STUDIES ON EPIDEMIOLOGY AND MANAGEMENT OF PHYTOPHTHORA BLIGHT (*Phytophthora drechsleri* Tucker f.sp. *cajani*) OF PIGEONPEA [*Cajanus cajan* (L.) Millsp.]

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M. Sc. (Ag.)

DOCTOR OF PHILOSOPHY IN AGRICULTURE
(PLANT PATHOLOGY)

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BY

JADESHA. G
M. Sc. (Ag.)

THESIS SUBMITTED TO THE
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2014

CERTIFICATE

Mr. JADESHA. G has satisfactorily prosecuted the course of research and that thesis entitled"**STUDIES ON EPIDEMIOLOGY AND MANAGEMENT OF PHYTOPHTHORA BLIGHT (*Phytophthora drechsleri* Tucker f.sp. *cajani*) OF PIGEONPEA [*Cajanus cajan* (L.) Millsp.]**"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 his for a degree of any University.

Date: **(Dr. P. NARAYAN REDDY)**

Place: Hyderabad Chairperson

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON EPIDEMIOLOGY AND MANAGEMENT OF PHYTOPHTHORA BLIGHT (*Phytophthora drechsleri* Tucker f.sp. *cajani*) OF PIGEONPEA [*Cajanus cajan* (L.) Millsp.]"** submitted in partial fulfilment of the requirements for the degree of"**Doctor of Philosophy in Agriculture**"of the AcharyaN.G.RangaAgriculturalUniversity, Hyderabad is a record of the bonafide original research work carried out by Mr. **JADESHA. G** 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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DECLARATION

I, **JADESHA. G,**hereby declare that the thesis entitled **"STUDIES ON EPIDEMIOLOGY AND MANAGEMENT OF PHYTOPHTHORA BLIGHT (*Phytophthora drechsleri* Tucker f.sp. *cajani*) OF PIGEONPEA [*Cajanus cajan* (L.) Millsp.]"** submitted to the **AcharyaN.G.RangaAgriculturalUniversity**for the degree of **Doctor of Philosophy in Agriculture**is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier in any manner.

Place: **Hyderabad** **(JADESHA. G)**

Date: **I.D. No. RAD/11-28**

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LIST OF SYMBOLS AND ABBREVATIONS

%	:	per cent
@	:	at the rate of
>	:	More than
<	:	Less than
a.i	:	Active Ingredient
AP	:	Andhra Pradesh
cm	:	centimeter
CO_2	:	Carbon Dioxide
cv.	:	Cultivar
DAS	:	Days after sowing
e.g.	:	for example
et al.	:	and others
etc.	:	and so on
Fig.	:	Figure
f.sp.	:	Forma species
g	:	Gram
h	:	Hour
ha	:	hectare
HPR	:	Host Plant Resistance
HSD	:	Honesty SignificantDifference
ICRISAT	:	International Crops Research Institute for the Semi-Arid Tropics
ICP	:	ICRISAT Pigeonpea germplasm
ICPL	:	ICRISAT Pigeonpea breeding line
ICPH	:	ICRISAT Pigeonpea hybrid
IDM	:	Integrated Disease Management
IPCC	:	Intergovernmental panel on climate change
i.e,	:	that is
KA	:	Karnataka
kg	:	Kilogram
kg $acre^{-1}$	:	Kilogram per acre
kg ha^{-1}	:	Kilogram per hectare
l	:	litre

m	:	Metre
m^2	:	Metre square
mg	:	milligram
MH	:	Maharasthra
ml	:	milliliter
mm	:	millimeter
µm	:	Micrometer
MP	:	Madhya Pradesh
No.	:	Number
OTC	:	Open Top Chambers
^oC	:	Degree Centigrade
PDA	:	Potato Dextrose Agar
Pdc	:	*Phytophthora drechsleri* f. sp. *cajani*
PDI	:	Per cent disease incidence
Pf	:	*Pseudomonas fluorescens*
pH	:	Hydrogen ion concentration
ppm	:	Parts Per Million
psi	:	pounds per square inch
r	:	Correlation Co-efficient
R^2	:	Multiple Regression
RBD	:	Randomized Block Design
RH	:	Relative Humidity
SMC	:	Soil Moisture Content
Spp.	:	Species
ST	:	Seed treatment
t	:	Tonne
viz.	:	namely

Abstract

Author	:	**JADESHA. G**
Title of the thesis	:	**STUDIES ON EPIDEMIOLOGY AND MANAGEMENT OF PHYTOPHTHORA BLIGHT (*Phytophthora drechsleri* Tucker f. sp.*cajani*) OF PIGEONPEA [*Cajanus cajan* (L.) Millsp.]**
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ABSTRACT

Phytophthora blight of Pigeonpeaincited by *Phytophthora drechsleri* Tucker f. sp.*cajani*is a potentially important disease of Pigeonpea after fusarium wilt (*Fusarium udum*Butler) and Pigeonpea sterility mosaic disease(Pigeonpea sterility mosaic virus).

Periodical survey conducted at ICRISAT fields during *kharif* 2012 and 2013 indicated that in *kharif* 2012 disease incidence ranged from 0.0 to 59.0 per cent whereas in *kharif*2013 disease incidence ranged from 0.0 to 55.0 per cent.Disease incidence was correlated with weather parameters, among all, cumulative rainfall, maximum temperature and maximum relative humidity showed positive correlation. Further, regression analysis showeda linear positive relationship between per cent disease incidence with cumulative rainfall and maximum relative humidity.

Roving survey conducted in Deccan Plateau of India during *kharif* 2012 and 2013, indicated, occurrence of disease throughout Deccan Plateau of India ranging from 0.0 to 54.4 per cent in *kharif*2012, whereas 0.0 to 33.0 per cent in *kharif*2013. Disease occurrence was observed irrespective of cropping system, soil types and cultivars.

Influence of different media, temperature, relative humidity and carbon dioxide were tested on growth and sporulation of *P. drechsleri* f. sp. *cajani.* Tomato juice agar medium, temperature of 30 ^{o}C, relative humidity of more than 70 per cent and elevated carbon dioxidelevel of 550 and 700 ppm supported maximum growth of the pathogen, whereas sporulation of pathogen was not supported by any of the media, temperature and relative humidity. Therefore, different methods proposed by various research workers for production of sporangia and zoospores were studied against the *P. drechsleri* f. sp.*cajani.* Modification of V8 broth method, where V8 broth substituted withdiluted tomato juice broth could induce abundant sporangia and zoospores within 24 hours of incubation and alsoproduced antheridium, oogonium and oospore on 48 hours after incubation.

The effect of different range of temperature, relative humidity and CO_2levels was also studied on production of sporangia and zoospores using diluted tomato juice

broth method. Temperature of 30 ^{o}C, relative humidity of 100 per cent and 550 ppm of CO_2showed maximum number of zoospores production.

Influence of weather parameters (temperature, relative humidity and CO_2) and edaphic parameters (soil type, soil moisture and duration of flooding) were assessed on incidence of Phytophthora blight under greenhouse conditions using the soil mixing and soil drenching method of inoculation. Temperature range of 25, 30, 35 and 40 °C, relative humidity of 95 and 100 per cent and elevated CO_2levels (550 and 700 ppm) showed highest disease incidence in both the inoculation methods. Further, red loamy soil and flooding condition showed maximum disease incidence.

Different methods of inoculation, inoculum quantity and plant age were studied to induce the disease in greenhouse conditions. Soil mixing method of inoculation, 10 per cent of inoculum quantity and 10-15 day old seedlings were found to be the best in inducing the disease under greenhouse conditions.

Forty three Pigeonpea breeding lines were screened in sick plot at Sehore, Madhya Pradesh, of which ICPL 99044, 99055, 99099 showed the resistant reaction against the disease. Further, 19 Pigeonpea genotypes were screened at ICRISAT, nine of them showed moderate resistant reaction. Further, reaction of 59 Pigeonpea improved lines assessed at ICRISAT, under natural conditions during the *kharif* 2013, 38 lines did not show any incidence, whereas ICPL 20092, Kamika and*Cajanus scarabeiodes*showed theincidence of more than 80 per cent. Confirmation of these resistant improved lines need to done under artificial inoculations in greenhouse and field conditions.

Fungicide mefenoxam and metalaxyl were tested against the *P. drechsleri* f. sp. *cajani* under *in vitro*condition and 100 per cent of mycelial inhibition was observed at concentration of 2.0 µg/ml with both fungicides, whereas 100 per cent inhibition of zoospore induction was achieved at 2.0µg/ml and 5.0 µg/ml concentration of mefenoxam and metalaxyl respectively.

Five isolates of *Trichoderma*and four isolates of *Pseudomonas*were evaluated against *P. drechsleri* f. sp. *cajani* of which*Trichoderma* isolate-3and *Pseudomonas*isolate-1gave highest mycelial inhibition of 80.52 and71.90 per cent respectively.Further compatibility of *Trichoderma* isolate-3 with mefenoxam and metalaxyl was tested and up to 1000 ppm concentration of the test fungicides were found compatible with *Trichoderma* isolate-3.

Influence of fungicides and bioagents on incidence of Phytophthora blight under greenhouse and field conditions at ICRISAT was studied using three varieties (ICP 87119, ICP 8863 and ICP 7119). In all three varieties, the minimum disease was observed in mefenoxam seed treatment alone and was at par with seed treatment with mefenoxam plus *Trichoderma*isolate-3, Mefenoxam plus *Pseudomonas*isolate-1 and metalaxyl seed treatment alone,indicated the fungicides dosage can be reduced by supplementing *Trichoderma* and *Pseudomonas* without compromising the efficacy to manage the disease.

Introduction

Chapter I

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.], is the fourth most important food legume in the world after dry bean (*Phaseolus vulgaris* L.), field pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.). In India, Pigeonpea is the second most importantfood legume crop after chickpea. It provides a high quality diet for human consumption as a main source of protein, especially for vegetarian population of the Indian subcontinent. It is grown under a wide range ofcropping systems in the Deccan Plateau (DP) in India(Reddy *et al*. 1998).Globally, Pigeonpea is cultivated over about 5.32 million ha, adding 4.24 million tonnes of grain to the global food basket(Factfish, 2012). In India, it is cultivated over3.89 million ha, accounting 3.02 million tonnes of grain and in Andhra Pradesh it is cultivated in about 0.48 million ha, with 0.25 million tonnes of grain (Indiastat, 2013).

Pigeonpea is susceptible to many diseases and insectpests but only a few of them are economically important (Nene *et al*. 1996; Vishwa Dhar *et al*. 2004). Phytophthora blight caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* is a potentially important disease of Pigeonpea in India after wiltand sterility mosaic disease(Kannaiyan *et al*. 1984). Chauhan *et al*.(2002) reported Phytophthora blight asmost important production constraint in Northeast India. The disease caused heavy plant mortality at seedling and vegetative stages, resulting in poor plant stand and lower yield (Mishra and Shukla, 1987).

The first suspected occurrence of Phytophthora blight on Pigeonpea in India was reported in 1966 by Williams *et al*. (1968). Since its appearance, the disease had spread to most Pigeonpea growing areas in Asia (Pal *et al*. 1970; Williams *et al*. 1975), Africa, America (Kannaiyan*et al*. 1984), Australia (Wearing and Birch, 1988), Dominican Republic, Kenya, Panama and Puerto Rico (Nene *et al*. 1996). Pande *et al*. (2006) reported 14.0 per cent incidence of Phytophthora blight in Rangareddy, Mahbubnagar, Nizamabad and Medak districts of Andhra Pradesh. Recurrence of Phytophthora blight as a major threat to Pigeonpea production and productivity in the Deccan Plateau of India was reported irrespective of cropping system, soil type and cultivars (Sharma *et al*. 2006; Pande *et al*. 2011). Further, the disease has been spreading at an alarming rate all over the Pigeonpea growing tracts during the last 15 years. The knowledge of weather parameters *viz.* temperature, relative humidity, carbon dioxide, soil type, soil

moisture on disease development is a prerequisite to predict the occurrence of the disease.

Adoption of the integrated disease management technology is essential for economical and effective control of Phytophthora blight of Pigeonpea. Moderate levels of host plant resistance can be combined with other cultural practices and/or application of antagonist bioagents with minimum dosage of fungicide for control of Phytophthora blight.

The occurrence of *Phytophthora* in flooded soils has been attributed to requirements of the pathogen for high soil moisture (Zentmyer, 1980). Indeed, evidence exists which indicated that soil-water status can exert a determining influence on several epidemiologically important stages in the life cycle of *Phytophthora* spp. Members of this genus reproduce by formation of sporangia which, under proper conditions, germinate to release free-swimming zoospores. The zoospores of *Phytophthora* are chemotactic and can swim to plant roots to establish new infections. It is advised that, Pigeonpea should be sown in well-drained soil and not subject to flooding. Proper drainage of fields to reduce the rate and extent of buildup of inoculum, and sowing of crop on raised beds, avoidance of sowing of Pigeonpea in low lying fields are suggested to reduce Phytophthora blight incidence.

The preliminary step for exploiting host plant resistance is the development of reliable and repeatable techniques for large scale screening of germplasm and breeding lines and use of resistant cultivars is considered to be the best way of controlling the disease. Several techniques suitable for Phytophthora blight resistance screening under field and green house conditions have been reported (Pal *et al.* 1970; Kannaiyan *et al.* 1981; Nene *et al.* 1981; Reddy *et al.* 1990). In addition information on inoculation technique, inoculum density and plant age and its susceptibility to disease is necessary to develop the screening method to identify stable resistance sources.

The use of fungicides has become inevitable in the management of plant diseases particularly in absence of availability of resistant varieties.Among the phenylamides (acylanilides) group of chemicals, metalaxyl was most widely used against the *Phytophthora* spp. (Erwin and Ribeiro, 1996).Mefenoxam is the active isomeric form of metalaxyl. The active isomeric form comprised 50 per cent of metalaxyl whereas, mefenoxam is 100 per cent active isomer.Mefenoxam has been used as a seed treatment to control *Phytophthora* spp. (Smiley *et al.* 1996; Dorrance and McClure, 1999; Babadoost and Islam, 2002;Mbong *et al*. 2013)

Though management of disease with chemicals is highly effective, the growing concern on pesticide toxicity has warned for searching alternatives. In this direction, biological control is considered as an eco-friendly and good alternative for sustainable agriculture to overcome the problems of public concern associated with pesticides and pathogens resistant to chemical pesticides (Akhtar and Siddiqui, 2008).The development of pathogenic strains resistant to pesticides, has now become a major problem throughout the world, may be minimized by the use of biocontrol agents (Utkhede and Smith 1992). Hence, in recent years, the use of bioagentshas gained more importance. These antagonistic organisms act on the pathogen by different mechanisms *viz*. competition, lysis, antibiosis, siderophore production and hyperparasitism (Vidyasekaran, 1997).

Trichoderma species showed antagonistic activity against *Phytophthora* spp.(Weindling, 1932; Baker and Cook, 1974;Pugeg and Ian, 2006; Ezziyyani *et al.,* 2007; Srivastava and Mall, 2008; Singh and Dubey, 2010). Considerable attention has been paid worldwide on *Pseudomonas fluorescens* for their antifungal antagonism and plant growth promoting activities of *P. fluorescens*has been reported to be inhibitory against *Phytophthora* spp. (*Yan et al*. 2002; Thanh *et al*. 2009 and Guo *et al*. 2009b).

The shift in the variables of climate, their unpredictable occurrence, is creating outbreaks of Phytophthora blight epidemics. Despite the extensive investigations on *Phytophthora* spp. on other hosts, the information on epidemiology, resistance mechanism and integrated disease management in Pigeonpea is scanty. A thorough understanding of these aspects aid in devising management strategies for Phytophthora blight.

The management of Phytophthora blight is essential to provide increased andstable Pigeonpea yields throughout the Pigeonpea growingregions. Hence, integrated disease management program suitable for adoption by resource poor farmers need to be developed. Keeping the situation as mentioned in the above paragraphs, the present investigation was taken up with the following objectives.

1. Assessment of spatial and temporal incidence of Phytophthora blight of Pigeonpeain major Pigeonpea growing pockets in India
2. Morphological and physiological studies of *Phytophthora drechsleri* f. sp.*cajani*.
3. Study the epidemiology of Phytophthora blight of Pigeonpea
4. Management of Phytophthora blight disease of Pigeonpea

Review of Literature

Chapter II

REVIEW OF LITERATURE

2.1. History and Distribution

Phytophthora blight disease of Pigeonpea caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* has been comprehensively reviewed by many researchers (Kannaiyan *et al.* 1984; Williams*et al*. 1975). The first suspected occurrence of Phytophthora blight on Pigeonpea in India was reported by Williams *et al*. (1968) in 1966 at Indian Agricultural Research Institute, New Delhi. Since then, the disease had spread to most Pigeonpea growing areas in Asia (Pal *et al.* 1970; Williams *et al.* 1975), Africa, America (Kannaiyan *et al.* 1984), Australia (Wearing and Birch, 1988), Dominican Republic, Kenya, Panama, and Puerto Rico (Nene *et al.* 1996). Thereafter, the incidence of the disease and its economic importance has been reported by many researchers in India.

2.2. Economic importance

Phytophthora blight is an important disease of Pigeonpea in India causing serious losses.Though the disease is sporadic in nature, occasionally it assumes epidemic proportions in places of heavy and frequent rainfall leading to mortality of young plants (Kannaiyan *et al*. 1984; Kannaiyan *et al*. 1980; Willams *et al*. 1975). Pal *et al*. (1970) estimated yield losses up to 98 per cent as the affected plants dry up rapidly. Further in South India, Reddy and Sheila, (1994) reported 100 per cent yield loss in short-duration Pigeonpea varieties.

The disease has been reported as an important production constraint in Northeast India particularly in low lying poorly drained fields (Kannaiyan *et al*. 1984; Mishra and Shukla, 1987). Bajpai and Tripathi (1999) opined that the disease is a serious concern in Pigeonpea as the mortality occurs at seedling stage. Losses due to disease increased many folds (84 %) under favorable weather conditions, especially in short duration and highly susceptible variety (Birendra and Dubey, 2005). Recently, the recurrence of Phytophthora blight as a major threat to Pigeonpea production and productivity in the Deccan Plateau of India irrespective of cropping system, soil types and cultivars has been reported by Sharma *et al*. 2006; Pande *et al*. 2011.

2.3. Prevalence of the disease

The classical survey conducted by Kannaiyan, *et al*. (1984) implied that thedisease occurred in major Pigeonpea growing areas of Asia, Africa and America and

in eleven states of India. Disease incidence of about 26.3 per cent was reported in West Bengal by Kannaiyan *et al*.1984. However a mild incidence of 2.73 per cent was noticed by Mishra and Shukla, 1987 in Uttar Pradesh.Reddy *et al*. (1990) reported a severe epidemic of Phytophthora blight in experimental plots at ICRISAT, Patancheru. Further, Chauhan *et al.* (2002) and Srivastava *et al.* (2008) reported varying proportion of disease incidence in Uttar Pradesh and Gujarat.

The survey conducted by Sharma *et al*. (2006) in Deccan Plateau during the crop season, revealed a widespread incidence of Phytophthora blight on improved and local cultivars grown in different intercropping systems and majority of improved cultivars grown at research farms were found susceptible to Phytophthora blight (>10% disease incidence). The Phytophthora blight incidence was more on improved cultivars (11.9-26.5%) than on local or traditional cultivars (5.0-9.3%). Among improved cultivars grown by farmers, ICP 8863 (Maruti) had the least Phytophthora blight incidence (11.9%) followed by BDN-2 (20.3 %), BDN-7 (22.6%) and BDN-1 (26.5%). Pigeonpea intercropped with groundnut, blackgram and coriander had less disease incidence (≤10%).

2.4. Disease symptoms

Phytophthora blight symptoms have been studied in detail by Williams *et al*. (1975), Reddy *et al.* (1990) and Singh *etal.* (1992). Symptoms of the Phytophthora blight have been described as stem rot (Pal *et al.* 1970), stem blight (Williams *et al,* 1975; Amin *et al.* 1978; Kannaiyan *et al.* 1980), stem canker (Kaiser and Melendez, 1978) and root rot (Wearing and Birch, 1988).

Pal *et al*. (1970) described that the symptoms of the disease in the field is usually recognized when the seedlings are about two week-old and the affected plants dry up rapidly, causing total loss to the crop.The lesions are brown to dark brown lesions distinctly marked from dark green healthy portions on the stem at ground level or a few inches higher affecting both leaves and stems. The disease appear as soon as the seedlings emerge and can go unnoticed as the small germinated seedlings collapse on the ground with damping off type of symptoms.

Rapid wilting of the plant parts above the invasion site, desiccation and upward rolling of leaflets, usually without chlorosis, withering of petioles and small stems, dark-brown to black necrotic lesions encircling the stem at the base or upto a meter or more above soil level were observed (Williams *et al*. 1975). Nene *et al*. (1979) described that lesions at the base often extend 15-20 cm up the stem and, appear on the

main stem, branches, or petioles, which usually have definite margins, and initially have a plane surface but later extend slightly several centimeters in each direction from the apparent invasion site.

At emergence the infected seedlingsshow crown rot symptom which topple down and dry. Older seedlings show water soaked lesions on leaflets which later become necrotic with brown dark lesions on main stem, branches and petioles and lesions usually girdle the stems drying theportions above the lesions. Finally stems breakdown due to wind (Faujdar and Oswalt,1992). Vishwa Dhar *et al*. (2005) reported that the characteristic symptoms of the disease are water-soaked lesions on the leaves and slightly sunken lesions on stem and petioles. Lesions girdle the stem and the foliage dries up.

Pande *et al*. (2011) observed that the seedlings become infected with Phytophthora blight as soon as they emerge. The symptoms first appear as water soaked lesions on the primary and trifoliate leaves which become necrotic within a week when humidity is 80 per cent and temperature 20-30 ^{o}C. The leaflet lesions are circular to irregular in shape and can be as large as 1 cm in diameter. Stem symptoms appear as brown to dark brown lesions distinctly different from healthy green portions on main stem, branches and petioles. The lesions on stem and branches increases rapidly and extend up to 20 cm, girdlingthe stem which cracks and further dries up. It was also observed that the lesions on stem are swollen into cankerous structures with thickened or raised margins. Infected stem and branches break easily due to wind. Phloem vessels show smoky gray colored discoloration and xylem vessels remain healthy.

2.5. Causal organism

2.5.1. Identification of the pathogen

Williams *et al*. (1968) first isolated the pathogen from wilted Pigeonpea plants with stem canker symptoms at New Delhi, India. Pal *et al*. (1970) identified the pathogen causing Phytophthora blight as *Phytophthora drechsleri* Tucker var. *cajani* Pal. However Amin *et al*. (1978) identified the Phytophthora blight pathogen as *Phytophthoracajani* Amin.

Kannaiyan *et al*. (1980) designated the fungus isolated from Pigeonpea as a forma species of *Phytophthora drechsleri,* in order to confirm the identity of the pathogen.He carried the detailed investigations in the laboratory of Dr .D.C. Erwin at the University of California, Riverside with five isolates[P2 (Hyderabad), P3 (New Delhi), P4 (Kanpur), P5 (Kalyanpur) and P6 (Deeg)] of the pathogen collected in

India.Based on the shape and size of sporangia, oogonium and oospore formation, temperature requirement and pathogenicity tests he named it as*P. drechsleri* Tucker f. sp. *cajani*. Recent literature of the disease indicated that Phytophthora blight of Pigeonpea is incited by *Phytophthora drechsleri* Tucker f. sp. *cajani*.

2.5.2. Morphological characteristics of the pathogen

Kannaiyan *et al.* (1980) observed that the mycelium was intracellular, branched, hyaline, filamentous to slender, aerial mycelium white and fluffy, coenocytic when young but later septate with thick plugs and abundant hyphal swellings of 13-15 µm in diameter with tube like projections. Sporangia were of proliferating type and the size ranged from 42-83µm × 28-48 µm (average 61.8 µm × 37.3 µm). The stalk of the sporangia were either narrowly tapered or widened somewhat at the base of the sporangium. Oogonium and oospore size showed little variation (19-29 to 34-44 µm). Formation of chlamydospores was not observed on any media at different temperatures.

The mycelium of*Phytophthora drechsleri*was reported as homothallic (Tucker, 1931) or heterothallic (Waterhouse and Blackwell, 1963; Kannaiyan *et al.* 1980). Oogonia of *P. drechsleri*f. sp. *cajani* were spherical to globose with 23.4µm-37.0 µm size and the thickness of oogonial wall was 2.4µm-4.8 µm (Pal *et al*. 1970). Amphigynous type of antheridia formed with in 2 to 3 week of *P. drechsleri*f. sp. *cajani* cultures in Lima bean agar medium (Amin *et. al*. 1978). Oogonia was smooth, spherical, thick walled, yellow to brown, and measured 24µm to 37 µm in diameter; while the antheridia were amphigynous and measured 12.5 to 19.0µm x 10 to 17 µm. Oospores were spherical or globose, smooth, thick walled (about 1.2 µm in width) and 20 to 32 µm in diameter (Singh and Chauhan, 1988).

2.6. Physiological characteristics of *Phytophthora* spp.

2.6.1. Influence of media

Potato dextrose agar (PDA), oatmeal dextrose agar (OMDA), lima bean agar (LBA), cornmeal agar (CMA), oat meal agar (OMA), V-8 Juice Agar (V8JA) and Pigeonpea seed meal agar (PSMA) wereused for growing, multiplying and isolating and assay of the genus *Phytophthora* from infected plant parts, infected crop debris and soil by Amin *et al.* 1978; Ribeiro, 1978; Kannaiyan *et al.* 1980; Erwin *et al.* 1981; Sheila *et al.* 1983.

Ribeiro, (1978) used V8 juice agar medium with calcium carbonate ($CaCO_3$) for growing and multiplying various species of *Phytophthora*. Nene *et al*. (1979)tested five different growth media *viz*. cornmeal agar (CMA), CMA + pimaricin-vancomycin-

PCNB, potato-dextrose agar, V-8 juice-agar, and Pigeonpea stem extract-dextrose-agar for growth of five isolates of *P. drechsleri*f. sp. *cajani*and found that CMA supported maximum growth.

Sheila *et al*. (1983) made attempts to find a simple and an equally efficient substitute for the V-8 juice medium, and reported thatPigeonpea seed meal medium was as efficient as V-8 juice media in supporting the growth of *P. drechsleri*f. sp. *cajani*. On the otherhand Dhingra and Sinclair (1995) have reported tomato juice agar medium as the best medium for the growth and sporulation of many *Phytophthora* spp. Further, Bisht and Nene (1988) reported that amendment of PDA with fungicides *viz*., 20 ppm Benlate, 20 ppm hymexazol, 10 ppm mycostatin, 20 ppm pentachloro-nitro-benzene, 5 ppm pimaricin, 200 ppm vancomycin, and 10 ppm rifamycin for selectiveisolation of *P. drechsleri*f. sp. *cajani*.

2.6.2. Influence of temperature and relative humidity on *Phytophthora* spp.

Of various environmental factors, temperature has been reported to have greatest influence on growth, reproduction and pathogenesis of *Phytophthora* spp. (Sujkowski, 1987; Sing and Chauhan, 1988; Matheron and Matejka, 1992). The influence of temperature on mycelial growth of different *Phytophthora* spp. has been reported (Waterhouse and Blackwell, 1963; Zentmeyer *et al*. 1976; Zentmeyer, 1981; Sujkowski, 1987; Simpfendorfer *et al*. 2001; Dirac and Menge, 2002).

Understanding the influence of temperature on mycelial development of *Phytophthora* spp. is an essential step in the development of best management practices of the disease. The temperature in any given environment must be permissive to mycelial development and growth for the respective *Phytophthora* spp. to support disease progress (Duniway, 1983). The parallel effects of temperature on mycelial growth and the development of Phytophthora diseases have been described by Fawcett, 1936; Zentmeyer *et al*. 1976; Zentmeyer, 1981; Duniway, 1983; Matheron and Matejka, 1992.

Pal and Grewal, (1975) reported 30 ^{o}C is the optimum temperature for the growth of *P. drechsleri* f. sp. *cajani*. However the fungus was viable up to 133 days at 15 °C, but the optimum temperature for the growth of pathogen was between 27 ^{o}C to 33 ^{o}C (Nene *et al*. (1979). Further Mishra *et al*. (2010) observed maximum vegetative growth at 30 ^{o}C.

Waterhouse and Blackwell (1963) reported25 – 28 ^{o}C as optimum temperature for mycelial growth of*Phytophthora citricola*, for*P. citrophthora* 26 ^{o}C (Dirac and

Menge, 2002) and *P. parasitica* at 30 – 31 ^{o}C (Matheron and Matejka, 1992; Dirac and Menge, 2002).

Phytophthora palmivora is a high-temperature organism with an optimum of 30 °C for mycelial growth (Zitko *et al*. 1991). Pao-Jen Ann (2000) reported *Phytophthoraparasitica* showedmaximum radial growth of mycelium at temperatures of 36-37 ^{o}C whereas *P. citrophthora* at 32-33 ^{o}C while *P. palmivora* at 35 ^{o}C.

Sahu *et al*. (2000) found that an optimum temperature of 30 °C was suitable for the growth of *Phytophthora colocasiae*followed by 25 °C but it did not grow at 5 ^{o}C and 40 °C. *Phytophthora cryptogea* was observed to have optimum mycelial growth at 20 °C-25 °C (Hardham, 2001). Similarly Nelson *et al*. (2011) reported that *Phytophthora colocasiae* grew well between 20-25 °C and most rapidly at 27-30 °C. Minimum and maximum temperatures for growth were 10 °C and 35 °C.

Studies on the influence of temperature on sporangial production by water-borne *Phytophthora* spp. is an important prerequisite for developing disease predictive models in the field. The influence of temperature on the rate of sporangia formation in *Phytophthora* spp. has been documented by Gooding and Lucas, 1959; Duniway, 1983; Matheron and Matejka, 1992; Timmer *et al*. 2000. Limited viability of sporangia at relative humidity of less than 100 per cent was reported by Glendinning *et al*. 1963; Warren and Colhoun 1975 and Minogue and Fry 1981; Rotem and Cohen 1974.

Trujillo, (1965) reported that *Phytophthora colocasiae* sporulation occurrednormally in the zone of active fungal growth under optimum conditions with a relative humidity of 100 per cent and temperature of 20-22 °C. He also observed that sporulation could take place at the margin of a lesion in less than 3 hours. Hausbeck and Lamour (2004) observed that sporangial production of *Phytophthora capsici* on cucumber fruits was greater at 60 and 80 per cent relative humidity than at >90 per cent.

Rotem and Cohen, (1974) found that sporangia were able to infect potato following exposure to conditions of 50 and 80 per cent RH for 48 h. Sporangia exposed to 30 ^{o}C were short-lived but still caused infection after 8 h at 80% RH and 6 h at 50%. These results suggested that sporangia may remain viable following long exposure to dry conditions and that RH may be more important in survival at higher temperatures. These findings were supported by Minogue and Fry (1981), who found that an increase in RH extended the half-life of sporangia only at the highest temperature tested (30 ^{o}C), and that sporangia had a half-life of 5 to 6 hours under moderate conditions (15 to 20 ^{o}C, 40 to 88 % RH).

Sporangia of *P. cinnamomi*were produced in large numbers when the soil is just below saturation and infection typically occured when soil temperatures were at 15 to 28 ^{o}C (Kuhlman, 1964). Many other *Phytophthora* spp. also had similar soil moisture and temperature requirements (Duniway, 1979; Hendrix and Campbell, 1973).

2.7. Techniques for the induction of sporangia and zoospores

Asexual developmental stages are crucial for plant infection and disease development (Hardham, 2001). *Phytophthora* spp. have a complex asexual life cycle with distinct multiple infectious propagules which include the mycelium, sporangia, and zoospore cysts (de Souza*et al*. 2003). Sporangia production is an important stage in the life cycle of *Phytophthora* spp. providing an opportunity for increase in infective units and increased disease incidence (Matheron and Matejka, 1992). Sporangia germinated either indirectly by releasing zoospores or directly by germ tubes thus enhancing plant infection (Matheron and Matejka, 1992; Timmer 2000).

According to Amin *et al*. (1978) sporangia formed when diseased Pigeonpea stem sections were surface sterilized and placed in distilled water for 36 to 48 hours but the sporangial production was not observed at 15-25 ^{o}C in culture medium. In contrast, Pal and Grewal, (1984) reported the formation of sporangia of *P. drechsleri*f. sp. *cajani* on oat meal agar medium after 30-35 days at temperature of 25 to 30 °C, at a pH of 6.0 to 6.5 with an incubation period of 4-8 days. Minimum, optimum and maximum temperature for the germination of zoospores after their release from zoosporangia were found to be 10°C, 25-27°C and 35 °C respectively.

Kannaiyan *et al*. (1980) described a brief technique for the production of sporangia and zoospore of *P. drechsleri*f. sp. *cajani*. Sporangia of *P. drechsleri*f. sp. *cajani*were not produced in agar or liquid nutrient media at any temperature and relative humidity, but were produced when the culture was transferred to sterile water at the tip of the sporangiophores which had hyphal stalks devoid of hyphal swellings. Each sporangium released 8 to 20 zoospores and became nonmotile after 2 to 5 hours, encysted, and usually germinated with one or more germ tubes sometimes forming a microsporangium (Singh and Chauhan, 1988).

Singh, and Chauhan, (1988) reported the occurrence of sexual phase in the host tissue and sparse oospore formation in old cultures, hence a technique has been developed in which abundant mature oospores were formed on the leaflets of Pigeonpea and also on glass slides using zoospores and mycelial discs. Maximum numbers of oospores were formed when incubated at 25 ^{o}C for 36h.

Birendra and Dubey,(2005) obtained sporangia of *P. drechsleri*f. sp. *cajani* by placing 5 mm mycelial discs of 5 days old culture in Petri dishes containing 10 ml of sterilized tap water at 25 ^{o}C. In addition, a wide range of media and different salt solutionswere proposed by many authors to induce sporangial production in *Phytophthora* spp. as given below.

Protocols proposed for the induction of sporangia and zoospore

S.No.	Method	Pathogen	Reference
Different media			
1	Bean seed medium	*Phytophthora meadii, P. cactorum, P.heveae, P. capsici, P. hibernalis* and *P. syringae*	(Peries and Fernando, 1972) (Bywater and Hickman, 1959)
2	Frozen pea medium	*Phytophthora*spp.	(Dance *et al*. 1975)
3	Hemp Seed agar/broth	*P. cinnamoni*	(Schmitthenner, 1973)
4	Prune juice broth	*P. citrophthora, P. parasitica and P. coctorum*	(Fawcett and Klotz, 1934)
5	Sun flower, Pea, Corn &Soybean seed media	*P. fragariae*	(Felix,1962)
6	Alfalfa stem media	*P. parasitica* and *P. citrophthora*	(Klotz and DeWolfe, 1960)
7	*Eucalyptus sieberi* seedling media	*P. drechsleri*	(Gerrettson-Cornell, 1975)
Inorganic salts solutions			
8	Petri solution	*P. sojae*	(Petri, 1917)
9	Wills mineral salt solution	*P. parasitica* var. *nicotianae*	(Wills, 1954b)
10	Salt solution	*P. cinnamomi*	(Chen and Zentmyer, 1970)
11	Synchronous method	*P. cinnamomi*	(Zaki and Zentmyer, 1976)
12	Cellophane over layer technique	*P. cinnamomi*	(Hwang *et al.*1975)
13	Salt solution	*P. cinnamomi*	(Halsall, 1977)
14	Plant nutrient solution	*P. megasperma* f. sp. *medicaginis*	(Hoagland and arnon,1950)
15	KNO_3 solution	*P. citrophthora*	(Schiffman and Cohen, 1968)
16	Soil extract	*P. cinnamomi*	(Zentmyer and Marshell, 1959)
17	Bacterial method (*Chromobacterium cinnamomi*)	*P. cinnamomi*	(Zentmyer, 1965)

2.8. Epidemiology and disease development

Chemical industry and plant breeders develop the 'weapons', but the epidemiologists set the strategy (Vanderplank, 1963). Epidemiological studies generate lot of information on different aspects of disease development. This processed data helps in developing appropriate strategies for plant disease management.

Different environmental factors such as temperature, water relations, physical and chemical conditions, and interacting combinations of these factors have been reported to influence Phytophthora pathogenesis (Duniway, 1983). Moreover, the geographic distribution and seasonality of Phytophthora diseases are thought to depend on the constraints of any of these environmental factors on any or all of the life cycle stages (Duniway, 1983).

2.8.1. Effect of temperature and relative humidity

Moisture and temperature are factors known to be of critical importance in governing infectivity of various *Phytophthora* spp. to different hosts (Duniway, 1983; Gerlach *et al*. 1976; Grove and Boal, 1991; Grove *et al*. 1985; Hau and Kranz, 1990; Timmer *et al*. 2000).

High humidity coupled with the disease may cause rapid development of stem rot of Pigeonpea. (Pal *et al*. 1970). Cloudy weather accompanied by intermittent rains followed by mean temperatures of 25±1 °C favored infection and development of Phytophthora blight. The disease occurred during rainy and cloudy weather and when day temperatures are less than 28 ^{o}C (Reddy *et al*.1990). Reddy *et al*. (1991) found that Phytophthora blight outbreak usually occurred when there was a decrease in day temperatures of the previous week and difference between maximum and minimum temperatures were the least and high rainfall and cloudy weather.

Epidemics of Phytophthora brown rot of citrus generally follow extended periods of high rainfall (Cohen and Knorr, 1960; Graham *et al*. 1998; Whiteside, 1970). Timmer and Zitko, (2000) reported optimum temperatures of 27 to 30°C for infection and development of Phytophthora brown rot of citrus. Gerlach *et al*. (1976) found that *P. citrophthora* penetrated leaves of *Pieris japonica* at favorable temperatures of 20 to 30 °C.

Infection and subsequent disease development of Phytophthora blightof pigeonpea was occurred when day temperature was<28°C coupled with rain on 7 consecutive days prior to disease onset in 1987 and on 5 of 7 days in 1988 (Reddy *et al.* 1991). The loss due to Phytophthora blight can be quite high in seasons of high rainfall

and under waterlogging conditions when infection occured at wide range of temperature of 10-35 ^{o}C, A leaf wetness period of 12 hrs was found essential for infection and disease development (Masood *et.al*. 2005).

In Pigeonpea, Phytophthora blight could spread fast during the rainy daysAgarwal *et al*. (2002). The infection index and plant mortality due to blight were positively correlated with rainfall, rainy days and relative humidity, and negatively correlated with maximum temperature. The disease suppressed with the cessation of rains, resulting in the survival of infected plants, which developed knots at the infection site on stems. Sporangia were observed on infected leaves and stems under cloudy atmosphere with high humidity, especially in the morning. The sporangia were detected during the period with low maximum temperature (24.0-28.5 ^{o}C), high relative humidity (88-100 %) and 100 % rainy days. The period of higher infection indices coincided with the detection of sporangia. The oospores were detected on infected stems but not on leaves after the cessation of rains. Formation of oospores was positively correlated with maximum temperature (28-30 ^{o}C), and negatively correlated with minimum temperature (20-22 ^{o}C), relative humidity (76-93%) and rainfall.

The weather data and Phytophthora blight incidence collected in India from 2000-2010 showed higher incidence of Phytophthora blight when maximum temperature (28-40 ^{o}C), minimum temperature (12-24 ^{o}C); relative humidity 75-96% was coupled with 300 mm rain fall within a week (Sharma *et al*. 2006; Pande and Sharma, 2010).

In pepper,Phytophthora fruit rot incidence and lesion length were greatest when inoculated fruits were incubated at 27 °C (Biles *et al*. 1995). Cucumbers incubated with *Phytophthora capsici* at 35 °C did not produce any symptoms even though pathogen produced maximum growth at 35 °C in culture medium (Erwin and Ribeiro 1996; Stanier *et al* 1971). Granke and Hausbeck (2010b) reported that *P. capsici* infected the cucumber fruit at 15 to 30 °C temperature and severity of disease was higher at 25 °C at 4 days post inoculation (dpi) and diameter of water-soaking lesions and pathogen growth increased as the relative humidity increased. Further, Huq (2011) reported that severity of leaf rot of betel vine caused by *Phytophthora parasitica* reached maximum when average temperature, relative humidly and rainfall gradients were 29.6 ^{o}C, 94.6 % and 13.4 mm respectively.

Late blight of potato developed on potato foliage under conditions when ambient relative humidity is above 90 per cent (Lacey, 1967; Rotem *et al*. 1971). The effects of relative humidity on development of Phytophthora fruit rot of cucumber caused by *P. capsici* were investigated in controlled growth chamber and the results

indicated that lesions formed on cucumbers incubated at all relative humidity tested (35%, 60%, 70%, 80%, and 100%), and the diameter of water-soaking and pathogen growth increased as the incubation relative humidity increased (Granke and Hausbeck, 2010b).

Suheri and Price (2000) and Shakywar *et al*. (2012) reported that maximum sporangia germination, zoospore formation and penetration of taro leaves by *Phytophthora colocasiae* was at 21-26 ^{o}C temperature, 90-100% maximum average relative humidity and frequent cumulative rainfall.

Warm temperature ranging between 25 to 30 ^{o}C in combination with high relative humidity of >70 per cent was essential/optimum conditions for maximum disease development of leaf blight and fruit rot (*Phytophthora nicotianae* var. *nicotianae*) of bell pepper (Bhardwaj, 1983; Jia, 1992). The disease always appeared just after the onset of monsoons due to the presence of abundant rainfall, high RH and warm weather (Bhardwaj, 1983). High incidence of the disease caused by *Phytophthora* favoured by heavy rainfall, excessive moisture, irrigation and hot weather (Bowers *et al*. 1990; Tang *et al*. 1992).

2.8.2.Effect of elevated CO_2 Level

Concentrations of CO_2 has increased markedly since the inception of the industrial revolution, and will continue to climb well into the 21st century, CO_2 is expected to double the preindustrial levels by 2050 (Prather & Ehhalt, 2001; Vingarzan, 2004; IPCC, 2007).

Elevated CO_2 levels may increase plant productivity due to enhanced C-fixation (Kimball, 1983; Poorter, 1993), suppressed photorespiration (Valle *et al*. 1985) and/or suppressed dark respiration (Gifford *et al*. 1985; Bunce, 1990; Amthor, 1991). The increased CO_2 levelon the other hand has caused increased disease in many plants.

Chakraborty *et al*. (1998) reported that out of ten biotrophic pathogens studied, six pathogens could enhance disease severity while four pathogens reduced the disease severity at high CO_2 level. Similarly out of 15 necrotrophic pathogens, nine pathogens could increase disease severity, whereas four pathogens reduced the disease severity and two remained unchanged.

Hibberd *et al*. (1996a) showed that the penetration of *Erysiphe graminis*was reduced at 700 ppm CO_2 in barley leaves. The reduced rates of primary penetration led to increased resistance with the production of papillae and accumulation of silicon at the site of appressorial penetration. However, the increased stomatal conductance at

high $CO_2$was responsible for reduction of infection by *E. graminis* in barley (Hibberd *et al*. 1996b).

Colletotrichum gloeosporioides showed delayed or reduced conidial germination, germtube growth, and appressorium production when inoculated onto susceptible *Stylosanthes scabra* plants under increased CO_2 (Chakraborty *et al*. 2000b). Jwa and Walling (2001) reported that tomato plants showed a tolerance against *Phytophthora parasitica* at elevated CO_2.This might be due to the effect of carbon dioxide concentration on the transcription or post-translational turnover of PR proteins, or through increased photosynthesis and water use efficiency. In soybean, elevated CO_2 significantly reduced downy mildew disease severity by 39–66% across 3 years. In contrast, elevated CO_2 significantly increased brown spot severity in all 3 years, but the increase was small in magnitude (Eastburn *et al*. 2010).

Despite abundant evidence about the direct effects of atmospheric change on plants, we still need a thorough understanding of their impact on plant diseases in agricultural ecosystems to properly predict and plan for disease pressure under future climatic conditions (Manning and Tiedemann, 1995; Coakley *et al*. 1999; Runion, 2003; Scarascia-Mugnozza *et al*. 2005; Garrett *et al*. 2006; Chakraborty *et al*. 2008).

2.8.3. Soil moisture

Soil moisture is considered as an important factor for disease development specially Phytophthora blight. Howeverthere are contradicting reports available in literature. Williams *et al*. (1975) related high incidence of Phytophthora blight of Pigeonpea in poor soil surface drainage, on the other hand Singh and Chauhan (1985) found Phytophthora blight developing to an epidemic level in well drained soils. However, Pande *et al*. (2009) reported an outbreak of Phytophthora blight in well drained, partially drained and temporarily water logged fields irrespective of cropping systems, soil types and crop cultivars in Deccan Plateau, India.

Many authors are of the opinion that, low lying, poorly drained soil was conducive for the incidence of Phytophthora blight as it promote high soil moisture (Kannaiyan *et al*. 1984; Mishra and Shukla 1987 and Chauhan *et al*. 2002; Masood *et al*. 2005; Sharma *et al*. 2006; Pande *et al*. 2011).

The water holding capacity of soil is a function of soil profile. Few authors proposed a correlation between soil profile and *Phytophthora* incidence. Goodall, (1962) correlated incidence of Phytophthora root rot in avocado with soil profile characteristics. Their research outcome implied recent alluvial soils are the safest

whilethe old terrace soils are the most hazardous to disease development. The texture of the soil also influenced on diseaseincidence, where loam, sandy loam and clay showed the incidence of 20, 17 and 3 per cent respectively. Further, Masood *et al*. (2005) reported thatPhytophthora blight disease appeared both in vertisols and alfisols.

Literature depicts flooding in many host pathogen system increases the disease incidence by favouring the induction of sporangia and movement of zoospore and modifying host to susceptible condition. Hence proper drainage of Pigeonpea fields and growing piogeonpea in well elevated or well drained fields can be advised.

Sowing of crop on raised beds and proper drainage of fields are the suggestive agronomic practices to reduce Phytophthora blight incidence, since water logging is believed to predispose Pigeonpea crop to Phytophthora blight under favorable environmental conditions (Singh and Chauhan, 1985; Reddy *et.al*. 1991; Sharma *et al*. 2006).

2.9. Integrated disease management

Adoption of the integrated disease management (IDM) technology is essential for economical and effective control of Phytophthora blight of Pigeonpea. Moderate levels of host plant resistance (HPR) can be combined with other cultural practices and/or application of minimum dosage of fungicide for control of Phytophthora blight.

The best option to control Phytophthora blight is not to sow Pigeonpea in fields or areas of the field, which are prone to waterlogging. The losses due to the disease can be minimized if the crop is raised on ridges or broad beds than on flat beds, as the disease incidence is higher if a film of water surrounds the base of the plant. Seed dressing with fungicides such as metalaxyl (1g a.i. kg^{-1} seed) is very useful in minimizing the losses caused by the disease (Kannaiyan and Nene, 1984).

Under low levels of disease pressure, these individual components may help in managing the disease. But under high levels of disease pressure, integration of these components is necessary to keep the disease under control. Selecting a tolerant cultivar, seed treatment with fungicides and sowing the crop on ridges were found effective to keep the disease under economic threshold level conditions.

2.9.1. Host plant resistance

2.9.1.1. Effect of inoculum density

Inoculum density on plant infection is important before attempting to elucidate quantitatively the overall interactions of the host, pathogen and the environment because of the complexity of factors influencing infection and then disease

development (Mitchell, 1978). A range of inoculum densities should be selected so as to characterize better relationship between inoculum density and infection (Fraedrich *et al*. 1989; McIntyre and Taylor 1976; Milholland *et al*. 1989).

Mitchell *et al*. (1978) reported higher infection and mortality of cuttings of watercress with increasing levels of zoospores per plant. The inoculum load required for plant mortality was higer than the infection. The results of this study are in general agreement with the work of Gooding and Lucas, (1959) with *P. parasitica* var. *nicotianae* on tobacco, *P. palmivora* on papaya (Kliejunas and KO, 1974; Ko, and Chan, 1974; Ramirez and Mitchell, 1975). Charles*et al*. (1995) reported that in pepper fruit rot incidence and severity increased significantly with the increase in zoospore concentration.

Yannis and Michael, (2001) studied the effect of inoculum density on disease severity in alfalfa, maize, sorghum and sugarbeet plants and concluded that the incremental root length of all plants decreased and root discoloration increased as inoculum concentration of the pathogen increased. Similar results were observed by other workers on *Phytophthora* spp. (Lipps and Bruehl 1980, Mitchell *et al*. 1978, Palloix *et al*. 1988).

2.9.1.2. Effect of plant age

The relationship of the age of a plant and its susceptibility to disease helps to obtain valid results of host plant resistance and management of disease.

Williams *et al*. 1975 reported that Pigeonpea plants are susceptible to Phytophthora stem blight from seedling to the mature fruit stage.Mishra and Shukla, (1986a) reported the susceptibility of the plants was directly proportional to their age,though the pathogen was able to infect the plants at all growth stages, but its incidence varied according to age of the plant. The disease incidence was maximum (100 per cent) at the age of 15 days and declined gradually with increase in the age of the plants.

Older plants of all Pigeonpea cultivars showed less plant mortality than younger plants irrespective of *P. drechsleri*f. sp. *cajani* isolate used for inoculationSarkar *et al*. (1992). They also observed significant variation in plant mortality, particularly in the 30-day-old plants of the test genotypes. However, death of older plants may occur due to progress of early infections under conducive weather conditions (Masood *et al.* 2005).

Further, Pande and Sharma (2011)observed the susceptibility of Pigeonpea to Phytophthora blight irrespective of growth stage both in the field and greenhouse. Under field conditions, it is not uncommon to observe plants dying even after 60 days and disease symptoms continue expressing from seedling stage to flowering and beyond. In such cases it is possible that infection might have occurred at an early age, and that the disease progresses slowly, killing the plants later.

Similar correlation of plant age and incidence was also documented in other *Phytophthora* spp. and hosts.Many authors opine that maturity of plants induces resistance to phtophthora infection. Kim *et al*. (1989) demonstrated the expression of age-related resistance in pepper plants infected with the *Phytophthora capsici*. Dorrance and McClure (2001) observed lack of ontogenic resistance expression in seeds and seedlings of soybean cultivars to infection by *Phytophthora sojae.* The amount of disease was limited when inoculated with *Phytophthora sojae*at seedling growth stages.

2.9.1.3. Screening techniques

The preliminary step for exploiting host plant resistance (HPR) is the development of reliable and repeatable techniques for large scale screening of germplasm and breeding lines. Several techniques suitable for Phytophthora blight resistance screening under field and green house conditions have been reported (Pal *et a.* 1970; Kannaiyan *et al.* 1981; Nene *et al.* 1981; Reddy *et al.* 1990).

Pal *et al*. (1970) used a 'leaf scar' method to inoculate 30 to 60 day-old plants. The inoculum multiplied on potato dextrose agar, was applied at the point of attachment of a leaf after its removal. Kannaiyan *et al.* (1981) reported a pot-culture drench inoculation technique, in which 5 to 10 day old seedlings were inoculated by drenching with mycelial suspension of *P. drechsleri*f. sp. *cajani*. A sick-soil method for large scale screening of Pigeonpeagermplasm proved very effective (Singh *et al*, 1992).

Mishra and Shukla (1986a) reported that the incidence of Phytophthora blight was maximum (100 %) in seedlings inoculated at 15 days, and declined with increasing plant age to a minimum (25 %) at 120 days. A new technique was proposed by Singh *et al.* (2000) which is applicable at all stages of plant growth. A higher blight infection was recorded using the detached leaf technique (100 %) compared with 74.9 % with the spray method. In this technique, inoculum was multiplied on Pigeonpea dal medium (40 g/liter water) medium and a 3 mm diameter mycelial mat was placed at a height of 15 cm up the seedling, where the leaf had been removed. To eliminate the possibility of drying up of the inoculum, the inoculated portion was wrapped with 15 mm of

cellophane tape. Infection at the seedling stage and maturity was 100 % and 83.3 %, respectively.

The components and procedures of the field screening of Pigeonpea genotypes for Phytophthora blight resistance were standardized at ICRISAT by Nene *et al.* (1981), Planting of test material with a 30cm rowspace and inter planting a susceptible cultivar (ICP 2376 and/or ICP 7119) to serve as an indicator line after every 2-4 rows. The collar region of one monthold plants was inoculated with mycelial mats of the fungus after mixing with carborandum.

Reddy *et al*. (1990) developed a diseased debris field inoculation technique. In this technique, a well leveled alfisol was selected and Phytophthora blight susceptible cultivars (ICP 2376 and ICP 7119) are sown as closely as possible (30 × 10 cm) on flat beds preferably before the monsoon rain arrives. When the plants were about one month old, approximately 250 kg of diseased plant debris (Pigeonpea stems with Phytophthora blight lesions collected during the previous season and stored dry in the field shelter) were scattered all over the field. During rain-free days, sprinkler irrigation was liberally provided.

2.9.1.4. Resistance sources

The use of resistant cultivars is considered to be the best way of controlling the disease. Pal *et al*. (1970) were the first to carry out screening of Pigeonpea genotypes for resistance to Phytophthora blight. The results implied that line AS-3, 2357 showed resistant reaction.The nature of resistance to Phytophthora blight in Pigeonpea was studied by Gupta *et al*. (1997) with seedlings of parental, F1, F2 and backcross generations of 8 Pigeonpea crosses (6 between susceptible and resistant genotypes, and 2 between susceptible parents). The results indicated a monogenic dominant nature of resistance and the presence of some minor genes were involved in resistance expression. Resistance in ICP 7065 and ICP 2376 with the P2 isolate was controlled by a single dominant gene (Sharma *et al*. 1995).

Kannaiyan*et al*. (1981) screened 2,835 Pigeonpea (*Cajanus cajan*) accessions and cultivars and seven *Atylosia* spp. under glasshouse and field condition for resistance to *P. drechsleri*f. sp. *cajani*. Seventy seven germplasm accessions, three cultivars, and two species of *Atylosia* were found to be resistant under field conditions.

Nene *et al*. (1980) screened 3419 germplasm lines and identified 122 as resistant and to obtain combined resistance he developed a multiple-disease screening nursery and identified several lines with resistance to more than one disease.

LinesICP3753, ICP7182 and ICP 7273)showed resistant to both wilt and Phytophthora blight, while lines ICP-934, 4765, 4866,5656, 6974, 7185, 7232, 7269, 7273, 7414, 8101, 8127, 8132, 8139, 8151 and 8147 were resistant to sterility mosaic and Phytophthora blight. The line ICP-7273 was resistant to wilt, sterility mosaic and Phytophthora blight.

Bhargava and Gupta (1983) evaluated 46 lines in the field at Sehore (Madhya Pradesh) and noticed a fair degree of resistance in most of the late maturing lines. Some Pigeonpea lines, ICPL 161, METH 12, COMP-1, ESR-6, Pant A-3, Pant A-83-14, GAUT 82-58, ICPL 366 and DPPA 85-11, exhibited field resistance at Pantnagar, Uttar Pradesh (Singh *et al*. 1985).

Reddy *et al*. (1991) reported some medium and long duration lines, such as KPBR 80-2-1, KPBR 80-2-2 and ICP 9252, expressed field resistance to most of the Indian isolates of *P. drechsleri*f. sp. *cajani* in adult plants. However, these lines subsequently became susceptible within 2-3 weeks of sowing. Singh *et al*. (1985) investigated 258 Pigeonpea genotypes to locate field resistance against *P. drechsleri*f. sp. *cajani*. They placed 93.5% entries in the highly susceptible category compared with only 7 resistant genotypes (BDN 627, Sehore 197, Sehore 197-1, ICPL 187-1, ICPL 84052, ICPL 84023 and ICPL 88009). Mishra and Shukla (1986b); Reddy *et al*. (1998) reported cultivation of resistant genotypes KPBR 79-1 and KPBR 5786 were the best in recording lessPhytophthora incidence in Pigeonpea.

Evaluation of wild species of Pigeonpea, indicated that a few accessions of *Cajanus platycarpus* (ICWP 61, ICWP 66 and ICWP 67) have shown high levels of resistance against prevailing isolates of *P. drechsleri* f. sp. *cajani* (Masood *et.al.* 2005). Pande *et al*. (2006) found varying levels of resistance among the improved lines and wild *Cajanus* species. They reported *C. sericeus* was highly resistant (≤ 10% plant mortality), while *C. scarabaeoides* was moderately resistant (≤ 20%) and *C. cajanifolius* was susceptible (≥ 40%) under natural Phytophthora blight epiphytic conditions at ICRISAT during the 2005 rainy season. Chaudhary and Dhar, 2008 evaluated 739 germplasm, breeding lines and selections of short, medium and long duration Pigeonpea in a sick field for five years revealed 20 selections as moderately resistant, while 51germplasm, breeding lines and selections were tolerant and remaining 668 lines were susceptible and none of the Pigeonpea line was resistant.

Pande and Sharma (2009) evaluated a large number of wild species of *Cajanus*, newly developed Pigeonpea lines and hybrids, vegetable type Pigeonpea germplasm

and minicore under natural infection conditions found that most of them succumb to *P. drechsleri*f. sp. *cajani* isolate with 40 % plant mortality.

Studies conducted in different Pigeonpea growing areas particularly in India, identified several sources of resistance to Phytophthora blight (Pal *et al.* 1970; Kannaiyan *et al.* 1980; Bhargava and Gupta, 1983; Singh and Chauhan, 1985; Mishra and Shukla, 1986b; Sharma *et al.* 1995; Reddy *et al.* 1990). However, most of lines identified as resistant by various researchers were later found susceptible to *P. drechsleri*f. sp. *cajani* under natural epidemic conditions in Deccan Plateau (Sharma *et al.* 2006).

Frequent evolution of new pathotypes and co-existence of more than one pathotype at a location has becomedifficult in developing resistant genotypes against Phytophthora blight. However, a few genotypes found resistant to Phytophthora blight are listed below.

Genotypes	**Remarks**	**Source**
ICPs 7916, 12055, 12114, 12161, 13126, 15511, 7265, 6523, 15530, 7719, 7889, 8914, 9189, 10002, 12029, 7925, 12034, 12068, 12142, 12799, 12842, 13103, 13162, 13229, 13241, 13271, 13438, 13599, 13613, 13828, 13852, 14104, 15142, 15452	Resistant in the field and greenhouse	Pande *et al.* 2011
HPL 24-47, ICP 11376-5, ICP 11975, ICP 12730, ICP 12751, ICP 12755, ICPL 20093, ICPL 20096, ICPL 20099, ICPL 20100, ICPL 20101, ICPL 20104, ICPL 20105, ICPL 20109, ICPL 20114, ICPL 20115, ICPL 20122, ICPL 20124, ICPL 20125, ICPL 20126, ICPL 20127, ICPL 20128, ICPL 20135, ICPL 20136, ICPL 93179, ICPL 99044, KPBR 80-2-1, KPL 96053, KPBR 80-2-2-1	Resistant under natural epiphytotic conditions	Pande *et al.* 2006

2.9.2. Chemical management

2.9.2.1. Effect of fungicides against growth of *Phytophthora* spp. under *in vitro*

Agarwal (1987) tested thirteen fungicides *in vitro* against *Phytophthora drechsleri*f. sp. *cajani*using poisoned food technique. Among them zineb, copper oxychloride, metalaxyl, thiram and captan inhibited mycelial growth completely even at 250 ppm concentration. Mancozeb checked the growth completely at 1000 ppm.

The effect of ridomil against *Phytophthora parasitica* var *nicotianae*was studied byReddy and Nagarajan (1980) andfound significant reduction in growth and sporulation of fungus at 0.1, 0.2, 0.3 and 0.4% concentration. Metalaxyl was found effective in inhibiting growth and sporangial formation of *Phytophthora* spp. (Sastry, 1982). Subramanyam (2009) reported that metalaxyl MZ at 250 ppm recorded 100 per cent inhibition of radial growth of *P. capsici.* Spraying and drenching with metalaxyl MZ @ 1.25 g/litre reduced the population of *P. capsici* in the soil and increased the survival of pepper vines.

*Phytophthora megasperma*f. sp. *glycinea*, *P. cinnamomi*, *P. nicotianae* and *P. palmivora* are very sensitive to metalaxyl (Coffey and Bower, 1984). Mycelial growth, sporangium formation, zoospore motility, and zoospore cyst germination of *Phytophthora capsici* was differentially affected by azoxystrobin, dimethomorph, fosetyl-Al, fluazinam, and metalaxyl (Matheron and Porchas, 2000).

2.9.2.2. Effect of fungicides on disease incidence caused by *Phytophthora* spp under *in vivo*.

Melalaxyl was found effective against *Phytophthora* spp. (Papavizas *et al*. 1979).Significant control of Phytophthora blight of Pigeonpea (>90%) was achieved with metalaxyl (1.75 g a.i kg^{-1} seed) in a greenhouse experiment (Chaube *et al.* 1984; Kannaiyan and Nene, 1984; Agarwal, 1987; Bisht and Nene, 1988).

Nene *et al*. (1979) evaluated seed dressing with ridomil at five concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 per cent and results showed that seedling blight did not occur with seed dressings of 0.4 and 0.5%. However, 15 days after the second inoculation, seedlings recorded an incidence of 10.4 and 6.8 per cent. These results clearly indicate that seed dressing with ridomil gave complete control of blight diseaseatleast up to 3 weeks.

A seed dressing formulation of metalaxyl was evaluated under greenhouse and field conditions for the control of Pigeonpea blight caused by *P. drechsleri*f. sp. *cajani* Significant control of blight (over 90 %) was achieved with metalaxyl (1.75 g a.i. /kg seed) in a greenhouse trial for 30 days after planting. However, the fungicide was found ineffective against the disease in field tests (Kannaiyan, and Nene 1984).

Agarwal, (1987) selected seven fungicides and tested against disease in sick plot, and found that metalaxyl when sprayed at 30^{th} and 45^{th} day after sowing showed maximum reduction in stem blight incidence in Pigeonpea. The incidence of Phytophthora blight of Pigeonpea can be reduced substantially with foliar sprays of

metalaxyl more so in the field resistant genotypes than in susceptible genotypes (Bisht and Nene, 1988).

Metalaxyl was reported to be effective fungicide against *Phytophthora parasitica* var. *nicotianae* causing black shank of tobacco (Vasilakakis *et al*. 1979) and *P.infestans* on tomato plants (Cohen *et al*. 1979). The systemic fungicide metalaxyl and Fosetyl Al both as foliar spray and soil drench were found effective against *Phytophthora capsici* in field conditions (Ramachandran and Sarma, 1985). Ramachandran *et al*. (1988) opined that under field conditions metalaxyl was highly effective in suppressing soil population of *Phytophthora palmivora*. Mefenoxam also provided excellent protection against development of stem and fruit lesions of fruit rot of pepper (Parra and Ristaino, 1998; Ristaino and Johnston, 1999).

Metalaxyl was efficient both *in vitro* and *in vivo* against *Phytophthora capsici* (Ramachandran and Sarma, 1989; Ramachandra *et al*. 1990b). Ramachandran and Sarma (1990) evaluated systemic fungicides *viz.* metalaxyl, fosetyl Al, oxadixyl and propamocarb for their bio-efficacy on different phases of *Phytophthora capsici,*metalaxyl, ziram and fosetyl Al were superior in reducing the disease. Fullerton and Tyson (2004) reported successful control of taro leaf blight with fungicides metalaxyl and phosphorus acid. Ridomil plus, Ridomil Gold were also effective in managing the taro blight disease caused by *Phytophthora colacasia* (Mbong *et al*. 2013)

2.9.3. Biological control

Biological control is eco-friendly and a good alternative for pesticides in sustainable agriculture to overcome the problems of public concern associated with pesticides and pathogens resistant to chemical pesticides (Akhtar and Siddiqui, 2008).

The development of pathogenic strains resistant to pesticides has now become a major problem throughout the world. This may be minimized by the use of biocontrol agents (Utkhede and Smith 1992). Use of biological control has emerged as an important alternative in managing soil borne plant diseases (Bowen and Rovira 1999; Whipps 1997).

Fungal bioagent*Trichoderma*was studied primarily for its ability to control plant disease through antagonism, rhizosphere competence, enzyme production, induction of defense response in plants, metabolism of germination stimulants, and beneficial growth of the host following root colonization (Weindling, 1932, 1934, 1937; Wright, 1956; Lindsey and Baker 1967; Elad, *et al*. 1981; Elad, *et al*. 1987; Benhamou, and

Chet, 1993; Zimand *et al*.1996; Bailey and Lumsden 1998; Gams and Bissett 1998; Washington *et al*. 1999; Howell, 2003).

Trichoderma spp. was found to be antagonistic to *Phytophthora parasitica* (Weindling, 1932) and several strains of *Phytophthora arecae* (Thomas, 1939). Nambiar and Sarma (1977) isolated *Trichoderma* spp. from roots of black pepper vines and noted that the lysis of mycelium of *Phytophthora capsici*was due to over growth of antagonist. *Trichoderma* spp. as biological agent against betel vine wilt was reviewed by Tiwari and Mehrotra (1968); root rot of avacado (Zentmeyer, 1963, 1967) and against many other Phytophthora diseases by Baker and Cook 1974.

Howell and Stipanovic (1983) isolated and described a new antibiotic, gliovirin, from *Trichoderma virens* that was inhibitory to *Pythium* spp.and a *Phytophthora*spp. Similarly, Washington *et al*. (1999), Smith *et al*. (1990) and Harman, (2000) reported antagonistic activities of *Trichoderma* spp. against *Phytophthora* spp. to reduce the diseases caused by them.

Trichoderma harzianum and *T. viride* have been used as biological control agents against diseases of black pepper caused by *Phytophthora capsici* both in the main field and in the nursery (Anandaraj and Sarma 1995). *Trichoderma* spp. were also used for the management of root rot of coorg mandarin caused by *Phytophthora* sp. (Sawant *et al*. 1995) and root rot caused by *Phytophthora capsici* in bell pepper (Ahmed *et al*. 1999).

Use of *T. hamatum* was found effective against Phytophthora foot rot of pepper (Anandaraj *et al*. 1995*).* Effectiveness of *T. harzianum* and other species in controlling soil borne pathogens has been reviewed by Chet *et al*. 1987. Both *T. viride* and *T. harzianum* overgrew and suppressed the growth of *P. capsici*(Subramanyam, 2009). Anith and Manomohandas (2001) reported that *Trichoderma harzianum* significantly reduced the incidence of *Phytophthora capsici* in black pepper. *T. viride* with FYM treatment effectively reduced the incidence of foot rot of black pepper (Kannan and Revathy, 2002).

Rajan *et al*. (2002) screened *Trichoderma* spp. both *in vitro* and *in vivo*and found effective in managing the foot rot disease of black pepper. *Trichoderma* spp. applications reduced soil populations of *P. cactorum* and reduced leather rot incidence (76.6%) compared with the untreated control (Porras *et al*. 2007).*T. viride* and *T. harzianum* inhibited the mycelial growth of the *P. drechsleri*f. sp. *cajani* with 65.9 and 62.5 % respectively (Srivastava and Mall, 2008)

Singh and Dubey, (2010) reported that *Pseudomonas fluorescens* and *Bacillus subtilis*, and *Trichoderma viride* and *Trichoderma hamatum* were effective against *P. drechsleri*f. sp. *cajani*under *invitro* . Mycelium of *P. drechsleri*f. sp. *cajani*was lysed by *P. fluorescens* and *B. subtilis*, whereas *Trichoderma* species overgrew in dual culture

Combined application of fungal and bacterial antagonists were more efficient in managing soil borne plant diseases (Duffy *et al*. 1996; Xhang *et al*. 1996; Nagtazaam *et al*. 1998). *Pseudomonas* spp. have been used extensively as biological control agents against many soil borne plant pathogens in several crops (Weller, 1988; Whipps, 1997).

Bacillus spp. and *Pseudomonas fluorescens* were used for controlling *Phytophthora* spp. (*Yan et al*. 2002; Thanh *et al*. 2009). *P. fluorescens* controlled Phytophthora blight of bell pepper under greenhouse and field conditions (Guo *et al*. 2009b). Liu *et al*. (2014) reported that *Bacillus subtilis* AR12, *Bacillus subtilis* SM21, and *Chryseobacterium sp*. R89—(BBC), could control mixed diseases, including Ralstonia wilt, Phytophthora blight and Meloidogyne root-knot on bell pepper with high biocontrol efficacy and yield increase under greenhouse and field conditions.

2.9.4. Compatibility of bioagents with chemical fungicides

Though the use of biocontrol agents offers an environmentally, economic and safer alternative for fungicides (Chet, 1987),they have not attained efficiencies matching those of currently available fungicides under all environmental conditions. Therefore, it is necessary to develop methods to enhance the efficiency of biocontrol agents and to attain consistency in their performance over a wide range of agroclimatic conditions.

Integrated management of disease with fungicides and biocontrol agents is possible only if they are compatible. Investigation were carried out by many workers (Papavizas, 1985; Sawant and Mukhopadhyay, 1991; Singh and Dubey,2010) to identify organisms resistant or tolerant to fungicides, to manipulate biocontrol agents genetically to develop tolerance to fungicides and to evaluate such altered organism in combination with fungicides for disease control under field conditions.

Trichoderma harzianum was tolerant to metalaxyl as exemplified by uninhibited growth, sporulation and spore germination up to 1000 ppm level of the fungicides (Sawant and Mukhopadhyay, 1991). Literature on successful management of disease in field by integration of biocontrol agents and fungicidal seed/ soil treatments is available

Pseudomonas fluorescens was found compatible with metalaxyl, mancozeb + metalaxyl, captan, thiram and carbendazim. *T. viride* and *T. hamatum* were compatible

at 0.6 and 0.3% of metalaxyl and 0.3% of mancozeb + metalaxyl, whereas, *B. subtilis* was compatible with metalaxyl only at 0.3%. Growth and sporulation of *Trichoderma* sp. and *T. viride* was compatible at 1000 ppm of metalaxyl and 100 ppm of mancozeb + metalaxyl. However, *T. hamatum* was sensitive at higher levels of treatment (Singh and Dubey,2010).

Materials and Methods

Chapter III

MATERIAL AND METHODS

Location of work

The present investigations were carried out in the laboratory, greenhouse and field during 2012-13 and 2013-14. All the laboratory and greenhouse experiments were conducted at Legumes Pathology, International Crops Research Institute for the Semi-Arid tropics (ICRISAT), Patancheru, Hyderabad,India whereas field experiments were conducted at ICRISAT, Patancheru, and Department of Plant Pathology, RAK College of Agriculture, Sehore, Madhya Pradesh.

Legumes Pathology, International Crops Research Institute for the semi-arid tropics, Patancheru is situated at 17.53^{o} North latitude, 78.27^{o} East longitude at an Altitude of 545 MSL, and RAK, College of Agriculture,Sehore is situated at 23.11^{o} North latitude, 77.03^{o} East longitude at an 502 MSL

3.1. Assessment of distribution of disease

A survey was conducted to record the occurrence and distribution of Phytophthora blight of Pigeonpea in 15 districts of the four major Pigeonpea growing states of India *viz.* Andhra Pradesh, Karnataka, Maharashtra, and Madhya Pradeshduring *kharif*2012 and 2013 (Fig.3.1).Periodical survey was also conducted in the fields of ICRISAT to document the incidenceof Phytophthora blight during *kharif*and *rabi*season2012 and 2013.

In each of the selected fields, five plots of 10m x 10m area were demarked randomly in a field of one acre. In each plot, observation on Phytophthora blight was made based on typical symptoms (Williams *et al*. 1975) and per cent disease incidence (PDI) was calculated based on total number of plants present and number of plants showing typical Phytophthora blightsymptoms in each plot. The average incidence of Phytophthora blight in each field was worked out after taking the per cent disease incidence values of all the five plots in one field.

$$\text{Per cent disease incidence} = \frac{\text{Total number of infected plants in each plot}}{\text{Total number of plants in each plot}} \times 100$$

$$\text{Average per cent disease incidence of field} = \frac{\text{Sum of the per cent incidence in individual plot}}{\text{Number of plots surveyed}}$$

$$\text{Average per cent disease incidence of a location} = \frac{\text{Sum of the average per cent incidence in individual fields}}{\text{Total number of fields surveyed}}$$

3.2. Effect of weather parameters in relation to incidence of the disease

An attempt was made to study the effect of weather factors in relation to per cent disease incidence. The weather parameters of ICRISAT location was correlated to periodical incidence of the disease at same location by using the Karl Pearson's correlation coefficient (r) as given below.

$$r = \frac{\sum_{i=1}^{n} (X_i - \overline{X})(Y_i - \overline{X})}{\sqrt[nn]{\sum_{i=1} (X_i - \overline{X})^2 - \sum_{i=1} (Y_i - \overline{Y})^2}}$$

Where,

X and Y are two variables

$\overline{X}$: Mean of X

$\overline{Y}$: Mean of Y

r : Karl Pearson's correlation coefficient

Further, the data were subjected to multiple linear regression analysis to find out thelinearity of the independent variables for prediction.

3.2.1. Disease prediction models

The weekly disease severity was graphically analyzed for estimation of diseasedevelopment and to predict the intensity. The 2nddegree polynomial model was used to estimate the disease progression.

$$Yx = a + b_1X + b_2 X^2 + b_3X^3$$

Where,

Yx : Expected disease severity at time x

X: Time interval in days

a : Intercept

b_i : Coefficient

Where, aand bi are intercept and regression coefficients, respectively for Yxindicated expected disease severity at time x of seven days interval (Cox and Hinkley, 1979; Snedecor and Cochran, 1994).

3.3. Collection of infected samples

Pigeonpea plants showing the typical Phytophthora blight symptoms were collected, packed in labeled polythene bags and brought to the laboratory for isolation of the pathogen.

3.4. Isolation of*Phytophthoradrechsleri*f. sp. *cajani*on different media

The plants depicting typical symptoms of Phytophthorablight were selected from field during the survey and brought to the laboratory. Isolation of fungus was done according to tissue segment method (Rangaswamy, 1958). Stem bits consisting of 50 per cent infected and 50 per cent healthy were surface sterilized using 1 per cent sodium hypochlorite (NaOCl) for 60 seconds and then washed in sterile water thrice. The stem bits were blot dried and plated on petriplate containing different media *viz.* oat meal agar, corn meal agar, V-8 juice agar, Pigeonpea seed meal agar, potato dextrose agar, tomato juice agar and V8 PARP (Pimaricin, Ampicillin, Refampcilin and PCNB) medium,(Composition of medium is given in Appendix-1)and incubated at30^{o}Cand the plates were observed regularly for the growth of the pathogen. Confirmation of pathogen was done by cultural and morphological characteristics as described by Gupta *et al.* 1997.

3.4.1. Identification and maintenance of*Phytophthoradrechsleri*f. sp. *cajani*

The stem bits were surface sterilizedand then washed in sterile water thrice, blot dried and plated on petriplate containing V8 PARP (Pimaricin, Ampicillin, Refampcilin and PCNB) medium, and incubated at 30 ^{o}C for three days. The initial growth of fungus was subcultured on new plate containing V8 Juice agar medium and allowed to grow for 7 days. A total of two isolates were obtained from ICRISAT fields.

Pure culture of the fungus was obtained by hyphal tip culture method (Tutte, 1969). The upper portion of a 6 mm plug of actively growing mycelium from 7 day old culture was suspended into a 1.5 ml microfuge tube containing 1 ml of sterile distilled water (SDW). Three to four drops of suspension was transferred to the surface of a water agar plate and uniformly spread with sterile glass spreaders. This technique

forced the single hyphal bit to ramify through the water agar. After 24 hrs, plates were inspected under a dissecting microscope at 10x magnification and a single hyphal branch was transferred to V8 juice agar plates.

The pathogen was identified based on cultural and morphological characteristics and confirmed by comparing the morphological features, growth patterns and morphological features of sporangia, oogonia, antheridia and hyphal swellings with species descriptions reported in literature (Gupta *et al.* 1997). Morphology of the fungus was observed under Olympus CX41 phase contrast microscope with Q image micropublisher 5.0 RTV digital camera. On confirming the pathogen, the fungus wassubcultured and maintained on V8 juice agar at 15°C for 2-3 weeks. Virulence of the pathogen was maintained by transferring the pathogen through host after every 30 days.

3.5. Establishing the pathogenicity

Pathogenicity of both isolates was established by proving the Koch's postulates on the highly susceptible cultivar ICP 7119 under greenhouse conditions.

3.5.1. Raising of seedlings

Susceptible cultivar (ICP 7119) of Pigeonpea was grown in the plastic pots (12 cm) filled with a mixture of sterilizedalfisol comprising of 60% sand, 33% clay and 7% silt (Kannaiyan*et al*. 1981) kept in a greenhouse maintained at 28-30 ^{o}C for 10 days. Before sowing, seeds are surface sterilized using 2% sodium hypochlorite for 2 minutes, and rinsed in sterile water in order to wash off sodium hypochlorite.

3.5.2. Inoculum preparation

Mass multiplication was done by transferring fungal disc of 6 mm diameter to 100 ml sterilized V8 juice broth in 250 ml of flasks and incubated at 30 ^{o}C with 12 hours of alternate light (2000 Lux) and dark condition for 2 weeks.

3.5.3. Inoculation

Inoculation of fungus was done by soil drenching method of inoculation (Kannaiyan*et al*. 1981).The fungus was macerated (mycelial mat + broth) in a blender for 1-2 min by operating the blender intermittently, and the suspension was diluted to get a final volume of 100 ml from each flask. Ten day old seedlings were inoculated by pouring 100 ml of inoculum around the base of the seedlings in a pot, and the pots were watered 3-4 times at 2-3 h intervals daily.Initial symptoms of Phytophthora blightwere observed after 24 hours after inoculation.

Each pot containing 10 seedlings was considered as one replication and was replicated thrice.

3.5.4. Disease assessment

Disease was assessed according to per cent disease incidence at the beginning of the incubation period and upto 7 days after inoculation. Per cent disease incidence (PDI) was calculated as given below

$$\text{Per cent disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants}} \times 100$$

The organism was reisolated from the artificially inoculated Pigeonpea plant showing typical Phytophthora blight symptoms and the culture thus obtained was compared with the original culture for morphology and colony characters for confirmation.

To evaluate the virulence of the two isolates, per cent disease incidence was calculated according to the formula of per cent disease incidence and virulent isolate was used throughout the study.

3.6. Physiological characterization of *P. drechsleri*f. sp. *cajani*

Experiments were conducted to determine suitable media, temperature, relative humidity and CO_2 for growth and sporulation of *P. drechsleri*f. sp. *cajani*.

3.6.1. Influence of different media on growth of *P. drechsleri*f. sp. *cajani.*

Twelve solid media *viz.* oat meal agar, Pigeonpea sand flour agar, corn meal agar, tomato juice agar, potato dextrose agar, chickpea seed agar, czapek-dox agar,carrot agar, potato sucrose agar, potato glucose agar and water agar, and three liquid media *viz.*potato dextrose broth, tomato juice broth and V8 juice broth were used for observing the growth of the fungus (Composition of solid and liquid medium are given in Appendix-1).The media and broth were prepared according to the manufacturer instructions (HiMedia, India) and compositions (Dhingra and Sinclair, 1995). All the media and broth were sterilized at 1.1 kg/cm^2 pressure and 121.5 °C for 20 min. Each Petri dish was poured with 20 ml sterilized medium whereas 100 ml sterilized broth was poured in 250 ml conical flask.Discs of a 6 mm diameter of *P. drechsleri*f. sp. *cajani*isolate (Pdc-1) were taken from7dayold pre-cultured petridishes on V8 juice agar with the help of a cork borer and inoculated to petridishes and conical flaskscontaining different media and broth. After inoculation, petridishes andconical

flasks were incubated at 30 °C (Mishra*et al*. 2010). The diameter of pathogen was recorded on solid medium in millimeters in two directions at right angles to each other. In liquid broth, mycelial growth of *P. drechsleri*f. sp. *cajani*was harvested in pre weighed moistureless Whatmanfilter paper No. 42, oven dried at 60 °C and weighed again to record mycelial growth in milligrams. The mycelial growth and average colony diameterwas recordedand calculated as described by Keith and Phillip (1971). The growth was measured at an interval of 24 hours, until the plate was covered completely. The sporulation of the pathogen on different media was studied at an interval of 5 daysupto 20 days.

3.6.2. Effect of temperature on growth and sporulation of *P. drechsleri*f. sp. *cajani*on solid and liquid media

An experiment was conducted to know the optimum temperature for growth of *P. drechsleri*f. sp. *cajani*at a temperature range of *viz*. 5, 10, 15, 20, 25, 30, 35 or 40 °C using the tomato juice agar medium and V8 juice broth as described by (Mishra*et al*. 2010). Seven day old culture was inoculated in petriplate or conical flask and incubated at different temperatures. The experiment was replicated thrice for each temperature. Observations on radial growth in diameter, mycelial weight and sporulation of *P. drechsleri*f. sp. *cajani*were recorded as explained in earlier section 3.6.1.

3.6.3. Influence of relative humidity on growth and sporulation of *P. drechsleri*f. sp. *cajani*

The influence of relative humidity on growth of pathogen was assessed in tomato juice agar and V8 broth at a relative humidity of *viz*. 50, 60, 70, 80, 85, 90, 95 and 100 per cent. The inoculation, incubation, replications, observation and other growth conditions were same as explained in earlier section 3.6.1.

3.6.4. Influence of CO_2 on growth and sporulation of *P. drechsleri*f. sp. *cajani*

The mycelial growth and sporulation of the test pathogen as influenced by different levels of $CO_2$was assessed using tomato juice agar medium and V8 juice broth. The different levels of CO_2*viz.* 350, 550 and 700 ppm were adjusted in incubators and the experiment was set up as mentioned in section 3.6.1.

3.6.5. Induction of sporangia and zoospores of *P. drechsleri*f. sp. *cajani*

Production of sporangia and zoosporesof*P. drechsleri*f. sp. *cajani*were not found irrespective of media and range of temperature and relative humidity used. Hence, to induce the sporangia and zoospores various protocols proposed by different research workers for *Phytophthora* spp. were tested with the following methods.

S. No.	Method	Reference
1	KNO_3 solution	Schiffman-Nadel and Cohen (1968)
2	Sun flower, Pea, Corn and Soybean seed media	Satour and Butler(1968)
3	Frozen pea medium	Dance *et al*. (1975)
4	Flooding on cultures	Ribeira and Baumer, (1973)
5	V8 broth	Kannaiyan *et al*. (1992)
6	Rape seed extract agar	Satour and Butler, (1968)

The above methods did not produce the sporangia and zoospores and a modified method of V8 brothdeveloped in our laboratory resulted in abundant sporangia and zoospores production as described below.

3.6.5.1. Protocol for induction of sporangia and zoospores using diluted tomato juice broth

Five day old culture of *P. drechsleri* f. sp. *cajani* (Plate3.1a) was used for induction of sporangia and zoospores. Mycelial disc of 6 mm diameter from five day old culture of *P. drechsleri* f. sp. *cajani* grown on tomato juice agar medium was cut using a cork borer (Plate3.1b) and transferred to each petri platecontaining 15 ml of diluted tomato juice broth (Plate3.1c). The broth was decanted, washed the mycelial bits with sterile distilled water and 15 ml of sterile distilled water was added (Plate3.1d). Induction of sporangia was initiated after 12 hours and abundant production was observed within 24-36 hours primarily towards the margin of the colony (Plate 3.1e) and zoospores were released within 12 hours.About 100-150 zoospores are released from each sporangia (Plate 3.1f: 3.1g).The sequence of release of zoospores from zoosporangia was enumerated (Plate 3.2).The homothallic nature of *P. drechsleri* f. sp. *cajani* was observed with the formation of male and female gametangia called oogonium and antheridium (Plate3.1h) and formation of oospores through amphigynous sexual reproduction (Plate 3.1i). To determine the zoospore load, one drop of spore suspension was placed on a haemocytometer and the number of

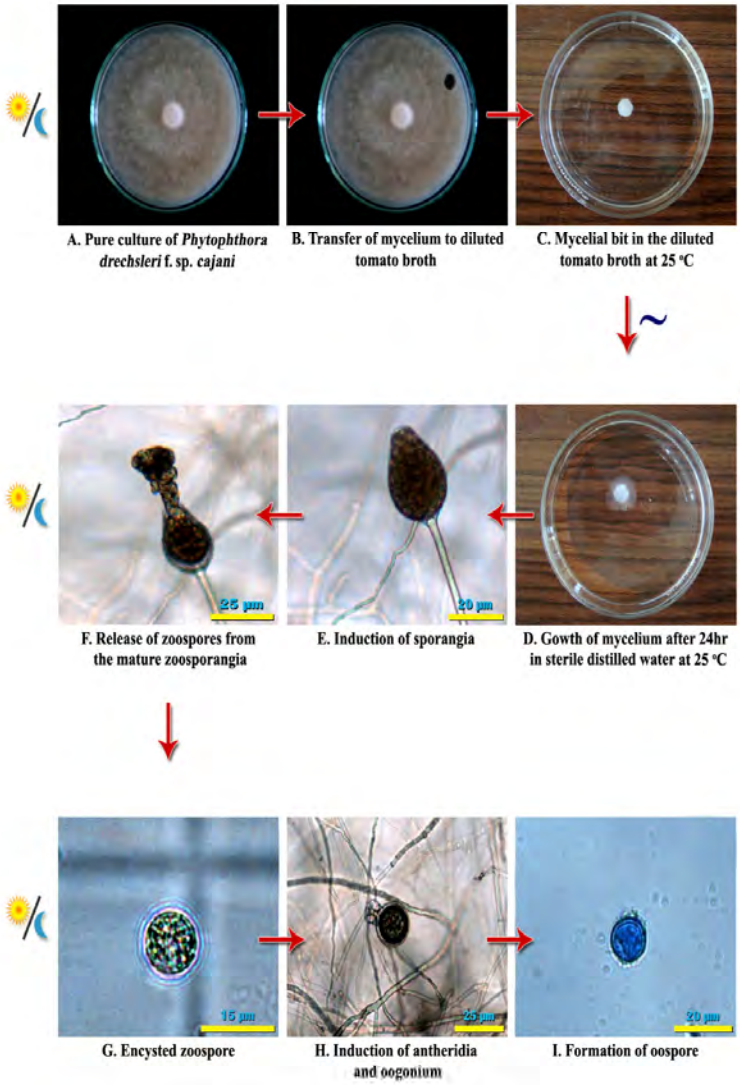


Plate 3.1. Induction of sporangia and zoospores in *Phytophthora drechsleri* f. sp. *cajani* on diluted tomato juice broth.

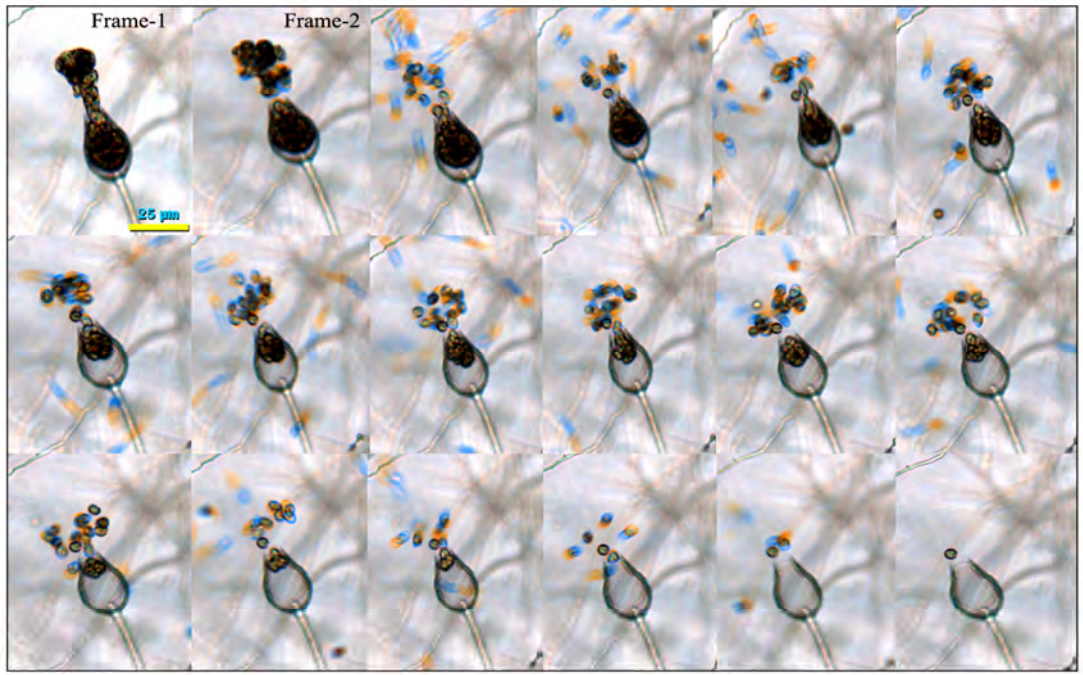


Plate 3.2. Sequence of zoospore release from sporangium of *Phytophthora drechsleri* f. sp. *cajani*.

Note: In frame 1 the emitted zoospores are retained in the membranous sac.
In frame 2 the membranous sac was broken and zoospores were released.
Bar=25µm.

zoospores was counted in 5 squares. The number of zoospores per ml was calculated with a haemocytometer, using the formula given by Pathak (1984) as follows

$$\text{Number of spores per ml} = \frac{N \times 1000}{X}$$

Where:

N = Total No. of spores counted/No. of squares,

X = Volume of mounting solution between the cover glass and above the squares counted.

3.6.5.2. Isolation of single zoospore and proving its pathogenicity

The isolation of singlezoospores was done using the method of Thakur*et al*. (1998) with diluted tomato juice method. The fresh zoospores were harvested from water suspension and diluted (1 to 2 zoospores/field of microscope) suspension (0.5 ml) was spread uniformly over the surface of sterile water agar medium (1%) in petri plates and the excess suspension was drained off. Single, well-isolated zoospores were marked on water agar using a dummy objective under the microscope (10×). Single zoospores were picked up with the help of a flat-tipped needle and transferred onto the petriplate containing tomato juice agar and the growth was observed in petriplate. Further, pathogenicity of suspension (1000 zoospore/ml) of 100 ml was tested using a susceptible Pigeonpea variety ICP 7119 at 10 day old seedling under greenhouse conditions. The growth condition for seedling and observation, replications remained same as in earlier section 3.5.

3.6.6. Influence of temperature, relative humidity and carbon dioxide on production of zoospores in diluted tomato juice broth

An experiment was conducted to determine the effect of various range of temperatures (20, 25, 30, 35, 40 °C), relative humidity (50, 75, 85, 95, 100%) and CO_2 (350, 550, 700 ppm) for induction of zoospores using the diluted tomato juice broth method. The range of different temperature, relative humidity and CO_2levels was maintained constant in each incubator and the experimental procedure and observation was followed as explained in section 3.6.5.1.

3.7. Study the epidemiology of Phytophthora blight of Pigeonpea

3.7.1. Influence of temperature on disease development

The effect of two inoculation methods *i.e.* soil mixing and soil drenching on Phytophthora blight development was assessed at different range of temperatures on susceptible variety ICP 7119. Soil mixing method of inoculation was done as described by Pande *et al*. (2012), where inoculum multiplied by transferring 6 mm disc growth of *P. drechsleri* f. sp. *cajani*to 100 gram of sterilized Pigeonpea sand flour medium in 250 ml flasks and incubated at 30 ^{o}C with 12 hours of alternate light (2000 Lux) and dark condition for 2 weeks. Hundred grams of inoculum multiplied on Pigeonpea sand flour medium was directly mixed into the soil without disturbing the roots of the seedling on 10 day old seedlings in the pot. Pots were watered 3-4 times at 2-3 h intervals daily to create adequate soil moisture. Soil drenching method of inoculation, raisingof seedlings, and replications remained same as mentioned in section 3.5. The inoculated pots were incubated at 5, 10, 15, 20, 25, 30, 35, or 40 °C (14/10 hours of day/night photoperiod and 2000 Lux of light intensity and 80 per cent relative humidity) (Granke and Hausbeck, 2010b). The incidence disease was recorded in each treatment separately at 24 hours interval.

3.7.2. Effect of relative humidity on disease development

The influence of relative humidity on disease development was studied as described by Granke and Hausbeck, 2010b using the susceptible variety ICP 7119 and soil drenching and soil mixing method of inoculation with relative humidity *viz.* 50, 60, 70, 80, 85, 90, 95 and 100 per cent was used in the present experiment. The growth conditions, multiplication of inoculum, method of inoculation, replications and observation of disease were same as explained in section 3.5 and 3.7.1.

3.7.3. Effect of CO_2on disease development

Effect of elevated CO_2 on incidence of disease was determined by using the susceptible variety ICP 7119. This experiment was conducted in incubators and Open Top Chambers (OTC) separately. Three levels of CO_2 was maintained comprising of an ambient (350 ppm) and two elevated levels (550 and 700 ppm). The growth conditions, multiplication of inoculum, method of inoculation, replications and observation of disease were same as explained in section 3.5 and 3.7.1.

3.7.4. Effect of soil type

The effect of soil type on development of disease was conducted by sowing susceptible variety ICP7119 in black clay and red loamy soil under pot conditions and incubated in greenhouse. The set of experimental details were same as mentioned in 3.5 and 3.7.1.

3.7.5. Effect of soil moisture

An experiment was conducted with soil moisture regimes, *viz*. 60 per cent, 80 per cent, 100 per cent moisture at field capacity and flooding to study the development of disease under controlled conditions using susceptible Pigeonpea cultivar ICP7119. Uninoculated control was maintained for each soil moisture level. Deionized water was used for maintaining the soil moisture content in each treatment. Multiplication of inoculum, inoculation methods, growth conditions and replications were same as explained in section 3.5 and 3.7.1.

The soil moisture content (SMC) was determined as per the procedure of Sharma and Pande, (2013) using the gravimetric method on oven-dry basis. The soil sample was saturated and later soil moisture was removed by oven drying (100–110°C) until the weight remains constant. The samples were removed from the oven and brought to room temperature and weighed again. The difference in weight was considered as amount of moisture in the soil. The available SMC in the soil was calculated by the following formula

$$\text{Soil moisture content (\%)} = \frac{\text{Saturated soil weight} - \text{Oven dry soil weight}}{\text{Oven dry soil weight}} \times 100$$

The four levels of SMC (60%, 80%, 100% and flooding) was adjusted by maintaining the constant weight by regular weighing and replacing the moisture deficit in each pot by watering (Suriachandraselvan and Seetharaman, 2003).

3.7.6. Effect of flooding

To know the role of pre and post flooding on disease development, an experiment was conducted using susceptible variety ICP 7119. The treatments were imposed as described by Barta, (1986) and mentioned below

T1- Pre inoculation flooding for two days

T2- Pre inoculation flooding for four days

T3- Post inoculation flooding for 1 day

T4- Post inoculation flooding for 2 days

T5- Post inoculation flooding for 3 days

T6- Post inoculation flooding for 4 days

T7- Post inoculation flooding for 5 days

T8- Flooding uninoculated and

T9- Nonflooding

Each pot containing 10 seedlings constituted one replication and the experiment was replicated thrice.Growing of seedlings and inoculum multiplication and method of inoculation were same as explained in section 3.5 and 3.7.1.The data on per cent disease incidence was calculated.

3.8. Integrated disease management for Phytophthora blight of Pigeonpea

3.8.1. Host plant resistance

3.8.1.1. Influence of inoculation techniques on disease development

An experiment was conducted to identifythe inoculation technique forestablishment and development of Phytophthora blight in Pigeonpea by the following inoculation methods.

i. Spray inoculation of mycelial suspension

ii. Soil drenching with mycelial suspension

iii. Soil mixing with mycelial inoculum

iv. Stem inoculation with mycelial inoculums and

v. Stem staging with mycelial bits

Two isolates of *P. drechsleri*f. sp. *cajani*(Pdc-1 and Pdc-2) were used in all inoculation techniques on highly susceptible variety ICP 7119. Growth conditions, replication and disease assessment was same as in section 3.5.

i. Spray inoculation of mycelial suspension

Seven day old culture disc of 6 mm diameter of actively expanding mycelium of *P. drechsleri*f. sp. *cajani*isolates were transferred to 100ml of autoclaved V8 broth in 250 ml flasks and incubated for 2 weeks at 30^{o}C. The mycelial mats were then removed and macerated with 100 ml of sterile distilled water. Mycelialsuspension was sprayed on 10 day old Pigeonpea seedlings with an automizer @ 100 ml/ pot. An uninoculated control was also maintained by spraying with sterile distilled water (Nene *et. al*. 1981).

ii. Soil drenching with mycelial suspension

Ten day old seedlings were soil drenched with 100 ml mycelial suspension per pot. The mycelial suspension was prepared as explained earlier and incubated in greenhousefor 7 days at 25 ± 2^{o}C. The pots were watered 3 times a day to maintain the adequate moisture for disease development(Kanniyan *et. al*. 1981).

iii. Soil mixing with mycelial inoculums

a. Inoculum preparation

Inoculum preparation and inoculation was done as described by Pande *et al*. (2012). Pure culture of *P. drechsleri*f. sp. *cajani*was isolated from Pigeonpea plants infected with Phytophthora blight on specific medium V8 PARP (Pimaricin, Ampicillin, Rifampicin, Pentachloronitro benzene). *P. drechsleri*f. sp. *cajani*was subcultured on sterilized tomato juice agar by pouring20 ml medium into each petri dishes and incubated at 30 ^{o}C for 1 week. Mass multiplication was done by transferring 6 mm discs of the fungus growth to 100 gram of sterilized Pigeonpea sand flour medium in 250 ml flasks and incubated at 30 ^{o}C with 12 hours of alternate light (2000 Lux) and dark condition for 2 weeks. The medium was sterilized at 121.5 ^{o}C for 20 minutes.

b. Inoculation

Hundred grams of inoculum multiplied on Pigeonpea sand flour medium was directly mixed into the soil without disturbing the roots of the seedling on 10 day old seedlings in the pot. Pots were watered 3-4 times at 2-3 h intervals daily to create adequate soil moisture.

iv. Stem inoculation with mycelial inoculums

Multiplication of inoculum was done in Pigeonpea sand flour medium as explained earlier. Carborandum powder wasrubbed on the collar region (base) of 15 day old plants of Pigeonpea (ICP 7119) and inoculated with 2 grams of inoculum and incubated in greenhouse. Control plants weremaintained by mock inoculation.

v. Stem staging with mycelial bits

The base of the stem of Pigeonpea seedlings (15 day) were slightly injured with scalpel and the mycelia growth of 10 mm of 7 day old culture was staged with cellophane tape and incubated. Control plants were subjected to the same treatment but without mycelium.

3.8.1.2. Standardization of inoculum density

Among the inoculation techniques tested, the best inoculation method was selected to quantify the optimum inoculum density to establish the disease. The pathogen was mass multiplied on both Pigeonpea sand flour medium andV8 broth media as explained in section 3.5. and3.7.1.Each pot containing 1 kg of pot mixture was inoculated using soil mixing method and soil drenching method separately at the rate of 1, 2.5, 5, 7.5, 10 and 12.5 per cent of soil weight. Pots were maintained in greenhouse as explained in section 3.5.The disease incidence was estimated in each treatment at different days of inoculation and per cent disease incidence was calculated.

3.8.1.3. Influence of plant age on disease development

The plant age and its susceptibility to disease was determined by inoculating the highly susceptible variety ICP 7119 as described by Sarkar*et al*. (1992). Sowing of ICP 7119 was taken at different days to obtain plants of all stages *viz*. 10, 20, 25, 35, 45, 60, 75, 90, 105 and 120 at one time and inoculation through soil mixing and soil drenching method was done at a time and data on disease incidence was calculated at different days after inoculation. Multiplication of inoculum, inoculation method, growth conditions and replications are same as explained in section 3.5. and 3.7.1.

3.8.1.4. Screening of Pigeonpea genotypes against *P. drechsleri*f. sp. *cajani*

NineteenPigeonpeagenotypesand43 Pigeonpea Wilt and Sterility Mosaic Disease Nursery (PWSMDN) lines were screened in field condition at ICRISAT, Patancheru and Sehore, Madhya Pradesh respectively.The components and procedures of the field screening of Pigeonpea genotypes for Phytophthora blight resistance method was adopted as described by Nene *et al*. (1981). The genotypes and PWSMDN lines were planted at 30 cm distance in a row and susceptible cultivar ICP 7119 was planted after every two rows. The collar region (base) of 1-month-old plants were inoculated with mycelialinoculum grown on Pigeonpea seed flour medium after mixing with carborandum powder. The field was flooded immediately after inoculation and frequently irrigated twice a week, if dry weather prevailed. Typical blight symptoms appeared in about 10 days. Plants which did not show symptoms were reinoculated after one month. The percentage of blighted seedlings was calculated based on number of infected plants to total number of plants (Chauhan*et al.* 2002). Based on disease incidence the lines were categorized as per the scale described by Reddy and Jain (1989) where

Per cent disease incidence (%)	Category
0-10	Resistant
11-20	Moderately Resistant
21-50	Moderately Susceptible
51-80	Susceptible
81-100	Highly Susceptible

Pigeonpea genotypes screened under field condition at ICRISAT, Patancheru, Hyderabad.

S. No.	Genotype
1	ICPL 288
2	ICP 4135
3	ICP 9174
4	ICP 87119
5	ICP 8863
6	JA-4
7	ICP 11302
8	ICP 580
9	ICP 11290
10	BDN 2
11	ICPL 161
12	Bahar
13	KPBR 80-2-1
14	ICP 113
15	ICP 11304
16	UPAS 120
17	ICP 339
18	ICP 2376
19	ICP 7119

Pigeonpea wilt and sterility mosaic disease nursery lines screened in sick plot at Sehore, Madhya Pradesh.

S. No.	Nursery lines
1	ICP 11376
2	ICP 12012
3	ICP 12728
4	ICP 12739
5	ICP 12752
6	ICP 13361

7	ICPL 94062
8	ICPL 20095
9	ICPL 20119
10	ICPL 20123
11	ICPL 20124
12	ICPL 20135
13	ICPL 20136
14	ICPL 20137
15	ICPL 87051
16	ICPL 90011
17	ICPL 96053
18	ICPL 96061
19	ICPL 99008
20	ICPL 99009
21	ICPL 99044
22	ICPL 99048
23	ICPL 99055
24	ICPL 99091
25	ICPL 99095
26	ICPL 99098
27	ICPL 99099
28	ICPL 99100
29	ICP 2376
30	ICP 8863
31	ICP 7119
32	BDN 2
33	ICP 9174
34	ICPL 87119
35	KPBR-80-2-1
36	Bahar
37	ICP 113
38	ICP 4135
39	ICP 11290
40	ICP 11302
41	ICPL 161
42	ICP 580
43	ICP 339

3.8.2. Chemical management

3.8.2.1. *In vitro* efficacy of fungicides against the growth of *P. drechsleri*f. sp. *cajani*

The efficacy of metalaxyl and mefenoxam at different concentrations *viz*.0, 0.10, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0, 5.0, and 10 µg/ml of active ingredient were tested against *P. drechsleri*f. sp. *cajani*by Poisoned food technique (Grover and Moore, 1962). The required concentrations of chemicals were prepared and incorporated into sterilized, cooled tomato juice agar. Twenty ml of cooled medium was poured into 90 mm sterilized petridishes and all the plates were inoculated with actively growing mycelial disc of 6mm diameter *P. drechsleri*f. sp. *cajani.* The experiment was replicated thrice. These plates were incubated at 30 ^{o}C for seven days, and colony diameter was recorded in two directions for each individual plate and averaged. Per cent inhibition of mycelial growth over control (PIOC) was calculated by using the formula of Vincent (1947).

$$PIOC = \frac{(C- T)}{C} \times 100$$

PIOC = Per cent inhibition of mycelium over control

C= Growth of mycelium in control

T = Growth of mycelium in fungicidal treatment

3.8.2.2. Efficacy of fungicides against the induction of zoospores using diluted tomato broth method under *in vitro* condition

Two fungicides mefenoxam and metalaxyl were used to study their efficacy on the induction of zoospore using diluted tomato broth method. Mycelial plugs of 6 mm diameter were cut from actively growing margins of the seven day old culture and transferred into the petriplate containing diluted tomato juice and the inoculated plates were incubated under alternate light and dark condition at 25°C for 24 hours. Later the broth was replaced by the sterile distilled water previously amended with mefenoxam or metalaxyl at 0, 0.10, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0, 5.0, 10 µg/ml and the plates were incubated under alternate light and dark conditions at 25 °C for 24 hours. The number of zoospores induced were calculated using haemocytometer and calculated by the formula given by Pathak (1984) and per cent reduction of zoospore production was calculated by using the formula of Vincent (1947).

$$PIOC = \frac{(C- T)}{C} \times 100$$

PIOC = Per cent reduction of zoospores production over control

C= Number of zoospores in control

T = Number of zoospores in fungicidal treatment

3.8.3. Biological control

3.8.3.1.Isolation of fungal antagonistic microorganisms from soil

Soil samples collected from root zone of healthy Pigeonpea plants at ICRISAT fields were pooled and representative samples were drawn. The soil samples were subjected to serial dilution technique as described below.

Ten grams of soil was transferred aseptically into a 250 ml conical flask, containing 90 ml of sterile distilled water and the contents were mixed thoroughly by shaking for five minutes. Ten ml of the aliquot was drawn and transferred to 90 ml sterile distilled water. The suspension was shaken for one minute, and diluted to 10^{-2} and $10^{-4}$and further used for isolation of fungal bioagents. Twenty ml of molten (40°C) *Trichoderma*specific medium(Elad and Chet, 1983) was poured in a series of Petri plates. One ml suspension from respective dilution was transferred aseptically into Petri plates containing the medium separately. The plates were rotated manually for uniform distribution of the suspension in medium and allowed to solidify. The plates were incubated at 30 °C for seven days and observed for development of fungal colonies. The colonies with characteristic growth of *Trichoderma*spp. were observed under the microscope and confirmed with the morphological characters as described by Rifai (1969). The isolates of the bioagents were designated as below.

S. No.	**Source of isolate (Field Number)**	**Designation**
1	BP-15	*Trichoderma*isolate-1
2	RL-17	*Trichoderma*isolate-2
3	BP-5	*Trichoderma*isolate-3
4	BP-14a	*Trichoderma*isolate-4
5	DHF-04	*Trichoderma*isolate-5

3.8.3.2.Isolation of bacterial antagonistic microorganisms from soil

The soil adhering to the healthy root systems of Pigeonpea were washed off thoroughly. Roots were cut from the tip into 2-3 cm bits. The root segments were further washed in distilled water and blotted to remove the moisture. One gram of the root material was transferred to 100 ml sterile water blank in a 250 ml conical flask and

shaken for 20 minutes at 250 rpm in a rotary shaker to dislodge bacteria adhering to the root surface. Similarly, one gram of rhizosphere soil was mixed thoroughly in 100 ml sterile water and subjected to serial dilution agar plate technique (Aneja, 2002). Suitable dilutions (10^{-5} and 10^{-6}) of rhizosphere soil were plated on King's B medium (King *et al*. 1954). The plates were incubated at room temperature (28±2°C) for 24-48 h (Aneja, 2002).

Colonies on petri plates were observed under UV light on a transilluminator. The colonies fluorescing under UV light were picked up, purified and preserved at -80°C with 50 per cent glycerol. The isolates of the bioagents were designated as follows.

S. No.	Source of isolate (Field Number)	Designation
1	DHF-04	*Pseudomonas*isolate-1
2	BP-14a	*Pseudomonas* isolate-2
3	BP-15	*Pseudomonas* isolate-3
4	RL-17	*Pseudomonas* isolate-4

3.8.3.3. Evaluation of antagonists against *P. drechsleri*f. sp. *cajani*under *in vitro* conditions

The antagonistic activity of *Trichoderma* isolates was tested on tomato juice agar medium against *P. drechsleri* f. sp. *cajani*(Singh and Dubey, 2010). A culture disc of 6 mm diameter of 6 dayold fungal antagonist was placed 1 cm away from the periphery of a petri plate on solidified sterilized medium. The *P. drechsleri*f. sp. *cajani*of 6dayold culture discs of 6 mm were inoculated at exactly the opposite side of fungal antagonist of the same plate by leaving 1 cm gap. Similarly antagonistic activity of *Pseudomonas* isolates was tested on tomato juice agar medium against *P. drechsleri* f. sp. *cajani*(Gupta *et al*. 2001). A culture disc of 6 mm diameter of 6 dayold culture of test pathogen was placed in the centre of plates containing tomato juice agar medium. A loopful 24 hour of old culture of *Pseudomonas*isolate was then streak inoculated at either sides of pathogen disc at a distance of 2 cm apart. The fungal pathogen culture inoculated centrally on petri platesand uninoculated by *Pseudomonas*isolate served as control.The inoculated Petri plates were incubated at 27 ± 2 ^{o}C for seven days. Each treatment was replicated four times. The growth of the test pathogen was recorded. Per cent inhibition over control was calculated using the formula given by Vincent (1947).

$$PIOC = \frac{(C-T)}{C} \times 100$$

PIOC = Per cent inhibition of mycelium over control.

C= Growth of mycelium in control.

T = Growth of mycelium in treatment.

3.8.3.4. Mass multiplication of *Trichodermasp*. culture for field application

*Trichoderma*isolate-3 was multiplied on molasses yeastmedium (molasses 30 ml + yeast 5 g + water 100 ml) prepared in conical flasks and sterilized at 1.1kg/cm^2 for 20 min. Culture of *Trichoderma*isolate-3 was inoculated in different flasks by taking a fungal discfrom seven day old culture and incubated for ten days (Raguchander*et al*. 1997).

The fungal biomass and broth were mixed with talc powder at 1:2 ratio. The mixture was airdried and mixed with carboxy methyl cellulose (CMC) @ 5g/kg of the product and then packed in polythene bags and used in seed treatment for managing the disease.

3.8.4. Compatibility of*Trichoderma*isolate-3 against metalaxyl and mefenoxam

The compatibility of metalaxyl and mefenoxam was tested with*Trichoderma*isolate-3 at 0, 500, 1000, 2000, 3000 and 4000µg/ml concentrations by poisoned food technique. The sterilized and cooled tomato juice agar was incorporated with required concentration of fungicides. This medium was poured into sterile petriplates and allowed to solidify. Actively growing 6 mm culture disc of *Trichoderma*isolate-3was inoculated in the center of petriplate under aseptic condition. Each treatment was replicated thrice. The plates were incubated at room temperature 27 ± 1 ^{o}C till control plates were completely covered with mycelium. Inhibition of mycelial growth of *Trichoderma*isolate-3over control was calculated using formula given by Vincent (1947).

$$PIOC = \frac{(C- T)}{C} \times 100$$

PIOC = Per cent inhibition of mycelium over control

C= Growth of mycelium in control

T = Growth of mycelium in treatment

3.8.5.Influence of fungicides and bioagents on incidence of Phytophthora blight undergreenhouse

Effect of fungicides (mefenoxam and metalaxyl) and bio agents (*Trichoderma*isolate-3. and *Pseudomonas* isolate-1)were tested in greenhouse conditions using three varieties of Pigeonpea (ICP 87119, ICP 7119 and ICP 8863).Seeds were treated with fungicides and bioagents before 30 minutes of sowing and were allowed to dry in shade. Inoculum production, inoculation, growth conditions, replications and disease assessment were similar as explained in earlier section 3.5. Details of treatments are given below.

Treatment	Details
T1	Mefenoxam (2 gram of a.i. kg^{-1} of seed)
T2	Mefenoxam (1 gram of a.i. $kg^{-1}$of seed) + *Trichoderma*isolate-3 (4 gram $kg^{-1}$of seed)
T3	Mefenoxam (1 gram of a.i. kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 gram kg^{-1} of seed)
T4	*Trichoderma*isolate-3 (4 gram $kg^{-1}$of seed)
T5	*Pseudomonas* isolate-1 (10 gram $kg^{-1}$of seed)
T6	*Trichoderma*isolate-3 (4 gram $kg^{-1}$of seed) + *Pseudomonas* isolate-1 (10 gram kg^{-1} of seed)
T7	Metalaxyl seed treatment (2 gram of a.i. $kg^{-1}$of seed)
T8	Inoculated control and
T9	Uninoculated control

3.8.6.Management of Phytophthora blight under field conditions using fungicides and bioagents

Two fungicides mefenoxam and metalaxyl and bio agents *Trichoderma*isolate - 3 and *Pseudomonas*isolate-1were evaluated in the field during *kharif* 2013 at RL-17 at ICRISAT, Patancheru against *P. drechsleri* f. sp. *cajani*. Treatments of experiments were the same as under greenhouse experiment and multiplications of inoculum, method of inoculation, were similar as explained in earlier section 3.5. Data on disease incidence was collected up to 120 days after inoculation and the details of the field layout and experiments are furnished below.

S.No.	Particulars	ICRISAT, Patancheru, Hyderabad
1	Design	RBD
2	Replications	3
3	Plot size	4 ×1.5 meter
4	Spacing	75 × 10 cm
5	Number of treatments	9
6	Variety	ICP 87119, ICP 7119 and ICP 8863
7	Date of planting	25.06.2013
8	Time of inoculum applied	09.07.2013

3.9. Statistical analysis

The data were statistically analyzed (Gomez and Gomez, 1984) using the SAS 9.2 version developed by the SAS institute, NC, USA. Lab experiments were carried out using Completely Randomized Design (CRD) while the field experiments were conducted using Randomized Block Design (RBD). The percentage values of the disease incidence were transformed to arcsine. Data was subjected to analysis of variance (ANOVA) at two significant levels ($P< 0.05$ and $P< 0.01$) and means were compared by Tukey's Honesty significant difference (HSD).

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

Pigeonpeais commonly known as redgram or arhar.The seeds are used as dal which are rich in protein (21%), iron and iodine. They are also rich in essential amino acids like lysine, tyrosine, cystine and arginine. The green pods are used as vegetable. The pod husk and leaves after threshing serve as a valuable fodder for cattleand also used in stock feed rations. Pigeonpeawhen rotated with cereal crops increases the yield of cereals by enhancing soil nitrogen and helps in breaking the disease cycle of important cereal pathogens. Because of its tolerance to heat and drought, it is suitable for low-fertility soils.Globally, Pigeonpea is cultivated in 5.32 million ha, adding 4.24 million tonnes of grain to the global food basket. India alone accounts 72.50 per cent of area with 62.54 per cent of production (Factfish, 2012)

Phytophthora blight is caused by *Phytophthora drechsleri* Tucker f. sp.*cajani.* The first suspected occurrence of Phytophthora blight on Pigeonpea in India was reported in 1966 by Williams *et al*. (1968). Since then, the disease had spread to most Pigeonpea growing areas in Asia (Pal *et al*. 1970; Williams *et al*. 1975), Africa, America (Kannaiyan *et al*. 1984), Australia (Wearing and Birch, 1988), Dominican Republic, Kenya, Panama and Puerto Rico (Nene *et al*. 1996). Recently, the recurrence of Phytophthora blight was reported and has become a major threat to Pigeonpea production and productivity in the Deccan Plateau of India irrespective of cropping system, soil types and cultivars (Sharma *et al.* 2006: Pande *et al.* 2011). Information on worldwide losses caused by Phytophthora blight is not available, but there is no doubt that the disease is gaining importance and devastatingthe crop particularly due to changing pattern in total rainfall in the Semi-Arid Tropics where Pigeonpea is being cultivated primarily as a rainfed crop. The effect of Phytophthora blight on grain yield depends on the appearance of the disease during the crop growth period which largely depend on weather conditions and inoculum levels of the pathogen.

Scanty information is available on physiological characteristics of *P. drechsleri*f. sp.*cajani*, epidemiology and disease development, and strategies to manage the disease made an impetus to undertake the present investigation with these objectives in view.The present investigations were carried out to understand the spatial and temporal distribution of disease, morphological and physiological characteristics of *P. drechsleri* f. sp. *cajani* andto study the epidemiological aspects of disease and to

develop suitable management practices. The results of various experiments are presented in the foregoing line and discussed with pertinent literature.

4.1. Periodical survey of Phytophthora blight of Pigeonpea at ICRISAT, Patancheru

Periodical incidence of disease was recorded at research farm of ICRISAT during the *kharif*2012 and 2013 and the results are presented in Table 4.1.

The disease incidence varied during both the years of study. Fifteen Pigeonpea fields were surveyed at regular interval during *kharif* 2012 and the results indicated thatthe disease incidence ranged from 0.0 to 59.0 per cent with an average incidence of 7.47 per cent. Among all the fields surveyed, the disease incidence was observed only in three fields. Field No. BP-15B showed the highest disease incidence (59.0 %), followed by BP-05 (38.0 %) and RCW-01 (15.0 %),while the rest of fields did not show any disease incidence. Whereas during *kharif* 2013, twenty fourPigeonpea fields were surveyed and the disease incidence ranged from 0.0 to 55.0 per cent with an average incidence of 7.25 per cent. Field No. BP-14C showed the highest incidence of 55.0 per cent followed by RL-17 (32.0 %) and remaining 18 fields did not show anydisease incidence.

The disease incidence occurred irrespective of the soil type and Pigeonpea genotypes grown in both the years. The variation in disease incidence in different Pigeonpea fields may be due to varied distribution of soil inoculum and inoculum potential of the pathogen and varieties cultivated with different genetic makeup.

4.1.1. Correlation and multiple regressions of weather parameters with disease incidence at ICRISAT, Patancheru

Disease prediction models play a vital role in the prediction and forecasting of disease, which is a prerequisite for deployment of cost effective management practices. In the present study, disease prediction models were developed by using per cent disease incidence as dependent variable. Experiment conducted during *kharif* 2012 and 2013 showed highly significant correlation coefficient (r) and coefficient of determination (R^2) between Phytophthora blight disease incidence and weather parameters Table 4.2: Fig. 4.1. A simple linear regression disease prediction model was developed depicting maximum correlation with given PDI. Our model

Table 4.1.Periodical incidence of Phytophthora blight disease of Pigeonpea during *kharif* 2012 and 2013 at ICRISAT, Patancheru, India.

S. No.	Field Number	Soil Type	Per cent disease incidence at different days after sowing*				
			30	60	90	120	150
***kharif*2012**							
1	RCW-01	Red	08.0^{c} (16.2)	09.0^{c} (17.2)	10.0^{c} (18.1)	12.0^{c} (19.9)	15.0^{c} (22.2)
2	BP-05	Black	21.0^{b} (26.4)	24.0^{b} (28.2)	28.0^{b} (30.5)	35.0^{b} (34.3)	38.0^{b} (35.8)
3	BP-15B	Black	36.0^{a} (34.8)	40.0^{a} (36.8)	44.0^{a} (38.7)	57.5^{a} (44.8)	59.0^{a} (45.5)
***kharif*2013**							
1	RCE 23 A	Red	12.5^{b} (20.5)	19.0^{b} (25.32)	22.0^{c} (28.25)	22.0^{c} (28.25)	22.0^{c} (28.25)
2	DHF 04	Red	5.3^{d} (12.77)	7.1^{d} (15.35)	9.0^{f} (17.26)	11.3^{e} (19.70)	14.3^{d} (20.79)
3	BP 14 A	Black	11.0^{c} (18.49)	13.8^{c} (20.90)	17.1^{d} (23.68)	27.0b^{c} (30.22)	29.8^{b} (31.63)
4	BP 14 B	Black	0.0^{e} (0.57)	0.0^{e} (0.57)	15.0^{e} (21.40)	17.4^{d} (23.54)	21.0^{c} (26.42)
5	BP 14 C	Black	11.0^{c} (18.73)	18.0^{b} (24.19)	48.3^{a} (41.28)	52.0^{a} (42.69)	55.0^{a} (44.11)
6	RL 17	Red	20.0^{a} (25.27)	26.0^{a} (29.09)	29.0b (31.12)	31.0b (32.47)	32.0^{b} (33.30)

*Mean of five replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's HSD.
Figures in parentheses are arcsine transformed values.

Table 4.2. Correlation of weather parameters with incidence of Phytophthora blight disease at ICRISAT, Patancheru, India.

S. No.	Weather Parameters	Correlation coefficient (r)											
		kharif - 2012				*kharif* - 2013							Pooled data of *kharif* 2012 and 2013
		RCW 01	BP05	BP15B	Pooled	RCE23A	DHF04	BP14A	BP 14B	BP14C	RL17	Pooled	
1	Rainfall	0.91**	0.98**	0.99**	0.98**	0.82*	0.97**	0.97**	0.93**	0.95*	0.83*	0.96**	0.95**
2	Temperature maximum	0.93**	0.86*	0.78	0.84*	0.78	0.58	0.61	0.51	0.68	0.74	0.70	0.48
3	Temperature minimum	-0.90**	-0.91**	-0.94**	-0.93**	-0.32	-0.68	-0.69	-0.70	-0.57	-0.38	-0.57	-0.42
4	RH maximum	-0.64	-0.41	-0.33	-0.41	0.49	0.41	0.33	0.54	0.58	0.41	0.51	0.92**
5	RH minimum	-0.99**	-0.99**	-0.97**	-0.99**	-0.68	-0.84*	-0.92**	-0.80	-0.80	-0.71	-0.83*	-0.54

* Significant at 5 per cent probability level
** Significant at 1 per cent probability level

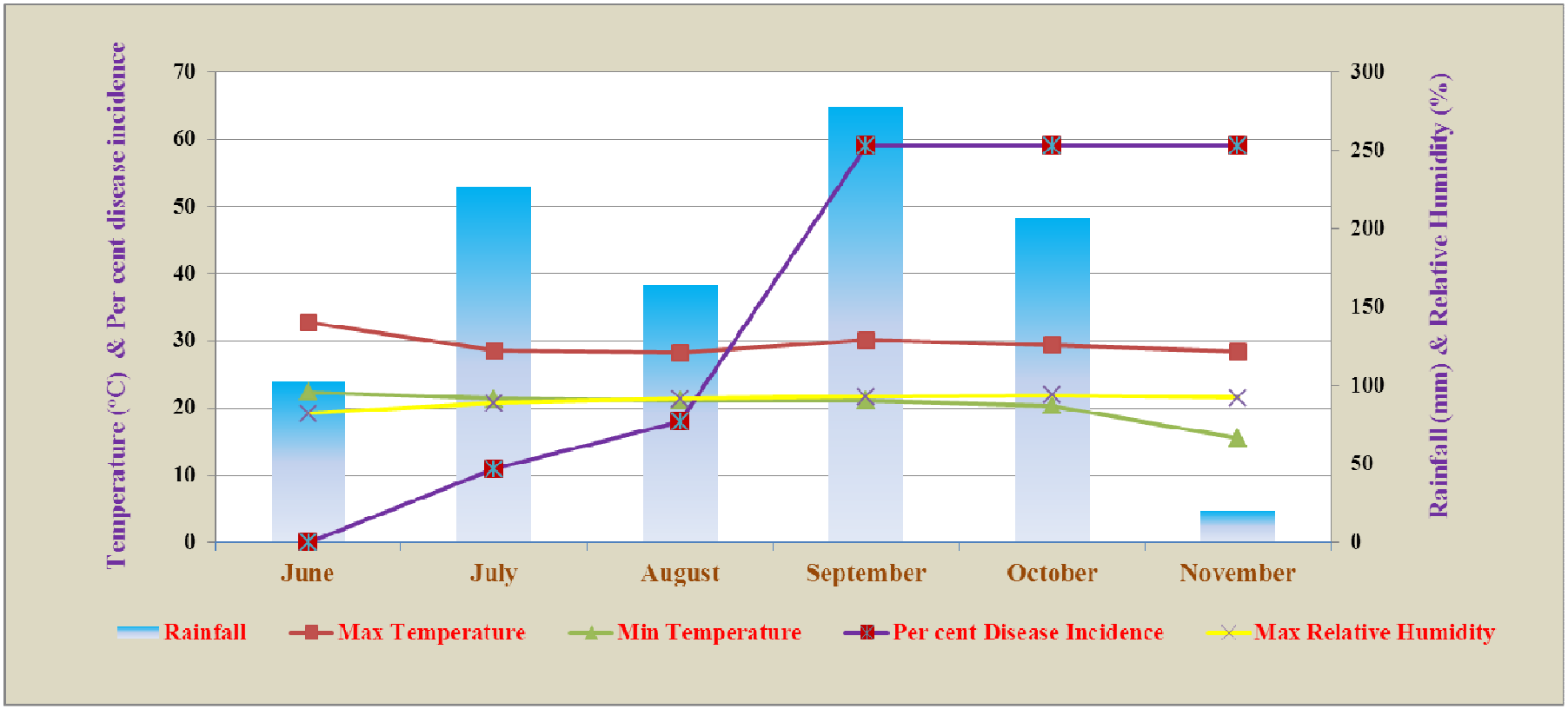


Figure 4.1. Influence of weather parameters on disease incidence of Phytophthora blight of Pigeonpea during 2013 at ICRISAT, Patancheru.

helps to predict the incidence of disease which is dependent on maximum R^2 and it can fit anywhere from PDI taken at 30 to 150 DAS.

Correlation of incidence of the disease and weather parameters for *kharif*, 2012 and 2013 indicated that cumulative rainfall, average maximum temperature and average maximum relative humidity showed positive correlation, whereas average minimum temperature and average minimum relative humidity showed negative correlation. However cumulative rainfall and average maximum relative humidity had significant positive correlation at both 0.01 and 0.05 probability level, while average minimum relative humidity had negative correlation.

Regression equation showed that there was a linear positive relationship between per cent disease incidence, cumulative rainfall and average maximum relative humidity, whereas negative relationship with average maximum temperature (Table 4.3). During the above period high rainfall caused water stagnation which favoured the multiplication and spread of inoculum of *P. Drechsleri* f.sp. *cajani*. Similar observations were made by Kannaiyan *et al*. 1984; Mishra and Shukla 1987 and Chauhan *et al*. 2002; Masood *et al*. 2005; Sharma *et al*. 2006; Pande *et al*. 2011that low lying, poorly drained soil with high soil moisture was conducive for the incidence of Phytophthora blight. Agarwal *et al*. (2002) reported that, sporangia were formed during the period with low maximum temperature (24.0-28.5 ^{o}C), high relative humidity (88-100 %) and 100 % rainy days.

4.2. Occurrence and distribution of Phytophthora blight in major Pigeonpea growing pockets of India

Phytophthora blight is an important disease of Pigeonpea in India causing serious losses, but occasionally assumes epidemic proportions in places of heavy and frequent rainfall leading to mortality of young plants (Willams *et al*. 1975: Kannaiyan *et al*. 1984). Recently, Pande *et al*. (2011) proposed the recurrence of Phytophthora blight as a major threat to Pigeonpea production and productivity in the Deccan Plateau of India irrespective of cropping system, soil type and cultivars.

In this context a roving survey was conducted in major Pigeonpea pockets of Indiato record the occurrence and distribution of Phytophthora blight of Pigeonpea in major Pigeonpea growing states *viz*. Andhra Pradesh, Karnataka, Maharashtra during *kharif*2012 and Andhra Pradesh, Karnataka, Maharashtra and Madhya Pradesh during *kharif*2013.

Table 4.3. Multiple regression equation for disease incidence of Phytophthora blight in relation to weather parameters.

S. No.	*kharif* -2012			*kharif* -2013		
	Field No.	**Multiple linear regression equation**	**R^2**	**Field No.**	**Multiple linear regression equation**	**R^2**
1	RCW 01	$Y = 212.6 + 0.06 X_1 - 0.21 X_2 - 2.19 X_3$	0.98	RCE23A	$Y = -73.05 + 0.07 X_1 + 2.51 X_2 + 0.08 X_3$	0.92
2	BP05	$Y = 404.8 + 0.15 X_1 - 1.29 X_2 - 3.90 X_3$	0.98	DHF 04	$Y = 6.13 + 0.07 X_1 + 0.37 X_2 - 0.20 X_3$	0.98
3	BP 15B	$Y = 656.9 + 0.23 X_1 - 3.11 X_2 - 5.98 X_3$	0.94	BP 14A	$Y = 49.32 + 0.15 X_1 + 1.29 X_2 - 0.99 X_3$	0.99
4	-	-	-	BP 14B	$Y = -62.07 + 0.12 X_1 + 0.08 X_2 + 0.56 X_3$	0.94
5	-	-	-	BP 14C	$Y = -214.67 + 0.26 X_1 + 3.23 X_2 + 1.26 X_3$	0.98
6	-	-	-	RL 17	$Y = -38.04 + 0.11 X_1 + 3.22 X_2 - 0.51 X_3$	0.91
Pooled 2012		$Y = -37.39 + 0.08 X_1 + 0.74 X_2 + 0.29 X_3$	0.99	Pooled 2013	$Y = -55.33 + 0.13 X_1 + 1.78 X_2 + 0.03 X_3$	0.97
Pooled 2012 and 2013 : $R^2 = 0.94$: $Y = 67.20 + 0.10 X_1 - 1.64 X_2 + 0.26 X_3$						

Y= Per cent disease incidence
X_1 = Rainfall
X_2 = Temperature maximum
X_3= Relative humidity Maximum

In *kharif* 2012, the Phytophthora blight incidence across the fields in Andhra Pradesh, Karnataka and Maharashtra ranged from 1.0 – 26.7 per cent, 6.01 – 54.4 per cent and 0.8 – 46.2 per cent respectively (Table 4.4). In Maharashtra, Akola showed the highest incidence of 32.85 per cent (Plate 4.1) followed by Bidar (30.21 %), while least incidence was recorded in Adilabad (1.0 %). During*kharif* 2013,the Phytophthora blight incidence ranged from 0.0 – 9.1 per cent, 0.0 -33 per cent, 0.0 – 9.1 per cent and 7.5 – 25.14 per cent in four states *viz.*Andhra Pradesh, Karnataka, Maharashtra and Madhya Pradesh respectively. In Madhya Pradesh, Sehore showed highest disease incidence (15.96 %) followed by Bellary (11.58 %) of Karnataka, whereas Yavatmal, Amravati, Jalna, Parbhani, Jalgaon and Latur districts of Maharashtra did not show any disease incidence. In Andhra Pradesh, Ranga Reddy district showed 5.8 per cent disease incidence followed by Mahbubnagar with 2.91 per cent (Table 4.5).

The survey revealed that the disease incidence varied from locality to locality because of environmental conditions especially rainfall, cropping patterns of the location and distribution of inoculum in the soil, inoculum potential of the pathogen and existence of the physiological races in the pathogen.

4.3. Symptoms

Infected young seedlings showed crown rot symptoms soon after their emergence, later collapsed and died within 4 days. In older plants, i.e. on 1 to 2 month old plants, water soaked lesions of varied size and shape appeared on the primary and trifoliate leaves (Plate 4.2a). As the disease progressed, the entire foliage blighted (Plate 4.2b). Brown, dark brown or black lesions appeared on the collar region above the ground level on the main stem and branches (Plate 4.2c: 4.2d). During severe infection, the lesions increased in size and encircled the stems causing them to shrink. The stem above the lesion girdled dried out and broke at the infection site (Plate 4.2e). In late infections, lesions on stems developed into cankers or galls and the infected bark cracked. (Plate 4.2f). Williams *et al*. (1975); Nene *et al*. (1979); Vishwa Dhar *et al*. (2005) and Pande *et al*. (2011) observed similar type of symptoms in the field and under greenhouse conditions.

4.4. Isolation of *Phytophthora drechsleri* f. sp. *cajani*

Most *Phytophthora* spp. were difficult to isolate from infected plant tissues due to the rapid growth rate of other saprophytic fungi. Successful isolation of

Table 4.4.Occurrence and distribution of Phytophthora blight disease during *kharif* 2012 in major Pigeonpea growing areas of India.

S. No.	District	Location	Variety	Stage of crop (DAS)	Soil Type	Cropping pattern	*PDI (%)	Mean PDI (%)
Andhra Pradesh								
1	Adilabad	Dongragoan	Maruti	30	Black clay	Pigeonpea: Cotton (1:6)	1.00^{i} (5.73)	1.00^{d} (5.73)
2	Rangareddy	Tandur	Local	60	Red sandy	Mono cropping	2.00^{hi} (8.10)	14.35^{b} (21.74)
3		Agganoor	Local	55	Red loam	Mono cropping	26.70^{c} (29.77)	
Karnataka								
4	Bidar	Kalasdar	Maruti	60	Black loam	Pigeonpea: Soybean (2:6)	6.01^{g} (14.1)	30.21^{a} (31.74)
5		Kolampur	Local	60	Black loam	Pigeonpea: Green gram (1:1)	54.40^{a} (43.45)	
Maharashtra								
6	Hingoli	Andharwad	Maruti	40	Red clay	Pigeonpea: Soybean (3:6)	11.00^{f} (18.97)	11.00^{b} (18.97)
7	Akola	Akola	Local	30	Black loam	Monocropping	19.50^{d} (25.36)	32.85^{a} (33.15)
8		Kolambi	Local	30	Black loam	Pigeonpea: Soybean (1:5)	46.20^{b} (39.70)	
9	Amravati	Dhanora	Local	35	Black clay	Pigeonpea: Green gram (1:6)	4.20^{gh} (11.50)	5.77^{c} (13.77)
10		Javara	Local	40	Black clay	Pigeonpea: Soybean (1:6)	1.20^{i} (6.28)	
11		Shirpur	Maruti	40	Black clay	Pigeonpea: Soybean (1:6)	11.90^{f} (19.74)	

Table 4.4. (Cont.).

S. No.	District	Location	Variety	Stage of crop (DAS)	Soil Type	Cropping pattern	*PDI (%)	Mean PDI (%)
12	Yavatmal	Uttar Wadhona	Local	40	Black clay	Pigeonpea: Cotton (2:8)	6.50^{g} (14.49)	11.68^{b} (19.60)
13		Saykhed (Umri)	Local	50	Black sandy	Pigeonpea: Cotton (2:10)	28.00^{c} (30.51)	
14		Pimpalgaon	Local	50	Black clay	Pigeonpea: Cotton (1:6)	0.80^{i} (5.12)	
15		Yerad	Local	60	Black loam	Pigeonpea: Cotton (1:10)	12.50^{f} (30.51)	
16		Wadafali	Local	40	Reddish black	Pigeonpea: Soybean (1:6)	10.60^{f} (18.62)	

*Mean of five replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's HSD.
Figures in parentheses are arcsine transformed values.

Table 4.5.Occurrence and distribution of Phytophthora blight during *kharif* 2013 in major Pigeonpea growing areas of India.

S. No.	District	Location	Variety	Stage of crop (DAS)	PDI (%)	Mean PDI (%)
Andhra Pradesh						
1	Rangareddy	Mahbapur	Local	30	9.10^{ef} (17.23)	5.80^{d} (13.80)
2		ARS Tandur	Maruti	35	2.50^{j} (9.06)	
3	Mahbubnagar	Kodangal	Maruti	40	8.90^{ef} (17.03)	2.90^{e} (9.76)
4		Kondareddy Palli	Maruti	35	0.0^{k} (0.57)	
5		Nettur	Maruti	30	0.0^{k} (0.57)	
6		Rawalpally	Local	40	2.70^{j} (9.42)	
Karnataka						
7	Glubarga	Glubarga	ICPH2740	40	5.30^{igh} (13.03)	8.90^{c} (17.10)
8		Dongargaon	Asha	40	12.50^{e} (20.24)	
9	Bidar	Bidar	ICPH2671	35	8.30^{f} (16.44)	5.80^{d} (13.80)
10		Kaud gaon	BSMR736	35	3.30^{ij} (10.41)	
11	Bellary	Kalkamba	Asha	30	0.0^{k} (0.57)	
12		Laxmipura	Asha	35	0.0^{k} (0.57)	

Table 4.5. (Cont.).

S. No.	District	Location	Variety	Stage of crop (DAS)	PDI (%)	Mean PDI (%)
13	Bellary	Yallapura cross	Maruti	35	19.00[d] (25.03)	11.58[b] (19.52)
14		Kurugodu	Maruti	30	8.00[fg] (16.13)	
15		Badnatti	Ahsa	30	33.0[a] (33.22)	
16		Yerangali	Local	40	29.0[ab] (31.07)	
17		Somalapura	Local	40	3.67[ij] (10.89)	
18		Veerapura	Local	40	0.0[k] (0.57)	
Maharashtra						
19	Latur	Tongari	Local	40	0.0[k] (0.57)	0.0[f] (0.57)
20		Latur	Maruti	40	0.0[k] (0.57)	
21	Jalgaon	Nirmal Pachor	Local	50	0.0[k] (0.57)	0.0[f] (0.57)
22	Parbhani	Parbhani	BSMR 736	50	0.0[k] (0.57)	0.0[f] (0.57)
23		Takli	Asha	50	0.0[k] (0.57)	
24	Aurangabad	ARS Badnapur	Asha	50	9.10[ef] (17.23)	9.10[c] (17.30)
25	Jalna	Jalna	Maruti	45	0.0[k] (0.57)	0.0[f] (0.57)

Table 4.5. (Cont.).

S. No.	District	Location	Variety	Stage of crop (DAS)	PDI (%)	Mean PDI (%)
26	Buldhana	Chikkili	Local	40	5.17^{ih} (12.89)	2.59^{e} (9.21)
27		Hathini	Maruti	50	0.0^{k}	
28	Akola	Akola	BSMR 853	50	5.13^{ih} (12.83)	2.57^{e} (9.18)
29		Kharb	Local	45	0.0^{k} (0.57)	
30	Amravati	Durgapur	Maruti	50	0.0^{k} (0.57)	0.0^{f} (0.57)
31		Amravati	Asha	40	0.0^{k} (0.57)	
32	Yavatmal	Yavatmal	Asha	50	0.0^{k} (0.57)	0.0^{f} (0.57)
Madhya Pradesh						
33	Sehore	Auti pura	Local	40	21.03^{dc} (23.36)	15.96^{a} (22.94)
34		Shahapur Kodiya	Local	40	10.17^{ef} (18.21)	
35		Baliya keli	Local	40	7.50^{fgh} (15.66)	
36		Sehore	Local	40	25.14^{bc} (28.87)	

*Mean of five replications.

Means followed by a common letter are not significantly different at 5% level by Tukey's HSD.

Figures in parentheses are arcsine transformed values.

Plate 4.1. Disease incidence of Phytophthora blight in Pigeonpea field at Akola, Maharashtra.

A. Healthy and infected plants of Pigeonpea

B. Stem blight symptoms of Phytophthora blight

A. Water soaked area

B. Leaf blight symptom

C. Stem blight (Initial stage)

D. Stem blight (Advanced stage)

E. Breaking of stem

F. Stem gall

Plate 4.2. Symptoms of Phytophthora blight disease of Pigeonpea.

Phytophthora spp. can be achieved by the use of selective media that either slow down the growth or inhibit the growth of otherfungi and bacteria.

Seven media *viz.*oat meal agar, corn meal agar, V-8 juice agar, Pigeonpea seed meal agar, potato dextrose agar, tomato juice agar and V8 PARP (Pimaricin, Ampicillin, Refampcilin and PCNB) medium were screened for isolation of *P. drechsleri*f. sp.*cajani*from the infected stem bits. Among all V8 PARP (Pimaricin, Ampicillin, Refampcilin and PCNB) medium was efficient in supporting the growth of *P.drechsleri*f. sp.*cajani*the pathogen from the infected stem bits which reduced the bacterial contamination due to the action of antibiotics present in the medium and also due to PCNB which reduced the growth of saprophytes. The present study was in accordance with finding of Jeffers and Martin (1986); Ferguson& Jeffers(1999).

4.4.1. Morphological characterization of *P. drechsleri* f. sp. *cajani*

The colonies of *P. drechsleri* f. sp. *cajani* were dull white colour with flat mycelium on tomato juice agar medium (Plate 4.3a). Mycelium was hyaline, branched, coenocytic filamentous (Plate 4.3b). Hyphal swelling was common and it was terminal and/or intercalary (Plate 4.3c).

The sporangia (Plate 4.3e) were of proliferating type (Plate 4.3f) and sporangiaphore were simple sympodial (Plate 4.3d) which emerged externally from the base of previous sporangium (Plate 4.3g) and produced a new sporangium. Caducity of sporangia was absent because sporangia did not detach at maturity. Production of zoospores by sporangia was completed within few hours, and about 70-110 zoospores were released per sporangia (Plate 4.3h).

Phytophthora drechsleri f. sp. *cajani* is homothallic and produced the male and female gametangia called oogonium and antheridium (Plate 4.3i). Amphigynous type of sexual reproduction was observed and produced a sexual spore called oospore (Plate 4.3j). The asexual and sexual characteristics of *P. drechsleri* f. sp. *cajani* are represented in Table 4.6. Similar observations were made by Williams *et al*. (1968), Kannaiyan *et al.* (1980) and Singh *et al.* (1992).

4.5. Establishing the pathogenicity

Ten day old seedlings of susceptible variety ICP 7119 were artificially inoculatedwith Pdc-1 isolate of *P. drechsleri* f. sp. *cajani*by soil drenching method. The first symptom on the seedling appeared as water soaked lesion on primary and

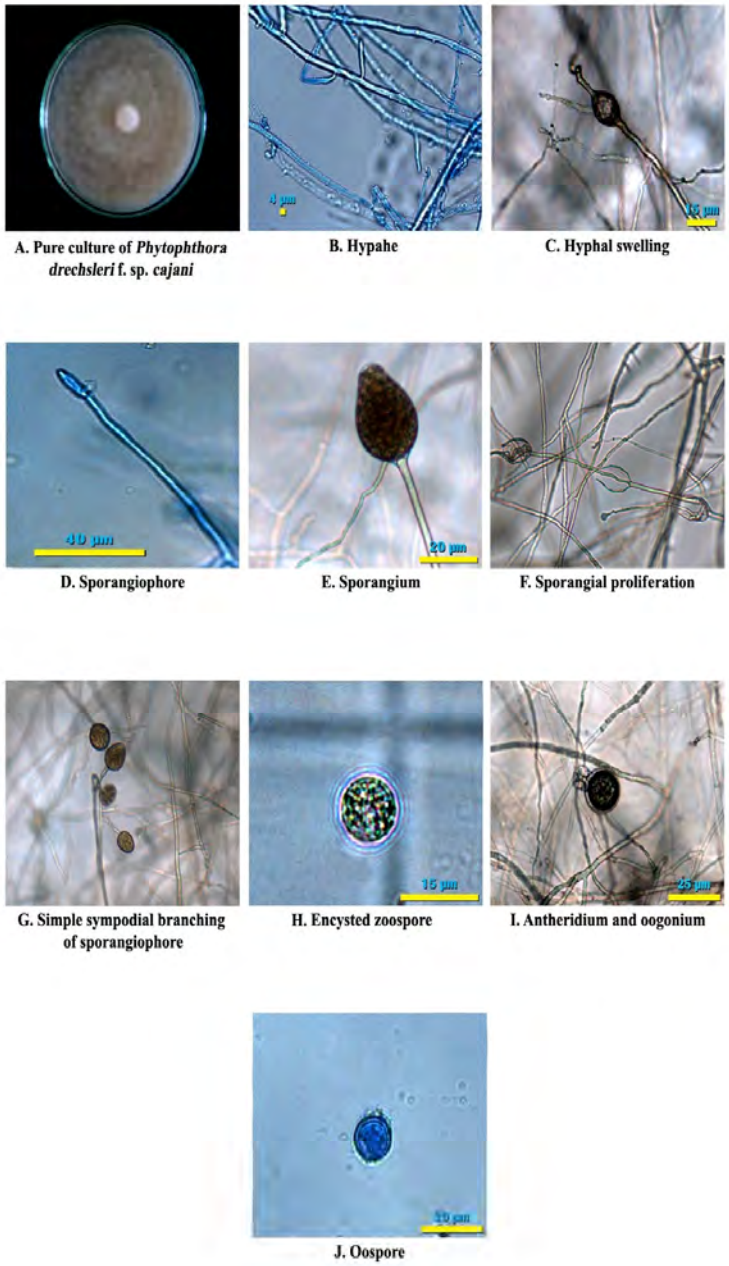


Plate 4.3. Morphological characteristics of *Phytophthora drechsleri* f. sp. *cajani* of Pigeonpea.

Table 4.6. Morphological characters of *Phytophthoradrechsleri* f. sp. *cajani* isolates on tomato juice agar medium.

S. No.	Particulars	Morphological characteristics*	
Asexual characteristics		**Pdc-1Isolate**	**Pdc-2 Isolate**
1	Mycelium	Hyaline, Coenocytic and branched	Hyaline, Coenocytic and branched
2	Breadth of hyphae (µm)	4.07	4.02
3	Hyphal swelling	Intercalary and terminal	Intercalary and terminal
	a. Length (µm)	17.9	15.6
	b. Breadth (µm)	13	10.8
4	Sporangiophore	Simple sympodium	Simple sympodium
	a. Length (µm)	83	100
	b. Breadth (µm)	3.6	3.8
5	Sporangia shape	Oviod-obpyriform and non-pedicellate	Oviod-obpyriform and non-pedicellate
6	Sporangia proliferation	Present	Present
7	Sporangia	Non-papilate	Non-papilate
	a. Length (µm)	33.1	33.7
	b. Breadth (µm)	21.2	19.6
	c. L:B ratio	1.56	1.72
	d. Sporangia exit pore Breadth (µm)	7.3	4.7
8	Zoospore Diameter (µm)	11.7	11.2
9	Caducity	No	No
10	Chlamydospores	Absent	Absent
Sexual characteristics			
11	Mating type	Homothallism	Homothallism
12	Type of sexual reproduction	Amphigynous	Amphigynous
13	Diameter of oogonium (µm)	23.1	20.8
14	Breadth of antheridium (µm)	9.3	5.8
	Length of antheridium (µm)	6.6	5.8
15	Oogonium stalk Length (µm)	7.8	6.8
16	Diameter of oospore (µm)	18.3	17.6

*Values are mean of three replications.

trifoliate leaves within 48 hours after inoculation. The seedlings showed characteristics symptoms of crown rot which completely collapsed and died within 7 days.Un-inoculated seedlings remained healthy throughout the experiment. Reisolations were done from infected plants and the morphological and cultural characters of the fungus were found similar to the original isolates.

4.6. Virulence of *P. drechsleri* f. sp. *cajani* isolates

Differentinoculation techniques were used for disease establishment using two isolates *viz.* Pdc-1 and Pdc-2 and the results are presented in Table 4.20 and Fig. 4.14. It is found that, IsolatePdc-1 was most virulent with disease incidence of 100 per cent on 7th day after inoculation, whereasPdc-2 isolate showeddisease incidence of 90.9 per cent. Hence the isolate Pdc-1 was used throughout the study.

4.7. Physiological characterization of*P. drechsleri* f. sp. *cajani*

4.7.1. Influence of different media on growth of *P. drechsleri* f. sp. *cajani*

The diversity in cultural and morphological characters of *P. drechsleri* f. sp. *cajani* was studied on twelve solid media at30 °Ctemperature.The radial growth of the fungus and sporulation were recorded and presented in Table 4.7. Among the media tested, tomato juice agar medium supported highest mycelial growth recording 90 mm and was on par with V8 juice agar medium (89.0 mm) on 6th day after incubation, followed by potato dextrose agar medium (84.20 mm) and the least growth was observed on carrot agar medium with 40.67 mm (Fig. 4.2: Plate 4.4).

The colour of the mycelium of *P. drechsleri* f. sp. *cajani* varied from dull white to cottony white growth whichwas flat to aerial with smooth to irregular margins.Mycelium was dull white with flat topography and smooth margin on oat meal agar, Pigeonpea seed meal agar, chickpea seed agar, czapek-dox agar and carrot agar medium. But rose petal type of growth was observed in V8 juice agar, potato dextrose agar and potato glucose agar medium. Whereas aerial type of growth was observed on tomato juice agar and potato sucrose agar medium. Further, irregular transparent and flat mycelium was observed on corn meal agar medium. However sporulation was not observed in any of the medium tested.

Taking the clue from results of solid medium, three liquid media were tested for mass multiplication of pathogen and the data are presented in Table 4.8. Among the three broth tested, V8 juice broth yielded highest wet mycelial weight (96.21 mg) and dry weight (2.19 mg) which was statistically on par with tomato juice broth with 94.17 mg and 2.09 mg wet and dry weight respectively, whereas potato broth

Table 4.20. Effect of inoculation technique on disease incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

Treatment	Pdc-1 Isolate							Pdc-2 Isolate						
	*Per cent Disease Incidence at different days after inoculation							*Per cent Disease Incidence at different days after inoculation						
	1	**2**	**3**	**4**	**5**	**6**	**7**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
T1	0.0^{a} (0.9)	25.9^{a} 29.3	36.2^{b} (34.9)	46.2^{b} (39.7)	57.2^{b} (44.7)	60.5^{b} (46.2)	$79.0^{b,c}$ (52.3)	0.0^{a} (0.9)	10.0^{b} (18.1)	26.9^{a} (29.9)	30.3^{b} (31.8)	50.5^{b} (41.7)	57.3^{b} (44.7)	$70.9^{b,d}$ (53.7)
T2	0.0^{a} (0.9)	0.0^{d} (0.9)	0.0^{e} (0.9)	0.0^{e} (0.9)	0.0^{d} (0.9)	0.0^{e} (0.9)	$0.0^{e,i}$ (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	$0.0^{a,i}$ (0.9)
T3	0.0^{a} (0.9)	20.3^{b} (25.9)	39.5^{a} (36.5)	55.9^{a} (44.1)	76.8^{a} (53.6)	100.0^{a} (71.8)	$100.0^{a,a}$ (71.8)	0.0^{a} (0.9)	12.7^{a} (20.4)	23.3^{b} (27.8)	49.2^{a} (35.1)	57.2^{a} (44.7)	87.2^{a} (59)	$90.9^{a,b}$ (65.9)
T4	0.0^{a} (0.9)	0.0^{d} (0.9)	17.3^{c} (23.9)	23.5^{c} (27.9)	23.7^{c} (28.0)	36.2^{c} (34.9)	$50.8^{c,e}$ (37.2)	0.0^{a} (0.9)	0.0^{a} (0.9)	10.3^{d} (18.4)	20.3^{c} (25.9)	23.7^{d} (28)	33.0^{d} (33.2)	$36.6^{d,h}$ (35.1)
T5	0.0^{a} (0.9)	10.7^{c} (18.7)	13.2^{d} (20.8)	17.1^{d} (23.8)	23.0^{c} (27.6)	30.4^{d} (31.8)	$46.4^{d,f}$ (31.8)	0.0^{a} (0.9)	9.8^{b} (18)	16.3^{c} (23.2)	21.9^{c} (26.9)	33.1^{c} (33.3)	36.4^{c} (35)	$40.3^{c,g}$ (36.9)
T6	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	$0.0^{a,i}$ (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	$0.0^{a,i}$ (0.9)

Treatment: T1- Soil drenching with mycelial suspension (100 ml/ pot)
T2- Spray inoculation of mycelialsuspension ((100 ml/ pot))
T3- Soil mixing with mycelial inoculum(100 gram/ pot)
T4- Stem inoculation with mycelial inoculum
T5- Stem staging with mycelial bits
T6- Control

*Mean of three replications
In a column, means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
In a row, means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
Figures in parentheses are arcsine transformedvalues.

Figure 4.2. Influence of different media on the growth of *Phytophthora drechsleri* f. sp. *cajani.*

Table 4.7. Influence of agar media on the growth and sporulation of *Phytophthora drechsleri* f. sp. *cajani.*

S. No.	Medium	Radial growth of mycelium at different days * (mm)						Mycelial characteristics	Sporulation *
		1	2	3	4	5	6		
1	Oat meal agar	7.53^{e}	24.27^{e}	30.67^{h}	40.53^{i}	46.33^{g}	52.17^{h}	Dull white and flat mycelium	Nil
2	Corn meal agar	6.93^{f}	23.67^{e}	31.07^{h}	39.97^{i}	45.63^{g}	53.23^{h}	Irregular transparent and flat mycelium	Nil
3	V8 juice agar	11.07^{b}	32.27^{b}	45.27^{b}	72.27^{b}	79.23^{b}	89.03^{a}	Dull white and flat, rose petal type mycelium	Nil
4	Pigeonpea seed meal agar	9.37^{c}	22.27^{f}	32.30^{g}	47.67^{f}	54.33^{e}	66.13^{f}	Dull white and flat mycelium	Nil
5	Chickpea seed agar	8.37^{d}	24.27^{e}	30.63^{h}	44.33^{h}	51.20^{f}	64.60^{g}	Dull white and scanty flat mycelium	Nil
6	Potato dextrose agar	11.23^{b}	30.27^{c}	44.63^{c}	64.47^{c}	72.17^{c}	84.20^{b}	Dull white and rose petal type mycelium	Nil
7	Czapek- Dox agar	7.03^{f}	25.33^{d}	33.33^{f}	45.83^{g}	54.33^{e}	70.33^{e}	Dull white and flat mycelium	Nil
8	Carrot agar	6.03^{g}	16.47^{g}	20.30^{i}	26.17^{j}	30.07^{h}	40.67^{i}	Dull white and flat mycelium	Nil
9	Tomato juice agar	13.83^{a}	36.33^{a}	48.33^{a}	75.33^{a}	81.50^{a}	90.00^{a}	Dull white and aerial mycelium	Nil
10	Potato sucrose agar	9.57^{c}	30.63^{c}	40.33^{d}	58.17^{d}	72.03^{c}	82.07^{c}	Cottony white and aerial mycelium	Nil
11	Potato glucose agar	9.33^{c}	31.63^{b}	39.63^{e}	52.20^{e}	67.17^{d}	75.63^{d}	Cottony white and rose petal type mycelium	Nil
12	Water agar	0.0^{h}	0.0^{h}	0.0^{h}	0.0^{h}	0.0^{h}	0.0^{h}	No growth	Nil

*Mean of three replications

Means followed by a common letter are not significantly different at 5% level by Tukey's HSD.

Table 4.8.Influence of liquid media on the growth and sporulation of *Phytophthora drechsleri* f. sp. *cajani.*

S. No.	Liquid broth medium	Weight of mycelium* (mg)		Sporulation*
		Wet	Dry	
1	Potato dextrose broth	72.01[b]	1.56[b]	No
2	V8 juice broth	96.21[a]	2.19[a]	No
3	Tomato juice broth	94.17[a]	2.09[a]	No

*Mean of six replications

Means followed by a common letter are not significantly different at 5% level by Tukey's HSD.

showed the least growth of the pathogen with 72.01 mg wet weight and 1.56 mg of dry weight. However,sporulation was absent in the broth media tested.

The results were in accordance with Ribeiro (1978), who reported that V8 juice agar as the best medium for the growth of many *Phytophthora* spp. Similarly, Dhingra and Sinclar (1995), stated tomato juice agar medium was ideal for growth and sporulation of *Phytophthora* spp. Lack of sporulation in *Phytophthora* spp. was probably due to a failure to meet some precise requirement mineral nutrition and temperature for this process to occur (Grant *et al*. 1984).

4.7.2. Effect of temperature on growth and sporulation of *P. drechsleri* f. sp. *cajani* on solid media and liquid broth

Temperature is one of the pre-requisite for the growth and sporulation of the fungus which plays an important role in infection and disease development. The growth of*P. drechsleri* f. sp. *cajani* was tested on tomato juice agar medium and V8 juice broth at different temperatures of 5, 10, 15, 20, 25, 30, 35 and 40 ^{o}C and the results are summarized in Table 4.9. As the days progressed the growth of mycelium on tomato juice agar medium increased at all the temperatures except 5, 10 and 40 ^{o}C. The maximum mycelial growth was observed at 30 ^{o}C (90.0 mm) followed by 25 ^{o}C (87.4 mm). Further at 15 and 20 ^{o}C the growth started from 2nd day after incubation whereas at temperature of 20 to 30 ^{o}C growth was initiated at 1st day itself (Fig. 4.3). However, the growth of the mycelium was less at 35 ^{o}C when compared to the growth at 15 to 30 ^{o}C. Pathogen did not show any growth when plates were incubated at temperature of 5, 10 and 40 ^{o}C.

Similar trend was observed when *P. drechsleri* f. sp. *cajani*was grown onV8 juice broth. Maximum fresh and dry weight of mycelium was observed at a temperature of 30 ^{o}C (101.4 and 5.4 mg respectively) followed by 25 ^{o}C (92.4 and 3.2 mg) and least at 35 ^{o}C (42.66 and 2.00 mg). Sporulation was not observedin both agar media and broth at all the temperatures tested.

The results concur with the findings of Tucker (1931) in *Phytophthoradrechsleri*. Further, Pal and Grewal (1975); Kannaiyan *et al*. (1980) and Mishra *et al*. (2010) reported maximum vegetative growth of *P. drechsleri* f. sp. *cajani* at 30 ^{o}C.

Figure 4.3. Effect of temperature on growth of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

Table 4.9. Influence of temperature on growth and sporulation of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro.*

S. No.	Temperature (°C)	Tomato juice agar medium								V-8 juice broth		
		Mycelial growth at different days* (mm)							Sporulation*	Mycelial weight* (mg)		Sporulation*
		1	2	3	4	5	6	7		Wet	Dry	
1	05	0.0^{d}	0.0^{f}	0.0^{f}	0.0^{f}	0.0^{f}	0.0^{f}	0.0^{f}	--	0.0^{f}	0.0^{e}	--
2	10	0.0^{d}	0.0^{f}	0.0^{f}	0.0^{f}	0.0^{f}	0.0^{f}	0.0^{f}	--	0.0^{f}	0.0^{e}	--
3	15	0.0^{d}	9.2^{d}	15.3^{d}	25.1^{d}	30.1^{d}	38.5^{d}	46.4^{d}	No	58.66^{d}	1.20^{d}	No
4	20	0.0^{d}	2.4^{e}	33.4^{c}	44.8^{c}	51.1^{c}	62.2^{c}	73.1^{c}	No	60.60^{c}	4.86^{b}	No
5	25	14.8^{b}	27.2^{b}	45.0^{b}	55.2^{b}	66.8^{b}	80.2^{b}	87.4^{b}	No	92.40^{b}	3.20^{b}	No
6	30	17.3^{a}	31.1^{a}	48.1^{a}	59.3^{a}	68.1^{a}	81.4^{a}	90.0^{a}	No	101.35^{a}	5.44^{a}	No
7	35	9.4^{c}	11.2^{c}	14.3^{e}	17.1^{e}	20.2^{e}	21.3^{e}	23.5^{e}	No	42.66^{e}	2.00^{c}	No
8	40	0.0^{d}	0.0^{d}	0.0^{f}	0.0^{f}	0.0^{f}	0.0^{f}	0.0^{f}	No	0.0^{f}	0.0^{e}	--

*Mean of three replications

Means followed by a common letter are not significantly different at 5% level by Tukey's HSD.

4.7.3. Influence of relative humidity on growth and sporulation of *P. drechsleri* f. sp. *cajani*

Relative humidity is another important epidemiological factor for influencing physiology of fungal growth and sporulation as well as the outbreak of the disease under field conditions. Effect of relative humidity (RH) on growth and sporulation of *P. drechsleri* f. sp. *cajani*was recorded and the results indicated that growth of fungus increased as relative humidity increased from 50 per cent to 100 per cent. However RH of 75 per cent to 100 per cent yielded the maximum growth (90.00 mm) of test fungus and found statistically at par with each other (Table 4.10). The RH of less than 75 per cent decreased growth while least growth was observed in RH 50 per cent with 82.0 mm (Fig. 4.4). Neither sporangia nor oospores were formed irrespective of the range of relative humidity tested.

Similar experiment was conducted to determine the influence of relative humidity on mycelial weight using the V8 juice broth. Results indicated that,with the increase in relative humidity there was a corresponding increase in mycelial growth of the pathogen. However, hundred per cent humidity was found to be statistically superior in increasing the wet weight (99.76 mg) and dry weight (5.26 mg) of the mycelium when compared to others. At a relative humidity of 95 per cent,a wet weight of 92.96 mg and dry weight of 4.64 mg was recorded, whereas least wet (29.06) and dry (0.52) weight was observed at 50 per cent. In addition, sporulation of pathogen was studied at different RH and results implied that induction of sporangia, zoospores and oospores was absent at all the relative humidity tested throughout the experiment.

The results are in accordance with finding of Granke (2011) who reported that maximum growth of *Phytophthora capsici* was observed at cent per cent relative humidity.

4.7.4. Influence of CO_2 on growth and sporulation of *P. drechsleri* f. sp. *cajani*

Phytophthora drechsleri f. sp. *cajani* was inoculated on tomato juice agar and incubated at different CO_2levels and observations on radial growth of mycelium was recorded and presented in Table 4.11. Results revealed that, the radial growth of the *P. drechsleri* f. sp. *cajani* increased at increased CO_2 levels. However, 550 ppm of CO_2 showed the highest mycelial growth recording 90.0 mm on 6^{th} day after incubation and was on par with 700 ppm (88.9 mm) and least growth was observed

Table 4.10. Influence of relative humidity on growth and sporulation of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro.*

S. No	Relative Humidity (%)	Tomato juice agar medium							V-8 juice broth		
		Mycelial growth at different days* (mm)						Sporulation*	Mycelial weight* (mg)		Sporulation*
		1	2	3	4	5	6		Wet	Dry	
1	50	14.0^{d}	33.0^{d}	40.0^{f}	56.3^{e}	75.0^{e}	82.0^{b}	No	28.06^{e}	0.52^{e}	No
2	55	14.1^{d}	34.4^{c}	43.0^{e}	57.0^{d}	75.0^{e}	82.0^{b}	No	28.42^{e}	0.56^{e}	No
3	60	14.4^{c}	34.4^{c}	43.0^{e}	57.0^{d}	75.0^{e}	83.1^{b}	No	29.34^{e}	0.58^{e}	No
4	65	14.4^{c}	36.4^{b}	51.2^{d}	62.0^{c}	79.3^{d}	85.0^{b}	No	29.46^{e}	0.59^{e}	No
5	75	14.4^{c}	36.7^{b}	53.0^{c}	69.0^{b}	85.0^{c}	90.0^{a}	No	40.66^{d}	1.32^{d}	No
6	85	14.5^{bc}	36.7^{b}	53.0^{c}	69.3^{b}	88.3^{b}	90.0^{a}	No	46.72^{c}	2.44^{c}	No
7	95	14.7^{ab}	36.7^{b}	53.3^{b}	70.0^{a}	90.0^{a}	90.0^{a}	No	92.96^{b}	4.64^{b}	No
8	100	14.8^{a}	37.7^{a}	59.0^{a}	70.0^{a}	90.0^{a}	90.0^{a}	No	99.76^{a}	5.26^{a}	No

*Mean of three replication

Means followed by a common letter are not significantly different at 5% level by Tukey's HSD

Table 4.11. Influence of CO_2 on growth and sporulation of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

S No.	CO_2 (ppm)	Radial growth of mycelium at different days* (mm)						Morphological characteristics	Sporulation*
		1	2	3	4	5	6		
1	350	9.2[c]	16.9[c]	26.7[c]	44.8[b]	56.6[c]	80.7[b]	Dull white with flat mycelium	Nil
2	550	10.8[a]	20.0[a]	35.0[a]	54.5[a]	73.5[a]	90.0[a]	Dull white with irregular andaerial mycelium	Nil
3	700	9.9[b]	17.9[b]	32.2[b]	53.7[a]	70.8[b]	88.9[a]	Dull white withirregular and flat mycelium	Nil

*Mean of five replications.

Means followed by a common letter are not significantly different at 5% level by Tukey's HSD.

Figure 4.4. Effect of relative humidity on growth of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

at 350 ppm (80.7 mm). Production of sporangia and or zoospores were absent at all CO_2 levels under study.

The colour of the mycelium was dull white in all levels of CO_2 levels, and the growth pattern of the *P. drechsleri* f. sp. *cajani* was irregular at 550 and 700 ppm of CO_2 levels compared to growth pattern at 350 ppm. Further, aerial type of mycelium was observed at 550 ppm whereas the mycelium was flat at 350 and 700 ppm of CO_2.

Results of the study are in accordance with Mitchell and Zentmyer, (1977a) where they reported that *Phytophthora* species tolerated well at elevated level of CO_2. Similarly, many researchers have reported that the elevated CO_2 was known to stimulate fungal pathogen growth rates, aggressiveness, and fecundity (Coakley *et al.* 1999; Hibberd *et al.* 1996a,b; McElrone *et al.* 2005; Chakraborty *et al*. 2000b).

4.7.5. Protocol for induction of sporangia and zoospores of *P. drechsleri* f. sp. *cajani*

Production of sporangia and zoospores is the principle means by which the numbers of infective units are increased and accounts for the rapid regeneration time of *Phytophthora* species. In the present study various protocols given by research workers were tried for induction of sporangia and zoospores of *P. drechsleri* f. sp. *cajani* and results are presented in Table 4.12.

The present study revealed that, Out of all the protocols studied, Pigeonpea seed flour medium produced only sparse amount of sporangia, antheridium oogonium and oospores. However based on our results on use of different media for growth of *P. drechsleri* f. sp. *cajani,*tomato juice agar produced maximum growth therefore, we further probed to develop a protocol for maximum production of sporangia and zoospores using diluted tomato juice broth.

The protocol given by Kanniyan *et al*. (1992) was modified by using tomato juice instead of V8 juice broth. To our surprise it was found that tomato juice could induce abundant sporangia and zoospores within 48 hours of incubation. The male and female gametangia *viz.*antheridium and oogonium and sexual spore oospore were formed within 72 hours of induction. The induction of sporulation of *P. drechsleri* f. sp. *cajani*using diluted tomato juice broth *in vitro* is first of its kind.

On observation we found that the most important factor involved in the process of sporulation by *P. drechsleri* f. sp. *cajani*is sudden reduction in food

Table 4.12.Production of sporangia and zoospores of *Phytophthora drechsleri* f. sp. *cajani* using different methods.

S.No.	Method	Asexual spores*		Male and female gametes*		Sexual spore*	Resting spore*
		Sporangia	Zoospore	Antheridium	Oogonium	Oospore	Chlamydospore
1	KNO_3 solution (Schiffman and Cohen, 1968)	-	-	-	-	-	-
2	Sun flower, Pea, Corn and Soybean seed media (Felix , 1962)	-	-	-	-	-	-
3	Frozen pea medium (Dance, 1975)	-	-	-	-	-	-
4	Flooding on cultures (Ribeira and Baumer, 1973)	-	-	-	-	-	-
5	V-8 broth (Kannaiyan *et al*. 1992)	-	-	-	-	-	-
6	Rape seed extract agar (Satour and Butler, 1968)	-	-	-	-	-	-
7	Pigeonpea seed flour medium	+	-	+	+	+	-
8	Diluted tomato juice	++	++	++	++	++	-

*Mean of five replications
- = Absent
+ = Present in sparse amount
++ = Present in abundant amount

supply which forced the fungus to sporulation stage instead of the vegetative stage and stimulation of sporangial production thus initiating the cycle of spore formation. The study is of great value in research on the Phytophthora blight of Pigeonpea, as it will greatly facilitate to study the pathogenand host pathogen interaction.

4.7.6. Influence of temperature, relative humidity and CO_2levels on induction of zoospores in diluted tomato juice broth

Phytophthora have a complex asexual life cycle with distinct multiple infectious propagules which include the mycelium, sporangia, and zoospore cysts (de Souza *et al.* 2003). Each of these asexual developmental stages is crucial for plant infection and disease development (Hardham, 2001).

Different environmental factors such as temperature, water relations, physical and chemical conditions, and interacting combinations of these factors have been reported to influence *Phytophthora* pathogenesis (`Duniway, 1983). Moreover, the geographic distribution and seasonality of Phytophthora diseases are thought to depend on the constraints of any of these environmental factors on any or all of the life cycle stages (Duniway, 1983). Of the environmental factors, temperature has been reported to have greatest influence on growth, reproduction and pathogenesis of *Phytophthora* spp. (Matheron and Matejka, 1992). Effects of temperature on soil-borne *Phytophthora* spp. have been reported (Timmer *et al*. 2000).

Studies on the influence of temperature on sporangial production by *Phytophthora* spp. is an important prerequisite for developing disease predictive models in the field. Hence an attempt made to study the impact of temperature (20, 25, 30, 35 and 40 ^{o}C) on sporulation of *P. drechsleri* f. sp. *cajani*using the tomato juice broth method. The results revealed that an increase in temperature from of 20 to 30 ^{o}C yielded an increase in number of zoospores. Maximum (183×10^3 per ml) number of zoospores were recorded at 30 ^{o}C but the production of zoospores decreased drastically at 35 ^{o}C while at 40 ^{o}C there was no production of zoospores (Table 4.13: Fig. 4.5).

Similar reports were made by Matheron and Matejka, 1992; Mizubuti and Fry, 1998 and Timmer *et al*. 2000.Peries and Fernando (1972) reported that a close relationship between temperature and spore production was observed, the greatest number being produced at 28 °C. Few sporangia were produced above 30 °C and

Table 4.13. Effect of temperature, relative humidity and CO_2 on induction of zoospores of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

Treatment	*Number of zoospores ($\times 10^3$) / ml
Temperature (^oC)	
20	112[c]
25	154[b]
30	183[a]
35	86[d]
40	00[e]
Relative Humidity (%)	
50	103[e]
75	152[d]
85	178[b]
95	181[c]
100	188[a]
CO_2 (ppm)	
350	159[b]
550	183[a]
750	172[a]

* Mean of twelve replications
Means followed by a common letter are not significantly different at 5% levelby Tukey's HSD

Figure 4.5. Influence of temperature on induction of zoospores of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

below 25 °C, showing that the ambient temperature has a more critical effect on reproduction than on growth.

Further experiments were also conducted to determine the impact of relative humidity (50, 75, 85, 95 and 100 %) and different CO_2 levels (350, 550 and 700 ppm) on induction of sporangia of *P. drechsleri* f. sp. *cajani*using the diluted tomato juice method under controlled incubator conditions.

The impact of relative humidity on induction of zoospores are presented in Table 4.13 and Fig. 4.6. The number of zoospores increased with increase in relative humidity. Relative humidity of 100 per cent induced highest number of zoospores (188 $\times 10^3$ per ml) and statistically superior over others, followed by 95 per cent (181 $\times 10^3$ per ml) and least number of zoospores was observed at 50 per cent with 103 $\times 10^3$ per ml.

These results were on par with the finding of Agarwal *et al*. (2002) where they found that the maximum number of sporangia was produced at high relative humidity (88-100 %). The relative humidity played great role in influencing the amount of sporangia and its longevity and also favoured the zoospore formation, liberation, viability and infection to host plants (Granke and Hausbeck, 2010b).

Similarly elevated CO_2 levels also influenced the induction of zoospores and the number of zoospores increased with increased level of CO_2 (Table 4.13).Of the three levels of CO_2 tested, maximum zoospores were induced at 550 ppm (183 $\times 10^3$) followed by 700 ppm (172 $\times 10^3$) and least (159 $\times 10^3$) was observed at 350 ppm (Fig 4.7).

Our findings are in accordance with Coakley *et al.*(1999); Hibberd *et al.* (1996a,b); McElrone *et al.*(2005) and Chakraborty *et al*. (2000b) who reported that the elevated CO_2 is known to stimulate fungal pathogen growth rates, aggressiveness, and fecundity.

4.8. Studies on epidemiology of Phytophthora blight of Pigeonpea

4.8.1. Influence of temperature on disease development

Temperature is a major environmental factor that regulates plant growth and development as well as its interaction with other organisms (Long and Woodward, 1988). It is alsoan important factor known to be of critical in governing infectivity of various *Phytophthora* spp. in different hosts (Duniway, 1983; Gerlach *et al*. 1976; Grove and Boal, 1991; Grove *et al*. 1985; Hau and Kranz, 1990; Timmer *et al*. 2000).

The impact of different temperatures were tested for disease development under incubator condition using soil mixing and soil drenching method of inoculation.

Figure 4.6. Influence of relative humidity on induction of zoospores of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

Figure 4.7. Influence of CO_2 on induction of zoospores of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

The results revealed that in both the inoculation methods the per cent disease incidence increased with increase in temperature (Table 4.14).

On perusal of data it was found that in soil mixing method of inoculation, the per cent disease incidence was 6.0 after 4 days of inoculation at 15 °C and gradually increased to 42.6 per cent, whereas at 20 °C PDI was 12.6 per cent from second day of inoculation and 62.6 per cent at sixth day after inoculation. However the disease incidence was observed from the first day of inoculation itself at temperature of 25 °C, 30 °C and 35 °C and attained 100 per cent PDI from fourth day of inoculation whereas at 40 °C PDI was 100 per cent from first day of inoculation itself.

A variation was observed in soil drenching method of inoculation the disease did not occur up to 20 °C when incubated for 6 days. PDI of 36.0 per cent was recorded 3 days after inoculation at 25 °C whereas the disease incidence was observed from first day of inoculation at 30 °C, 35 °C and 40 °C. Hundred per cent disease incidence was recorded at 30 °C and 35 °C at 6^{th} day after inoculation, whereas at 40 °C 100 per cent per cent disease incidence was recorded at 3^{rd} day after inoculation. Whereas, 5 and 10 °C did not induce any symptoms in both inoculation methods. Further,uninoculated plants at all the temperature levels remained healthy throughout the experiment.

Incubation period (Time between inoculation and expression of first symptoms) and temperature levels was inversely proportional to each other. Incubation period of 24 hours was observed in temperature of 25, 30, 35 and 40 °C whereas 48 hours at 20 °C followed by 72 hours in 15 °C (Fig. 4.8).

The results of the present study are in agreement with the finding of Timmer and Zitko, (2000), who reported optimum temperature of 27 to 30 °C for infection and development of Phytophthora brown rot of citrus. High temperature often inhibits disease resistance or plant immunity (Dropkin, 1969). The present study also indicated that disease incidence could be recorded even at 40 °C. Increased disease at high temperature levels could be because of decreased amount of defense in the plants. Correspondingly, Zhu *et al*. (2010) reported that an elevated growth temperature often inhibits plant defense responses and renders plants more susceptible to pathogens. However, the mechanism underlying this modulation are unknown.

Table 4.14. Effect of temperature on the disease incidence of Phytophthora blight of Pigeonpea under growth chamber conditions.

S. No.	Temperature (^{o}C)	Soil mixing method of inoculation							Soil drenching method of inoculation						
		Per cent disease incidence at different days after inoculation*							Per cent disease incidence at different daysafterinoculation*						
		1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean
1	5	0.0^{e} (0.9)	0.0^{f} (0.9)	0.0f (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{g} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{e} (0.9)	0.0^{e} (0.9)	0.0^{d} (0.9)	0.0^{c} (0.9)	0.0^{e} (0.9)
2	10	0.0^{e} (0.9)	0.0^{f} (0.9)	0.0^{f} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{g} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{e} (0.9)	0.0^{e} (0.9)	0.0^{d} (0.9)	0.0^{c} (0.9)	0.0^{e} (0.9)
3	15	0.0^{e} (0.9)	0.0^{f} (0.9)	0.0^{f} (0.9)	6.0c (14.0)	19.3c (25.3)	42.6c (38.0)	11.3f (19.3)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{e} (0.9)	0.0^{e} (0.9)	0.0^{d} (0.9)	0.0^{c} (0.9)	0.0^{e} (0.9)
4	20	0.0^{e} (0.9)	12.6^{e} (20.4)	29.3^{e} (31.3)	39.3^{b} (36.4)	59.3^{b} (45.7)	62.6b (47.1)	34.4e (34.0)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{e} (0.9)	0.0^{e} (0.9)	0.0^{d} (0.9)	0.0^{c} (0.9)	0.0^{e} (0.9)
5	25	29.3^{d} (31.3)	56.0^{d} (44.2)	82.6^{d} (56.5)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	78.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	36.0^{d} (34.8)	46.0^{d} (39.6)	69.3^{c} (50.1)	70.0^{b} (50.5)	36.9^{d} (35.2)
6	30	49.3c (41.2)	76.0^{c} (53.2)	89.3^{c} (60.2)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	85.8^{c} (58.2)	3.3^{c} (10.4)	16.0^{c} (23.0)	39.3^{c} (36.4)	59.3^{c} (45.7)	86.0^{b} (58.3)	100^{a} (71.8)	50.7^{c} (41.8)
7	35	89.3^{b} (60.2)	89.3^{b} (60.2)	95.8^{b} (64.9)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	95.8^{b} (64.8)	9.3^{b} (17.5)	62.6^{b} (47.1)	66.0^{b} (48.7)	79.3^{b} (54.8)	86.0^{b} (58.3)	100^{a} (71.8)	67.2^{b} (49.2)
8	40	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	42.6^{a} (38.0)	72.6^{a} (51.7)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	100^{a} (71.8)	85.9^{a} (58.2)

*Mean of three replications

Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).

Figures in parentheses are arcsine transformed values.

Figure 4.8. Effect of temperature on the disease incidence of Phytophthora blight of Pigeonpea under growth chamber conditions.

4.8.2. Effect of relative humidity on disease development

The influence of relative humidity on disease development under controlled incubator conditions was assessed using soil mixing and soil drenching method of inoculation and the results are presented in Table 4.15.

The per cent disease increased with the increase in relative humidity in both methods of inoculation. In soil mixing method, relative humidity of 100 and 95 per cent showed the highest disease incidence with 99.7 per cent and found statistically superior over others, followed by 85 and 75 per cent of RH with incidence of 94.7 %. A relative humidity of 50 per cent showed low incidence of 37.6 % per cent. Similar results were obtained in the soil drenching method of inoculation (Fig. 4.9).

Incidence of *Phytophthora* increased at high relative humidity because high humidity favoured the increase in the number of sporangia and aid in zoospore liberation, viability and infection ofhost. The results were in accordance with Pal *et al*. 1970 who opined increased Phytophthora stem rot of Pigeonpea was due to increased relative humidity.Lacey (1967) and Rotem *et al*. (1971) reported late blight of potato developed on potato foliage under conditions when ambient relative humidity is above 90 per cent. Similarly Granke and Hausbeck (2010b) reported increased relative humidity increased the incidence of Phytophthora rot of cucumber fruit.

4.8.3. Effects of CO_2 on disease development

Since, global CO_2 concentration has been steadily increasing, we need to predict the effect of elevated CO_2 concentration on plant disease development. Chakraborty *et al*. (1998) reported thatout of ten biotrophic pathogens studied, disease severity enhanced in six pathogens and reduced in four pathogens at high CO_2 level. Whereas out of 15 necrotrophic pathogens, disease severity increased in nine pathogens, reduced in four pathogens and remained unchanged in the other two. In this junction, we studied the influence of elevated CO_2 concentration on Phytophthora blight disease under incubators and Open Top Chambers (OTC) separately.

The results of present investigation revealed that, increase in CO_2 levels increased the disease incidence in both conditions (Table 4.16). The maximum disease incidence was observed at 550 ppm in both cases recording 100 per cent incidence. This was at par with 700 ppm yielding 100 and 96.9 per cent in incubators and OTC, respectively. The lowest disease incidence was noticed in 350 ppm

Table 4.15. Effect of relative humidity on the disease incidence of Phytophthora blight of Pigeonpea under growth chamber conditions.

S.No	RH (%)	Soil mixing method of inoculation						Soil drenching method of inoculation					
		Per cent disease incidence at different days*						Per cent disease incidence at different days*					
		1	2	3	4	5	6	1	2	3	4	5	6
1	50%	0.0[a] (0.91)	0.0[e] (0.91)	0.0[f] (0.91)	25.1[e] (28.8)	37.7[e] (35.6)	37.6[e] (35.6)	0.0[a] (0.91)	0.0[e] (0.91)	0.0[e] (0.91)	14.9[f] (22.1)	20.8[f] (26.2)	21.6[e] (26.8)
2	55%	0.0[a] (0.91)	6.3[d] (14.4)	23.2[e] (27.7)	33.2[d] (33.3)	44.7[d] (39.0)	50.6[d] (41.7)	0.0[a] (0.91)	11.6[d] (19.5)	14.5[d] (21.8)	26.9[e] (29.9)	46.6[e] (39.9)	60.3[d] (46.1)
3	60%	0.0[a] (0.91)	18.5[c] (24.7)	37.4[d] (35.5)	53.1[c] (42.9)	77.5[c] (54.4)	83.6[c] (63.1)	0.0[a] (0.91)	12.8[d] (20.5)	15.7[d] (22.7)	25.0[e] (28.8)	58.1[d] (45.1)	70.7[c] (52.6)
4	65%	0.0[a] (0.91)	26.0[b] (29.4)	43.2[d] (38.3)	56.4[c] (44.4)	76.5[c] (53.9)	83.6[c] (63.1)	0.0[a] (0.91)	17.0[c] (23.7)	28.2[c] (30.6)	37.3[d] (35.4)	57.5[d] (44.8)	70.7[c] (50.8)
5	75%	0.0[a] (0.91)	27.3[b] (30.1)	69.2[c] (50.1)	89.7[b] (60.4)	89.7[b] (60.5)	94.7[b] (63.9)	0.0[a] (0.91)	24.6[b] (28.6)	46.9[b] (40)	59.6[c] (45.8)	72.3[c] (51.5)	78.8[b] (55.6)
6	85%	0.0[a] (0.91)	27.1[b] (30.0)	69.2[c] (50.1)	89.7[b] (60.4)	89.7[b] (60.4)	94.7[b] (63.9)	0.0[a] (0.91)	25.5[b] (29.1)	46.9[b] (40.0)	72.8[b] (51.8)	82.0[b] (56.2)	82.4[b] (56.4)
7	95%	0.0[a] (0.91)	42.3[a] (37.9)	84.4[b] (57.4)	93.1[a] (62.7)	99.7[a] (70.7)	99.7[a] (70.7)	0.0[a] (0.91)	30.5[a] (31.9)	50.1[a] (41.5)	74.3[a] (52.5)	95.0[a] (64.1)	95.5[a] (64.6)
8	100%	0.0[a] (0.91)	45.0[a] (39.1)	94.8[a] (64.0)	93.1[a] (62.7)	99.7[a] (70.7)	99.7[a] (70.7)	0.0[a] (0.91)	30.2[a] (31.7)	50.1[a] (41.5)	74.8[a] (52.7)	93.2[a] (62.8)	95.5[a] (64.6)

*Mean of three replications

Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).

Figures in parentheses are arcsine transformed values.

Table 4.16. Effect of CO_2 on disease incidence of Phytophthora blight of Pigeonpea under controlled conditions.

S. No.	Treatment	Open Top Chamber (OTC) condition							Growth chambers						
		*Per cent disease incidence at different days after inoculation							*Per cent disease incidence at different days after inoculation						
		1	2	3	4	5	6	7	1	2	3	4	5	6	7
1	350 ppm	0.0^{a} (0.9)	13.3^{c} (21.7)	28.3^{c} (31.2)	42.5^{c} (38.3)	56.5^{c} (44.6)	72.5^{c} (52.1)	90.1^{b} (61.1)	0.0^{a} (0.9)	10.2^{a} (19.3)	16.6^{c} (23.9)	36.5^{c} (35.2)	67.5^{c} (49.8)	72.6^{c} (52.3)	89.3^{b} (60.9)
2	550 ppm	0.0^{a} (0.9)	19.2^{b} (26.2)	46.6^{b} (40.3)	70.0^{b} (51.0)	80.9^{a} (55.8)	90.1^{a} (63.1)	100^{a} (71.8)	0.0^{a} (0.9)	10.3^{a} (19.5)	30.5^{b} (32.4)	51.3^{a} (42.6)	71.2^{b} (50.9)	86.7^{b} (59.2)	100^{a} (71.8)
3	700 ppm	0.0^{a} (0.9)	24.0^{a} (28.9)	52.2^{a} (42.9)	71.0^{a} (51.3)	76.6^{b} (53.8)	88.6^{b} (60.7)	96.9^{a} (68.1)	0.0^{a} (0.9)	9.6^{a} (18.8)	31.5^{a} (32.9)	48.5^{b} (41.2)	72.6^{a} (52.2)	88.7^{a} (60.3)	100^{a} (71.8)
4	Control	0.0^{a} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{a} (0.9)	0.0^{b} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{c} (0.9)

*Mean of three replications

Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).

Figures in parentheses are arcsine transformed values.

Figure 4.9. Influence of relative humidity on the disease incidence of Phytophthora blight of Pigeonpea under growth chamber conditions.

recording 89.3 and 90.1 per cent in incubator and OTC respectively. (Fig. 4.10: Plate 4.5).

The results are in accordance with the findings of Thompson and Drake, (1994); Kobayashi *et al.*(2006); Eastburn *et al.*(2009) and Andrew *et al.*(2010)who reported increased amount of disease at elevated CO_2level in many plant pathogen interactions. The increased severity of the disease may be attributed to stimulation of fungal pathogen growth rates, aggressiveness, and fecundity at elevated level of CO_2 (Coakley *et al.* 1999; Hibberd *et al.* 1996 a,b; McElrone *et al.* 2005 and Chakraborty *et al*. 2000b).

4.8.4. Effect of soil type

The effect of soil type on development of disease was assessed by soil mixing and soil drenching method of inoculation on black and red sandy loam soil.

Among the soil types, red loamy soil was statistically superior over black clay soil with incidence of 93.33 per cent in soil mixing and 80.0 per cent in soil drenching method of inoculation (Table 4.17). Black clay soil showed a disease incidence of 43.33 per cent and 36.66 per cent in soil mixing and soil drenching method of inoculation. On the other hand soil mixing method of inoculation was found statistically superior over soil drenching method in both red loamy 93.3 per cent and black clay soil 43.3 per cent, whereas soil drenching method showed 80.0 and 36.6 per cent in red loamy and black clay soil respectively. (Fig. 4.11). Theincubation period was two days in red loamy soil whereas three days in black clay soil irrespective of inoculation method used.

The water holding capacity of soil is a function of soil profile. Few authors proposed a correlation between soil profile and Phytophthora incidence. Goodall (1962) correlated incidence of Phytophthora root rot in avocado with texture of the soil. Incidence of the disease was high in sandy loam soil compared to clay soil as sandy loamy soil aid in movement of zoospores and establish a new infections. However, Masood *et al*. (2005) reported that the Phytophthora blightdisease appeared both in alfisols and vertisols.

4.8.5. Effect of soil moisture

Soil moisture status is an important factor in stimulating *Phytophthora* spp. (Wilcox and Mircetich, 1985a; Bowers *et al*. 1990; Cafe-Filho and Duniway, 1995). Poor soil aeration and prolonged soil saturation promote sporangia formation and

Table 4.17. Effect of soil type on incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

S. No.	Treatment	IP* (days)	Per cent disease incidence at different days after inoculation**						
			1	2	3	4	5	6	7
1	Red loamy soil – Soil drenching	2	0.00[a] (0.91)	20.00[a] (25.7)	39.67[a] (36.6)	43.33[a] (38.4)	53.39[b] (43.0)	66.51[b] (48.9)	80.00[b] (55.2)
2	Red loamy soil – Soil mixing	2	0.00[a] (0.91)	13.33[b] (21.0)	39.67[a] (36.6)	43.44[a] (38.4)	69.67[a] (50.3)	87.07[a] (58.9)	93.33[a] (62.9)
3	Black clay soil– Soil drenching	3	0.00[a] (0.91)	0.00[c] (0.91)	6.67[b] (14.8)	13.33[b] (21.0)	23.99[d] (28.2)	27.31[d] (30.1)	36.66[d] (35.1)
4	Black clay soil – Soil mixing	3	0.00[a] (0.91)	0.00[c] (0.91)	3.33[c] (10.5)	13.37[b] (21.0)	26.65[c] (29.5)	33.33[c] (33.5)	43.33[c] (38.4)
5	Red loamy soil - Soil drenching- Mock inoculation	-	0.00[a] (0.91)	0.00[c] (0.91)	0.00[d] (0.91)	0.00[c] (0.91)	0.00[e] (0.91)	0.00[e] (0.91)	0.00[e] (0.91)
6	Red loamy soil - Soil mixing - Mock inoculation	-	0.00[a] (0.91)	0.00[c] (0.91)	0.00[d] (0.91)	0.00[c] (0.91)	0.00[e] (0.91)	0.00[e] (0.91)	0.00[e] (0.91)
7	Black clay soil - Soil drenching - Mock inoculation	-	0.00[a] (0.91)	0.00[c] (0.91)	0.00[d] (0.91)	0.00[c] (0.91)	0.00[e] (0.91)	0.00[e] (0.91)	0.00[e] (0.91)
8	Black clay soil - Soil mixing - Mock inoculation	-	0.00[a] (0.91)	0.00[c] (0.91)	0.00[d] (0.91)	0.00[c] (0.91)	0.00[e] (0.91)	0.00[e] (0.91)	0.00[e] (0.91)

* IP-Incubation period

**Mean of three replications

Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).

Figures in parentheses are arcsine transformed values.

Figure 4.10. Effect of CO_2 on disease incidence of Phytophthora blight of Pigeonpea under growth chamber conditions.

Figure 4.11. Effect of soil type on incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

Open Top Chambers

Control

350 ppm

550 ppm

700 ppm

Plate 4.5. Effect of different levels of CO_2 on disease incidence of Phytophthora blight of Pigeonpea in open top chamber facility.

zoospore release required for growth, reproduction and dissemination of Phytophthora (Agrios, 2005).

In this connection, four soil moisture regimes, *viz*. 60 per cent, 80 per cent, 100 per cent of field capacity and flooding condition was studied on the development of disease under greenhouse condition and data on incidence of disease is presented in Table 4.18.

The results indicated that with the increase in soil moisture there was an increase in incidence of the disease. The incubation period decreased withan increase in soil moisture at both the inoculation methods (Fig. 4.12). Flooding condition was found statistically superior in both inoculation methods with 100 per cent disease incidence followed by 100 per cent moisture of field capacity with disease incidence of 50.3 per cent in soil mixing and 15.0 per cent in soil drenching method of inoculation. However the disease incidence was not observed at 80 and 60 per cent moisture in case of soil drenching method, whereas 25.1 and 22.5 per cent disease incidence was observed in soil mixing method respectively (Plate 4.6).

Peries and Fernando (1972) reported that the sporangia of *Phytophthora meadii*induced andliberated zoospores only in the presence of free water and zoospores germinated poorly and produced little germ-tube growth on dry surfaces, even under highly humid conditions (approaching 100 % RH). Our study also showed that flooding condition showed 100 per cent disease incidence which helped in production of sporangia and movement of zoospores.

4.8.6. Influence of flooding duration

Phytophthora diseases are multicyclic, which means that inoculum may amplify rapidly when the congenial environmental conditions prevails, the most important of which is the presence of free water. The increase in inoculum from low, often undetectable levels to high levels within a few days or weeks is caused by the rapid production of sporangia and zoospores at the surface of infected plant tissues. The short regeneration time and great reproductive capacity imply that *Phytophthora* diseases may develop epidemically when the soil remains excessively wet for prolonged periods (Erwin and Ribeiro, 1996).

An experiment was conducted to determine the role of pre and post flooding in disease development under pot culture condition on susceptible cultivar ICP 7119. Flooding was imposed two and four days before inoculation and one to five days

Table 4.18. Effect of soil moisture on incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

S. No.	SMC* (%)	Soil mixing method of inoculation												
		Per cent Disease Incidence at different days**												
		IP (Hour)	1	2	3	4	5	6	8	10	12	14	16	18	20
1	60	72	0.0^{b} (0.9)	0.0^{b} (0.9)	0.0^{b} (0.9)	0.6^{d} (2.9)	1.9^{d} (4.6)	10.6^{c} (18.7)	14.4^{c} (21.6)	20.6^{b} (26.1)	20.6^{b} (26.1)	20.6^{c} (26.1)	22.5^{c} (27.3)	22.5^{c} (27.3)	22.5^{c} (0.9)
2	80	72	0.0^{b} (0.91)	0.0^{b} (0.91)	0.0^{b} (0.91)	2.8^{c} (9.4)	6.6^{c} (14.6)	12.8^{bc} (20.5)	20.1^{b} (25.7)	21.2^{b} (26.5)	21.2^{b} (26.5)	24.6^{c} (28.4)	25.1^{c} (28.8)	25.1^{c} (28.8)	25.1^{c} (28.8)
3	100	60	3.1^{b} (5.8)	10.0^{b} (9.9)	19.4^{b} (14.2)	27.1^{b} (25.5)	28.4^{b} (26.5)	36.5^{b} (34.7)	40.2^{b} (37.3)	40.2^{b} (37.3)	40.4^{b} (37.7)	50.3^{b} (43.0)	50.3^{b} (43.0)	50.3^{b} (43.0)	50.3^{b} (43.0)
4	Flooding	30	9.4^{a} (15.5)	30.1^{a} (27.8)	50.6^{a} (37.2)	69.4^{a} (46.9)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)
5	Control	0	0.0^{b} (0.9)	0.0^{b} (0.9)	0.0^{b} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)
S. No	SMC* (%)	Soil drenching method of inoculation												
		Per cent Disease Incidence at different days**												
		IP (Hour)	1	2	3	4	5	6	8	10	12	14	16	18	20
1	60	0	0.0^{a} (0.9)	0.0^{b} (0.9)	0.0^{b} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)
2	80	0	0.0^{a} (0.9)	0.0^{b} (0.9)	0.0^{b} (0.9)	0.0^{c} (0.9)	0.0c (0.9)	0.0c (0.9)	0.0c (0.9)	0.0c (0.9)	0.0c (0.9)	0.0c (0.9)	0.0^{c} (0.9)	0.0c (0.9)	0.0c (0.9)
3	100	96	0.0^{a} (0.9)	0.0^{b} (0.9)	0.0^{b} (0.9)	2.5b (9.1)	5.0^{b} (12.8)	15.0^{b} (22.2)	15.0^{b} (22.2)	15.0^{b} 22.3)	15.0^{b} (22.3)	15.0^{b} (22.3)	15.0^{b} (22.3)	15.0^{b} (22.3)	15.0^{b} (22.3)
4	Flooding	48	0.0^{a} (0.9)	22.5a (27.3)	45.1a (39.2)	77.4a (53.3)	82.4a (56.4)	97.4a (56.4)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} (71.3)	100^{a} 71.3)
5	Control	0	0.0^{a} (0.9)	0.0^{b} (0.9)	0.0^{b} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)	0.0^{c} (0.9)

*SMC –Soil moisture content, **Mean of three replications, Figures in parentheses are arcsine transformed values.
Means followed by a common letter are not significantly different at 5% level by Tukey's (HSD)

Soil drenching method of inoculation **Control** **Soil mixing method of inoculation**

SMC : Soil moisture content at field capacity

Plate 4.6. Influence of soil moisture levels on disease incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

Figure 4.12. Effect of soil moisture on disease incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

after inoculation and the per cent disease incidence was recorded and presented in Table 4.19.

Among the pre and post inoculation flooding, post inoculation flooding was statistically superior over pre inoculation flooding in both the inoculation methods. However, post inoculation flooding for 3, 4, and 5 days was superior over other flooding treatments with incidence of 100 per cent in both inoculation methods followed by post inoculation flooding for 2 days with incidence of 59.0 and 65.3 per cent in soil mixing and soil drenching method respectively. Least incidence of the disease was observed in pre inoculation flooding for two days with 44.7 and 43.5 per cent in soil mixing and soil drenching method of inoculation respectively and it is on par with pre inoculation flooding for four days with 46.5 and 45.7 per cent in soil mixing and soil drenching method of inoculation respectively. (Fig.13).

The results are in agreement with the finding of Duniway (1977) in Phytophthora root rot in safflower, Baker and Macdonald (1981) in Phytophthora root and crown rot of Rhododendron and Barta, (1986) in Phytophthora root rot of alfalfa. Similarly Kuan and Erwin (1980), reported post inoculation flooding treatments were much more effective than pre inoculation flooding in increasing the Phytophthora root rot of alfalfa. Flooding either increases the production and dispersion of inoculum or negatively affects root metabolism, thus facilitating infection and growth within the diseased tissue.

The occurrence of *Phytophthora* in flooded soils has been attributed to requirements of the pathogen for high soil moisture (Zentmyer, 1980). Indeed, evidence exists which indicates that soil-water status can exert a determining influence on several epidemiologically important stages in the life of *Phytophthora* spp. Members of this genus reproduce by formation of sporangia which, under proper conditions, germinate to release free-swimming zoospores. The zoospores of *Phytophthora* are chemotactic and can swim to plant roots to establish new infections.

Excess irrigation and rainfall are considered to be the most important factors that increase the severity and spread of *Phytophthora* incited diseases. In turn, the duration of free water in soil is the most important environmental factor in the development of disease caused by *Phytophthora* because it is during this time that propagules proliferate and infect (Erwin and Ribeiro, 1996). In addition, zoospores, travel in the soil in irrigation water, rainfall run-off and movement of soil.

Table 4.19.Effect of flooding duration on incidence of Phytophthora blight of Pigeonpea under greenhouse conditions .

Treatment	Soil mixing method of inoculation							Soil drenching method of inoculation						
	Per cent disease incidence at different days after inoculation*							Per cent disease incidence at different days after inoculation*						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T-1	0.0[a] (0.9)	20.3[c] (25.9)	34.7[d] (34.1)	34.7[d] (34.1)	39.7[g] (36.6)	42.7[d] (38.1)	44.7[d] (39.0)	0.0[a] (0.9)	17.2[c] (23.8)	27.7[d] (30.4)	32.2[e] (32.8)	39.1[e] (36.3)	43.5[e] (38.4)	43.5[f] (38.4)
T-2	0.0[a] (0.9)	20.7[c] (26.26)	34.1[d] (33.8)	39.1[c] (36.3)	43.5[f] (38.4)	46.5[d] (39.8)	46.5[d] (39.8)	0.0[a] (0.9)	17.8[b] (24.2)	29.7[d] (31.5)	31.7[e] (32.5)	37.7[e] (35.6)	45.7[e] (39.5)	45.7[f] (39.5)
T-3	0.0[a] (0.9)	24.7[b] (28.6)	37.2[c] (35.4)	40.2[c] (36.9)	40.2[f] (36.9)	46.4[d] (39.8)	49.7[c] (41.3)	0.0[a] (0.9)	15.2[c] (22.4)	27.8[d] (30.4)	31.0[e] (32.1)	40.3[e] (36.9)	56.1[d] (44.2)	56.1[e] (44.2)
T-4	0.0[a] (0.9)	24.6[b] (28.6)	31.2[e] (32.3)	40.4[c] (37.0)	49.9[e] (41.4)	59.0[c] (45.5)	59.0[b] (45.5)	0.0[a] (0.9)	18.5[b] (24.7)	37.2[c] (35.4)	46.9[d] (40.0)	59.1[d] (45.6)	65.2[c] (48.3)	65.3[d] (48.3)
T-5	0.0[a] (0.9)	27.9[a] (30.5)	49.8[a] (41.4)	68.4[b] (49.7)	77.8[c] (54.1)	90.7[b] (61.1)	100.0[a] (71.3)	0.0[a] (0.9)	15.3[c] (22.4)	49.8[a] (41.4)	65.9[c] (48.6)	84.1[b] (57.3)	93.4[b] (62.9)	100.0[a] (71.3)
T-6	0.0[a] (0.9)	24.7[b] (28.6)	50.2[a] (41.5)	68.5[b] (49.8)	81.1[b] (55.7)	100.0[a] (71.3)	100.0[a] (71.3)	0.0[a] (0.9)	18.5[a] (24.7)	49.8[a] (41.4)	68.4[b] (49.7)	81.2[c] (55.8)	100.0[a] (71.3)	100.0[a] (71.3)
T-7	0.0[a] (0.9)	27.9[a] (30.5)	43.5[b] (38.4)	81.2[a] (55.8)	93.4[a] (62.9)	100.0[a] (71.3)	100.0[a] (71.3)	0.0[a] (0.9)	18.2[a] (24.5)	46.2[b] (39.7)	74.2[a] (52.4)	90.6[a] (61.0)	100.0[a] (71.3)	100.0[a] (71.3)
T-8	0.0[a] (0.9)	0.0[d] (0.9)	0.0[f] (0.9)	0.0[f] (0.9)	0.0[h] (0.9)	0.0[e] (0.9)	0.0[e] (0.9)	0.0[a] (0.9)	0.0[e] (0.9)	0.0[e] (0.9)	0.0[f] (0.9)	0.0[f] (0.9)	0.0[f] (0.9)	0.0[g] (0.9)
T-9	0.0[a] (0.9)	0.0[d] (0.9)	0.0[f] (0.9)	0.0[f] (0.9)	0.0[h] (0.9)	0.0[e] (0.9)	0.0[e] (0.9)	0.0[a] (0.9)	0.0[e] (0.9)	0.0[e] (0.9)	0.0[f] (0.9)	0.0[f] (0.9)	0.0[f] (0.9)	0.0[g] (0.9)

Treatment: T1- Pre inoculation flooding for two days T5- Post inoculation flooding for 3 days

T2- Pre inoculation flooding for four daysT6- Post inoculation flooding for 4 days

T3- Post inoculation flooding for 1 days T7- Post inoculation flooding for 5 days

T4- Post inoculation flooding for 2 days T8- Flooding un inoculated

T9- Non-Flooding

*Mean of three replications

Means followed by a common letter are not significantly different at 5% level by Tukey's (HSD)

Figures in parentheses are arcsine transformed values

T1- Pre inoculation flooding for two days
T2- Pre inoculation flooding for four days
T3- Post inoculation flooding for 1 days
T4- Post inoculation flooding for 2 days
T5- Post inoculation flooding for 3 days
T6- Post inoculation flooding for 4 days
T7- Post inoculation flooding for 5 days
T8- Flooding uninoculated and
T9- Non Flooding

Figure 4.13. Effect of flooding duration on incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

To conclude, flooding conditions predispose the Pigeonpea to the Phytophthora blight hence, Pigeonpea should be sown in well-drained soil and not subject to flooding, proper drainage of fields to reduce the rate and extent of buildup of inoculum, and sowing of crop on raised beds, avoidance of sowing of Pigeonpea in low laying fields are the suggestive agronomical practices to reduce Phytophthora blight incidence.

4.9. Management of Phytophthora blight disease of Pigeonpea

4.9.1. Host plant resistance

4.9.1.1. Influence of inoculation techniques on disease development

The preliminary step for exploiting host plant resistance (HPR) is the development of reliable and repeatable techniques for large scale screening of germplasm and breeding lines. Five different methods of inoculation *viz.*soil drenching with mycelial suspension, spray inoculation of mycelialsuspension, soil mixing with mycelial inoculum, stem inoculation with mycelial inoculum and stem staging with mycelial bits were tested for development of disease in the greenhouse conditions using ten day old susceptible cultivar ICP 7119 using two isolates of *P. drechsleri* f. sp. *cajani* and the results are presented in Table 4.20.

Among the five methods of artificial inoculation, soil mixing of mycelial inoculum was found to be the best with cent per cent disease incidencein Pdc-1 isolate and 90.9 per cent in Pdc-2 isolate, followed by the soil drenching of mycelial suspension 79.0 per cent (Pdc-1) and 70.9 per cent (Pdc-2). However, spray inoculation of mycelial suspension did not induce any infection in both the isolates(Fig. 4.14).Whereas stem inoculation with mycelial inoculum induced 50.8 and 36.6 per cent disease incidence in Pdc-1 and Pdc-2 isolate respectively. The uninoculated control was healthy throughout the experiment.

Upon inoculation of mycelial inoculum to soil induction of sporangia and zoospores in free water takes place and then zoospores will disperse with aid of free water and infect the plants, However under dry conditions sporangia will germinate by germ tube and infect the plants. Results are in accordance with the findings of Kannaiyan *et al.* (1981).

4.9.1.2. Standardization of inoculum quantity

The quantity of inoculum should be selected so as to characterize better relationship between inoculum concentration and infection (Fraedrich *et al*. 1989; McIntyre and Taylor 1976; Milholland *et al*. 1989). Quantityof inoculum is bound

Table 4.20. Effect of inoculation technique on disease incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

Treatment	Pdc-1 Isolate							Pdc-2 Isolate						
	*Per cent Disease Incidence at different days after inoculation							*Per cent Disease Incidence at different days after inoculation						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T1	0.0[a] (0.9)	25.9[a] 29.3	36.2[b] (34.9)	46.2[b] (39.7)	57.2[b] (44.7)	60.5[b] (46.2)	79.0[b,c] (52.3)	0.0[a] (0.9)	10.0[b] (18.1)	26.9[a] (29.9)	30.3[b] (31.8)	50.5[b] (41.7)	57.3[b] (44.7)	70.9[b,d] (53.7)
T2	0.0[a] (0.9)	0.0[d] (0.9)	0.0[e] (0.9)	0.0[e] (0.9)	0.0[d] (0.9)	0.0[e] (0.9)	0.0[e,i] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a,i] (0.9)
T3	0.0[a] (0.9)	20.3[b] (25.9)	39.5[a] (36.5)	55.9[a] (44.1)	76.8[a] (53.6)	100.0[a] (71.8)	100.0[a,a] (71.8)	0.0[a] (0.9)	12.7[a] (20.4)	23.3[b] (27.8)	49.2[a] (35.1)	57.2[a] (44.7)	87.2[a] (59)	90.9[a,b] (65.9)
T4	0.0[a] (0.9)	0.0[d] (0.9)	17.3[c] (23.9)	23.5[c] (27.9)	23.7[c] (28.0)	36.2[c] (34.9)	50.8[c,e] (37.2)	0.0[a] (0.9)	0.0[a] (0.9)	10.3[d] (18.4)	20.3[c] (25.9)	23.7[d] (28)	33.0[d] (33.2)	36.6[d,h] (35.1)
T5	0.0[a] (0.9)	10.7[c] (18.7)	13.2[d] (20.8)	17.1[d] (23.8)	23.0[c] (27.6)	30.4[d] (31.8)	46.4[d,f] (31.8)	0.0[a] (0.9)	9.8[b] (18)	16.3[c] (23.2)	21.9[c] (26.9)	33.1[c] (33.3)	36.4[c] (35)	40.3[c,g] (36.9)
T6	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a,i] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a,i] (0.9)

Treatment: T1- Soil drenching with mycelial suspension (100 ml/ pot)
T2- Spray inoculation of mycelialsuspension ((100 ml/ pot))
T3- Soil mixing with mycelial inoculum(100 gram/ pot)
T4- Stem inoculation with mycelial inoculum
T5- Stem staging with mycelial bits
T6- Control

*Mean of three replications
In a column, means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
In a row, means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
Figures in parentheses are arcsine transformedvalues.

Figure 4.14. Effect of inoculation techniques on disease incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

to influence the disease incidence andinfection takes place only when minimum inoculum potential of the pathogen is present in the soil. The best inoculation methods *viz*. soil mixing of mycelial inoculum and soil drenching of mycelial suspension were further selected to quantify the optimum inoculum quantity to establish the disease.

In the present study, it was observed that with the increase in quantity of inoculum there was substantial increase in the infection in both the inoculation methods (Table 4.21) Among the inoculum quantity tested, 12.5 per cent and 10.0 per cent of inoculum was found to induce maximum disease incidence with 100 per cent in soil mixing method, while in soil drenching method it was 85.3 per cent, whereas at 5.0 and 7.5 per cent of inoculum quantity the disease was 3.3 and 83.3 per cent in soil mixing while the disease incidence was 6.6 and 50.0 per cent in soil drenching method. Further the quantity of 1.0 and 2.5 per cent of inoculum did not induce the disease on inoculated plants and were as good as control uninoculated plants (Fig. 4.15). Hence, 10 per cent of inoculum was used to carryout the experiment.

The amount of initial inoculum determines the amount of disease. The disease increased with the increase in a amount of inoculum quantityand at certain stage addition of extra inoculum did not increase the amount of disease. This could be due to saturation of all the infection courts Van der Plank (1975). In this study both inoculum of 10.0 and12.5 per cent has shown the same disease incidence. Further it is concluded that, use of 10.0 per cent of inoculum is optimum for inducing the disease.

4.9.1.3. Influence of plant age on disease development

The relationship of the age of a plant and its susceptibility to a disease helps in screening of genotypes against the pathogen to obtain valid results of host plant resistance. Study was conducted to find out the susceptible stage of the Pigeonpea crop for maximum incidence of the disease from seedling stage to flowering stage. All the stages were inoculated by using the soil mixing and soil drenching method of inoculation on susceptible cultivar ICP 7119.

Ontogenetically determined resistance (also known as adult plant resistance) is a type of quantitatively inherited resistance that develops during plant maturation (Boyle and Aust, 1997). Results in Table 4.22 and Plate 4.7 indicated that all the age of plants were susceptible to the disease in both the methods of inoculation. Though

Table 4.21. Effect of inoculum concentration on disease incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

Treatment	Soil mixing method of inoculation							Soil drenching method of inoculation						
	Per cent disease incidence at different days after inoculation*							Per cent disease incidence at different days after inoculation*						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
T1	0.0[a] (0.9)	0.0[c] (0.9)	0.0d (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[a] (0.9)	0.0[b] (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[c] (0.9)	0.0[c] (0.9)	0.0[d] (0.9)
T2	0.0[a] (0.9)	0.0[c] (0.9)	0.0d (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[a] (0.9)	0.0[b] (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[c] (0.9)	0.0[c] (0.9)	0.0[d] (0.9)
T3	0.0[a] (0.9)	0.0[c] (0.9)	0.0d (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[c] (0.9)	3.3[c] (4.1)	0.0[a] (0.9)	0.0[c] (0.9)	0.0d (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[c] (0.9)	6.6[c] (5.5)
T4	0.0[a] (0.9)	0.0[a] (0.9)	3.3[c] (4.1)	33.3[b] (11.7)	56.6[c] (15.4)	60.0[b] (15.9)	83.3[b] (25.9)	0.0[a] (0.9)	0.0[b] (0.9)	3.3[b] (4.1)	13.3[c] (7.6)	30.0[b] (11.2)	33.3[b] (11.7)	50.0[b] (23.6)
T5	0.0[a] (0.9)	15.6[a] (8.2)	33.3[a] (17.6)	52.3[a] (36.8)	63.8[b] (46)	90[a] (51.2)	100[a] (62.06)	0.0[a] (0.91)	13.3[a] (7.6)	25.6[a] (16.2)	40.2[b] (25.8)	55.3[a] (35)	69.6[a] (39.4)	85.2[a] (47.5)
T6	0.0[a] (0.9)	13.3[b] (21.8)	29.4[b] (32.4)	52.5[a] (42.7)	68.5[a] (48.5)	91[a] (61.3)	100[a] (71.3)	0.0[a] (0.9)	14.2[a] (20.6)	25.2[a] (28.9)	42.3[a] (37)	55.4[a] (42.7)	70.1[a] (50.1)	85.3[a] (58.8)
T7	0.0[a] (0.9)	0.0[a] (0.9)	0.0[c] (0.9)	0.0d (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[a] (0.9)	0.0[b] (0.9)	0.0[c] (0.9)	0.0[d] (0.9)	0.0[c] (0.9)	0.0[c] (0.9)

Treatment: T1-1.0 per cent of inoculum to soil weight
T2-2.5 per cent of inoculum to soil weight
T3-5.0 per cent of inoculum to soil weight
T4-7.5 per cent of inoculum to soil weight
T5- 10 per cent of inoculum to soil weight
T6- 12.5 per cent of inoculum to soil weight
T7- Uninoculated control

*Mean of three replications
Means followed by a common letter are not significantly different at 5% level by Tukey's HSD.
Figures in parentheses are arcsine transformedvalues.

Figure 4.15. Effect of inoculum quantity on development of Phytophthora blight of Pigeonpea under greenhouse conditions.

Table 4.22. Effect of plant age on incidence of Phytophthora blight in Pigeonpea under greenhouse conditions.

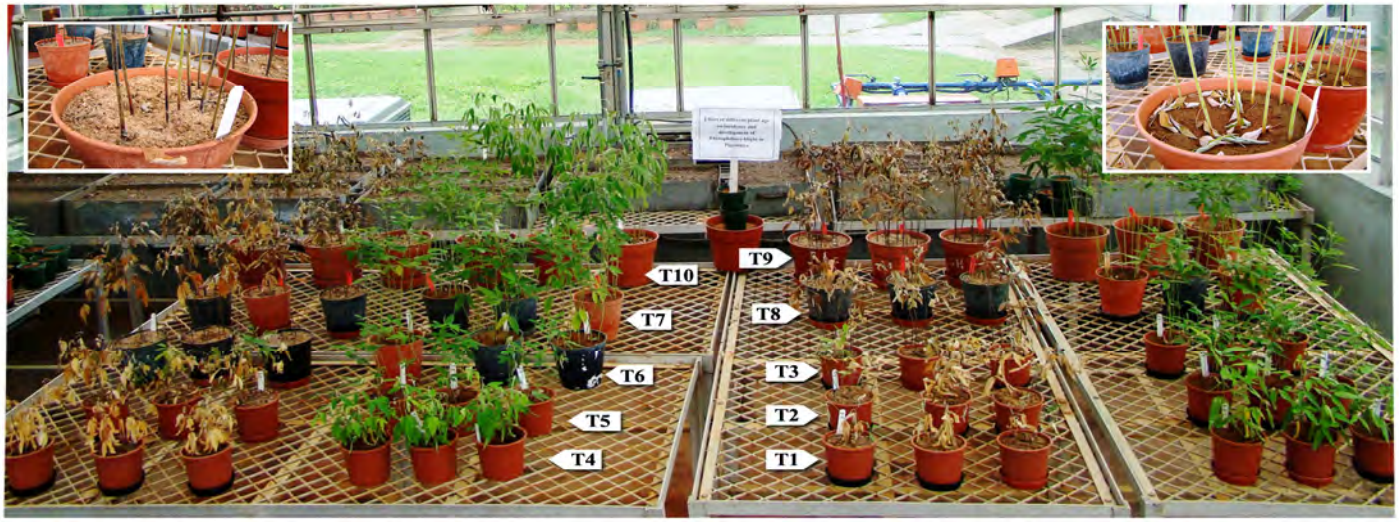
S.No.	Plant age (DAS)*	Soil mixing method of inoculation							Soil drenching method of inoculation						
		Per cent disease incidence at different days after inoculation**							Per cent disease incidence at different days after inoculation**						
		1	2	3	4	5	6	7	1	2	3	4	5	6	7
1	120	0.0^{a} (0.91)	10.9^{c} (18.9)	23.0^{b} (27.6)	56.3^{b} (44.3)	70.9^{bc} (50.8)	88.8^{a} (59.9)	100.0^{a} (71.3)	0.0^{a} (0.91)	0.0^{e} (0.91)	16.3^{d} (23.2)	46.7^{d} 39.9)	62.7^{e} (47.2)	80.9^{d} (55.6)	100.0^{a} (71.3)
2	90	0.0^{a} (0.91)	6.4^{f} (14.5)	16.3^{c} (23.2)	39.7^{d} (36.6)	65.1^{d} (48.2)	81.8^{d} (56.1)	100.0^{a} (71.3)	0.0^{a} (0.91)	6.2^{d} (14.2)	16.3^{d} (23.2)	40.0^{f} (36.8)	62.7^{e} (47.2)	80.6^{d} (55.5)	100.0^{a} (71.3)
3	75	0.0^{a} (0.91)	9.9^{d} (18)	23.0^{b} (27.6)	63.0^{a} (47.3)	71.3^{bc} (51)	85.3^{b} (57.9)	100.0^{a} (71.3)	0.0^{a} (0.91)	0.0^{e} (0.91)	9.7^{e} (17.8)	55.9^{b} (44.1)	72.7^{ab} (51.7)	84.8^{a} (57.6)	100.0^{a} (71.3)
4	60	0.0^{a} (0.91)	9.1^{e} (17.3)	23.0^{b} (27.6)	46.3^{c} (39.8)	63.2^{e} (47.4)	82.8^{c} (56.6)	100.0^{a} (71.3)	0.0^{a} (0.91)	13.0^{a} (20.7)	23.0^{b} (27.6)	46.0^{d} (39.6)	68.6^{cd} (49.8)	79.2^{e} (54.8)	100.0^{a} (71.3)
5	45	0.0^{a} (0.91)	6.2^{f} (14.2)	16.3^{c} (23.2)	63.0^{a} (47.3)	72.2^{ab} (51.4)	84.8^{b} (57.7)	100.0^{a} (71.3)	0.0^{a} (0.91)	10.9^{b} (18.9)	23.0^{b} (27.6)	56.0^{b} (44.2)	66.2^{de} (48.7)	80.9^{d} (55.6)	100.0^{a} (71.3)
6	30	0.0^{a} (0.91)	13.0^{a} (20.7)	23.0^{b} (27.6)	63.0^{a} (47.3)	73.0^{a} (51.8)	81.4^{d} (55.9)	100.0^{a} (71.3)	0.0^{a} (0.91)	11.0^{b} (19)	26.2^{a} (29.5)	52.6^{c} (42.7)	63.6e (47.6)	79.6^{e} (55)	100.0^{a} (71.3)
7	25	0.0^{a} (0.91)	9.7^{d} (17.8)	16.3^{c} (23.2)	56.4^{b} (44.3)	66.4^{d} (48.8)	80.3^{e} (55.3)	100.0^{a} (71.3)	0.0^{a} (0.91)	6.2^{d} (14.2)	17.6^{cd} (24.1)	56.0^{b} (44.2)	71.0^{bc} (50.9)	83.9 (57.2)	100.0^{a} (71.3)
8	20	0.0^{a} (0.91)	12.0^{b} (19.8)	26.6^{a} (29.7)	63.0^{a} (47.3)	71.0^{bc} (50.9)	85.5^{b} (58)	100.0^{a} (71.3)	0.0^{a} (0.91)	11.0^{b} (19)	23.6^{b} (28)	62.6^{a} (47.1)	75.0^{a} (52.8)	80.9^{db} (55.6)	100.0^{a} (71.3)
9	15	0.0^{a} (0.91)	13.3^{a} (20.9)	26.3^{a} (29.5)	56.4^{b} (44.3)	72.1^{ab} (51.4)	81.5^{d} (55.9)	100.0^{a} (71.3)	0.0^{a} (0.91)	9.9^{c} (18)	23.6^{b} (28)	56.0^{b} (44.2)	70.3^{bc} (50.6)	81.0^{d} (55.7)	100.0^{a} (71.3)
10	10	0.0^{a} (0.91)	10.9^{c} (18.9)	26.3^{a} (29.5)	56.4^{b} (44.3)	70.5^{c} (50.7)	83.6^{c} (57)	100.0^{a} (71.3)	0.0^{a} (0.91)	9.9^{c} (18)	19.7^{c} (25.5)	43.3^{e} (38.3)	57.3^{f} (44.8)	82.2^{c} (56.3)	100.0^{a} (71.3)
11	Control	0.0^{a} (0.91)	0.0^{g} (0.91)	0.0^{d} (0.91)	0.0^{e} (0.91)	0.0^{f} (0.91)	0.0^{f} (0.91)	0.0^{b} (0.91)	0.0^{a} (0.91)	0.0^{e} (0.91)	0.0^{f} (0.91)	0.0^{g} (0.91)	0.0^{g} (0.91)	0.0^{f} (0.91)	0.0^{a} (0.91)

* DAS-Days after sowing

**Mean of three replications

Means followed by a common letter are not significantly different at 5% level by Tukey's (HSD).

Figures in parentheses are arcsine transformedvalues.



Inoculated **Control** **Inoculated** **Control**

T1- 10 day old
T2- 15 day old
T3- 20 day old
T4- 25 day old
T5- 30 day old
T6- 45 day old
T7- 60 day old
T8- 75 day old
T9- 90 day old
T10- 120 day old

Plate 4.7. Effect of plant age on disease incidence of Phytophthora blight of Pigeonpea under greenhouse conditions.

100 per cent infection was observed on 7th day of inoculation, the progress of disease was gradual at all days after inoculation except at 4th day of inoculation where the increase in disease incidence was almost 30 per cent when compared to other days after inoculation. In the present study, it is indicated lack of ontogenic resistance expression in Pigeonpea against *P. drechsleri* f. sp. *cajani.* The results are in accordance with findings ofWilliams *et al*. (1975); Masood *et al.* (2005). Similarly Pande and Sharma (2011) reported that, Pigeonpea susceptible to Phytophthora blight irrespective of growth stage both in the field and greenhouse conditions. Lack in expression of resistance to pathogen is due to biochemical composition of plants, and/or genetically determined factors, have yet to be determined.

4.9.1.4. Screening of Pigeonpea genotypes against *P. drechsleri* f. sp. *cajani*

Several sources of resistance to Phytophthora blight were identified by various researchers *viz.*Pal *et al*. (1970); Kannaiyan *et al*. (1980); Bhargava and Gupta(1983); Singh and Chauhan(1985); Mishra and Shukla (1986); Sharma *et al*. (1995) and Reddy *et al*. (1990). However, most of these lines were later found susceptible to *P. drechsleri* f. sp. *cajani* under natural epidemic conditions in Deccan Plateau of India (Sharma *et al.* 2006).This could be due to frequent evolution of new pathotypes and coexistence of more than one pathotype at one location. In this connection, we have screened the existing Pigeonpea genotypes and improved Pigeonpea Wilt and Sterility Mosaic Disease resistant Nursery (PWSMDN) using stem inoculation method. In addition the reaction of improved Pigeonpea breeding lines under natural ecosystem to Phytophthora blight at research farm of ICRISAT, Patancheru was determined during the periodical survey conducted in *kharif* 2013.

4.9.1.4.1. Screening of Pigeonpea genotypes against *P. drechsleri* f. sp. *cajani* at ICRISAT, Patancheru

Nineteen Pigeonpea genotypeswere screened in research farm (RL-17) of ICRISAT, Patancheru,usingstem inoculation method and the results are presented in Fig. 4.16. Results revealed that, ICP 9174 showed the least disease incidence (12.2 %), followed by ICP 8863 (14.3 %) whereas, highest incidence was recorded by genotype ICPL 161 (52.5 %) and was on par with genotype ICP 7119 (52.3 %) (Table 4.23). Based on disease reaction, the genotypeswere grouped as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible. Among the 19 Pigeonpea genotypes, nine genotypes (ICP 9174, ICP 8863, JA-4, ICP 11302, ICP 11290, BDN 2, Bahar, KPBR 80 2 1, ICP 2376) showed the moderately resistant

Table 4.23. Screening of Pigeonpea genotypes against Phytophthora blight disease under field conditions at ICRISAT, Patancheru.

S. No.	Genotypes	Per cent disease incidence at different days after inoculations*				Disease Reaction
		05	10	20	40	
1	ICPL 288	8.3^{cd} (16.5)	17.2^{f} (23.8)	17.2^{ef} (23.8)	21.0^{f} (26.4)	Moderately Susceptible
2	ICP 4135	21.3^{i} (26.5)	21.9^{g} (26.9)	24.3^{g} (28.4)	25.5^{g} (29.1)	Moderately Susceptible
3	ICP 9174	5.8^{ab} (13.8)	10.8^{cd} (26.9)	12.0^{ab} (28.4)	12.2^{a} (29.1)	Moderately Resistant
4	ICP 11290	10.7^{def} (13.8)	25.7^{hi} (18.8)	27.0^{gh} (19.9)	27.8^{g} (20.1)	Moderately Susceptible
5	ICP 8863	6.4^{abc} (14.5)	7.6^{a} (15.8)	9.7^{a} (17.8)	14.3^{abc} (21.7)	Moderately Resistant
6	JA-4	12.7^{h} (20.4)	12.7^{de} (20.4)	13.2^{bc} (20.8)	16.3^{b} (23.2)	Moderately Resistant
7	ICP 11302	12.5^{efg} (20.3)	17.6^{f} (24.1)	18.2^{f} (24.5)	19.1^{def} (25.1)	Moderately Resistant
8	ICP 580	21.1^{i} (26.4)	24.3^{gh} (28.4)	25.8^{gh} (29.3)	26.7^{g} (29.8)	Moderately Susceptible
9	ICP 87119	7.3^{bc} (15.5)	9.5^{bc} (17.6)	14.2^{bcde} (21.6)	16.6^{cd} (23.4)	Moderately Resistant
10	BDN 2	4.8^{a} (12.5)	8.3^{b} (16.5)	16.2^{def} (23.1)	19.7^{ef} (25.5)	Moderately Resistant
11	ICPL 161	16.2^{h} (23.1)	43.3^{k} (38.3)	47.3^{j} (40.2)	52.5^{i} (42.6)	Susceptible
12	Bahar	12.7^{efg} (20.5)	14.0^{e} (21.5)	15.8^{cdef} (22.8)	16.8^{cdeg} (23.6)	Moderately Resistant

Table 4.23. (Cont.).

S. No.	Genotypes	Per cent disease incidence at different days after inoculations*				Disease Reaction
		05	10	20	40	
13	KPBR 80 2 1	10.0^{de} (18.1)	11.8^{de} (19.7)	13.5^{bcd} (21.1)	15.6^{bc} (22.7)	Moderately Resistant
14	ICP 113	24.2^{i} (28.3)	25.9^{hi} (29.3)	25.9^{g} (29.3)	28.7^{g} (30.9)	Moderately Susceptible
15	ICP 11304	14.0^{gh} (21.5)	28.2^{ij} (30.6)	31.1^{hi} (32.2)	36.3^{h} (34.9)	Moderately Susceptible
16	UPAS 120	21.5^{i} (26.6)	27.9^{i} (30.5)	32.3^{i} (32.8)	35.0^{h} (34.2)	Moderately Susceptible
17	ICP 339	22.3^{i} (27.2)	31.4^{j} (32.4)	31.8^{i} (32.6)	36.3^{h} (34.9)	Moderately Susceptible
18	ICP 2376	13.2^{fgh} (20.8)	17.1^{f} (23.8)	19.0^{f} (25.1)	19.7^{ef} (25.5)	Moderately Resistant
19	ICP 7119	35.4^{j} (34.5)	44.3^{k} (38.8)	45.5^{j} (39.4)	52.3^{i} (42.5)	Susceptible

*Mean of three replications.
Means followed by a common letter are not significantly different at 5% level byTukey's (HSD).
Figures in parentheses are arcsine transformedvalues.

Figure 4.16. Screening of Pigeonpea genotypes against Phytophthora blight disease under field conditions at ICRISAT, Patancheru.

reaction, whereas eight genotypes (ICPL 288, ICP 4135, ICP 87119, ICP 580, ICP 113, ICP 11304, UPAS 120, ICP 339) showed moderately susceptible reaction and two genotypes (ICPL 161 and ICP 7119) showed susceptible reaction. None of the genotypes showed highly resistant reaction.

4.9.1.4.2. Screening of Pigeonpea Wilt and Sterility Mosaic Disease resistant Nursery (PWSMDN) lines at Phytophthora blight sick plot, Sehore, Madhya Pradesh, India

Forty three lines of PWSMDN were screened in Phytophthora blight sick plot at Sehore location. The disease incidence ranged from 7.3 per cent to 62.3 per cent (Fig. 4.17). Two genotypes ICPL-99044 and ICPL 99055 showed least disease incidence with 7.3 per cent, followed by genotype ICPL 99099 (9.4 %) and highest incidence was observed in genotype ICP 12752 with 62.3 per cent (Table 4.24). Three genotype *viz*. ICPL99044, 99055, 99099 showed resistant reaction, whereas 21 genotype (ICPL94062, 20095, 20119, 20123, 20124, 96053, 99009, 99048, 99095, 99098, 87119, 161 and ICP 2376, 8863, 9174, 113, 4135, 11290, 13361. BDN 2, KPBR-80-2-1) showed moderately resistant reaction, 15 genotype(ICPL 20135, 20136, 20137, 90011, 96061, 99091, 99100 and ICP 7119, 11302, 580, 339, 11376, 12012 and 12739, Bahar) showed moderately susceptible reaction, and four genotype(ICPL 99008 and 87051 ICP 12728 and 12752) showed susceptible reaction. While, none of the genotypes showed highly susceptible reaction.

4.9.1.4.3. Reaction of Pigeonpea genotypes to Phytophthora blight during *kharif*-2013 at ICRISAT, Patancheru under natural ecosystem

Reaction of 57 Pigeonpea genotypes was assessed at research farm (BP- 14A) at ICRISAT, Patancheru under natural environmental condition. The disease incidence ranged from 0.0 to 100 per cent (Table 4.25). Among the 57 genotypes, 38 (ICPL 20338 DT, ICPL 20326 NDT, MN 1, MN 5, MN 8, ICPL 87051, 88034, 96053, 6058, 96061, 98008, 92016, 87154, 84023, ICPA 2209, 2043, 2047, 2048, 2092, 2199, ICPH 2363, 2671, 2740, 3461, 3933, 3762, 3477, 3492, 4503, ICP 5028, Maruti, Asha, Lakshmi, Abhaya, Sarita, Durga, Jagriti, Prasada) showed resistant reaction, whereas six genotypes (ICPA 2039, 2089, ICPH 2438, 2364, ICPL 20340 DT, ICPL 85030) showed moderately resistant reaction and 10 genotypes (ICPH 2433, 2751, ICPL 20325 NDT, ICPL 87091, 88039, 97250, 99004,UPAS 120, Pragati, *Cajanus cajanifolius*) showed the moderately susceptible reaction. However genotype ICPL 20092, Kamika, *Cajanus scarabeiodes* showed highly susceptible reaction.

Table 4.24. Screening of improved breeding lines against Phytophthora blight of Pigeonpea in sick plot at Sehore, Madhya Pradesh.

S. No.	Breeding lines	Per cent disease incidence*	Disease reaction
1	ICP 11376	36.8^{op} (35.2)	Moderately Susceptible
2	ICP 12012	48.9^{r} (41.0)	Moderately Susceptible
3	ICP 12728	57.2^{s} (44.7)	Susceptible
4	ICP 12739	28.9^{mn} (31.0)	Moderately Susceptible
5	ICP 12752	62.3^{s} (47.0)	Susceptible
6	ICP 13361	15.5^{dfgh} (22.6)	Moderately Resistant
7	ICPL 94062	13.3^{cdef} (22.6)	Moderately Resistant
8	ICPL 20095	11.1^{bc} (20.8)	Moderately Resistant
9	ICPL 20119	16.8^{fghi} (19.1)	Moderately Resistant
10	ICPL 20123	15.9^{fghi} (23.5)	Moderately Resistant
11	ICPL 20124	15.3^{dfgh} (22.8)	Moderately Resistant
12	ICPL 20135	$45.2q^{r}$ (39.2)	Moderately Susceptible
13	ICPL 20136	29.6^{mn} (31.4)	Moderately Susceptible
14	ICPL 20137	$40.2p^{q}$ (36.9)	Moderately Susceptible
15	ICPL 87051	59.5^{s} (45.7)	Susceptible
16	ICPL 90011	34.1^{no} (33.8)	Moderately Susceptible
17	ICPL 96053	15.4^{dfgh} (22.5)	Moderately Resistant
18	ICPL 96061	44.6^{qr} (39.0)	Moderately Susceptible
19	ICPL 99008	57.0^{s} (44.6)	Susceptible
20	ICPL 99009	13.7^{cdefg} (21.2)	Moderately Resistant
21	ICPL 99044	7.3^{a} (15.5)	Resistant
22	ICPL 99048	14.7^{defgh} (22.0)	Moderately Resistant
23	ICPL 99055	7.3^{a} (15.4)	Resistant
24	ICPL 99091	23.6^{kl} (27.6)	Moderately Susceptible

Table 4.24. (Cont.).

S. No.	Breeding lines	Per cent disease incidence*	Disease reaction
25	ICPL 99095	17.1^{ghi} (23.8)	Moderately Resistant
26	ICPL 99098	14.8^{defgh} (22.0)	Moderately Resistant
27	ICPL 99099	9.4^{ab} (17.5)	Resistant
28	ICPL 99100	33.0^{no} (33.2)	Moderately Susceptible
29	ICP 2376	20.0^{ijk} (25.7)	Moderately Resistant
30	ICP 8863	11.6^{bcd} (19.5)	Moderately Resistant
31	ICP 7119	24.9^{lm} (28.7)	Moderately Susceptible
32	BDN 2	16.6^{fghi} (23.4)	Moderately Resistant
33	ICP 9174	16.6^{fghi} (23.3)	Moderately Resistant
34	ICP 87119	12.0^{bcde} (19.8)	Moderately Resistant
35	KPBR-80-2-1	17.3^{ghi} (23.9)	Moderately Resistant
36	Bahar	24.9^{lm} (28.7)	Moderately Susceptible
37	ICP 113	18.8^{hij} (24.9)	Moderately Resistant
38	ICP 4135	14.5^{cdefg} (21.8)	Moderately Resistant
39	ICP 11290	14.7^{defgh} (22.0)	Moderately Resistant
40	ICP 11302	22.3^{jkl} (27.2)	Moderately Susceptible
41	ICPL 161	16.9^{fghi} (23.6)	Moderately Resistant
42	ICP 580	24.9^{lm} (28.7)	Moderately Susceptible
43	ICP 339	37.9^{op} (35.7)	Moderately Susceptible

Mean of three replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
Figures in parentheses are arcsine transformedvalues.

Figure 4.17. Screening of improved breeding lines against Phytophthora blight of Pigeonpea in sick plot at Sehore, Madhya Pradesh.

Table 4.25.Reaction of Pigeonpea genotypes against Phytophthora blight disease during *kharif* 2013 under natural field condition at ICRISAT,Patancheru.

S. No.	Genotype	Per cent disease incidence at different days after sowing*				Disease Reaction
		30	60	90	130	
1	ICPA 2039	0.7^{b} (4.8)	15.11^{m} (22.3)	18.4^{k} (24.6)	17.8^{j} (24.2)	Moderately Resistant
2	ICPA 2089	1.1^{bc} (6.0)	9.7^{ij} (17.9)	15.0^{j} (22.3)	16.0^{i} (23.0)	Moderately Resistant
3	ICPH 2438	0.0^{a} (0.9)	12.8^{kl} (20.5)	13.5^{h} (21.1)	14.3^{h} (21.7)	Moderately Resistant
4	ICPH 2363	1.0^{b} (5.6)	2.5^{bcde} (9.0)	4.5^{ef} (12.1)	4.5^{d} (12.1)	Resistant
5	ICPH 2364	3.6^{e} (10.9)	8.1^{hi} (16.4)	13.9^{i} (21.4)	16.5^{i} (23.3)	Moderately Resistant
6	ICPH 2433	7.6^{f} (15.8)	29.3^{o} (31.2)	37.6^{q} (35.6)	40.0^{o} (36.7)	Moderately Susceptible
7	ICPL 20338 DT	0.0^{a} (0.9)	4.7^{g} (12.4)	4.7^{ef} (12.4)	4.7^{d} (12.4)	Resistant
8	ICPL 20340 DT	0.0^{a} (0.9)	11.6^{jk} (19.5)	12.6^{h} (20.4)	14.5^{h} (21.9)	Moderately Resistant
9	ICPL 20325 NDT	3.2^{de} (10.2)	26.8^{o} (29.8)	31.6^{o} (32.5)	32.9^{n} (33.2)	Moderately Susceptible
10	ICPL 20326 NDT	0.0^{a} (0.9)	4.6^{fg} (12.3)	4.6^{de} (12.3)	4.6^{d} (12.3)	Resistant
11	MN 1	0.0^{a} (0.9)	1.7^{b} (7.4)	1.8^{b} (7.8)	1.8^{b} (7.8)	Resistant
12	MN 5	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
13	MN 8	2.9^{de} (9.8)	2.9^{bc} (9.8)	2.9^{b} (9.8)	2.9^{b} (9.8)	Resistant

Table 4.25. (Cont.).

S. No.	Genotype	Per cent disease incidence at different days after sowing*				Disease Reaction
		30	60	90	130	
14	Sarita	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
15	Pragati	0.0[a] (0.9)	22.0[n] (27.0)	22.0[l] (27.0)	26.1[lm] (29.4)	Moderately Susceptible
16	Durga	0.0[a] (0.9)	2.0[bc] (8.1)	2.0[b] (8.1)	2.0[b] (8.1)	Resistant
17	Jagriti	0.0[a] (0.9)	4.0[efg] (11.4)	4.0[c] (11.4)	4.0[c] (11.4)	Resistant
18	ICPL 87091	0.0[a] (0.9)	15.8[lm] (22.8)	17.7[k] (24.1)	23.8[k] (28.1)	Moderately Susceptible
19	ICPL 88039	12.6[g] (20.4)	13.5[kl] (21.1)	25.6[m] (29.2)	26.9[m] (29.9)	Moderately Susceptible
20	ICPL 88034	0.0[a] (0.9)	2.0[c] (8.2)	2.0[b] (8.2)	3.1[c] (10.1)	Resistant
21	ICPL 98008	1.9[d] (7.8)	1.9[b] (7.8)	1.9[b] (7.8)	1.9[b] (7.8)	Resistant
22	Prasada	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
23	ICPL 92016	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
24	ICPL 87154	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
25	ICPL 85030	0.0[a] (0.9)	8.3[hi] (16.5)	8.3[g] (16.5)	10.6[g] (18.7)	Moderately Resistant
26	ICPL 84023	0.0[a] (0.9)	7.0[h] (15.1)	7.0[g] (15.1)	7.5[f] (18.7)	Resistant

Table 4.25. (Cont.).

S. No.	Genotype	Per cent disease incidence at different days after sowing*				Disease Reaction
		30	60	90	130	
27	ICPA 2209	0.0^{a} (0.9)	1.8^{bc} (7.7)	1.8^{b} (7.7)	1.8^{b} (7.7)	Resistant
28	ICPA 2043	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
29	ICPA 2047	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
30	ICPA 2048	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
31	ICPA 2092	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
32	ICPA 2199	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
33	ICPH 2671	2.6^{de} (9.2)	3.0^{cdef} (9.9)	4.8^{f} (12.6)	5.3^{e} (13.29)	Resistant
34	ICPH 2740	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
35	ICPH 2751	0.0^{a} (0.9)	18.3^{mn} (24.5)	18.3^{k} (24.5)	23.9^{k} (28.2)	Moderately Susceptible
36	ICPH 3461	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
37	ICPH 3933	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
38	ICPH 3762	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant
39	ICPH 3477	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	Resistant

Table 4.25. (Cont.).

S. No.	Genotype	Per cent disease incidence at different days after sowing*				Disease Reaction
		30	60	90	130	
40	ICPH 3492	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
41	ICPH 4503	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
42	Maruti	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
43	Asha	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
44	Lakshmi	1.7[cd] (7.4)	1.7[b] (7.4)	1.7[b] (7.4)	1.7[b] (7.4)	Resistant
45	Abhaya	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	0.0[a] (0.9)	Resistant
46	ICP 5028	3.6[e] (10.8)	3.6[defg] (10.8)	3.6[d] (10.8)	5.4[e] (13.3)	Resistant
47	ICPL 96058	0.0[a] (0.9)	1.7[b] (7.4)	1.7[b] (7.4)	1.7[b] (7.4)	Resistant
48	ICPL 96061	0.0[a] (0.9)	1.9[bc] (7.9)	1.9[b] (7.9)	1.9[b] (7.9)	Resistant
49	ICPL 96053	2.3[de] (8.6)	2.3[bcd] (8.6)	4.5[ef] (12.2)	4.5[d] (12.2)	Resistant
50	ICPL 87051	2.4[de] (9.1)	4.9 (12.7)	7.3[g] (15.5)	7.3[f] (15.5)	Resistant
51	ICPL 97250	0.0[a] (0.9)	29.8[o] (31.5)	29.8[n] (31.5)	39.7[o] (36.6)	Moderately Susceptible
52	ICPL 99004	0.0[a] (0.9)	18.3[mn] (24.5)	18.3[k] (24.5)	25.0[kl] (28.8)	Moderately Susceptible

Table 4.25. (Cont.).

S. No.	Genotype	Per cent disease incidence at different days after sowing*				Disease Reaction
		30	60	90	130	
53	Kamika	25.0^{h} (28.8)	59.4^{r} (45.7)	83.7^{t} (57.1)	100.0^{r} (71.8)	Highly Susceptible
54	ICPL 20092	11.2^{g} (19.2)	44.8^{q} (39.0)	74.8^{s} (52.7)	100.0^{r} (71.8)	Highly Susceptible
55	*Cajanus cajanifolius*	6.0^{f} (14.0)	21.2^{n} (26.5)	33.6^{p} (33.5)	43.4^{p} (38.4)	Moderately Susceptible
56	UPAS 120	13.2^{g} (20.8)	19.8^{n} (25.6)	32.9^{op} (33.5)	33.0^{n} (38.4)	Moderately Susceptible
57	*Cajanus scarabeiodes*	21.7^{h} (26.8)	38.6^{p} (36.1)	64.6^{r} (48.0)	82.1^{q} (56.2)	Highly Susceptible

*Mean of three replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's (HSD).
Figures in parentheses are arcsine transformed values

Unlike other major plant pathogen systems of crop plants, detailed investigations have not been undertaken on the infection process of *P. drechsleri* f. sp. *cajani* on the Pigeonpea plant and on the biochemical and genetic basis of Phytophthora blight resistance in Pigeonpea. Preliminary investigations suggest that phenolic constituents of leaves and stems increased after inoculation in resistant varieties while they decreased in the Phytophthora blight susceptible variety of Pigeonpea (Pal and Grewal, 1975). It appears that there may be stimulation of host defense reaction due to infection in the resistant variety while such mechanism may be absent in the susceptible one. Resistance identified so far needs to be reconfirmed under epidemiologically sound disease development environment and with the emergence of new pathotypes of *P. drechsleri* f. sp. *cajani*.

4.9.2. Chemical management

The use of fungicides has become an inevitable method in the management of plant diseases particularly in absence of available resistant varieties. Metalaxyl was introduced in 1977 and used to control plant diseases caused by oomycetes (Ionnou and Grogan, 1984; Schwinn and Staub, 1995). Among the phenylamides (acylanilides) group of chemicals metalaxyl was most widely used against the *Phytophthora* spp, (Erwin and Ribeiro, 1996).

Mefenoxam has been used as a seed treatment to control damping-off caused by *Phytophthora* and *Pythium* spp. in soybean (Dorrance and McClure, 1999), and wheat (Smiley *et al.* 1996). Bradford *et al*. (1988) used mefenoxam as a seed-treatment to improve muskmelon seedling emergence. Mefenoxam is the active isomeric form of metalaxyl. The active isomeric form comprised 50 per cent of metalaxyl; whereas mefenoxam is 100 per cent active isomer. To our knowledge, this is the first report on evaluating the effectiveness of mefenoxam as seed treatment for the control of *P. drechsleri*f. sp.*cajani* causing Phytophthora blight of Pigeonpea.

4.9.2.1. *In vitro* efficacy of fungicides against the growth of *P. drechsleri* f. sp. *cajani*

Mefenoxam and metalaxyl were tested at different concentrations of active ingredient against mycelial growth of *P. drechsleri* f. sp. *cajani*. The results (Table 4.26: Plate 4.8) revealed that, with the increase in concentration of fungicides from 0.1 to 10 µg/ml of medium there was reduction in growth of the pathogen *P. drechsleri* f. sp. *cajani.* Maximum per cent inhibition over control was found at a concentration of 2.0, 3.0, 5.0 and 10 µg/ml of media. However fungicide mefenoxam was superior in reducing the growth of *P. drechsleri* f. sp. *cajani* when compared to metalaxyl at 0.25,

Table 4.26. Effect of fungicides on the growth of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

S. No.	Concentration (a.i. µg/ml)	Mycelial growth (mm)		Per cent inhibition over control*	
		Mefenoxam	Metalaxyl	Mefenoxam	Metalaxyl
1	0.0	$90.0^{g,g}$	$90.0^{g,g}$	$0.0^{g,a}$ (0.9)	$0.0^{g.a}$ (0.9)
2	0.10	$78.3^{f,a}$	$80.5^{f,a}$	$12.9^{f,a}$ (20.6)	$10.6^{f,a}$ (18.6)
3	0.25	$46.3^{e,a}$	$55.3^{e,b}$	$48.6^{e,a}$ (40.7)	$38.5^{e,b}$ (36.0)
4	0.50	$27.3^{d.a}$	$33.3^{d,b}$	$69.6^{d,a}$ (50.3)	$62.9^{d,b}$ (47.3)
5	0.75	$13.4^{c,a}$	$21.3^{c,b}$	$85.1^{c,a}$ (57.6)	$76.3^{c.b}$ (53.4)
6	1.0	$7.7^{b,a}$	$9.5^{b,b}$	$91.5^{b,a}$ (61.6)	$89.5^{b,b}$ (60.3)
7	2.0	$0.0^{a,a}$	$0.0^{a,a}$	$100.0^{a,a}$ (71.8)	$100.0^{a,a}$ (71.8)
8	3.0	$0.0^{a,a}$	$0.0^{a,a}$	$100.0^{a,a}$ (71.8)	$100.0^{a,a}$ (71.8)
9	5.0	$0.0^{a,a}$	$0.0^{a,a}$	$100.0^{a,a}$ (71.8)	$100.0^{a,a}$ (71.8)
10	10	$0.0^{a,a}$	$0.0^{a,a}$	$100.0^{a,a}$ (71.8)	$100.0^{a,a}$ (71.8)

*Mean of three replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
Figures in parentheses are arcsine transformedvalues.

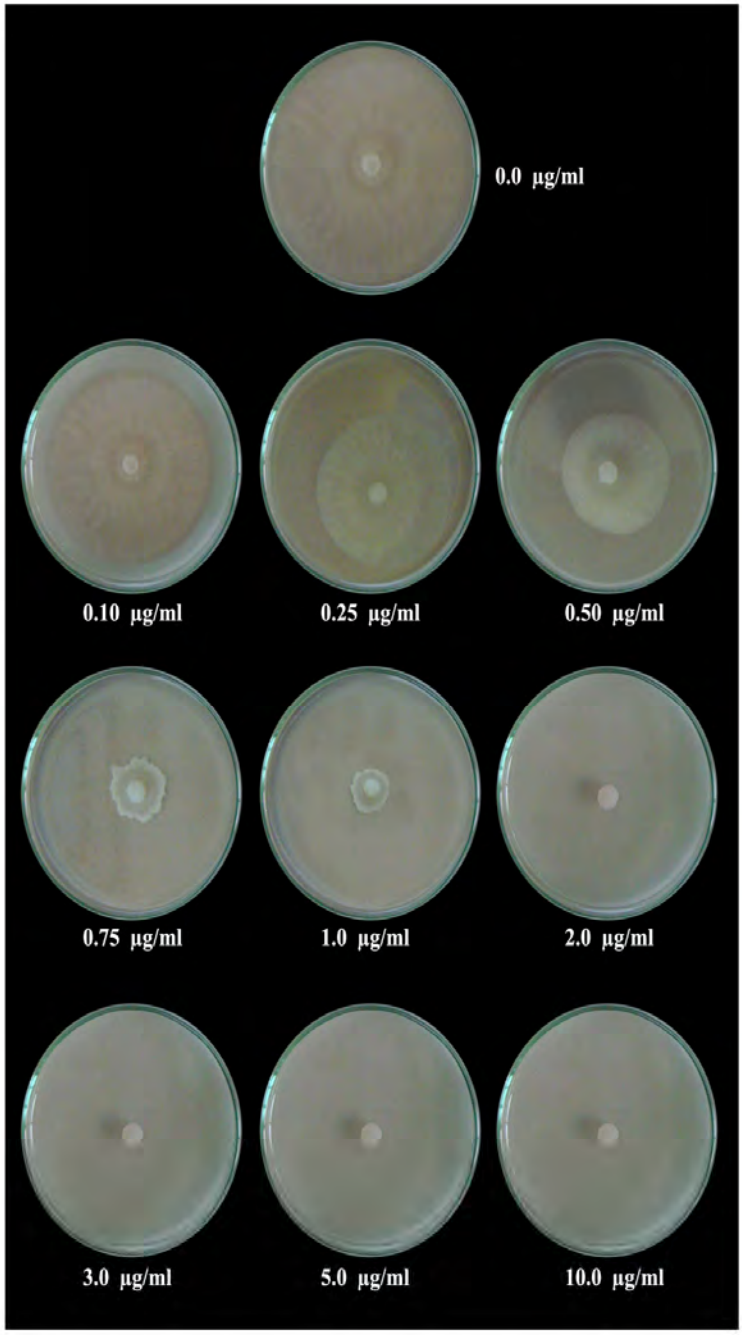


Plate 4.8. Effect of mefenoxam fungicide on growth of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

0.5, 0.75 and 1.0 concentration. However, per cent inhibition over control of mefenoxam was almost on par with metalaxyl at concentration of 2.0, 3.0, 5.0 and 10.0 µg/ml (Fig. 4.18).

Though literature on mefenoxam to control *P. drechsleri*f. sp.*cajani*is not available, the findings of Birendra and Dubey(2005)showed that metalaxyl proved to be most toxic and completely inhibited the growth of *P. drechsleri*f. sp.*cajani* in less than 1.0 a.i µg/ml. Similarly Chaube *et al.* (1987) also achieved 100 per cent inhibition of the growth of *P. drechsleri*f. sp.*cajani* with metalaxyl at 0.5 a.i µg/ml. The research finding of Cohen *et al*. (1979); Sozzi and Staub, (1987); Fernando and Linderman, (1994) showed the efficacy of metalaxyl to *Phytophthora* spp.

4.9.2.2. *In vitro* efficacy of fungicides against the induction of zoospores using diluted tomato broth method

Mefenoxam and metalaxyl at different concentrations of active ingredient were tested against induction of zoospores of *P. drechsleri* f. sp. *cajani* by using the diluted tomato broth method. A significant difference was observed between metalaxyl and mefenoxam at all concentrations of each fungicide tested (Table 4.27: Fig. 4.18). Both the fungicides mefenoxam and metalaxyl inhibited 100 per cent induction of zoospores at 5 and 10 µg/ml concentrations when compared to other concentration.Further mefenoxam was statistically superior over metalaxyl in inhibiting the induction of zoospores at concentration of 0.1, 0.25, 0.50, 0.75, 1.0, 2.0 and 3.0 µg/ml, however both fungicides were on par at concentration at 5.0 and 10.0 µg/ml. Results are in accordance with Chaube *et al.* (1987) who observed inhibition of the sporulation and sporangial germination of *P. drechsleri*f. sp.*cajani* with metalaxyl at 2.0 a.i µg/ml.

4.9.3. Biological control

The ever growing concern of pesticides toxicity has forced researchers to search for alternative to chemicals for management. In this context biological control agents play an important role as they have multifaceted mode of action *viz.* competition, lysis, antibiosis, siderophore production and hyperparasitism (Vidyasekaran, 1997). Formulations of antagonistic organisms are available at cheaper rate and these organisms when once introduced into the soil survive for a longer period. There is also circumstantial report that native antagonists are more efficient than introduced antagonists (Kulkarni and Sagar, 2006).

The development of pathogenic strains resistant to pesticides, which has now become a major concern throughout the world, can be minimized by the use of biocontrol agents

Table 4.27.Effect of fungicides on induction of zoospore of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro.*

S. No.	Concentration (a.i µg/ml)	Number of zoospore* (10^3ml^{-1})		Per cent inhibition over control*	
		Mefenoxam	Metalaxyl	Mefenoxam	Metalaxyl
1	0.0	$182.3^{i,a}$	$183.7^{i,a}$	$0.0^{i,a}$ (0.9)	$0.0^{i,a}$ (0.9)
2	0.10	$150.0^{h,a}$	$160.3^{h,b}$	$17.7^{h,a}$ (24.2)	$5.5^{h,b}$ (13.4)
3	0.25	$144.0^{g,a}$	$149.0^{g,b}$	$21.0^{g,a}$ (26.4)	$12.2^{g,b}$ (13.4)
4	0.50	$128.0^{f,a}$	$136.0^{f,b}$	$29.8^{f,a}$ (26.4)	$25.7^{f,b}$ (20.0)
5	0.75	$110.3^{e,a}$	$117.7^{e,b}$	$39.5^{e,a}$ (31.5)	$30.7^{e,b}$ (29.2)
6	1.0	$96.3^{d,a}$	$112.7^{d,b}$	$47.2^{d,a}$ (36.5)	$33.6^{d,b}$ (32.0)
7	2.0	$79.3^{c,a}$	$98.0^{c,b}$	$56.5^{c,a}$ (40.2)	$42.2^{c,b}$ (33.5)
8	3.0	$28.0^{b,a}$	$34.7^{b,b}$	$84.6^{b,a}$ (44.4)	$79.6^{b,b}$ (33.5)
9	5.0	$0.0^{a,a}$	$0.0^{a,a}$	$100.0^{a,a}$ (71.8)	$100.0^{a,a}$ (71.8)
10	10	$0.0^{a,a}$	$0.0^{a,a}$	$100.0^{a,a}$ (71.8)	$100.0^{a,a}$ (71.8)

*Mean of three replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
Figures in parentheses are arcsine transformedvalues.

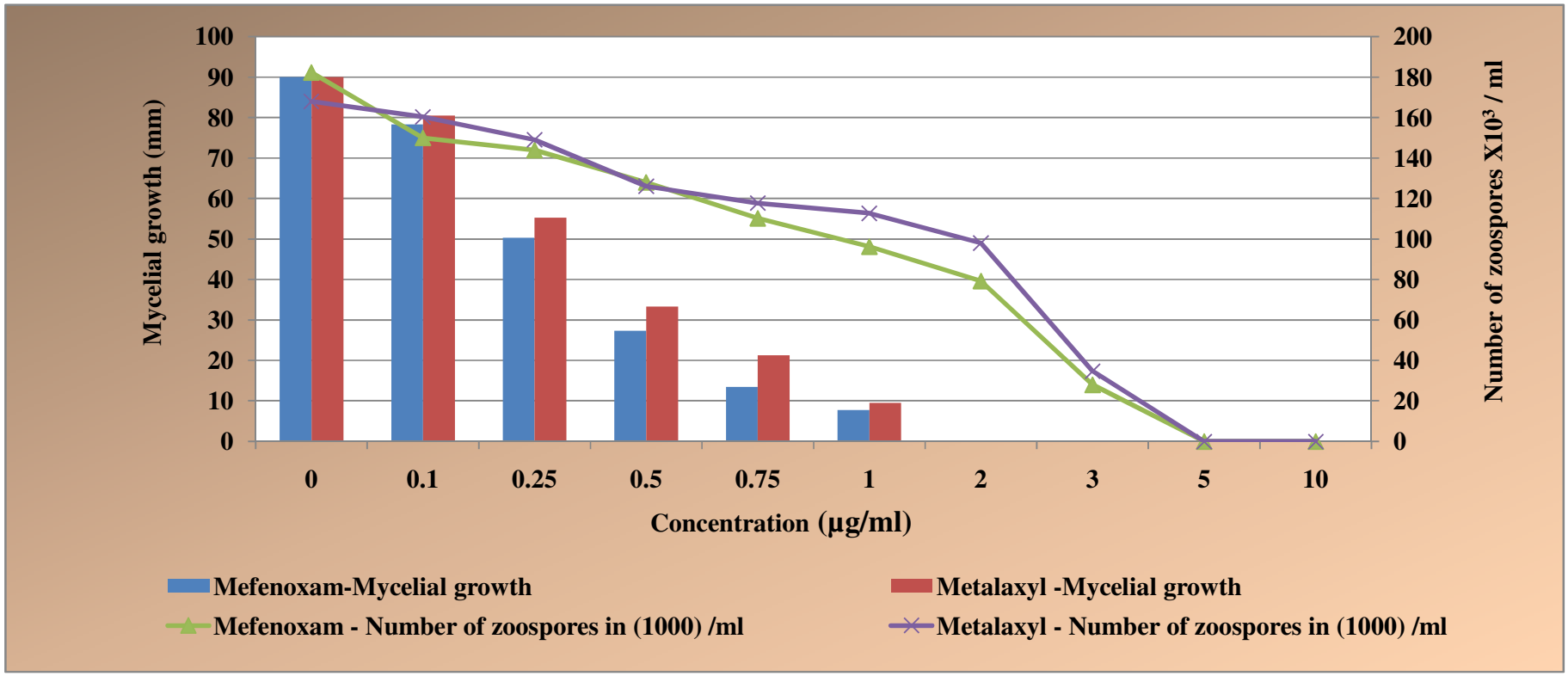


Figure 4.18. Effect of fungicides on the growth of mycelium and induction of zoospores of *Phytophthora drechsleri* f. sp. *cajani* of Pigeonpea.

(Utkhede and Smith, 1992)and has emerged as an important alternative in managing soil borne plant diseases (Bowen and Rovira, 1999; Whipps, 1997).

4.9.3.1. *In vitro* evaluation of *Trichoderma*isolates against *P. drechsleri* f. sp. *cajani*

Management of soil-borne plant diseases and increase in yield by the use of bioagents is well documented (Chet, 1987; Kumarand Dubey, 2001; Dubey, 2006). Efficacy of five isolates of *Trichoderma* were evaluated (Table 4.28) against *P. drechsleri* f. sp. *cajani* by dual culture technique. Among five isolates, *Trichoderma* isolate-3 gave highest inhibition in growth with 80.52 per cent (Plate 4.9) followed by *Trichoderma* isolate-4 (75.48 %). The least inhibition (60.85 %) of the fungus was observed in *Trichoderma* isolate-1 (Fig. 4.19).

Similar results were reported with antagonistic activity of *Trichoderma* and results are in accordance with findings of Weindling, (1932); Baker and Cook (1974); Anandaraj and Sarma (1995); Srivastava and Mall (2008). Similarly, Singh and Dubey, 2010 reported that, *Trichoderma* species overgrew on the mycelium of *P. drechsleri*f. sp.*cajani* and caused lysis.

Phytophthora species belongs to Oomycetes, a group of fungi characterized by having a cellulosic cell wall. The *Trichoderma* species produces cellulase in addition to other cell wall degrading enzymes, glucanase and chitinase. Production of mycolytic enzymes explains their ability to lyse fungal hyphae.

4.9.3.2. *In vitro* evaluation of *Pseudomonas* isolates against *P. drechsleri* f. sp. *cajani*

Fluorescent Pseudomonads has been paid considerable attention worldwide as a Plant Growth Promoting Rhizobacteria (PGPR), which facilitates the best alternative to chemicals for the eco-friendly biological control of soil and seed borne pathogens. In the present study (Table 4.29) four isolates of *Pseudomonas* were evaluated for their efficacy against *P. drechsleri* f. sp. *cajani* through dual culture technique. Results revealed that (Fig. 4.20: Plate 4.10) *Pseudomonas* isolate-1 gave maximum inhibition (71.90 %) followed by *Pseudomonas* isolate-4 (63.90 %). While least inhibition of the fungus was observed in *Pseudomonas* isolate-2 (54.30 %).

Results are in accordance with the finding of Singh and Dubey, (2010)who reported that *Pseudomonas fluorescens* produced maximum inhibition zone and lysed the mycelium of *P. drechsleri* f. sp. *cajani.* Production of some kind of lytic substance and secondary metabolites such as siderophore, antibiotics and volatile compounds by *P. fluorescens* may be responsible for the inhibition of *P. drechsleri* f. sp. *cajani* (Utkhede 1984). Yan *et al*. (2002) and Thanh *et al*. (2009) used *Bacillus* spp. and *Pseudomonas*

Table 4.28. Effect of *Trichoderma* isolates on growth of *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

S. No.	*Trichoderma* isolates	Growth of pathogen* (mm)	Per cent inhibition over control
1	*Trichoderma* isolate-1	35.23[d]	60.85[d] (46.34)
2	*Trichoderma* isolate -2	28.40[c]	68.44[c] (49.76)
4	*Trichoderma* isolate -3	17.53[a]	80.52[a] (55.43)
3	*Trichoderma* isolate -4	22.07[b]	75.48[b] (52.99)
5	*Trichoderma* isolate -5	22.95[b]	74.5 (52.5)[b]
6	Control	90.00[e]	0.00[e] (0.9)

*Mean of three replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
Figures in parentheses are arcsine transformedvalues.

Table 4.29. Effect of *Pseudomonas* isolates on growth of *Phytophthoradrechsleri* f. sp. *cajani* under *in vitro*.

S. No.	*Pseudomonas* isolates	Growth of pathogen* (mm)	Per cent inhibition over control
1	*Pseudomonas* isolate -1	25.32[a]	71.90[a] (51.3)
2	*Pseudomonas* isolate -2	41.12[d]	54.30[c] (43.4)
3	*Pseudomonas* isolate -3	38.32[d]	57.40[d] (44.8)
4	*Pseudomonas* isolate -4	32.50[b]	63.90[b] (47.70)
5	Control	90.00[e]	0.00[e] (0.9)

*Mean of three replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).
Figures in parentheses are arcsine transformedvalues.

Figure 4.19. Effect of *Trichoderma* isolates on growth of *Phytophthora drechsleri* f. sp. *cajani* of Pigeonpea under *in vitro.*

Figure 4.20. Effect of *Pseudomonas* isolates on *Phytophthora drechsleri* f. sp. *cajani* of Pigeonpea under *in vitro*.

Plate 4.9. Effect of *Trichoderma* isolates against *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

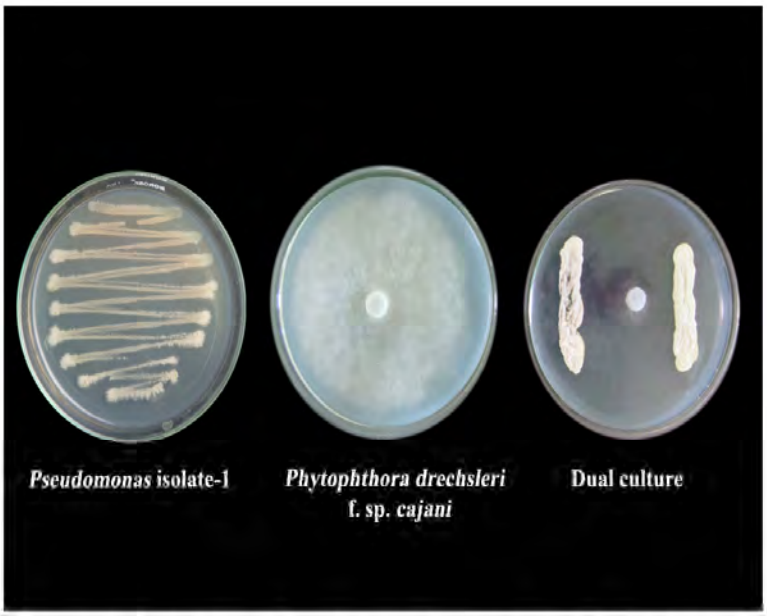


Plate 4.10. Evaluation of *Pseudomonas* isolates against *Phytophthora drechsleri* f. sp. *cajani* under *in vitro*.

fluorescens for controlling *Phytophthora* spp. Guo *et al*. (2009b) reported that *P. fluorescens* controlled Phytophthora blight of bell pepper under greenhouse and field conditions.

4.9.3.3. Compatibility of *Trichoderma* isolate-3 against Metalaxyl and Mefenoxam

Biological control is often not highly effective when deployed as a single control strategy for soil and seed-borne diseases. Since fungicides may have deleterious effects on the pathogen as well as the antagonist, an understanding of the effect of fungicides on the pathogen and the antagonist, would provide information on the selection of selective fungicides and fungicide resistant antagonists. An antagonist insensitive to the chemical may be combined for extending the duration of protection and effectiveness. The idea of combining biocontrol agents (BCA) with fungicides for the development or establishment of desired microbes in the rhizosphere (Papavizas and Lewis, 1981). Further, the antagonism of BCA was influenced by the addition of fungicides (Kay and Stewart, 1994; Naar and Kecskes, 1998). Many authors reported the compatibility of fungicides with biocontrol agents in various crops (Utkhede and Koch, 2002; Senthilvel *et al*. 2004; Anand *et al*. 2007).

The present study was conducted to determine the compatibility of *Trichoderma* isolate-3with six different concentrations of mefenoxam and metalaxyl and the results are presented in Table 4.30.Among the six concentrations tested, three concentrations *viz.* 0, 500 and 1000 µg/ml of mefenoxam and metalaxylwas found compatible with *Trichoderma* isolate-3 whereas the other three concentrations inhibited the growth of *Trichoderma* isolate-3. The least mycelial growth of *Trichoderma* isolate-3was observed in case of mefenoxam (33 mm) and metalaxyl (29.7 mm) at concentration of 4000 µg/ml when compared to control at five days after incubation. Over all, from the results it was concluded that up to 1000 ppm concentration, the test fungicides were compatible with *Trichoderma* isolate-3. Singh and Dubey, (2010) reported the compatibility of *Trichoderma* species with metalaxyl, but the growth of *Trichoderma* was insensitive to metalaxyl at the concentration of 3000 ppm. Similar results were reported by Bharadwaj and Gupta (1987) and Sawant and Mukhopadhyay, (1990).

The results of the present study concluded that, *Trichoderma* species tolerated metalaxyl and mefenoxam up to 1000 ppm, which suggested the possibility of integration of these fungicides with bioagents for the effective management of Phytophthora blight of Pigeonpea.

Table 4.30. Compatibility of *Trichoderma* isolate-3with Mefenoxam and Metalaxyl under *in vitro*.

S. No.	Concentration (µg/ml of culture media)	Mefenoxam	Metalaxyl
		Mycelial growth* (mm)	Mycelial growth* (mm)
1	0.00	90.0[a]	90.0[a]
2	500	90.0[a]	90.0[a]
3	1000	90.0[a]	90.0[a]
4	2000	85.3[b]	80.0[b]
5	3000	58.0[c]	53.7[c]
6	4000	33.0[d]	29.7[d]

*Mean of three replications.
Means followed by a common letter are not significantly different at 5% level by Tukey's(HSD).

4.9.4. Management of Phytophthora blight of Pigeonpea with fungicides and bioagents

Despite the effectiveness of chemicals in management of *Phytophthora,* their efficacy under epidemic is questionable. Further, there has been a growing concern about excess usage of fungicides and its toxic effect on environment. The reliance of biocontrol agents seems to be effective in managing the soil borne disease. Species of *Trichoderma* and *Pseudomonas* have been very effective in curtailing soil borne diseases by multiple mode of action.

Biological control is a good alternative for sustainable agriculture to overcome the problems of public concern associated with pesticides and pathogens resistant to chemical pesticides and to become eco-friendly (Akhtar and Siddiqui, 2008).

An attempt was made to manage the Phytophthora blight disease by using fungicides mefenoxam and metalaxyl and effective bioagents (*Trichoderma* isolate-3 and/or *Pseudomonas* isolate-1) and combination of both bioagents and reduced dose of fungicides with three varieties Pigeonpea *viz.* ICP 87119, ICP 8863 and ICP 7119.

The experiment was conducted in both greenhouse and field condition (Field No. RL-17) at ICRISAT, Patancheru during *kharif* 2013. The treatments were imposed as seed treatment. Control was maintained throughout the experiment. The data on per cent disease was calculated and the results presented in Table. 4.31 and 4.32.

The results on efficacy of fungicides and bioagents against the Phytophthora blight disease incidence under greenhouse conditions are presented in Table 4.31: Fig. 4. 21.

In ICP 87119, T-1 (Mefenoxam seed treatment) showed the least incidence with 9.3 per cent and was on par with the other treatments *viz*. T-2 (Reduced dose of mefenoxam + *Trichoderma*isolate-3), T-3 (Reduced dose mefenoxam + *Pseudomonas* isolate-1) and T-7 (Metalaxyl seed treatment) with disease incidence of 9.5, 10.3 and 9.8 per cent respectively. Further, seed treatment with *Trichoderma* isolate-3 (28.4 %), *Pseudomonas* isolate-1 (30.9 %) and combination of *Trichoderma* isolate-3 and*Pseudomonas* isolate-1(27.9%) showed less disease incidence compared to inoculated control (42.1 %). Response of ICP 7119 and ICP 8863 varieties to fungicides and bioagents were found similar to variety ICP 87119.

Efficacy of fungicides and bioagents against the Phytophthora blight disease under field condition at ICRISAT are presented in Table 4.32: Fig. 4.22.

Table 4.31. Management of Phytophthora blight disease using fungicides and bioagents under greenhouse conditions at ICRISAT, Patancheru.

S. No.	Treatment	Per cent disease incidence in different varieties*			Per cent reduction over control*		
		ICP87119	ICP7119	ICP8863	ICP87119	ICP7119	ICP8863
T1	Mefenoxam (2 g kg^{-1} of seed)	9.3^{b} (17.5)	14.2^{b} (21.6)	9.7^{b} (17.9)	77.9^{b} (54.2)	79.8^{b} (55.1)	78.3^{b} (54.3)
T2	Mefenoxam (1 g kg^{-1} of seed) +*Trichoderma*isolate-3 (4 g kg^{-1} of seed)	9.5^{b} (17.7)	15.1^{b} (22.3)	10.1^{b} (18.2)	77.4^{b} (53.9)	78.5^{b} (54.4)	77.4^{b} (53.9)
T3	Mefenoxam (1 g kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)	10.3^{b} (18.4)	15.0^{b} (22.2)	11.0^{b} (19.0)	75.5^{b} (53.0)	78.6^{b} (54.5)	75.3^{b} (52.9)
T4	*Trichoderma* isolate-3 (4 g kg^{-1} of seed)	28.4^{c} (30.7)	44.9^{c} (39.1)	32.0^{c} (32.7)	32.5^{c} (33.0)	36.0^{c} (34.8)	28.3^{c} (30.7)
T5	*Pseudomonas* isolate-1 (10 g kg^{-1} of seed)	30.9^{c} (32.1)	45.2^{c} (39.2)	32.5^{c} (33.0)	26.6^{c} (29.7)	35.6^{c} (34.6)	27.1^{c} (30.0)
T6	*Trichoderma* isolate-3 (4 g kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)	27.9^{c} (30.5)	44.7^{c} (39.0)	29.5^{c} (31.4)	33.7^{c} (33.6)	36.3^{c} (34.9)	33.9^{c} (33.7)
T7	Metalaxyl seed treatment (2 g kg^{-1} of seed)	9.8^{b} (18.0)	15.1^{b} (22.3)	10.3^{b} (18.4)	76.7^{b} (53.6)	78.5^{b} (54.4)	76.9^{b} (53.7)
T8	Inoculated control	42.1^{d} (37.8)	70.2^{d} (50.5)	44.6^{d} (39.0)	0.0^{d} (0.9)	0.0^{d} (0.9)	0.0^{d} (0.9)
T9	Uninoculated control	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	100.0^{a} (71.8)	100.0^{a} (71.8)	100.0^{a} (71.8)

*Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by Tukey's(HSD)

The figures in parentheses are arcsine transformed

Table 4.32. Management of Phytophthora blight disease using fungicides and bioagents under field conditions at ICRISAT, Patancheru.

S. No.	Treatment	Per cent disease incidence in different varieties*			Per cent reduction over control*		
		ICP 87119	ICP7119	ICP8863	ICP87119	ICP7119	ICP8863
T1	Mefenoxam (2 g kg^{-1} of seed)	21.2^{b} (26.5)	26.3^{b} (29.6)	23.9^{b} (28.1)	47.4^{b} (40.3)	50.6^{b} (41.7)	42.0^{b} (37.7)
T2	Mefenoxam (1 g kg^{-1} of seed) +*Trichoderma*isolate-3 (4 g kg^{-1} of seed)	21.9^{b} (26.9)	27.9^{b} (30.5)	24.3^{b} (28.4)	45.7^{b} (39.5)	47.6^{b} (40.3)	41.0^{b} (37.2)
T3	Mefenoxam (1 g kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)	23.7^{b} (28.0)	27.2^{b} (30.1)	24.1^{b} (28.3)	41.2^{b} (37.3)	48.9^{b} (40.9)	41.5^{b} (37.5)
T4	*Trichoderma* isolate-3 (4 g kg^{-1} of seed)	30.1^{c} (31.7)	36.3^{c} (34.9)	30.2^{d} (31.7)	25.3^{c} (29.0)	31.8^{c} (32.6)	26.7^{d} (29.8)
T5	*Pseudomonas* isolate-1 (10 g kg^{-1} of seed)	30.7^{d} (32.0)	38.2^{c} (35.9)	30.8^{d} (32.1)	23.8^{d} (28.1)	28.2^{c} (30.6)	25.2^{d} (28.9)
T6	*Trichoderma* isolate-3 (4 g kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)	30.4^{c} (31.8)	35.2^{c} (34.4)	31.3^{c} (32.3)	24.6^{c} (28.5)	33.8^{c} (33.7)	24.0^{c} (28.2)
T7	Metalaxyl seed treatment (2 g kg^{-1} of seed)	23.6^{b} (28.0)	25.2^{b} (29.8)	23.2^{c} (27.7)	41.4^{b} (37.5)	52.6^{d} (42.7)	43.7^{b} (38.5)
T8	Inoculated control	40.3^{e} (36.9)	53.2^{d} (42.9)	41.2^{e} (37.3)	0.0^{e} (0.9)	0.0^{e} (0.96)	0.0^{e} (0.9)
T9	Uninoculated control	0.0^{a} (0.9)	0.0^{a} (0.9)	0.0^{a} (0.9)	100.0^{a} (71.8)	100.0^{a} (71.8)	100.0^{a} (71.8)

*Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by Tukey's(HSD)

The figures in parentheses are arcsine transformed

Treatments: T1- Mefenoxam (2 g kg^{-1} of seed)
T2- Mefenoxam (1 g kg^{-1} of seed) + *Trichoderma* isolate-3 (4 g kg^{-1} of seed)
T3- Mefenoxam (1 g kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)
T4- *Trichoderma* isolate-3 (4 g kg^{-1} of seed)
T5- *Pseudomonas* isolate-1 (10 gkg^{-1} of seed)
T6- *Trichoderma* isolate-3 (4 g kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)
T7- Metalaxyl seed treatment (2 g kg^{-1} of seed)
T8- Inoculated control
T9- Uninoculated control

Figure 4.21. Management of Phytophthora blight disease using fungicides and bioagents under greenhouse conditions at ICRISAT, Patancheru.

Treatments: T1- Mefenoxam (2 g kg^{-1} of seed)
T2- Mefenoxam (1 g kg^{-1} of seed) + *Trichoderma* isolate-3 (4 g kg^{-1} of seed)
T3- Mefenoxam (1 g kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)
T4- *Trichoderma* isolate-3 (4 g kg^{-1} of seed)
T5- *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)
T6- *Trichoderma* isolate-3 (4 g kg^{-1} of seed) + *Pseudomonas* isolate-1 (10 g kg^{-1} of seed)
T7- Metalaxyl seed treatment (2 g kg^{-1} of seed)
T8- Inoculated control and T9- Uninoculated control

Figure 4.22. Management of Phytophthora blight disease using fungicides and bioagents under field conditions at ICRISAT, Patancheru.

Perusal of data indicated that the per cent disease incidence was low in all the treatments in variety ICP 87119 followed by ICP 8863 and high incidence on ICP 7119. In ICP 87119, the treatment T-1 (Mefenoxam seed treatment) showed the least incidence with 21.2 per cent and was on par with the other treatments *viz*. T-2 (Reduced dose of mefenoxam + *Trichoderma*isolate-3), T-3 (Reduced dose mefenoxam + *Pseudomonas* isolate-1) and T-7 (Metalaxyl seed treatment) with disease incidence of 21.9, 23.7 and 23.6 per cent respectively. Further, seed treatment with *Trichoderma* isolate-3, *Pseudomonas* isolate-1 and combination of *Trichoderma* isolate-3 and*Pseudomonas* isolate-1 showed disease incidence of 30.1, 30.7 and 30.4 per cent respectively. Whereas inoculated control sowed the disease incidence of 40.3 per cent.

In variety ICP 7119 and ICP 8863, treatment T-7 (Metalaxyl seed treatment) showed the least incidence of disease and found on par with the other treatments *viz.*T-1 (Mefenoxam seed treatment), T-2 (Reduced dose of mefenoxam + *Trichoderma*isolate-3) and T-3 (Reduced dose mefenoxam + *Pseudomonas* isolate-1), indicating the fungicides dosage can be reduced by supplementing with *Trichoderma* and *Pseudomonas* withoutcompromising the efficacy. The results in all the three varieties were consistent with greenhouse indicating the stability of treatments in field conditions.

Hubballi, (2014) reported that the efficacy of *Pseudomonas* integrated with a reduced dose of azoxystrobin to a half of the normal dose effectively reduced the incidence of leaf blast to the tune of 86.4 per cent and neck blast up to 84.4 per cent.

In a similar study, Anand *et al.* (2010) observedmaximum reduction of anthracnose and powdery mildewin chilli when half dose of azoxystrobin was mixed with*Pseudomonas*. Further, Kishore *et al.* (2005) found that the combination of *P. aeruginosa*and chlorothalonil (500 µg/ml) reduced the severityof late leaf spot in groundnut when compared tochlorothalonil (2000 µg/ml) alone. Theseresults suggested that the dosage of fungicides can bereduced by more than 50% without compromising theefficiency of disease control.

The possible reason of managing the disease by chemicals and bioagents attributed to the biochemical mode of action of mefenoxam and metalaxyl involves inhibition of RNA synthesis. Metalaxyl is highly inhibitory to sporangium formation, and also reduces chlamydospore and oospore formation (Cohen and Coffey 1986). It also has a high level of persistence within the plant, which can prevent colonization of leaf tissue by mycelium because it inhibits the growth of hyphae (Erwin and Ribeiro 1996).

Inhibitory action of metalaxyl against *Phytophthora* spp. has been reported by Papavizas *et al*. (1979). Significant control of Phytophthora blight of Pigeonpea (>90%) was achieved with metalaxyl (1.75 g a.i kg^{-1} seed) in a greenhouse experiment (Chaube *et al.* 1984; Kannaiyan and Nene, 1984; Agarwal, 1987; Bisht and Nene, 1988). Fullerton and Tyson (2004) reported that successful control of taro leaf blight with metalaxyl and phosphoric acid. Further, Mbong *et al*. (2013) reported, Ridomil plus, Ridomil Gold effectively managed taro blight disease caused by *Phytophthora colacasia.*

Trichoderma viride has been found to retard the radial growth of *P. infestans*. This antagonistic mode of action of *Trichoderma* could be attributed to the production of antibiotics and fungal cell wall degrading enzymes (Chutrakul *et al*. 2008; Sharma *et al*. 2009). Mycoparasitic action of *Trichoderma* in this study suggests that it has good potential in controlling *P. drechsleri*f. sp.*cajani*. Similar action of *Trichoderma* strains was reported against related pathogens, *Phytophthora cinnamomi* (Pugeg and Ian, 2006) and *Phytophthora capsici* (Ezziyyani *et al*. 2007).

Various mechanisms are involved in disease suppression by soil borne fluorescent pseudomonads, *viz.* production of siderophores, HCN and antibiotics, competition, induction of systemic resistance etc. Root colonization by the introduced organism plays an important role in biological control efficacy (O'Sullivan and O'Gara 1992; Dowling and O'Gara 1994; Bowen and Rovira 1999). Similarly, Torres-Rubio *et al.* (2000) reported *P. fluorescens* inhibited *P. infestans* to an extent of 74%. The clear inhibition zone that was observed in the dual plate is suggestive of production of antagonistic metabolites by *P. fluorescens*.

Potential use of biological control agents as replacements or supplements for chemical fungicides has been addressed in many reports (Weindling, 1932, 1934, 1937; Wright, 1956; Lindsey and Baker 1967;Elad, *et al*. 1981; Elad, *et al*. 1987; Benhamou, and Chet, 1993; Zimand *et al.* 1996; Bailey, and Lumsden 1998; Gams and Bissett 1998; Washington *et al*. 1999; Howell, 2003).

Studies also showed that the integration of bio-agents and fungicides were found to be effective against several diseases caused by Oomycetes fungi such as stem and root rot (*Phytophthora vignae*) of cowpea (Fernando and Linderman 1994), crown and root rot (*Phytophthora cactorum*) of apple (Utkhede 1987).

Summary and Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

Phytophthora blight of Pigeonpea, incited by *Phytophthora drechsleri* Tucker f. sp. *cajani* is potentially important disease of Pigeonpea after Fusarium wilt and Pigeonpea sterility mosaic disease.

Periodical survey conducted at ICRISAT fields during *kharif* 2012 and 2013 indicated that in *kharif* 2012 disease incidence ranged from 0.0 to 59.0 per cent with an average incidence of 7.47 per cent, whereas during *kharif* 2013 disease incidence ranged from 0.0 to 55.0 per cent with an average incidence of 7.25 per cent. There was a correlation between per cent disease incidence with weather parameters *viz.* cumulative rainfall, maximum temperature, minimum temperature, maximum relative humidity and minimum relative humidity. Among all weather parameters, cumulative rainfall, maximum temperature and maximum relative humidity showed positive correlation, whereas minimum temperature and minimum relative humidity showed negative correlation. Further, regression analysis showed a linear positive relationship between per cent disease incidence with cumulative rainfall and maximum relative humidity. Correlation and regression analysis suggested that cumulative rainfall and maximum relative humidity plays an important role in occurrence of disease.

In addition to periodical survey, roving survey was conducted in Deccan Plateau of India during *kharif* 2012 and 2013, and disease was presented throughout the Deccan Plateau of India from 0.0 to 54.4 per cent in *kharif* 2012 and 0.0 to 33.0 per cent in *kharif* 2013. Disease occurrence was recorded irrespective of cropping system, soil type and cultivars.

The symptoms of disease appeared at all the stages of plant. Infected young seedlings showed crown rot symptoms soon after their emergence, later collapsed and died within 4 days. In older plants, *i.e.* on 1 to 2 month old plants, water soaked lesions of varied size and shape appeared on the primary and trifoliate leaves. As the disease progressed, the entire foliage blighted. Brown, dark brown or black lesions appeared on the collar region above the ground level on the main stem and branches. During severe infection, the lesions increased in size and encircled the stems causing them to shrink. The stem above the lesion girdled, dried out and broke at the infection site. In late infections, lesions on stems developed into cankers or galls and the infected bark cracked.

Seven media *viz.* oat meal agar, corn meal agar, V-8 juice agar, pigeonpea seed meal agar, potato dextrose agar, tomato juice agar and V8 PARP (Pimaricin, Ampicillin, Refampcilin and PCNB) medium were screened for isolation of *P. drechsleri* f. sp. *cajani* from the infected stem bits. Among all media tested, V8 PARP (Pimaricin, Ampicillin, Refampcilin and PCNB) medium was efficient in supporting the growth of the pathogen.

The colonies of *P. drechsleri* f. sp. *cajani* were dull white with flat mycelium on tomato juice agar medium. Mycelium was hyaline, branched, coenocytic and filamentous. Hyphal swelling was common and it was terminal and/or intercalary. The sporangia were of proliferating type and sporangiaphores were simple sympodial. Caducity of sporangia was absent. About 70-110 zoospores were released per sporangium. It is homothallic and produced the male and female gametangia called oogonium and antheridium. Amphigynous type of sexual reproduction was observed and produced a sexual spore called oospore.

Virulence study of two isolates of *P. drechsleri* f. sp. *cajani* showed that, isolate Pdc-1 recorded highest per cent disease incidence as compared to isolate Pdc-2, Hence isolate Pdc-1 was used throughout the study.

Out of the twelve media tested for growth and sporulation of the pathogen, tomato juice agar medium supported highest mycelial growth recording 90 mm and was on par with V8 juice agar medium (89.0 mm) on 6^{th} day after incubation, followed by potato dextrose agar medium (84.20 mm) and the least growth was observed on carrot agar medium with 40.67 mm. Among the three broth tested, V8 juice broth yielded highest wet mycelial weight (96.21 mg) and dry weight (2.19 mg) which was statistically on par with tomato juice broth with 94.17 mg and 2.09 mg wet and dry weight respectively, whereas potato broth showed the least growth of the pathogen with 72.01 mg wet weight and 1.56 mg of dry weight. However, sporulation was absent in both solid and liquid media tested.

Influence of temperature on mycelial growth indicated that, as the days progressed the growth of mycelium on tomato juice agar medium increased at all the temperatures except 5, 10 and 40 ^{o}C. The maximum mycelial growth was observed at 30 ^{o}C (90.0 mm) followed by 25 ^{o}C (87.4 mm) and least growth was at temperature of 35 ^{o}C (23.5 mm). Similar trend was observed when *P. drechsleri* f. sp. *cajani* was grown on V8 juice broth. Maximum fresh and dry weight of mycelium was observed at a temperature of 30 ^{o}C (101.4 and 5.4 mg respectively) followed by 25 ^{o}C (92.4 and 3.2

mg) and least at 35 ^{o}C (42.66 and 2.00 mg). Sporulation was not observed in both agar media and broth at all the temperatures tested. The results of the present study indicated that, pathogen can grow in wide range of temperatures, however 30 ^{o}C was found superior in supporting the growth of pathogen. Whereas, temperature had no effect on sporulation either on tomato juice agar or V8 juice broth.

Effect of relative humidity (RH) on growth of the *P. drechsleri* f. sp. *cajani* revealed that growth of fungus increased as relative humidity increased from 50 per cent to 100 per cent. However RH of 75 per cent to 100 per cent yielded the maximum growth (90.00 mm) of test fungus and found statistically on par with each other. The RH of less than 75 per cent decreased growth while least growth was observed in RH 50 per cent with 82.0 mm. Irrespective of range of relative humidity sporangia and zoospores were not formed. From the result it is concluded that, RH of minimum 70 per cent can support maximum growth of the pathogen, but had no influence on induction of sporangia and zoospores.

The effect of different levels of CO_2 on radial growth of the *P. drechsleri* f. sp. *cajani* revealed that the radial growth of the pathogen increased at increased CO_2 levels. However, 550 ppm of CO_2 showed the highest mycelial growth recording 90.0 mm and was on par with 700 ppm (88.9 mm) and least growth was observed at 350 ppm (80.7 mm). Production of sporangia and zoospores were absent at all CO_2 levels.

Several protocols proposed by various researchers against *Phytophthora* spp. were tried for induction of sporangia and zoospores of *P. drechsleri* f. sp. *cajani*, only pigeonpea seed flour medium produced sparse amount of sporangia, antheridium, oogonium and oospores. A modified method of V8 juice broth with diluted tomato juice broth could induce abundant sporangia and zoospores with in 24 hours of incubation, further male and female gametangia *viz.* antheridium and oogonium and oospores were formed within 48 hours of incubation. The induction of sporulation of *P. drechsleri* f. sp. *cajani* using diluted tomato juice broth *in vitro* is first of its kind.

The effect of temperature, relative humidity and elevated level of CO_2 on induction of zoospores using diluted tomato broth method were studied and the results indicated that an increase in temperature from of 20 to 30 ^{o}C yielded an increase in number of zoospores. Maximum (183×10^3 per ml) number of zoospores was recorded at 30 ^{o}C but the production of zoospores decreased drastically at 35 ^{o}C, while at 40 ^{o}C there was no production of zoospores. Zoospore quantity and relative humidity are directly proportional to each other, further relative humidity of 100 per cent induced highest number of zoospores (188×10^3 per ml) and statistically superior over others,

followed by 95 per cent (181 × 10^3 per ml), and least number of zoospores was observed at 50 per cent with 103 × 10^3 per ml. Elevated CO_2 levels had positive correlation with induction of zoospores, Of the three levels of CO_2 tested, maximum zoospores were induced at 550 ppm (183 × 10^3) followed by 700 ppm (172 × 10^3) and least (159 × 10^3) was observed at 350 ppm. From the study it is concluded that temperature, relative humidity and CO_2 levels play an important role in induction of zoospores in diluted tomato broth method, with, highest number of zoospores produced at 30 ^oC temperature, 100 per cent relative humidity and 550 ppm of CO_2.

Temperature is a critical factor known to be governing infectivity of various *Phytophthora* spp. In the present study, incidence of disease occurred in wide range of temperatures from 15 to 40 °C, whereas at 5 and 10 °C disease incidence was not observed. Hundred per cent per cent disease incidence was recorded at 30 °C and 35 °C at 6th day after inoculation, whereas at 40 °C 100 per cent per cent disease incidence was recorded at 3rd day after inoculation. Further, incubation period and temperature were inversely proportional to each other. The disease increased with increase in temperature, which may be attributed to reduced activation of defense mechanism in host plant at increased temperature.

The effect of RH on disease incidence was studied and the results suggest that, the per cent disease increased with the increase in relative humidity in both methods of inoculation, this may be due to high humidity favouring the increase in the amount of sporangia and longevity of sporangia and aid in zoospore liberation, viability and infection of the host plant.

Effect of elevated level of CO_2 was assessed against disease incidence and the disease incidence increased at elevated level of CO_2 compared to 350 ppm at both incubator and OTC conditions, which may be due to stimulation of fungal pathogen growth rates, aggressiveness and fecundity.

Impact of soil type on disease incidence indicated that high level of disease incidence was observed in red loamy soil compared to black clay soil in both the inoculation methods. Increased incidence in red loamy soil is due to free movement of zoospores and establish the new infections.

The effect of different soil moisture regimes were studied to determine the effect on incidence of disease and the results showed that with the increase in soil moisture there was an increase in incidence of the disease. The incubation period decreased with increased amount of soil moisture at both the inoculation methods.

Flooding condition was found statistically superior in both inoculation methods with of 100 per cent disease incidence followed by 100 per cent moisture of field capacity. However the disease incidence was not observed at 80 and 60 per cent moisture in case of soil drenching method, whereas 25.1 and 22.5 per cent disease incidence was observed in soil mixing method respectively. From the experiment it is concluded that incidence of disease is favoured by flooding condition than any moisture level at field capacity due to induction of sporangia and free movement of zoospores and modify host susceptibility to the pathogen.

Pre and post inoculation flooding was assessed on disease development and results revealed that, post inoculation flooding was superior over pre inoculation flooding. Post inoculation flooding for 3, 4, and 5 days was superior over other flooding treatments with incidence of 100 per cent in both inoculation methods. Hence, post inoculation flooding has great role in disease development and minimum three days are required to induce 100 per cent disease incidence.

Among five artificial inoculation methods tested with two isolates of the pathogen to induce the disease, soil mixing of mycelial inoculum was found to be the best, followed by soil drenching of mycelial suspension. Spray inoculation of mycelial suspension did not produce any infection in both the isolates.

With the increase in quantity of inoculum there was an increase in disease incidence. Whereas in 10.0 and 12.5 per cent of inoculum was found to induce high disease incidence in both soil mixing and soil drenching method of inoculation, whereas 1.0 and 2.5 per cent of inoculum could not induce disease. Hence, 10.0 per cent of inoculum density is optimum to induce the disease.

Pigeonpea plants of different ages (10 to 120 day old plants) were inoculated with *P. drechsleri* f. sp. *cajani*, and the result indicated that all the age of plants were susceptible to the disease and showed 100 per cent incidence in both the inoculation methods, However, seedling stage of 10-15 day old plants may be selected for artificial inoculation, as it will reduce the quantity of inoculum required to screen the Pigeonpea genotypes.

Screening of Pigeonpea genotypes at ICRISAT, Patancheru, revealed that, genotype ICP 9174 showed the least disease incidence with 12.2 per cent, followed by genotype ICP 8863 (14.3 %) whereas, highest incidence was recorded by genotype ICPL 161 (52.5 %) and was on par with genotype ICP 7119 with 52.3 per cent. Among the 19 Pigeonpea genotypes, nine genotypes (ICP 9174, ICP 8863, JA-4, ICP 11302,

ICP 11290, BDN 2, Bahar, KPBR 80 2 1, ICP 2376) showed the moderately resistant reaction, whereas eight genotypes (ICPL 288, ICP 4135, ICP 87119, ICP 580, ICP 113, ICP 11304, UPAS 120, ICP 339) showed moderately susceptible reaction and two genotypes (ICPL 161 and ICP 7119) showed susceptible reaction and none of the genotypes showed highly resistant reaction.

Screening of Pigeonpea genotypes at Sehore, Madhya Pradesh, revealed that, three improved lines *viz.* ICPL 99044, 99055, 99099 showed resistant reaction, and 21 lines showed moderately resistant reaction.

Reaction of 57 Pigeonpea genotypes was assessed at ICRISAT research farm under natural ecosystem and the results showed that 38 genotypes did not show any disease incidence, whereas, ICPL 20092, Kamika and *Cajanus scarabeiodes* recorded a disease incidence of more than 80 per cent. However, genotypes which did not show any incidence need to be tested by artificial inoculation in greenhouse and field conditions to confirm the resistance.

Fungicides mefenoxam and metalaxyl assayed against the pathogen under *in vitro* conditions revealed that mefenoxam was statistically superior over metalaxyl in reducing the growth and inhibition of zoospore. A concentration of 2.0 µg/ml inhibited 100 per cent of mycelial growth in both the fungicides, whereas 100 per cent inhibition in induction of zoospores was achieved at 2.0µg/ml and 5.0 µg/ml concentration of mefenoxam and metalaxyl respectively.

Five isolates of *Trichoderma* and four isolates of *Pseudomonas* were evaluated against the growth of *P. drechsleri* f. sp. *cajani*, of which, *Trichoderma* isolate-3 (80.52 %) and *Pseudomonas* isolate-1 (71.90 %) gave highest growth inhibition followed by *Trichoderma* isolate-4 (75.48 %) and *Pseudomonas* isolate-4 (63.90 %). The least growth inhibition was in *Trichoderma* isolate-1 (60.85 %) and *Pseudomonas* isolate-2 (54.30 %). Hence, *Trichoderma* isolate-3 and *Pseudomonas* isolate-1 were used in further studies to manage the disease in greenhouse and field conditions.

The compatibility of *Trichoderma* isolate-3 with fungicides mefenoxam and metalaxyl were evaluated by poisoned food technique and results revealed that concentration of 0.0, 500 and 1000 µg/ml was found to be compatible with *Trichoderma* isolate-3 and it can be concluded that *Trichoderma* isolate-3 was compatible at 1000 ppm concentration of both mefenoxam and metalaxyl.

The influence of fungicides and bioagents on the incidence of Phytophthora blight under greenhouse was studied and the results implied that as the days advances the disease increased in all the three varieties. In all three varieties, the minimum disease was observed in mefenoxam seed treatment alone and was on par with seed treatment with mefenoxam plus *Trichoderma* isolate-3, mefenoxam plus *Pseudomonas* isolate-1 and metalaxyl seed treatment alone. It is concluded that reduced dosage of fungicides can be supplemented with *Trichoderma* or *Pseudomonas* without compromising the efficacy. The results of field experiment in all the three varieties were consistent with greenhouse studies indicating the stability of treatments in field conditions.

SCOPE FOR FUTURE RESEARCH

From the present investigations it is understood that the disease is prevalent in all the Pigeonpea growing areas and future research work may be formulated on the following aspects.

1. Development of weather based predictive model for occurrence of disease with long term disease and weather datasets.
2. Study the variability in pathogen under varying climatic conditions
3. Validation of resistant genotypes obtained in this study in multi-location trials
4. Study the mechanism of resistance in the resistant genotypes
5. Production and characterization of toxin by the *Phytophthora drechsleri* f. sp. *cajani.*

Literature cited

LITERATURE CITED

Agarwal, S.C. 1987. Fungicidal control of stem blight of Pigeonpea caused by *Phytophthora drechsleri* Tucker f. sp. *cajani*. *Indian Journal of Plant Protection*. 15: 35-37.

Agarwal, S.C., Gupta, R.K., and Prasad, K.V.V. 2002. *In vivo* sporulation of *Phytophthora drechsleri* f. sp. *cajani* causing blight in Pigeonpea. *Indian Journal of Pulse Research.* 15(2): 195-196.

Agrios, G.N. 2005. *Plant Pathology*, Academic Press, San Diego, CA. 635-636.

Ahmed, A. S., Sanchez, C. P., Egea, E., Candela, M. 1999. Evaluation of *Trichoderma harzianum* for controlling root rot caused by *Phytophthora capsici* in pepper plants. *Plant Pathology*. 48: 58-65.

Akhtar, M.S. and Siddiqui, Z.A. 2008. *Arbuscular mycorrhizal* fungi as potential bioprotectants against plant pathogens. *Mycorrhizae Sustainable Agriculture and Forestry.* Library of Congress. Springer Science. 61-97.

Amin, K.S., Baldev, B., Williams, F.J. 1978. *Phytophthora cajani,* a new species causing stem blight on *Cajanus cajan*. *Mycologia.* 70: 171-176.

Amthor, J.S. 1991. Respiration in a future, higher CO_2 world. *Plant Cell and Environment.* 14: 13-20.

Anand, T., Prakasam, V., Chandrasekaran, A., Samiyappan, R., Karthikeyan, G. and Saravanan, A. 2007. Compatibility of azoxystrobin (Amistar 25 SC) with biocontrol agents. *Pestology*. 31: 21-24.

Anand, T., Chandrasekaran, A., Kuttalam, S. Senthilraja, G and Samiyappan, R. 2010. Integrated control of fruit rot and powdery mildew of chilli using the biocontrol agent *Pseudomonas fluorescens* and a chemical fungicide. *Biological Control.* 52: 1-7.

Anandaraj, M., Sarma, Y. R. 1995. Diseases of black pepper (*Piper nigrum*) and their management. *Journal of Spices and Aromatic Crops*. 4: 17-23.

Anandaraj, M., Sarma, Y.R. and Venugopal, M.N. 1995. Biological control of foot rot of black pepper. In Anandraj, M. and Peter (Eds)- *Biological Control of Diseases of Spices,* Indian Institute of Spice Research, Calicut. 120.

Andrew, J., McElrone, Jason, G. Hamilton, Anthony, J. Krafnick, Mihai Aldea, Rachel, G. Knepp, Evan, H., DeLucia. 2010. Combined effects of elevated CO_2 and natural climatic variation on leaf spot diseases of redbud and sweet gum trees. *Environmental Pollution.* 158: 108–114.

Aneja, K.R. 2002. *Experiments in Microbiology, Plant Pathology, Tissue culture and Mushroom production technology*, New Age International (P) Ltd., New Delhi.

Anith, K.N. and Manomohandas, T.P. 2001. Combined application of *Trichoderma harzianum* and *Alcaligenes sp*. strain AMB 8 for controlling nursery rot disease of black pepper. *Indian Phytopathology.* 54 (3): 335-339.

Babadoost, M. and Islam, S.Z. 2002. Fungicide seed treatment effects on seedling damping off of pumpkin caused by *Phytophthora capsici*. *Plant Disease*. 87: 63-68.

Bailey, B. A., and Lumsden R. D. 1998. Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens. In. *Trichoderma* and *Gliocladium*: *Enzymes, Biological Control and Commercial Applications*. G. E. Harman and C . P. Kubicek, eds. Taylor and Francis, London. 185-204.

Bajpai, C.C. and Tripathi, H.S. 1999. Agricultural Reviews. Karnal. 20: 63-67.

Baker, E.F. and Cook, R.J. 1974. *Biological Control of Plant Pathogens*, W.H. Freeman, San Fransisco. 443.

Baker, M. S., and MacDonald, J. D. 1981. Predisposing effects of soil moisture extremes on the susceptibility of rhododendron to Phytophthora root and crown rot. *Phytopathology.* 71: 831-834.

Barta, A.L. 1986. Interaction between flooding stress and Phytophthora root rot among alfalfa cultivars. *Plant disease* 70: 310-313.

Benhamou, N., and Chet, I. 1993. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: Ultrastructure and gold cytochemistry of the mycoparasitic process. *Phytopathology*. 83: 1062-1071.

Bharadwaj, S. S., and Gupta, P.K., 1987. *In vitro* antagonism of *Trichoderma* species against fungal pathogens associated with rhizome rot of ginger. *Indian Journal of Mycology and Plant Pathology*. 5: 41-42.

Bhardwaj, S.S. 1983. Studies on fruit rot and leaf blight of bell pepper (*Capsicum annuum* L.) caused by *Phytophthora nicotianae* var. *nicotianae* Waterhouse. Ph.D Thesis, Department of Mycology and Plant Pathology, HPKV, Solan. 114.

Bhargava, P.K., Gupta, R.K., 1983. Reaction of Pigeonpea varieties to Phytophthora stem necrosis in field. *JNKVV Research journal.* 17: 301-303.

Biles, C. L., Bruton, B. D., Wall, M. M., and Rivas, M. 1995. *Phytophthora capsici* zoospore infection of pepper fruit in various physical environments. *Proceeding Okla Academic sciences.* 75: 1-5.

Birendra S. and Dubey, S.C. 2005. Variability in *Phytophthora drechsieri* f. sp. *cajani* in response to host differentials and fungicides. *Indian Phytopathology*. 58 (1): 57-62.

Birendra, S., and Dubey, S.C. 2005. Cultural and Morphological in *Phytophthora drechsleri* f. sp. *cajani* causing Pigeonpea blight. *Annual of plant protection sciences.* 13(2): 508-510.

Bisht, V.S., Nene, Y.L., 1988. A selective medium for *Phytophthora drechsleri* f. sp. *cajani* causing Pigeonpea blight. *International Pigeonpea Newsletter.* 8: 12-13.

Bowen, G. D., and Rovira, A. D. 1999. The rhizosphere and its management to improve plant growth. *Advances in Agronomy*. 66: 1-10.

Bowers, J.H., Sonoda, R.M., Mitchell, D.J., 1990. Path coefficient analysis of the effect of rainfall variables on the epidemiology of Phytophthora blight of pepper caused by *Phytophthora capsici. Phytopathology.* 80: 1439-1446.

Boyle, C. and Aust, H.J. 1997. Ontogenetically determined resistance (adult plant resistance). In: Hartleb Heitefuss R, Hoppe, HH (eds). *Resistance of Crop Plants Against Fungi*. Fischer Verlag, Jena. 254-271.

Bradford, K. J., May, D. M., Hoyle, B. J., Skibinski, Z. S., Scott, S. J., and Tyler, K. B. 1988. Seed and soil treatments to improve emergence of muskmelon from cold or crusted soils. *Crop Science.* 28: 1001-1005.

Bunce, J.A. 1990. Short and long term inhibition of respiratory carbon dioxide efflux by elevated carbon dioxide. *Annals of Botany*. 65: 637-642.

Bywater, J., and Hickman, C.J. 1959. A new variety of *Phytophthora erythroseptica* which causes a soft rot of pea roots. *Transactions of the British Mycological Society.* 42: 513-524.

Cafe-Filho, A.C., Duniway, J.M., 1995. Effects of furrow irrigation schedules and host genotype on Phytophthora root rot of pepper. *Plant Disease*. 79: 39-43.

Chakraborty, S., Murray, G.M., Magarey, P.A., Yonow, T., O'Brien, R., Croft, B.J., Barbetti, M.J., Sivasithamparam, K., Old, K.M., Dudzinski, M.J., Sutherst, R.W., Penrose, L.J., Archer, C., Emmett, R.W. 1998. Potential impact of climate change on plant diseases of economic significance to Australia. *Australasian Plant Pathology.* 27: 15-35.

Chakraborty, S., Pangga, I.B., Lupton, J., Hart, L., Room, P.M., Yates, D. 2000b. Production and dispersal of *Colletotrichum gloeosporioides* spores on *Stylosanthes scabra* under elevated CO_2. *Environmental Pollution*. 108: 381-387.

Chakraborty, S. Luck, J. Hollaway, G. 2008. Impacts of global change on diseases of agricultural crops and forest trees. CAB Reviews: *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. 3: 1-15.

Charles L. B. Benny D. B. Marisa M. W and Melinda R. 1995. *Phytophthora capsici* zoospore infection of pepper fruit in various physical environments. *Proceeding Okla Academic sciences*. 75: 1-5.

Chaube, H.S., Singh, U.S., and Sinha, P.K. 1984. Translocation and persistence of metalaxyl in Pigeonpea. *Indian Journal of Agriculture Sciences*. 54 (9): 777-779.

Chaube, H.S., V.K. Razdon and U.S. Singh 1987. Effect of metalaxyl on growth, sporulation and sporangial germination of *Phytophthora drechsleri* f. sp. *cajani*. *Internenational Pigeonpea News letter*. 6: 59-61.

Chauhan, V.B., Singh, V.B. and Singh, A.K. 2002. Evaluation of Pigeonpea genotype for resistance to Phytophthora blight. *International Chickpea and Pigeonpea Newsletter.* 9: 42.

Chauhan, V.B., Singh, V.B. and Singh, A.K. 2002. Status of Phytophthora blight of Pigeonpea in eastern Uttar Pradesh. *Annuals of plant protection sciences.* 10(2) : 402-404.

Chen, D.W., and Zentmyer, G.A. 1970. Production of sporangia by *Phytophthora cinnamomi* in axenic culture. *Mycologia*. 62: 397- 402.

Chet, I., 1987. *Trichoderma* application, mode of action and potential as a biocontrol agent of soil borne plant pathogenic fungi, In: *Innovative approaches to plant disease control* (Ed. Chet. I.). J. Wiley and Sons Inc. New York, USA. 137-160.

Chet, I., Harman, G.E. and Baker, R. 1987. *Trichoderma hamatum* its hyphal interactions with *Rhizoctonia solani* and *Pythium* sp. *Microbial ecology*. 7: 29-38.

Chaudhary, R.G and Dhar, V. 2008. Resistant sources against Phytophthora blight in Pigeonpea. *Indian Phytopathology*. 61(3): 359.

Chutrakul, C., Alcocer, M., Bailey, K and Peberdy, J.F. 2008. The production and characterization of Trichotoxin peptaibols by *Trichoderma* sp. *Chemistry and Biodiversity*. 5: 1694-1706.

Coakley, S.M., Scherm, H., Chakraborty, S. 1999. Climate change and plant disease management. *Annual Review of Phytopathology*. 37: 399–426.

Coffey, M.D. and Bower, L.A. 1984. *In vitro* variability among isolates of six *Phytophthora* species in response to metalaxyl. *Phytopathology*. 74: 502-506.

Cohen, M., and Knorr, L. C. 1960. Winter outbreaks of citrus brown rot in Florida. *Plant Disease Report.* 44: 578-579.

Cohen, Y., M. Reuveni and H. Eyal. 1979. The systemic antifungal activity of ridomil against *Phytophthora infestans* on tomato plants. *Phytopathology.* 69: 645-649.

Cohen, Y. and Coffey, M.D. 1986. Systemic fungicides and the control of oomycetes. *Annual Review of Phytopathology.* 24: 311-338.

Cox, D. R. and Hinkley, D. V. 1979. *Theoretical Statistics.* Chapman & Hall/ CRC Press. Florida. 458.

Dance, M.H., Newhook, FJ., Cole, J.S. 1975. Bioassay of *Phytophthora* spp. in soil. *Plant Disease report*. 59: 523.527.

De Souza, J.T., Arnould, C., Deuvolt, C., Lemanceau, P., Gianinazzi-Pearson, V., and Raaijmakers, J.M. 2003. Effect of 2, 4-diacetylphloroglucinol on pythium: cellular responses and variation in sensitivity among propagules and species. *Phytopathology*. 93: 966-975.

Dhingra, O.D. and Sinclair, J.B. 1995. *Basic Plant Pathology Methods.* CRC Press, Inc. Boca Raton, Florida. 132-163.

Dirac, M.F., and Menge, J.A. 2002. High temperatures are not responsible for lack of infection of citrus roots by *Phytophthora citrophthora* during summer, but suppressive soil microorganisms may inhibit infection by *Phytophthora citrophthora*. *Plant and Soil.* 241: 243-249.

Dorrance, A. A., and McClure, S. A. 1999. Evaluation of seed treatment fungicides for the control of Phytophthora root rot on roundup ready soybean cultivars. *Fungicide and nematicide tests*. 54: 357.

Dorrance, A. E. and McClure, S. A. 2001. Beneficial effects of fungicide seed treatments for soybean cultivars with partial resistance to *Phytophthora sojae Plant Disease*. 85 (10): 1063-1068.

Dowling, D. N., O'Gara, F. 1994. Metabolites of Pseudomonas involved in biological control of plant diseases. *Tibtech.* 12: 133–141.

Dropkin, V. 1969. The necrotic reaction of tomatoes and other hosts resistant to Meloidogyne: reversal by temperature. *Phytopathology*. 59: 1632-1637.

Dubey, S.C., 2006. Integrating bioagent with plant extract, oil cake and fungicide in various mode of application for the better management of web blight of urdbean. *Archives of Phytopathology and Plant Protection*. 39: 341-351.

Duffy, B. K., Simon, A., Weller, D. M. 1996. Combination of *Trichoderma koningii* and *Fluorescent pseudomonads* for controlling take all on wheat. *Phytopathology.* 86: 188-194.

Duniway, J. M. 1977. Predisposing of effect of water stress on the severity of Phytophthora root rot in safflower. *Phytopathology*. 67: 884-889.

Duniway. J. M. 1979. Water relations of water molds. *Annual Review of Phytopathology.* 17: 431-460.

Duniway, J. M. 1983. Effects of temperature, concentration, age, and algaecides on *Phytophthora capsici* zoospore infectivity. In. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao. *Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology*. *Plant Disease*. 94: 54-60.

Duniway, J. M. 1983. Role of physical factors in the development of Phytophthora diseases. In. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao. *Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology*. *Plant Disease*. 94: 175-187.

Eastburn, D., Degennaro, M., DeLucia, E.H., Dermody, O., McElrone, A.J. 2009. Elevated atmospheric CO_2 and ozone alter soybean diseases at SoyFACE. *Global Change Biology.* 1365-2486.

Eastburn, D. M. Melissa, M. Degennaro, Evan H. Delucia, Dermody, O and Mcelrone, A. J. 2010. Elevated atmospheric carbon dioxide and ozone alter soybean diseases at SoyFACE. *Global Change Biology.* 16: 320-330

Elad, Y. and Chet, T., 1983, Improved selective media for isolation of *Trichoderma* and *Fusarium* spp. *Phytoparasitica*. 11: 56-58.

Elad, Y., Chet, I., and Henis, Y. 1981. Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma harzianum*. *Plant Soil.* 60: 245-254.

Elad, Y., Sadowsdy, Z., and Chet, I. 1987. Scanning electron microscopical observations of early stages of interaction of *Trichoderma harzianum* and *Rhizoctonia solani*. *Transactions of the British Mycological Society.* 88: 259-263.

Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora diseases worldwide*. American Phytopathological Society, St. Paul, MN.

Erwin, D.C., Bartnici-Garcia, S., Taso, P.H., 1981. *Phytophthora: Its Biology, Taxonomy, Ecology and Pathology.* American Phytopathology Society, St. Paul. Minnesota, USA. 392.

Ezziyyani, M., Requena, M.E., Egea-Gilabert, C and Candela, M.E. 2007. Biological control of Phytophthora root rot of pepper using *Trichoderma harzianum* and

Streptomyces rochei in combination. *Journal of Phytopathology.* 155: 342-349.

Factfish.com. *Pigeon peas, production quantity (tons) - for all countries.* 2012. http://www.factfish.com/statistic/pigeon+peas,+production+quantity

Faujdar, S and Oswalt, D. L. 1992. *Pest Management in Pigeonpea*. Skill development Series. 12. ICRISAT. 23.

Fawcett, H.S. 1936. *Citrus diseases and their control.* McGraw-Hill Book Co., Inc. New York. 656.

Fawcett, H.S., and Klotz, L.J. 1934. A procedure for inducing the production of sporangial and swarm stages in certain species of *Phytophthora. Phytopathology.* 24: 693-394.

Felix, E.L. 1962. Culture media for sporangial production of *Phytophthora fragariae* (Abstr.). *Phytopathology*. 52: 9.

Fernando, W.G.D. and R.G. Linderman 1994. Chemical control of stem and root-rot of cowpea caused by *Phytophthora vignae*. *Plant Disease.* 78: 967-971.

Fraedrich, S.W., Tainter, F.H. and Miller, A.E. 1989. Zoospore inoculum density of *Phytophthora cinnamomi* and the infection of lateral root tips of short leaf and loblolly pine. *Phytopathology*. 79: 1109-1113.

Fullerton, R. A. and Tyson J. L. 2004. The biology of *Phytophthora colocasiae* and implications for its management and control. *Hort -Research, Auckland*. 1: 7.

Ferguson, A.J., and Jeffers, S.N. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. *Plant Disease.* 83: 1129-1136.

Gams, W., and Bissett, J. 1998. Morphology and identification of *Trichoderma*. In ed. Taylor and Francis, London. C. P. Kubicek and G. E. Harman. *Trichoderma* and *Gliocladium: Basic Biology, Taxonomy and Genetics*. 3-34.

Garrett, K. A., Dendy, S.P., Frank, E.E., Rouse, M.N., Travers, S.E. 2006. Climate change effects on plant disease: genomes to ecosystems. *Annual Review of Phytopathology.* 44: 490-509.

Gerlach, W. W. P., Hoitink, H. A. J., and Schmitthenner, A. F. 1976. *Phytophthora citrophthora* on Pieris japonica: Infection, sporulation, and dissemination. *Phytopathology.* 66: 302-308.

Gerrettson-Cornell, 1975. Notes on the formation of sporangia in *Phytophthora drechsleri* Tucker. *Australasian Plant Pathology Society Newsletter*. 4: 32-33.

Gifford, R.M., Lambers, H. and Morrison, J.L. 1985. Respiration of crop species under CO_2 enrichment. *Physiologia Plantarum.* 63: 351-356.

Glendinning, D., J. A. Macdonald, and J. Grainger. 1963. Factors affecting the germination of sporangia in *Phytophthora infestans*. *Transactions of the British Mycological Society.* 46: 595-603.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures in Agricultural Research.* John Wiley & Sons. New York: 643.

Goodall, G. E. Hansen, D. M. and Burns, R. M. 1962. Santa barbara county avocado root rot soil survey. *California Avocado Society, Yearbook* . 46: 63-71.

Gooding G.V., and Lucas, G.B. 1959. Factors influencing sporangial formation and zoospore activity in *Phytophthora parasitica* var. *nicotianae*. *Phytopathology*. 49: 277-281.

Gooding, G. V., and Lucas. G. B. 1959. Effect of inoculum on the severity of tobacco black shank. *Phytopathology.* 49: 274-276.

Graham, J. H., Timmer, L. W., Drouillard, D. L., and Peever, T. L. 1998. Characterization of *Phytophthora* spp. causing outbreaks of citrus brown rot in Florida. *Phytopathology*. 88: 724-729.

Granke, L.L., Hausbeck, M.K. 2010b. Effects of temperature, humidity, and wounding on development of Phytophthora rot of cucumber fruit. *Plant Disease.* 94 (12): 1417–1424.

Granke, L.L. 2011. *Effects of environmental conditions on Phytophthora capsici dispersal and disease development.* Bibliobazaar press. Charleston, South Carolina. 36.

Grant, B. R., Griffith, J. M., Irving. H. R., and Radda. M. 1984. An improved method for the synchronous production of zoospores from *Phytophthora palmivora*. *Experimental Mycology*. 8: 382-385.

Grove, G. G., and Boal, R. J. 1991. Influence of temperature and wetness duration on infection of immature apple and pear fruit by *Phytophthora cactorum*. *Phytopathology.* 81: 1465-1471.

Grove, G. G., Madden. L. V., Ellis, M. A., and Schmitthenner, A. F. 1985. Influence of temperature and wetness duration on infection of immature strawberry fruit by *Phytophthora cactorum*. *Phytopathology.* 75: 165-169.

Grover R.K., Moore J.D. 1962. Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa. Phytopathology.* 52: 876- 880.

Guo, J. H., Xue, Q. Y., & Liu, H. X. 2009b. Shu Dekang as a brand name. Patent application number: ZL7823197.

Gupta, A.K., Singh, I.S., Reddy, M.V., and Bajpai, G.C. 1997. Genetic resistance of P3 isolate to Phytophthora blight of Pigeonpea. *Euphytica.* 95: 73-76.

Gupta, C.D., Dubey, R.C., Kang, S.C and Maheshwari, D.K. 2001. Antibiotic mediated necrotrophic effect of *Pseudomonas* GRC2 against two fungal plant pathogens. *Current Sciences*. 81: 91-94.

Halsall, D.M. 1977. Effect of certain cations on the formation and infectivity of *Phytophthoa* zoospores. *Canadian Journal of Microbiology*. 23: 1002- 1010.

Hardham, A.R. 2001. The cell biology behind the *Phytophthora* pathogenicity. *Australasian Plant Pathology.* 30: 91-98.

Harman, G. E. 2000. Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T- 22. *Plant Disease*. 84: 377-393.

Hau, B., and Kranz, J. 1990. Mathematics and statistics for analyses in epidemiology. In. Ed. J. Kranz. Springer-Verlag, Berlin. *Epidemics of Plant Diseases: Mathematical Analyses and Modeling.* 12-52.

Hausbeck, M. K., and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. *Plant Disease*. 88. 1292-1303.

Hendrix, F.F., and Campbell, W.A. 1973. *Pythium* as plant pathogens. *Annual review of Phytopathology.* 11: 77-98.

Hibberd, J.M., Whibread, R. and Farrar, J.F. 1996. Effect of 700 µmol mol^{-1} CO_2 and infection with powdery mildew on the growth and carbon partitioning of barley. *New Phytolo*gy. 134: 309-315.

Hibberd, J.M., Whibread, R. and Farrar, J.F. 1996b. Effect of elevated concentrations of CO_2 on infection of barley by *Erysiphe graminis*. *Physiological Molecular Plant Pathology.* 48: 37-53.

Hoagland, D. R. and Arnon, D.I. 1950. The water culture method for growing plants without soil. *California's Agricultural Experiment Station circular number*. 347. 32.

Howell, C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease*. 87: 4-10.

Howell, C. R., and Stipanovic, R. D. 1983. Gliovirin, a new antibiotic from *Gliocladium virens* and its role in the biological control of *Pythium ultimum*. *Canadian Journal of Microbioogy*. 29: 321- 324.

Hubballi, M. and Rabindran, R. 2014. Morpho-genotyping of *Pyricularia oryzae* isolates inciting rice blast disease and its Management. *Journal of Mycology and Plant Pathology*. 44 (1): 46-53.

Huq, M, I. 2011. Studies on the Epidemiology of leaf rot and leaf spot diseases of betel vine (*Piper Betle* L.). *Bangladesh Journal of Scientific and Industrial Research.* 46(4): 519-522.

Hwang, S.C., Ko, W.H., and Aragaki, M. 1975. A simplified method of sporangial production by *Phytophthora cinnamami. Mycologia*. 67: 1233-1234.

IndiaStat.Com. India's comprehensive statistics information. *Agricultural production*. 2013. http://www.indiastat.com/agriculture/2/agriculturalproduction/225/stats.aspx.

IPCC. 2007. Climate change. 2007. Synthesis report. Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change [Core writing team, pachauri, R.K and reisinger, A.(eds)]. IPCC, Geneva, Switzerland. 104.

Ionnou, N., and Grogan, R. G. 1984. Control of Phytophthora root rot of processing tomato with thazol and metalaxyl. *Plant Disease*. 68: 429-435.

Jeffers, S.N., and Martin, S.B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease.* 70: 1038-1043.

Jia, J.S. 1992. On the chilli pepper Phytophthora blight and its control in Xinjiang. *Acta Phytophylacica Sciences*. 22: 257-263.

Jwa, N. S. and Walling, L. L. 2001. Influence of elevated CO_2 concentration on disease development in tomato. *New Phytologist.* 149: 509-518.

Kaiser, W.J., Melendez, P.L., 1978. A Phytophthora stem canker disease of Pigeonpea in Puerto Rico. *Plant Disease Reporter*. 62: 240-242.

Kannaiyan, J and Rebeiro, O K and Erwin, D C and Nene, Y L. 1980. Phytophthora blight of Pigeonpea in India. *Mycologia.* 72 (1): 169-181.

Kannaiyan, J., Nene, Y.L., Raju, T.N. and Sheila, V.K. 1981. Screening for resistance to Phytophthora blight of Pigeon Pea. *Plant Disease*. 61-62.

Kannaiyan, J and Nene, Y L and Reddy, M V., Ryan, J G and Raju, T N. 1984. Prevalence of Pigeonpea diseases and associated crop losses in Asia, Africa and the Americas. *Tropical Pest Management.* 30 (1): 62-71.

Kannaiyan, J, and Nene. Y.L. 1984. Efficacy of Metalaxyl for control of Phytophthora blight of Pigeonpea. *Indian Phytopathology*. 37(3): 506-510.

Kannaiyan J., Sheila, V.K. and Nene, Y.L. 1992. A technique for *Phytophthora drechsleri* f. sp. *cajani*. Sporangia and zoospores production. *International Chickpea and Pigeonpea Newsletter.* 1: 36-37.

Kannan, R. and Revathy, N. 2002, Biological management of foot rot of pepper caused by *Phytophthora capsici*. *Plant Disease Research*. 17(1): 127-130.

Kay, S.J. and Stewart, A. 1994. The effect of fungicides on fungal antagonists of onion white rot and selection of dicarboximide-resistant biotypes. *Plant Pathology*. 43: 863-871.

Keith, F. Jensen and Phillip, E. and Reynolds. 1971. Response of *Fusarium solani* to fluctuating temperatures. U.S.D.A. Forest service research paper NE-210. 1971.

Kim, Y. J., Hwang, B. K., and Park, K. W. 1989. Expression of age-related resistance in pepper plants infected with *Phytophthora capsici*. *Plant Disease*. 73: 745-747.

Kimball, B.A. 1983. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agronomy Journal*. 75: 779-789.

King, E.O., M.K. Ward, and D.E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine.* 44: 301-307.

Kishore, G.K, Pande, S. and Podile, A.R. 2005. Management of late leaf spot of groundnut with chlorothalonil-tolerant isolates of *Pseudomonas aeruginosa. Plant Pathology.* 54: 401-408

Kliejunas, J. T., and KO. W. H. 1974. Effect of motility of *Phytophthora palmivora* zoospores on disease severity in papaya seedlings and substrate colonization in soil. *Phytopathology.* 64: 426-428.

Klotz, L.J. and DeWolfe, T.A. 1960. Production and use of zoospores suspensions of *Phytophthora* spp. for investigations on disease of citrus. *Plant Disease Report*. 44: 572-573.

Ko, W. H., and Chan. M. J. 1974. Infection and colonization potential of sporangia, zoospores and chlamydospores of *Phytophthora palmivora* in soil. *Phytopathology* 64: 1307-1309.

Kobayashi, T., Ishiguro, K., Nakajima, T., Kim, H.Y., Okada, M., Kobayashi, K. 2006. Effects of elevated atmospheric CO_2 concentration on the infection of rice blast and sheath blight. *Phytopathology.* 96: 425–431.

Kuan, T. L., and Erwin, D. C. 1980. Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. *Phytopathology.* 70: 981-986.

Kuhlman, E.G. 1964. Survival and pathogenicity of *Phytophthora cinnamomi* in several western Oregon soils. *Forest Sciences*. 10: 151–158.

Kulkarni, S. and Sagar, S. D., 2006, Biological control of plant diseases- An indian perspective. In Ed. Ashok V. Gadewar and B.P. Singh. *Plant Protection in New Millennium. Vol. II.* Satish Serial Publishing, New Delhi. 163-196.

Kumar Deepak and Dubey, S.C. 2001. Management of collar rot of pea by the integration of biological and chemical method. *Indian Phytopathology*. 54: 62-66.

Lacey, J. 1967. The role of water in the spread of *Phytophthora infestans* in the potato crop. *Annual Applied biology.* 59: 245-255.

Lindsey, D. L., and Baker, R. 1967. Effect of certain fungi on dwarf tomatoes grown under gnotobiotic conditions. *Phytopathology.* 57: 1262-1263.

Lipps, P.E. and Bruehl, G.W. 1980. Infectivity of *Pythium* spp. zoospores in snow rot of wheat. *Phytopathology.* 70: 723-726.

Liu, H. X, Shi, M. L. YuMing. L, Luo, L. X. Li, J. Q and Guo. J. H. 2014. Biological control of Ralstonia wilt, Phytophthora blight, Meloidogyne root-knot on bell pepper by the combination of *Bacillus subtilis* AR12, *Bacillus subtilis* SM21 and *Chryseobacterium* sp. R89. *European Journal of Plant Pathology.* 139: 107-116.

Long, S.P., Woodward, F.I. 1988. *Plants and temperature*. Cambridge, England: Society for Experimental Biology. 133.

Manning, W.J., Tiedemann, A.V. 1995. Climate change: potential effects of increased atmospheric carbon dioxide (CO_2), ozone (O_3), and ultraviolet-B (UV-B) radiation on plant diseases. *Environmental Pollution.* 88: 219-245.

Masood, A, Singh, B.B., Kumar, S. and Vishwa Dhar. 2005. Advances in Pigeonpea research. *Indian Society of Pulses Research and Development*, IIPR, Kanpur, India. 229-261.

Matheron, M. E., and Porchas, M. 2000. Impact of azoxystrobin, dimethomorph, fluazinam, fosetyl-Al, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. *Plant Disease*. 84: 454-458.

Matheron, M.E., and Matejka, J.C. 1992. Effects of temperature on sporulation and growth of *Phytophthora citrophthora* and *P. parasitica* and development of foot and root rot on citrus. *Plant Disease.* 76: 1103-1109.

Mbong G. A, Fokunang C N, Lum A. Fontem, Bambot M. B, Tembe E.A. 2013. An overview of *Phytophthora colocasiae* of cocoyams: A potential economic

disease of food security in Cameroon. *Discourse Journal of Agriculture and Food Sciences*. 1(9): 140-145.

McElrone, A.J., Reid, C.D., Hoye, K.A., Hart, E., Jackson, R.B., 2005. Elevated CO_2 reduces disease incidence and severity of a red maple fungal pathogen via changes in host physiology and leaf chemistry. *Global Change Biology*. 11(10): 1828-1836.

McIntyre, J.L. and Taylor, G.S. 1976. Screening tobacco seedlings for resistance to *Phytophthora parasitica* var. *nicotianae*. *Phytopathology*. 66: 70-73.

Milholland, R.D., Cline, W.O. and Daykin, M.E. 1989. Criteria for identifying pathogenic races of *Phytophthora fragariae* on selected strawberry genotypes. *Phytopathology.* 79: 535-538.

Minogue, K. P., and W. E. Fry. 1981. Effect of temperature, relative humidity and rehydration rate on germination of dried sporangia of *Phytophthora infestans. Phytopathology.* 71: 1181-1184.

Mishra, A.N and Shukla, P. 1987. Prevalence of Phytophthora blight of Pigeonpea in Uttar Pradesh. *Indian Phytopathology*. 40: 56-58.

Mishra, A.N., and Shukla, P. 1986a. Relation between the age of Pigeonpea plant and its susceptibility to Phytophthora blight. *Indian Journal of Mycology and Plant Pathology.* 16: 292.

Mishra, A.N., and Shukla, P. 1986b. Screening of Pigeonpea germplasms against Phytophthora blight. *Farm Science Journal.* 11(1-2): 84-85.

Mishra, V. Rashmi, G. Srivastava, K.C. and Srivastava, N. 2010. Effect of culture media, temperature and pH on growth of *Phytophthora drechsleri* f. sp. *cajani. Annuals of Plant Protection Sciences.* 18 (1): 164-166.

Mitchell, D.J. Zentmyer, G.A. 1971a. Effect of oxygen and carbon dioxide tensions on growth of several species of *Phytophthora*. *Phytopathology.* 58: 787-791.

Mitchell, D. J., Kannwischer, M. E. and Moore, E. S. 1978. Relationship of numbers of zoospores of *Phytophthora cryptogea* to infection and mortality of watercress. *Phytopathology*. 68: 1446-1448.

Mitchell, D.J. 1978. Relationships of inoculum levels of several soil borne species of *Phytophthora* and *Pythium* to infection of several hosts. *Phytopathology.* 68: 1754-1759.

Mizubuti, E.S.G. and Fry, W.E. 1998. Temperature effects on developmental stages of isolates from three clonal lineages of *Phytophthora infestans. Phytopathology.* 88: 837-843.

Naar, Z. and Keeskes, M. 1998. Antagonism of *Trichoderma atroviride* and *Trichoderma viride* strains against *Sclerotinia minor* as influenced by mancozeb, benomyl and vinclozolin. *Acta Phytopathologica et Entomologica Hungarica*. 33: 123-130.

Nagtazaam, M. P. M., Bollen, G. J., Termorshizen, A. J. 1998. Efficacy of *Talaromyces flavus* alone or in combination with other antagonists in controlling *Verticillium dahliae* in growth chamber experiments. *Journal of Phytopathology.* 146: 165- 173.

Nambiar, K.K.N. and Sarma, Y.R., 1977, Wilt diseases of black pepper. *Journal of Plant Crops*. 5: 102-103.

Nelson. S, Brooks, F and Teves G. 2011. Taro leaf blight in hawai. Information bulletin, college of tropical agriculture and human resources, *Plant Disease.* 71: 1-14.

Nene. Y.L., Kannaiyan, J., Harware, M.P., and Reddy, M.V. 1979. Review of the work done at ICRISAT on soil-borne diseases of pigeonpea and chickpea, ICRISAT. 15-25.

Nene, Y. L. Kannaiyan, J. and Reddy, M. V. 1980. *Proceedings of the International Workshop on Pigeonpeas. Volume 1*. 121-122.

Nene, Y.L, Kannaiyan, J., Reddy, M.V., 1981. *Pigeon pea disease resistance screening techniques*. Information Bulletin. (9). ICRISAT, Patancheru, Andhra Pradesh, India. 5-14.

Nene, Y. L., Sheila, V. K. and Sharma, S. B. 1996. *A World List of Chickpea and Pigeonpea Pathogens*. 5th ed. ICRISAT, Patancheru, India. 27.

O'Sullivan, D. J., O'Gara, F. 1992. Traits of *fluorescent Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiology Review*. 56: 662–676.

Pal, M., Grewal, J.S. and Sarbhoy, A.K. 1970. A new stem rot of arhar caused by *Phytophthora. Indian Phytopathology*. 23: 583-587.

Pal, M. and Grewal, J.S. 1975. Physiological studies on *Phytophthora drechsleri* var. *cajani*. *Indian Phytopathology*. 28(4): 479-482.

Pal, M., Grewal, J.S., 1975. Resistance of Pigeonpea to Phytophthora blight total phenolic content. *Indian Phytopathology*. 28: 559-560.

Pal, M., Grewal, J.S., 1983. Chemical control of Phytophthora blight of Pigeonpea. *Indian Phytopathology*. 36: 380-381.

Pal, M and Grewal, J.S. 1984. Zoosporangia formation in *Phytophthora drechsleri* var. *cajani* the causal organism of Phytophthora blight of Pigeonpea. *Indian Phytopathology*. 37(1): 69-74.

Palloix, A., Daubeze, A.M. and Pochard, E. 1988. Phytophthora root rot of pepper: influence of host genotype and pathogen strain on the inoculum density–disease severity relationships. *Journal of Phytopathology*. 123-25.

Pande, S., Pathak, M., Sharma, M., Narayana Rao, J., Anil Kumar, P., Madhusudan Reddy, D., Benagi, Mahalinga, D., Zhote, D.D., Karanjkar, P.N. and Eksinghe, B.S. 2006. Outbreak of Phytophthora Blight of Pigeonpea in the Deccan Plateau of India. *ejournal.icrisat.org*. 2: 1-3.

Pande, S., Sharma, M., 2009. Status of resistance to Phytophthora blight in the improved Pigeonpea lines and hybrids at ICRISAT, Patancheru. *Legumes Pathology Progress Report*, ICRISAT, Patancheru, Andhra Pradesh. India.

Pande, S., Sharma, M., 2010. Climate change: potential impact on chickpea and Pigeonpea diseases in the rainfed semi-arid tropics (SAT). In: 5th International Food Legumes Research Conference (IFLRC V) & *7th European Conference on Grain Legumes* (AEP VII), Antalya, Turkey. 26-30.

Pande, S. Sharma, M, Mangla, U. N. Ghosh, R, Sundaresan, G. 2011. Phytophthora blight of Pigeonpea [*Cajanus cajan* (L.) Millsp.]: An updating review of biology, pathogenicity and disease management. *Crop Protection.* 30: 951-957.

Pande, S., Sharma, M., Guvvala, G. and Telangre, R. 2012. High throughput phenotyping of Pigeonpea diseases: Stepwise identification of host plant resistance. *Information bulletin*, ICRISAT. 93: 19-20.

Pao-Jen Ann. 2000. Phytophthora diseases of some ornamental foliage plants as new records in Taiwan. *Plant Pathology Bulletin.* 9: 47-52.

Papavizas, G. C., Schweak, F.W. Locke, J.C. Lewis, J.A. 1979. Systemic fungicides for controlling of Phytophthora root rot and damping off of soybean. *Plant disease*. 63: 708-711.

Papavizas, G.C. and Lewis, J.A. 1981. Introduction and augmentation of microbial antagonists for the control of soil borne plant pathogens. In. Ed. G.C. Papavizas, Allanheld, Osmum, New York. *Biological Control in Crop Production*. 305-322.

Papavizas, G.C., 1985, *Trichoderma* and *Gliocladium*: Biology, Ecology and their potential for biological control. *Review of Phytopathology*. 23: 23-54.

Parra, G., and Ristaino, J. 1998. Insensitivity to Ridomil Gold (mefenoxam) found among field isolates of *Phytophthora capsici* causing Phytophthora blight on bell pepper in North Carolina and New Jersey. *Plant Disease*. 82: 711.

Pathak, V.N. 1984. *Laboratory Manual of Plant Pathology*. Second edition. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 11–12.

Peries, O. S., and Fernando T. M., 1972. Studies on the biology of *Phytophthora meadii*. *Rubber Research Institute Ceylon.* 49: 13-27.

Petri, L. 1917. Research on the morphology and biology of *Blepharospora cambivora*, from chest. *Atti. R. Accad. Lincei, Rend. Cl. Sci. Fis. Mat. Nat. Ser.* 5: 297-299.

Poorter, H. 1993. Interspecific variation in the growth response of plants to elevated CO_2: a search for functional types. In. Ed. Korner C, Bazzaz F.A., San Diego, C.A. USA. Academic Press. *Carbon dioxide, populations, and communities.* 212.

Porras, M.; Barrau, C.; Arroyo, F.T.; Santos, B.; Blanco, C. & Romero, F. 2007. Reduction of *Phytophthora cactorum* in strawberry fields by *Trichoderma* spp. and soil solarization. *Plant Disease.* 91: 142- 146.

Prather, M. and Ehhalt, D. 2001. Atmospheric chemistry and greenhouse gases. In. Ed. Houghton J.T. The Scientific Basis. Cambridge University Press, New York, NY, USA. *Climate Change.* 239-287.

Pugeg, I.N.A and Ian, D.G. 2006. Mycoparasitic and antagonistic inhibition on *Phytophthora cinnamomi* by microbial agents isolated from manure composts. *Plant Pathology*. 5: 291- 298.

Raguchander, T., Rajappan, K. and Samiappan, R. 1997. Evaluating methods of application of biocontrol agent in the control of mungbean root rot. *Indian Phytopathology*. 50(2): 229-234.

Rajan, P.P., Sarma, Y.R. and Anandaraj, M. 2002. Management of foot rot disease of black pepper with *Trichoderma* sp. *Inidan Phytopathology*. 55(1): 34-38.

Ramachadran, N. and Sarma, Y.R. 1985. Efficacy of three systemic fungicides in controlling *Phytophthora* infection of black pepper. *Indian Phytopathology*. 38: 160-162.

Ramachandran, N., Sarma, Y.R., Anandaraj, M. and Jose Abraham. 1988. Effect of climatic factors on *Phytophthora* leaf infection in black pepper grown in arecanut black pepper mixed cropping system. *Journal of Plantation Crops.* 16: 140-148.

Ramachandran, N. and Sarma, Y.R. 1989. Effect of pH on *in vitro* activity of Fosetyl-Al on *Phytophthora palmivora* MF4. *Annuals of Applied Biology.* 114: 34-35.

Ramachandran, N. and Sarma, Y.R. 1990. Bio-efficacy of systemic fungicides against *Phytophthora* infection in black pepper (*Piper nigrum* L.). *Journal of Plant Crop*. 20: 79- 84.

Ramachandran, N., Sarma, Y.R. and Anandaraj, M. 1990b. Management of *Phytophthora* infection in black pepper. In. Ed. Y.R. Sarma and T. Premkumar. Workshop on pepper research. NRCS, Calicut, India. *Diseases of black pepper*. 158-174.

Ramirez, B. N., and Mitchell. D. J. 1975. Relationship of density of chlamydospores and zoospores of *Phytophthora palmivora* in soil to infection of papaya. *Phytopathology*. 65: 780-785.

Rangaswami, G. 1958. An agar blocks technique for isolating soil microorganisms with special reference to Pythiaceous fungi. *Science and Culture*. 24: 85.

Reddy, T.G.N. and Nagarajan, K. 1980. Evaluation of germplasm and chemical control of *Phytophthora parasitica* var. *nicotianae* on tobacco. *Workshop on Phytophthora Diseases of Tropical Cultivated Plants.* 100-185.

Reddy, M. V and Jain, K. C. 1989. Recent advances in breeding for disease resistance in Pigeonpea. In: *New Frontiers in Pulses Research and Development: Proceedings of National Symposium*. ICRISAT, Patancheru, India.

Reddy, M. V., Nene, Y.L., Raju, T.N., Sheila, V.K., Nandita Sarkar, Remanandan, P., and Amin, K.S. 1990. Disease debris field inoculation technique for Phytophthora blight of Pigeonpea. *International Pigeonpea Newsletter.* 12: 25-26.

Reddy, M.V., Sharma, S.B., Nene, Y.L., Hall, S.D. and Sheila, V.K. 1990. Pigeonpea disease management. In Ed. Nene. Y.l. , Hall., S.D., and Sheila, V.K., *The Pigeonpea*. Oxford, U.K. 349.

Reddy, M.V., Sarkar, N., Nene, Y.L., Raju, T.N., 1991. Predisposing factors for Phytophthora blight of Pigeonpea. *Indian Phytopathol*ogy. 268-270.

Reddy, M.V. and Sheila, V.K. 1994. Phytophthora blight of Pigeonpea: Present status and future priorities. *International Journal of Pest Management.* 40(1): 98-102.

Reddy, M. V., Raju, T. N. and Lenne, J. M. 1998. Diseases of Pigeonpea. In. Ed. D.J. Allen and J.M. Lenne. CAB International, Wallingford, UK. *The Pathology of Food and Pasture Legumes.* 517-558.

Ribeiro, O.K., 1978. *A Sourcebook of the Genus Phytophthora.* In: Cramer, J., Lehre, W. (Eds.), Germany. 420.

Rifai, M. A. 1969. A revision of the genus *Trichoderma*. Mycological Papers, Common Wealth Mycological Institute. KEW Survey, London, U. K. 116: 1-56.

Ristaino, J. B., and Johnston, S. A. 1999. Ecologically based approaches to management of Phytophthora blight on bell pepper. *Plant Dis*ease. 83: 1080-1089.

Rotem, J., Cohen, Y., and Putler, J. 1971. Relativity of limiting and optimum inoculum loads, wetting durations, and temperatures for infection by *Phytophthora infestans*. *Phytopathology.* 61: 275-278.

Rotem, J., and Cohen. Y. 1974. Epidemiological patterns of *Phytophthora infestans* under semi-arid conditions. *Phytopathology.* 64: 711-714.

Runion, G.B. 2003. Climate change and plant pathosystems – future disease prevention starts here. *New Phytologist.* 159: 531-533.

Sahu A.K., Maheshwari, S.K., Sriram. S. and Mishra, R.S. 2000. Effect of temperature and pH on the growth of *Phytophthora colocasiae*. *Annuals of Plant Protection Sciences.* 8: 112-114.

Sarkar, N., Nene, Y.L., Reddy, M.V., and Sheila, B.K. 1992. Influence of plant age on susceptibility of Pigeonpea to Phytophthora blight. *Indian Phytopathology*. 45(4): 426-429.

Sastry, M.N.L. 1982. Studies on species of *Phytophthora* affecting plantation crops in Karnataka with special reference of koleroga of arecanut and black pepper. Ph.D. thesis. University of Agricultural Sciences, Bangalore.

Satour, M.M. and Butler, E.E. 1968. Comparative morphological and physiological studies of the progenies from interspecific matings of *Phytophthora capsici. Phytopathology*. 58: 183-192.

Sawant, I. S. and Mukhopadhyay, A. N., 1990, Control of damping off of sugar beet by seed treatment with metalaxyl. *Indian Phytopathology.* 43: 408-413.

Sawant, I. S. and Mukhopadhyay, A. N. 1991. Integration of metalaxyl with *Trichoderma harzianum* for the control of Pythium damping-off in sugarbeet. *Indian Phytopathology.* 43: 535-541.

Sawanth, I. S., Sawanth, S. D., *Nayana, K. A.* 1995. Biological control of Phytophthora root rot of coorg mandarin (*Citrus reticulata*) by *Trichoderma* species grown on coffee waste. *Indian Journal of Agricultural Sciences*. 65: 842–846.

Scarascia-Mugnozza, G, De Angelis, P and Sabatti, M. 2005. Global change and agroforest ecosystems: adaptation and mitigation in a FACE experiment on a poplar plantation. *Plant Biosystems.* 139: 255-264.

Schiffman-Nadel, M., and Cohen, E. 1968. Method for production of sporangia of *Phytophthora cithrophthora. Phytopathology*. 58-550.

Schmitthenner, A.F. 1973. Isolation and identification methods for *Phytophthora and Pythium. In. Proceeding of First Woody Ornamental Disease Workshop.* University of Missouri. 94-110.

Schwinn, F., and Staub, T. 1995. *Oomycetes fungicides: Modern Selective Fungicides: Properties, Applications, Mechanisms of Actions.* Gustav Fischer Verlag, New York. 323-346.

Senthilvel, V., Marimuthu, T. and Raguchander, T. 2004. Compatibility of azoxystrobin 25 SC with biocontrol agents. *Pestolology*. 28: 61-64.

Shakywar, R.C., Pathak, S.P., Pathak, M., Singh, A.K., 2012. Evaluation of taro (*Colocasia esculenta* var. *antiquorum*) genotypes against leaf blight (*Phytophthora colocasiae* Racib.) under eastern Uttar Pradesh condition. *Hort Flora Research Spectrum* 1(2): 184-186.

Sharma, M., Pande, S., Pathak, M., Narayana Rao, Anilkumar, P., Madhusudhan, D., Benagi, V.I., Mahalinga, D.M., Zhote, K.K., Karanjkar, P.N., and Eksinghe, B.S. 2006. Prevalence of Phytophthora blight in the Deccan Plateau in India. *Plant Pathology Journal.* 22(4): 309-313.

Sharma, K., Kumar, M and Misra, R.M. 2009. Morphological, biochemical and molecular characterization of *Trichoderma harzianum* isolates for their efficacy as biocontrol agents. *Journal of Phytopathology.* 157: 51-56.

Sharma, M. and Pande, S. 2013. Unravelling effects of temperature and soil moisture stress response on development of dry root rot [*Rhizoctonia bataticola* (Taub.)] Butler in Chickpea. *American Journal of Plant Sciences.* 4: 584-589.

Sharma, Y.R., Harjindar, S.,Gurdip, S., and Sidhu, P.S. 1995. Screening of Pigeonpea germplasm for resistance to Phytophthora stem blight. *Plant Disease Research.* 10(1): 102-103.

Sheila, V.K., Nene, Y.L., and Kannaiyan, J. 1983. A simple culture medium for *Phytophthora drechsleri* f. sp. *cajani*. *Indian Phytopathol*ogy. 36(1): 152-154.

Simpfendorfer, S., Harden, T.J., and Murray, G.M. 2001. Effect of temperature and pH on the growth and sporulation of *Phytophthora clandestina*. *Australasian Plant Pathology.* 30: 1-5.

Singh, B.V., Pandya, B.P., and Rathi, Y.P.S. 1985. Field screening of Pigeonpea lines for resistance to Phytophthora blight at Pantnagar, India. *International Pigeonpea Newsletter.* 4: 34-35.

Singh, U.P., Chauhan, V.B., 1985. Relationship between field levels and light and darkness on the development of Phytophthora blight of Pigeonpea (*Cajanus cajan* (L.) Millsp.) *Phytopathology.* 114: 160-167.

Sing, U.P., and Chauhan, V.B. 1988. Effect of temperature on germination of zoospores of *Phytophthora drechsleri* f. sp. *cajani*. *Indian Phytopathology.* 41: 80-85.

Sing, U.P., and Chauhan, V.B. Singh, U.S., Mukhopadhyay, A.N. Kumar, J. and Chaube, H.S. 1992. Phytophthora blight of pigeon pea. In Ed. Singh, U.S., Mukopadhya, A. N., Kumar, S. Eaglewood cliffs, New Jersy, UAS. *Plant diseases of international importance*. 375-387.

Singh, B., Chaturvedi, S.N. and Singh, R.H. 2000. Technique for mass multiplication of inoculums and inoculation of *Phytophthora dreehsleri* f. sp. *cajani* causing Pigeonpea blight. *Annuals of Plant Protection Sciences*. 8: 179-182.

Singh, B and Dubey, S. C. 2010. Bioagent based integrated management of Phytophthora blight of Pigeonpea. *Archives of Phytopathology and Plant Protection*. 43 (9): 922-929.

Smiley, R. W., Patterson, L. M., and Rhihart, K. E. L. 1996. Fungicide seed-treatment effects on emergence of deeply planted winter wheat. *Journal of Production Agriculture*. 9: 564-570.

Smith, V. L., Wilcox, W. F., and Harman, G. E. 1990. Potential for biological control of Phytophthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. *Phytopathology.* 80: 880-885.

Snedecor, G. W. and Cochran, W. G. 1989. *Statistical Methods*, Eighth Edition, Iowa State University Press. Ames, Iowa. 334-336.

Sozzi, D. and T. Staub 1987. Accuracy of methods to monitor sensitivity of *Phytophthora infestans* to phenylamide fungicides. *Plant Disease*. 71: 422-425.

Srivastav, M, Srivastaval, R.K. and Mall, T.P. 2008. Present scenario of Phytophthora stem blight disease of Pigeon pea in Bahraich district. *Indian Phytopath*ogy. 61(1) :128-129.

Srivastava. M. and Mall. T.P. 2008. Efficacy of *Trichoderma* species on *Phytophthora dresceleri* f. sp. *cajani* of Pigeon pea. *Annuals of Plant Protection Sciences.* 16(1): 162-164.

Stanier, R.Y., Doudoroff, M., Addberg, E.A., 1971. *General Microbiology*, third ed. Macmillian, London.

Subramanian, K., 2009, Integrated disease management of foot rot of black pepper caused by *Phytophthora capsici*. *Karnataka Journal of Agricultural Sciences*. 22(2): 444-447.

Suheri, H. and Price, T.V. 2000. Infection of onion leaves by *Alternaria porri* and *Stemphylium vesicarium* disease development in controlled environments. *Plant Pathology.* 49: 375-382.

Sujkowski, L.S. 1987. Seasonal variation in sporulation of *Phytophthora infestans*. *Journal of Phytopapology.* 117: 357- 361.

Suriachandraselvan. M. and Seetharaman, K. 2003. Factors influencing susceptibility of sunflower to charcoal rot disease caused by *Macrophomina phaseolina*. *Journal of Mycololgy and Plant Pathology.* 33(2): 252-256.

Tang, D.Z., Sun, Y.B., He, S.Q. and Li, Y.Q. 1992. Study on infection cycle, epidemic factors and the control of Phytophthora blight of honey dew. *Acta Phytophylacica Sinica* 19: 71-275.

Thakur, R. P., Pushpavathi, B. and Rao, V. P. 1998. Virulence characterization of single-zoospore isolates of *Sclerospora graminicola* from pearl millet. *Plant Disease*. 82(7): 747-751.

Thanh, D. T., Tarn, L. T. T., Hanh, N. T., Tuyen, N. H., Bharathkumar, S., Lee, S. V., & Park, K. S. 2009. Biological control of soil borne diseases on tomato, potato and black pepper by selected PGPR in the greenhouse and field in Vietnam. *The Plant Pathology Journal.* 25: 263-269.

Thomas, K.M., 1939, Detailed administration report of the Government Mycologist. Madras for the year -1938-39. 30.

Thompson, G.B., Drake, B.G., 1994. Insects and fungi on a C3 sedge and a C4 grass exposed to elevated atmospheric CO_2 concentrations in open-top chambers in the field. *Plant, Cell and Environment.* 17: 1161-1167.

Timmer, L.W., Zitko, S.E., Gottwald, T.R. and Graham, J.H. 2000. Phytophthora brown rot of citrus: Temperature and moisture effects on infection, sporangium production, and dispersal. *Plant Disease*. 84: 157-163.

Tiwari, D.P. and Mehrotra, R.S. 1968. Rhizosphere and rhizoplane studies of Piper betle L. with special reference to biological control of root rot disease. *Bulletin of Indian Phytopathological Society*. 4: 79-89.

Torres-Rubio, M.G., Valencia-Plata, S.A., Bernal-Sastillo, J and Martinez-Nieto, P. 2000. Isolation of *Enterobacteria*, *Azotobacter* spp. and *Pseudomonas* spp.,

producers of Indole-3-acetic acid and siderophores, from Colombian rice rhizosphere. *Revista Latinoamericana de Microbiologia.* 42: 171-176.

Trujillo, E. E. 1965. The effect of humidity and temperature of Phytohpthora blight of taro. *Phytopathology*. 55: 183-188.

Tucker, C. M. 1931. Taxonomy of the genus *Phytophthora* de Bary. *research bulletin modern agriculture experiment station.* 153. 208.

Tutte, J. 1969. *Plant pathological methods Fungi and bacteria.* Burgess publishing company. Minnesota. USA. 229.

Utkhede, R.S. 1984. Antagonism of isolates of *Bacillus subtilis* to *Phytophthora cactorum. Canadian Journal of Botany*. 62: 1032-1035.

Utkhede, R.S. 1987. Chemical and biological control of crown and root-rot of apple caused by *Phytophthora cactorum*. *Canadian Journal of Plant Pathology*. 9: 295-300.

Utkhede, R.S. and Smith, E.M. 1992. Promotion of apple tree growth and fruit production by the EBW-4 strain of *Bacillus subtilis* in apple replant disease soil. *Canadian Journal of Microbiology*. 38(12): 1270-1273.

Utkhede, R. S. and Koch, C. A. 2002. Chemical and biological treatments for control of gummy stem blight of greenhouse cucumber. *European Journal of Plant Pathology*. 108: 443-448.

Valle, R. Mishoe, J.W., Jones, J.W. and Allen, L.H. 1985. Transpiration rate and water use efficiency of soybean leaves adapted to different CO_2 environments. *Crop Science.* 25: 477-482.

Van der Plank, J. E. 1963. *Plant disease: Epidemics and control.* Academic Press, New York.

Van der Plank, J. E. 1975. *Principles of plant infection*. Academic Press, New York. 216.

Vasilakakis, C.H.O., Hadzistavros. C.S. and Sterguinopolus, G.N. 1979. The use of systemic fungicides in soil application for the control of *Phytophthora parasitica* var. *nicotianae* in tobacco field in Greece, a preliminary report. *Coresta Phytopathology and Agronomy Study Group Report*. 201-207.

Vidhyasekaran, P., Sethuraman, K., Rajappan, K., and Vasumathi, K., 1997, Powder formulation of *Pseudomonas fluorescens* to control Pigeonpea wilt. *Biological Control*. 8: 166-171.

Vingarzan, R. 2004. A review of surface ozone background levels and trends. *Atmospheric Environment.* 38: 3431-3442.

Vincent, J.M.1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 150: 850-853.

Vishwa Dhar, Singh, R. A. and Gurha, S. N. 2004. Integrated disease management in pulse crops. In. Ed. Ali Masood, B.B. Singh, Shiv Kumar and Vishwa Dhar, Indian Society of Pulses Research and Development, IIPR, Kanpur, India. *Pulses in New Perspective*. 325-344.

Vishwa Dhar, Reddy, M. V. and Chaudhary, R. G. 2005. Major diseases of Pigeonpea and their management. In. Ed. Ali Masood, and Shiv Kumar. IIPR, Kanpur, India. *Advances in Pigeonpea Research*. 229-261.

Warren, R. C., and Colhoun, J. 1975. Viability of sporangia of *Phytophthora infestans* in relation to drying. *Transactions of the British Mycological Society.* 64: 73-78.

Washington, W. S., Engleitner, S., Boontjes, G. and Shanmuganathan, N. 1999. Effect of fungicides, seaweed extracts, tea tree oil, and fungal agents on fruit rot and yield in strawberry. *Australian Journal of Experimental Agriculture*. 39: 487-494.

Waterhouse, G.M and Blackwell. 1963. *Key to the species of Phytophthora de Bary.* Mycological Paper. 92. Commonwealth Mycological Institute, Kew, England. 1-21.

Wearing, A.H., Birch, J. 1988. Root rot of Pigeonpea. *International Pigeonpea News letter.* 8: 13.

Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology.* 22: 837-845.

Weindling, R. 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology* 24: 1153-1179.

Weindling, R. 1937. The isolation of toxin substances from the culture filtrates of *Trichoderma* and *Gliocladium*. *Phytopathology.* 27: 1175-1177.

Weller, D. M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology*. 26: 379-407.

Whipps, J. M. 1997. Developments in biological control of soil borne plant pathogens. *Advances in Botanical Research*. 26: 1-134.

Whiteside, J. O. 1970. Factors contributing to the restricted occurrence of citrus brown rot in Florida. *Plant Disease Report*. 54: 608-611.

Wilcox, W.F. and S.M. Mircetich. 1985a. Effects of flooding duration on the development of Phytophthora root and crown rots of cherry. *Phytopathology.* 75: 1451-1455.

Williams, F J., Amln, K.S. and Baldev, S. 1975. Phytophthora stem blight of *Cajanus cajan*. *Phytopathology*. 65: 1029-1030.

Williams, F.J., Grewal, J.S., Amin, K.S. 1968. Serious and new diseases of pulse crops in India in 1966. *Plant Disease Report*. 52: 300-304.

Wills, W. H. 1954b. Sporangium formation of *Phytophthora parasitica.* var. *nicotiana. Journal of Elisha Mitchell Science Society*. 70: 235-243.

Wright, J. M. 1956. Biological control of a soil-borne *Pythium* infection by seed inoculation. *Plant Soil.* 8: 132-140.

Xhang, J. C., Howell, C. R., Starr, J. I. 1996. Suppression of *Fusarium* colonization of cotton roots and fusarium wilt by seed treatment with *Gliocladium virens* and *Bacillus subtilis. Biocontrol Science and Technology.* 6: 175-187.

Yan, Z., Reddy,M. S., Ryu, C.M.,Mc. Inroy, J.A.,Wilson,M. A., & Kloepper, J. W. 2002. Induced systemic protection against tomato late blight elicited by plant growth promoting rhizobacteria. *Phytopathology.* 92: 1329-1333.

Yannis R. and Michael W. D. 2001. Effects of inoculum density, plant age and temperature on disease severity caused by pythiaceous fungi on several plants. 30(1): 67-76.

Zaki, A.I. and Zentmyer, G.A. 1976. An approach to synchoronous myeclail growth for sporangium production of *Phytophthora cinnamomi*. *Phytophthora newsletter*. 4: 31-32.

Zentmyer, G.A. and Marshell, L.A. 1959. Factors affecting the sporangial production by *Phytopthota cinnamomi* (Abstr.). *Phytopathology*. 49: 556.

Zentmyer, G.A., 1963, Biological control of Phytophthora root rot of avocado with alfa alfa meal. *Phytopathology*. 53v 4383-4385.

Zentmyer, G.A. 1965. Bacterial stimulation of sporangium production in *Phytopthota cinnamomi. Science*. 150: 1178-1179.

Zentmyer, G.A., 1967, Recent advances in control of soil fungi. F.A.O. *Plant Protection Bulletin.* 15: 1-5.

Zentmeyer, G.A., Leary, J.V., Klure, L.J., and Grantham, G.L. 1976. Variability in growth of *Phytophthora cinnamomi* in relation to temperature. *Phytopathology.* 66: 982-986.

Zentmyer. G.A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Monograph 10. The American Phytopathological Society, St. Paul, MN. 96.

Zentmeyer, G.A. 1981. The effect of temperature on growth and pathogenesis of *Phytophthora cinnamomi* and on growth of its avocado host. *Phytopathology.* 71: 925-928.

Zimand, G., Elad, Y., and Chet, I. 1996. Effect of *Trichoderma harzianum* on *Botrytis cinere*a pathogenicity. *Phytopathology.* 86: 1255-1260.

Zitko, S. E., Timmer, L. W., and Sandler, H. A. 1991. Isolation of *Phytophthora palmivora* pathogenic to citrus in Florida. *Plant Disease*. 75: 532-535.

Zhu, Y., Qian, W. and Hua, J. 2010. Temperature modulates plant defense responses through NB-LRR proteins. *PLoS Pathogens*. 6(4):1-12.

APPENDIX A

1. Tomato juice agar

Tomato Juice 200 ml

$CaCO_3$ 2 g

Agar - 20 g

Distilled water - 1000 ml

Take a one liter beaker and 200 ml of tomato juice and add 2 gram of $CaCO_3$ then add 20 gram of agar and make up to volume of 1000 ml.

2. V8 PARP (Pimaricin Ampicillin Rifampicin Pentachloronitro benzene)

Pimaricin - 400 µl/l

Ampicillin - 250 mg/l

Rifampicin - 1 ml/l

Pentachloronitro benzene (PCNB) - 5 ml/l

V8 juice - 100 ml

CaCO3 - 2 g

Dextrose - 20 g

Agar - 20 g

Distilled water - 900 ml

Preparation of PARP

Pimaricin - Prepare 2.5 % aqueous solution of pimaricin.

Rifampicin - 10 mg Rifampicin diluted in 1 ml DMSO

PCNB - 1 gm PCNB diluted in 200 ml ethanol.

Provide gentle heating in water bath at 70 ^{o}C.

Suspend 44.3 g of V8 agar in 100 ml distilled water and sterilize it. Add PARP in media at the time of pouring.

3. V8 juice agar medium

V8 juice agar medium is a product of the Campbell soup co. and it contains juices of tomato, carrot, celery, beet parsley, lettuce, spinach, watercress, vitamin C, and salts.

V8 juice - 100 ml

CaCO3 - 2 g

Dextrose - 20 g

Agar - 20 g

Distilled water - 900 ml

Add this composition in distilled water, dissolve the chemicals and sterilize at 121 ^{o}C, 15 lb pressure for 20 min. V8 agar consists of 20 % V8 juice, 0.2 % calcium carbonate,

and 2 % Difco agar, adjusted to pH 6.0-6.5 with sodium hydroxide. Cleared V8 broth contains V8 juice (100 ml), calcium carbonate (4 g), and distilled water (100 ml). Centrifuged this at 5000 g for 20 min, and filter the supernatant through Whatman no.1 filter paper. Dilute this up to 10 fold with glass distilled water, adjust pH to 6.0-6.5 and sterilize by autoclave at 15 psi for 20 min.

4. Pigeonpea sand- flour medium

Sand - 90 g

Pigeonpea - 10 g

Distilled water - 20 ml

Take a 250 ml conical flask and add 10 g Pigeonpea flour with 90 g riverbed sand and add 20 ml distilled water to the flask.

5. Potato dextrose agar (PDA)

Peeled, sliced potatoes - 200 g

Agar - 20 g

Dextrose - 20 g

Distilled water - 1000 ml

Boil sliced potatoes in distilled water at 110 ^{o}C for 10 min, collect the juice and add 20 g of dextrose and 20 g of agar to one liter of distilled water. Pour the PDA into flasks plugged with cotton plug and sterilize by autoclaving at 121 ^{o}C at 15 lb pressure for 20 minutes. Before pouring PDA into petri plate, add streptomycin (antibiotic) with the concentration of 0.75 g per liter in order to avoid contamination of media.

6. Potato dextrose broth (PDB)

Peeled potatoes - 200 g

Dextrose - 20 g

Distilled water - 1000 ml

Cut 200 g of peeled potatoes into small equal parts and add distilled water to it and boil for 10 minutes in an oven. Add 20 g of dextrose to 1 liter of the potato extract. Pour the PDB into flasks plugged with cotton plug and sterilize by autoclaving at 121 ^{o}C at 15 lb pressure for 20 minutes.

7. *Trichoderma* selective medium

Magnesium sulphate (MgSo4) - 0.2 g

Dipotassium Hydrogen phosphate (K2HPO4) - 0.9 g

Ammonium nitrate (NH3NO3) - 1.0g

Potassium chloride (KCL) - 0.15g

Glucose (C6H12O6) - 3.0 g

Metalaxyl - 0.3 g

Pentachloronitrobenzene (PCNB) - 0.2 g

Rose Bengal - 0.15g

Chloromphenicol - 0.25 g

Agar - 20.0 g

Distilled water -1000ml

8. King' B- medium

Protease peptone - 20.0 g

Di-potassium hydrogen phosphate (K_2HPO_4) - 2.5g

Glycerol - 15.0 ml

Magnesium sulphate (Mg $SO_4.7H_2O$) - 6.0 g

Agar - 20.0 g

Water (distilled) - 1000 ml

PH - 7.2

9. Oat meal agar

Oat flakes 60 g

Agar-agar 20 g

Distilled water 1000 ml

Oat flakes were boiled in 500 ml of distilled water for 20 min and the extract was filtered through a muslin cloth. Agar-agar was melted separately in 500 ml of distilled water. Both the solutions were mixed thoroughly. The volume was made up to 1000 ml with distilled water and sterilized.

10. Corn (maize) meal agar

Maize 30 g

Agar-agar 20 g

Water 1000 ml

Maize meal was boiled in distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in distilled water. The corn extract was mixed in the molten agar and volume was made up to 1000 ml with distilled water and sterilized.

11. Czapek's agar

Sucrose -30 g

Sodium nitrate (NaNO3) - 2 g

Potassium dihydrogen phosphate (K_2HPO_4) - 1 g

Magnesium sulphate ($MgSO_4.7H_2O$) - 0.5 g

Potassium chloride (KCl)- 0.5 g

Ferrous sulphate ($FeSO_4.7H_2O$)- 0.01 g

Agar-agar 20 g

Distilled water 1000 ml

Agar-agar was melted in 500 ml of distilled water. All the ingredients were mixed thoroughly. The volume was made up to 1000 ml and sterilized.

12. Tomato juice broth

Tomato Juice 200 ml

$CaCO_3$ 2 g

Distilled water - 1000 ml

Take a one liter beaker and 200 ml of tomato juice and add 2 gram of $CaCO_3$ then add 20 gram of agar and make up to volume of 1000 ml.

13. Diluted tomato juice broth

Tomato Juice 50 ml

$CaCO_3$ 2 g

Distilled water - 1000 ml

Take a one liter beaker and 200 ml of tomato juice and add 2 gram of $CaCO_3$ then add 20 gram of agar and make up to volume of 1000 ml.

14. Potato sucrose agar

Peeled, sliced potatoes - 200 g

Agar - 20 g

Sucrose - 20 g

Distilled water - 1000 ml

Boil sliced potatoes in distilled water at 110 ^oC for 10 min, collect the juice and add 20 g of sucrose and 20 g of agar to one liter of distilled water. Pour the PDA into flasks plugged with cotton plug and sterilize by autoclaving at 121 ^oC at 15 lb pressure for 20 minutes. Before pouring PDA into petri plate, add streptomycin (antibiotic) with the concentration of 0.75 g per liter in order to avoid contamination of media.

15. Potato glucose agar

Peeled, sliced potatoes - 200 g

Agar - 20 g

Glucose - 20 g

Distilled water - 1000 ml

Boil sliced potatoes in distilled water at 110 ^oC for 10 min, collect the juice and add 20 g of glucose and 20 g of agar to one liter of distilled water. Pour the PDA into flasks

plugged with cotton plug and sterilize by autoclaving at 121 ^{o}C at 15 lb pressure for 20 minutes. Before pouring PDA into petri plate, add streptomycin (antibiotic) with the concentration of 0.75 g per liter in order to avoid contamination of media.

16. Chickpea seed agar

Chickpea seed - 250 g

Agar – 15 g

Sucrose – 20 g

Distilled water - 1000 ml

Wash chickpeas seed in tap water and then soak in distilled water overnight. Decant the water and mesh the seed. Add 1 liter of water and steam fir 1 hour. Filet through cheesecloth. Dissolve the 15 gram of agar and 20 gram of sucrose, separately, and then add to the filtrate. Make the volume to 1 liter.

16. Carrot agar

Sliced carrot – 20 g

Agar – 20 g

Distilled water - 1000 ml

Soak the 20 gram of sliced carrot for 1 hour in 1 liter of water and then boil for 5 minute. Filter and add 20 gram of agar.

APPENDIX B

Weekly meteorological data recorded at ICRISAT, Patancheru during 2012

Standard week	Rainfall (mm)	Temperature (^{o}C)		Relative humidity (%)	
		Maximum	Minimum	Maximum	Minimum
30	33.20	29.19	21.87	88.43	70.00
31	12.00	28.82	21.46	89.56	71.00
32	14.90	30.39	21.73	89.29	65.00
33	4.40	30.23	21.67	87.43	60.71
34	8.40	29.98	22.44	89.43	68.70
35	55.39	28.82	22.00	92.00	76.14
36	28.00	29.12	21.89	91.43	71.43
37	15.59	29.23	21.55	92.29	66.56
38	9.19	30.00	21.98	94.29	64.85
39	5.59	30.89	21.01	95.00	57.57
40	73.50	29.35	21.50	95.70	73.70
41	0.00	31.23	16.60	93.00	43.42
42	0.30	31.01	16.53	92.29	40.85
43	0.00	30.33	18.67	94.56	50.57
44	38.20	27.51	18.41	87.70	61.14
45	0.00	29.14	17.28	95.14	52.14
46	0.00	28.21	10.66	92.56	40.42
47	0.00	29.66	16.03	96.14	47.71
48	0.00	29.89	16.19	96.56	45.71
49	0.00	30.16	15.24	93.29	45.28
50	0.00	31.82	14.99	87.29	34.42
51	0.00	28.94	11.49	94.43	39.71
52	0.00	28.60	12.51	90.75	37.63

APPENDIX C

Weekly meteorological data recorded at ICRISAT, Patancheru during 2013

Standard week	Rainfall (mm)	Temperature (^{o}C)		Relative humidity (%)	
		Maximum	Minimum	Maximum	Minimum
30	57.00	27.85	21.44	88.29	73.70
31	28.60	27.96	20.80	91.00	70.00
32	52.60	28.26	21.26	91.43	71.70
33	87.79	27.85	21.42	94.85	77.29
34	3.60	28.60	20.66	88.14	65.14
35	0.80	29.26	21.48	92.70	70.29
36	51.10	30.10	21.01	93.29	63.14
37	48.89	30.19	21.76	95.43	72.14
38	177.59	29.39	20.96	94.00	73.00
39	0.00	30.82	20.91	89.70	59.14
40	24.39	29.60	21.44	93.14	68.29
41	69.40	29.92	21.00	94.70	69.14
42	5.79	31.21	19.89	91.70	51.00
43	107.59	26.80	20.82	96.85	80.00
44	0.00	29.87	18.46	94.14	54.42
45	0.00	28.53	15.16	92.70	50.14
46	0.00	27.96	13.02	90.29	37.71
47	18.69	28.03	16.19	93.00	55.42
48	2.00	28.44	16.05	93.85	53.28
49	0.00	27.62	12.18	95.29	45.28
50	0.00	28.60	8.31	94.29	30.00
51	0.00	28.10	10.83	92.56	36.00
52	0.00	26.55	12.42	93.62	46.38