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Bioefficacy of Bioagents and Fungicides against *Macrophomina phaseolina* (Tassi) Goid. Causing Charcoal Rot Disease in Sorghum Crop

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ABSTRACT

Macrophomina phaseolina causing charcoal rot of sorghum is an aggravated disease in dry land areas when accompanied with severe drought conditions. The prolong survival structure of the pathogen such as microsclerotia in soil and debris made difficult to manage the disease. Therefore, the present study was conducted on evaluation of bioagents and fungicides against the pathogen under *in vitro*. Among eight different bioagents tested by using dual culture method, Arka microbial consortium at 3000 ppm (100%) *P. fluorescens* (84.52%) and waste decomposer (76.34%) significantly reduced the mycelial growth of the fungi. Among seven selected fungicides tested by using poisoned food technique, carbendazim, thiophanate methyl, carbendazim 12 % + mancozeb 63% WP (SAAF), metalaxyl M4 + mancozeb 64%, 75% WP (Ridomil) and thiophanate methyl 45 % + pyraclostrobin 5% FS (Xelora) exhibited hundred per cent inhibition of the mycelial growth of *M. phaseolina* followed by captan (93.20%). Whereas, copper oxy chloride was found to be ineffective in controlling the disease.

Keywords Charcoal rot, *Macrophomina*, Bioagents, Fungicides, *In vitro.*

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INTRODUCTION

Charcoal rot is a major disease in the dry sorghum-growing regions of Asia, Africa, Americas and Australia. It is caused by *Macrophomina phaseoli* (Maubl.), which was first reported in India (Uppal 1931). Further, it has been reported as *Macrophomina phaseolina* (Tassi) Goid (Edmunds 1962). The disease is relatively more severe and destructive on high yielding sorghum cultivars when grain filling coincides with low soil moisture in hot dry weather.

In India, the disease is rarely severe during the monsoon season, but has been reported in epidemic form in *rabi,* which cause heavy crop loss (Bhagwat 1975). It has been estimated up to 48.6% loss in seed weight at Dharwad (Anahosur and Rao 1977). Depending upon the cultivars, weather conditions and disease severity, yield losses ranged from 15.18 to 54.59% (Anahosur and Patil 1983).

The pathogen *M. phaseolina* has a wide host range and associated with high soil temperature from 30 ^{0}C to 40 ^{0}C and low soil moisture content (Arora and Pareek 2013). It survives in the form of sclerotia as a resting structure on the stalks left over in the field after harvesting and dispersed by the movement of soil, air, water, farm machineries, tractor and contaminated seeds (Kaur *et al*. 2012). Hence, it is difficult to be managed by virtue of its long survival mechanism, vast distribution in soil and wider host range. Therefore, *in vitro* efficacy of bioagents on pathogen coupled with the selected fungicides for charcoal rot disease can be made feasible for effective management strategies.

MATERIALS AND METHODS

Isolation of the pathogen

Macrophomina phaseolina associated with charcoal rot was isolated from infected stalks of sorghum plants by using tissue isolation method on potato dextrose agar (PDA) medium, which were collected from severely infected field (Hagari). In order to confirm the identity of the fungus, the microsclerotia was observed under the high power (40X) microscope.

Evaluation of bioagents and fungicides

Eight bioagents such as *Trichoderma viride, T. harzianum, Pseudomonas fluorescens, Bacillus subtilis, B. thuringiensis, Metarhizium anisopliae, Azotobacter tropicalis* + *Bacillus aryabhattai* + *Pseudomonas taiwanensis* (Arka microbial consortium at 1000, 2000 and 3000 ppm) and Waste decomposer were tested against the pathogen by using dual culture method.

Dual culture method

Approximately 20 ml molten PDA was poured into each of 90 mm diameter sterilized Petri plates. Following solidification, 5 mm bit of the pathogen and antagonist T*richoderma* isolates were placed on PDA surface at equidistant from each other (minimum 2 cm apart). The control plates were inoculated by placing one bit of the pathogen in center. Three replications were maintained for each treatment. The plates were incubated at 28 ± 2 °C.

Poisoned food technique

Seven fungicides viz., Carbendazim 50% WP, copper oxychloride 50% WP, captan 50% WP, thiophanate methyl 75% WP, carbendazim 12% + mancozeb 63% WP (SAAF), metalaxyl MZ + mancozeb 64%, 75% WP (Ridomil) and thiophanate methyl 45% + pyraclostrobin 5% FS (Xelora) were evaluated (at 500, 1000 and 2000 ppm each) by *in vitro* against *M. phaseolina,* using poisoned food technique (Nene and Thapliyal1993). Based on active ingredient, the required quantity of each test fungicide was calculated and mixed thoroughly with autoclaved and cooled (40 °C) PDA medium separately in a conical flask to obtain desired concentrations of 500, 1000 and 2000 ppm. Then poured (20 ml/plate) aseptically in Petri plates (90 mm dia) and allowed to solidify at room temperature. Later, all the plates were inoculated aseptically with a week old actively growing pure culture of *M. phaseolina* (5 mm disc) and incubated at 28 ±2 °C. Petri plates containing only PDA (without any fungicide) and inoculated with the culture disc of the test pathogen were maintained as control.

Per cent mycelial growth inhibition of the test pathogen with the test bioagents and fungicides over control was calculated by applying the following formula (Vincent 1947);

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

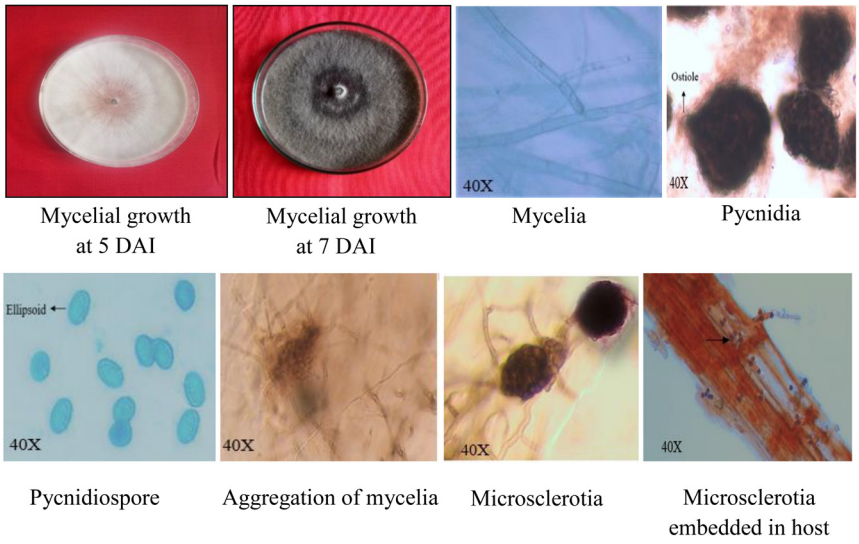

Fig 1. Morphology of *Macrophomina phaseolina.*

RESULTS AND DISCUSSION

Pathogen

M. phaseolina grew profusely on PDA medium. Initially the hyphae were dirty white in color, which later turned to black i.e., seven days after incubation at 28 ± 2^0C. Branches from the main hyphae are generally formed at right angle on parent hyphae with constriction at the point of origin. Microsclerotia are formed by a compact mass of hardened fungal mycelium, which are dark brown to black and oval to oblong in shape. Pycnidia are dark brown, sub-globose and rarely observed under natural conditions. Pycnidiospores are hyaline, single celled and ellipsoid in shape (Fig. 1).

Efficacy of bioagents against *M. phaseolina*

The observations on mycelial growth and its inhibition revealed that, all the bioagents were significantly superior over the control in inhibiting mycelial growth of the pathogen (Table 1 and Fig. 2). Among them, Arka microbial consortium at 3000 ppm showed hundred per cent inhibition, which might be due to symbiosis and increases the colonization of beneficial microbes in rhizosphere which is helpful in supplementing the nutrients to the plants such as nitrogen, potassium, phosphorous and zinc and imparts resistance (Krishna and Nataraj 2019).

Next best bioagent was *P. fluorescens* (84.52%), as it produces phytohormones, HCN, IAA, siderophores and induction of systemic resistance in plants (Shruthi 2017). Which is followed by waste decomposer at 3000 ppm (76.34%), AMC at 2000 ppm (69.65%), *T. harzianum* (67.43%), *B. subtilis*

Table 1. Efficacy of bioagents against *M. phaseolina*. * Mean of three replications. Figures in parenthesis are arcsine value.

Tr. No.	Bioagents	Mycelial inhibition*(%)
T_1	*Trichoderma viride*	29.40 (32.81)
T_2	*Trichoderma harzianum*	67.43 (55.18)
T_3	*Pseudomonas fluorescens*	84.52 (66.80)
T_4	*Bacillus subtilis*	56.53 (48.73)
T_5	*Bacillus thurengensis*	30.02 (33.20)
T_6	*Metarrhiziun anisopliae*	26.92 (31.23)
T_7	Arka microbial consortium (1000 ppm)	45.50 (42.40)
T_8	Arka microbial consortium (2000 ppm)	69.65 (56.55)
T_9	Arka microbial consortium (3000 ppm)	10. (90.00)
T_{10}	Waste decomposer (3000 ppm)	76.3 (60.87)
T_{11}	Control	-
	SEm ±	0.41
	CD @ 1%	1.20

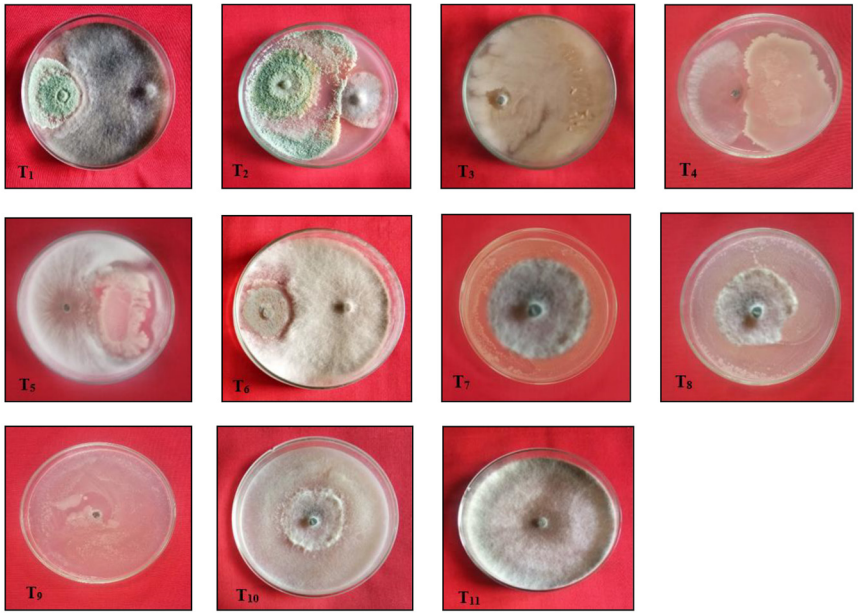

Fig. 2. Efficacy of bioagents against *M. Phaseolina.*

T_1: *Trichoderma viride*
T_2: *T. harzianum*
T_3: *P. fluorescens*
T_4: *Bacillus subtilis*
T_5: *B. thurengensis*
T_6 : *Metarrhizium anisopliae*
T_7 : Arka microbial consortium (1000 ppm)
T_8 : Arka microbial consortium (2000 ppm)
T_9 : Arka microbial consortium (3000 ppm)
T_{10} : Waste decomposer (3000 ppm)
T_{11} : Control

(56.53 %) and AMC at 1000 ppm (45.50%). Whereas, *B. thuringiensis* (30.02%) was on par with *T. viride* (29.40%).

Metarhizium anisopliae was found to be least effective (26.92%) as it is an entomopathogenic and reported fungitoxic against *M. phaseolina* (Dara *et al.* 2018).

Mechanism of waste decomposer is due to compost microbial communities such as *Trichoderma* spp., *Gliocladium virens, Flavobacterium balustinum, Pseudomonas putida* and *Xanthomonas maltophilia* are involved in reducing the activity of the pathogen (Mehta *et al.* 2014). *Bacillus* sp. were known to produce diffusible and volatile antibiotics, siderophore, IAA, phosphate solubilization and nutrient competition which imparts antagonism against *M. phaseolina* (Rajesh *et al*.2013).

T. harzianum and *T. viride* suppressed the mycelial growth of the fungus, this antagonism might be due to coiling and disintegration of hyphae of the test pathogen which results in loss of competitive saprophytic ability (Naik and Sen 1995). In the present study *T. viride* was found to be less effective. It might be due to abiotic stress such as temperature (≥37 ^{0}C) resulted in delayed growth and sporulation compared to *T. harzianum*, which has good growth at 37 ^{0}C and supported by earlier workers (Leo *et al.* 2011 and Vithya *et al.* 2018).

Efficacy of fungicides against *M. phaseolina*

All the fungicides tested were found statistically non-significant in inhibiting the mycelial growth. Among these five fungicides such as carbendazim, thiophanate methyl, carbendazim 12% + mancozeb 63% WP (SAAF), metalaxyl M4 + mancozeb 64%,

Table 2. Efficacy of fungicides against mycelial growth and inhibition of *M. phaseolina.* *Mean of three replications. Figures in parenthesis are arcsine value.

Tr. No.	Treatments	Mycelial inhibition * (%) Concentrations (ppm) 500	1000	2000	Mean
T_1	Carbendazim 50%WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
T_2	Copper oxychloride 50%WP	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
T_3	Captan 50 %WP	91.11(72.69)	94.07(75.91)	94.44(76.36)	93.20(74.98)
T_4	Thiophanate methyl 75 % WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
T_5	Carbendazim 12%+Mancozeb 63% WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
T_6	Metalaxyl M 4+Mancozeb 64% (75%) WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
T_7	Thiophanate methyl 45%+ Pyraclostrobin 5% FS	100(90.00)	100(90.00)	100(90.00)	100(90.00)
T_8	Control	-	-	-	-
		Non-significant			

75% WP (Ridomil) and thiophanate methyl 45% + pyraclostrobin 5% FS (Xelora) showed hundred per cent inhibition of mycelial growth of *M. phaseolina* at 500, 1000 and 2000 ppm (Table 2 and Fig. 3). The inhibition by carbendazim and thiophanate methyl attributed to fungistatic action and appears to bind to an unspecified site on tubulin and suppress microtubule assembly dynamic. This results in cell cycle arrest at the G2/M phase and an induction of apoptosis (Thelingwani *et al.* 2009) and mancozeb inactivates the sulfhydryl groups of amino acids and enzymes within fungal cells resulting in disruption of lipid metabolism, respiration and production of adenosine phosphate (Gullino *et al*. 2010).

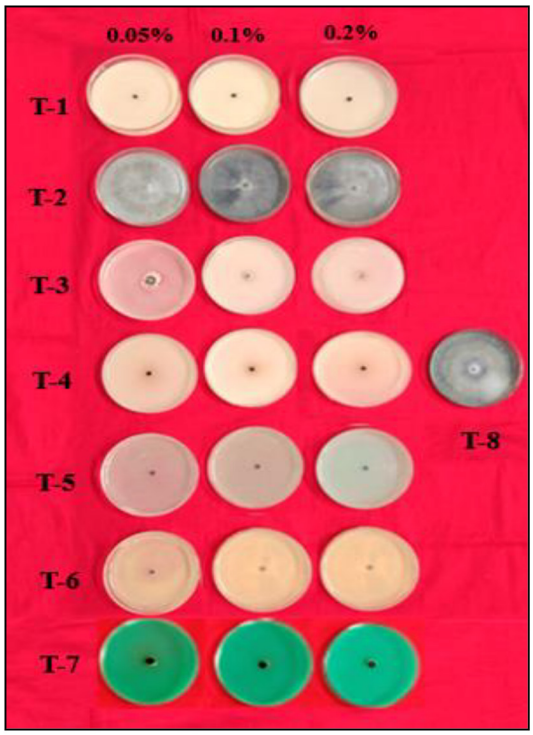

Fig. 3. Efficacy of fungicides against *M. phaseolina.*

T_1: Carbendazim 50% WP
T_2: Copper oxychloride 50% WP
T_3: Captan 50% WP
T_4: Thiophanate methyl 75% WP
T_5: Carbendazim 12% + Mancozeb 63% (75%) WP
T_6: Metalaxyl M4 + Mancozeb 64% WP
T_7: Thiophanate methyl 64% + Pyraclostrobin 5% FS
T_8: Control

Metalaxyl-M inhibits the mycelial growth of the pathogen by targeting the RNA polymerase enzymes of fungi and interrupt its transcription process. Pyraclostrobin is a carbamate ester which play an important role in mitochondrial cytochrome-bc1 complex inhibitor (Nene and Thapliyal 1993). Next best fungicide was captan (at 500, 1000 and 2000 ppm) with mycelial growth inhibition of 93.20 per cent, because it acts as a multisite activity fungicide, which has been known to inhibit fungal mitochondrial respiration by non-specifically interacting with proteins containing thiol moiety (Yang *et al.* 2011).

CONCLUSION

From the present investigations Arka microbial consortium at 3000 ppm, *P. fluorescens* and waste decomposer among different bioagents and all selected fungicides except copper oxychloride were found to be significantly effective in controlling the disease.

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