1	0.04	+++	+++	+++	+++	+++	+++	++	+++	+++
	2	PO 3	0.09	++	++	++	+	++	++	+	++	++
	3	PO 4	0.19	++	++	+	+	++	++	+	+	+
	4	PO 5	0.22	-	-	-	-	+	+	-	-	-
	5	PO 6	0.24	-	-	-	-	-	+	-	-	-
	6	PO 7	0.26	+	+	+	+	+	+	-	-	-
	7	PO 8	0.29	-	-	+	-	+	+	-	-	-
	8	PO 9	0.31	+	+	+	-	-	+	-	-	+
3	1	PO 1	0.04	+++	+++	+++	+++	+++	+++	+++	+++	++
	2	PO 2	0.06	+++	+++	+++	+++	+++	+++	+++	+++	+
	3	PO 3	0.09	+++	+++	+++	+++	+++	+++	+++	++	++
	4	PO 4	0.19	+	+++	++	++	++	+++	++	+	+
	5	PO 5	0.22	-	+	-	-	-	+	-	-	-
	6	PO 6	0.24	+	+	+	++	++	++	++	-	-
	7	PO 7	0.26	-	++	+	+	-	-	-	-	-
	8	PO 8	0.29	-	+	-	-	-	-	-	-	-
4	1	PO 1	0.04	+++	+++	+++	+++	+++	+++	+++	+++	++
	2	PO 3	0.09	+++	+++	+++	+++	+++	+++	+++	+++	++
	3	PO 4	0.19	+	++	++	+++	+++	+++	++	+	+
	4	PO 5	0.22	-	+	+	+	-	+	+	-	-
	5	PO 6	0.24	+	+	+	+	+	+	-	-	-
5	1	PO 1	0.04	+++	+++	+++	+++	+++	+++	-	-	-
	2	PO 3	0.09	+++	+++	+++	+++	+++	+++	-	-	-
	3	PO 4	0.19	-	+	+	+	+	+	-	-	-
	4	PO 5	0.22	+	++	+	++	++	++	-	-	-
	5	PO 6	0.24	+	++	+	++	++	+	-	-	-
	6	PO 7	0.26	-	+	-	-	-	+	-	-	-
6	1	PO 1	0.04	+++	+++	+++	+++	+++	+++	++	++	+
	2	PO 3	0.09	+++	+++	+++	+++	+++	+++	-	-	-
	3	PO 4	0.19	+	++	++	++	+	++	-	-	-
	4	PO 5	0.22	++	++	+	++	++	++	-	-	-
	5	PO 6	0.24	-	+	-	-	-	-	-	-	-
7	1	PO 1	0.04	+++	+++	+++	+++	+++	+++	+++	+++	+
	2	PO 3	0.09	+++	+++	+++	+++	+++	+++	+++	+++	++
	3	PO 4	0.19	+	++	++	++	+	+	-	-	-
	4	PO 5	0.22	++	-	++	++	+	++	-	-	-
	5	PO 6	0.24	-	++	-	-	++	+	-	-	-

+ Presence of band **-** Absence of band **++/+++** Band intensity

***dpi** days post inoculation **Rf** Relative front

Treatment details are given below table 4.34

Table 4.39. Effect of bioformulations on polyphenol oxidase (PPO) activity in groundnut plants

Treatments	Sampling intervals (U)								
	0 dpi*	**1 dpi**	**2 dpi**	**3 dpi**	**4 dpi**	**5 dpi**	**6 dpi**	**7 dpi**	**Mean**
T1	0.14	0.48	1.41	1.05	0.92	0.84	0.87	0.74	0.81
T2	0.27	0.61	1.57	1.22	1.10	1.03	1.03	0.90	0.97
T3	0.66	1.00	1.66	1.31	1.20	1.14	1.14	0.99	1.14
T4	1.07	1.19	1.94	1.61	1.45	1.41	1.40	1.26	1.42
T5	0.94	1.28	2.21	1.85	1.71	1.63	1.66	1.33	1.58
T6	1.32	1.65	2.96	2.62	2.47	2.44	2.43	1.75	2.21
T7	0.07	0.32	0.73	0.39	0.27	0.21	0.18	0.65	0.35
T8	0.03	0.24	0.65	0.33	0.22	0.17	0.15	0.58	0.30
T9	0.03	0.20	0.53	0.24	0.12	0.03	0.05	0.52	0.22
Mean	0.50	0.77	1.52	1.18	1.05	0.99	0.99	0.97	-

Factors	CD (0.01)	S.Em ±	CV (%)
Treatments	0.009	0.003	6.10
Sampling intervals	0.009	0.003	
Interaction	0.027	0.010	

***dpi –** days post inoculation **U –** $\Delta OD_{420nm} min^{-1} g^{-1}$ FW mg^{-1} protein

Treatment details are given below table 4.34

Table 4.40. Zymogram showing banding pattern of polyphenol oxidase isozymes in bioformulation treated groundnut plants challenged with *S. rolfsii*

dpi*	Band no.	Isoform	Rf**	Treatments								
				T1	T2	T3	T4	T5	T6	T7	T8	T9
0	1	PPO 1	0.07	++	++	++	++	++	++	++	+	+
	2	PPO 3	0.11	+	++	++	+	+	+	+	+	-
	3	PPO 5	0.21	+	+	+	+	+	+	+	+	-
	4	PPO 7	0.29	-	+	+	+	+	+	+	+	-
1	1	PPO 1	0.07	++	++	+	+	++	++	+	+	+
	2	PPO 2	0.09	++	++	++	++	++	++	-	+	-
	3	PPO 3	0.11	+	+	+	+	++	++	+	-	-
	4	PPO 5	0.21	+	+	+	+	++	++	-	-	-
	5	PPO 7	0.29	+	+	+	+	++	+	-	-	-
	6	PPO 8	0.36	-	-	-	-	+	+	-	-	-
2	1	PPO 1	0.07	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	PPO 2	0.09	+	+	+	+	+	+	+	-	-
	3	PPO 3	0.11	++	++	++	++	++	++	+	-	-
	4	PPO 5	0.21	++	++	++	++	++	++	-	-	-
	5	PPO 6	0.25	+	++	++	++	++	++	-	-	-
	6	PPO 7	0.29	+	+	++	++	++	+	-	-	-
	7	PPO 9	0.36	+	++	++	++	++	+	-	-	-
	8	PPO 10	0.38	+	+	+	+	+	+	-	-	-
	9	PPO 11	0.43	+	+	+	+	+	+	+	+	-
	10	PPO 12	0.51	+	+	+	+	+	+	-	-	-
	11	PPO 13	0.64	+	+	+	+	+	+	+	+	-
3	1	PPO 1	0.07	+++	+++	+++	+++	+++	+++	+++	++	++
	2	PPO 2	0.09	-	-	-	-	+	+	-	-	-
	3	PPO 3	0.11	+	+	+	+	++	++	+	+	+
	4	PPO 5	0.21	+	+	+	+	+	+	-	-	-
	5	PPO 6	0.25	-	-	-	-	+	+	-	-	-
	6	PPO 7	0.29	-	+	+	+	+	+	-	-	-
	7	PPO 9	0.36	++	++	++	+	++	++	-	+	-
	8	PPO 10	0.38	+	-	+	+	+	+	-	-	-
	9	PPO 11	0.64	+	+	+	+	+	+	+	+	-
4	1	PPO 1	0.07	+++	+++	+++	+++	+++	+++	+++	+	++
	2	PPO 2	0.09	+	++	++	++	++	++	-	-	-
	3	PPO 3	0.11	+	++	+	+	+	+	+	+	+
	4	PPO 4	0.14	+	+	+	+	+	+	+	+	-
	5	PPO 5	0.21	++	++	++	++	++	++	+	-	-
	6	PPO 7	0.29	+	+	+	+	+	+	-	-	-
	7	PPO 9	0.36	-	-	-	-	-	+	-	-	-
	8	PPO 10	0.38	-	-	-	-	-	+	-	-	-
	9	PPO 11	0.64	+	+	+	+	+	+	-	-	-
	10	PPO 12	0.69	+	-	+	-	+	+	-	-	-
	11	PPO 13	0.70	+	-	+	-	+	+	-	-	-
5	1	PPO 1	0.07	+++	+++	+++	+++	+++	+++	+++	-	-
	2	PPO 2	0.09	+	+	+	+	+	+	-	-	-
	3	PPO 3	0.11	++	++	++	++	++	++	-	-	-
	4	PPO 4	0.14	-	+	-	+	+	+	-	-	-
	5	PPO 5	0.21	+	+	+	+	+	+	-	-	-
	6	PPO 7	0.29	+	+	-	+	+	+	-	-	-
	7	PPO 9	0.36	-	+	+	+	+	+	-	-	-
6	1	PPO 1	0.07	+++	+++	+++	+++	+++	+++	+	+	+
	2	PPO 3	0.09	+++	+++	+++	+++	+++	+++	+	+	-
	3	PPO 4	0.14	++	++	-	++	++	+	-	-	-
	4	PPO 5	0.21	+	+	+	-	+	+	-	-	-
	5	PPO 7	0.29	+	+	+	+	+	+	-	-	-
	6	PPO 9	0.36	+	+	+	+	+	+	+	+	-
7	1	PPO 1	0.07	+++	+++	+++	+++	+++	+++	+	+	+
	2	PPO 3	0.11	+	+	++	++	++	++	-	-	-
	3	PPO 4	0.14	-	-	-	+	+	+	-	-	-
	4	PPO 5	0.21	+	+	-	-	+	+	-	-	-

+ Presence of band **-** Absence of band **++/+++** Band intensity

***dpi** days post inoculation **Rf** Relative front

Treatment details are given below table 4.34

Table 4.41. Effect of bioformulations on catalase (CAT) activity in groundnut plants

Treatments	Sampling intervals (U)								
	0 dpi*	**1 dpi**	**2 dpi**	**3 dpi**	**4 dpi**	**5 dpi**	**6 dpi**	**7 dpi**	**Mean**
T1	0.08	0.15	2.81	2.04	1.60	1.12	1.36	1.12	1.29
T2	0.37	0.43	2.08	1.23	0.92	0.73	1.44	0.41	0.95
T3	0.51	0.57	2.84	1.95	1.69	1.26	1.50	1.52	1.48
T4	0.44	0.53	2.93	2.07	1.74	1.20	0.54	1.27	1.34
T5	0.79	0.85	3.19	2.39	2.02	1.53	1.78	1.19	1.72
T6	0.91	1.47	3.79	2.97	2.26	2.14	1.98	1.77	2.16
T7	0.11	0.18	1.91	0.60	0.43	0.27	0.37	0.30	0.52
T8	0.01	0.07	0.87	0.03	0.04	0.84	0.03	0.02	0.24
T9	0.02	0.03	0.72	0.05	0.05	0.69	0.04	0.04	0.21
Mean	0.36	0.48	2.35	1.48	1.19	1.09	1.00	0.85	-

Factors	CD (0.01)	S.Em ±	CV (%)
Treatments	0.015	0.005	8.70
Sampling intervals	0.016	0.006	
Interaction	0.048	0.017	

***dpi –** days post inoculation

U – µmole H_2O_2 $min^{-1}g^{-1}$ FW mg^{-1} protein

Treatment details are given below table 4.34

Table 4.42. Zymogram showing banding pattern of catalase isozymes in bioformulation treated groundnut plants and challenged with *S. rolfsii*

dpi*	Band no.	Isoform	Rf**	Treatments								
				T1	T2	T3	T4	T5	T6	T7	T8	T9
0	1	CAT 1	0.07	+	+	+	+	+	+	+	+	-
1	1	CAT 1	0.07	+	+	+	+	+	+	+	+	-
	3	CAT 3	0.57	+	+	+	+	+	+	-	-	-
2	1	CAT 1	0.07	+	+	+	+	+	+	+	+	-
	2	CAT 2	0.25	+	+	-	-	+	+	-	-	-
	3	CAT 3	0.57	+	+	+	+	+	+	-	-	-
3	1	CAT 1	0.07	+	+	+	+	+	+	+	+	+
	2	CAT 2	0.25	-	-	-	-	+	+	-	-	-
	3	CAT 3	0.57	+	+	+	+	+	+	-	-	-
4	1	CAT 1	0.07	+	+	+	+	+	+	-	-	-
	2	CAT 2	0.25	+	+	-	-	+	+	-	-	-
	3	CAT 3	0.57	+	+	+	+	+	+	-	-	-
5	1	CAT 1	0.07	+	+	-	-	+	+	-	-	-
	2	CAT 2	0.25	+	+	-	+	+	-	-	-	-
	3	CAT 3	0.57	+	+	+	+	+	+	-	-	-
6	1	CAT 1	0.07	+	+	+	+	+	+	-	-	-
	3	CAT 3	0.57	-	-	-	-	+	+	-	-	-
7	1	CAT 1	0.07	-	+	+	+	+	+	+	+	+

\+ Presence of band - Absence of band

***dpi** days post inoculation **Rf Relative front

Treatment details are given below table 4.34

Table 4.43. Effect of bioformulations on *β*-1,3-glucanase activity in groundnut plants

Treatments	Sampling intervals (U)								
	0 dpi*	**1 dpi**	**2 dpi**	**3 dpi**	**4 dpi**	**5 dpi**	**6 dpi**	**7 dpi**	**Mean**
T1	5.55	6.80	12.07	11.31	11.35	9.48	6.27	5.54	8.55
T2	6.03	7.28	12.60	11.70	11.90	9.99	6.34	6.05	8.99
T3	7.13	8.32	13.62	12.89	12.89	11.06	7.68	7.10	10.09
T4	7.48	8.69	13.93	13.25	13.24	11.40	7.03	6.17	10.15
T5	5.90	13.32	18.05	17.76	17.80	15.97	11.60	10.51	13.86
T6	7.15	16.29	22.93	21.54	20.81	19.02	14.15	13.08	16.87
T7	7.08	8.29	11.90	11.49	10.80	10.24	6.61	5.58	9.00
T8	3.48	6.89	11.37	10.35	8.30	9.63	3.69	3.04	7.09
T9	3.80	5.01	9.93	8.42	7.74	6.47	4.20	4.04	6.20
Mean	5.96	8.99	14.04	13.19	12.76	11.47	7.51	6.79	-

Factors	CD (0.01)	S.Em ±	CV (%)
Treatments	0.036	0.013	7.12
Sampling intervals	0.038	0.013	
Interaction	0.113	0.040	

***dpi –** days post inoculation **U –** µmole glucose $min^{-1}g^{-1}$ FW mg^{-1} protein

Treatment details are given below table 4.34

Table 4.44. Effect of bioformulations on chitinase activity in groundnut plants

Treatments	Sampling intervals (U)								
	0 dpi*	**1 dpi**	**2 dpi**	**3 dpi**	**4 dpi**	**5 dpi**	**6 dpi**	**7 dpi**	**Mean**
T1	0.70	1.88	4.31	3.14	2.60	1.80	0.90	0.69	2.01
T2	1.50	2.29	4.40	3.69	2.85	2.24	1.14	0.78	2.36
T3	1.42	2.23	4.41	3.60	2.71	2.67	1.59	1.23	2.48
T4	2.02	2.69	4.69	4.02	3.53	2.82	1.71	1.33	2.85
T5	2.14	2.68	4.92	4.19	3.61	3.17	1.79	1.32	2.98
T6	2.18	3.08	5.67	5.08	4.15	3.63	2.36	2.00	3.52
T7	0.66	1.46	2.41	1.79	2.25	1.45	0.86	1.02	1.49
T8	0.21	1.05	2.46	2.35	1.32	1.06	0.53	0.43	1.18
T9	0.32	0.97	1.45	1.69	1.18	0.74	0.61	0.59	0.94
Mean	1.24	2.04	3.86	3.28	2.69	2.18	1.28	1.04	-

Factors	CD (0.01)	S.Em ±	CV (%)
Treatments	0.033	0.012	6.31
Sampling intervals	0.035	0.013	
Interaction	0.105	0.038	

***dpi –** days post inoculation **U –**µmole N-acetyl-D-glucosaminemin^{-1}g^{-1} FW mg^{-1} protein

Treatment details are given below table 4.34

Table 4.45. Effect of bioformulations on stem rot severity in groundnut under field conditions

Treatment	Disease severity (%)																	
	Location I						Location II						Pooled					
	15 dpi*	30 dpi	45 dpi	60 dpi	75 dpi	Mean	15 dpi	30 dpi	45 dpi	60 dpi	75 dpi	Mean	15 dpi	30 dpi	45 dpi	60 dpi	75 dpi	Mean
T1	22.08 (28.03)**	47.50 (43.57)	62.92 (52.49)	66.25 (54.48)	70.00 (56.79)	53.75 (47.07)	24.17 (29.45)	52.08 (46.19)	62.92 (52.49)	72.08 (58.11)	75.83 (60.55)	57.42 (49.36)	23.13 (28.76)	49.79 (44.90)	62.92 (52.51)	69.17 (56.30)	72.92 (58.67)	55.59 (48.23)
T2	19.58 (26.27)	50.00 (45.00)	64.17 (53.23)	69.17 (56.27)	73.75 (59.18)	55.33 (47.99)	25.00 (30.00)	51.67 (45.96)	67.50 (55.24)	76.25 (60.83)	82.08 (64.96)	60.50 (51.40)	22.29 (28.19)	50.84 (45.50)	65.84 (54.26)	72.71 (58.54)	77.92 (62.00)	57.92 (49.70)
T3	19.58 (26.27)	50.42 (45.24)	64.17 (53.23)	67.92 (55.50)	70.83 (57.31)	54.58 (47.51)	19.17 (25.96)	48.75 (44.28)	58.75 (50.04)	67.50 (55.24)	72.08 (58.11)	53.25 (46.73)	19.38 (26.13)	49.59 (44.78)	61.46 (51.65)	67.71 (55.40)	71.46 (57.73)	53.92 (47.14)
T4	20.42 (26.86)	45.42 (42.37)	54.17 (47.39)	58.75 (50.04)	63.33 (52.73)	48.42 (43.88)	27.08 (31.36)	49.58 (44.76)	60.83 (51.26)	65.00 (53.73)	70.83 (57.31)	54.66 (47.68)	23.75 (29.18)	47.50 (43.59)	57.50 (49.34)	61.88 (51.90)	67.08 (55.02)	51.54 (45.81)
T5	20.42 (26.86)	45.42 (42.37)	55.42 (48.11)	60.00 (50.77)	63.33 (52.73)	48.92 (44.17)	29.17 (32.69)	47.50 (43.57)	59.17 (50.28)	65.42 (53.98)	69.58 (56.53)	54.17 (47.41)	22.92 (28.62)	45.00 (42.15)	56.05 (48.50)	61.25 (51.53)	64.79 (53.63)	50.00 (44.89)
T6	16.67 (24.09)	30.42 (33.47)	42.08 (40.44)	50.42 (45.24)	52.08 (46.19)	38.42 (37.95)	20.00 (26.57)	36.67 (37.27)	45.42 (42.37)	52.08 (46.19)	55.42 (48.11)	41.92 (40.10)	18.54 (25.52)	33.55 (35.41)	43.75 (41.43)	51.25 (45.74)	53.75 (47.17)	40.17 (39.05)
T7	17.08 (24.41)	42.50 (40.69)	52.92 (46.67)	57.08 (49.07)	60.00 (50.77)	45.83 (42.26)	22.08 (28.03)	41.25 (39.96)	50.42 (45.24)	60.83 (51.26)	66.67 (54.74)	48.25 (43.85)	21.25 (27.46)	43.34 (41.19)	52.92 (46.70)	60.42 (51.04)	65.00 (53.76)	48.59 (44.03)
T8	31.25 (33.99)	63.75 (52.98)	72.50 (58.37)	78.75 (62.55)	81.67 (64.65)	65.58 (54.51)	25.42 (30.27)	60.83 (51.26)	70.00 (56.79)	77.50 (61.68)	83.75 (66.23)	63.50 (53.25)	28.34 (32.18)	62.29 (52.14)	71.25 (57.60)	78.13 (62.15)	82.71 (65.46)	64.54 (53.91)
T9	2.08 (8.30)	9.58 (18.03)	24.17 (29.45)	27.92 (31.89)	31.67 (34.24)	19.08 (24.38)	7.50 (15.89)	13.33 (21.42)	23.75 (29.17)	27.08 (31.36)	27.92 (31.89)	19.92 (25.95)	4.79 (12.65)	11.46 (19.79)	23.96 (29.32)	27.50 (31.64)	29.80 (33.10)	19.50 (25.30)
Mean	18.80 (25.01)	42.78 (40.41)	54.72 (47.71)	59.58 (50.65)	62.96 (52.73)	-	22.18 (27.80)	44.63 (41.63)	55.42 (48.10)	62.64 (52.49)	67.13 (55.38)	-	20.49 (26.52)	43.71 (41.05)	55.07 (47.92	61.11 (51.58)	65.05 (54.06)	-

Factors	Location I			Location II			Pooled		
	CD (0.05)	S.Em.±	CV (%)	CD (0.05)	S.Em.±	CV (%)	CD (0.05)	S.Em.±	CV (%)
Treatments	0.77	0.27	11.50	0.35	0.12	11.10	1.07	0.54	11.20
dpi	0.57	0.21		0.26	0.09		0.81	0.41	
Interaction	1.73	0.62		0.79	0.28		2.41	1.23	

***dpi** – days post pathogen inoculation **Figures in the parenthesis are arc sine transformed values

T1 – ST+SA of talc formulation of *Bacillus* sp. (B1), **T2** – ST+SA of talc formulation of *Bacillus* sp. (B1) (with chitin), **T3** – ST+SA of talc formulation of *Trichoderma* sp. (T1), **T4** – ST+SA of talc formulation of *Trichoderma* sp. (T1) (with chitin), **T5** – ST+SA of talc formulation of *Bacillus* sp. + *Trichoderma* sp. (T1+B1), **T6** – ST+SA of talc formulation of *Bacillus* sp. + *Trichoderma* sp. (T1+B1) (with chitin), **T7** – Chemical control , **T8** – Inoculated control, **T9** – Un-inoculated control

Table 4.46. Effect of bioformulations on stem rot incidence in groundnut under field conditions

Treatment	Disease incidence (%)																	
	Location I						Location II						Pooled					
	15 dpi*	30 dpi	45 dpi	60 dpi	75 dpi	Mean	15 dpi	30 dpi	45 dpi	60 dpi	75 dpi	Mean	15 dpi	30 dpi	45 dpi	60 dpi	75 dpi	Mean
T1	34.05 (35.66)**	43.39 (41.22)	54.49 (47.62)	56.59 (48.83)	57.18 (49.19)	49.14 (44.50)	34.37 (35.89)	43.25 (41.13)	54.75 (47.78)	55.60 (48.28)	56.71 (48.94)	48.94 (44.40)	34.21 (35.81)	43.32 (41.18)	54.62 (47.68)	56.10 (48.53)	56.95 (49.02)	49.04 (44.44)
T2	33.58 (35.39)	44.40 (41.80)	53.25 (46.89)	54.42 (47.57)	55.00 (47.90)	48.13 (43.91)	34.68 (36.07)	42.82 (40.89)	53.82 (47.22)	55.22 (48.03)	56.91 (49.01)	48.69 (44.24)	34.13 (35.77)	43.61 (41.35)	53.54 (47.05)	54.82 (47.79)	55.96 (48.44)	48.41 (44.08)
T3	32.98 (35.06)	42.28 (40.58)	50.95 (45.57)	54.02 (47.34)	54.29 (47.50)	46.90 (43.21)	23.59 (27.63)	42.72 (40.82)	50.51 (45.32)	52.93 (46.72)	54.54 (47.65)	44.86 (41.63)	28.29 (32.15)	42.50 (40.71)	50.73 (45.44)	53.48 (47.02)	54.42 (47.56)	45.88 (42.58)
T4	33.35 (35.28)	41.28 (39.99)	49.37 (44.66)	54.55 (47.65)	54.83 (47.82)	46.68 (43.08)	33.29 (35.25)	41.44 (40.09)	47.30 (43.47)	52.26 (46.33)	54.51 (47.63)	45.76 (42.55)	33.32 (35.27)	41.36 (40.04)	48.34 (44.07)	53.41 (46.98)	54.67 (47.70)	46.22 (42.81)
T5	31.39 (34.06)	40.99 (39.82)	43.23 (41.12)	49.14 (44.53)	50.59 (45.36)	43.07 (40.98)	31.38 (34.06)	40.29 (39.38)	42.77 (40.84)	47.41 (43.54)	49.31 (44.63)	42.23 (40.49)	30.94 (33.81)	39.31 (38.84)	42.86 (40.91)	48.03 (43.89)	49.29 (44.62)	42.09 (40.41)
T6	25.37 (30.23)	32.77 (34.90)	36.16 (36.98)	40.68 (39.64)	42.04 (40.43)	35.40 (36.44)	25.51 (30.30)	32.34 (34.65)	37.99 (38.06)	41.71 (40.23)	43.56 (41.31)	36.22 (36.91)	25.44 (30.31)	32.56 (34.81)	37.08 (37.53)	41.20 (39.95)	42.80 (40.88)	35.82 (36.70)
T7	30.49 (33.50)	38.32 (38.22)	42.94 (40.95)	48.65 (44.25)	49.27 (44.61)	41.93 (40.31)	30.83 (33.74)	39.92 (39.20)	42.73 (40.83)	47.17 (43.40)	48.56 (44.20)	41.84 (40.27)	31.11 (33.92)	40.46 (39.52)	42.98 (40.99)	48.16 (43.96)	49.58 (44.78)	42.46 (40.63)
T8	38.21 (38.19)	52.66 (46.56)	63.30 (53.01)	79.74 (63.31)	80.68 (64.02)	62.92 (53.02)	38.48 (38.35)	54.96 (47.89)	73.54 (59.15)	77.70 (61.86)	78.31 (62.30)	64.60 (53.91)	38.35 (38.28)	53.81 (47.21)	68.42 (55.84)	78.72 (62.56)	79.50 (63.11)	63.76 (53.40)
T9	5.20 (13.03)	10.04 (18.44)	14.34 (22.22)	16.35 (23.83)	16.65 (24.05)	12.52 (20.31)	4.98 (12.76)	10.18 (18.54)	16.16 (23.71)	15.35 (22.99)	17.01 (24.36)	12.74 (20.47)	5.09 (13.05)	10.11 (18.55)	15.25 (23.00)	15.85 (23.47)	16.83 (24.23)	12.63 (20.46)
Mean	29.40 (32.27)	38.46 (37.95)	45.34 (42.11)	50.46 (45.22)	51.17 (45.65)	-	28.57 (31.56)	38.66 (38.07)	46.62 (42.93)	49.48 (44.60)	51.05 (45.56)	-	28.99 (32.04)	38.56 (38.02)	45.98 (42.50)	49.97 (44.91)	51.11 (45.59)	-

Factors	Location I			Location II			Pooled		
	CD (0.05)	S.Em.±	CV (%)	CD (0.05)	S.Em.±	CV (%)	CD (0.05)	S.Em.±	CV (%)
Treatments	1.93	0.68	8.90	2.33	0.82	7.92	1.41	0.71	12.30
dpi	1.43	0.51		1.79	0.61		1.05	0.53	
Interaction	4.31	1.53		5.20	1.85		3.16	1.61	

***dpi** – days post pathogen inoculation **Figures in the parenthesis are arc sine transformed values

Treatment details are given below table 4.45

Table 4.47. Effect of bioformulations on stem discolouration and pod rot in groundnut under field conditions

Treatments	Stem discolouration at harvest (%)			Pod rot at harvest (%)		
	Location I	Location II	Pooled	Location I	Location II	Pooled
T1	9.58 (17.97)*	8.68 (17.11)	9.13 (17.60)	5.06 (13.00)	5.44 (13.39)	5.25 (13.25)
T2	9.17 (17.60)	9.02 (17.47)	9.10 (17.56)	6.24 (14.45)	5.82 (13.95)	6.03 (14.22)
T3	6.62 (14.90)	7.23 (15.59)	6.93 (15.27)	2.65 (9.36)	3.86 (11.31)	3.26 (10.40)
T4	7.39 (15.78)	7.61 (15.81)	7.50 (15.90)	2.97 (9.56)	3.17 (10.26)	3.07 (10.10)
T5	5.26 (13.24)	5.32 (13.33)	5.29 (13.30)	2.01 (8.07)	1.50 (6.97)	1.76 (7.62)
T6	3.67 (11.00)	3.43 (10.68)	3.55 (10.87)	1.32 (6.52)	1.04 (5.86)	1.18 (6.24)
T7	4.67 (12.42)	5.98 (14.10)	5.33 (13.35)	2.72 (9.46)	1.62 (7.17)	2.17 (8.48)
T8	14.51 (22.39)	16.49 (23.82)	15.50 (23.20)	8.61 (17.05)	9.68 (17.99)	9.15 (17.61)
T9	1.60 (7.19)	0.92 (5.50)	1.26 (6.45)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	6.94 (14.72)	7.19 (14.82)	7.07 (14.83)	3.51 (9.72)	3.57 (9.66)	3.54 (9.77)

	Location I		Location II		Pooled	
	Discolouration	Pod rot	Discolouration	Pod rot	Discolouration	Pod rot
CD (0.05)	2.45	2.43	3.53	2.43	0.94	0.82
S.Em.±	0.82	0.81	1.17	0.81	0.32	0.28
CV (%)	9.60	14.50	13.8	14.5	11.60	15.42

*Figures in the parenthesis are arc sine transformed values

Treatment details are given below table 4.45

Table 4.48. Effect of bioformulations on mortality due to stem rot of groundnut under field conditions

Treatment	Mortality (%)																	
	Location I						Location II						Pooled					
	15 dpi*	30 dpi	45 dpi	60 dpi	75 dpi	Mean	15 dpi	30 dpi	45 dpi	60 dpi	75 dpi	Mean	15 dpi	30 dpi	45 dpi	60 dpi	75 dpi	Mean
T1	0.00 (0.00)**	15.86 (23.36)	30.84 (33.57)	35.58 (36.50)	37.08 (37.43)	23.87 (26.17)	0.00 (0.00)	29.28 (32.77)	37.07 (37.51)	45.05 (42.17)	48.81 (44.34)	32.04 (31.36)	0.00 (0.00)	22.57 (28.38)	33.96 (35.66)	40.32 (39.44)	42.95 (40.97)	27.96 (28.89)
T2	0.00 (0.00)	14.73 (22.49)	33.81 (35.54)	37.93 (38.01)	38.51 (38.35)	25.00 (26.88)	0.00 (0.00)	27.32 (31.53)	38.88 (38.58)	42.52 (40.71)	46.72 (43.13)	31.09 (30.79)	0.00 (0.00)	21.03 (27.31)	36.35 (37.09)	40.23 (39.38)	42.62 (40.77)	28.05 (28.91)
T3	0.00 (0.00)	14.36 (22.28)	29.99 (33.16)	32.63 (34.66)	35.64 (36.63)	22.52 (25.35)	0.00 (0.00)	24.50 (29.57)	35.70 (36.67)	40.76 (39.69)	44.80 (42.03)	29.15 (29.59)	0.00 (0.00)	19.43 (26.17)	32.85 (34.98)	36.70 (37.30)	40.22 (39.38)	25.84 (27.57)
T4	0.00 (0.00)	12.27 (20.46)	27.39 (31.29)	35.59 (36.48)	36.18 (36.82)	22.29 (25.01)	0.00 (0.00)	18.80 (25.69)	30.65 (33.55)	37.72 (37.80)	38.83 (38.47)	25.20 (27.10)	0.00 (0.00)	15.54 (23.22)	29.02 (32.61)	36.66 (37.280	37.51 (37.78)	23.75 (23.40)
T5	0.00 (0.00)	13.61 (21.60)	22.37 (28.19)	27.47 (31.55)	29.02 (32.59)	18.49 (22.79)	0.00 (0.00)	13.05 (21.13)	21.46 (27.55)	26.35 (30.82)	27.83 (31.84)	17.74 (22.27)	0.00 (0.00)	13.33 (21.42)	21.92 (27.93)	26.91 (31.26)	28.43 (32.23)	18.12 (22.57)
T6	0.00 (0.00)	9.04 (17.44)	15.13 (22.90)	19.21 (25.99)	19.49 (26.18)	12.57 (18.50)	0.00 (0.00)	11.76 (20.05)	17.28 (24.58)	22.59 (28.35)	24.20 (29.41)	15.17 (20.48)	0.00 (0.00)	10.40 (18.82)	16.21 (23.75)	20.90 (27.22)	21.85 (27.88)	13.87 (19.53)
T7	0.00 (0.00)	12.44 (20.61)	19.71 (26.31)	27.14 (31.37)	28.74 (32.37)	17.61 (22.13)	0.00 (0.00)	13.21 (21.29)	19.98 (26.51)	27.33 (31.47)	32.48 (34.65)	18.60 (22.78)	0.00 (0.00)	12.83 (21.00)	19.85 (26.47)	27.24 (31.47)	30.61 (33.61)	18.11 (22.51)
T8	0.00 (0.00)	21.53 (27.65)	43.56 (41.31)	53.58 (47.09)	54.75 (47.78)	34.68 (32.77)	0.00 (0.00)	21.13 (27.36)	42.50 (40.69)	52.49 (46.46)	57.01 (49.10)	34.63 (32.72)	0.00 (0.00)	21.33 (27.52)	43.03 (41.01)	53.04 (46.76)	55.88 (48.40)	34.66 (32.74)
T9	0.00 (0.00)	2.00 (8.10)	5.94 (13.79)	10.02 (18.43)	10.61 (18.98)	5.71 (11.86)	0.00 (0.00)	2.45 (8.63)	6.25 (14.07)	10.16 (18.59)	10.44 (18.84)	5.86 (12.03)	0.00 (0.00)	2.23 (8.58)	6.10 (14.30)	10.09 (18.53)	10.53 (18.94)	5.79 (12.07)
Mean	0.00 (0.00)	12.87 (20.44)	25.42 (29.56)	31.02 (33.34)	32.22 (34.13)	-	0.00 (0.00)	17.94 (24.22)	27.75 (31.08)	33.89 (35.12)	36.79 (36.87)	-	0.00 (0.00)	15.41 (22.49)	26.59 (30.42)	32.45 (33.92)	34.51 (35.55)	-

Factors	Location I			Location II			Pooled		
	CD (0.05)	SEm±	CV (%)	CD (0.05)	SEm±	CV (%)	CD (0.05)	SEm±	CV (%)
Treatments	2.39	0.85	14.10	1.95	0.69	10.61	1.65	0.59	13.30
dpi	1.78	0.63		1.45	0.51		1.23	0.44	
Interaction	5.36	1.91		4.36	1.55		3.69	1.32	

*__dpi__ – days post pathogen inoculation **Figures in the parenthesis are arc sine transformed values

Treatment details are given below table 4.45

Table 4.49. Effect of bioformulations on growth parameters of groundnut under field conditions

Treatments	Location I					Location II					Pooled				
	Germ (%)	Plant height (cm)	Nod./ plant	Oil content (%)	Protein content (%)	Germ (%)	Plant height (cm)	Nod./ plant	Oil content (%)	Protein content (%)	Germ (%)	Shoot length (cm)	Nod./ plant	Oil content (%)	Protein content (%)
T1	70.83	48.23	144.15	46.77	23.32	73.96	30.22	120.20	46.26	25.67	72.40	39.23	132.18	46.52	24.50
T2	70.83	47.97	170.68	46.70	23.72	73.96	31.62	136.48	46.52	26.74	72.40	39.80	153.58	46.61	25.23
T3	73.96	50.00	192.10	46.99	24.59	77.08	30.35	109.82	47.15	26.24	75.52	40.18	150.96	47.07	25.42
T4	71.25	49.72	230.97	47.48	24.14	74.38	32.77	140.42	46.98	27.21	72.82	41.25	185.70	47.23	25.68
T5	72.92	51.55	285.13	47.42	25.22	76.04	35.78	137.83	47.51	28.14	74.48	43.67	211.48	47.47	26.68
T6	78.33	55.77	251.77	48.90	26.44	79.58	39.68	178.45	48.81	28.75	78.96	47.73	215.11	48.86	27.60
T7	75.42	51.65	159.40	47.58	24.75	78.54	33.03	105.07	46.80	27.09	76.98	42.34	132.24	47.19	25.92
T8	70.42	50.12	125.68	46.22	22.76	73.54	28.35	99.55	45.96	26.85	71.98	39.24	112.62	46.09	24.31
T9	70.92	49.23	153.63	47.99	24.78	72.39	33.13	112.03	48.42	25.34	71.66	41.18	132.83	48.21	25.06
Mean	72.76	50.47	190.39	47.34	24.30	75.50	32.77	126.65	47.16	26.89	74.13	41.62	158.52	47.25	25.60
CD (0.05)	3.72	3.23	53.07	1.15	1.21	1.23	5.48	23.15	2.01	1.86	1.41	1.52	17.34	1.08	0.51
S.Em.±	1.33	1.01	17.70	0.38	0.43	3.45	1.83	7.72	0.67	0.62	0.42	0.53	6.03	0.17	0.17
CV (%)	7.90	9.70	16.10	7.41	6.80	6.53	9.70	10.60	10.51	6.51	6.41	6.60	14.20	3.51	3.90

Treatment details are given below table 4.45

Table 4.50. Effect of bioformulations on yield and yield related parameters of groundnut under field conditions

Treatments	Location I						Location II						Pooled					
	Pods/ plant	100 kernel weight (g)	Shelling (%)	Pod yield (kg/ha)	Biomass yield (kg/ha)	B:C ratio	Pods/ plant	100 kernel weight (g)	Shelling (%)	Pod yield (kg/ha)	Biomass yield (kg/ha)	B:C ratio	Pods/ plant	100 kernel weight (g)	Shelling (%)	Pod yield (kg/ha)	Biomass yield (kg/ha)	B:C ratio
T1	13.87	34.10	63.22	1202.36	1750.00	1.98	11.18	32.03	60.93	1291.25	1277.78	2.13	12.53	33.07	62.08	1246.81	1513.89	2.06
T2	13.57	33.60	63.42	1235.35	1673.61	2.04	11.17	32.05	62.44	1263.33	1402.78	2.08	12.37	32.83	62.93	1249.34	1538.20	2.06
T3	15.07	33.07	66.40	1264.86	1847.22	2.08	11.10	34.02	63.24	1241.53	1409.72	2.05	13.09	33.55	64.82	1253.20	1628.47	2.07
T4	14.10	34.33	67.22	1327.71	2236.11	2.19	13.38	34.25	66.08	1398.54	1763.89	2.30	13.74	34.29	66.65	1363.13	2000.00	2.25
T5	15.58	36.00	71.25	1780.49	2638.89	2.94	14.95	36.91	69.71	1734.17	2020.83	2.86	15.27	36.04	70.48	1757.33	2329.86	2.90
T6	16.88	38.37	74.30	1888.26	2798.61	3.11	16.50	38.53	72.87	1834.10	2298.61	3.03	16.69	38.45	73.59	1861.18	2548.61	3.07
T7	12.77	35.05	66.33	1622.50	2500.00	2.67	12.08	36.23	66.91	1480.28	2201.39	2.44	12.43	35.64	66.62	1551.39	2350.70	2.56
T8	11.23	32.83	58.27	1177.99	1527.78	1.94	10.03	31.48	60.53	1079.17	1256.94	1.78	10.63	32.16	59.40	1128.58	1392.36	1.86
T9	13.13	35.13	62.92	1466.67	2986.11	2.42	12.18	34.40	64.33	1515.00	2437.50	2.50	12.66	34.77	63.63	1490.84	2711.81	2.46
Mean	14.02	34.72	65.93	1440.69	2217.59	-	12.51	34.34	65.23	1426.37	1785.49	-	13.27	34.53	65.58	1433.53	2001.54	-
CD (0.05)	3.13	2.29	4.92	42.33	221.56	-	2.10	1.63	3.14	80.55	141.53	-	1.33	1.42	1.41	55.23	96.11	-
S.Em.±	1.04	0.76	1.64	15.12	79.13	-	0.67	0.54	1.04	28.77	50.56	-	0.41	0.52	0.49	19.23	31.33	-
CV (%)	12.90	9.30	8.32	11.39	12.80	-	9.20	5.70	5.31	8.50	11.60	-	8.60	5.39	6.90	10.22	12.33	-

Treatment details are given below table 4.45

Table 3.2. List of isolates of *S. rolfsii* used for molecular diversity through RAPD

S. No.	Isolate	MCGs
1	SrKa-1	MCG 1
2	SrKa-5	MCG 2
3	SrKa-20	MCG 3
4	SrTs-1	MCG 4
5	SrTs-10	MCG 5
6	SrKa-12	MCG 6
7	SrAp-2	MCG 7
8	SrAp-10	MCG 8
9	SrMh-1	MCG 9
10	SrMh-6	MCG 10
11	SrTn-1	MCG 11
12	SrTn-5	MCG 12
13	SrGj-1	MCG 13
14	SrGj-3	MCG 14
15	SrGj-6	MCG 15

Table 3.3. List of RAPD primers used for molecular diversity study of isolates of *S. rolfsii*

S. No.	Primer	Primer Sequence (5´- 3´)
1	489	CGC ACG CACA
2	485	AGA ATA GGGC
3	467	AGC ACG GGCA
4	418	GAG GAA GCTT
5	438	AGA CGG CCGG
6	482	CTA TAG GCCG
7	626	CCA AGC CCGG
8	638	GCG GTG ACTA
9	OPA 02	TGC CGA GCTG
10	OPA 20	GTT GCG ATCC
11	OPA 10	GTGATCGCAG
12	OPA 04	AAT CGG GCTG
13	OPB 07	GGT GAC GCAG
14	OPB 11	GTA GAC CCGT
15	OPB 06	TGC GCC CTTC
16	OPB 18	CCA CAG CAGT
17	OPB 02	TGA TCC CTGG
18	OPB 17	AGG GAA CGAG
19	OPE 16	GGT GAC TGTG
20	OPZ 19	GTG CGA GCAA
21	OPH 19	CTG ACC AGCC
22	OPT 18	GAT GCC AGAC
23	OPH 20	GGG AGA CATC
24	UBC 90	GGG GGT TAGG
25	UBC 85	GTG CTC GTGC
26	GLB 12	CCT TGA CGCA
27	GLB 15	GGA AGG TGTT
28	GLL 04	GAC TGC ACAC
29	GLL 05	ACG CAG GCAC
30	GLL 12	GGG CGG TACT

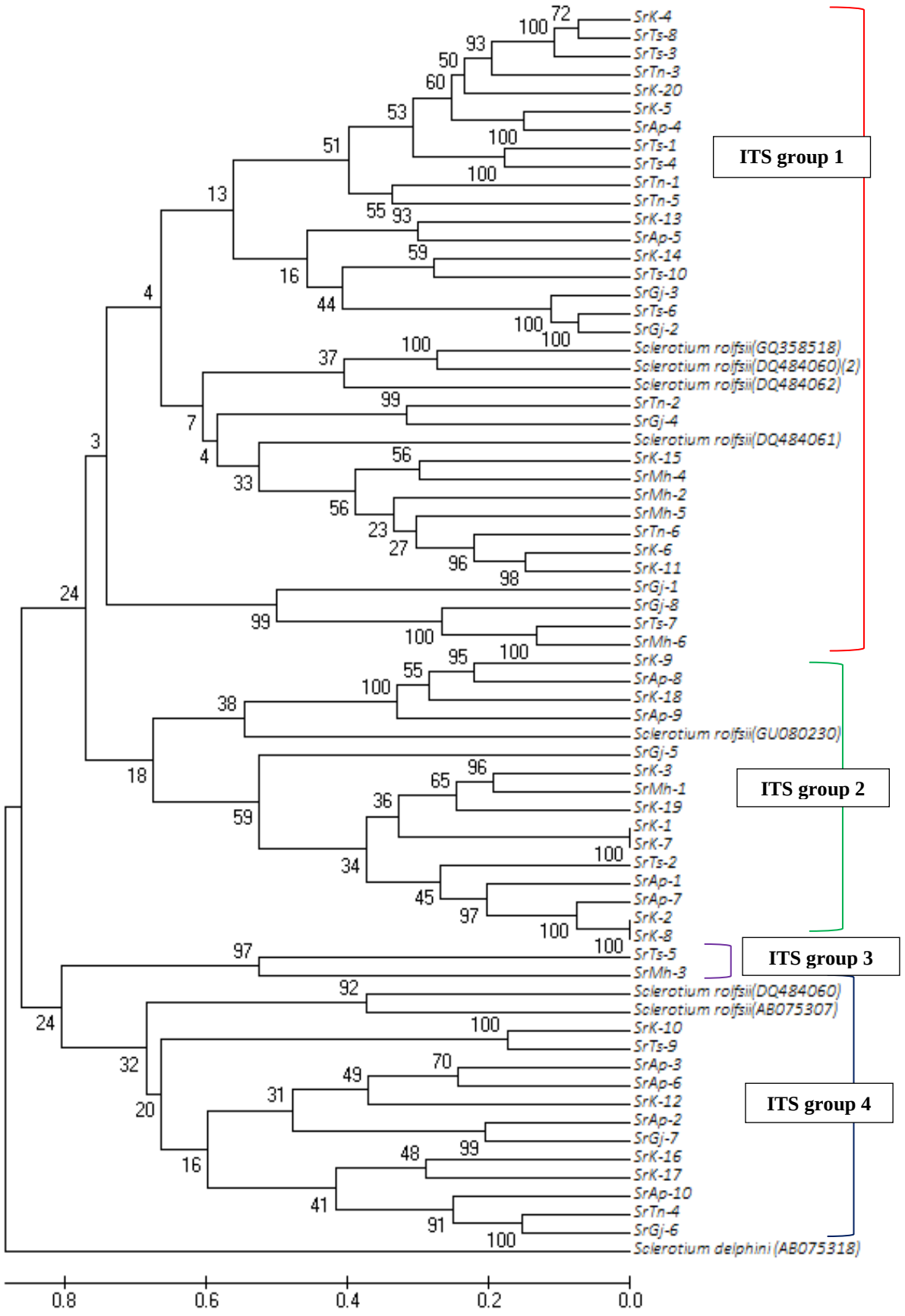

Fig. 4.1. Phylogeny of ITS 18S rDNA sequences of 60 isolates of *S. rolfsii* using the unweighted pair group method (UPGMA)

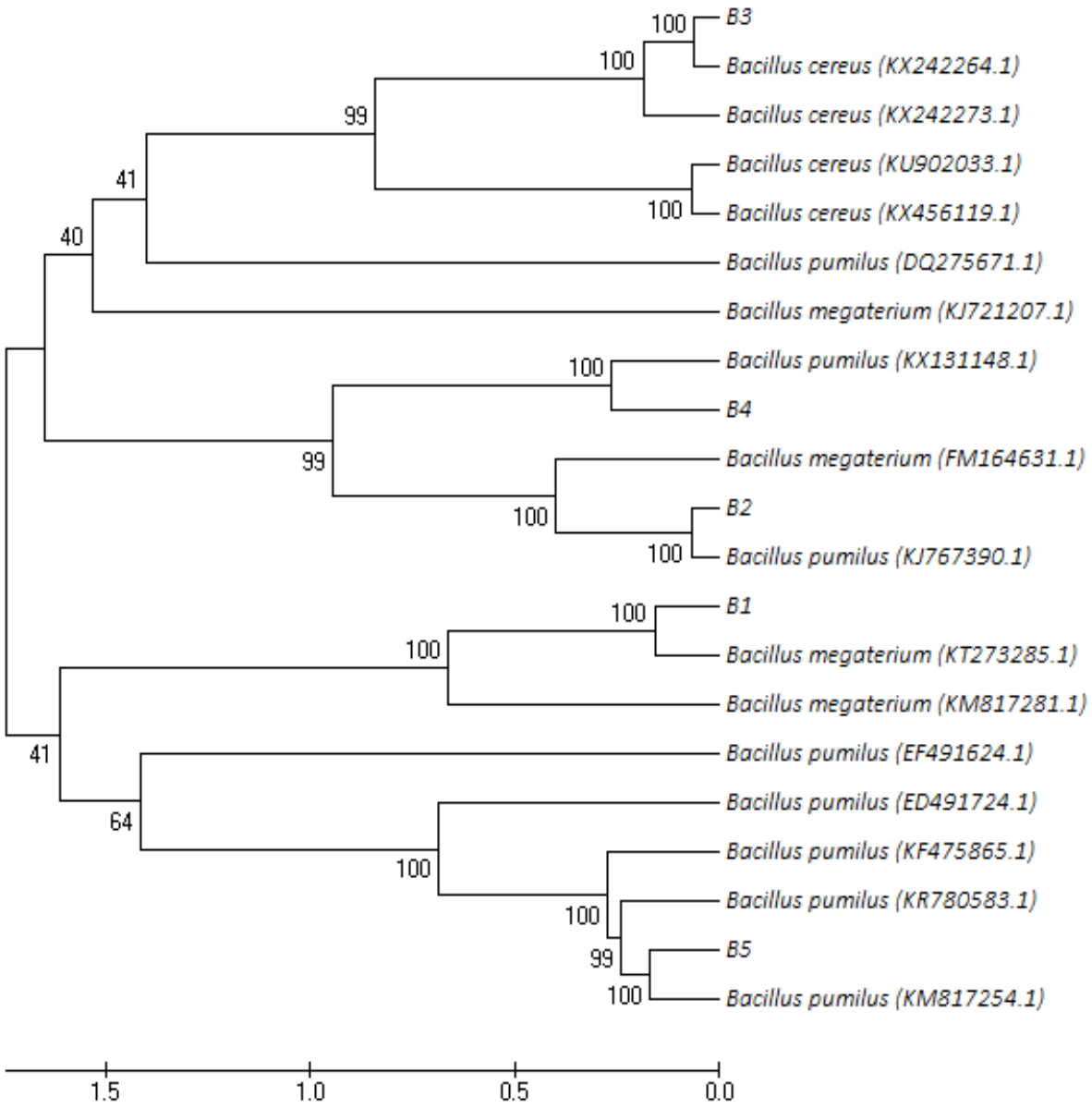

Figure 4.6. Phylogeny of 16S rDNA sequences of five potential isolates of *Bacillus* sp. using UPGMA

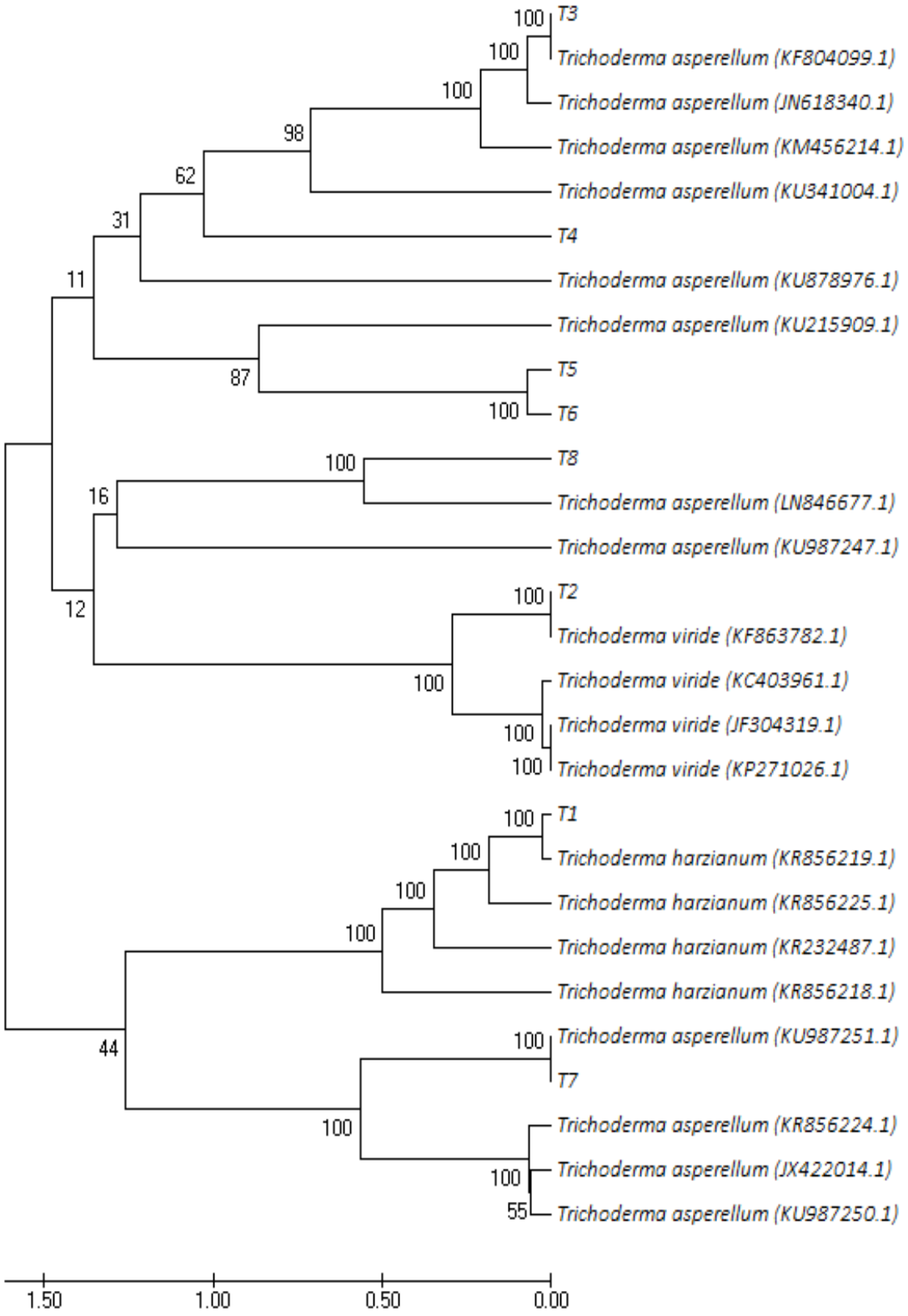

Figure 4.4. Phylogeny of 18S rDNA sequences of eight potential isolates of *Trichoderma* sp. using UPGMA

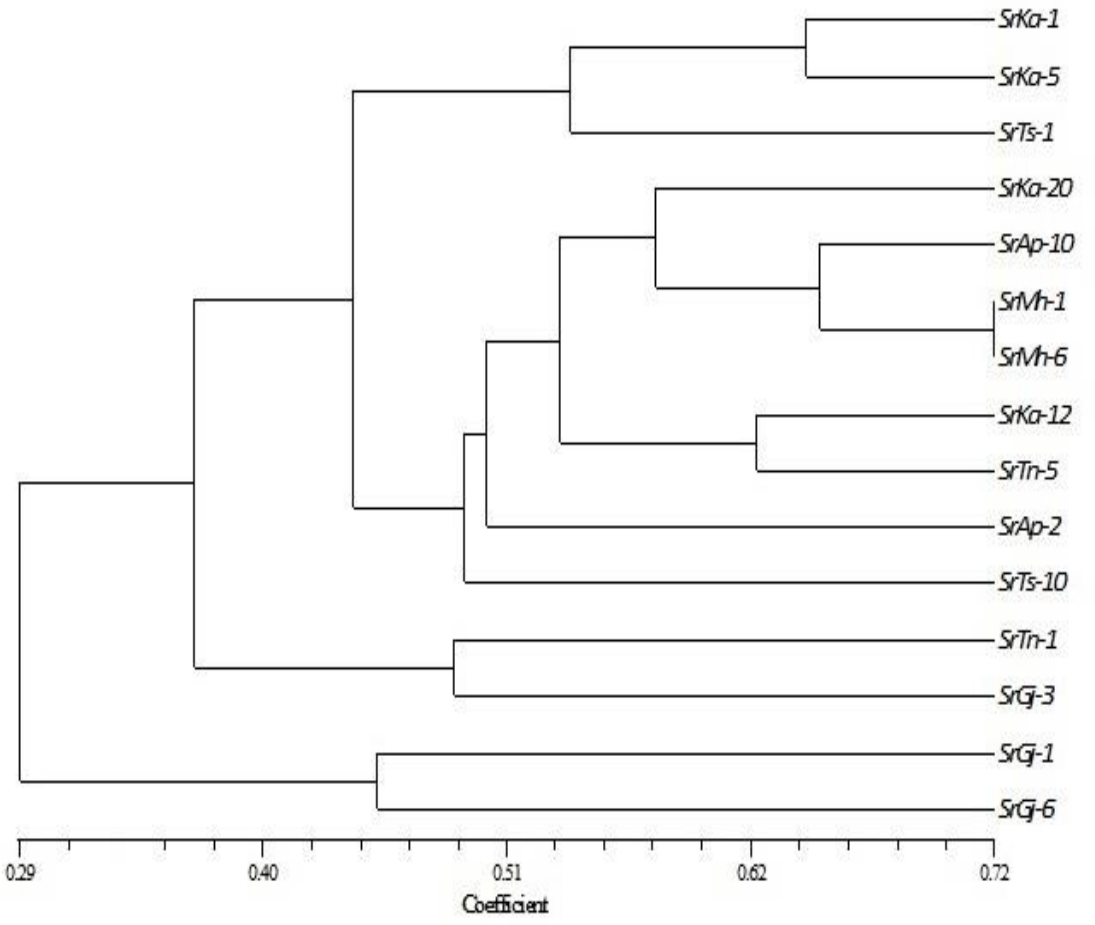

Figure 4.2. UPGMA generated dendrogram of RAPD analysis representing genetic distance among isolates *S. rolfsii*

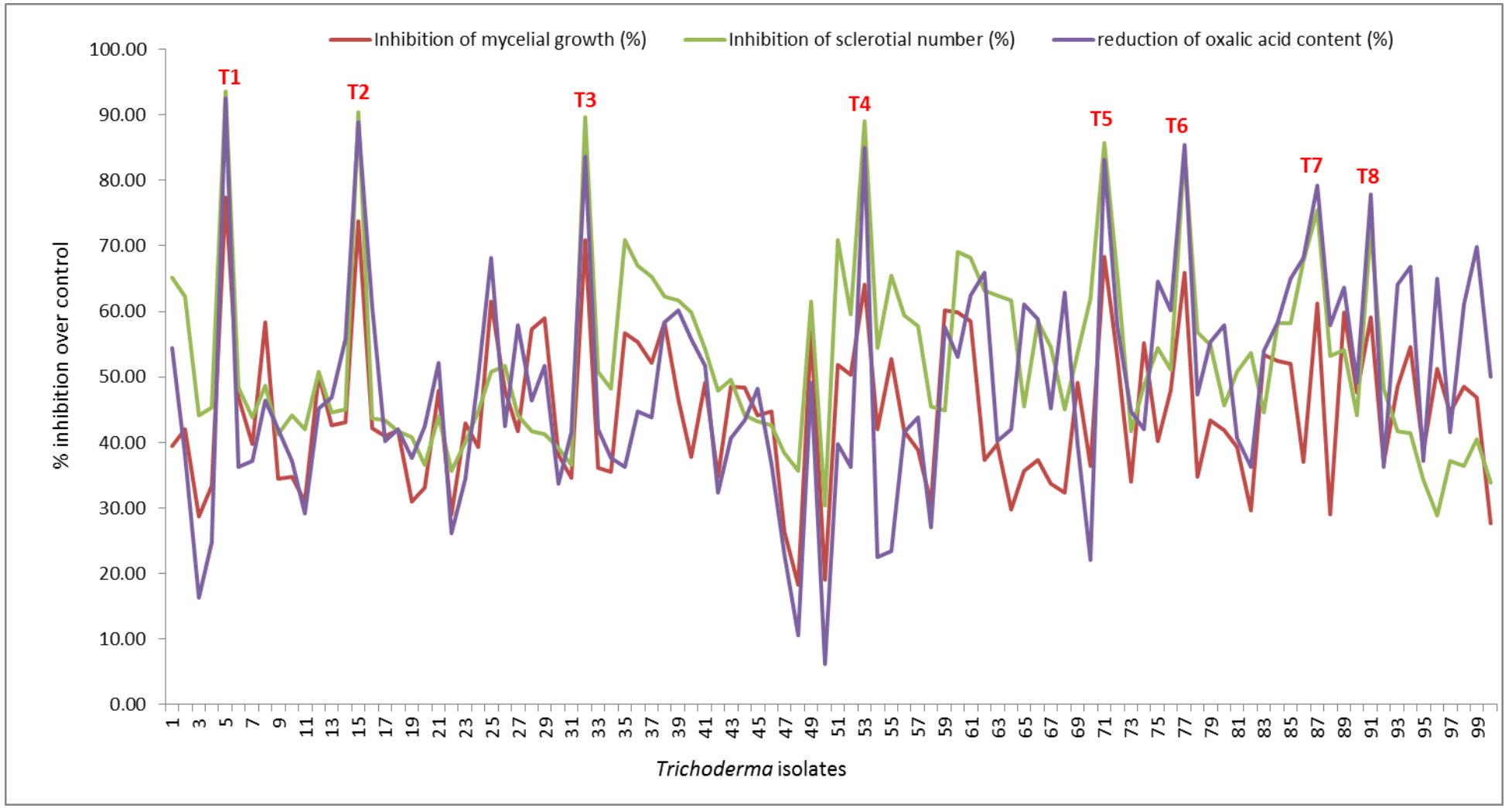

Figure 4.3. Preliminary screening of 100 isolates of *Trichoderma* sp. against the virulent isolate of *S. rolfsii*

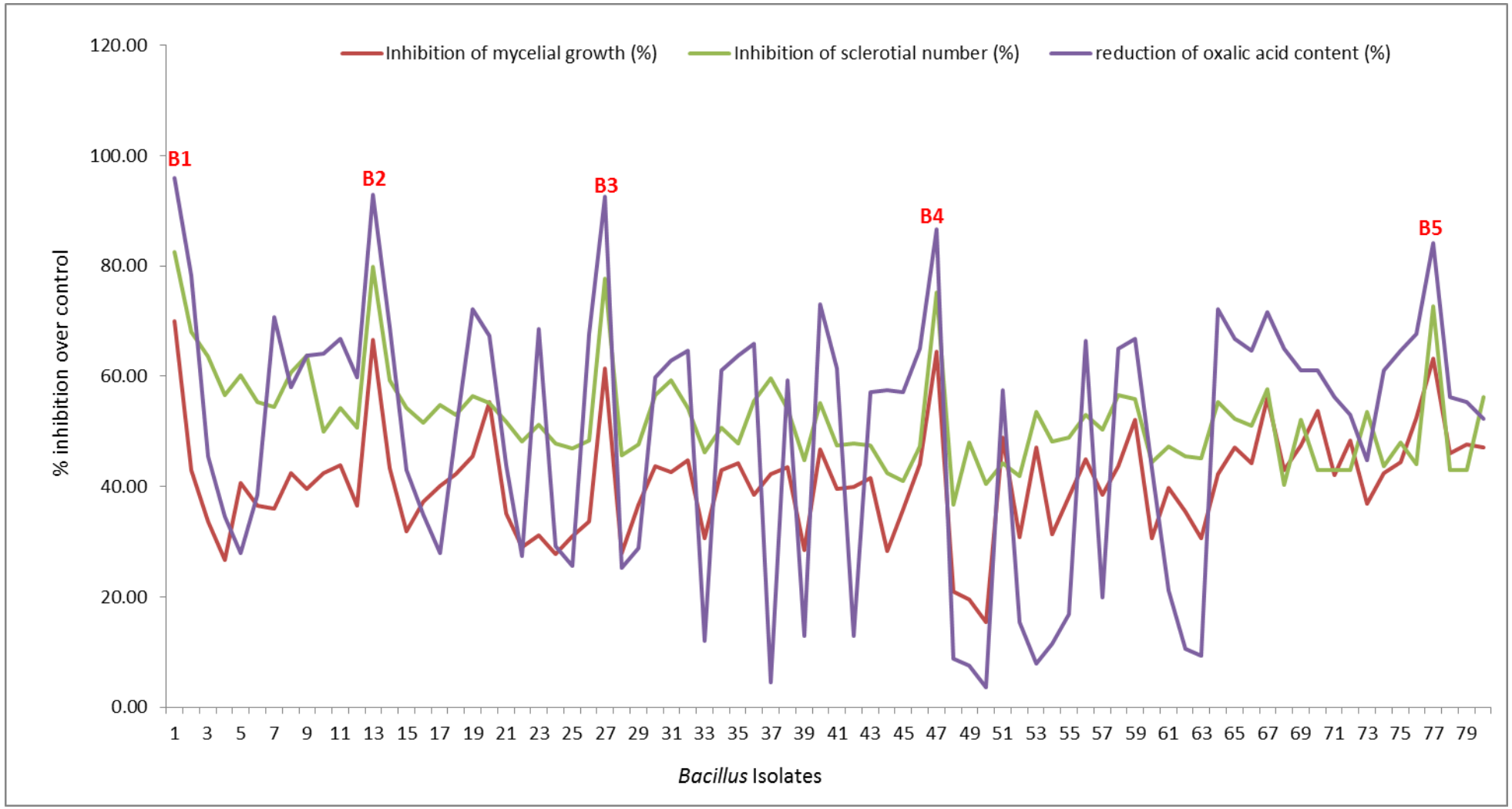

Fig. 4.5. Preliminary screening of 80 isolates of *Bacillus* sp. against the virulent isolate of *S. rolfsii*

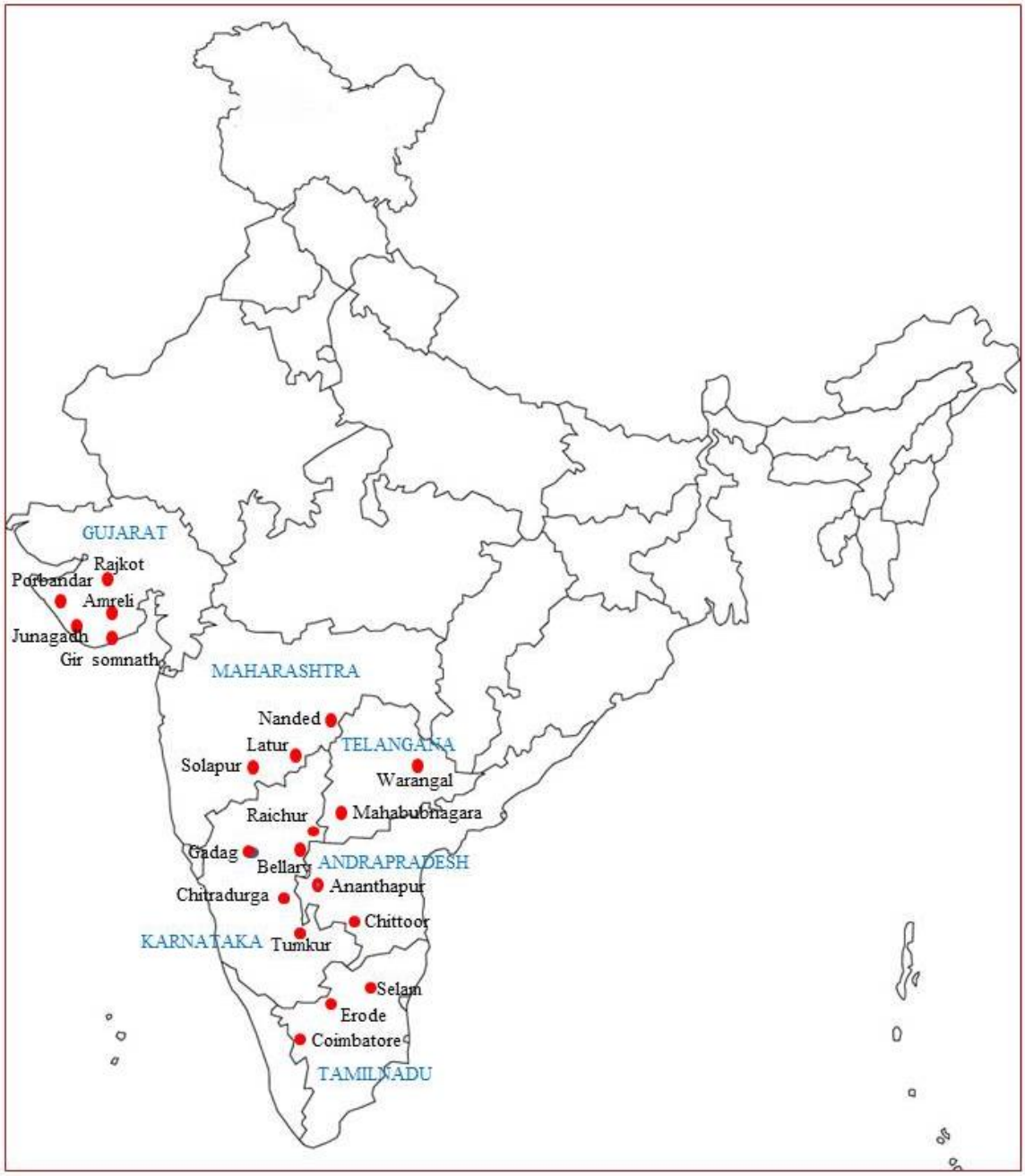

Fig. 3.1. Map showing the groundnut growing districts of India surveyed

SUMMARY AND CONCLUSIONS

Chapter V

SUMMARY AND CONCLUSIONS

Groundnut (*Arachis hypogaea* L.) is an annual legume and an important oilseed crop grown in India. Of the various diseases inciting groundnut, the stem rot caused by *S. rolfsii* is a potential threat to groundnut production (Tiwari *et al*., 2004). The *S. rolfsii* causes severe damage during any stage of crop growth (Cilliers *et al*., 2000; Ganesan *et al*., 2007) and attacks all parts of the plant but the stem infection is the most common and serious with yield losses over 25% (Mayee and Datar, 1988). In the present investigation efforts were made to know about various aspects like prevalence and distribution of stem rot of groundnut in major growing areas of India, collection and characterisation of isolates of *S. rolfsii,* screening of indigenous biocontrol agents against *S. rolfsii,* characterisation of systemic resistance inducing ability of potential biocontrol agents in groundnut against *S. rolfsii,* and finally evaluation of these bioformulations against stem rot under glasshouse and field conditions. The results obtained are summarized below.

The stem rot of groundnut exhibited the primary symptoms like browning and wilting of leaves and branches which were still attached with the plant. The fungus preferentially infected stem by forming a whitish mycelial mat around the stem which was later spread over the soil and around the basal canopy of the plant. In advanced stage the fungus produced sclerotia at the infected area which were like mustard seeds in size and colour. In later stage the entire plant was killed or only few branches were affected. Infected pods were completely covered with white mycelial growth and in severe cases rotting of pods were observed.

During *kharif,* 2013 the incidence of stem rot in major growing areas of India was ranged from 11.23 to 55.40%. Further, the Gujarat state recorded the highest mean stem rot incidence of 28.86% and the lowest mean incidence was recoded in Telangana (20.65%). Similar trend was observed during *kharif,* 2014 wherein the overall incidence of stem rot in major groundnut growing areas of India was ranged from 10.11 to 59.33%. Among the different states, Gujarat recorded highest mean incidence of 27.62% and lowest mean incidence was recorded in Telangana (22.39%). Further, higher incidence of stem rot was observed in all the districts of Gujrat and parts in Maharashtra and Tamil Nadu where the crop was grown in black soils with susceptible groundnut cultivars (TMV 2, JL 24, GG 20, and GG 11) continuously in a sole cropping pattern.

Totally 60 isolates of *Sclerotium rolfsii* were collected from major groundnut growing areas of India. Among them, 20 isolates were from Karnataka, 10 each from Telangana and Andhra Pradesh, 6 each from Maharashtra and Tamil Nadu and 8 from Gujarat.

For *in vitro* production of oxalic acid by *S. rolfsii*, the Richards's broth was found to be the best supporting medium. Further, the 60 of isolates of *S. rolfsii* exhibited wide variation in the amount of oxalic acid production under *in vitro* conditions and was ranged from 0.64 to 2.85 mg/ml of culture filtrate.

For the glasshouse studies employing artificial inoculation of *S. rolfsii*, the inoculum level of 15g per 7" pot was found most suitable and effective.

All the 60 isolates of *S. rolfsii* in the study were found pathogenic. Further, there was great variation among the isolates for virulence levels on three groundnut cultivars. The isolates *viz.,* SrKa-12, SrAp-2, SrTn-3 and SrTn-4 exhibited the mean incubation period of more than 8 days, induced no permanent wilting, mean disease severity of less than 60% and mean mortality of less than 25%, hence were categorized into less virulent. Similarly, the remaining 56 isolates exhibited the mean incubation period of less than 8 days, days to permanent wilting of less than 16 days, mean disease severity of more than 60% and mean mortality of more than 25%, hence were categorized into highly virulent.

Additionally, there was a positive correlation found between the amount of oxalic acid produced under *in vitro* conditions and the virulence of the isolates. In the study highly virulent isolates produced significantly highest amount of oxalic acid which was ranged from from 0.99 mg/ml in SrTn-2 to 2.85 mg/ml in SrGj-3, whereas the less virulent isolates produced least amount of oxalic acid which was ranged from 0.64 mg/ml in SrTn-3 and SrTn-4, to 0.78 mg/ml in SrAp-2.

Culturally the 60 isolates of *S. rolfsii* were found diverse. The growth rate of the isolates tested exhibited wide range (0.66 to 1.29 mm/hr). In regard to biomass production the isolates exhibited wide variation ranging from 6.82 to 14.62 mg/day. All the isolates of *S. rolfsii* under study produced sclerotia on PDA medium. Most of the isolates produced the colonies which were raised at ends (n=27) followed by flat type (n=20) and raised type (n=13) colonies. As per mycelial growth type, most of the isolates were found highly profuse in growth (n=36) and few were profuse (n=24).

Likewise, the 60 isolates were also found diverse morphologically. Wide variation in days to produce (4 to 17 days) and days for maturation (7 to 23 days) of sclerotia was found among the isolates. Similar type of variation was found with respect to 100 sclerotial weight (0.12 to 1.19 g), number of sclerotia per plate (52 to 910), pattern of sclerotia produced in petri dish (scattered (*n*=38), peripheral (*n*=16) and central (*n*=6)), colour of sclerotia (brown (*n*=25), dark brown (*n*=20) and light brown (*n*=15)) and size of sclerotia (0.15 mm to 2.81 mm). Interestingly, there was no correlation found between the cultural and morphological variability with virulence of isolates.

The 60 isolates of *S. rolfsii* were found genetically diverse as only 5.78% of combinations in mycelial compatibility study were found compatible while remaining combinations (94.22%) were antagonistic. Thus, based on mycelial compatibility, 15 MCGs were found among the 60 isolates of *S. rolfsii*. Further, the majority of MCGs contained the isolates from same state. However, few MCGs contained one isolate from geographically adjoining state. Further, the isolates collected from same state did not fall under single MCG instead they were distributed into 2 to 5 MCGs.

The molecular identity of 60 isolates of *S. rolfsii* was performed by amplification and sequencing of ITS-rDNA region of these isolates and confirmed as *S. rolfsii.* Further, the phylogenetic analysis of the ITS-rDNA sequences revealed four main groups in phylogenetic tree wherein most of the isolates were clustered into ITS group 1 (*n*=30), followed by ITS group 2 (*n*=15), ITS group 4 (*n*=12) and ITS group 3 (*n*=2) indicating the relative uniformity in *S. rolfsii* population.

Additionally, the ITS-rDNA sequencing did not give detailed insight into the intraspecific diversity. Hence, to assess the same, the 15 isolates of *S. rolfsii* (one random isolate each from 15 MCGs) were subjected to diversity study using 30 RAPD primers. Further, the cluster analysis grouped the isolates into two clusters wherein cluster I comprised of most isolates while cluster II contained two isolates (SrGj-6 and SrGj-1). Looking to the style of grouping of isolates, there was no defined correlation found between the genetic diversity of isolates and their geographical origin.

There was a significant variability found among the 60 isolates of *S. rolfsii* with regard to sensitivity to commonly used fungicides in groundnut cultivation. Among the four fungicides tested, Tebuconazole and Azoxystrobin were found to be highly effective in inhibiting the growth of *S. rolfsii* under laboratory conditions. Whereas the Carbendazim and Thiram were found little less effective. Among the fungicides tested the Carbendazim had recorded higher resistance factor (1.12). Further, the higher resistance factor of

Carbendazim was noted in isolates collected from all states except for Gujarat isolates (1.03). Additionally, the higher resistance factor of Tebuconazole was recorded in isolates collected only from Gujarat (1.11) than the other state isolates. Furthermore, the higher resistance factors of fungicides Carbendazim and Tebuconazole were region specific and were probably due to their routine usage in groundnut cultivation at the respective locations. However, to state clearly the resistance development in *S. rolfsii* isolates against commonly used fungicides in groundnut cultivation, a detailed investigation need to be conducted.

For selection of potential biocontrol agents against stem rot of groundnut, totally 100 isolates of antagonistic fungi and 80 isolates of antagonistic bacteria were isolated from the groundnut rhizosphere soil. Both antagonistic fungal and bacterial antagonists were subjected to preliminary screening against SrGj-3, the virulent isolate of *S. rolfsii* to test their biocontrol ability. From the preliminary screening, eight and five most potential isolates of fungal and bacterial antagonists were selected for further studies.

Molecular identification of eight most potential isolates (T1 to T8) of fungal antagonists was performed by ITS-rDNA amplification, sequencing, and confirmed as *Trichoderma* sp. In phylogenetic tree the T1 isolate was closely clustered with *T. harzianum*, T2 with *T. viride* and remaining isolates to *T. asperellum* reference strains. Likewise, molecular identification of five potential isolates (B1 to B5) of bacterial antagonists was performed by 16S rRNA amplification, sequencing, and confirmed as *Bacillus* sp. In phylogenetic tree, the B1 isolate was closely clustered with *B. megaterium*, B3 to *B. cereus* and remaining isolates with *B. pumilus* reference strains.

Under *in vitro* evaluation the T1 isolate of *Trichoderma* sp. and the B1 isolate *Bacillus* sp. was found most effective against virulent isolate of *S. rolfsii* (SrGj-3). Further, these isolates found highly compatible with the commonly used fungicides and among with each other. Additionally, the bioformulations of T1 and B1 isolates with or without chitin alone or in combination exhibited appreciable extents of plant growth parameters (germination %, shoot length, root length, biomass, root volume, vigour index-I and vigour index-II) in TMV 2 groundnut cultivar under *in vitro* conditions. Hence, these isolates were further tested for their ability to induce systemic resistance in groundnut against stem rot under glasshouse conditions.

The talc based bioformulations of T1 and B1 isolates with or without chitin alone or in combination through seed treatment and soil application induced the systemic resistance in groundnut plants against stem rot under glasshouse in the form of higher activity of

defense enzymes (PAL, peroxidases, polyphenol oxidase and catalase), PR proteins (chitinase and β-1,3 glucanase) and defense chemicals (total phenol).

Further, among the bioformulation treatments, the combined application of T1 and B1 bioformulations with chitin recorded significantly highest mean protein content (43.17 mg g^{-1} FW) followed by combined application of T1 and B1 without chitin (37.19 mg g^{-1} FW). There was sharp increase in total protein content in bioformulation applied on 2^{nd} day dpi and persisted up to 5^{th} dpi.

The similar trend was observed with respect to total phenol content wherein the combined application of T1 and B1 fortified with chitin rerecorded significantly highest mean total phenol content (2.30 mg g^{-1} FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (1.94 mg g^{-1} FW mg^{-1} protein). On 2^{nd} dpi sharp increase in total phenol content was noted and persisted up to 7^{th} dpi with slight decline in above mentioned treatments.

Likewise, significantly highest mean PAL activity was observed with combined application of T1 and B1 with chitin (9.31 µmole trans-cinammic acid $min^{-1}g^{-1}$ FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (7.97 µmole trans-cinammic acid $min^{-1}g^{-1}$ FW mg^{-1} protein). Amongst the sampling intervals sharp increase in PAL activity was noticed on 2^{nd} dpi and persisted up to 6^{th} dpi in all bioformualtions treated plants compared to all controls.

Similarly, the significantly highest mean peroxidase activity (2.53 $\Delta OD_{470nm}min^{-1}g^{-1}$ FW mg^{-1} protein) was recorded in combined application of T1 and B1 with chitin followed by combined application of T1 and B1 without chitin (1.99 $\Delta OD_{470nm}min^{-1}g^{-1}$ FW mg^{-1} protein). Among the sampling intervals sharp increase in peroxidase activity was noticed on 2^{nd} dpi and persisted up to 5^{th} dpi in bioformualtions treated groundnut plants.

In addition, the native PAGE analysis of peroxidase activity revealed the expression of nine isoforms *viz.*, PO 1, PO 2, PO 3, PO 4, PO 5, PO 6, PO 7, PO 8 and PO 9 in different bioformulation applied groundnut plants. Further, on 2^{nd} and 3^{rd} dpi the combined application of T1 and B1 bioformulation with chitin induced the expression of eight isoforms followed by combined application T1 and B1 without chitin which induced only five isoforms. In uninoculated, inoculated, and chemical control poor induction of peroxidase isoforms was noted.

With respect to polyphenol oxidase (PPO) the similar trend was observed wherein the significantly highest mean PPO activity was observed with combined application of T1 and

B1 with chitin amendment (2.21 $\Delta OD_{420nm} min^{-1} g^{-1}$ FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin amendment (1.58 $\Delta OD_{420nm} min^{-1} g^{-1}$ FW mg^{-1} protein). Further, on 2nd dpi significantly sharp increase in PPO activity was observed in bioformulation treated groundnut plants and was persisted up to 5th dpi.

Added to it, the native PAGE analysis of polyphenol oxidase activity revealed the induction of about 13 isoforms *viz.*, PPO 1, PPO 2, PPO 3, PPO 4, PPO 5, PPO 6, PPO 7, PPO 8, PPO 9, PPO 10, PPO 11, PPO 12, and PPO 13 in different bioformulation treatment. The isoforms PPO1, PPO3, PPO5 and PPO7 were found to be constitutive as they were expressed in all treatments at all sample intervals and the isoforms PPO 2, PPO 4, PPO 6, PPO 8, PPO 9, PPO 10, PPO 11, PPO 12, and PPO 13 were specific and were induced as a result of pretreatment with bioformulations. Further, the highest expression of PPO isoforms were noted on 2nd, 3rd and 4th dpi in combined application of T1 and B1 with chitin *i.e.*, 11, 9, and 11 isoforms respectively.

Similar observations were recorded with respect to catalase activity. The combined application of T1 and B1 with chitin recorded significantly highest mean catalase activity (2.16 µmole H_2O_2 $min^{-1} g^{-1}$ FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (1.72 µmole H_2O_2 $min^{-1} g^{-1}$ FW mg^{-1} protein). Further, significantly sharp increase in catalase activity was found on 2nd dpi and persisted up to 4th in bioformulation treatments.

Additionally, the native PAGE analysis of catalase activity revealed the varied number of induction of catalase isoforms in bioformulation applied groundnut plants. Among the isoforms induced, the CAT 1 was found to be constitutive as it was expressed in all treatments and at all sample intervals and the isoforms CAT 2 and CAT 3 were found to be specific and induced as a result of bioformulation pre-treatment. In the study, on 2nd, 3rd, 4th and 5th dpi all three catalase isoforms CAT 1, CAT 2 and CAT 3 were expressed in combine application of T1 and B1 bioformulation with or without chitin.

The T1 and B1 isolates were found effective inducers of PR proteins (β-1,3 glucanase and chitinase). In these lines, the significantly highest mean activity of β-1,3 glucanase was recorded with combined application of T1 and B1 with chitin (16.87 µmole glucose $min^{-1} g^{-1}$ FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (13.86 µmole glucose $min^{-1} g^{-1}$ FW mg^{-1} protein). Further significantly sharp increase in β-1,3 glucanase activity was found on 2nd dpi and persisted up to 5th dpi in different bioformulation treatments. Further, the combined application of T1 and B1 with chitin was also recorded significantly highest mean chitinase activity (3.52 µmole N-acetyl-D-

glucosaminemin^{-1}g^{-1} FW mg^{-1} protein) followed by combined application of T1 and B1 without chitin (2.98 µmole N-acetyl-D-glucosaminemin^{-1}g^{-1} FW mg^{-1} protein). Here to significantly sharp increase in chitinase activity was noted on 2nd dpi and persisted up to 6th dpi in different bioformulation treatments. Thus, the T1 and B1 isolates were found most efficient inducers of systemic resistance against *S. rolfsii* in groundnut. Hence their efficacy against stem rot was further evaluated under glasshouse and field conditions.

The bioformulations of T1 and B1 with or without chitin alone or in combination through seed treatment and soil application found effective against stem rot of groundnut under glasshouse conditions. Wherein, the combined application of T1 and B1 fortified with chitin found most promising and noted significantly lowest mean disease severity (33.20%), incidence (60.73%) and mortality (13.33%) which was at par with chemical control (with 35.70%, 64.86% and 14.06% of mean disease severity, incidence and mortality respectively) followed by the combined application of T1 and B1 without chitin (with 38.67%, 67.81% and 19.69% of mean disease severity, incidence and mortality respectively).

The similar effect of bioformulations of T1 and B1 with or without chitin against stem rot of groundnut was observed in field conditions at two locations *viz.*, locations-I, ICRISAT, Patancheru and Location-II, PJTSAU, Rajendranagar during *kharif*, 2016.

In these lines, at location-I, the combined application of T1 and B1 bioformulation with chitin recorded significantly lowest mean disease severity (38.42%) followed by chemical control (45.83%). Similarly, at location-II, significantly least mean disease severity was observed with combined application of T1 and B1 bioformulation with chitin (41.92%) followed by chemical control (48.25%). The similar trend was noted in pooled data. Additionally the combined application of T1 and B1 bioformulation without chitin was found to be next best treatment and was at par with chemical control.

Likewise, with respect to disease incidence the combined application of T1 and B1 bioformulation with chitin was recorded significantly lowest mean disease incidence at both the locations (35.40% and 36.22% at location-I and location-II respectively) and similar trend was observed in pooled data (35.82%). Further, the combined application of T1 and B1 bioformulation without chitin recorded next lowest mean disease incidence at location-I (43.07%), location-II (42.23%) and in pooled data (42.09%) and was at par with chemical control.

Further, the similar effect was noted with respect to stem discoloration and pod rot wherein the T1 and B1 bioformulation mixture with chitin recorded the significantly lowest

mean stem discoloration and pod rot at both the locations and in the pooled data. Further, the T1 and B1 bioformulation mixture without chitin was found to be next best treatment and was at par with chemical control.

Similarly, the mixture of T1 and B1 bioformulation with chitin found superior in reducing mortality induced by *S. rolfsii* and recorded significantly least mean mortality at both the locations (12.57% at location-I and 15.17% at location-II) and similar trend was observed in pooled data (13.87%). Further, the T1 and B1 bioformulation mixture without chitin was found to be next best treatment and was at par with chemical control.

The bioformulation application in groundnut under field conditions had positive effect on growth and yield related traits at both the locations and similar trend was noted in pooled data. Thus, the mixture of T1 and B1 bioformulation with chitin recorded significantly highest pod yield of 1888.26 kg/ha at location-I, 1834.10 kg/ha at location-II and 1861.18 kg/ha in pooled data followed by mixture of T1 and B1 bioformulation without chitin (with 1780.49 kg/ha, 1734.17 kg/ha and 1757.33 kg/ha at location-I, location-II and in pooled data respectively). Further, the above treatments recorded higher B:C ratio compared to other bioformulation treatments.

CONCLUSIONS

From current study following conclusions were made,

- The roving survey conducted during *kharif,* 2013 and *kharif,* 2014 revealed the incidence of stem rot in major groundnut growing areas of India and was ranged from 11.23 to 55.40% and 10.11 to 59.33% respectively.

- All the districts of Gujrat, parts in Maharashtra and Tamil Nadu recorded comparatively higher incidence where the crop was grown in black soils with susceptible cultivars (TMV 2, JL 24, GG 20, and GG 11) continuously as sole crop.

- The medium Richards's broth was found to be best supporting medium for *in vitro* oxalic acid production by *S. rolfsii.* Further, the 60 of isolates exhibited wide variation in *in vitro* oxalic acid production.

- The 15g inoculum level per 7" pot was found most effective and suitable for glasshouse studies employing artificial inoculation of pathogen.

- Based on virulence reaction on three susceptible groundnut cultivars, the 60 isolates of *S. rolfsii* were grouped into two category, highly virulent (n=56) and less virulent (n=4). Further, there was a positive correlation found between the amount of oxalic acid produced *in vitro* and virulence of the isolates.

- The 60 isolates of *S. rolfsii* were found diverse with respect to cultural and morphological characters. Further, the cultural and morphological variability of *S. rolfsii* isolates was not correlated with the virulence of isolates.

- Based on ITS-rDNA sequencing and phylogeny the 60 isolates of *S. rolfsii* found relatively uniform.

- The intraspecific diversity among 15 isolates of *S. rolfsii* (one random isolate each from 15 MCGs) studied using RAPD did not had definite correlation between the genetic diversity, MCGs and geographical origin of isolates of *S. rolfsii.*

- Among the fungicides tested against *S. rolfsii* isolates *in vitro,* Tebuconazole and Azoxysrobin were found to be highly effective followed by Carbendazim and Thiram.

- The fungicides Carbendazim and Tebuconazole were recorded higher resistance factor and was happened to be region specific. Further, the development of region specific resistance factor was probably due to their routine usage in groundnut cultivation at the respective locations.

- The T1 isolate of *Trichoderma* sp. and the B1 isolate of *Bacillus* sp. exhibited significantly highest biocontrol characters against virulent isolate of *S. rolfsii* and were found highly compatible with the commonly used fungicides in groundnut cultivation and with each other.

- The isolates T1 and B1 found highly effective in inducing systemic resistance against *S. rolfsii*. Further, the individual application talc formulations of T1 and B1 induced least defense response compared to combined application of talc formulations of T1 and B1.

- The chitin amendment of bioformulation of T1 and B1 isolates enhanced their resistance inducing capacity against *S. rolfsii*.

- Among the various defense responses induced by T1 and B1 isolates against *S. rolfsii,* the activity of polyphenol oxidase was significantly highest with more number of isoforms inductions.

- The individual application talc formulations of T1 and B1 was found least effective in controlling the stem rot of groundnut under glasshouse and field conditions compared to combined application of talc formulations of T1 and B1. Further, the chitin amendment of bioformulation of T1 and B1 isolates enhanced their efficacy against *S. rolfsii*.

- The isolates T1 and B1 induced substantial amount of growth and yield attributing parameters in groundnut under field conditions. Further, the combined application was superior to the individual application of T1 and B1 isolates bioformulations.

Future lines of work

- Need to conduct a detailed investigation on characterization of resistance development in *S. rolfsii* against commonly used fungicides in groundnut
- As the oxalic acid is major pathogenicity factor of *S. rolfsii* the research should focus on identification of groundnut genotypes which possess the oxalic acid detoxifying compounds or resistance genes which codes for enzyme/product which nullify the effect of oxalic acid.
- The *S. rolfsii* is known to suppress the polyphenol oxidase (PPO) in host during pathogenesis hence there is a need to elucidate the exact mechanism of suppression
- Metabolomics study with focus on characterization of metabolite with oxalic acid detoxifying ability.
- Elucidation of exact pathway of oxalic acid production by *S. rolfsii* which in turn help to design management strategy against the pathogen.

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