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Efficacy of new fungicides and bioagents against grain mold fungi

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Sorghum [*Sorghum bicolor* (L.) Moench] is a vital life-sustaining food crop for human being as well as for livestock in many parts of world. It is one of the major staple foods for the world's poorest and insecured people. There are several factors responsible for low yield of *Kharif* Sorghum in Maharashtra. Among these factors, diseases are major constraint for low yield. The five major disease problem in order of importance are grain mold, charcoal rot, downy mildew, anthracnose and sorghum viral disease .Among these diseases grain mold fungi occurs at maturity stage on sorghum hybrids of Maharashtra state is important. Initial mold symptoms appear as white or grey mycelial growth on rachis, glumes and anthers. The grain become discolored and various discolorations such as black (*Curvularia* spp), pink (*Fusarium* spp.*),* grey *(Alternaria* spp.*)* are observed. The major fungal pathogens viz. *F. moniliforme, C. lunata* are responsible for grain quality detoriation in Grain mold therefore mangement of these pathogens by the new fungicides is the need of research and not much work was done earlier on chemical and biological management. Considering these asapects seven fungicides evaluated *in vitro* against *F. moniliforme, C. lunata.* However, of the fungicides tested, Carbendazim 12% + Mancozeb 63%, Difenconazole, Hexaconazole, Propiconazole were found antifungal or fungistatic with significant inhibition of mycelial growth of the test pathogen. Five fungal antagonists and two bacterial antagonists evaluated *in vitro* were found fungistatic effective against *F. moniliforme, C. lunata.* However, out of the bioagents viz*., T. viride, T. harzianum* and *T. koningii* were found effective with significant inhibition of mycelial growth of the test fungus

Key words: Fungicides, bioagents, *g*rain mold fungi, management, sorghum

INTRODUCTION

Sorghum cultivated area 6.21 lakh hectare in Maharashtra with production 3.68 lakh tonnes and productivity 594 kg/ha (Anon, 2015-16). Nutritionally sorghum grain contains about 10-12 per cent protein, 3 per cent fat, 70 per cent carbohydrate and 2 per cent crude fiber.

Kharif (wet) sorghum hybrids though high yielding are associated with few drawbacks such as susceptibility to pests and diseases with poor grain quality. Addition to this in the years of prolonged rainfall at the time of crop maturity, occurrence of is used to describe the diseased appearance of sorghum grains resulting from infection by one or more pathogenic/ saprophytic fungi affects quality of grain leading to poor market value. Among the sorghum diseases grain mold fungi are infected heavily due to rain in the September-October months at maturity crop stage of sorghum hybrids in Maharashtra state.

The losses due to mold in the hybrid sorghum are up to 50 per cent in highly susceptible cultivar, yield losses reach up to 30 to 100 per cent. Grain mold results in reduction of market value, germination and acceptability of harvested grains for human consumption. Seven fungicides in (500, 1000, 1500 ppm) concentration were evaluated *in vitro* against *Fusarium moniliforme* and *Curvularia lunata* exhibited a wide range of mycelial growth and inhibition. All the bioagents evaluated exhibited fungi-

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static/antifungal activity against *Fusarium moniliforme* and *Curvularia lunata* results in significant inhibition of its growth over untreated control.

MATERIALS AND METHODS

In vitro evaluation of fungicides and bioagents

Fungicides

Eight fungicides were evaluated (systemic @ 500, 1000, 1500 ppm) *in vitro* against grain mold pathogens *Fusarium moniliforme* and *Curvularia lunata* by poisoned food technique, 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^{0}C) Potato Dextrose Agar medium (PDA) separately in conical flasks to obtain desired concentrations of 500, 1000, 1500. 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 *F. monilforme* and *C. lunata.* The culture disc was placed on PDA in inverted position in the centre of the Petri plate and plates were incubated at 28$\pm$2^{0}C. Petri plates filled with plain PDA (without any fungicide) and inoculated with the culture disc of the test pathogens *F. monilforme* and *C. lunata* were maintained as control (untreated).

Observations on radial mycelial growth/colony diameter of the pathogen was 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, 1947).

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

where,
C = Growth (mm) of test fungus in untreated control plate
T = Growth (mm) of test fungus in treated plates

Bioagents

Five fungal antagonists *viz*., *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. koningii* and *Gliocladium virens* and two bacterial antagonists viz., *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* against *F. monilforme* and *C. lunata* applying Dual culture technique.Seven days old cultures of the test bioagents and the test pathogen (*F. moniliforme, C. lunata*) grown on agar media were used for the study. The culture disc (5mm) of the test pathogen and bioagent were cut out with sterilized cork borer, from a week old culture. Then two culture discs, one each of the test opposite with each other on solidified PDA medium in Petri plates and plates were incubated at 28$\pm$2^{0}C.Three plates/ treatment/replication were maintained. PDA plates inoculated only with culture disc of the test pathogen were maintained as untreated control.

Observations on linear mycelial growth of the test pathogens and bioagent 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.

Statistical analysis

$$\text{Percent growth Inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Experimental details

Design	:	C.R.D.
Replications	:	Three
Treatments	:	Eight

The data obtained of all the experiments was subjected to the statistical analysis. The percentage values were transformed into arcsine values. The standard error (SE) and critical difference (C.D.) at level P = 0.05 were worked out and results obtained were compared statistically. All the statistical analysis was done using VNMKV-STAT statistical programmer at Central Computer Laboratory, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

RESULTS AND DISCUSSION

In vitro efficacy of fungicides on growth of Fusarium moniliforme

Treatment details

Table 1: Chemicals and bioagents used in the experiment

	Treatment No	Name Chemical or Bioagent	Source
Chemical			
	T_1	Carbendazim	Bavistin50 WP
	T_2	Chorothalonil	Ishaan 75WP
	T_3	Carbendazim 12% + Mancozeb 63%	SAAF 75 WP
	T_4	Difenconazole	Score 25 EC
	T_5	Hexaconazole	Contaf 5 EC
	T_6	Propiconazole	Tilt 25 EC
	T_7	Azoxystrobin	Amistar 23 % SC
	T_8	Control	
Bioagent			
	T_1	*Trichoderma viride*	Local strain taken from Deptt of Plant Pathology,VNMKV,Parbhani
	T_2	*T. harzianum*	-do-
	T_3	*T. hamatum*	-do-
	T_4	*T. koningii*	-do-
	T_5	*G. virens*	-do-
	T_6	*P. fluorescens*	-do-
	T_7	*B. subtilis*	-do-
	T_8	Control	

Table 2: *In vitro* efficacy of fungicides on growth of grain mold fungi

Treat	Name of chemical / doses	Growth inhibition (%)							
		F.moniliforme				*C.lunata*			
		500 PPM	1000 PPM	1500 PPM	Mean	500 PPM	1000 PPM	1500 PPM	Mean
T_1	Carbendazim	82.5	86.1	88.5	85.7	21.7	47.2	69.4	46.1
T_2	Chlorothalonil	75.0	81.2	87.8	81.3	30.6	69.4	78.3	59.4
T_3	Carbendazim 12% + Mancozeb 63 %	100	100	100	100	87.2	100	100	95.7
T_4	Difenconazole	91.7	100	100	97.2	100	100	100	100
T_5	Hexaconazole	94.4	100	100	98.1	100	100	100	100
T_6	Propiconazole	100	100	100	100	100	100	100	100
T_7	Azoxystrobin	59.4	65.9	71.8	65.7	66.1	71.1	73.9	70.4
T_8	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	S.E.±	0.24	0.21	0.23	-	0.24	0.20	0.18	-
	C.D. @ 5 %	0.99	0.88	0.95	-	1.00	0.84	0.77	-

Seven fungicides @500, 1000, 1500 ppm concentration were evaluated *in vitro* against *Fusarium moniliforme* exhibited a wide range of mycelial growth and inhibition of the test pathogen (Table 2, Fig. 1, 2, 3 and 9).

Results revealed that all the 7 fungicides tested at 500, 1000 and 1500 ppm significantly inhibited mycelial growth of *F.moniliforme* over untreated control (0.00 %). Further, the percent mycelial growth inhibition was increased with increase in concentration of the fungicide tested.

At 500 ppm fungicide (Table 2, Fig. 1 and 9) mycelial growth inhibition of the test pathogen was ranged from 59.42 % (azoxystrobin) to 100 % (carbendazim + mancozeb, propiconazole). However, fungicide carbendazim + mancozeb,

Table 3 : *In vitro* efficacy of bioagents on growth of *Fusarium moniliforme* and *Curvularia lunata*

Treat. No.	Name of Bioagent	Colony diameter (mm)	Inhibition (%)	Colony diameter (mm)	Inhibition (%)
T_1	*Trichoderma viride*	12.4	86.2	17.9	80.0
T_2	*T.harzianum*	17.9	80.0	24.0	73.3
T_3	*T. hamatum*	26.3	70.7	40.0	55.5
T_4	*T. koningii*	15.7	82.5	26.3	70.7
T_5	*Gliocladium virens*	65.2	27.4	47.5	47.2
T_6	*Pseudomonas fluorescens*	67.3	25.1	62.5	30.5
T_7	*Bacillus subtilis*	48.2	46.4	58.5	35.0
T_8	control (untreated)	90.0	0.00	90.0	0.00
	S.E.±	0.27	0.30	0.44	0.49
	C.D. @ 5 %	1.11	1.23	1.80	2.01

propiconazole was found best which was inhibited cent percent (100 %) mycelial growth. The second and third best fungicide found were hexaconazole (94.44 %), difenconazole (91.67 %). this was followed by carbendazim (59.42 %).

At 1000 ppm fungicides (Table 2, Fig. 2 and 9) mycelial growth inhibition was increased compared to 500 ppm and it was ranged from 65.94 % (azoxystrobin) to 100 % (carbendazim + mancozeb, difenconazole, hexaconazole, propiconazole).

However, fungicides carbendazim + mancozeb, difenconazole, hexaconazole and propiconazole caused cent per cent (100 %) mycelial inhibition. These were followed by the fungicides *viz.*, carbendazim (86.11 %) and chlorothalonil (81.24 %), whereas azoxystrobin was found least effective (65.94 %).

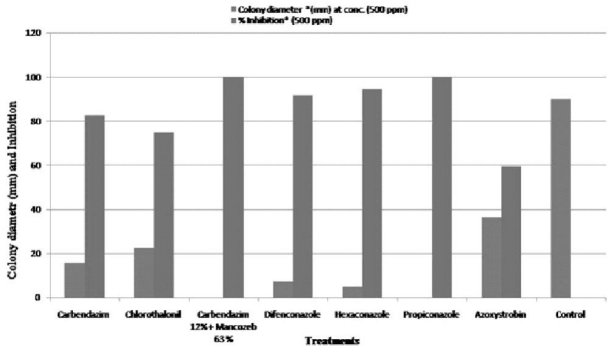

Fig. 1 : *In vitro* efficacy of fungicides at 500 ppm against mycelial growth and inhibition of *Fusarium moniliforme*

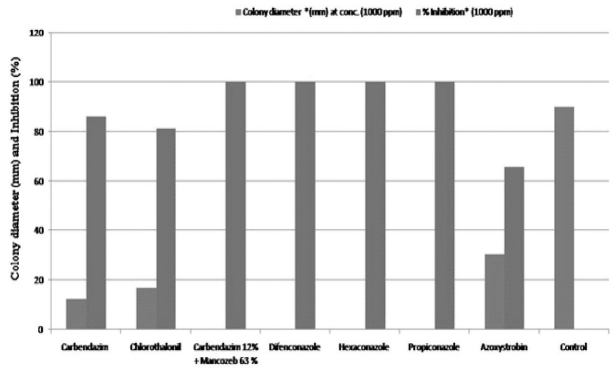

Fig. 2 : *In vitro* efficacy of fungicides at 1000 ppm against mycelial growth and inhibition of *Fusarium moniliforme*

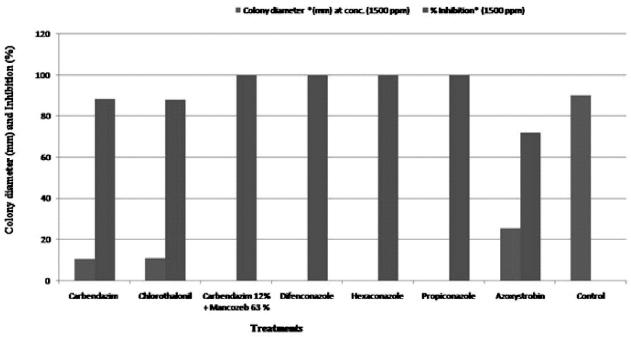

Fig. 3 : *In vitro* efficacy of fungicides at 1500 ppm against mycelial growth and inhibition of *Fusarium moniliforme*

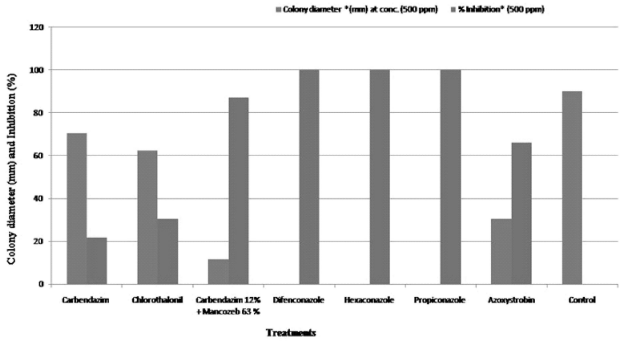

Fig. 4 : *In vitro* efficacy of fungicides at 500 ppm against mycelial growth and inhibition of *Curvularia lunata*

At 1500 ppm fungicide (Table 2, Fig. 3 and 9), mycelial growth inhibition was increased compared to 500 ppm, 1000 ppm and it was ranged from 71.83 % (azoxystrobin) to 100 % (carbendazim + mancozeb, difenconazole, hexaconazole and propiconazole). However, fungicides carbendazim + mancozeb, hexaconazole, difenconazole, hexaconazole and propiconazole were inhibited cent percent growth (100 %) mycelial. This was followed by the fungicides *viz.,* carbendazim (88.52 %), chlorathalonil 87.83 %. Whereas, azoxystrobin was found least effective (71.83 %).

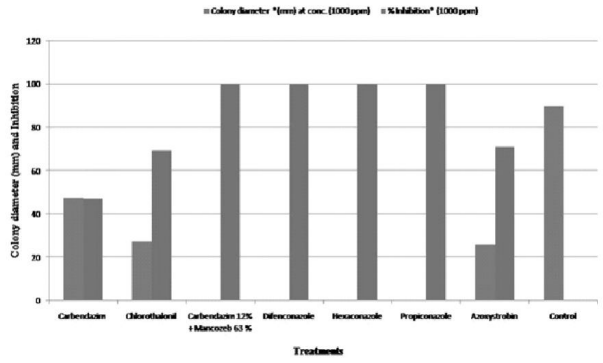

Fig. 5 : *In vitro* efficacy of fungicides at 1000 ppm against mycelial growth and inhibition of *Curvularia lunata*

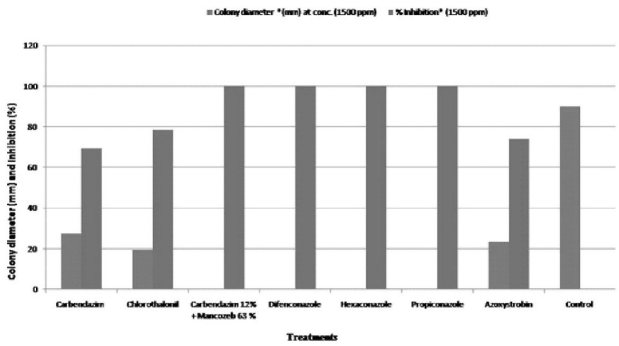

Fig. 6 : *In vitro* efficacy of fungicides at 1500 ppm against mycelial growth and inhibition of *Curvularia lunata*

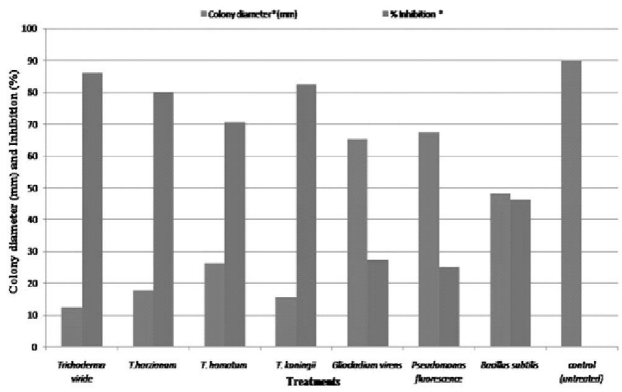

Fig. 7 : *In vitro* efficacy of bioagents against mycelial growth and inhibition of *Fusarium moniliforme*

Thus, all the fungicides tested were found fungistatic against *Fusarium moniliforme* and significantly inhibited its mycelial growth over untreated control. However fungicide found most effective in the order merit were carbendazim + mancozeb, difenconazole, hexaconazole, propiconazole, cabendazim, chlorathalonil and azoxystrobin.

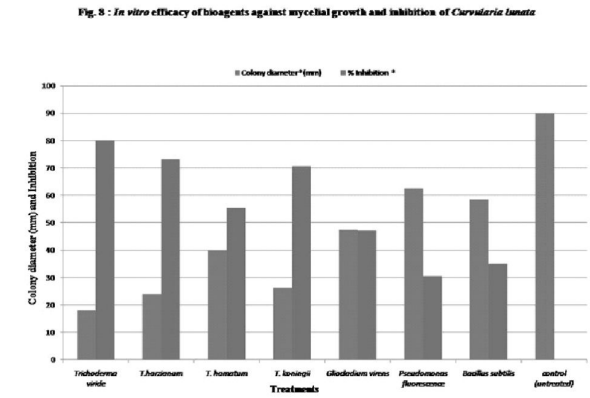

Fig. 8 : *In vitro* efficacy of bioagents against mycelial growth and inhibition of *Curvularia lunata*

Similar type of results were against *F.molniliforme* infecting sorghum and many other crops were reported earlier by several workers. Fungicides *viz.,* carbendazim + mancozeb, difenconazole, hexaconazole, propiconazole, carbendazim,

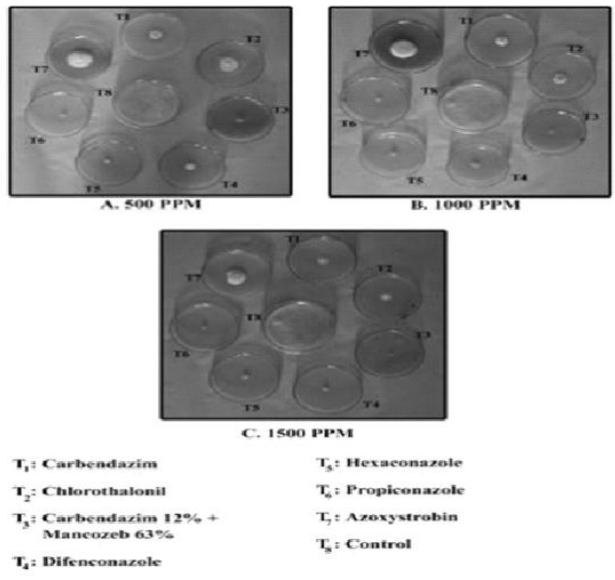

Fig. 9 : *In vitro* efficacy of the fungicides (500,1000,1500 ppm against mycelial growth and inhibitions of l.moniliforme

chlorathalonil, azoxystrobin were reported to cause significant mycelial growth inhibition of *Fusarium moniliforme* earlier by several workers Kadam (1997), Kakade (1999), Allen *et al.* (2004) evaluated benomyl, difensaconazole, hydrogen dioxide,

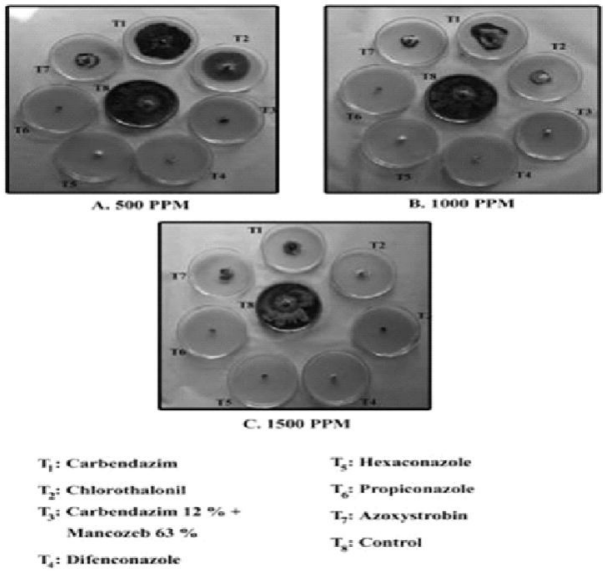

Fig. 10 : *In vitro* efficacy of the fungicides (500,1000,1500 ppm against mycelial growth and inhibitions of *C.lunata*

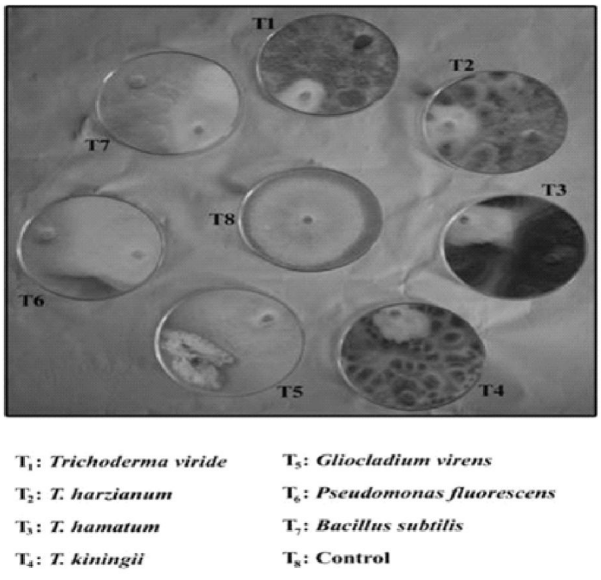

Fig. 11 : *In vitro* efficacy of the fungicides (500,1000,1500 ppm against mycelial growth and inhibitions of *C.lunata*

mancozeb and thiabendazole for their ability to inhibit the growth of four species of *Fusarium and* to enhance long-leaf pine seed germination., Pan *et al.* (2007) and Wabale and Chahuan (2010) reported that topsin-M 75% WP (500, 1000 and 1500 ppm) and emisan 6% WP (3000 ppm) were highly fungotoxic in growth inhibition up to 7 days of incubation against *F. moniliforme.* Maximum inhibition in mycelial growth was observed in the hexaconozole at 1000 ppm followed by other fungicides at the same concentration (Salma Begum *et al*. 2015).

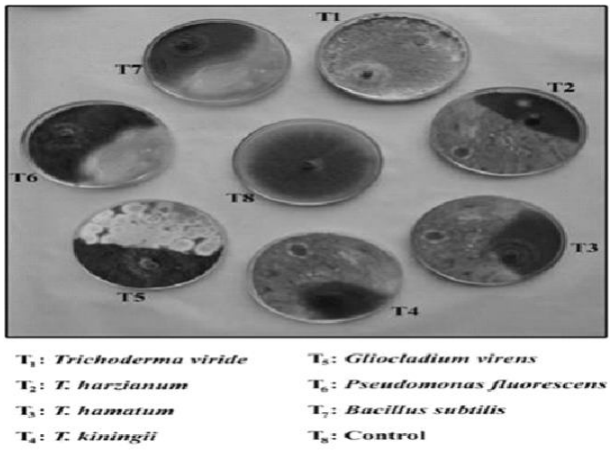

Fig. 12 : *In vitro* efficacy of the Bioagents against mycelial growth and inhibitions of *C.lunata*

In vitro efficacy of fungicides on growth of Curvularia lunata

Seven fungicides at (500, 1000, 1500 ppm) concentrations were evaluated *in vitro* against *Curvularia lunata* exhibited a wide range of mycelial growth and inhibition of the test pathogen. The results obtained are presented in the Table 2, Fig. 4, 5, 6 and 10.

Results revealed that all the 7 fungicides tested at 500, 1000 and 1500 ppm significantly inhibited mycelial growth of *Curvularia lunata* over untreated control (0.00 %). Further, the percent mycelial growth inhibition was increased with increase in concentration of the fungicide tested.

At 500 ppm concentration fungicide (Table 2, Fig. 4 and 10) mycelial growth inhibition of the test pathogen was ranged from 21.7% (carbendazim) to 100% (carbendazim + mancozeb, propiconazole). However, fungicide difeconazole, hexaconazole, propiconazole were found best and inhibited cent percent (100 %) mycelial growth. The second and third best fungicide were found carbendazim + mancozeb (87.2 %) and azoxystrobin (66.1 %) followed by chlorothalin (30.56 %) whereas carbendazim was found least effective (21.7 %).

At 1000 ppm concentration fungicide (Table 2, Fig.5 and 10) mycelial growth inhibition was increased as compared to 500 ppm and it was ranged from 47.2% (carbendazim) to 100 % (carbendazim + mancozeb, difenconazole, hexaconazole, propiconazole). However, fungicides carbendazim + mancozeb, hexaconazole, difenconazole and propiconazole caused cent per cent (100 %) mycelial inhibition. This was followed by the fungicide *viz.,* azoxystrobin (71.1 %) whereas chlorothalonil and carbendazim was found least effective (47.2%).

At 1500 ppm fungicide (Table 2, Fig 6 and 10) mycelial growth inhibition was increased as compared to 500 ppm, 1000 ppm and it was ranged from 69.4% (carbendazim) to 100 (carbendazim + mancozeb, difenconazole, hexaconazole and propiconazole). However, fungicide carbendazim + mancozeb, hexaconazole, difenconazole, propiconazole caused cent per cent (100 %) mycelial inhibition. This was followed by the fungicides viz., azoxystrobin (73.8%) and chlorathalonil (78.3%) whereas carbendazim was found least effective (69.4%).

Thus, all the fungicides tested were found fungistatic against *Curvularia lunata* and significantly inhibited its mycelial growth over untreated control. However, fungicides found most effective in the order merit were hexaconazole, diffeconazole, propiconazole, carbendazim + mancozeb, azoxystrobin, chlorathalonil and cabendazim.

Similar fungistatic effect of the test fungicides against *Curvularia lunata* infecting sorghum and many other crops were reported earlier by several workers. Fungicides viz., hexaconazole, difenconazole, propiconazole, carbendazim + mancozeb, azoxystrobin, chlorathalonil, carbendazim were reported to cause significant mycelial growth inhibition of *Curvularia lunata* earlier by several workers viz., Allen *et al.* (2004) evaluated benomyl, difensaconazole, hydrogen dioxide, mancozeb and thiabendazole for their ability to inhibit the growth of four species of *Fusarium and* to enhance long-leaf pine seed germination(Pan *et al.* 2007). Maximum inhibition in mycelial growth was observed in the hexaconozole at 1000 ppm followed by other fungicides at the same concentration (Salma Begum *et al*. 2015) .

In vitro efficacy of fungicides on growth of Curvularia lunata

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In vitro efficacy of bioagents on growth of Fusarium moniliforme and Curvularia lunata

The results obtained on mycelial growth and inhibition of *F.moniliforme* and *Curvularia lunata* with five fungal and two bacterial agents are presented in Table 3 and depicted in Fig. 7,8,11 and 12, results revealed that all the bioagents evaluated exhibited fungistatic/antifungal activity against *Fusarium moniliforme* and *Curvularia lunata* with significant inhibited their growth over untreated control.

Among the bioagent/antagonist tested *Trichoderma viride* was found most effective with significantly least mycelial growth (12.4 mm) with highest mycelial growth inhibition (86.2 %) of *F.moniliforme* also least mycelial growth (17.9mm) with highest mycelial growth inhibition (80.0%) of *C.lunata* as compared to control. The second and third best antagonists were found *Trichoderma koningii*, *Trichoderma harzianum* against *F.moniliforme* with least mycelial growth of 15.7 mm and 17.9 mm and inhibition 82.5 % and 80.06% respectively whereas the second and third best antagonists were found *Trichoderma harzianum*, *Trichoderma koningii* against *C.lunata* with least mycelial growth of 24.0mm and 26.3mm and inhibition 73.3% and 70.7% respectively. These were followed by *Trichoderma hamatum*, *Gliocladium virens*, *Pseudomonas fluorescens*, *Bacillus subtilis* (26.3 and 40.0mm , 65.2 and 47.5mm,67.3 and 58.5mm,48.2 and 62.5mm) and (70.7 and 55.5%, 27.4 and 47.2%, 25.1 and 35.0%, 46.4 and 30.5%) of mycelial growth and it's inhibition of *F.moniliforme and C.lunata* respectively. *Pseudomonas fluorescens* was found comparatively less effective with maximum mycelial growth 67.36 and 62.5mm and minimum mycelial inhibition 25.1 and 30.5% against *F.moniliforme and C.lunata* respectively.

Results of the present study on antifungal activity of the *Trichoderma viride*, *Trichoderma harzianum, Trichoderma virens* and two bacterial antagonist viz., *Pseudomonas fluorescens* against *Fusarium moniliforme* are in conformity with those reported earlier by several workers. Raut (1999), Bhuvaneshwari and Rao (2001),Lambhate *et al* (2002), Sharma and Chandel (2003), Chirme and Padule (2005), Suryawanshi (2005), Kumar *et al.* (2007) reported *T. viride* as the most effective antagonist. Asha *et al*. (2011) evaluated *in vitro* ten isolates of *P. fluorescens* against *F. oxysporum*, causing wilt in tomato. Salma Begum *et al*. (2015) tested the antagonistic effect of four biocontrol agents in controlling the growth and sporulation of *Fusarium moniliforme, in vitro* for management of damping- off in cabbage seedlings*.*

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