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Evaluation of Current Biological and Chemical Control Methods under Climate Change to Better Manage Stem Rot Disease in Groundnut

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ABSTRACT

Background: Around 90% of global groundnut production takes place in semi-arid tropic (SAT) regions, highlighting its importance as a key oilseed and food crop that delivers essential nutrients for human consumption. However, climate change poses significant threats to both the yield and quality of groundnut products in these areas.

Methods: During the *rabi* seasons of 2022-23 and 2023-24, potential Trichoderma and Bacillus isolates were collected from rhizospheric soils in Telangana at the Groundnut Pathology Laboratory, ICRISAT, Patancheru. These isolates were assessed for their antagonistic effectand effectiveness of fungicides against *Sclerotium rolfsii* at different carbon dioxide levels (400 ppm, 550 ppm and 700 ppm) in CO_2 incubators.

Result: Results showed that *Trichoderma harzianum* (T3) achieved 73.88% and 65.55% inhibition of the pathogen's radial growth at 700 ppm and 550 ppm CO_2 levels, respectively. Meanwhile, *Trichoderma viride* (T1) exhibited 62.10% inhibition at 400 ppm CO_2. *Bacillus tequilensis* (B2) exhibited the strongest activity, reducing radial growth by 68.32% at 700 ppm. At 550 ppm, *Bacillus velezensis* (B4) achieved the highest radial growth inhibition, with a 65.55% reduction. At 400 ppm, *Bacillus cereus* (B5) demonstrated the greatest inhibition, reducing radial growth by 56.10%. Notably, 100% inhibition of *Sclerotium rolfsii* was recorded with both tebuconazole and thiram across all three CO_2 levels. Azoxystrobin showed 93.05%, 86.38% and 80.55% inhibition at 700 ppm, 550 ppm and 400 ppm CO_2 levels, respectively. Overall, the biocontrol activity of the fungal and bacterial bioagents increased with rising carbon dioxide levels, as did the effectiveness of the fungicides.

Key words: Bacillus, Carbon dioxide, Groundnut, *Sclerotium rolfsii*, Trichoderma.

INTRODUCTION

Peanut, scientifically referred to as *Arachis hypogea* L., is among the significant oilseed crops in the globe. China is the key grower, following India, United Statesand Nigeria (Groundnut Outlook, Agricultural Market Intelligence Centre, PJTSAU, 2019).

Globally, peanut is cultivated on approximately 29.58 million hectares, producing a total output of 48.74 million tonnes (FAOSTAT, 2019). In India, peanuts are cultivated across 4.7 million hectares, producing a remarkable 9.3 million tonnes (INDIASTAT, 2019). In Telangana, groundnut farming spans approximately 0.13 million hectares, yielding 0.30 million tonnes, with a significant production rate of 2,364 kg per hectare (Directorate of Economics and Statistics, 2019).

Groundnut productivity is being affected by several abiotic and biotic stresses which include poor soil fertility, moisture stress, viral diseases, collar rot and stem rot (Vamshi *et al*., 2025). Peanut crop is vulnerable to various diseases caused by viruses, nematodes, bacteria and fungi, all of which adversely affect pod yield and fodder quality. Among these, stem rot, caused by *Sclerotium rolfsii* Sacc, is particularly troublesome. This disease significantly reduces both the quality and output of peanut production and is regarded as one of the most damaging diseases for this crop, causing approximate annual yield losses ranging from 10 to 25 per cent (Sturgeon, 1986).

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Stem rot was first discovered by Peter Rolfs on tomato plants in 1892, resulting in a severe 70% reduction. The fungal hyphae show an upward development on the plant's surface, surrounded by a cotton-like, white mycelium that spreads both internally and externally, especially near the soil level. In its early stage, the fungus forms many small, spherical, evenly sized white sclerotia, which darken to brown as the fungus matures (Kwon and Park, 2002).

In India, the occurrence of stem rot is especially widespread in states such as Karnataka, Madhya Pradesh, Gujarat, Maharastra, Andhra Pradesh, Orissa and Tamil Nadu. In heavily infected fields, damage can be alarmingly high, exceeding 80% (Mehan and McDonald, 1990).

According to the latest report from the Intergovernmental Panel on Climate Change (IPCC), it has emphasizedthat greenhouse gases (GHG) have significantly risen due to human activities since 1750. These alterations will not only influence the cultivation and growth of diverse crops but also affect the reproduction, dispersal and intensity of many plant pathogens. Climate change, coupled with anthropogenic factors like water, air and soil pollution, the remote introduction of non-native species and urban expansion, will affect plant diseases. These global climatic shifts affect all three components of the disease triangle: the host, the pathogen and the environment (IPCC, 2007).

Chemical management, particularly the application of fungicides like difenconazole, chlorothalonil and tebuconazole (Cilliers *et al*., 2003), is the initial strategy for managing stem rot disease. However, the widespread use of these chemicals can result in soil contamination and the development of resistance in pathogen. For instance, the United States Environmental Protection Agency has identified tebuconazole as a probable cancer causing agent for human beings (Cui *et al*., 2018). Additionally, research has shown that tebuconazole can leach from soil into water systems via outflow, with concentrations in US streams often ranging from 0.010 to 0.115 mg/L (Bradley *et al*., 2017). Consequently, the adoption of innovative biological control agents with strong antagonistic properties is seen as a more eco-friendly approach for managing peanut stem rot disease (Whipps, 2004).

Bio-control offers a viable and environmentally friendly substitute to fungicides (Djordje *et al*., 2018). Several isolates, including Pseudomonas (Liu *et al*., 2022), Trichoderma (Motlagh *et al*., 2022), Bacillus (Li, 2018; Chen *et al*., 2020; Yang *et al*., 2017) and Streptomyces (Jacob *et al*., 2018), have demonstrated effective antagonistic properties against *Sclerotium rolfsii*, considerably decreasing the intensity and occurrence of stem rot in pot trials.

Extensive research highlights the effectiveness of *Trichoderma* species, such as *T. gamsii, Trichoderma atroviride*, *T. koningii*, *T. harzianum*, *T. polysporum*, *T. virens*, *T. asperellum* and *T. Hamatum*, as Biological control organisms for managing different soil-borne pathogens, including *Aspergillus, Rhizoctonia, Phytophthora*, *Sclerotium*, *Pythium* and *Fusarium* (Sharma and Prasad, 2018; Javaid *et al*., 2018; Moosa *et al*., 2017; Ingale and Patale, 2019).

Furthermore, multiple findings have demonstrated that the antagonism strength of fungal and bacterial agents, such as *Pseudomonas, fluorescens* and Trichoderma isolates, can be improved when paired with the organic amendments (Jangir *et al*., 2020; Karthikeyan *et al*., 2006; Vengadesh kumar *et al*., 2019). As a result, an attempt was made to identify the most efficient fungicide and biocontrol agent for managing *S. rolfsii* under different carbon dioxide concentrations.

MATERIALS AND METHODS

Isolation of *Trichoderma* and *Bacillus* species from peanut rhizosphere soil

During the *rabi* seasons of 2022-23 and 2023-24, species of *Trichoderma* and *Bacillus* were isolated at the Groundnut Pathology Laboratory, ICRISAT, Patancheru, from rhizosphere soils obtained from various groundnut-producing regions across Telangana. The plants were gently removed to preserve the integrity of the root systems and the soil attached to the roots was gathered. Ten grams of this root zone soil were placed in a 250-milliliter Erlenmeyer flask containing 100 mL of distilled water. One milliliter of the resulting 10^{-3} dilution was transferred to a Petri dish containing a Trichoderma-specific medium to isolate the fungal antagonists. Similarly, one milliliter of aliquots from the 10^{-5} and 10^{-6} dilutions was added to sterile Petri dishes with *Bacillus* specific medium(BSM). The plates were incubated for 24 hours at 27°C. Bacillus isolates were purified on nutrient agar medium using the streak plate technique (Rangaswami, 1993), while *Trichoderma* isolates were cultured on potato dextrose agar. A total of 21 isolates of *Bacillus* and 23 isolates of *Trichoderma* were obtained from the peanut root zone soil. Light microscopy was used to examine the morphology of the cultures and pure cultures of bio-control agents were maintained at 4°C on the appropriate agar slants for storage.

Screening of potential *Trichoderma* isolates against *S. rolfsii* under different carbon-dioxide levels

Potential *Trichoderma* isolates, designated T1 to T5, were assessed against a virulent strain of *Sclerotium rolfsii* using a dual culture method (Vidhyasekaran and Muthamilan, 1999). The effectiveness of each isolate was evaluated by measuring its suppressive effect on the radial expansion of the pathogen. For the assay, 6 mm mycelial plugs from actively growing colonies of both Trichoderma isolates and the pathogen were placed opposite each other, about 5 cm apart, on Petri dishes containing solidified PDA medium. Appropriate controls were set up and the plates were incubated in CO_2 incubators at different concentrations (400 ppm, 550 ppm and 700 ppm). The performance of the *Trichoderma* species was determined by measuring

the percentage inhibition of the pathogen's radial growth relative to the control plate, using the following formula:

$$I = \frac{C - T}{C} \times 100$$

Where,

I= Percentage inhibition compared to control.

C= Radial growth of *Sclerotium rolfsii* in the control plates.

T= Radial growth of *Sclerotium rolfsii* in the presence of *Tichoderma* isolates.

Screening of potential *Bacillus* isolates against *S. rolfsii* under different carbon-dioxide levels

Potential *Bacillus* isolates, designated B1 to B5, were tested against a virulent strain of *Sclerotium rolfsii* using a dual culture technique (Vidhyasekaran and Muthamilan, 1999). The effectiveness of each isolate was assessed by measuring its suppressive effect on the pathogen's radial expansion. In this experiment, each Bacillus isolate was separately streaked on one half of a Petri dish containing potato dextrose agar, while a 6 mm mycelial plug from the virulent *S. rolfsii* strain was placed on the opposite half. Appropriate controls were set up and the plates were incubated in CO_2 incubators at varying concentrations (400 ppm, 550 ppm and 700 ppm). The activity of the Bacillus isolates was evaluated by measuring the percentage inhibition of the pathogen's radial growth compared to the control plate, using the following formula.

$$I = \frac{C - T}{C} \times 100$$

Where,

I= Percentage inhibition compared to control.

C= Radial growth of *Sclerotium rolfsii* in the control plates.

T= Radial growth of *Sclerotium rolfsii* in the presence of *Tichoderma* isolates.

Sensitivity of *S. rolfsii* isolates to commonly used fungicides under varying carbon dioxide levels

The susceptibility of *Sclerotium rolfsii* isolates to four fungicides commonly employed in peanut cultivation Azoxystrobin 23.8 SC (Amistar), Carbendazim 50 WP (Bavistin), Tebuconazole 2 DS (Raxil) and Thiram 75 WP was evaluated using the poison food method at both the recommended and half the recommended concentrations. The fungicides were measured according to their specified doses and blended with potato dextrose agar medium just before being poured into Petri dishes. Appropriate controls plates were maintained without any fungicide. Mycelial plugs, 6 mm in diameter, were taken from the edges of five days old actively growing cultures of each isolate and positioned in the center of both fungicide-treated and untreated PDA plates. The plates were then incubated at various CO_2 concentrations (400 ppm, 550 ppm and 700 ppm) in CO_2 incubators. Colony diameters were measured once full growth of the isolates was observed on the control plates.

Statistical analysis

Per cent data was converted into arc sin values and square root transformed values. Fischer's method of analysis of variance was used for analysis and interpretation of the data (Gomez and Gomez, 1984). Other statistical analysis *viz*., OP STAT online statistical analysis program developed by Hissar Agricultural University, IBM SPSS and MS-excel were used to analyze the data.

RESULTS AND DISCUSSION

Influence of CO_2 levels on the biocontrol traits of potential *Trichoderma* isolates against *S. rolfsii*

Five potential Trichoderma isolates were assessed against a highly virulent isolate of *Sclerotium rolfsii* (SrPWp) using a dual culture method at three different carbon dioxide levels (400, 550 and 700 ppm) (Table 1, Fig 1, Fig 2 and Fig 3).

The results showed that *Trichoderma harzianum* (T3) achieved the highest percentage of radial growth inhibition (73.88%) of the pathogen at 700 ppm, followed closely by *Trichoderma viride* (T2) (72.77%) and another isolate of *Trichoderma viride* (T1) (69.99%), which were statistically similar. The next best bioagents were *Trichoderma hamatum* (T4) (68.77%) and *Trichoderma harzianum* (T5) (69.88%).

At 550 ppm, *Trichoderma harzianum* (T3) again recorded the highest inhibition (65.55%), followed by

Table 1: Influence of CO_2 levels on potential isolates of *Trichoderma* spp. against *Sclerotium rolfsii* in CO_2 incubators.

Trichoderma isolate	Per cent inhibition of radial growth over control		
	400 ppm	550 ppm	700 ppm
Trichoderma viride (T1)	60.55 (51.093)*	64.44 (53.400)	69.99 (56.827)
Trichoderma viride (T2)	62.10 (52.010)	64.77 (53.630)	72.77 (58.610)
Trichoderma harzianum (T3)	61.66 (51.787)	65.55 (54.080)	73.88 (59.307)
Trichodermahamatum (T4)	55.55 (48.193)	61.66 (51.740)	68.77 (56.027)
Trichoderma harzianum (T5)	58.88 (50.177)	65.00 (53.763)	69.88 (56.737)
Factors	CD (0.01)	S.Em ±	CV (%)
CO_2 levels	1.78	0.61	4.44
Isolates	2.30	0.79	
Interaction	3.98	1.38	

Trichoderma harzianum (T5) (65.00%), *Trichoderma viride* (T2) (64.77%) and *Trichoderma viride* (T1) (64.44%), all of which were on par with one another.

At 400 ppm, *Trichoderma viride* (T1) exhibited the highest inhibition (62.10%), followed closely by *Trichoderma harzianum* (T3) (61.66%) and *Trichoderma viride* (T2) (60.55%), which were also comparable.

Overall, the results indicate that as carbon dioxide levels increased from 400 ppm to 700 ppm, there was a significant increase in the inhibition of the pathogen by the Trichoderma isolates.

Trichoderma harzianum inoculated seedlings showed diminished growth at CO_2 concentrations of 1000-1200 ppm. This is further supported by microbial population studies of the root zone from treated seedlings after ninty days, indicating that bacteria, particularly *Pseudomonas putida*, were more impervious to elevated carbon-dioxide levels, succeeded by *Bacillus subtilis*. In contrast, fungal agent *Trichoderma harzianum* was more susceptible to higher CO_2 levels (1000-1200 ppm) than the bacterial agents. This may be due to the formation of endospores in bacterial agents in response to CO_2-induced stress, unlike fungal bioagents.

Vamshi *et al*. (2025) reported that *T. viride* and *Bacillus cereus* were particularly effective in inhibiting the radial growth of *S. rolfsii* in dual culture. Louaileche *et al*. (1993) found that when D12 was cultured with CO_2 supplementation, the final cell yield was significantly

Fig 2: Dual culture assay of potential isolates of *Trichoderma* spp. against virulent isolate of *Sclerotium rolfsii* at 550 ppm CO_2 level.

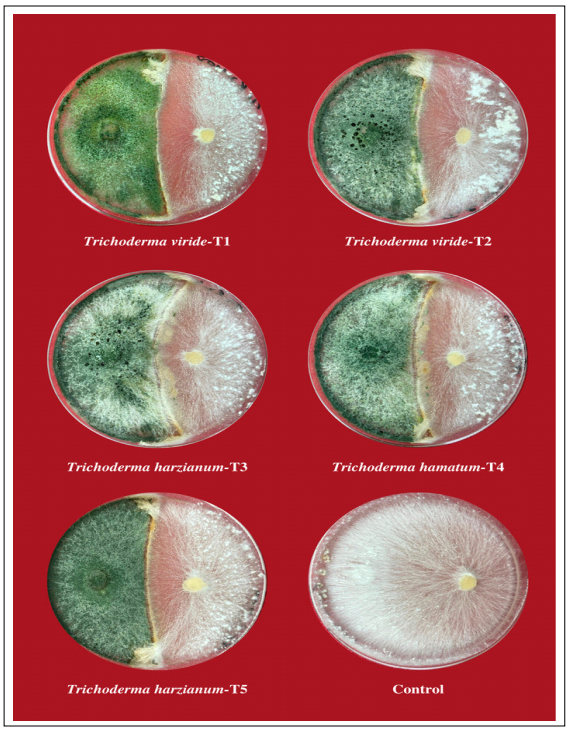

Fig 1: Dual culture assay of potential isolates of *Trichoderma* spp. against virulent isolate of *Sclerotium rolfsii* at 400 ppm CO_2 level.

Fig 3: Dual culture assay of potential isolates of *Trichoderma* spp. against virulent isolate of *Sclerotium rolfsii* at 700 ppm CO_2 level.

greater compared to cultures without CO_2. Similarly, Macueley and Griffin (1969) observed that the activity of *Trichoderma* isolates and *Gibberell azeae* in soil was enhanced at elevated CO, concentrations.

Influence of CO_2 levels on the biocontrol traits of potential *Bacillus* isolates against *S. rolfsii*

The evaluation of five potential Bacillus isolates-*Bacillus velezensis* (B1), *Bacillus tequilensis* (B2), *Bacillus velezensis* (B3), *Bacillus velezensis* (B4) and *Bacillus cereus* (B5)-against *Sclerotium rolfsii* in a dual culture method revealed that Bacillus isolates were as effective as the Trichoderma isolates in combating the pathogen. Among the isolates tested, *Bacillus tequilensis* (B2) demonstrated the highest potency, achieving a radial growth reduction of 68.32% at 700 ppm, followed closely by *Bacillus cereus* (B5) (67.77%) and *Bacillus velezensis* (B4) (66.66%), all of which were statistically similar (Table 2, Fig 4, Fig 5 and Fig 6).

At 550 ppm, *Bacillus velezensis* (B4) recorded the highest percentage inhibition of radial growth (65.55%), followed by *Bacillus cereus* (B5) (62.22%) and *Bacillus tequilensis* (B2) (60.55%), with these three isolates also being on par with one another.

At 400 ppm, *Bacillus cereus* (B5) exhibited the greatest inhibition of radial growth (56.10%), followed by *Bacillus velezensis* (B4) (54.99%) and *Bacillus tequilensis* (B2) (52.77%), which were again statistically similar.

Fig 5: Dual culture assay of potential isolate of *Bacillus* spp against virulent isolate of *Sclerotium rolfsii* at 550 CO_2 level.

Fig 4: Dual culture assay of potential isolate of *Bacillus* spp against virulent isolate of *Sclerotium rolfsii* at 400 ppm CO_2 level.

Fig 6: Dual culture assay of potential isolate of *Bacillus* spp against virulent isolate of *Sclerotium rolfsii* at 700 ppm CO_2 level.

Overall, the results indicate that as the carbon dioxide levels increased from 400 ppm to 700 ppm, there was a significant increase in the inhibition of the pathogen by the Bacillus isolates.

Comparable outcomes were reported by Enfors and Molin (1980), who noted a 50% reduction in the growth rate of *Pseudomonas fragi* at 0.5 atmosphere CO_2, with similar declines observed in *Bacillus cereus* at 1.3 atmosphere and *Streptococcus cremoris* at 8.6 atmosphere. Eklund (1984) further indicated that the growth rates of *Bacillus subtilis* and *Pseudomonas aeruginosa* were progressively inhibited at CO_2 concentrations up to 40%, while *E. coli* and *B. cereus* experienced up to 80% suppression. This assists the broader observation that increasing atmospheric carbon-dioxide levels can promote entire plant growth (Mulholland *et al*., 1998; McKee *et al*., 1995; Long *et al*., 1996). In this study, it was also observed that higher carbon-dioxide concentrations in the control chambers hindered development and plant height more than in treated treatments. However, *Pseudomonas putida* and *Bacillus subtilis* inoculated plants were more resilient to elevated CO_2 levels, resulting in better growth of the seedlings.

Response of *Sclerotium rolfsii* isolates to widely applied fungicides

A total of four fungicides thiram, carbendazim, tebuconazole and azoxystrobin were evaluated for their efficacy against *S. rolfsii* using poisoned food technique at recommended and half the recommended concentrations across three carbon dioxide levels (400 ppm, 550 ppm and 700 ppm), with results presented in (Fig 7, Fig 8, Fig 9 and Fig 10).

Among the fungicides screened, thiram and tebuconazole achieved 100% inhibition of *S. rolfsii* at both the recommended and half-recommended dosages across all three carbon dioxide levels. At 700 ppm, azoxystrobin demonstrated 93.05% and 88.33% inhibition at the recommended and half-recommended concentrations, respectively, followed by carbendazim, which recorded 88.88% and 75.83% inhibition at the same concentrations.

At 550 ppm, azoxystrobin showed 86.38% and 82.21% inhibition at the recommended and half-recommended concentrations, respectively, while carbendazim recorded 85.55% and 45.66% inhibition at the same concentrations.

At 400 ppm, azoxystrobin exhibited 80.55% and 73.32% inhibition at the recommended and half-recommended

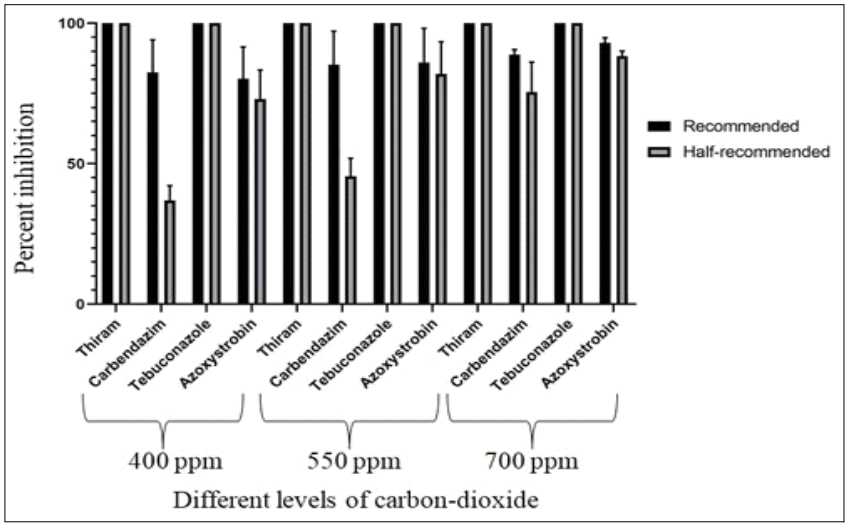

Fig 7: Sensitivity of isolates of *S. rolfsii* to commonly used fungicides in CO_2 incubators.

Table 2: Influence of CO_2 levels on potential isolates of *Bacillus* spp. against *Sclerotium rolfsii* in CO_2 incubators.

Bacillus isolate	Per cent inhibition of radial growth over control		
	400 ppm	550 ppm	700 ppm
Bacillus velezensis (B1)	51.11 (45.63)*	59.44 (50.47)	65.55 (54.09)
Bacillus tequilensis (B2)	52.77 (46.59)	60.55 (51.09)	68.32 (55.760)
Bacillus velezensis (B3)	49.44 (44.68)	57.21 (49.15)	61.66 (51.75)
Bacillus velezensis (B4)	54.99 (47.87)	65.55 (54.06)	66.66 (54.73)
Bacillus cereus (B5)	56.10 (48.50)	62.22 (52.15)	67.77 (55.41)
Factors	CD (0.01)	S.Em ±	CV (%)
CO_2 levels	1.44	0.50	3.82
Isolates	1.86	0.64	
Interaction	3.23	1.12	

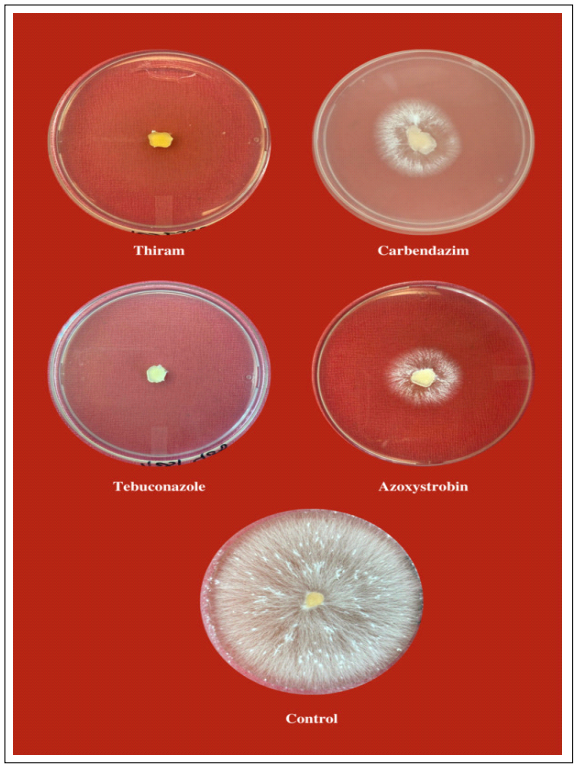

Fig 8: Sensitivity of virulent isolate of *Sclerotium rolfsii* with fungicides at 400 ppm CO_2 level.

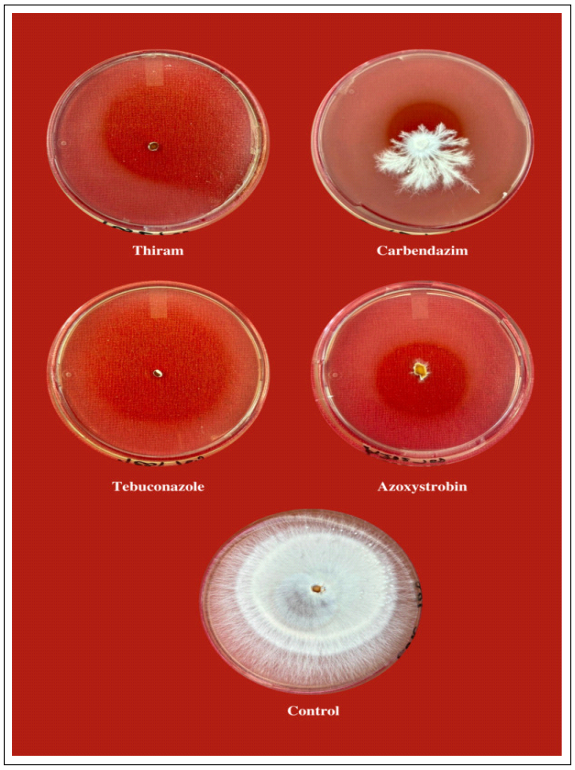

Fig 10: Sensitivity of virulent isolate of *Sclerotium rolfsii* with fungicides at 700 ppm CO_2 level.

Fig 9: Sensitivity of virulent isolate of *Sclerotium rolfsii* with fungicides at 550 ppm CO_2 level.

concentrations, respectively, followed closely by carbendazim, which recorded 82.77% and 37.07% inhibition.

Overall, the results indicate that as carbon dioxide levels increased from 400 ppm to 700 ppm, the effectiveness of the fungicides against *S. rolfsii* also increased. The findings indicate that both tebuconazole and thiram were highly efficient at recommended and half the recommended dosages across all carbon dioxide concentrations.

Gilardi *et al*. (2017) demonstrated that the efficacy of fungicides like azoxystrobin and mancozeb increased by 15.3% and 20.6%, respectively, under CO_2 concentrations of 800-850 ppm and temperatures between 23-26°C, compared to their performance under standard CO_2 conditions.

CONCLUSION

Trichoderma harzianum (T3) exhibited 73.88% and 65.55% inhibition of radial growth of the pathogen at carbon dioxide levels of 700 ppm and 550 ppm, respectively. *Trichoderma viride* (T1) demonstrated 62.10% inhibition at 400 ppm. Complete inhibition (100%) of *Sclerotium rolfsii* was achieved with tebuconazole and thiram at all three carbon dioxide levels. *Azoxystrobin* recorded 93.05%, 86.38% and 80.55% inhibition at 700 ppm, 550 ppm and 400 ppm,

respectively. *Carbendazim* showed 88.88%, 85.55% and 82.77% inhibition at the same carbon dioxide levels. Overall, the biocontrol activity of both fungal and bacterial agents increased with higher carbon dioxide concentrations and the effectiveness of fungicides also improved as carbon dioxide levels rise.

Conflict of interest

The authors declare no conflict of interests.

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