

Original Research Article

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Antagonistic Potential of Bio-agent and Botanicals against Banded Leaf and Sheath Blight of Maize (*Zea mays* L.) caused by *Rhizoctonia solani* (Kuhn)

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ABSTRACT

Banded Leaf and Sheath Blight (BSBL) of maize caused by *Rhizoctonia solani* f. sp. *sasakii* Exner is one of the devastating soil-borne disease of maize in India. An experiment was conducted to evaluate the effect of botanicals and bio-control agent *in vitro* and *in situ* which led to give direction to manage the pathogen in ecofriendly manner. The antagonistic effect of *Trichoderma harzianum* was assayed *in vitro* by employing dual culture method, which was found effective in the inhibition of mycelial growth (61.1 %) of *Rhizoctonia solani*. Five botanicals viz., *Neem* (*Azadirachta indica*), *Garlic* (*Allium sativum*), *Tulsi* (*Ocimum tenuiflorum*), *Bhang* (*Cannabis sativa*) and *Onion* (*Allium cepa*) each @ 5, 10 and 15 % were evaluated *in vitro* against *R. solani* following poisoned food technique. Among all the plant extracts tested, garlic was found highly effective in reducing the radial growth of *R. solani* i.e., 62.67 % followed by *neem* (61.93 %), *tulsi* (60.00 %), *onion* (48.33 %) and least by *bhang* (42.85 %) at 15 % concentration. Carbendazim 50 WP as treated check was tested @ 0.1, 0.2 and 0.3 % concentration, which recorded excellent inhibitory activity against *R. solani* (100 %). The botanicals and *Trichoderma harzianum* found effective *in vitro* were tested as foliar spray, soil treatment and seed treatment respectively against banded leaf and sheath blight of maize under field conditions during *kharif* season 2019. Among all the treatments, soil treatment with *Trichoderma harzianum* was observed superior over other treatments giving maximum reduction (63.30 %) in disease severity followed by *neem* (66.16 %), *garlic* (67.67 %), *tulsi* (68.19 %), *onion* (69.25 %) and *bhang* (71.08 %) as compared to treated check i.e., 40.26 %. *Trichoderma harzianum* has given maximum increase in grain yield (4.44 t/ha) and 100 grains weight (36.03 g).

Keywords

Banded leaf and sheath blight, *Bhang*, *Garlic*, *Neem*, *Onion*, *Rhizoctonia solani*, *Trichoderma harzianum*, *Tulsi*

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Introduction

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agroclimatic conditions. Globally, maize is known as 'Queen of Cereals' because it has the highest

genetic yield potential among the cereals. The multiple utilities of maize as a 'food', 'fodder' and 'feed' makes it further more demand friendly and insulates it against low demand situations. These unique characteristics of maize make the crop a suitable crop candidate for enhancing

farmer's income and livelihoods in India. The production and productivity of maize in India is declining because of many biotic and abiotic stresses. Among different fungal diseases affecting maize production, banded leaf and sheath blight (BSLB) induced by *Rhizoctonia solani* f. sp. *Sasakii* causes significant grain yield loss, depending on disease severity, which varies between 11 % to 40 %, even to 100 % on some cultivars in some warm and humid regions, where the conditions are favorable for the pathogen. (Madhavi *et al.*, 2011; Izhar and Chakraborty 2013; Gao *et al.*, 2014). The pathogen is known to be soil-borne and their sclerotia found in the plant and soil.

Presently, meagre information is available on its sustainable management and is generally treated by chemical applications. However, their field application may not always be desirable. The persistence and injudicious use of chemicals is discouraged owing to their toxic effect on non-target organism, the undesirable change they inflict upon the environment and due to development of resistance strain of pathogen against various chemical fungicides.

Keeping in view drawback of chemical control of plant diseases, the use of botanicals and bio-control agent in management of plant diseases is gaining importance. Biological and botanical control appear to be the best solution for long term sustainability and efficient management of this kind of soil-borne diseases which can significantly minimize the disease.

Use of plant products in disease management is a current approach which is ecofriendly and also getting hold of popularity because of its advantages over chemicals. Bio-degradability of these plant extracts is very fast without any residual effect on plant and are easily absorbed by the plants and also cost effective.

The occurrence of natural substance in these plants with having antifungal properties were reported and tested against broad range of microorganism, which can cause a severe damage to most of vital crops. Biocontrol agent, *Trichoderma harzianum* effectively control banded leaf and sheath blight through production of antibiotics, lytic enzyme (Sharma *et al.*, 2002) and also through the mechanism of hyphal coiling and penetration of the hyphae of *Trichoderma* in the hyphae of host fungus (Priya and Upadhyay 2017). In view of the increasing importance of banded leaf and sheath blight disease and the growing environmental concern, the present investigation was carried out against BLSB of maize through botanicals and biocontrol agent.

Materials and Methods

***In vitro* experiment**

The experiment was conducted in completely randomized design (CRD) in laboratory condition. Five plant extracts, namely, *neem* (*Azadirachta indica*) leaf extract, garlic (*Allium sativum*) bulb extract, *tulsi* (*Ocimum tenuiflorum*) leaf extract, *bharg* (*Cannabis sativa*) leaf extract and onion (*Allium cepa*) bulb extract each at 5, 10 and 15 % concentrations and a chemical, carbendazim 50 WP as check at 0.1, 0.2 and 0.3 % concentrations were tested for their efficacy against the pathogen *Rhizoctonia solani* *in vitro* using poison food technique on potato dextrose agar (PDA) medium.

The selected fresh leaves and bulbs of healthy plants were collected and washed thoroughly with clean water and dried. Plant leaves and bulbs were grounded in a pestle and mortar by adding same proportion of sterilized distilled water in weight by volume method (1:1 w/v). The plant extracts were filtered through double layered muslin cloth. The filtrates thus

obtained and the fungicide were thoroughly mixed in the PDA medium to required concentrations. Twenty ml of amended medium was poured in 90 mm sterilized Petri dishes and allowed to solidify. Mycelial disc of 5 mm from seven days old actively growing culture was inoculated at the center of the Petri plate and then incubated at $28 \pm 2^\circ\text{C}$. Control was maintained without any treatment. Five replications were maintained for each treatment and data was recorded at 72 hours after inoculation till full growth in control plate. Per cent inhibition of mycelial growth were calculated by the formula given by Vincent (1947).

$$I = (C - T / C) \times 100$$

Where,

I = Per cent inhibition of mycelial growth

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

The antagonistic microorganism, *Trichoderma harzianum* was tested *in vitro* for its antagonism against *Rhizoctonia solani* by dual culture technique. Twenty ml of PDA was poured into sterile Petri plates. *Trichoderma harzianum* was evaluated by inoculating the pathogen at one side of the petriplate and the antagonist inoculated exactly on the opposite side of the same plate by leaving a space of 3 to 4 cm. In control plate, only test pathogen was placed in a similar manner on a fresh PDA plate. Five replications were maintained and incubated at $28 \pm 2^\circ\text{C}$. Antagonistic activity was tested after full growth in control plate. The readings were transformed into percentage inhibition of radial growth (PIRG) using the formula developed by Dickinson (1976).

$$\text{PIRG} = (R_1 - R_2 / R_1) \times 100$$

Where,

R_1 = Radius of *R.solani* colony in control plate

R_2 = Radius of *R.solani* colony in dual culture plate.

Efficacy of biocontrol agent and botanicals on banded leaf and sheath blight disease of maize under field condition

In order to check the effect of botanicals and bio-agent on banded leaf and sheath blight of maize under field conditions, field experiment was laid out in randomized block design (RBD) with three replications. *Trichoderma harzianum* was soil treated and three foliar sprays of botanicals were given at an interval of 10 days. Treatments were imposed after appearance of the first disease symptoms. Observations on disease incidence, disease severity and plant height were recorded using 1-5 disease rating scale as suggested by Ahuja and Payak (1983) at 10 days interval and yield data were obtained after the harvest on physiological maturity.

Results and Discussion

In vitro* evaluation of bio-agent and botanicals against *Rhizoctonia solani* f. sp. *sasakii

The results presented in table (1) and depicted in figure (1) revealed that all the botanicals tested were significantly effective in inhibiting the growth of pathogen over control. The results indicated that increase in per cent inhibition was variably in proportion to increase in the concentration of the plant extracts. Among different plant extracts tested, garlic at 15 per cent showed maximum inhibition (62.67 %) followed by *neem* (61.93 %), *tulsi* (60.00 %), onion (48.33 %) and least effective was *bhang* (42.85 %).

However, at 15 % concentration the treatments (T₂ and T₃) and (T₂ and T₄) were non-significant and statistically at par with each other.

Sharma *et al.*, (2018) and Sonakar *et al.*, (2014) reported the maximum percentage of growth inhibition of *R. solani* with garlic which corroborates the present findings.

The results presented in table (2) and depicted in figure (2) revealed that *T. harzianum* exhibited fungistatic activity and significantly inhibited the mycelial growth with inhibition per cent of 61.1 % against *Rhizoctonia solani*.

In the present investigation, *Trichoderma harzianum* inhibited mycelial growth of *R. solani* up to 61 per cent in dual culture which corroborates the findings of De Mello and Faull (2000); Agarwal and Kotasthane (2007) and Rajput *et al.*, (2016), that *T. harzianum* significantly inhibited mycelial growth of *R. solani*. Similarly, Rajput and Zacharia (2017) reported *T. harzianum* reduce the colony growth of *R. solani* by dual culture technique, which confirms the present results.

Evaluation of bio-agent and botanicals on banded leaf and sheath blight disease of maize under field condition

Effect on disease incidence

Perusal of data in table no. 3 (Fig no.-3) reveal minimum disease incidence recorded in *Trichoderma harzianum* at 45, 55 and 65 days after sowing (50.55 %, 64.47 %, 73.44 % respectively) followed by, *Neem* (52.48 %, 66.41 %, 76.08 % respectively), *Garlic* (53.81 %, 67.64 %, 77.18 % respectively), *Tulsi* (60.10 %, 69.02 %, 80.34 % respectively), *Onion* (63.95 %, 71.58 %, 84.26%) and *Bhang* (65.22 %, 75.24 %, 88.32 % respectively) as compared to treated check (30.16 %, 35.29 % and 38.44 % respectively)

and untreated check (70.76 %, 78.38 % and 99.01 % respectively).

However, at 45 DAS the treatments (T₂ and T₃) were found non-significant and statistically at par with each other. At 55 DAS the treatments (T₁ and T₂) and (T₂ and T₃) were found non-significant and statistically at par with each other. At 65 DAS the treatments (T₂ and T₃) were found non-significant and statistically at par with each other.

Effect on percent disease index (PDI)

Perusal of data in table no. 4 (Fig no.- 4) reveal minimum disease severity recorded in *Trichoderma harzianum* at 45, 55 and 65 days after sowing (40.41 %, 54.54 %, 63.30 % respectively) followed by, *Neem* (42.15 %, 57.65 %, 66.16 % respectively), *Garlic* (44.18 %, 58.51 %, 67.67 % respectively), *Tulsi* (46.71 %, 60.14 %, 68.19 % respectively), *Onion* (49.25 %, 62.08 %, 69.25%) and *Bhang* (51.78 %, 65.51 %, 71.08 % respectively) as compared to treated check (35.05 %, 37.15 % and 40.26 % respectively) and untreated check (55.34 %, 66.74 % and 80.97 % respectively).

However, at 55 DAS the treatments (T₂ and T₃) were found non-significant and statistically at par with each other. At 65 DAS the treatments (T₂ and T₃), (T₃ and T₄) and (T₄ and T₆) were found non-significant and statistically at par with each other.

The results are in agreement with the findings of Meena *et al.*, (2003); Rajput and Zacharia (2017); Pandey and Upadhyay (2000) and Yadav *et al.*, (2005) that *Trichoderma harzianum* as soil application was found effective in disease reduction. The present findings were supported by Rajput and Zacharia (2017) and Kansal *et al.*, (2008) who found that *Neem* extract was effective in reducing disease caused by *Rhizoctonia solani*.

Effect on plant height (cm)

Perusal of data in table no. 5 (Fig no.- 5) reveal maximum plant height recorded in *Trichoderma harzianum* at 45, 55 and 65 days after sowing (41.76 cm, 99.00 cm, 154.02 cm respectively) followed by, *Neem* (40.80 cm, 93.26 cm, 148.69 cm respectively), *Garlic* (40.06 cm, 92.86 cm, 147.14 cm respectively), *Tulsi* (39.20 cm, 88.43 cm, 143.96 cm respectively), *Onion* (38.95 cm, 83.93 cm, 138.78 cm) and *Bhang* (37.90 cm, 79.46 cm, 134.52 cm respectively) as compared to treated check (43.80 cm, 108.80 cm and 163.44 cm respectively) and untreated check (37.10 cm, 63.83 cm and 118.82 cm respectively).

However, at 45 DAS the treatments (T_2 and T_3) and (T_4 and T_6) were found non-

significant and statistically at par with each other. At 55 DAS the treatments (T_2 and T_3) were found non-significant and statistically at par with each other. At 65 DAS the treatments (T_2 and T_3) were found non-significant and statistically at par with each other.

Effect on number of cobs

Perusal of data in table no. 6 (Fig no.-6) reveal maximum number of cobs recorded in *Trichoderma harzianum* (18.33) followed by, *Neem* (17.00), *Garlic* (15.67), *Tulsi* (15.00), *Onion* (14.33), *Bhang* (13.00) as compared to treated check (19.00) and untreated check (10.00). However, the treatments (T_1 and T_2), (T_2 and T_3), (T_3 and T_4), (T_3 and T_6) and (T_4 and T_6) were found non-significant and statistically at par with each other.

Table.1 Effect of botanicals on mycelial growth of *Rhizoctonia solani* in vitro

Treatments no.	Treatments	Radial growth (mm)** of <i>Rhizoctonia solani</i> after 72 hours					
		5 % concentration	Inhibition (%)	10 % concentration	Inhibition (%)	15 % concentration	Inhibition (%)
T_0	Control (untreated check)	90	0	90	0	90	0
T_2	<i>Neem</i>	66.31	26.27 (31.11) *	44.59	50.39 (45.52)	34.52	61.93 (51.53)
T_3	<i>Garlic</i>	47.63	47.44 (43.51)	42.27	52.67 (46.55)	33.34	62.67 (52.36)
T_4	<i>Tulsi</i>	70.14	22.16 (28.38)	48.66	46.53 (41.32)	36.44	60.00 (50.77)
T_5	<i>Bhang</i>	77.55	13.61 (21.22)	59.07	34.4 (35.67)	51.39	42.85 (40.69)
T_6	<i>Onion</i>	76.54	14.48 (22.63)	56.63	37.43 (37.64)	46.13	48.33 (44.03)
		0.1 % concentration	Inhibition (%)	0.2 % concentration	Inhibition (%)	0.3 % concentration	Inhibition (%)
T_7	Carbendazim (treated check)	0	100	0	100	0	100
	<i>S.Em</i> ±	0.704		0.716		0.815	
	<i>C.D</i> (5%)	2.16		2.19		2.50	

*Figures in the parentheses indicates arc sine transformed values.** Mean of five replicates

Table.2 Effect of *Trichoderma harzianum* on mycelial growth of *Rhizoctonia solani*

Treatment no.	Treatment Name	Radial growth (mm)*	Inhibition % *
T_0	Control	90	0
T_1	<i>Rhizoctonia solani</i> + <i>Trichoderma harzianum</i>	35	61.1
<i>S.Em</i> (\pm)		0.408	
<i>C.D</i> (5%)		1.65	

*Mean of five replicates

Table.3 Effect of *Trichoderma harzianum* and botanicals on disease incidence of banded leaf and sheath blight on maize under field condition

Treatment no.	Treatment	**Disease incidence (%)		
		45 DAS	55 DAS	65 DAS
T_0	Control (untreated check)	70.76 (57.17) *	78.38 (62.58)	99.01 (84.29)
T_1	<i>Trichoderma harzianum</i>	50.55 (45.29)	64.47 (53.55)	73.44 (58.95)
T_2	<i>Neem</i>	52.48 (46.61)	66.41 (54.39)	76.08 (61.21)
T_3	Garlic	53.81 (46.78)	67.64 (55.18)	77.18 (61.89)
T_4	<i>Tulsi</i>	60.10 (50.77)	69.02 (56.29)	80.34 (63.72)
T_5	<i>Bhang</i>	65.22 (53.85)	75.24 (60.27)	88.32 (69.91)
T_6	Onion	63.95 (52.83)	71.58 (57.82)	84.26 (66.89)
T_7	Carbendazim (treated check)	30.16 (33.58)	35.29 (36.81)	38.44 (38.29)
	<i>CD</i> (5%)	0.680	0.866	1.142
	<i>S.Em</i> (\pm)	0.222	0.283	0.373

Table.4 Effect of *Trichoderma harzianum* and botanicals on percent disease index (%) of banded leaf and sheath blight on maize under field condition

Treatment no.	Treatments	**Percent disease index (%)		
		45 DAS	55 DAS	65 DAS
T_0	Control (untreated check)	55.34 (48.10) *	66.74 (54.57)	80.97 (63.94)
T_1	<i>Trichoderma harzianum</i>	40.41 (39.29)	54.54 (47.52)	63.30 (52.53)
T_2	<i>Neem</i>	42.15 (40.69)	57.65 (49.31)	66.16 (54.70)
T_3	Garlic	44.18 (42.02)	58.51 (49.66)	67.67 (55.37)
T_4	<i>Tulsi</i>	46.71 (42.76)	60.14 (51.00)	68.19 (56.11)
T_5	<i>Bhang</i>	51.78 (46.03)	65.51 (53.79)	71.08 (57.82)
T_6	Onion	49.25 (44.71)	62.08 (52.42)	69.25 (56.48)
T_7	Carbendazim (treated check)	35.05 (36.57)	37.15 (37.76)	40.26 (39.58)
	<i>C.D</i> (5%)	0.695	0.735	0.806
	<i>S.Em</i> (\pm)	0.227	0.240	0.263

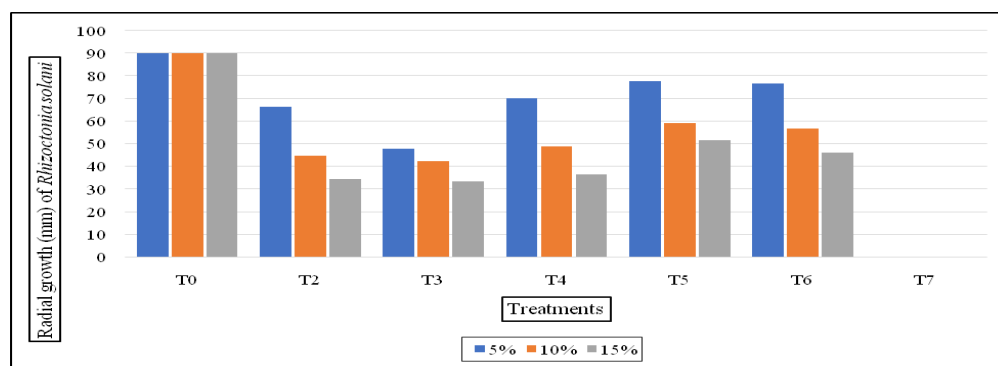
Table.5 Effect of *Trichoderma harzianum* and botanicals on plant height (cm) of maize at different days interval under field condition

Treatments no.	Treatments	*Plant height (cm)		
		45 DAS	55 DAS	65 DAS
T ₀	Control (untreated check)	37.10	63.83	118.82
T ₁	<i>Trichoderma harzianum</i>	41.76	99.00	154.02
T ₂	Neem	40.80	93.26	148.69
T ₃	Garlic	40.06	92.86	147.14
T ₄	Tulsi	39.20	88.43	143.96
T ₅	Bhang	37.90	79.46	134.52
T ₆	Onion	38.95	83.93	138.78
T ₇	Carbendazim (treated check)	43.80	108.80	163.44
	<i>S.Em</i> ±	0.257	0.373	1.025
	C.D (5%)	0.79	1.14	3.138

Table.6 Effect of *Trichoderma harzianum* and botanicals on yield of maize under field condition

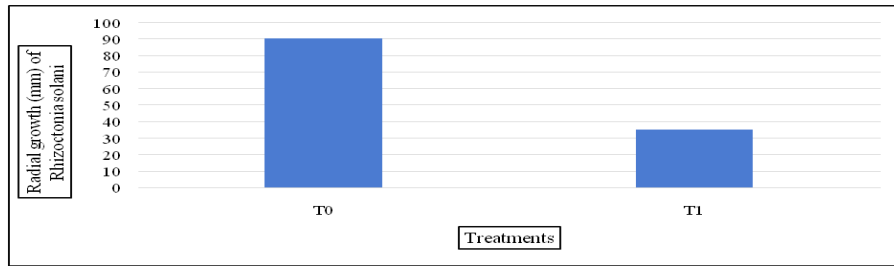
Treatments	*Number of cobs	Test weight (g)	*Yield (t/ha)
Control (untreated check)	10.00	27.13	2.35
<i>Trichoderma harzianum</i>	18.33	36.03	4.44
Neem	17.00	35.23	4.26
Garlic	15.67	34.36	4.06
Tulsi	15.00	34.03	3.84
Bhang	13.00	32.77	3.56
Onion	14.33	33.56	3.78
Carbendazim (treated check)	19.00	37.50	4.57
<i>S.Em</i> ±	0.48	0.35	0.04
C.D (5%)	1.47	1.07	0.12

Fig.1 Effect of botanical extracts at different concentrations on mycelial growth of *Rhizoctonia solani*



T₀– Control; T₂– Neem; T₃– Garlic; T₄– Tulsi; T₅ – Bhang; T₆ – Onion; T₇ – Carbendazim (treated check)

Fig.2 Comparative radial growth (mm) of *Rhizoctonia solani* after 3 days as affected by *Trichoderma harzianum*



T₀ - *Rhizoctonia solani*; T₁ - *R. solani* + *Trichoderma harzianum*

Fig.3 Effect of *Trichoderma harzianum* and botanicals on disease incidence (%) of banded leaf and sheath blight of maize under field condition

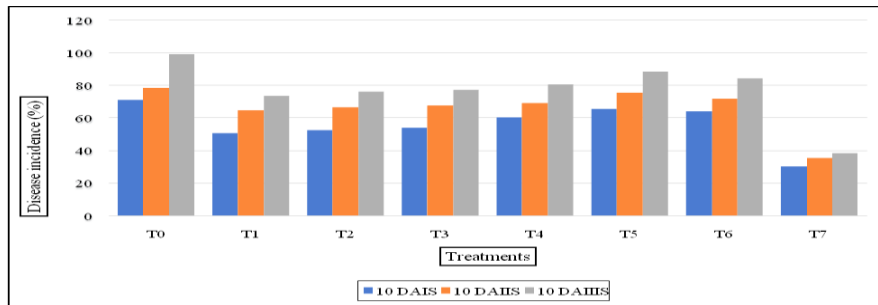


Fig.4 Effect of *Trichoderma harzianum* and botanicals on percent disease index (%) of banded leaf and sheath blight on maize under field condition

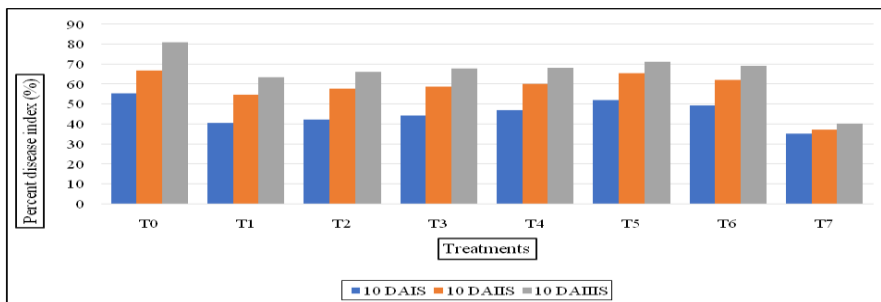


Fig.5 Effect of *Trichoderma harzianum* and botanicals on plant height (cm) of maize at different days interval under field condition

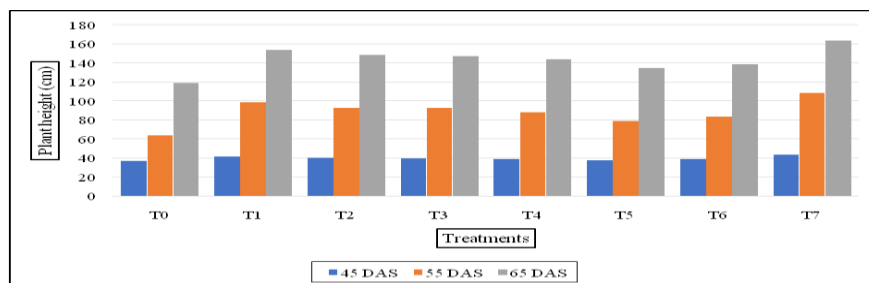


Fig.6 Effect of *Trichoderma harzianum* and botanicals on number of cobs of maize after harvesting under field condition

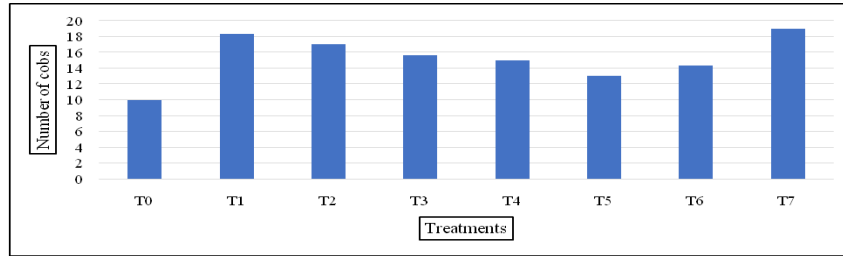


Fig.7 Effect of *Trichoderma harzianum* and botanicals on test weight (g) of maize grains (g) under field condition

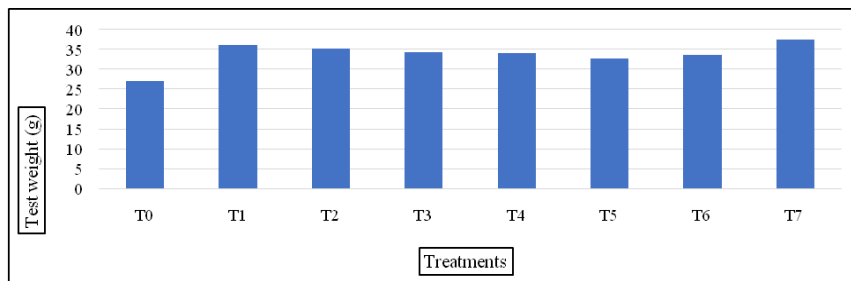


Fig.8 Effect of *Trichoderma harzianum* and botanicals on grain yield (t/ha) of maize under field condition

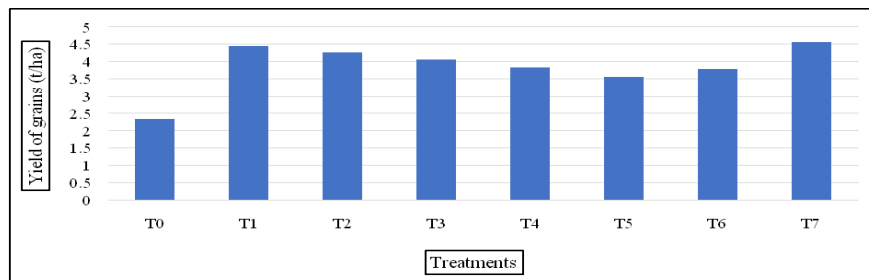
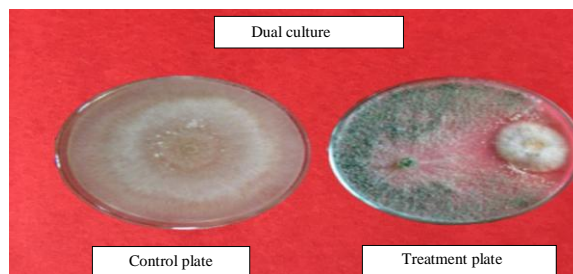


Plate.1 Antagonistic action of *Trichoderma harzianum* on the mycelial growth of *Rhizoctonia solani*



Control plate – *Rhizoctonia solani*; Treatment plate – *R. solani*+ *T. harzianum*

Effect on test weight (g)

Perusal of data in table no. 6 (Fig no.-7) reveal maximum test weight of grains recorded in treatment *Trichoderma harzianum* (36.03 g) followed by *Neem* (35.23 g), Garlic (34.36 g), *Tulsi* (34.03 g), Onion (33.56 g), *Bhang* (32.5 g) in comparison to treated check (37.5 g) and untreated check (27.13 g). However, the treatments (T₁ and T₂), (T₂ and T₃), (T₃ and T₄), (T₃ and T₆), (T₄ and T₆) and (T₅ and T₆) were found non-significant and statistically at par with each other.

Effect on grain yield (t/ha)

Perusal of data in table no. 6 (Fig no.- 8) reveal maximum yield recorded in treatment *Trichoderma harzianum* (4.44 t/ha) followed by, *Neem* (4.26 t/ha), Garlic (4.06 t/ha), *Tulsi* (3.84 t/ha), Onion (3.78 t/ha), *Bhang* (3.56 t/ha) in comparison to treated check (4.57 t/ha) and untreated check (2.35 t/ha). However, the treatments (T₄ and T₆) were found non-significant and statistically at par with each other.

In conclusion based on the results *Trichoderma harzianum* and Garlic recorded maximum inhibition of mycelial growth of *Rhizoctonia solani* *in vitro* condition. *In situ* condition, *T. harzianum* and *Neem* were found the most effective treatments showing minimum disease intensity and produced maximum yield as compared to other treatments except carbendazim which was taken as treated check. *T. harzianum* also recorded highest cost benefit ratio.

Since, chemicals have many harmful effects on the environment as well as the human health, they would be considered as better as they are eco-friendly and can also be recommended to the farmers for the efficient management of disease. The present research findings are limited to one crop season under

Prayagraj agroclimatic conditions as such more trials are required in future to validate the findings.

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