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RESEARCH PAPER

Bioefficacy of fungicides and bioagents against *Macrophomina phaseolina* causing charcoal rot in maize

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Abstract : In recent years *Macrophomina phaseolina* causing charcoal rot of maize is more problematic in maize growing parts of Maharashtra. Present investigation was taken on evaluation of fungicides and bio-agents against *M. phaseolina* under laboratory condition and pot culture. Under laboratory condition, nine fungicides and six bio- agents were evaluated against *M. phaseolina* by poison food technique and dual culture method, respectively. Among fungicides Carbendazim 63 % + Mancozeb 12% and Carbendazim alone recorded maximum inhibition of (100 %) mycelial growth. Among the bio-agents tested *Trichoderma harzianum* was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (63.33 %) of *M. phaseolina.* Under pot culture study, as soil application and seed treatment, among the fungicides, carbendazim + Mancozeb was found most effective. However, among bioagents *Trichoderma harzianum* was remarkably manage the charcoal rot.

Key Words : Maize, Bio agent, Fungicide, Charcoal rot, *Macrophomina phaseolina*

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro climatic conditions. Globally maize is known as "Queen of Cereals" because it has the highest genetic yield potential among the cereals.

In India maize is the third most important food crops after rice and wheat and also it is referred as corn in North America originated in Central Mexico. It is a superior cereal crop regarding the total production globally and productivity.

Maize is one of the world's important food crop containing starch (71-72%), protein (9-10 %), fat (4-45 %), fibre (9-10 %), sugar (2-3 %) and minerals or ash (1.4 %) on dry matter basis.

Maize has been cultivated in an area of 117 M ha^{-1} with production of 967 MT and productivity of 5.5 MT ha^{-1}. In India it has been grown in an area of 86.73 lakh ha with production of 222.5 lakh tones and average productivity of 2566 kg ha^{-1} (Anonymous, 2019).

Maize has a significant potential for doubling farmer's income as it generates better income and provides gainful employment. However, productivity of

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crop is adversely affecting by ubiquitous incidence of diseases.

It has been estimated that about 13.2 per cent of the economic production of maize is lost annually due to diseases in India (Dhillon and Prasanna, 2001).

Charcoal rot, popularly known as Post flowering stalk rots is the most serious, destructive and widespread disease in maize. Most of the commercially grown cultivars have shown a high level of disease incidence during grain filling stage.

The major pathogen responsible for the disease is *Macrophomina phaseolina* (Murali *et al.*, 2013). The extent of loss in grain yield ranged from 25-32.2 per cent and along with decrease in fodder quality (Mukesh Kumar *et al.*, 1996).

Hence present investigation was undertaken on evaluation of fungicides and bio-agents were against charcoal rot disease to find out the efficient fungicides and bio-agents for the better management of charcoal rot of maize disease**.**

MATERIAL AND METHODS

Collection, isolation and proving pathogenicity of *M. phaseolina:*

Charcoal rot infected samples were collected from AICRP on Maize, MPKV, Rahuri and farmers field and pathogen was isolated by standard tissue isolation method described by Ashraf *et al*. (2015). Pathogen was transferred to PDA slants and stored at 4±1 °C for further studies. Isolated pathogen was proved the pathogenicity by applying Koch's postulates on susceptible maize cv. G25.

***In vitro* evaluation of fungicides:**

Efficacy of nine fungicides was evaluated (at half of concentration than recommended and at recommended concentration) *in vitro* against *M. phaseolina*, by poisoned food technique (Nene and Thapliyal, 1993), using PDA as basal culture medium. Based on active ingredient, the requisite quantity of each test fungicide was calculated and mixed thoroughly with autoclaved and cooled (40^0C) Potato Dextrose Agar medium (PDA) separately in conical flasks to obtain desired concentrations of fungicides. Fungicide amended PDA medium was then poured (20 ml/plate) aseptically in Petri plates (90 mm dia.) and allowed to solidify at room temperature.

For each test fungicide and its test concentration, three plates / treatment / replication were maintained and replicated thrice. After solidification of the medium, all the plates were inoculated aseptically with a 5 mm culture disc obtained from a week old actively growing pure culture of *M. phaseolina*. The culture disc was placed on PDA in inverted position in the center of the Petri plate and plates were incubated at 28 ± 2^0C. Petri plates filled with plain PDA (without any fungicide) and inoculated with the culture disc of the test pathogen were maintained as control (untreated).

Observations on radial mycelial growth/colony diameter of the pathogen were recorded at 24 hrs. interval and continued till the untreated control plate was fully covered with mycelial growth of the test pathogen. Per cent mycelial growth inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying the following formula (Vincent, 1927).

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

where,

C = Growth (mm) of test fungus in untreated control plate

T = Growth (mm) of test fungus in treated plates

***In vitro* evaluation of bioagents:**

Four fungal antagonists *viz*., *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T koningii* and two bacterial antagonists *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* against *M. phaseolina*, applying dual culture technique (Dennis and Webster, 1971).

Seven days old culture of the test bio-agents and the test pathogen (*M. phaseolina*) grown on agar media were used for the study. The culture discs (5 mm) of the test pathogen and bio-agent were cut out with sterilized cork borer, from a weak old culture. Then two culture discs, one each of the test pathogen and bio-agent were placed aseptically at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates and plates were incubated at 28 + 2°C. Three plates/ treatment/ replication were maintained. PDA plates inoculated only with culture disc of test pathogen were maintained as untreated control.

Observations on linear mycelial growth of the test pathogen and bio-agent were recorded at an interval of 24 hours and continued till untreated control plate was fully covered with mycelial growth of the test pathogen.

Per cent inhibition of the test pathogen over untreated control was calculated by applying the following formula (Arora and Upadhyay, 1978).

$$\text{Per cent growth inhibition (I)} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Evaluation of fungicides and bioagents in pot culture:

A pot culture experiment on effect of fungicides and biocontrol agents on *M. phaseolina* was conducted under glass house condition by using variety G-25 susceptible to charcoal rot.

The inoculum of the test pathogen *M. phaseolina* was mass cultured on crushed cotton seeds and added to soil @ 100 g per kg of soil. Prior to use, plastic pots were disinfected 5 per cent copper sulphate. The *Macrophomina* culture was also added to the sterilized soil for fungicide trial. The seeds of G-25 were sown in the pots containing *Macrophomina* sick soil. In this trial, the seeds were treated with carbendazim + mancozeb, carbendazim, mancozeb, thiram (dry seed treatment) and *T. harzianum* and *T. viride* (wet seed treatment). The treated seed were sown in pots containing *Macrophomina* sick soil. Six seeds were sown in each pot. The pots were watered lightly and kept in glass house. The observations of the rot were recorded at 30, 45, 60 and 90 days after sowing.

Results and Discussion

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Collection, isolation and proving pathogenicity of *M. phaseolina:*

Applying tissue isolation technique, the test pathogen was isolated aseptically from the naturally charcoal rot affected maize stem basal portion on Potato dextrose agar (PDA) medium. After 2-3 days of incubation, black mycelial mat was developed on the PDA plates and after 7-8 days of incubation microsclerotia were developed in the plates. Test pathogen was aseptically sub-cultured purified and maintained on agar slant tube in refrigerator for further studies.

Pathogenicity of *M. phaseolina* was proved by soil inoculation (sick soil) with pure culture of *M. phaseolina* on charcoal rot susceptible maize cv. G 25, in pot under screen house conditions.

From these, artificially diseased maize basal portion, the pathogen was re-isolated and incubated at 28 ± 2^{0}C. After 2-3 days of incubation, dark colored mycelial mat developed and about 7-8 days of incubation black colored microsclerotia were developed. Morphological and cultural characteristics were similar to that of the original test pathogens culture obtained from naturally diseased maize samples.

***In vitro* evaluation of fungicides:**

All the nine fungicides (systemic, non-systemic and combi) at different conc. (at half of concentration than recommended and at recommended concentration) evaluated *in vitro* against M. *phaseolina* exhibited a wide range of mycelial growth and inhibition of the test pathogen. The results obtained are presented in Table 1.

Mycelial growth:

A total of nine fungicides evaluated in vitro against *M. phaseolina* exhibited a wide range of mycelial growth and inhibition of the test pathogen.

Results (Table 1 and Fig. 1) revealed, that at half of concentration than recommended, radial mycelial growth of the test pathogen ranged from 00.00 mm (carbendazim + mancozeb) to 88.66 mm (copper hydroxide). However, significantly least mycelial growth was recorded with the fungicide carbendazim + mancozeb (00.00 mm). It was followed by fungicides *viz*., carbendazim (9.33 mm), thiram (11.33 mm), captan (32.00 mm), mancozeb (41.33 mm), copper oxychloride (76.33) thiophanate methyl (86.66 mm). Fungicides propineb and copper hydroxide

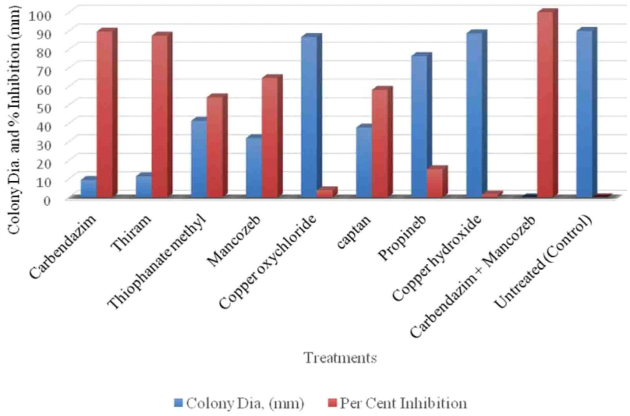

Fig. 1 : Efficacy of different fungicides against *M. phaseolina* under *in vitro* condition at lower concentration

were found comparatively less effective with maximum mycelial growth of 87.66 mm and 88.66 mm, respectively (Plate 1).

At recommended concentration, results showed that (Table 1 and Fig. 1), radial mycelial growth of the test pathogen ranged from 00.00 mm (carbendazim + mancozeb) to 88.00 mm (copper hydroxide) as against 90.00 mm inn untreated control. However, in treatment of fungicides, carbendazim + mancozeb and carbendazim there was no any fungus growth observed. This was followed by fungicides thiram (11.00 mm), captan (13.66 mm), propineb (34.66 mm), mancozeb (40.00 mm) and copper oxychloride (70.00mm). Fungicides thiophanate methyl (82.66 mm) and copper hydroxide (88.00 mm) showed comparatively maximum mycelial growth (Plate 2).

Mycelial growth inhibition:

Result (Table 1 and Fig. 1) revealed that all the 9 fungicides tested significantly inhibited mycelial growth *of M. phaseolina*, over untreated control.

At half of concentration than recommended, per cent mycelial growth inhibition of the test pathogen ranged from 1.48 per cent (copper hydroxide) to 100 per cent (carbendazim + mancozeb). However, significantly higher mycelial growth inhibition was recorded with carbendazim + mancozeb (100 %). The second and third best fungicides found were carbendazim (89.63 %), thiram (87.41 %), respectively. This was followed by fungicides viz., mancozeb (64.44 %), captan (58.15 %), thiophanate methyl (54.07 %), propineb (15.18 %), copper oxychloride (3.71 %) and copper hydroxide (1.48 %).

At recommended concentration (Table 1 and Fig. 1), mycelial growth inhibition was more as compared to half concentration than the recommended and it was ranged from 2.22 per cent (copper hydroxide) to 100 per cent (carbendazim + mancozeb and carbendazim alone). However, cent per cent inhibition was recorded with fungicides carbendazim + mancozeb and carbendazim. It was followed by fungicides thiram (87.77 %), mancozeb (84.82 %), captan (61.48 %), thiophanate methyl (55.55 %), propineb (22.22 %), copper oxychloride (8.15 %) and copper hydroxide (2.22 %).

Thus, all the fungicides tested were found fungistatic

Table 1 : ***In vitro*** **efficacy of different fungicides against mycelial growth and inhibition of** ***M. phaseolina***

Tr. No.	Treatments	Concentration (ppm)	Mean Col. Dia. (mm) after 7 DAI*	Mean inhibition zone (mm)	Percent inhibition over control
T_1	Carbendazim	500	9.33	80.67	89.63 (71.21)
		1000	00.00	90.00	100.00 (90.00)
T_2	Thiram	1000	11.33	78.67	87.41 (69.21)
		2000	11.00	79.00	87.77 (69.53)
T_3	Thiophanate methyl	500	41.33	48.67	54.07 (47.33)
		1000	40.00	50.00	55.55 (48.18)
T_4	Mancozeb	1250	32.00	58.00	64.44 (53.39)
		2500	13.66	76.34	84.82 (67.06)
T_5	Copper oxychloride	1500	86.66	3.34	3.71 (11.10)
		3000	82.66	7.34	8.15 (16.58)
T_6	Captan	1250	37.66	52.34	58.15 (49.69)
		2500	34.66	55.34	61.48 (51.63)
T_7	Propineb	1250	76.33	13.67	15.18 (22.93)
		2500	70.00	20.00	22.22 (28.12)
T_8	Copper hydroxide	1250	88.66	1.34	1.48 (6.98)
		2500	88.00	2.00	2.22 (8.56)
T_9	Carbendazim + Mancozeb	1000	00.00	90.00	100.00 (90.00)
		2000	00.00	90.00	100.00 (90.00)
T_{10}	Control	-	90.00	00.00	00.00 (00.00)
			90.00	00.00	00.00 (00.00)
	S.E.±		0.55		
			0.98		
	C.D. (P=0.05)		1.65		
			2.93		

*Average of three replications, DAI= Days After Inoculation, Col. = Colony, Dia. = Diameter, Conc. = Concentration, Av. =Average, Figures in parenthesis are arc sine transformed value

T_1:Carbendazim T_2: Thiram T_3: Thiophanate methyl
T_6: Captan T_7: Propineb T_8: Copper hydroxide

Plate 1 : A) Efficacy of different fungicides against *M. phaseolina* under *in vitro* condition at half of concentration than recommended

T_4: Mancozeb T_5: Copper oxychloride
T_9: Carbendazim + Mancozeb T_{10}: Untreated (Control)

Plate 2 : Efficacy of different fungicides against *M. phaseolina* under *in vitro* condition at recommended concentration

against *M. phaseolina* and significantly inhibited its mycelial growth over untreated control. However, fungicides found most effective in the order of merit were carbendazim 12 % + mancozeb 63 %, carbendazim, thiram, mancozeb, thiophanate methyl, propineb, copper oxychloride and copper hydroxide.

The results are in conformity with Chaudhary *et al*. (2017) and Khan and Sahi (2020).

In vitro evaluation of bioagents:

Results obtained on mycelial growth and inhibition of *M. phaseolina* with four fungal and two bacterial antagonists are presented in Table 2 and Fig. 2.

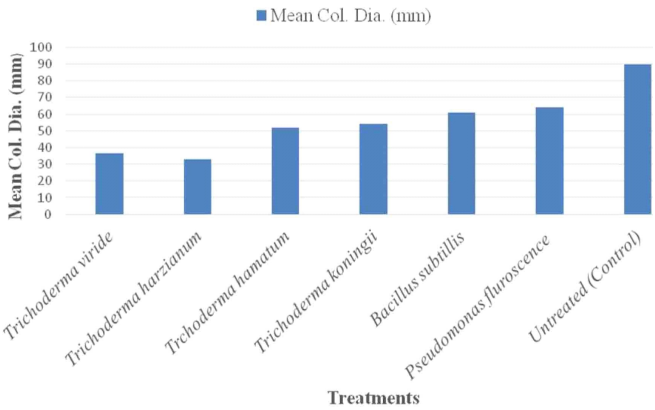

Fig. 2 : Mean colony diameter of *M. phaseolina* against bioagents

Table 2 : Evaluation of bioagents against *M. phaseolina* under *in vitro* condition

Tr. No.	Bio agent	Mean colony diameter in mm (DAI*)	Percent inhibition over control
T_1	*Trichoderma viride*	36.66	59.26 (50.33)
T_2	*Trichoderma harzianum*	33.00	63.33 (52.73)
T_3	*Trichoderma hamatum*	52.00	42.22 (40.52)
T_4	*Trichoderma koningi*	54.00	40.00 (39.23)
T_5	*Bacillus subtilis*	61.00	32.22 (34.58)
T_6	*Pseudomonas fluorescens*	64.00	28.88 (32.50)
T_7	Control	90.00	00.00 (00.00)
	S.E.±	0.59	
	C.D. (P=0.05)	1.81	

The mycelial growth of the test pathogen recorded at 7 days of incubation and per cent inhibition of mycelial growth of pathogen over control was calculated.

Radial mycelial growth:

At 7 days after incubation, mycelial growth of the test pathogen was ranged from 33.00 to 64.00 mm. Significantly least growth was recorded *with T. harzianum* (33.00 mm). This was followed by *Trichoderma viride* (36.66 mm), *T. hamatum* (52.00 mm), *T. koningii* (54.00 mm), *Bacillus subtilis* (61.00 mm), *Pseudomonas fluorescens* (64.00 mm), as compared to 90.00 mm growth in untreated control (Plate 3).

T_1:*Trichoderma viride*
T_2: *T. harzianum*
T_3: *T. hamatum*
T_4: *T. koningii*
T_5: *Bacillus subtilis*
T_6: *Pseudomonas fluorescens*
T_7: Untreated (Control)

Plate 3 : *In vitro* efficacy of the bioagents against mycelial growth and inhibition of *M. phaseolina*

Mycelial growth inhibition:

At 7 days after incubation, the per cent inhibition of the test pathogen with the bioagents tested was ranged from 28.88 to 63.33 per cent. However, significantly highest inhibition was recorded with *T. harzianum* (63.33 %). This was followed by *Trichoderma viride* (59.26 %), *T. hamatum* (42.22 %), *T. koningii* (40.00 %), *Bacillus subtilis* (32.22 %) and *Pseudomonas fluorescens* (28.88 %).

The average per cent inhibition of the test pathogen over control with all the treatments was ranged from *T. harzianum* (63.33 %) to *Pseudomonas fluorescens* (28.88 %) as against 00.00 % in untreated control. Significantly highest average per cent inhibition of the test pathogen was recorded with *T. harzianum* (63.33 %). This was followed by *T. viride* (59.26 %), *T. hamatum* (42.22 %) and *T. koningii* (40.00 %), *Bacillus subtilis* (32.22 %) and Pseudomonas fluorescens (28.88 %). Similar studies were done earlier by Gowdra *et al.* (2012) and Meena and Pandey (2015).

Evaluation of fungicides and bioagents against *M. phaseolina* in pot culture:

The results in respect of effect of fungicides and bioagents on charcoal rot of maize under glass house condition are presented in Table 3 and depicted Fig. 3. It is revealed that carbendazim + mancozeb and carbendazim alone effectively controlled the charcoal rot of maize by 87.67 and 85.00 per cent, respectively, followed by thiram (79.34 %) and mancozeb (76.67 %). Mancozeb seems to be less effective compared to other treatments. The charcoal rot incidence was 100 % in untreated control.

Among the bioagents, *Trichoderma harzianum* controlled the maize charcoal rot by 46.67 per cent as against 100 per cent disease incidence in untreated

Table 3 : Efficacy of fungicide and biocontrol agents against *Macrophomina phaseolina* in pot culture

Tr. No.	Treatment Name	Mean per cent charcoal rot*	Per cent charcoal rot control
T_1	Carbendazim + Mancozeb	12.33 (20.54)	87.67
T_2	Carbendazim	15.00 (22.77)	85.00
T_3	Thiram	20.66 (27.01)	79.34
T_4	Mancozeb	23.33 (28.86)	76.67
T_5	*Trichoderma harzianum*	53.33 (46.66)	46.67
T_6	*Trichoderma viride*	58.00 (49.58)	42.00
T_7	Control	100 (90.00)	00.00
	S.E.±	0.66	
	C.D. (P=0.05)	2.04	

(*) = Average of three replications
Figures in parenthesis are arcsine transformed values.

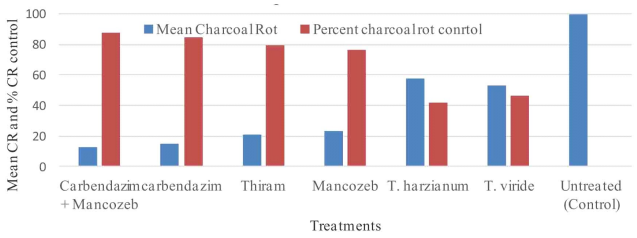

Fig. 3 : Evaluation of fungicides and bioagents against *M. phaseolina* in pot culture

control. *Trichoderma viride* was less effective as compared to *T. harzianum* which shows 42.00 per cent control of charcoal rot.

This clearly indicated that fungicides carbendazim + mancozeb and carbendazim alone were effective in controlling charcoal rot of maize in glass house condition.

Conclusion:

The importance of chemicals cannot be denied in disease management. In lieu of this, the efficacy of nine fungicides (Table 1) was tested *in vitro* at higher and lower concentrations. All the nine fungicides tested were found fungistatic against *M. phaseolina* and significantly inhibited its mycelial growth over untreated control.

At lower concentration, carbendazim + mancozeb (100 %) inhibit cent per cent mycelial growth of the test pathogen. The next best fungicide was carbendazim (89.63 %). It was followed by thiram (87.41 %), mancozeb (64.44 %), thiophanate methyl (54.07 %), propineb (15.18 %), copper oxychloride (3.71 %) and copper hydroxide (91.48 %).

While at higher concentration, carbendazim + mancozeb and carbendazim exhibited cent per cent mycelial growth inhibition.

Among the six biocontrol agents evaluated, *T. harzianum* was found most effective antagonist with significantly least mycelial growth (33.00 mm) and highest mycelial growth inhibition (66.33 %) of the test pathogen. The second and third best antagonists found were *T. viride* and *T. hamatum,* with second and third least mycelial growth of 36.66 mm and 52.00 mm and inhibition of 59.26 and 42.22 per cent respectively. *P. fluorescence* was found comparatively less effective with 64.00 mm mycelial growth and 28.88 per cent mycelial inhibition.

Under pot culture studies, as soil application and seed treatment, among the fungicides carbendazim + Mancozeb was found most effective with 87.67 per cent charcoal rot control. However, among bioagents *Trichoderma harzianum* was remarkably manage the charcoal rot.

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