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Integrated Management of Collar Rot of Chickpea (*Cicer arietinum*) Caused by *Sclerotium rolfsii* under Greenhouse Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Collar rot, one of the major soil borne diseases of chickpea incited by *Sclerotium rolfsii* causes significant economic losses in chickpea crop. The present study was undertaken to identify the eco-friendly management of this soil borne disease in chickpea. In this study, the efficacy of chemical,

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biocontrol and biofumigation to manage the chickpea collar rot disease was tested under Greenhouse conditions. Our results revealed that the treatments involving the sole application of chemical fungicide Tebuconazole 60 FS (as seed treatment or soil application) or in combination with other treatments resulted in 100% germination of chickpea seeds. Two treatments namely T_5 (SA of Tebuconazole 60 FS) and T_{12} (ST with Tebuconazole and SA of mustard seed cake) recorded 0.00% total mortality and hence found to be highly effective in protecting chickpea seeds from both pre emergence seed rot and post emergence seedling mortality.The results on the percent disease incidence revealed that, T_{10} (ST with *Trichoderma* spp and SA of mustard seed cake) provided maximum disease reduction of 81.93 per cent (PDI 12.04%) and 75.93 (PDI 24.07%) per cent over the control at 30 and 45 DAS respectively. This was followed by Treatment T_7 which exhibited 72.22 % (PDI 18.52%) and 69.45 % (PDI 30.55%) disease reduction over the control at 30 and 45 DAS respectively. Overall, the results of our study (considering both germination and percent disease incidence percentages), revealed that T_7 (ST and SA of *Trichoderma* spp.) was the most effective treatment in managing the collar rot disease of chickpeas.

Keywords: Chickpea; bengal gram; integrated management.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.), commonly called Bengal gram is the most important pulse crop in the world. It is the most prominent pulse crop in India, generating 13.63 million tonnes with a productivity of 1142 kg ha^{-1} in 11.1 million hectares area [1]. Despite the high production, chickpea yields are low due to many biotic and abiotic stresses. Among the various biotic stresses, soil borne plant pathogenic diseases are of economic importance affecting the crop right from germination stage till harvest.

Collar rot caused by *Sclerotium rolfsii* Sacc. is a devastating soil-borne disease of fungal origin [2]. This disease alone causes about 30% crop loss under both field and Greenhouse conditions and under conducive conditions, the loss may reach upto 95% [3]. *S. rolfsii* pathogen being known to cause disease in various crops tiling heavy toll in India [4] and globally [5] marks the significance of its management.

Managing soil-borne fungal pathogens is challenging due to their long-term survival capabilities and broad host range. Though the different fungicides are in practice, their use has been discouraged due to several drawbacks, which include groundwater pollution, presence of residues in food crops, adverse effects on non-target organisms, and the development of resistance to chemical fungicides, in addition to their high cost [6].

Given the disadvantages, alternative management practices need to be devised to effectively reduce the inoculum potential and the disease incidence while ensuring environmental safety. There is worldwide acceptance of ecologically safe, environmentally friendly methods for protecting crops from plant pathogens such as biocontrol, biofumigation etc. Among the various methods practised to manage *S. rolfsii*, the use of biocontrol agents gave promising results with either little or no hazardous effects on environment [7,8]. *Trichoderma* spp. are a widespread saprophytic, soil-inhabiting filamentous fungi that have the ability to antagonize various pathogenic thus reducing the disease incidence [9]. Mycoparasitism, production of cell-wall degrading enzymes and antifungal compounds, antibiosis and the competition for space and nutrients are the possible mechanisms of actions of these fungi against the fungal pathogens [10]. Keeping this in view, the present experiment was undertaken to find out the effectiveness of integrated use of chemical, bio control and bio-fumigation for the management of collar rot disease of chickpea caused by *S. rolfsii* under Greenhouse conditions.

2. MATERIALS AND METHODS

The present experiment was conducted under Greenhouse conditions during *Rabi* 2023-24 using the collar rot susceptible chickpea cultivar JG11. In this experiment, 15 treatments were imposed each of which was replicated thrice in a CRD design. The details of the treatments are as follows T_1: Seed treatment (ST) with *Trichoderma* spp. at 10 g/kg seed [11] T_2: Seed treatment with Tebuconazole 60 FS at 1ml/kg seed [11], T_3: T_1+T_2 (Seed treatment with both *Trichoderma* spp. and Tebuconazole 60 FS), T_4: Soil application (SA) of *Trichoderma* spp. at 10 g/pot at the time of sowing [12] , T_5: Soil application of Tebuconazole 60 FS at 0.1 % at the time of sowing , T_6: T_2+T_4 (Seed treatment with Tebuconazole 60 FS and soil application of *Trichoderma* spp.), T_7: T_1+T_4 (Both seed treatment and soil application of *Trichoderma* spp.) [13], T_8: Soil application (SA) of mustard seed cake at 25 g/kg soil 15 days before sowing [14], T_9: Biofumigation- soil incorporation of mustard plant material (60 days old) at one plant /pot 20 days before sowing [15], T_{10}: T_1+T_8 (Seed treatment with *Trichoderma* spp. and soil application of mustard seed cake), T_{11}: T_1+T_9 (Seed treatment with *Trichoderma* spp. and Biofumigation- Soil incorporation of mustard plant material), T_{12}: T_2+T_8 (Seed treatment with Tebuconazole 60 FS and soil application of mustard seed cake), T_{13}: T_2+T_9 (Seed treatment with Tebuconazole 60 FS and Biofumigation-soil incorporation of mustard plant material), T_{14}: Inoculated control (pots inoculated with *S.rolfsii*) and T_{15}: Un-inoculated control (pots not inoculated with *S.rolfsii*).

In this experiment, autoclaved soil was filled in sterilized plastic pots. *S. rolfsii* culture multiplied on sorghum grains [16] and was added to the soil at 20 g/ kg soil. The surface of seeds was sterilized with ethanol at 0.1 % for one minute followed by 3 consecutive washings in sterilized water and the seeds were sown. In each pot, 3 chickpea seeds of JG 11 were sown. Surface sterilized seeds were sown in inoculated sterilized soil without any treatment which served as inoculated control whereas surface sterilized seeds were sown in un -inoculated sterilized soil and without any treatment served as uninoculated control. The pots were watered as and when required. All the pots were maintained under uniform conditions.

The data on different parameters like germination percentage, pre-emergence seed rot, post-emergence seedling mortality, total mortality and percentage of disease incidence (at 15, 30 and 45 days after sowing) was recorded to find out the potentiality of different treatments on collar rot disease.

Percentage of seed germination, pre emergence seed rot and post-emergence seedling mortality were taken at 10 days after sowing by using the following formulae

a. $Germination\ (\%) = \frac{Number\ of\ seed\ germinated}{Total\ number\ of\ seed\ sown} \times 100$

b. $Pre-emergence\ seed\ rotting\%\ (PESR) =$

$$\frac{Number\ of\ ungerminated\ seeds}{Total\ number\ of\ seed\ sown} \times 100$$

c. $Post-emergence\ seedling\ mortality\%\ (PESM) =$

$$\frac{Number\ of\ infected\ seedlings}{Total\ number\ of\ seedlings} \times 100$$

Percent disease incidence (PDI) was calculated at 15, 30 and 45 days after sowing by using the formula [17].

$Percent\ disease\ incidence\ (PDI) =$

$$\frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants} \times 100$$

The data was statistically analyzed by using analysis of variance (ANOVA) appropriate for CRD.

3. RESULTS AND DISCUSSION

3.1 Germination and Pre Emergence Seed Rot (PESR)

The results (Table 1) of the experiment on the germination percentage and pre emergence seed rot (PESR) revealed that 100% germination was observed with sole application of chemical fungicide (T_2 - ST with Tebuconazole, T_5 - SA of Tebuconazole 60 FS) and in combination with other treatments like *Trichoderma* spp. (T_3 - ST with both *Trichoderma* spp. and Tebuconazole and T_6 - ST with Tebuconazole 60 FS and SA of *Trichoderma* spp.), Bio-fumigation with mustard seed cake (T_{12} - ST with Tebuconazole and SA of mustard seed cake) and mustard plant material (T_{13} - ST with Tebuconazole and incorporation of mustard plant material). The above six treatments were found to be effective in protecting the chickpea seeds from pre emergence seed rot with zero per cent PESR and 100% seed rot reduction over the inoculated control (33.33%). However, the above treatments were statistically equivalent to treatment T_7 (ST and SA of *Trichoderma* spp.) with 96.29% germination (5.56% PESR).

Out of all the treatments, treatment T_9 (Biofumigation – soil incorporation of mustard plant material) recorded minimum seed germination percent i.e 74.07% with 25.92% seed rot and found to be less effective treatment in managing the seed rot during germination stage of chickpea.

3.2 Post Emergence Seedling Mortality (PESM)

The data obtained on the post emergence seedling mortality (PESM) showed that all the treatments showed considerable variation. The results in the (Table 1, Fig. 1) revealed that four soil application of tebuconazole and Trichoderma treatments namely T_5 (SA of Tebuconazole 60 FS), T_7 (ST with *Trichoderma* spp. and SA of *Trichoderma* spp.), T10 (ST with *Trichoderma* spp and SA of mustard seed cake) and T12 (ST with Tebuconazole and SA of mustard seed cake) were found to be most effective in managing post emergence seedling mortality caused by *S. rolfsii* with zero per cent PESM and with cent per cent seedling mortality reduction over inoculated control (T_{14}-33.33%). This was followed by PESM of 6.02 % with 81.94% reduction over inoculated control recorded in treatment T_4 (SA of *Trichoderma* spp.).

Similar to the PESR %, treatment T_9 (Soil incorporation of mustard plant material) was found to be less effective in managing post emergence seedling mortality in comparison with other treatments with 19.84% PESM with 40.47 per cent PESM reduction over inoculated control.

3.3 Total Mortality

The results (Table 1, Fig. 1) obtained on total mortality were evaluated based on per cent seed rot incidence and post emergence seedling mortality in all the treatments. There was considerable variation observed among all the treatments on total mortality. Two treatments namely T_5 (SA of Tebuconazole 60 FS) and T_{12} (ST with Tebuconazole and SA of mustard seed cake) were found to effective (0.00%) and similar with un-inoculated control (T_{15}) with 100% total mortality reduction over inoculated control (T_{14} - 66.66%). These two treatments were found to be highly effective in protecting chickpea seeds from both PESR and PESM caused by *S. rolfsii*.

Similar to pre emergence seed rot and post emergence seedling mortality results treatment T_9 (soil incorporation of mustard plant material) was less effective (45.76 % total mortality) among different treatments.

Our results on per cent total mortality (PESR and PESM) were in agreement with the findings of Gowdar et al. [18] and Sunkad et al. [19] who reported that tebuconazole provides excellent control against soil borne fungal diseases including seed rot caused by *S. rolfsii.*

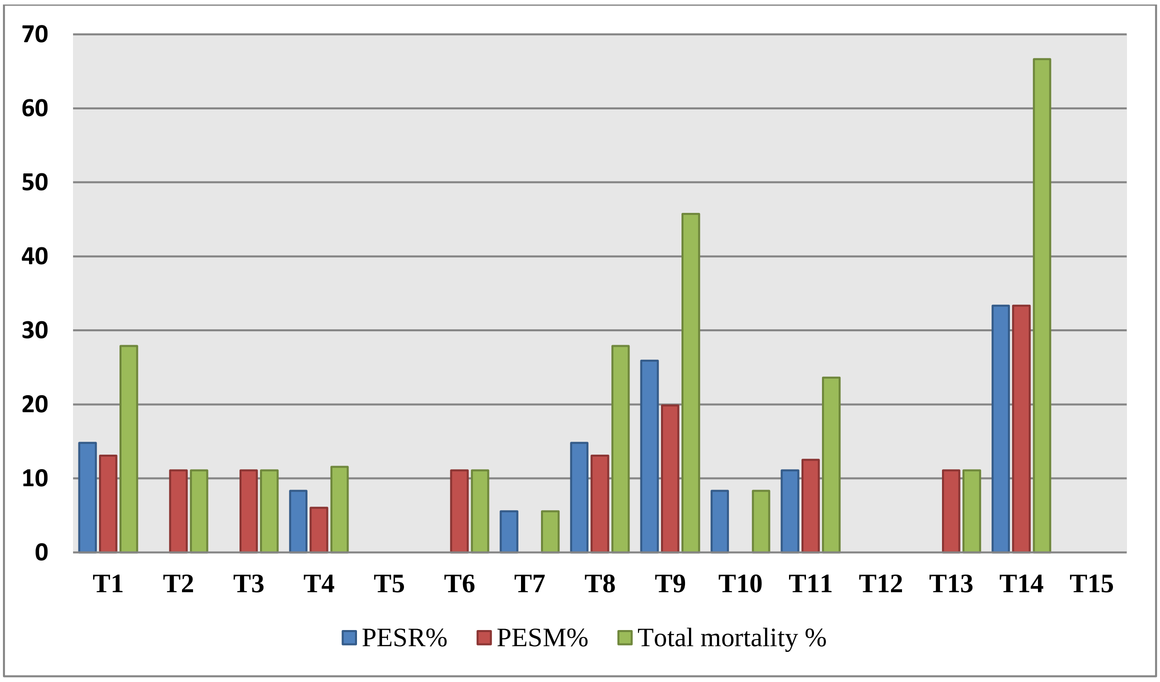

Fig. 1. Graph showing the PESR%, PESM % and Total mortality %

Table 1. Effect of different treatments on Germination%, PESR%, PESM% and total mortality

S.No.	Treatment	Germination %	Seed rot %	% Reduction over control	PESM%	% Reduction over control	Total Mortality%	% Reduction over control
1	T1 (ST with Trichoderma)	85.18 (67.61)*	14.81 (22.35)	55.56	13.09 (21.19)	60.73	27.91 (31.76)	58.13
2	T2 (ST with Tebuconazole)	100.00 (90.00)	0.00 (0.00)	100.00	11.11 (19.46)	66.66	11.11 (19.46)	83.33
3	T3 (T1+T2)	100.00 (90.00)	0.00 (0.00)	100.00	11.11 (19.46)	66.66	11.11 (19.46)	83.33
4	T4 (SA of Trichoderma)	92.59 (76.99)	8.33 (16.17)	75.00	6.02 (13.29)	81.94	11.57 (16.17)	82.64
5	T5 (SA of Tebuconazole)	100.00 (90.00)	0.00 (0.00)	100.00	0.00 (0.00)	100.00	0.00 (0.00)	100.00
6	T6 (T1+T5)	100.00 (90.00)	0.00 (0.00)	100.00	11.11 (19.46)	66.66	11.11 (19.46)	83.33
7	T7 (T1+T4)	96.29 (83.49)	5.56 (12.89)	83.32	0.00 (0.00)	100.00	5.56 (12.89)	91.66
8	T8 (Soil application of mustard seed cake)	85.18 (67.71)	14.81 (22.34)	55.56	13.09 (21.19)	60.73	27.91 (31.76)	58.13
9	T9 (Incorporation of mustard plant material)	74.07 (59.47)	25.92 (30.49)	22.23	19.84 (26.19)	40.47	45.76 (45.52)	31.35
10	T10 (T1+T8)	92.59 (76.99)	8.33 (16.17)	75.00	0.00 (0.00)	100.00	8.33 (13.78)	87.50
11	T11 (T1 + T9)	88.88 (70.49)	11.11 (19.46)	66.66	12.5 (20.69)	62.50	23.61 (29.06)	64.58
12	T12 (T2+ T8)	100.00 (90.00)	0.00 (0.00)	100.00	0.00 (0.00)	100.00	0.00 (0.00)	100.00
13	T13 (T2+T9)	100.00 (90.00)	0.00 (0.00)	100.00	11.11 (19.46)	66.66	11.11 (19.46)	83.33
14	T14 (Inoculated control)	66.66 (54.71)	33.33 (35.24)		33.33 (35.25)		66.66 (54.71)	
15	T15 (Un-inoculated control	100.00 (90.00)	0.00 (0.00)		0.00 (0.00)		0.00 (0.00)	
16	**C.D.**	9.15	5.54		3.66		5.64	
17	**SE(m)**	3.15	1.91		1.26		1.94	
18	**SE(d)**	4.46	2.70		1.78		2.75	
19	**C.V.**	6.89	20.49		12.42		13.46	

** Figures in parantheses are angular transformed values*

3.4 Percent Disease Incidence (PDI) of Collar Rot

The results presented in the Table 2, Figs. 2, 3 pertaining to per cent disease incidence of collar rot was recorded at 15, 30 and 45 days after sowing (DAS) varied significantly among all the treatments. The treatment (T_{15}) unioculated control not showed any symptoms at 15, 30 and 45 DAS, whereas treatment (T_{14}) inoculated control showed 100 per cent disease incidence at 45 days after sowing with complete drying of the plants, 50 per cent plants were infected at 15 DAS and 66.66 per cent disease incidence was recorded at 30 DAS which indicates that the concentration of inoculum applied was sufficient to cause infection at 15, 30 and 45 DAS.

Among all the treatments imposed in our investigation, T_{10} (ST with *Trichoderma* spp and SA of mustard seed cake) was found effective with maximum per cent disease reduction over the control 81.93 per cent and 75.93 per cent with minimum PDI of 12.04% and 24.07% at 30 and 45 DAS followed by Treatment T_7 exhibited 72.22 % (PDI 18.52%) and 69.45 % (PDI 30.55%) disease reduction over the control at 30 and 45 DAS. But at 15 DAS, the minimum PDI was recorded with treatment T_7 (ST with *Trichoderma* spp. And SA of *Trichoderma* spp.) over the treatment T_{10} (ST with *Trichoderma* spp and SA of mustard seed cake) with 11.11% and 12.04% respectively.

Our results were in conformity with the findings of Dutta et al. [20], who reported that soil application of mustard oil cake along with *Trichoderma harzianum* was found effective in management of basal stem rot disease in *Piper longum* caused by *S. rolfsii* with disease reduction up to 10.65%. Similarly Rafi et al. [21], stated that seed treatment with *T. harzianum* along with soil amendment with mustard cake was found to be effective against root infecting fungi in leguminous (peanut, chickpea) and non-leguminous crops (okra and sunflower). According to findings of Desai et al. [22] soil application of mustard oil cake at 2.0% enhances the growth of *Trichoderma* species and inhibits the germination of sclerotia of *S. rolfsii* at same concentration.

Among all the treatments imposed in the present experiment, treatment T_9 (soil incorporation of mustard plant material) showed minimum effect on collar rot disease reduction over inoculated control recorded at 30 days (32.13%) and 45 days (42.86%) after sowing, whereas T_6 (Seed treatment with Tebuconazole 60 FS and soil drenching with *Trichoderma* spp.) showed minimum (33.34 %) disease reduction over inoculated control at 15 days after sowing with 33.33 per cent disease incidence.

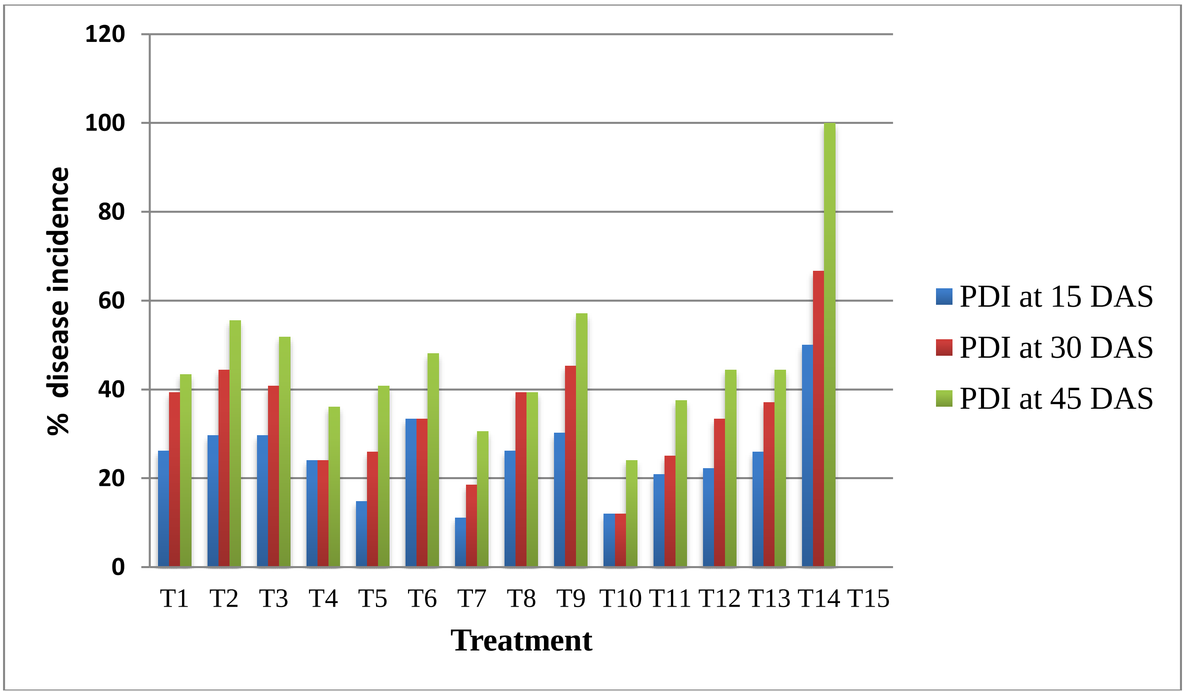

Fig. 2. Graph showing the Percent disease incidence (PDI) at 15, 30 and 45 DAS

Table 2. Effect of different treatments on Percent disease incidence (PDI) at 15, 30 and 45 DAS

S.No.	Treatment	PDI at 15 DAS	% Reduction over control	PDI at 30 DAS	% Reduction over control	PDI at 45 DAS	% Reduction over control
1	T1 (ST with Trichoderma)	26.19 (30.75)	47.62	39.27 (38.78)	41.09	43.43 (41.19)	56.66
2	T2 (ST with Tebuconazole)	29.63 (32.87)	40.74	44.44 (41.79)	33.33	55.55 (48.17)	44.45
3	T3 (T1+T2)	29.63 (32.87	40.74	40.74 (39.61)	38.88	51.85 (46.04)	48.15
4	T4 (SA of Trichoderma)	24.07 (29.36)	51.86	24.07 (29.36)	63.89	36.11 (36.91)	63.89
5	T5 (SA of Tebuconazole)	14.81 (22.35)	70.38	25.92 (30.49)	61.11	40.74 (39.61)	59.26
6	T6 (T1+T5)	33.33 (35.25)	33.34	33.33 (35.25)	50.00	48.14 (43.92)	51.86
7	T7 (T1+T4)	11.11 (19.46)	77.78	18.52 (25.23)	72.22	30.55 (33.49)	69.45
8	T8 (Soil application of mustard seed cake)	26.19 (30.76)	47.62	39.29 (38.79)	41.06	39.29 (38.79)	60.71
9	T9 (Incorporation of mustard plant material)	30.16 (33.29)	39.68	45.24 (42.25)	32.13	57.14 (49.08)	42.86
10	T10 (T1+T8)	12.04 (20.29)	75.92	12.04 (20.28)	81.93	24.07 (29.36)	75.93
11	T11 (T1 + T9)	20.83 (26.89)	58.34	25.00 (29.99)	62.49	37.50 (37.75)	62.50
12	T12 (T2+ T8)	22.22 (28.11)	55.56	33.33 (35.25)	50.00	44.44 (41.79)	55.56
13	T13 (T2+T9)	25.92 (30.49)	48.16	37.03 (34.43)	44.45	44.44 (41.79)	55.56
14	T14 (Inoculated control)	50.00 (44.99)		66.66 (54.71)		100.00 (90.00)	
15	T15 (Un-inoculated control	0.00 (0.00)		0.00 (0.00)		0.00 (0.00)	
16	**C.D.**	4.59		3.97		3.63	
17	**SE(m)**	1.58		1.37		1.25	
18	**SE(d)**	2.24		1.93		1.77	
19	**C.V.**	9.62		6.98		5.17	

** Figures in parantheses are angular transformed values*

Fig. 3. Management of Collar rot of chickpea under pot culture

In the present study, tebuconazole based treatments like soil application and seed treatment alone and incorporation with other treatments found effective in reduction of *S. rolfsii* infection on chickpea at the time germination (0.00%), but found less effective in reducing the collar rot incidence after 30 days (44.44%) and 45 days (55.55%) after sowing. Our results were similar to the findings of Gour and Sharma [23], who studied the effect of fungicides on groundnut root rot caused by *S. rolfsii* and reported that application of Folicur 250 EW (tebuconazole) showed zero per cent PDI at 3 DAS and 16.4 per cent PDI at 30 DAS on groundnut.

Among the different treatments, seed treatment with *Trichoderma* and soil application of *Trichoderma* (T_7) was found most effective treatment in reduction of seed rot (5.56%), post emergence seedling mortality (0.00%) and collar rot incidence (11.11%, 18.52% and 30.55% at 15, 30, 45 days after sowing respectively) caused by *S. rolfsii* in chickpea. Similar reports were also available on reduction of groundnut stem rot disease caused by *S. rolfsii* by seed treatment and soil application of *T. viridae* [24] and *T. harzianum* [13]. Recently Rani et al. [11] studied the integrated management of *S. rolfsii* incidence on groundnut and reported found seed treatment and soil application of *T. harzianum* more or less effective to tebuconazole application in disease suppression.

4. CONCLUSION

Our results revealed the scope of bio agents (*Trichoderma*) and their integration with other treatments which include biofumigation and chemical fungicides in managing the collar rot of chickpea. All the treatments imposed in our study were more or less effective in managing the collar rot of chickpea in comparison with inoculated control. Among all these treatments, treatment T_7 (Seed treatment and soil application of *Trichoderma* spp.) was found most effective in managing the collar rot of chickpea.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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