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Management of Charcoal Rot (*Macrophomina phaseolina*) of Fenugreek in Rajasthan

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ABSTRACT

Background: Charcoal rot of fenugreek caused by *Macrophomina phaseolina* is destructive disease of all fenugreek growing areas of Rajasthan, The disease is wide occurrence in sandy soil of Rajasthan, where the climatic conditions are dry and temperature remains high. Under severe infestation it cause 35.00-42.56% losses in yield. In the present investigation, our main emphasis was to find out some new fungicides alone and combination with bio-agent for management of charcoal rot of fenugreek.

Methods: Eight fungicides *viz*., captan 70%+ hexaconazole 5% WP, azoxystrobin 18.2% + difenaconazole 1.4% SC, chlorothalonil 75% WP, carbendazim 12% + mancozeb 63% WP, tebuconazole 50% + trifloxystrobin 25% WG, carboxin 37.5% + thiram 37.5% WP, copper oxychloride 50% WP and captan 70% WP were tested at different concentration of 100, 200, 300, 400 and 500 ppm against *M. phaseolina* using Poisoned Food Technique *in vitro.* The *in vivo* study was taken to evaluate the effect of one fungal and one bacterial bio-agent *viz*., *T. harzianum, P. fluorescens* was applied as seed treatment, soil dressing and soil application @ 5+5 g/kg and @ 10 kg/ha respectively and fungicides *viz*., tebuconazole 50%+ trifloxystrobin 25% @ 1.5 g/kg, carbendazim 12% + mancozeb 63% @ 2 g/kg and azoxystrobin 18.2% + difenaconazole 11.4% 2 ml/kg seed were used in different treatments. The experiment was conducted at Experimental farm COA, SKRAU, Bikaner during *Rabi* 2020-21 on most popular cv. Rmt-305 in RBD design with the application of seed treatment, soil dressing and foliar spray of different fungicides with bioagents at different concentrations against Charcoal Rot disease and compared with an untreated control.

Result: Among all the tested fungicides used in the present investigation, tebuconazole 50% + trifloxystrobin 25% WG found most effective in controlling the mycelium growth of pathogen. In field condition, it gave maximum disease control (85.72%) with highest grain yield (19.83 q/ha) when applied as seed treatment with tebuconazole 50% + trifloxystrobin 25% WG @ 1.5 g/kg seed + soil application of *T. harzianum* @ 10 kg/ha + SD with tebuconazole 50% + trifloxystrobin 25% WG. These treatments can provide an effective management of charcoal rot disease for fenugreek cultivators.

Key words: Bioagents, Charcoal rot, Fenugreek, Fungicides, *Macrophomina phaseolina.*

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) also known as *Methi* belongs to family *leguminoceae*. Fenugreek believed to be originated from South East Europe and West Asia is cultivated throughout India and other parts of world for leafy vegetables, condiment, medicinal purposes and fodder also. The leaves (per 100 g of edible portions) contain moisture (per 100 g of edible portions) 86.1 g, thiamine 0.05 mg, fat 0.9 g, calcium 360 mg, protein 4.4 g, oxalic acid 13 mg, fiber 1.1 g, iron 17.2 mg, potassium 51 mg, mineral 1.5 g, sulphur 167 mg, carbohydrates 6.00 g, vitamin A 6450 IU, magnesium 67 mg, nicotinic acid 0.7 mg, sodium 76.1 mg, vitamin C 54 mg, phosphorus 51 mg and chlorine 165 mg (Bose and Som, 1986).

India stands first in the production of fenugreek with total acreage of 1.20 lakh hectares and production of 1.88 lakh tonnes with average productivity of 1566.23 kg/ha. In India more than 80 per cent area and production of is contributed by Rajasthan state alone. In Rajasthan first rank in the production of fenugreek with total acreage of 45.31 thousand hectares and production of 62.89 thousand tonnes with average productivity of 1388.00 kg/ha (Anonymous, 2019-20).

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Fenugreek is cultivated in almost all districts of Rajasthan whereas Bikaner district stands first in production with total acreage of 7046 hectares and production of 7760 tonnes with productivity of 1101 kg/ha (Anonymous, 2018-19).

Fenugreek is infected by several fungal, bacterial and viral diseases. Major fungal diseases of fenugreek are cercospora leaf spot (*Cercospora traversian*), charcoal rot (*Macrophomina phaseolina* (Tassi) Goid.), wilt (*Fusarium oxysporum* Schlecht.), downy mildew (*Peronospora trigonellae* Gaum*),* rhizoctonia root rot (*Thanatephorus cucumeris* Kuhn), powdery mildew (*Leveillula taurica* (Lev.) Arm), root rot (*Sclerotinia trifoliorum* Sacc.) and rust (*Uromyces trigonellae* Pass.) *etc*.

Among all the fungal diseases of fenugreek, the most destructive is charcoal rot of fenugreek caused by *M. phaseolina* causing potential yield losses. Charcoal rot infected seeds act as an important source of primary inoculums for new areas. Plant stand affected due to pre and post emergence infection of crop. In pre emergence stage, the fungus causes seed rot and mortality of germinating seedling while in post emergence stage seedling get blightened due to soil and seed borne infection. The pathogen may infect almost all parts of plants *viz*., root, stem, branches, petioles, leaves and pods.

MATERIALS AND METHODS

Efficacy of fungicides against *M. phaseolina in vitro*

The efficacy of fungicides and combination products were tested by using 100, 200, 300, 400 and 500 ppm against *M. phaseolina in vitro*. Eight fungicides were evaluated in this study. These were T_1: captan 70% + hexacunazole 5% WP, T_2: azoxystrobin 18.2% + difenaconazole 11.4% SC, T_3: chlorothalonil 75% WP, T_4: carbendazim 12% + mancozeb 63% WP, T_5: tebuconazole 50% + trifloxystrobin 25% WG, T_6: carboxin 37.5% + thiram 37.5% WP, T_7: copper oxy chloride 50% WP and T_8: captan 70% WP.

The efficacy against mycelial growth was tested using Poisoned-Food-Technique (Nene and Thapliyal, 1973). Required quantities of fungicide was thoroughly mixed in melted PDA, just before pouring in sterilized petri dishes and were allowed to solidify for 12 hrs. Each plate was then inoculated with 5 mm disc of mycelial bit taken from the periphery of 10 days colony of *M. phaseolina* growing on PDA. The inoculated petri dishes were incubated at 25±1°C. Three plates were used for each treatments. serving as three replications. Colony diameter (two diagonals) was measured after 15 days of incubation. Medium without fungicide served as control. Per cent growth inhibition was calculated by Vincent's (1947) formula as follows:

$$\text{Per cent mycelium inhibition} = \frac{C - T}{C} \times 100$$

Where,

C= Mycelial growth of *M. phaseolina* in control (average of both diagonals).

T= Mycelial growth of *M. phaseolina* in treatment (average of both diagonals).

Efficacy of bioagents and fungicides against *M. phaseolina in vivo*

A field trial was conducted for the management of charcoal rot of fenugreek variety Rmt-305 using bio-agents and fungicides in *Rabi* season 2020-21. A most popular fenugreek cultivar Rmt-305 was used in this experiment. The fenugreek was sown on 29th October 2020. The crop was planted at 30 cm row to row and 10 cm plant to plant spacing. The gross plot size was 3.0 × 3.0 m^2. The experiment was laid out in randomized block design (RBD) with three replications. All other recommended practices required for cultivation of the crop were followed. Talc based formulations of one fungal and one bacterial bio-agent *viz*., *T. harzianum, P. fluorescens* were applied as seed treatment and soil application @ 5 + 5 g/kg and @10 kg/ha respectively and fungicide tebuconazole 50% + trifloxystrobin 25% @ 1.5 g/kg, carbendazim 12% + mancozeb 63% @ 2 g/kg and azoxystrobin 18.2% + difenaconazole 11.4% @ 2 ml/kg seed were used. Seed treatment of fungicides, seed and soil

Table 1: List of treatments.

Treatments	Dose
T_1: ST* with carbendazim 12% + mancozeb 63% WP	ST @ 2 g/kg seed
T_2: ST with tebuconazole 50% + trifloxystrobin 25% WG	ST @1.5 g/kg seed
T_3: ST with azoxystrobin 18.2% + difenaconazole 11.4% SC	ST @ 2 ml/kg seed
T_4: T_1 + Soil application of *T. harzianum* + SD* with T_1	SA @ 10 kg/ha
T_5: T_1 + Soil application of *P. fluorescens* + SD with T_1	SA @ 10 kg/ha
T_6: T_2 + Soil application of *T. harzianum*+ SD with T_2	SA @ 10 kg/ha
T_7: T_2 + Soil application of *P. fluorescens* + SD with T_2	SA @ 10 kg/ha
T_8: T_3 + Soil application of *T. harzianum* + SD with T_3	SA @ 10 kg/ha
T_9: T_3 + Soil application of *P. fluorescens* + SD with T_3	SA @ 10 kg/ha
T_{10}: ST with *T. harzianum*+ SA* of *T. harzianum*	ST-10 g/kg
+ SD of *T. harzianum* at 40 DAS*	SA-10 kg/ha + 2 g/L
T_{11}: ST with *P. fluorescens* + SA of *P. fluorescens*	ST-10 g/kg
+ SD of *P. fluorescens* at 40 DAS	SA-10 kg/ha + 2 g/L
T_{12}: Control	

ST*- Seed treatment, SA*- Soil application, SD*- Soil drenching, DAS*- Days after sowing.

treatment of the talc based bio-agent formulations was done. In case of control, seeds were sown in pathogen inoculated soil without any bio-agents and fungicides. The combination of the treatments and their doses were mentioned in Table 1. Observations on charcoal rot incidence were recorded periodically as well as the grain yield was recorded after harvesting of the crop.

Calculation and statistical analysis

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plant}}{\text{Number of plant observed}} \times 100$$

$$\text{Disease control (\%)} = \frac{\text{PDI in control - PDI in treatment}}{\text{PDI in control}} \times 100$$

$$\text{Dry wt. in (\%)} = \frac{\text{Dry wt. in treatment - Dry wt. in control}}{\text{Dry wt. of plants in control}} \times 100$$

The data of per cent disease incidence in all the experiments were transformed to their Arc sin values (Fisher and Yates, 1963). The statistical analysis of the data of all the laboratory experiments was done following completely randomized design. The data of field experiments were analysed following randomized block design (Cochran and Cox, 1957).

RESULTS AND DISCUSSION

Efficacy of fungicides against *M. phaseolina in vitro*

Eight (systemic, contact and combi) fungicides were tested at different concentration of 100, 200, 300, 400 and 500 ppm against *M. phaseolina* using Poisoned Food Technique *in vitro.* Table 2 and Plate 1 shows the effectiveness of all the tested fungicides. The data showed that the maximum mycelial growth inhibition was increased at different concentration of fungicides. tebuconazole 50% + trifloxystrobin 25% WG was found Cent percent inhibition of mycelial growth of *M. phaseolina* followed by captan 70% + hexaconazole 5%WP (2.93 mm) and carbendazim 12% + mancozeb 63% WP (10.26 mm) resulting in significantly reduction of mycelial growth of *M. phaseolina.* At 200 ppm concentration, growth of mycelium was not observed in tebuconazole 50% + trifloxystrobin 25% WG (0.00 mm) and captan 70%+ hexaconazole 5% WP (0.00 mm) followed by

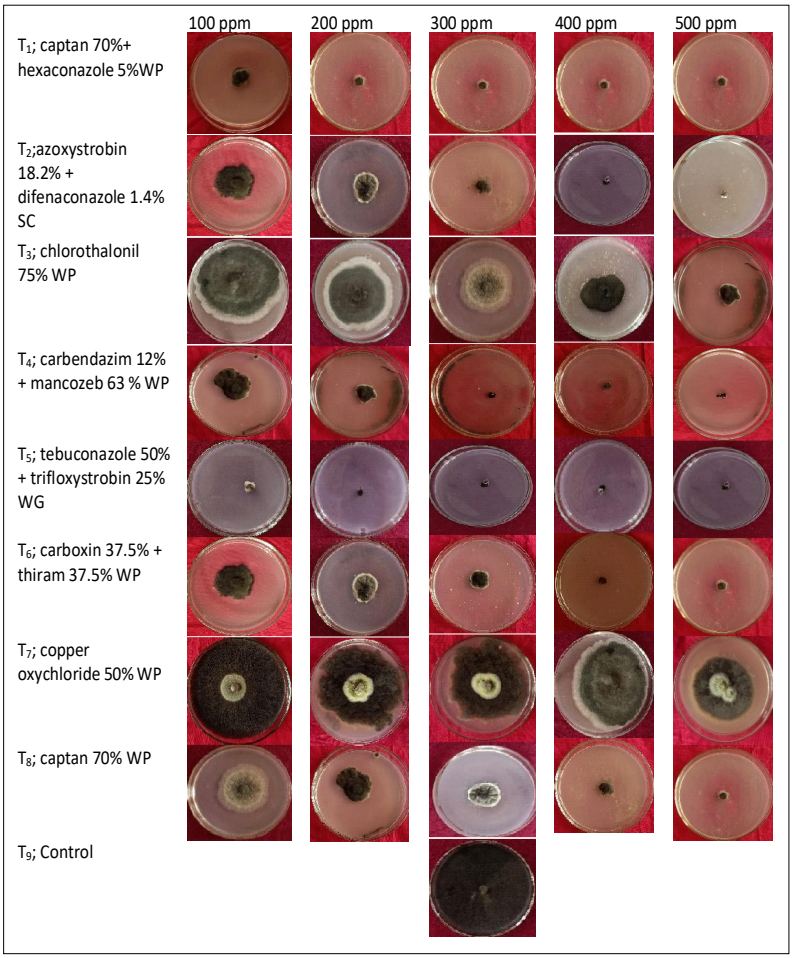

Plate 1: Ef!cacy of different fungicides on mycelial growth of *M. phaseolina* under *in vitro* condition.

carbendazim 12% + mancozeb 63% WP (5.84 mm). At 300 ppm concentration, tebuconazole 50% + trifloxystrobin 25% WG (0.00 mm), captan 70% + hexaconazole 5%WP (0.00 mm) and carbendazim 12% + mancozeb 63% WP (0.00 mm) inhibited Cent per cent mycelium growth. At 400 ppm and 500 ppm concentration, no mycelial growth of fungus was recorded in tebuconazole 50% + trifloxystrobin 25% WG, captan 70%+ hexaconazole 5%WP and carbendazim 12% + mancozeb 63% WP (0.00 mm).

Similarly, treatment Tebuconazole 50% + trifloxystrobin 25% WG was most effective at lowest concentrations *viz*., 100, 200 and 300 ppm. Similar, results was observed by Kumar *et al.* (2016) this study suggests the possibility to enhance the antifungal activity of fungicides Tebuconazole 50% + trifloxystrobin 25% WG towards the control of *M. phaseolina*. Bashir *et al.* (2017) The interaction between treatments and concentrations (T×C) showed that used concentrations 150 ppm, 250 ppm and 350 ppm of Nativo abundantly inhibit fungal colony growth up to 1.26 cm, 0.86 cm and 0.66 cm, respectively whereas the interaction between treatments and days expressed that after day ninth the minimum colony growth (1.23 cm) was observed for Nativo as compared to all other treatments.

Efficacy of bioagents and fungicides against *M. phaseolina in vivo*

The results showed in Table 3 indicate that the charcoal rot severity in fenugreek was effectively reduced by combination of bioagents and fungicides *viz.,* seed treatment with tebuconazole 50% + trifloxystrobin 25% WG @ 1.5 g/kg seed + soil application of *T. harzianum* @ 10 kg/ha + soil drenching with tebuconazole 50% + trifloxystrobin 25% WG (6.94%) followed by ST with tebuconazole 50% + trifloxystrobin 25% WG @ 1.5 g/kg seed + SA of *P. fluorescens* @ 10 kg/ha + SD with tebuconazole 50% + trifloxystrobin 25% WG (9.76%) and ST with carbendazim 12% + mancozeb 63% WP @ 2 g/kg seed + SA of *T. harzianum* @ 10 kg/ha + SD with carbendazim 12% + mancozeb 63% WP (13.22%) as compared to control where highest disease severity of 48.55% was recorded. Among the treatments highest disease severity was recorded in bioagents ST @ 10 g/kg seed with *P. fluorescens* + SA of *P. fluorescens* + SD *of P. fluorescens* (24.35%) followed ST with *T. harzianum* + SA of *T. harzianum* + SD *of T. harzianum* (21.22%) and ST with azoxystrobin 18.2% + difenaconazole 11.4% SC @ 2 ml/kg seed (18.31%).

The maximum disease control was recorded in seed treatment with tebuconazole 50% + trifloxystrobin 25% WG @ 1.5 g/kg seed + soil application of *T. harzianum* @ 10 kg/ha + soil drenching with tebuconazole 50% + trifloxystrobin 25% WG (85.72%) followed by ST with tebuconazole 50% + trifloxystrobin 25% WG @ 1.5 g/kg seed + SA of *P. fluorescens* @ 10 kg/ha + SD with tebuconazole 50% + trifloxystrobin 25% WG (79.85%) and ST with carbendazim 12% + mancozeb 63% WP @ 2g/kg seed + SA of *T. harzianum* @ 10 kg/ha + SD with carbendazim 12% + mancozeb 63% WP (72.77). Disease incidence of charcoal rot in fenugreek under field conditions and a view of experimental field are shown in Plate 2.

Table 2: Efficacy of different fungicides on mycelial growth of *M. phaseolina*under *in vitro* condition.

Treatments	Mycelial growth (mm)					Growth inhibition (%)				
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
T_1: Captan 70%+ hexaconazole 5%WP	2.93 (9.81)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	96.74	100.00	100.00	100.00	100.00
T_2: Azoxystrobin 18.2% + difenaconazole 1.4% SC	12.33 (20.54)	7.60 (15.97)	2.73 (9.45)	0.00 (0.00)	0.00 (0.00)	86.30	91.55	96.96	100.00	100.00
T_3: Chlorothalonil 75% WP	66.88 (54.84)	47.03 (43.27)	38.23 (38.17)	31.13 (33.89)	26.55 (30.99)	25.68	47.74	57.52	65.41	70.50
T_4: Carbendazim 12% + mancozeb 63% WP	10.26 (18.64)	5.84 (13.89)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	88.60	93.51	100.00	100.00	100.00
T_5: Tebuconazole 50% + trifloxystrobin 25% WG	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	100.00	100.00	100.00	100.00	100.00
T_6: Carboxin 37.5% + thiram 37.5% WP	14.24 (24.14)	8.50 (16.89)	4.10 (11.54)	0.00 (0.00)	0.00 (0.00)	84.17	90.55	95.44	100.00	100.00
T_7: Copper oxychloride 50% WP	90.00 (71.53)	76.68 (61.10)	64.20 (53.22)	56.04 (48.45)	46.29 (42.85)	0.00	14.80	28.66	37.73	48.56
T_8: Captan 70% WP	18.56 (25.48)	12.36 (20.53)	6.11 (14.23)	2.11 (8.20)	0.00 (0.00)	79.37	86.26	93.21	97.65	100.00
T_9: Control	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	-	-	-	-	-
S.Em±	0.57	0.66	0.64	0.43	0.31	-	-	-	-	-
CD (P= 0.05)	1.72	2.00	1.93	1.30	0.95	-	-	-	-	-

*Figures in parentheses are angular transformed values.

Grain yield

Maximum grain yield of fenugreek (19.83 q ha^{-1}) was obtained in seed treatment with tebuconazole 50% + trifloxystrobin 25% WG @ 1.5 g/kg seed + ST @ 10 kg/ha of *T. harzianum* @ 10kg/ha + SD @ 2g/L with tebuconazole 50% + trifloxystrobin 25% WG, followed by ST with

Table 3: Management of charcoal rot of fenugreek through different bio-agents and fungicides under *in vivo* condition.

Treatments	Dose	Disease severity (%)	Disease control (%)	Yield (q ha^{-1})
T_1: ST* with carbendazim 12% + mancozeb 63% WP	ST @ 2 g/kg seed	19.23 (25.93)*	60.39	14.96
T_2: ST with tebuconazole 50% + trifloxystrobin 25% WG	ST @1.5 g/kgseed	18.12 (25.11)	62.67	15.38
T_3: ST with azoxystrobin 18.2% + difenaconazole 1.4% SC	ST @ 2 ml/kg seed	20.31 (26.71)	58.16	14.56
T_4: T_1 + soil application of *T. harzianum* + SD* with T_1	SA @ 10 kg/ha	13.22 (21.20)	72.77	17.20
T_5: T_1 + soil application of *P. fluorescens* + SD with T_1	SA @ 10 kg/ha	15.03 (22.72)	69.04	16.63
T_6: T_2 + soil application of *T. harzianum* + SD with T_2	SA @ 10 kg/ha	6.94 (15.15)	85.72	19.83
T_7: T_2 + soil application of *P. fluorescens* + SD with T_2	SA @ 10 kg/ha	9.76 (18.00)	79.85	18.60
T_8: T_3 + soil application of *T. harzianum*+ SD with T_3	SA @ 10 kg/ha	15.98 (23.48)	67.08	16.42
T_9: T_3 + soil application of *P. fluorescens* + SD with T_3	SA @ 10 kg/ha	16.87 (24.19)	65.25	15.98
T_{10}: ST with *T. harzianum*+ SA* of *T. harzianum* + SD *of T. harzianum* at 40 DAS*	ST @ 10 g/kg SA @ 10 kg/ha + 2 g/L	21.22 (27.36)	56.29	13.67
T_{11}: ST with *P. fluorescens* + SA of*P.fluorescens* + SD of *P. fluorescens* at 40 DAS	ST @ 10 g/kg SA @ 10 kg/ha + 2 g/L	24.35 (29.50)	49.84	13.02
T_{12}: Control	-	48.55 (44.09)	0.00	12.50
S.Em±	-	1.52		1.56
CD (P=0.05)		4.50		4.62
CV (%)		10.45		11.83

Figures in parentheses are angular transformed values. ST- Seed treatment, SA*- Soil application, SD*- Soil Drenching, DAS*- Days after sowing.

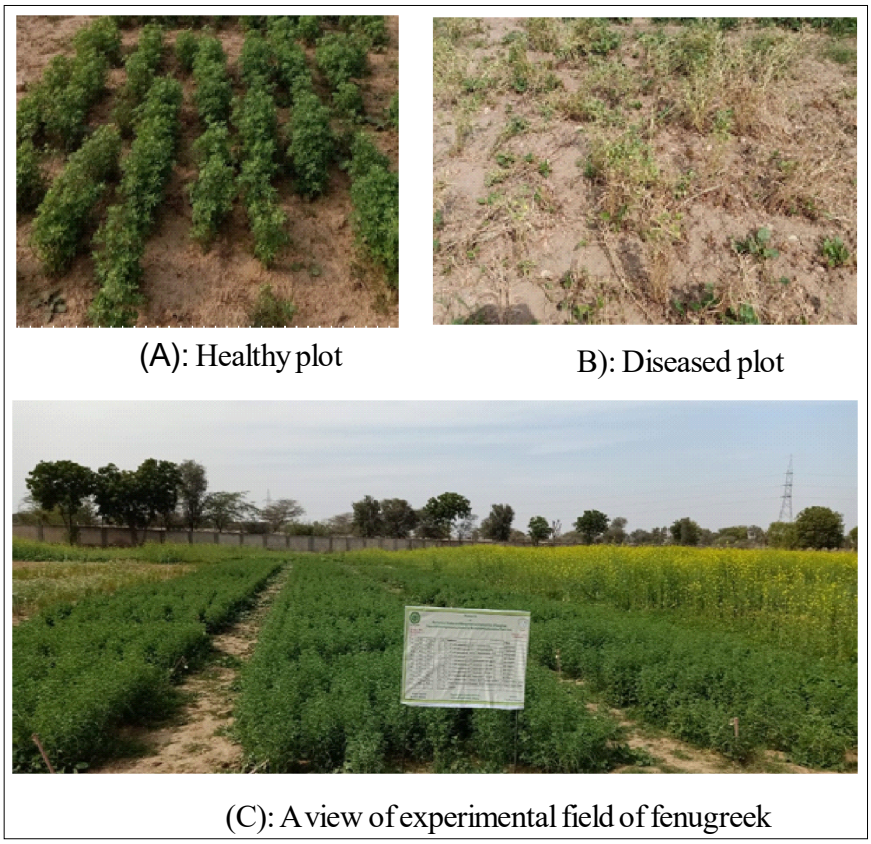

Plate 2: Disease incidence of fenugreek under field conditions and view of experimental field.

tebuconazole 50% + trifloxystrobin 25% WG @ 1.5 g/kg seed + SA @ 10 kg/ha of *P. fluorescens* @ 10 kg/ha + soil drenching @ 2 g/L with tebuconazole 50% + trifloxystrobin 25% WG (18.60 qha^{-1}) and ST with carbendazim 12% + mancozeb 63% WP @ 2 g/kg seed + SA of *T. harzianum* @ 10 kg/ha + SD with carbendazim 12% + mancozeb 63% WP (17.20 qha^{-1}). Similar findings was recorded by Meena *et al*. (2018) efficacy of Tebuconazole 50% + trifloxystrobin 25% WG, Carbendazim 12% + mancozeb 63% WP, *T. harzianum* and *P. fluorescens* were assessed under *in vivo* conditions. Bashir *et al.* (2017) observed that in the field conditions the treatment Nativo was found minimum disease incidence. These findings are very much similar with our findings.

CONCLUSION

It can be concluded unequivocally considering the results that in field conditions combination of bioagents and fungicide *viz.,* seed treatment with tebuconazole 50% + trifloxystrobin 25% WG @ 1.5 g/kg seed + SA of *T. harzianum* @ 10 kg/ha + SD @ 2 g/L with tebuconazole 50% + trifloxystrobin 25% WG was found most effective in managing the charcoal rot disease, lowest disease severity and maximum grain yield.

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Conflict of interest

All authors declared that there is no conflict of interest.

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