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Evaluation of Fungicides and *Trichoderma viride* against *Macrophomina phaseolina* Causing Charcoal Rot in Soybean

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ABSTRACT

Macrophomina phaseolina causes charcoal rot in economically important crops all over the world. Present investigation was taken on evaluation of seven fungicides viz., carbendaxim+mancozeb, captan, carboxin, thiophanate methyl, ridomil, tebuconazole and difeconazole and a bioagent *Trichoderma viride* against *M. phaseolina* under both *in vitro* and *in vivo* conditions. Among the fungicides, minimum mortality was recorded with carbendazim+mancozeb and this treatment was significantly superior over rest of the treatments, followed by thiophanate methyl. Maximum root length of soybean was recorded with carbendazim+mancozeb (12.08 cm) followed by *T. viride* (12.07 cm). From the results obtained, it was concluded that selected fungicides and biocontrol agent significantly reduced the growth of *M. phaseolina*.

Key words: Charcoal rot, biocontrol, fungicides, *Macrophomina phaseolina*, soybean

INTRODUCTION

Soybean (*Glycine max* L.) belongs to the family Leguminosae which contributes around 25% of the global edible oil as well as about two-thirds of the world's protein concentrate for livestock feeding (Rahman *et al.*, 2021). Soybean is a nitrogen-fixing, environmentally-friendly annual oilseed crop with an oil content of 18-23% (Hungria and Mendes, 2015). Because of its higher protein content (38-44%), soybean meal is used to make approximately 98% of aquaculture and livestock feeds. Soybean oil is used in the production of printing inks, soaps, insecticides, paints, linoleum and disinfectants (Zeffa *et al.*, 2020). *Macrophomina phaseolina* is a necrotrophic fungus that causes the soil- and seed-borne disease "charcoal rot" or "dry root rot" in soybean. This infection is called "charcoal" because it blackens the infected tissues with numerous black microsclerotia (Coser *et al.*, 2017). *M. phaseolina* grows faster in soil with high temperatures and low moisture content (Marquez *et al.*, 2021). The rolling and wilting of leaves, as well as their reduced size, are early morphological indicators of charcoal rot. Charcoal rot reduces annual soybean yield by approximately 50% (Mufti and Bano, 2019). At all stages, soybean [*Glycine max* (L.) Merr.] is susceptible to root and stem base rots caused by soil-borne pathogens (Lin *et al.* 2022). Effective and proficient use of chemicals and bio-control agents, therefore, may be potential to control the charcoal rot disease of soybean caused by *M. phaseolina* (Rahman *et al.*, 2021). Several scientists found that combined application of fungicide and biocontrol (*Trichoderma* spp.) was highly effective for managing the soil-borne as well as seed-borne plant pathogens during crop cultivation (Zin and Badaluddin, 2020). This combined package not only minimizes plant diseases but also improves soil health and ultimately crop production (Rahman *et al.*, 2021). Considering the aforesaid facts, the present research was undertaken to evaluate the effectiveness of bio-agent and fungicides against charcoal rot of soybean caused by *M. phaseolina*.

MATERIALS AND METHODS

Infected roots and basal stems of soybean plants were collected from the fields of Agronomy KVK, Durgapur, Maharashtra, and utilized to make the culture of *M. phaseolina* which was used in the current experiment. The diseased portion of the root and stem was chopped into 3 mm-sized pieces, surface-sterilized with 0.1% $HgCl_2$ solution for one minute, and then given three washes with sterilized distilled

water in order to isolate the pathogen. The bits were then put in Petri plates with previously poured hardened PDA media. The plates were incubated for seven days at 25±2°C *M. phaseolina* was recognized as the name of the isolated fungus.

Sorghum : sand medium was used for mass multiplication of the test pathogen (Fig. 1). It was prepared by mixing 100 g sorghum, 50 g dry sand and 50 ml distilled water in 250 ml capacity conical flask and autoclaved at 1.05 kg/cm² for 30 min. Autoclaved mixture was then inoculated with pure culture of *M. phaseolina.* The inoculated flasks were incubated at room temperature for two weeks. The prepared inoculum was mixed in pot soil to test the efficacy of fungicides and bioagents against *M. phaseolina.*

Two weeks old mass culture of *M. phaseolina* was mixed separately in soil 1:1000 (one part of inoculum and ten parts of sterilized soil) and pots were filled before four days of sowing the seeds. These pots were kept wet and in moist condition. Before sowing, the seeds were treated with fungicides viz, carbendazim+mancozeb, captan, carboxin, thiophnate methyl and one bioagent *T. viride*. The seeds without treatment served as control and for each treatment three replications were made. The seeds of soybean (JS-335) obtained from Plant Pathology Section were used. The pots were disinfected with 5% formalin solution. Soil solarization was done with the help of 10% formalin solution. Two weeks old growth of pathogen on sorghum : sand medium was mixed separately in upper 15 cm layer of soil, 72 h prior to sowing. The pots containing sterilized soil without inoculum served as control. Before sowing, the seeds of soybean were surface sterilized with 2% sodium hypochloride solution for 2 min followed by three washings with distilled sterile water. Twenty seeds were sown in pots and the pots without inoculum served as control treatment.

An experiment (pot culture) was conducted in randomized block design with three replications. The treatment details are given below:

T_1–Carbendazim+mancozeb (0.2%)
T_2–Thiophanate methyl (0.2%)
T_3–Captan (0.2%)
T_4–Ridomil (0.2%)
T_5–*Trichoderma viride* (6×10^5 CFU/ml)
T_6–Control

Observations were recorded on germination seeds up to 45 days after sowing and per cent mortality was calculated by using the formula. Poisoned food technique was used to evaluate the above-mentioned fungicides and bioagents against *M. phaseolina* (Karibasappa *et al.*, 2020). Potato Dextrose Agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flasks, autoclaved at 1.05 kg/cm² for 20 min. Then before solidification of medium different fungicides with desired concentration were incorporated aseptically in different flasks. These flasks were shaken thoroughly and poured in Petri plates at the rate 20 ml/ plate. Likewise three plates for each treatment were poured. One set of three plates was poured without any fungicide to serve as control. After solidification of medium, plates were inoculated with eight days old pathogens separately. The five mm mycelial discs selected from peripheral growth of the test pathogen by cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position so as to make the mycelial growth touch the surface medium. The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogen on medium was recorded and per cent inhibition was calculated as:

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

Where,

C=Mycelial growth in control (cm)
T=Mycelial growth in treatment (cm)

RESULTS AND DISCUSSION

In four days, fungal growth appeared from the cut ends of stem fragments. The fungal growth from the plate was transferred to potato dextrose agar slants, where it was kept pure. The fungus was identified based on the morphological features. *M. phaseolina* grew quickly and spread out quickly. At first, mycelium was fluffy and white, but it quickly turned brown with a white periphery, eventually turning black (Fig. 1). After 8-10 days, the sclerotial bodies were visible.

The bits of *M. phaseolina* were inoculated and incubated on PDA medium at room temperature. After 24 h, the mycelial growth started from fungus bit. The colour of the

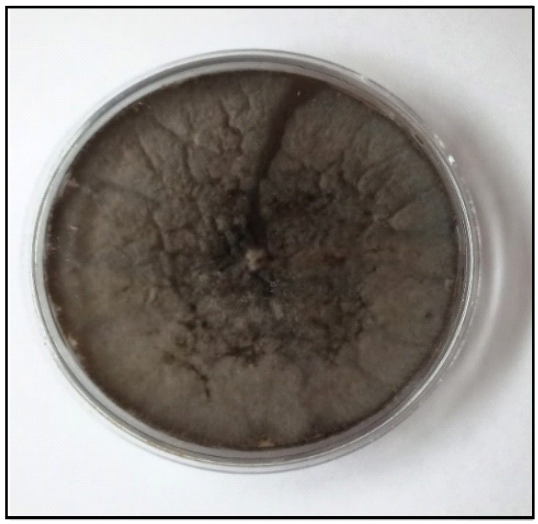

Fig. 1. Pure culture of *Macrophomina phaseolina*.

mycelium in the beginning was white, but after 48 h gradually it changed. The colour turned dark grey due to sclerotial formation. The mycelium was closely septated, branches raised at right angles to the parent hyphae. After third day hyaline and thick walled, hyphal from incubation, the fungus produced sclerotia. On 6th day, sclerotia were dark grey to black colour and irregular round in shape. 8th day old culture showed dark brown to dark grey colour of the colony. At high temperature, fungus growth covered the Petri plates in six to seven days and mycelial strands were oily in appearance and partially fluffy or aerial. But at low temperature did not show oil and fluffy growth. Fungus growth was close and horizontally parallel to the medium surface.

Soybean seeds of variety JS-335 were inoculated with *M. phaseolina*, initially the symptoms were noticed on aerial parts of the plant in the form of drooping off the tips of the plant and cotyledonous leaves. Patches of infected plants wilted and died prematurely. Seedlings could be infected from the moment they emerged through the early vegetative stages, but symptoms typically did not appear until the R5 (starting seed) to R7 (beginning maturity) growth stages. The leaves were yellow prior to wilting.

The pot culture experiment was conducted to record the mortality due to *M. phaseolina*. There was no mortality at 15 DAS but significant differences were noticed at 30 and 45 DAS control (Table 1). Fungicidal seed

Table 1. Per cent mortality of soybean seedlings due to charcoal rot at 15, 30 and 45 DAS in pot

Treatment	Conc. (%)	% Mortality				Average mortality	% Avg. reduction over control
		15 DAS	35 DAS	45 DAS	60 DAS		
Carbendazim+Mancozeb	0.2	0 (0.707)	0 (0.707)	0 (3.13)	19.74 (3.37)	4.9	71.29
Thiophanate methyl	0.2	0 (0.707)	0 (0.707)	17.59 (3.02)	18.55 (3.18)	9.03	47.10
Carboxin	0.2	0 (0.707)	17.08 (2.93)	18.59 (3.18)	20.38 (3.48)	14.01	40.20
Difeconazole	0.2	0 (0.707)	0 (0.707)	19.51 (3.34)	20.34 (3.47)	9.96	41.65
Captan	0.2	0 (0.707)	18.54 (3.18)	20.4 (3.48)	22.08 (3.76)	15.25	40.44
Ridomil	0.2	0 (0.707)	0 (0.707)	18.17 (3.11)	19.77 (3.38)	9.48	44.46
Tebuconazole	0.2	0 (0.707)	16.56 (2.85)	18.59 (3.18)	20.33 (3.47)	13.87	55.23
T. viride	6 x 105 cfu/ml	0 (0.707)	0 (0.707)	17.58 (3.02)	19.51 (3.34)	9.27	45.69
Control		0 (0.707)	21.2 (3.61)	22.89 (3.89)	24.21 (4.13)	17.07	-
S. Em		0.00	0.0138	0.003	0.014		
S. Ed		0.00	0.0195	0.0045	0.020		
C. D. (P=0.05)		0.00	0.0413	0.0095	0.042		
C. D. (P=0.01)		0.00	0.056	0.013	0.059		
C. V.			0.029	0.029	0.123		

Data in parantheses are arcsine transformed values.

treatment of soybean was found effective in improving germination by many workers (Sharma *et al.*, 2015; Karibasappa *et al.*, 2020; Kacsó *et al.*, 2022). Among the fungicides, minimum mortality was recorded with carbendazim+mancozeb (71.29%) and this treatment was significantly superior over rest of the treatments, followed by thiophanate methyl (47.10%). These observations are similar to the findings reported by Lokesh *et al.* (2020). However, at 45 DAS, there was no much difference in seedling mortality but remained same in these treatments. The differences were found to be significant over uninoculated control.

With regard to biocontrol agent, minimum mortality was observed in the *T. viride* treatment (45.69 %). Similar observations were recorded by Kumar *et al.* (2019), Mukhopadhyay and Kumar (2020) and Khan *et al.* (2021). This may be due to fact that *M. phaseolina* took 15 days to build the inoculum in soil and make effective for causing seedling mortality as also reported in soybean (Marquez *et al.,* 2021). Maximum germination percentage of soybean (84%) was found in combination of carbendazim+mancozeb followed by thiophanate methyl (64%; Bem Junior *et al.,* 2019). All seed treatments significantly increased the plant height as compared to control (9.41 cm). It was also clear that fungicidal seed treatment significantly increased the root length as compared to control (Molin *et al.,* 2022). The data indicated that there were significant differences in the root length due to fungicides and bioagent (Table 2). Maximum root length was recorded with carbendazim+mancozeb (12.08 cm) followed by *T. viride* (12.07 cm), respectively. The increase in root length might be siderophore production resulting in increased growth parameters. Similar observations were noted by Gowda *et al.* (2020). Seven fungicides viz., carbendaxim+ mancozeb, captan, carboxin, thiophanate methyl, ridomil, tebuconazole and difeconazole and a bioagent (*T. viride*) were screened at recommended concentration in the laboratory to check their efficacy against *M. phaseolina* by using poisoned food technique. Fungicides and biocontrol agent significantly reduced the growth of *M. phaseolina* (Table 3). The best fungicidal treatment was carbendazim+mancozeb (100%) showing complete inhibition of

Table 2. Plant height and germination percentage of soybean variety JS-335 at different fungicidal seed treatments

Treatment	Concentration	Germination (%)	Root length (cm)	Plant height (cm)
Carbendazim+Mancozeb	0.2	84 (66.42)	12.08	16.17
Thiophanate methyl	0.2	80 (63.43)	11.13	14.09
Carboxin	0.2	75.33 (60.22)	10.03	11.24
Difeconazole	0.2	64 (53.13)	9.15	9.08
Captan	0.2	75.33 (60.22)	11.16	12.16
Ridomil	0.2	50.66 (45.37)	8.09	10.03
Tebuconazole	0.2	50.66 (45.37)	10.08	9.10
T. viride	6 x 105 cfu/ml	86 (86.02)	12.07	14.26
Control		42 (40.39)	9.30	9.41
S. Em		0.0163	0.082	0.112
S. Ed		0.231	0.116	0.158
C. D. (P=0.05)		0.491	0.247	0.336
C. D. (P=0.01)		0.677	0.340	0.463
C. V.		3.48	1.379	1.658

Data in parantheses are arcsine transformed values.

Table 3. *In vitro* efficacy of fungicides and bioagent against *M. phaseolina*

Treatment	Concentration (%)	Radial mycelial growth (mm)				Per cent inhibition				Average inhibition
		3rd DAI	5th DAI	7th DAI	9th DAI	3rd DAI	5th DAI	7th DAI	9th DAI	
Carbendazim+Mancozeb	0.1+0.1	0.00	0.00	0.00	0.00	100	100	100	100	100
Thiophanate methyl	0.2	10.13	11.2	13.13	14.24	66.32	79.69	83.60	84.19	78.45
Carboxin	0.2	16.45	17.23	18.22	19.23	45.31	68.76	77.23	78.65	67.48
Difeconazole	0.2	25.24	26.32	27.38	28.45	16.09	52.28	65.80	68.42	50.64
Captan	0.2	27.13	28.19	29.24	30.34	44.00	48.90	63.48	66.32	55.67
Ridomil	0.2	17.19	18.25	19.32	20.39	42.85	66.92	75.86	77.36	65.74
Tebuconazole	0.2	14.33	15.42	16.49	17.57	52.36	72.03	79.4	80.49	71.07
T. viride	6 × 105 cfu/ml	23.46	25.33	25.75	28.57	22.00	54.07	68.13	68.28	53.12
Control		30.08	55.17	80.08	90.09	-	-	-	-	-
S. Em		0.018	0.013	0.018	0.018	-	-	-	-	-
S. Ed		0.025	0.018	0.026	0.026	-	-	-	-	-
C. D. (P=0.05)		0.054	0.039	0.056	0.056	-	-	-	-	-
C. D. (P=0.01)		0.074	0.054	0.076	0.077	-	-	-	-	-

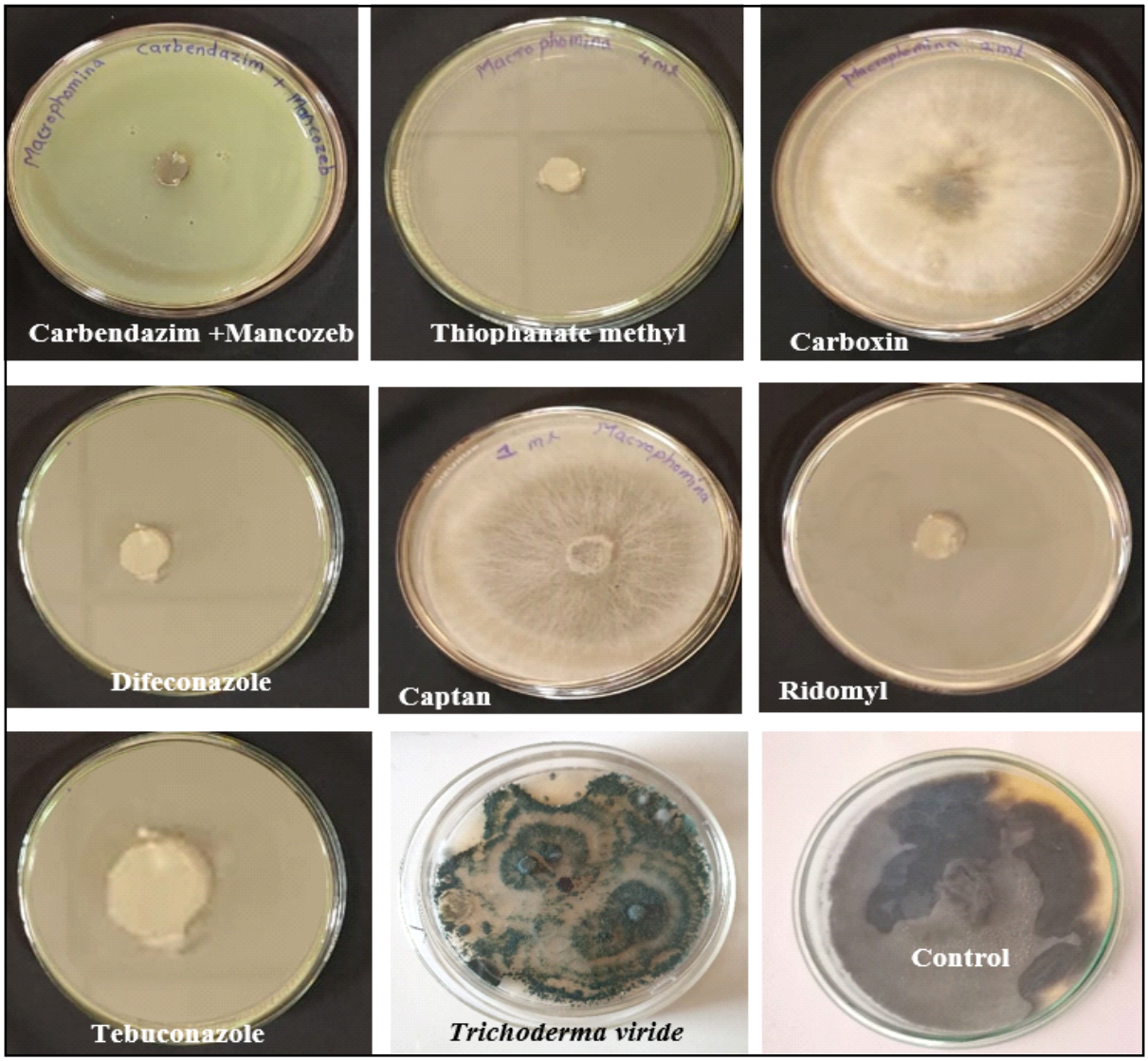

Fig. 2. *In vitro* evaluation of fungicides and bioagent against *M. phaseolina*.

mycelial growth followed by thiophanate methyl (78.45%) and tebuconazole (71.07%; Khaire *et al.*, 2018) as shown in Fig. 2. Mishra (2017) observed that carbendazim and mancozeb caused highest inhibition. Bioagent *T. viride* treatment recorded minimum radial mycelial growth at 3rd DAI 23.46 mm with 22% inhibition over control (Katyayani *et al.*, 2020). There was slight increase in the radial mycelial growth from 5th to 9th DAI with minimum per cent inhibition in the biocontrol agent treatment. The beneficial effect due to *T. viride* in controlling the *M. phaseolina* was reported by Asad e*t al.* (2014). Fungicides like difeconazole and captan were found less effective against test pathogen and this was followed by carboxin.

CONCLUSION

It is concluded that seed priming with fungicides and biocontrol agents plays vital role in increasing germination percentage and results in proper plant stand and healthy seedlings of soybean. Responsible management of diseases caused by *M. phaseolina* is essential, since the importance of this soil-borne fungus lies not only in the losses it causes but also in the impacts it has on the environment due to unsustainable management practices. Combined effect of carbendazim and mancozeb was found to be most effective for the management of charcoal rot of soybean under both *in vivo* and *in vitro* conditions showing minimum disease incidence and producing highest yield followed by thiophanate methyl and tebuconazole. These results suggested that the toxic effect of these fungicides inhibited maximum mycelium growth of test pathogen and provided management of charcoal rot of soybean.

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