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Management of dry root rot of mungbean caused by *Macrophomina phaseolina* using bioagents and fungicides

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Abstract
Mungbean is one of the most important pulse crops cultivated in India. In India, it is the third most important legume crop after chickpea and pigeon pea. It is cultivated in summer and kharif season in North and South India. Mungbean is a major source of high protein. As a leguminous crop it can fix-atmospheric nitrogen through symbiotic nitrogen fixation. Mungbean is being affected by various fungal, bacterial, and viral diseases but, dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. is considered as the extremely destructive disease in all the mungbean cultivating regions of the country. *Macrophomina phaseolina* is a soil-borne pathogen causes serious disease in several crops in India, diminishing crop yields. The present study on various aspects of the disease dry root rot caused by *Macrophomina phaseolina* in Mungbean was carried out for developing an effective and economical management strategies for its control. The seven culture medium tested, Potato dextrose agar was found to be most excellent medium for the growth of *Macrophomina phaseolina.* Maximum mycelium growth of dry root rot fungus *Macrophomina phaseolina* was recorded at temperature 30 ºC, and PH 6.5 independently. Three fungal biocontrol agents studied under *invitro*, *Trichoderma virens* was observed highly effective in inhibiting the mycelial growth of *Macrophomina phaseolina* with minimum mycelial growth (36.8 mm) and maximum growth inhibition (53.96%) followed by *Trichoderma harzianum*. Three different fungicides were evaluated under *invitro* condition, Carbendazim 12% + Mancozeb 63% WP (SAAF) was observed highly effective in inhibiting the mycelial growth of *Macrophomina phaseolina* and showed complete (100%) mycelial growth inhibition at 250 and 500ppm concentrations, respectively. In field conditions, Treatment (T_2) *Trichoderma harzianum* was found effective in managing the disease incidence followed by treatment (T_4) Carbendazim 12% + Mancozeb 63% WP (SAAF) at 30 DAS, 45 DAS and 60 DAS, respectively.

Keywords: Mungbean, dry root rot, *macrophomina phaseolina*, media, biocontrol agents, fungicides, *trichoderma* spp, management

Introduction
Mungbean [*Vigna Radiata* L.] also well-known as green gram belongs to family Leguminosae (P. Kumar & Gaur, 2020) [27]. Green gram cultivation in the country is largely focused in five states *viz*., Rajasthan, Maharashtra, Andhra Pradesh, Gujarat, and Bihar. Decandolle (1986) said that mungbean has been originated in India. According to Vavilov (1926), mungbean is a native of India and Central Asia (M. Kumar *et al*., 2020). It is cultivated in summer and kharif season in North and South India. In India, it is the third most important legume crop after chickpea and pigeon pea. Mungbean is a major source of high protein. It is obtained in diverse ways such as dal, halwa, snack and so many other preparations. As a leguminous crop it can fix-atmospheric nitrogen through symbiotic nitrogen fixation. It is also utilized as green manure crop. Ascorbic acid (Vitamin-C) is synthesized in sprouted seeds of mungbean. (R. Kumari & KS Shekhawat, 2012) [29].

Green gram is susceptible to several destructive diseases. Disease losses are responsible as the major biotic restriction to yield (Batzer *et al.*, 2022) [2]. Green gram is being affected by various fungal, bacterial, and viral diseases but, dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. Is considered as the extremely destructive disease in all the mungbean cultivating regions of the country. The disease is very extensive distributed around the Rajasthan state due to favourable environment and affects significant yield losses (P. Kumar & Gaur, 2020) [27]. The most important biotic causes consist of diseases such as yellow mosaic, anthracnose, powdery mildew, Cercospora leaf spot (CLS), dry root rot, halo blight, and tan spot, and insect-pests particularly bruchids, whitefly, thrips, aphids, and pod borers.

Abiotic stresses regarding mungbean production include waterlogging, salinity, heat, and drought stress. Dry root rot [*Macrophomina phaseolina* (Tassi) Goid] is an evolving disease of mungbean. Dry root rot involved 10-44% yield losses in mungbean production in India and according to reports 33-44% yield losses due to *Rhizoctonia* root rot (Nair *et al*., 2019) [38]. The main characteristic symptom of root rot was yellowing of the leaves and within two to three days, these leaves drop off. The plants may be shriveled within a week. On the stem, dark lesions may well be found on the bark at the ground stage. If the plants are removed out from the soil and evaluated the basal stem and main roots, shows root rot symptoms. In advance stage, scattered sclerotial bodies could be found on the altered tissues (M. Kumar *et al*., 2020) [26].

The management of dry root rot is extremely challenging as the pathogen is soil and seed-borne pathogen. The chemical fungicides currently suggested to control these diseases give protection for a limited period. The constant use of conventional fungicides may affect bioaccumulation of the toxic residues anyway giving rise to resistant strains. Increased public concern about pesticide application and the health hazards requires the exploitation of alternate techniques of disease management. Currently study on disease management across the world is about biological management or usage of combined treatment of bioagents, fungicide and biofertilizer. *Trichoderma* species are known antagonists, mostly in the soil and they are engaged in competition, antibiosis and hyperparasitic interactions, which creates them the most successful biocontrol agents even on foliar surfaces (M. Kumar *et al*., 2020) [26]. For the managing of dry root rot disease various approaches such as seed treatment with fungicides and bioagents, soil application of bioagent and fungicides were adopted Fungicides against *Macrophomina phaseolina* have produced satisfactory results, but more fungicide residue builds environmental and human health risks. The significance of biological management methods was reviewed to find a more environmentally safe method (Alyssa Swehla Sumner, 2017) [1]. Taking into consideration the significance of the disease, several management approaches were adopted *viz*., cultural, physical, biological, and chemical control techniques. When the disease appears in serious form, farmers usually use fungicides which do not prove highly effective. Apart from the excessive costs, chemical control may have deleterious effects on the environment and human. Hence, biological management recommends an economic and ecologically viable tactic towards disease management in the current agriculture as it is easier, safer to human beings, environment and nontarget organisms (S. S. and M. R. Khan, 2016) [23].

Materials and Methods

The current research work entitled "Management of dry root rot of mungbean caused by *Macrophomina phaseolina* using bioagents and fungicides" was conducted at Department of Plant Pathology and Agricultural Research Farm, School of Agriculture, Lovely Professional University, Phagwara, Punjab, during 2020-2022.

Pathogen culture

To study *Macrophomina phaseolina* for research, the fungus was brought from ITCC (Indian Type Culture Collection, Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi-110 012). For the final confirmation, microscopy was done to identify fungus from the brought culture. After the identification of the culture, slants were kept under refrigerator at 4 °C for mass culturing and further studies.

Physiological studies

The culture and colony morphology of *Macrophomina phaseolina* on different culture media

For cultural studies total 7 different culture media were used in the research. These 7 media was prepared and sterilised in an autoclave. Sterilized media was poured into 90 mm Petri plates. In each Petri plate 20ml media is poured and 3 replications of each media were made. After solidification of media, Petri plates are aseptically inoculated with *Macrophomina phaseolina* discs of 5mm, 7days old culture of *Macrophomina phaseolina* were used. After that Petri plates are incubated in BOD at 25 °C. Observations were made after 24 hours, 48 hours, and 72 hours. After 7 days. For cultural and morphological characterization colony diameter, colony growth, colony pattern, substrate colour, mycelium colour and structure and size of radial growth and sclerotia. Total 7 treatments were carried out (Table 1) with 3 replications and experimental design is CRD.

Table 1: List of different culture media used

Treatment No.	Treatments
T1	Potato Dextrose Agar
T2	Czapeks Dox Agar
T3	Malt Extract Agar
T4	Rose Bengal Agar
T5	Richards Synthetic Agar
T6	Oat Meal Agar
T7	Corn Meal Agar

Growth of *Macrophomina phaseolina* at different temperatures

To define the temperature which prefers the maximum mycelial growth of the pathogen various levels of temperature such as 20 °C, 25 °C, 30 °C, 35 °C and 40 °C were maintained in BOD incubator.

For each treatment of temperature three plates are inoculated as three replications. Plates were incubated at different temperatures after inoculation of 5mm inoculum disc of *Macrophomina phaseolina.*

Observations on colony diameter in each plate was recorded after 7days of inoculation. The data were analyzed statistically in CRD.

Growth of *Macrophomina phaseolina* at different PH levels

The pH of PDA media was adjusted before autoclaving with the help of HCL and NaOH using the digital pH meter.

After autoclaving the media was poured in the sterilized petri plates of three replications. The plates with pH 4.5, 5.5, 6.5, 7.5, and 8.5 were inoculated with the pathogen *Macrophomina phaseolina.*

Observations on colony diameter in each plate was recorded after 7days of inoculation. The data were analyzed statistically in CRD.

In vitro studies

The antagonist effect of *Trichoderma* spp. against

Macrophomina phaseolina causing dry root rot (_In vitro_)

Three biocontrol agents i.e., *Trichoderma* spp were used in this research (Table 2). All 3 bio agents were tested against the *Macrophomina phaseolina*. For the evaluation of these biocontrol agents, Dual culture technique is used.

PDA media was prepared. PDA media was poured into Petri plates and 5mm disc of biocontrol agent is placed on one side of the Petri plate and 5 mm disc of 7 days old culture of *Macrophomina phaseolina* was placed on the other side of the Petri plate. 3 replications of each biocontrol agent are made. These plates are kept in BOD at 25 °C. Observations of colony diameter is made after 48 hrs, respectively.

Percent inhibition of *Macrophomina phaseolina* was calculated by the formula given by Vincent, 1947.

Percent inhibition = C – T / C x 100

Where

C= Growth of test fungus in untreated control plates

T= Growth of test fungus in treated plates

Table 2: List of Biocontrol agents used

Treatment	Biocontrol agent
T1	*Trichoderma viride*
T2	*Trichoderma harzianum*
T3	*Trichoderma virens*
T4	Control

The effect of fungicides against _Macrophomina phaseolina_ causing dry root rot (_In vitro_)

Efficacy of three fungicides were used in the research (Table 3). These fungicides are used at concentration 100 ppm, 250 ppm, 500 ppm. Each conc was carried out in 3 replications with CRD experimental design. For the evaluation of these fungicides, Poison food technique is used.

Presterilized PDA media was kept in laminar air flow. 60ml PDA media was poured in 1st conical flask of 100 ppm chemical, 60 ml PDA media was poured in 2nd conical flask of 250 ppm chemical and in 3rd conical flask of 500 ppm chemical, 60 ml PDA media is added. Same is done for all the 3 chemicals. There 3 conical flasks of 100 ppm, 250 ppm and 500 ppm are further poured into Petri plates. Each of these is poured into 3 Petri plates and then 5 mm disc of Macrophomina phaseolina was placed in the centre of the Petri plate. Later these Petri plates are kept in BOD a 25 °C. Observations of colony diameter was recorded after 48 hours and respectively.

Percent inhibition of Macrophomina phaseolina was calculated by the formula given by Vincent, 1947. Percent inhibition= C -T/ C x 100

Where, C= Growth of test fungus in untreated control Petri plate T= Growth of test fungus in treated Petri plate.

Table 3: List of fungicides used

Treatment	Fungicide	Concentration
T1	Carbendazim 12% + Mancozeb 63% WP	100 ppm, 250 ppm, 500 ppm
T2	Azoxystrobin 11.4% + Difeconazole 18.2% W/W	100 ppm, 250 ppm, 500 ppm
T3	Hexaconazole 5% SC	100 ppm, 250 ppm, 500 ppm
T4	Control	

***In vivo* studies**

The efficacy of *Trichoderma* spp. and fungicides against _Macrophomina phaseolina_ causing dry root rot under field conditions

Seeds are treatment with 3 biocontrol agents and 3 fungicides (Table 5). The treatment was done as per the recommended dose (Table 4). Each treatment was carried out in replications and RBD experimental design is used.

The treated seeds are sown in the plot of size 3mx6m with spacing 45 cm Row to Row and 15 cm Plant to Plant. The intercultural operations such as irrigation, weeding, fertilizer application was given as per the requirement. Disease observation such as disease incidence and Percent disease index (PDI) was recorded after 30 DAS, 45 DAS and 60 DAS and calculated by the given disease rating scale and formula are as follows:

Disease rating scale

Grade	Disease Incidence	Disease reactions
1	0-10%	No infection on roots
3	10-25%	Very few small lesions on roots
5	25-50%	Lesions on roots clear but small, new roots free from infection
7	50-75%	Lesions on roots many, new roots generally free from lesions
9	>75%	Roots infected and completely discolored

(Nene *et al*, 1981), (Abawi Pastor-Corrales *et al*, 1987) [59]

PDI= Sum of all ratings/No. of ratings x maximum grade x 100

Table 4: Lists of treatments used in the field

Treatment No.	Treatments	Dosage
T1	*Trichoderma viridae*	2g/200 seeds
T2	*Trichoderma harzianum*	2g/200 seeds
T3	*Trichoderma virens*	2g/200 seeds
T4	Carbendazim 12% + Mancozeb 63% WP	0.2g/200g seeds
T5	Azoxystrobin 11.4% + Difeconazole 18.2% W/W	0.2ml/200g seeds
T6	Hexaconazole 5% SC	0.4ml/200g seeds
T7	Control	

Results and Discussion

The present study entitled “Management of dry root rot of mungbean caused by *Macrophomina phaseolina* using bioagents and fungicides” was conducted at Department of Plant Pathology and Agricultural Research Farm, School of Agriculture, Lovely Professional University, Phagwara, Punjab. The results recorded the present research on various aspects of dry root rot of mungbean are presented and discussed here under.

Physiological Studies

Effect of different culture media on mycelial growth of _Macrophomina phaseolina_

Seven different media with three replications for each were tested to check the culture growth and colony morphology of *Macrophomina phaseolina* to identify the best suitable growth medium for sclerotial production. The data presented in Table No. 5.

The results showed that among the seven culture medium tested Potato dextrose agar was found to be most excellent medium for the growth of *Macrophomina phaseolina* which was followed by Malt extract base. The next best suitable

growth medium is Rose Bengal agar base while remaining i.e., Oat meal agar, Czapeks dox agar, Richards synthetic agar and Corn meal agar indicated a significant difference in growth and sclerotial production.

After 7 days of incubation the maximum mycelial growth was recorded on Potato dextrose agar (77.8 mm). This was followed by Malt extract base (75.5 mm) and Rose Bengal agar base (74 mm). The lowest mycelial growth was observed on Oat meal agar, Czapeks dox agar, Richards synthetic agar and Corn meal agar.

The great sclerotial formation of *Macrophomina phaseolina* was found on Potato dextrose agar and Czapeks dox agar medium. Good sclerotial formation was observed on Richards synthetic agar, while moderate sclerotial formation was observed on Rose Bengal agar base.

The colony characteristics of the *Macrophomina phaseolina* differs in all the growing medium i.e., colony growth, colony pattern, colony colour and colony margin. Most common in the colony characteristics i.e., cottony white fluffy growth is found on Potato dextrose agar.

The present investigation correlates with (Sahi *et.al* 1992) [62] who observed that Potato dextrose agar (PDA) was the excellent medium for the growth of *Macrophomina phaseolina* and (Salunke *et al.*, 2009) [46] also found that out of all growth medium *Macrophomina phaseolina* showed maximum growth on PDA.

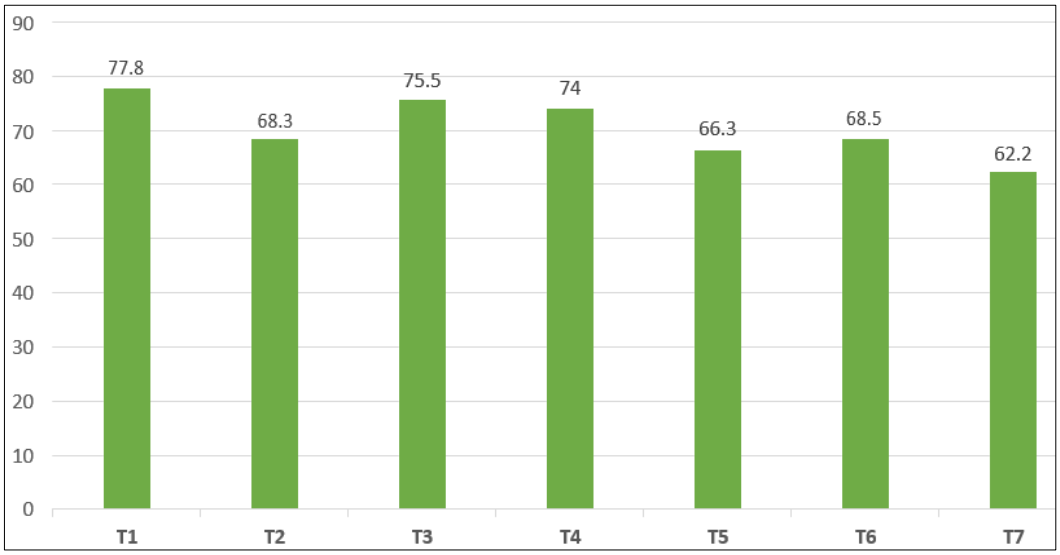
Here
T_1= Potato dextrose agar (At the centre)
T_2 = Czapeks dox agar (Bottom left)
T_3 = Malt extract base (Bottom middle)
T_4 = Rose Bengal agar base (Top left)
T_5 = Richards synthetic agar (Top middle)
T_6 = Oat meal agar (Top right)
T_7 = Corn meal agar (Bottom right)

Fig 1: Growth of *Macrophomina phaseolina* on different media

Table 5: Effect of different culture media on mycelial growth and cultural characteristics of *Macrophomina phaseolina.*

T. No.	Treatment	Colony diameter	Colony growth	Colony pattern	Colony color	Colony margin
T1	Potato dextrose agar	77.8	Fast	Fluffy and abundant	Dirty white	Regular
T2	Czapeks dox agar	68.3	Moderate	Flat and aerial	Light greenish	Irregular
T3	Malt extract base	75.5	Fast	Flat and dense	White	Smooth
T4	Rose Bengal agar base	74	Fast	Flat and light fluffy	Grayish	Regular
T5	Richards synthetic agar	66.3	Moderate	Submerged and Cottony	Light brown	Irregular
T6	Oat meal agar	68.5	Slow	Fluffy and feathery	Dirty white	Rough
T7	Corn meal agar	62.2	Very Slow	Flat and wooly	Light Black	Irregular
	C.D	3.176				
	SE (m)	1.037				
	SE (d)	1.467				
	C.V	2.552				

*= *Mean of three replications*



X axis = Different types of media
Y axis = Colony diameter (mm)

Where

T_1 = Potato dextrose agar T_2 = Czapeks dox agar T_3 = Malt extract base
T_4 = Rose Bengal agar base T_5 = Richards synthetic agar T_6 = Oat meal agar
T_7 = Corn meal agar

Graph 1: Growth of *Macrophomina phaseolina* on different media

Effect of different temperature on mycelial growth of *Macrophomina phaseolina*

The various temperatures levels such as 20 °C, 25 °C, 30 °C, 35 °C and 40 °C with a set of three petri plates have been adjusted in the BOD incubators to test out the best suitable temperature for the growth of *Macrophomina phaseolina*. The observations were recorded after 7 days of incubation. The data presented in Table No. 6.

The results showed that the *Macrophomina phaseolina* grows at all the temperature levels ranged from 20 °C – 40 °C and the data revealed that the fungus grows very well at 30 °C temperature.

Considerably the highest mycelial growth of the fungus *Macrophomina phaseolina* was observed at 30 °C temperature (87.5 mm) which was followed by 25 °C temperature (70.66 mm). The minimum mycelial growth was recorded at 20 °C (68.66 mm) which was followed by 35 °C (67.16) and 400 C (60.5) temperature levels.

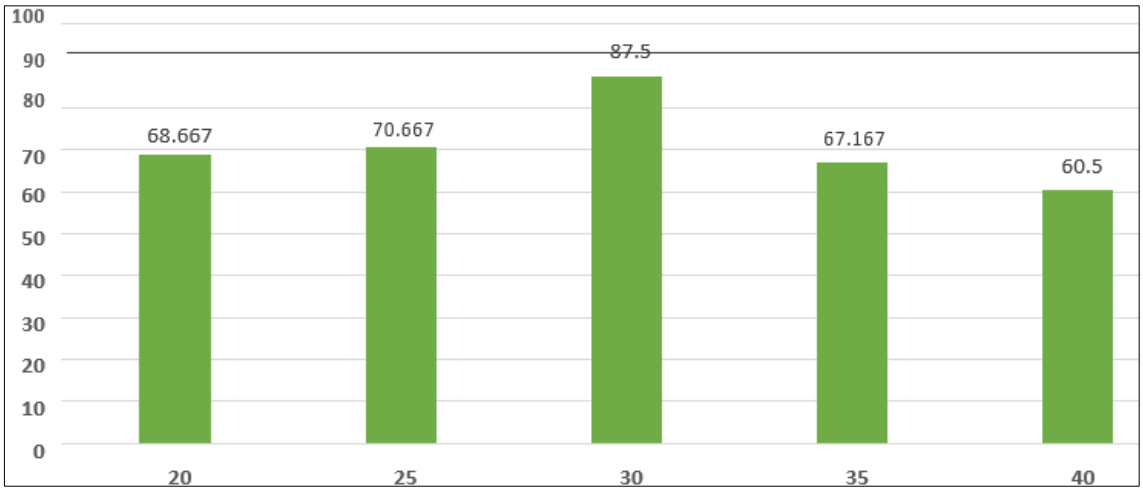
Similar results are in support with (Jha and Sharma, 2005) [63] who reported that optimum temperature required for *Rhizoctonia bataticola* was 30-35 °C for mycelial growth and (Sharma *et.al* 2004) [64] also found that high temperature ranges from 25-30 °C was favored the growth of *Macrophomina phaseolina*.

Here, 20 °C (Left 1st) 25 °C (Left 2nd) 30 °C (Center) 35 °C (Right 2nd) 40 °C (Right 1st)

Fig 2: Growth of *Macrophomina phaseolina* on different temperature

Table 6: Effect of different temperature on mycelial growth of *Macrophomina phaseolina*

Temperature (°C)	Mycelial growth (mm)			
	R1	R2	R3	Mean
20 °C	69	68	69	68.66
25 °C	71.5	70	70.5	70.66
30o °C	88.5	88	86	87.5
35 °C	66.5	67	68	67.16
40 °C	60	59	62.5	60.5
C.D				2.101
SE (m)				0.658
SE (d)				0.931
C.V				1.608



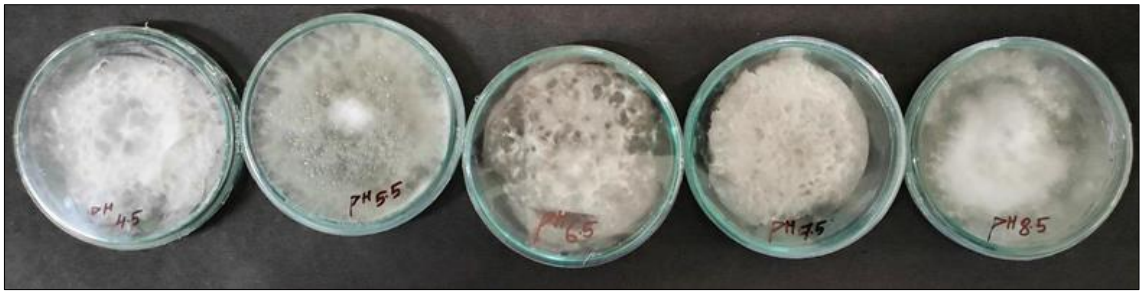
X axis: Different temperatures, Y axis: Mycelial growth (mm)

Graph 2: Growth of *Macrophomina phaseolina* on different temperatures

Effect of different pH levels on mycelial growth of *Macrophomina phaseolina*.

To understand the impact of PH on the growth *Macrophomina phaseolina*, the different PH levels such as pH 4.5, 5.5, 6.5, 7.5 and pH 8.5 with a set of three petri plates were adjusted with the help of PH meter. The observations were recorded after 7 days of incubation. The data presented in Table No. 7.

The results showed that the fungus *Macrophomina phaseolina* grows at all the PH levels ranged from 4.5 – 8.5 pH and the data revealed that the fungus grows very well at 5.5 pH level. Significantly the maximum mycelial growth of the fungus *Macrophomina phaseolina* was observed at PH 5.5 (87 mm) which was followed by pH 6.5 (75.5 mm). The lowest mycelial growth was recorded at pH 4.5 (71.16 mm) which was followed by pH 7.5 (67.5) and pH (52.33 mm) pH levels. The present findings are in support of (Khan *et.al*, 2012) [65] reported that maximum growth of *Macrophomina phaseolina* was found at pH 5.5 and also found that pH 6.0 is also good pH.

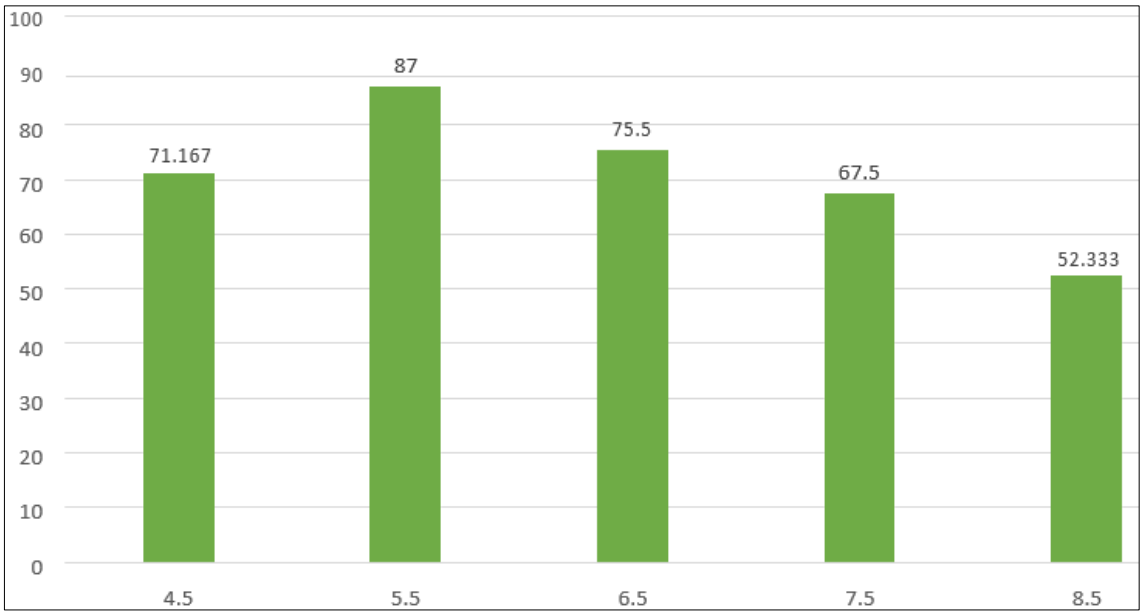


Here, pH 4.5 (left 1st) pH 5.5 (left 2nd) pH 6.5 (center) pH 7.5 (right 2nd) pH 8.5 (right 1st)

Graph 2: Growth of *Macrophomina phaseolina* on different pH

Table 7: Effect of different pH levels on mycelial growth of *Macrophomina phaseolina*.

pH level	Mycelial growth (mm)			
	R1	R2	R3	Mean
4.5	72.5	71	70	71.16
5.5	89.5	86	85.5	87
6.5	75.5	76.5	74.5	75.5
7.5	67.5	68	67	67.5
8.5	52	53	52	52.33
C.D				2.319
SE (m)				0.726
SE (d)				1.027
C.V				1.78



X axis: Different pH levels, **Y axis:** Mycelial growth (mm)

Graph 3: Growth of *Macrophomina phaseolina* on different Ph

Efficacy of *Trichoderma* spp. against *Macrophomina phaseolina* by Dual culture technique

Effect of three fungal biocontrol agents *viz*, *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma virens* were studied under *invitro* conditions for their antagonism against *Macrophomina phaseolina* by dual culture technique.

All the three antagonists significantly improved in inhibiting the mycelial growth of *Macrophomina phaseolina* over control. *Trichoderma virens* was observed highly effective in inhibiting the mycelial growth of *Macrophomina phaseolina* followed by *Trichoderma harzianum*.

The results presented in table 4.4 indicated that minimum mycelial growth (36.8 mm) and maximum growth inhibition (53.96%) was observed in *Trichoderma virens* which was followed by *Trichoderma harzianum* with mycelial growth (46.16mm) and growth inhibition (42.3%) and *Trichoderma viridae* shown highest mycelial growth (56.16mm) and lowest growth inhibition (27.3%).

Similar results are in support of (Lokesh & Benagi, 2007) [32] demonstrated the effectiveness of bioagents against *Macrophomina phaseolina* (Tassi) Goid. causing dry root rot of pigeon pea have been studied. In dual culture technique both *Trichoderma virens* and significantly reduced the mycelial growth of *Macrophomina phaseolina* by 78.22 percent.

T2 - *Trichoderma harzianum*

T3 - *Trichoderma virens*

Efficacy of *Trichoderma* spp. against *Macrophomina phaseolina* by Dual culture technique

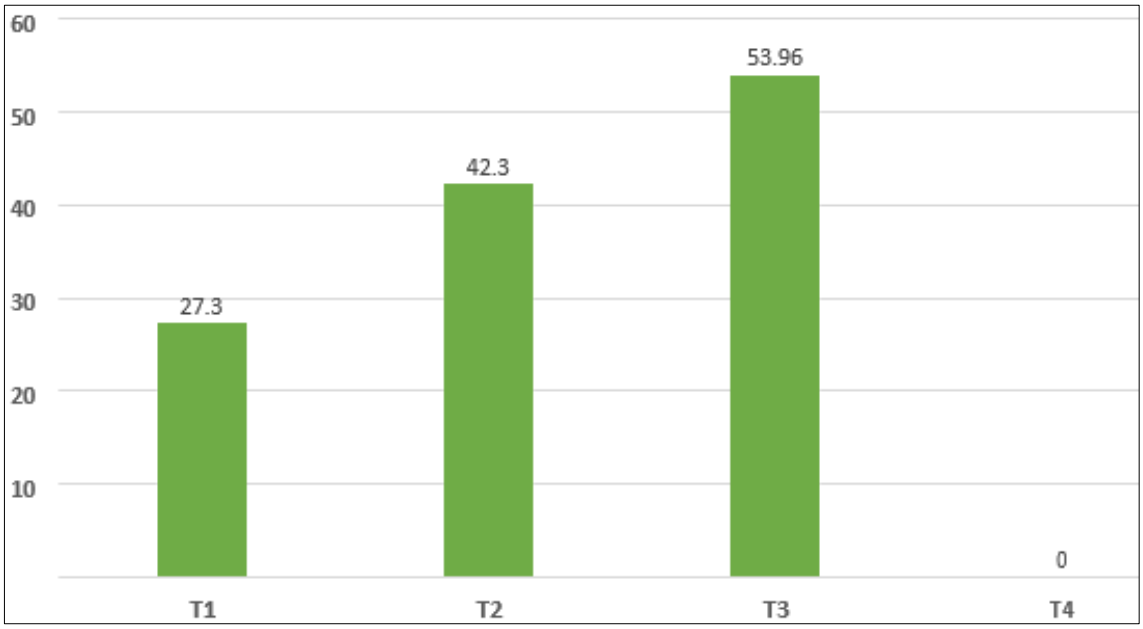
T1 - *Trichoderma viride*

T4 – *Macrophomina phaseolina* (Control)

Table 8: Effect of *Trichoderma* spp. against *Macrophomina phaseolina* by dual culture technique after 7 days of incubation at 30 + 1 °C

Treatment	Bio agent	Mycelial growth (mm)*	Percent Inhibition (%)
T_1	*Trichoderma viride*	58.16	27.3
T_2	*Trichoderma harzianum*	46.16	42.3
T_3	*Trichoderma virens*	36.83	53.96
T_4	Control	80	0
	C.D	1.265	
	SE (m)	0.382	
	SE (d)	0.540	
	C.V	1.196	

*= Mean of three replications



X axis: *Trichoderma* spp. Y axis: Percent Inhibition, Where,
T_1= Trichoderma viride
T_2= Trichoderma harzianum
T_3= Trichoderma virens
T_4= Control

Graph 4: Efficacy of *Trichoderma* spp. against *Macrophomina phaseolina* by Dual culture technique

4.2.5 Efficacy of fungicides against *Macrophomina phaseolina* by Poison food technique

The efficacy of three different fungicides i.e., Carbendazim 12% + Mancozeb 63% WP, Azoxystrobin 11.4% + Difeconazole 18.2% W/W and Hexaconazole 5% SC at three different concentrations i.e., 100 ppm, 250 ppm and 500 ppm were evaluated under *invitro* condition by poison food technique against *Macrophomina phaseolina*.

All three fungicides found most effective in inhibiting the mycelial growth of *Macrophomina phaseolina* and showed significantly superior in growth inhibition over control. Among them Carbendazim 12% + Mancozeb 63% WP (SAAF) was observed highly effective in inhibiting the mycelial growth of *Macrophomina phaseolina* and showed complete (100%) mycelial growth inhibition at 250 and 500 ppm concentrations, respectively.

The results presented in Table No. 8 indicated that lowest mycelial growth and highest growth inhibition of *Macrophomina phaseolina* over control was observed with Hexaconazole 5% SC at 500 ppm (25.3 mm and 68.33%) and Carbendazim 12% + Mancozeb 63% WP at 100 ppm (26.5 mm and 66.87%) which was followed by Azoxystrobin 11.4% + Difeconazole 18.2% W/W at 500 ppm (27 mm and 66.25%), Hexaconazole 5% SC at 250 ppm (31.5 mm and 60.62%), Azoxystrobin 11.4% + Difeconazole 18.2% W/W at 250 ppm (39.66 mm and 50.42%) respectively. Hexaconazole 5% SC and Azoxystrobin 11.4% + Difeconazole 18.2% W/W at 100 ppm was found least effective with mycelial growth (52 mm and 56.33 mm) and growth inhibition (35% and 29.58%) respectively.

Similar findings are in support with **(**Maruti1, Savitha, A. S.1, 2017) [36] informed that carbendazim 12% + mancozeb 63% WP showed cent percent (100%) inhibition and (HV Parmar, 2017) reported that carbendazim observed greatest with 95.23% mycelial growth inhibition. (Lokesh *et al.*, 2020) [31] proved that between systemic fungicides, significantly maximum average mycelial growth inhibition was observed with carbendazim (85.88%), followed by hexaconazole (75.29%) Among combined fungicides, considerably maximum percent growth inhibition over control was noted (88.24%) in carbendazim 12% + mancozeb 63%.

Efficacy of fungicides against *Macrophomina phaseolina* by Poison food technique

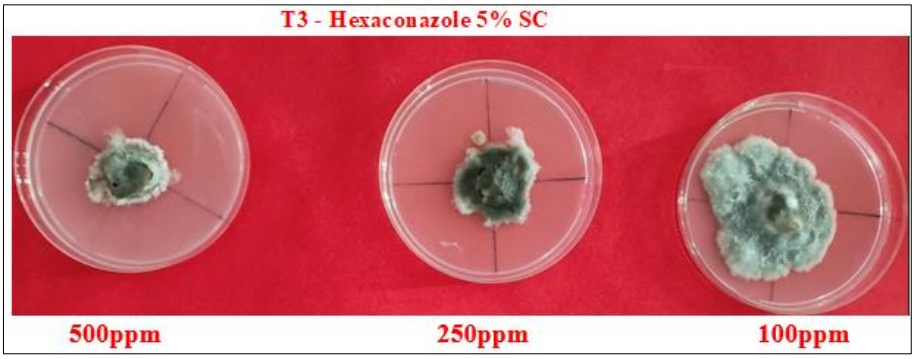
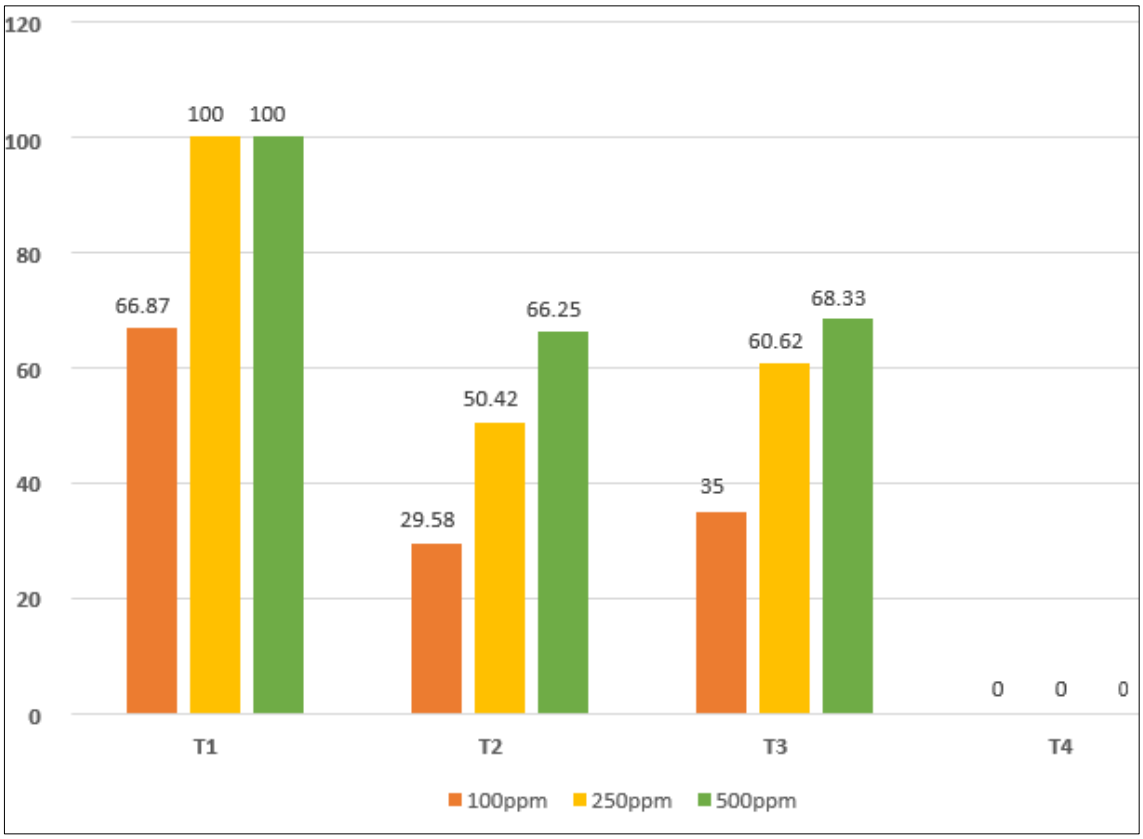


Table 9: Effect of fungicides against *Macrophomina phaseolina* by poisoned food technique after 7 days of incubation at 30± 1 °C

T. No.	Fungicides	Mycelial growth (mm)*				Percent Inhibition (%)			
		100 ppm	250 ppm	500 ppm	Mean	100 ppm	250 ppm	500 ppm	Mean
T1	Carbendazim 12% + Mancozeb 63% WP	26.5	0	0	8.83	66.87	100	100	88.95
T2	Azoxystrobin 11.4% + Difeconazole 18.2% W/W	56.33	39.66	27	40.99	29.58	50.42	66.25	48.75
T3	Hexaconazole 5% SC	52	31.5	25.3	36.27	35	60.62	68.33	54.65
T4	Control	80	80	80	80	0	0	0	0
	C.D	1.872	1.679	1.932					
	SE(m)	0.565	0.507	0.583					
	SE(d)	0.799	0.717	0.825					
	C.V	1.823	2.323	3.054					

*= Mean of three replications



X axis: Fungicides (Different concentrations)
Y axis: Percent Inhibition

Where,
T_1= Carbendazim 12% + Mancozeb 63% WP
T_2= Azoxystrobin 11.4% + Difeconazole 18.2% W/W
T_3= Hexaconazole 5% SC
T_4= Control

Graph 5: Efficacy of fungicides against *Macrophomina phaseolina* by Poison food technique

***In-vivo* evaluation of *Trichoderma* spp. and fungicides against dry root rot caused by M*acrophomina phaseolina* by seed treatment and soil application**

Three fungal biocontrol agents *viz*, *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma virens* and three fungicides i.e., Carbendazim 12% + Mancozeb 63% WP, Azoxystrobin 11.4% + Difeconazole 18.2% W/W and Hexaconazole 5% SC were evaluated under field conditions against dry root rot caused by *Macrophomina phaseolina* by seed treatment, soil application and foliar spray.

All the treatments done with bioagents (seed treatment and soil application) and fungicides (seed treatment and foliar spray) significantly controlled the dry root rot disease incidence of mungbean over control. Treatment (T2) *Trichoderma harzianum* was found effective in managing the disease incidence followed by treatment (T4) Carbendazim 12% + Mancozeb 63% WP (SAAF) at 30 DAS.

The results presented in Table No. 9 indicated that minimum disease incidence (15.55%, 22.95%, 33.32%) and maximum disease control (61.11%, 61.74%, 61.55%) was recorded at 30, 45 and 60 DAS in treatment (T2)i.e., *Trichoderma harzianum* done with seed treatment and soil application which was followed by treatment (T4) i.e., Carbendazim 12% + Mancozeb 63% WP (SAAF) done with seed treatment and foliar spray recorded (19.99%, 30.36%, 39.99) disease incidence and (50.01%, 49.39%, 53.85%) disease control at 30, 45 and 60DAS, respectively.

Other effective treatment in managing the dry root disease incidence were observed in treatment (T3) i.e., *Trichoderma virens* with 24.44% disease incidence and 38.88% disease control and treatment (T6) i.e., Hexaconazole 5% SC with 28.88% disease incidence and 27.7% disease control at 30 DAS which was followed by treatment (T1) i.e., *Trichoderma viride* with 33.32% disease incidence and 16.67% disease control and treatment (T5) with 37.03% disease incidence and 7.40% disease control at 30 DAS, respectively. Control was recorded highest percent disease incidence (39.99%, 59.99%, 86.66%) at 30, 45 and 60DAS over all the treatments.

Similar results are in support of (Hyder *et al.*, 2022) [11] reported that with all the tested fungal isolates, *Trichoderma harzianum* isolate showed improved effectiveness as biocontrol agent, (R. Lokesh, Y.B. Madagoudra, 2021) [40] showed that the seed treatment with *Trichoderma harzianum* @ 10g/kg seed reveals 100% disease reduction and 75 (Deepa, Sunkad *et al.*, 2018) [5] reported that the maximum decrease in dry root rot (77.60%) disease incidence was seen in the seed treatment with mancozeb 50% + carbendazim 25% WS @ 3.5 g/kg followed up by soil application (B. B. Thombre & Kohire, 2018) [57-58] demonstrated that the fungicide treatment of Carbendazim 12 WP + Mancozeb 63 WP (@ 0.2) recorded minimum average disease incidence.

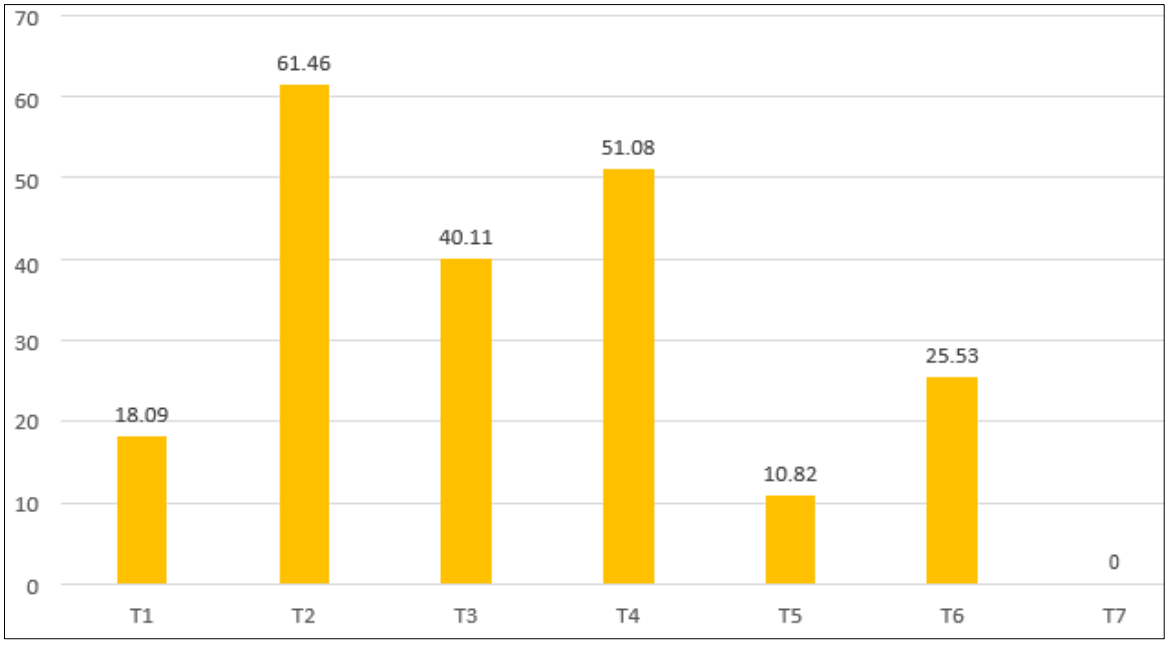
Treated Plot

Control Plot

Table 10: *In-vivo* evaluation of *Trichoderma* spp. and fungicides against dry root rot caused by M*acrophomina phaseolina* by seed treatment and soil application

T. No.	Treatment	PDI (%)				Percent Inhibition (%)			
		30DAS*	45DAS*	60DAS*	Mean	30DAS	45DAS	60DAS	Mean
T_1	*Trichoderma viride*	33.32	46.66	73.32	51.10	16.67	22.22	15.39	18.09
T_2	*Trichoderma harzianum*	15.55	22.95	33.32	23.94	61.11	61.74	61.55	61.46
T_3	*Trichoderma virens*	24.44	37.77	48.14	36.78	38.88	37.03	44.44	40.11
T_4	Carbendazim 12% + Mancozeb 63% WP	19.99	30.36	39.99	30.11	50.01	49.39	53.85	51.08
T_5	Azoxystrobin 11.4% + Difeconazole 18.2% W/W	37.03	51.10	77.77	55.30	7.40	14.81	10.25	10.82
T_6	Hexaconazole 5% SC	28.88	42.96	68.88	46.90	27.7	28.38	20.51	25.53
T_7	Control	39.99	59.99	86.66	62.21	0	0	0	0
	C.D	1.629	2.665						
	S.E(m)	0.523	0.885						
	S.E(d)	0.739	1.12						
	C.V	3.182	3.554						

*= Mean of three replications



X axis: Different treatments Y axis: Percent Inhibition
Where
T1= *Trichoderma viridae* T2= *Trichoderma harzianum* T3= *Trichoderma virens*
T4= Carbendazim 12% + Mancozeb 63% WP T5= Azoxystrobin 11.4% + Difeconazole 18.2% W/W,
T6= Hexaconazole 5% SC, T7= Control

Graph 6: Efficacy of *Trichoderma* spp. and fungicides

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