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Screening of Different Bioagents and Fungicides against Dry Root Rot of Chickpea Incited by *Macrophomina phaseolina* (Tassi) Goid

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ABSTRACT

Background: Dry root rot of chickpea caused by *Macrophomina phaseolina* (Tassi) Goid is a serious biotic constraint for chickpea production in Rajasthan. For the management of soil borne disease like dry root rot of chickpea, by using fungicides alone is not feasible due to environmental and health hazards. Hence integrated management of the disease by using bio-agents and fungicides is the best alternative. Therefore, in the present investigation, our main emphasis was to identify best fungicide and bio-agent for management of dry root rot in chickpea.

Methods: Eight fungicides and four bio-agents were evaluated against dry root rot pathogen (*M. phaseolina*) in lab as well as in field condition. The experiment was conducted at instructional farm, COA, SKRAU, Bikaner during *Rabi*-2019 on most popular cv. GNG-1581 in RBD design with the application of seed treatment and soil application of different bio-agents and fungicides at different concentrations against dry root rot disease and compared with an untreated control.

Result: Among all the fungicides used in the present investigation, tebuconazole 50% + trifloxystrobin 25% was found most effective in inhibiting the mycelial growth of the pathogen. Among all the bio-agents, *T. harzianum* was found most effective in inhibiting the mycelial growth of the pathogen. Under field condition, tebuconazole 50%+ trifloxystrobin 25% WG as seed treatment @ 1.5 g/kg along with *T. harzianum* @ 10 kg/ha as soil application gave maximum (83.76 %) disease control with highest pod yield (19.5 q/ha) and net return (Rs 39,826/ha). These treatments can provide an effective and economical management of dry root rot disease for chickpea cultivators.

Key words: Bio-agents, Chickpea, Dry root rot, Fungicides, *Macrophomina phaseolina*.

INTRODUCTION

Chickpea (*Cicer arietinum*) is an annual legume crop in temperate and sub-tropical regions. It belongs to family *Fabaceae*. Just after the bean, chickpea is the second most grown legume in the world. Southwest Asia (Turkey) is the originating centre and it is cultivated from ancient times both in Asia and European countries. India is the largest producer and consumer of chickpea in the world. India accounts approximately 75 per cent of world's chickpea production (Anonymous, 2016).

In world, chickpea is cultivated in 139 lakh ha with a production of 137.31 lakh tonnes, having average productivity of 982 kg ha^{-1}. Asia accounts for 89.2 per cent of chickpea area and comprises of 84.47 per cent of production. The countries growing chickpea which contribute about 90 per cent of total global production are India (71%) followed by Australia (3.63%), Pakistan (6.79%), Turkey (2.78%), Myanmar (2.75%) and Iran (4.25%) (FAOSTAT, 2014). Gram recorded highest production of 11.23 MT with a record productivity of 1,063 kg/ha in area of 10.56 Mha. Major 7 states contributing more than 90 per cent gram production are Madhya Pradesh (4.60 MT), Maharashtra (1.78 MT), Rajasthan (1.67 MT), Karnataka (0.72 MT) andhra Pradesh (0.59 MT), Uttar Pradesh (0.58 MT) and Gujarat (0.37MT) (Anonymous, 2016-17). One of the major crops grown in Rajasthan is chickpea that is grown in 14,86,163 ha with total production of 13,82,207 tonnes (Anonymous, 2016). If we look at the district-wise contribution to over all chickpea production in the state, Bikaner leads with a share of 13%, followed by Churu (9%), Jhunjhunu (9%), Hanumangarh (8%), Sri Ganganagar (8%), Jaipur (8%), Jaisalmer (5%), Sikar (5%), Ajmer (5%) and other remaining districts (30%) (Anonymous, 2015-16).

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Nearly 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and phytoplasma) will cause diseases in chickpea (Nene *et al.,*1996). These pathogens may be of bacteria, mycoplasma, fungi, nematodes and viruses which contain high genotypic variation. Commonly seen pathogens causing diseases in chickpea crop are *Ascochyta rabiei* found in 35 countries, *Fusarium oxysporum* f.sp. *ciceris* (32 countries), *Uromyces ciceris-arietini* (25 countries), bean leafroll virus (23 countries) and *Macrophomina phaseolina* (21 countries). Dry root rot is a major disease causing significant losses in chickpea that is 10-25 per cent losses (Pal, 1998). But it became severe in most of the chickpea cultivating areas and become reason for more than 50 per cent losses (Massoud and Kumar, 2001).

The fungicides are the most common tools for controlling plant disease. But they are not feasible for environment, health and it leads to environment hazard. Hence integrated management of the disease by using bio-agents and fungicides is the best alternative. Therefore, it is an urgent need to use some bio-agents and fungicides which are effective against dry root rot disease. Hence, keeping in view the importance of chickpea crop and potential threat of *Macrophomina phaseolina* in all the chickpea growing areas in the Rajasthan, the present investigation was undertaken to manage of this important disease through bio-agents and fungicides.

MATERIALS AND METHODS

Efficacy of bio-agents and fungicides were tested against *Macrophomina phaseolina* both in lab as well as in field condition.

Evaluation of the efficacy of bioagents against *Macrophomina phaseolina in vitro*

The fungal and bacterial bioagents (Table 1) were evaluated *in vitro* for testing their efficacy against *M. phaseolina* by dual culture method (Morton and Strouble, 1955) and paper disc inoculation method, respectively on PDA medium.

Per cent growth inhibition was calculated by following formula as; (Skidmore and Dickinson, 1976).

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

C = Mycelial growth of *M. phaseolina* in control (mm).
T = Mycelial growth of *M. phaseolina* in presence of antagonist (mm).

Evaluation of efficacy of fungicides against *M. phaseolina in vitro*

Eight fungicides (Table 2) were tested at a concentration of 100, 200, 300 and 500 ppm against *M. phaseolina* using poison food technique (Nene and Thapliyal, 1973) *in vitro*. Colony diameter (two diagonals) of pathogen was measured after 5 days of incubation. Medium without fungicide served as control. Per cent growth inhibition was calculated by formula (Skidmore and Dickinson, 1976).

Management of dry root rot of chickpea by bioagents and fungicides *in vivo*

A field trial was conducted for the management of dry root rot of chickpea using bioagents and fungicides during *Rabi* season 2019. A most popular chickpea cultivar GNG-1581 was used in this experiment. Seed treatment of fungicides and seed and soil treatment of the talc based bioagent formulations were done. In case of control, seeds were sown in pathogen inoculated soil without any bioagents and fungicides. Observations were taken periodically for the disease incidence.

12 treatments were conducted in the field using randomised block design and a plot size of 3 × 3 m^2. Each treatment was replicated thrice. The experiment was conducted under artificial soil infested conditions. For this purpose, sand maize meal inoculum of *M. phaseolina* was applied at 50 g per plot and mixed properly on top surface soil using a hand rack. Standard agronomic practices recommended for cultivation of chickpea crop in this region were followed. In case of control, the untreated seeds were sown. Observations on Dry Root Rot incidence were recorded periodically as well as the grain yield was recorded after harvesting the crop.

Field trial

In the view to study the efficacy of these biocontrol agents and fungicides as soil application, seed treatment alone and in combination against dry root rot incidence, field experiments were carried out during *rabi* 2019 at experimental farm, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. The chickpea crop was sown on 9th November 2019 with 30 × 10 cm row to row and plant to plant spacing.

Calculation and statistical analysis

Disease incidence (%) =

$$\frac{\text{No. of diseased plants}}{\text{Total no. of plants germinated}} \times 100$$

Disease control (%) =

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

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 analyzed following randomized block design (Cochran and Cox, 1957). Economics of each treatment was also computed.

RESULTS AND DISCUSSION

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

Efficacy of bioagents

The results presented in the Table 1 and Plate 1 shows that all the bioagents were significantly superior in controlling

the test fungus over the control. Among the tested fungal bioagents, maximum pathogen inhibition was resulted from *T. harzianum* (74.49%) followed by *T. viride* (64.25%), whereas in case of bacterial bioagents *P. fluorescens* (51.65%) and then *B. subtilis* (38.15). The observed results were very similar to the report of Meena *et al.* (2017) who observed antagonistic character of *Trichoderma* and other species isolated from infested soil by growing them on Czaper's dox agar medium in Petri dishes. According to them, Four fungi *Aspergillus niger*, *Trichoderma atroviride*, *T. harzianum*, *T. viride* showed different antagonistic characters inhibiting the pathogen.

The same trends were observed by Cherkupally *et al.* (2016). Who found that radial growth of *M. phaseolina* was inhibited to the maximum by *T. harzianum* under *in vitro* conditions. Similarly efficacy of *Trichoderma* spp. against pathogen causing brinjal root rot by dual culture method under *in vitro* conditions. Among the seven *Trichoderma* spp, *T. harzianum* inhibited maximum of 77.77%. Similar results were published by Sangappa and mallesh (2016). Thombre and Kohire, (2018) tested seven fungal antagonists *viz., Trichoderma viride*, *T. harzianum, T. hamatum*, *T. longibrachiatum, T. koningii*, *Gliocladium virens, Aspergillus niger* and two bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* under *in vitro* conditions against *M. phaseolina*. In these *T. harzianum, T. viride* and *P. fluorescens* showing 77.59%, 65.46%, 51.37% disease inhibition, respectively. Swamy *et al.* (2018) evaluated two isolates of the *Trichoderma* spp., four *Pseudomonas fluorescens* isolates, one *Pseudomonas putida* and one *Bacillus subtilis* for their efficacy. Among them maximum disease inhibition was resulted by *T. harzianum* (41.86%) followed by *T. viride* (39.07%), *P. fluorescens* (18.77%) and *B. subtilis* (14.85%). Our results are somewhat similar to these findings in efficacy of bioagents against *M. phaseolina* and these findings support our findings.

Efficacy of fungicides

Eight fungicides were evaluated at four concentrations, *viz.*, 100, 200, 300 and 500 ppm against *M. phaseolina* using poison food technique *in vitro*. The data presented in Table 2 and Plate 2 showed that all the eight fungicides caused significant reduction in mycelial growth as compared to

Table 1: Efficacy of bioagents against *M. phaseolina in vitro*.

Treatment	Mycelial growth (mm)	Growth inhibition (%)
T_1 - *T. harzianum*	22.96	74.49 (59.64)*
T_2 - *T. viride*	32.18	64.25 (53.27)
T_3 - *P. fluorescens*	43.52	51.65 (45.92)
T_4 - *B. subtilis*	55.67	38.15 (38.12)
T_5 - Control	90.00	0.00 (0.00)
S.Em±		0.34
CD @ 0.05		1.08
CV (%)		1.49

*Figures in parenthesis are angular transformed values.

Table 2: Efficacy of fungicides against *M. phaseolina in vitro*.

Treatment	Mycelial growth (mm) at conc.(ppm)				Growth inhibition (%) at conc.			
	100	200	300	500	100	200	300	500
T_1 - Captan 70% WP	71.26	46.68	27.90	21.02	20.83 (27.13)*	48.14 (43.91)*	69.02 (56.17)*	76.65 (61.09)*
T_2 - Thiophanate methyl 70% WP	8.93	5.89	0.00	0.00	90.08 (71.64)	93.46 (75.24)	100 (90.00)	100 (90.00)
T_3 - Chlorothalonil 75% WP	67.19	46.52	38.78	26.86	25.35 (30.19)	48.32 (44.01)	56.92 (48.96)	70.16 (56.92)
T_4 - Carbendazim 12% + mancozeb 63% WP	6.34	0.00	0.00	0.00	92.96 (74.62)	100 (90.00)	100 (90.00)	100 (90.00)
T_5 - Tebuconazole 50% + trifloxystrobin 25% WG	0.00	0.00	0.00	0.00	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_6 - Carboxin 37.5% + thiram 37.5% WS	15.19	8.37	3.28	0.00	83.13 (64.74)	90.7 (71.96)	96.36 (79.11)	100 (90.00)
T_7 - Copper oxychloride 50% WP	90.00	76.68	64.72	45.31	0.00 (0.00)	14.8 (22.55)	28.09 (31.98)	49.66 (44.78)
T_8 - Carbendazim 50% WP	24.30	18.10	9.05	0.00	73.00 (58.67)	79.89 (63.33	89.95 (71.50)	100 (90.00)
T_9 - Control	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.Em±					0.73	0.73	0.66	0.37
CD @ 0.05					2.20	2.19	1.99	1.11
CV (%)					2.74	2.27	1.86	0.94

*Figure in parenthesis are angular transformed values.

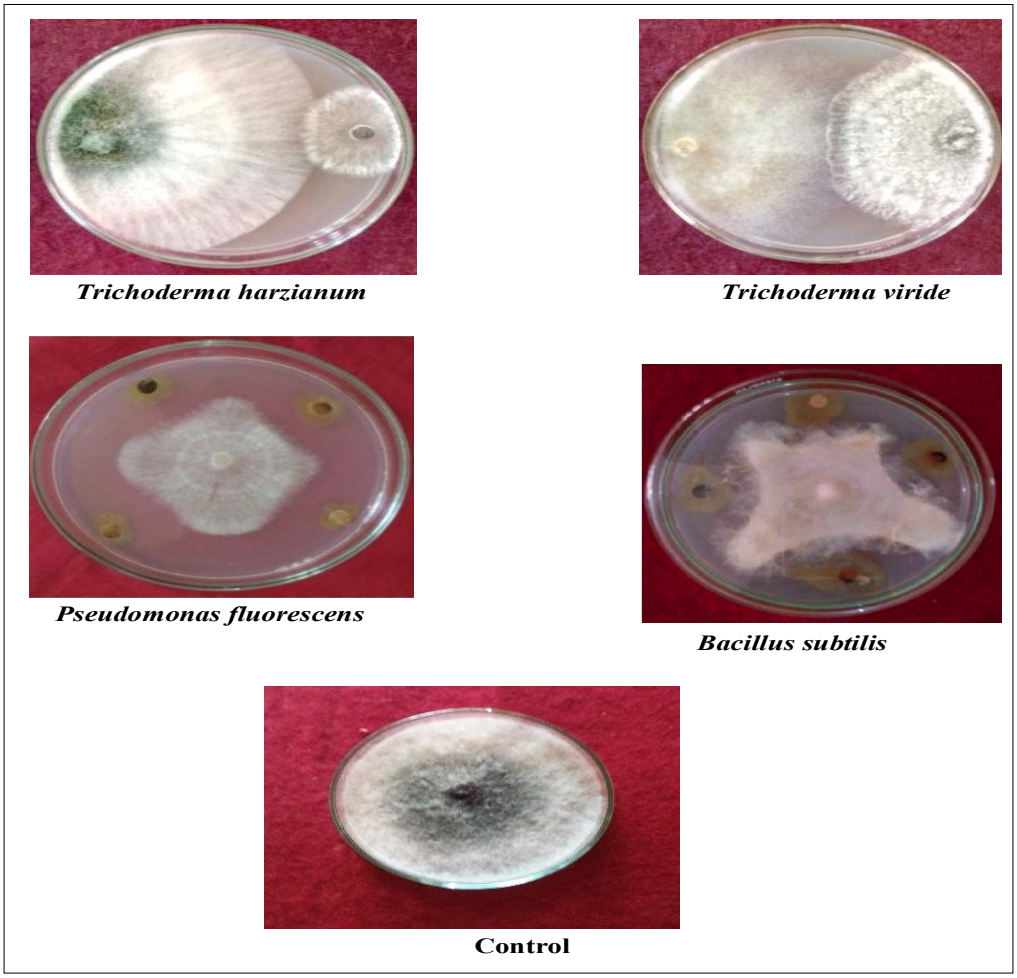


Plate 1: Efficacy of bioagents against *M. phaseolina in vitro*.

Table 3: Management of dry root rot of chickpea by bioagents and fungicides *in vivo*.

Treatment	Dose	Disease incidence (%)	Disease control (%)	Yield (q ha^{-1})
T_1 - Carbendazim 12% + mancozeb 63% WP	ST @ 2 g/kg seed	12.74 (20.28)*	64.33	14.10 (21.69)*
T_2 - Tebuconazole 50% + trifloxystrobin 25% WG	ST @ 1.5 g/kg seed	11.03 (18.85)	69.12	15.78 (23.16)
T_3 - Thiophanate methyl 70% WP	ST @ 2 g/kg seed	18.31 (24.27)	48.74	12.40 (20.40)
T_4 - T_1 + Soil application of *Trichoderma harzianum*	SA-10 kg/ha	7.43 (14.75)	79.19	18.72 (25.31)
T_5 - T_1 + Soil application of *Pseudomonas fluorescens*	SA-10 kg/ha	10.89 (18.04)	69.50	16.67 (23.16)
T_6 - T_2 + Soil application of *Trichoderma harzianum*	SA-10 kg/ha	5.80 (12.93)	83.76	19.50 (26.17)
T_7 - T_2 + Soil application of *Pseudomonas fluorescens*	SA-10 kg/ha	8.29 (15.54)	76.79	17.64 (24.70)
T_8 - T_3 + Soil application of *Trichoderma harzianum*	SA-10 kg/ha	10.03 (17.62)	71.92	16.98 (23.86)
T_9- T_3 + Soil application of *Pseudomonas fluorescens*	SA-10 kg/ha	12.72 (19.31)	64.38	14.54 (21.51)
T_{10} - ST with *Trichoderma harzianum* + SA of *T. harzianum*	ST-10 g/kg SA-10 kg/ha	11.50 (19.09)	67.80	16.20 (23.36)
T_{11} - ST with *Pseudomonas fluorescens* + SA of *P. fluorescens*	ST-10 g/kg SA-10 kg/ha	15.25 (22.43)	57.30	13.75 (21.59)
T_{12} - Control		35.72 (36.56)	0.00	10.30 (18.28)
S.Em±		1.37		1.27
CD @ 0.05		4.06		3.76
CV (%)		11.93		9.69

*Figure in parenthesis are angular transformed value.

control. Cent per cent growth inhibition was observed in treatment with Tebuconazole 50%+ Trifloxystrobin 25% WG at all the concentrations. Carbendazim 12%+ Mancozeb 63% inhibited cent per cent growth at three concentrations *viz.*, 200, 300 and 500 ppm, then Thiophanate methyl inhibited cent per cent growth at two concentrations (300 and 500 ppm). Most fungicides in this study were effective @ 500 ppm like Tebuconazole 50%+ Trifloxystrobin 25% WG, Carbendazim 12%+ Mancozeb 63%, Thiophanate methyl, Carboxin 37.5%+ Thiram 37.5% and Carbendazim 50% WP. But we have to recommend the lowest possible quantity of the effective fungicide to the farmers. So Tebuconazole 50%+ Trifloxystrobin 25% WG is effective (100% control) even at 100 ppm *viz*. is lowest concentration in the present study.

These results were similar with the report given by Meena *et al*. (2018) who tested tebuconazole 50%+ trifloxystrobin 25%WG and carbendazim 12%+ Mancozeb 63 % under *in vitro* conditions against *R. solani*. The results stated that tebuconazole 50%+ trifloxystrobin 25% WG fungicide reported maximum inhibition of mycelial growth at 100 ppm followed by carbendazim 12%+ mancozeb 63% WP. Sangappa and Mallesh (2016) revealed that cent per cent mycelial growth inhibition was seen in treatment with carbendazim at 0.05, 0.1 and 0.2% concentrations.

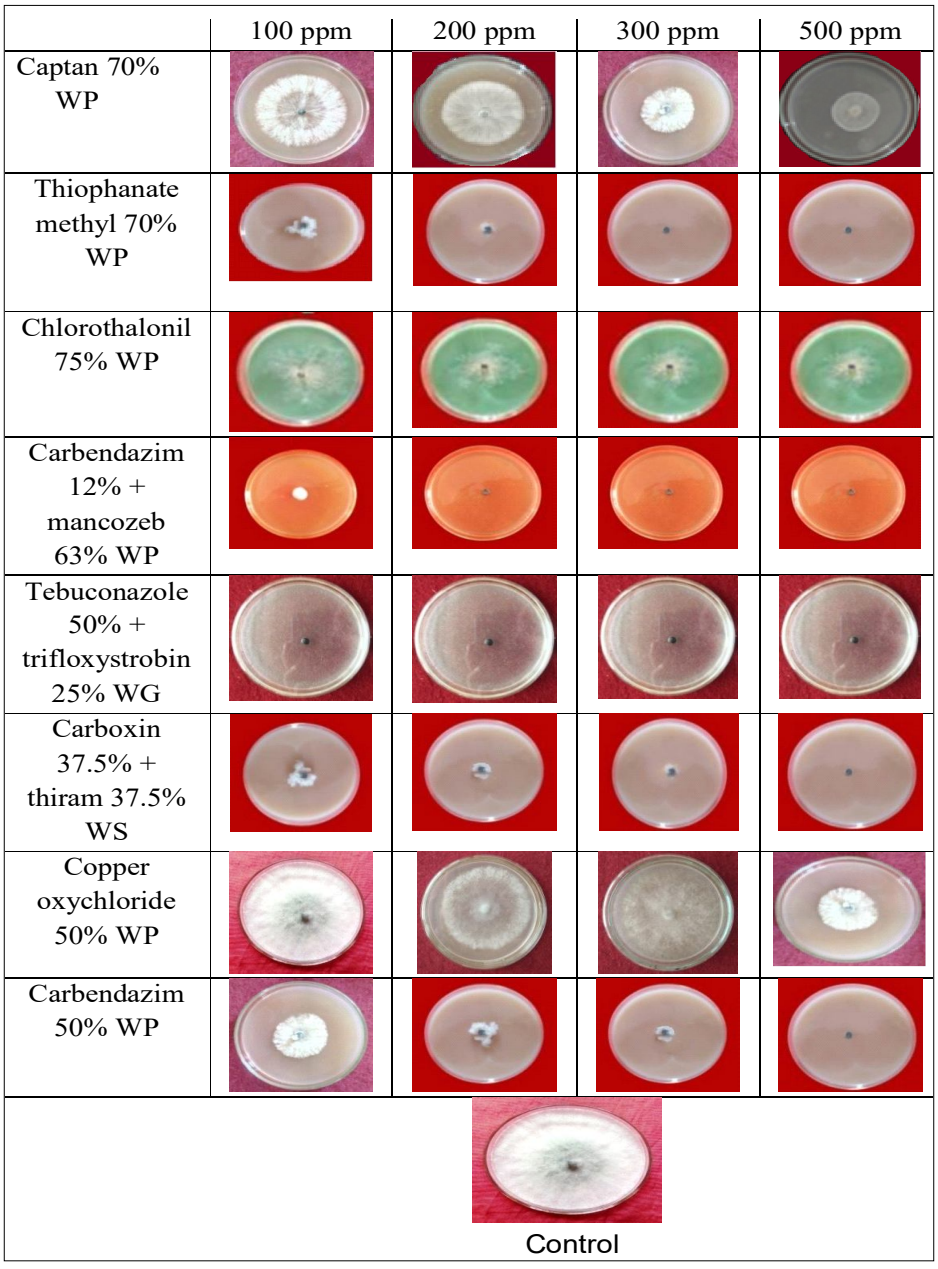


Plate 2: Efficacy of fungicides against *M. phaseolina in vitro*.

Kumar *et al*. (2024) found that tebuconazole 25.9% EC was most effective in inhibiting the mycelial growth of the pathogen followed by trifloxystrobin 25% + tebuconazole 50% WG.

Management of dry root rot of chickpea by bioagents and fungicides *in vivo*

The obtained results were displayed in the Table 3. These observations indicate that the dry root rot of chickpea incidence was greatly reduced by combined effect of chemical and biocontrol agents than the chemicals alone or biocontrol agents alone. Incidence of disease being reduced 83.76% in combined effect of bioagent and chemical that is tebuconazole 50%+ trifloxystrobin 25% WG as seed treatment @ 1.5 g/kg along with *T. harzianum*@ 10 kg/ha as soil application. This treatment was followed by combined application of carbendazim 12%+ mancozeb 63% WP @ 2 g/kg as seed treatment along with *T. harzianum* as soil application @ 10 kg/ha (79.19%). It was closely followed by tebuconazole 50%+ trifloxystrobin 25% WG as seed treatment with soil application of *P. fluorescens* @ 10 kg/ha. Similar results were obtained by Meena *et al*. (2018), Veena and Reddy (2016) and Lakhran and Ahir (2020).

Biological control of diseases through antagonists is helpful for inducing the long term disease resistance in the plants. Even though the biocontrol is somewhat slow in effect, long term disease resistance can be achieved by these. Chemicals will give immediate effect but they exhaust the nutritional capacity of the soil as well as cause pollution hazards. But presently a number of scientists are working on reduction of plant diseases with combined effect of chemicals as well as biocontrol agents. Antibiotics secreted by biocontrol agents can be utilized for inhibiting the pathogen in soil, so as to reduce the soil borne diseases caused by phytopathogens as stated by Harman *et al*. (2010) and Nawar (2008).

Yield

The yield results given in Table 3 revealed that grain yield was enhanced considerably with combined effect of fungicide as seed treatment and bioagents as soil application than the treatments containing fungicide or bioagent alone. The grain yield was highest (19.50 q ha^{-1}) in tebuconazole 50%+ trifloxystrobin 25% as seed treatment @ 1.5 g/kg seed plus *T. harzianum* as soil application @ 10 kg/ha and minimum 5.80 per cent disease incidence followed by carbendazim 12%+ mancozeb 63% as seed treatment @ 2 g/kg seed and *T. harzianum* as soil application @ 10 kg/ha (18.72 q ha^{-1} and 7.83% disease incidence). The control of soil borne plant pathogens and increase in yield of different crops after treatment with fungicides and bioagents have been reported by Elaigwu *et al*. (2017).

The economics computed on various treatments (Table 4) reveals that highest net gain (Rs 39,826 /ha) was obtained in the treatment T_6 followed by T_4 (Rs. 36,820 /ha). Similarly Nagamani *et al.,* (2011) conducted experiment on dry root rot of chickpea and reported that seed treatment with carbendazim @ 2 g/kg of seed+ seed treatment with *T. viride*

Table 4: Economics of different treatments of chickpea.

Treatment	Quantity of treatment (kg or lit./ha)	Cost of treatment (Rs/ha)	Labour cost (Rs/ha)	Total cost of treatment (Rs/ha)	Yield (q/ha)	Gross realization (Rs/ha)	Net realization over control (Rs/ha.)	Net gain (Rs/ha)
T_1- Carbendazim 12%+ mancozeb 63% WP	ST @ 2 g/kg seed	96	300	396	14.10	67,680	18,240	17,844
T_2- Tebuconazole 50%+ trifloxystrobin 25% WG	ST @ 1.5 g/kg seed	834	300	1134	15.78	75,744	26,304	25,170
T_3- Thiophanate methyl 70% WP	ST @ 2 g/kg seed	108	300	408	12.40	59,520	10,080	9,672
T_4- T_1 + Soil application of *Trichoderma harzianum*	SA-10 kg/ha	2596	1000	3596	18.72	89,856	40,416	36,820
T_5- T_1 + Soil application of *Pseudomonas fluorescens*	SA-10 kg/ha	2696	1000	3696	16.67	80,016	30,576	26,880
T_6- T_2 + Soil application of *Trichoderma harzianum*	SA-10 kg/ha	3334	1000	4334	19.50	93,600	44,160	39,826
T_7- T_2 + Soil application of *Pseudomonas fluorescens*	SA-10 kg/ha	3434	1000	4434	17.64	84,672	35,232	30,798
T_8- T_3 + Soil application of *Trichoderma harzianum*	SA-10 kg/ha	2608	1000	3608	16.98	81,504	32,064	28,456
T_9- T_3 + Soil application of *Pseudomonas fluorescens*	SA-10 kg/ha	2708	1000	3708	14.54	69,792	20,352	16,644
T_{10}- ST with *Trichoderma harzianum*+ SA of *T. harzianum*	ST-10 g/kg SA-10 kg/ha	2650	1000	3650	16.20	77,760	28,320	24,670
T_{11}- ST with *Pseudomonas fluorescens* + SA of *P. fluorescens*	ST-10 g/kg SA-10 kg/ha	2756	1000	3756	13.75	66,000	16,560	12,804
T_{12}- Control	-	-	-	-	10.30	49,440	-	-

Chickpea price Rs.4800/q., Carbendazim 12%+ mancozeb 63% WP = Rs 800/kg, Tebuconazole 50%+ trifloxystrobin 25% WG = Rs 9260/kg, Thiophanate methyl 70% WP = Rs 900 /kg, *Trichoderma harzianum* = Rs 250/kg, *Pseudomonas fluorescens* = Rs 260/kg.

@ 4 g/kg of seed + soil application of FYM fortified with T. viride recorded least disease incidence with highest yield and BC ratio. Manjunatha and Saifulla (2021) observed that fungicides and bioagents are effective for the the management of dry root rot of chickpea.

So by this present study, we can conclude that combined effect of chemicals and bioagents would be effective against the dry root rot disease in chickpea than the application of chemicals or bioagents alone.

CONCLUSION

It can be concluded that among the four bioagents, *T. harzianum* was found most effective in controlling the pathogen followed by *T. viride*. Their growth was superior than the causal organism and restricted the growth of the pathogen. Among the tested fungicides, tebuconazole 50% + trifloxystrobin 25% WG was found most effective against the mycelial growth of pathogen. Combined effect of fungicide as seed treatment and bioagent as soil application was most effective in controlling dry root rot of chickpea in field conditions. Among the twelve treatments, tebuconazole 50%+ trifloxystrobin 25% WG @ 1.5 g/kg seed and *T. harzianum* @ 10 kg/ha soil application yielded maximum grain yield (19.50 q ha^{-1}) and minimum disease incidence of 5.80 per cent. This treatment is better option of chickpea cultivators for enhancing the yield by managing dry root rot disease with maximum net return under field conditions.

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Conflict of interest

All authors declared that there is no conflict of interest.

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