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Management of Seed Borne Pathogens of Rice

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A B S T R A C T

Most of the rice diseases are transmitted by seeds which are the important limiting factor getting good quality seeds and good yield. Stored and freshly harvested rice seed of different varieties were collected from Agricultural farm of Orissa University of Agriculture and Technology, Bhubaneswar, Odisha to study the association of different fungal pathogens. The seeds were graded on the basis of intensity of discoloration. Among nine plant products evaluated against the pathogenic fungi *Fusarium fujikuroi*, *Curvurlaria lunata*, *Helminthosporium oryzae*, garlic extract alone and in combination with datura and neem leaf extract completely inhibited the growth of the fungi at 10% and 20% concentration. The biocontrol agents *Trichoderma viride* and *Bacillus subtilis* inhibited the growth of *F. fujikuroi* up to 78.11% and 78.7% respectively. *Pseudomonas fluorescens* inhibited 74.06% radial growth of *H. oryzae* which was more than *B. subtilis* (22.66% inhibition). 52.4% growth reduction was found by *T. viride* against *C. lunata* followed by *P. fluorescens* (51.8% reduction). Propiconazol 0.1%, Carbendazim+Mancozeb0.1%, Carboxin+Thiram 0.2% Tebuconazol 0.1% and copper oxychloride 0.3% completely inhibited the growth of all three pathogens.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple foods for more than half of the world's population (IRRI, 2006) and influences the livelihoods and economies of several billion people, and is grown in almost all the tropical and subtropical regions of the world.

Rice is cultivated in an area of 165 million hectares, the world rice production is now touching to 744.4 million tonnes (496.4 million tonnes, milled basis), (FAO 2014). Seed is the vital input in Agriculture. Good quality and viable seed is required for healthy and synchronous seedling which is prerequisite for successful crop establishment and uniform crop growth and development and finally the yield.

The three major aspects of seed quality are, a) genetic and physical purity, b) high germination percentage and vigor, and c) free from seed-borne diseases and insects (Seshu and Dadlani, 1988). Moreover, seeds of rice are imported and being sold without testing of health. Therefore, it is necessary to understand

the problem properly which affect the seed germination and take necessary steps need to be taken to overcome this huge amount of loss.

Materials and Methods

Collection of seed samples

Both stored and freshly harvested seed of different rice varieties were collected from Central Agricultural Research Farm, College of Agriculture, O.U.A.T, Bhubaneswar, Odisha during the Kharif season of 2014 and 2015.

Effect of various plant extracts on the growth inhibition of rice seed mycoflora

All the extracts were centrifuged at 10000 rpm for 10 minutes except garlic at 4000 rpm and the extracts were regarded as 100% and adjusted to different concentration as per need.

Poisoned food technique

The bio-efficacy of plant extracts were evaluated by poisoned food technique (Nene and Thapliyal, 1973). Ten, twenty ml of crude extracts were mixed with 90 and 80ml of molten potato dextrose agar medium so as to get 10% and 20% concentration respectively. Control was maintained without any plant extract.

The poisoned media was sterilized by autoclaving at 10 psi for 25 minutes. Twenty ml of media was poured into petri dish and allowed to solidify. Five mm culture disc was put on the middle of the solidified Petri Plate s. All the Plates were incubated at room temperature. Mycelia growth measurement was taken when maximum growth was observed in control plate. The efficacy was expressed as percentage inhibition of mycelia growth over control.

I= C−T/C×100 (Vincent, 1947) I = Percent inhibition C= Radial growth in control T = Radial growth in treatment

In-vitro Effect of various biocontrol agents on the growth inhibition of rice seed mycoflora

The efficacy of biocontrol agents were tested against casual organism by dual culture technique. Biocontrol agents like *Trichoderma viride, Trichoderma hamatum, and T. harzianum, Pseudomonas fluorescens* and *Bacillus subtillis* were tested against the isolated fungi by using dual culture technique.

In-vitro testing of various fungicides against seed borne pathogens of rice

Required amount of fungicides were added to pre sterilized potato dextrose agar medium. Twenty ml of poisoned medium was poured into sterilized Petri dishes. Mycelial discs of five mm from actively growing zone of ten days old culture were inoculated into each Plate and placed at the centre of Petri plate. Control was maintained without adding any fungicide. Each treatment was replicated thrice. The Plate s were incubated at 27±1°C temperature and radial growth of fungal mycelium was measured from both direction and radial growth was calculated. The data were analyzed statistically and efficacy of fungicide was expressed as percentage of inhibition of mycelia growth over control. The formula (Vincent, 1947) was used for calculation as mention above.

Results and Discussion

Efficacy of various plant extracts and their combination on the growth inhibition of different rice seed mycoflora

Nine plant extracts including combination of (garlic clove, datura and neem leaf extracts)

and neem oil were used for management of three seed borne pathogens of rice viz., *F. fujikuroi*, *H. oryzae* and *C. lunata* in the laboratory condition (Fig. 1a and b).

Efficacy of phyto extracts on the growth inhibition of *F. fujikuroi* on rice seed

The result indicated that there was significant differences among all the tested plant extracts and oil in both the 10% and 20% concentration were used against the pathogens. Garlic bulb extract alone and combination with datura and neem leaves extracts completely inhibited the radial growth of test pathogen in both 10 and 20% concentration.

Efficacy of phyto extracts on the growth inhibition of *H. oryzae* on rice seed

All the plant extract in both the concentration showed growth reduction of *H. oryzae*. Here also garlic bulb and combination with Datura and Neem leaves extracts completely inhibited the growth resulting 100% control. Neem leaves extracts (10%) induce least growth inhibition (4.58%) fallowed by onion bulb extract (10.68) Datura, Neem oil and Karanj, Turmeric exhibited similar pattern in controlling *F. fujikuroi*. But in 20% concentration datura, onion and neem oil exhibited similar pattern) (Table 1).

Onion bulb extracts and mint leaf extract, showed similar growth pattern with control treatment in 10% concentration. Datura, onion, neem oil and turmeric rhizome extracts exhibited similar growth inhibition in 20% concentration.

Efficacy of phyto extracts on the growth inhibition of *C. lunata* on rice seed

Significant differences were also observed among all plant extracts in reducing the radial growth of *C. lunata* in both 10% and 20% concentration. Here also garlic alone and combination with datura and neem leaf extracts control the test pathogen completely (100%). Mint, neem leaf and onion bulb extracts all (10 % concentration) grew in similar way as that of control Plate s. There was no significant difference observed in growth inhibition of the test pathogen among neem oil 45.47% and turmeric rhizome extracts 44.55% at (10%) concentration (Table 2).

The result indicated that there was significant differences among all the tested plant extracts and oil in both the 10% and 20% concentration were used against the pathogens. Garlic bulb extract alone and combination with datura and neem leaves extracts completely inhibited the radial growth of test pathogen in both 10 and 20% concentration.

Neem leaf extract (10%) recorded less growth inhibition (4.58%) followed by Onion bulb extract 10.68%. Asadi and Behroozin (1987) found similar result of growth inhibition *of F. solani, F.oxysporum and F. acuminatum* using Garlic bulb extract and Garlic bulb extract with datura and neem leaves completely inhibited the growth of *H. oryzae* resulting 100% control.

Onion bulb and Mint leaf extract showed similar growth pattern with control in 10% concentration. Lalitha *et al.*, (2014) conducted *in vitro* evaluation of essential oils of *P. nigram, S. album* against different seed borne pathogens and found 100% inhibition in radial growth of *Bipolaris oryzae*. In the current study 55.60% inhibition in radial growth was observed against *H. oryzae* (*B. oryzae*). The growth of *Curvularia lunata* was completely reduced by garlic bulb extract alone and combination with datura and neem leaf in both 10 and 20% concentration.

Efficacy of bio control agents against rice seed mycoflora

Efficacy of five bio control agents comprising *Trichoderma viride Trichoderma harzainum, Trichoderma hamatum, Pseudomonas fluorescens* and *Basillus subtilis* were evaluated against three seed mycoflora i.e. *F. fujikuroi, H. oryzae* and *C. lunata* in dual culture method. *Trichoderma viride* and *Bacillus subtilis* inhibited the growth *F. fujikuroi* up to 78.11%and 78.72% respectively. *T. hamatum* reduced the radial growth of *F. fujikuroi* (66.47%) which was less than *T. viride. Pseudomonas fluorescens* recorded highest (74.06%) inhibiting radial growth of *H. oryzae* which was more efficacious than *Bacillus sabtilis* (22.66% inhibition). Among three *Trichoderma sp., T. harzianum* had less bio control activity (27.1% reduction) with *T. hamatum* the highest (67.76% reduction). All the five bio control agents express reduced efficacy against *C. lunata*. Maximum 52.41% growth reduction was found by *T. viride* fallowed by *P. fluorescens (51.8%* reduction*).* Other three bio agents reduced the radial growth below 50% (Figure 2 and Table 3).

Bio efficacy of five bio control agents *Trichoderma viride*, *Trichoderma harzianum, Trichoderma hamatum, Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against three fungi i.e *Fusarium fujikuroi*, *Helminthosporium oryzae* and *Curvularia lunata* in dual culture method. *Trichoderma viride* and *Bacillus subtillis* inhibited the growth of *Fusarium fujikuroi* up to 78.11% and 78.7% respectively. *P. fluorescens* inhibited 74.6% radial growth of *H. oryzae* which was more than *Bacillus subtilis* (22.66% inhibition).

In inhibiting the radial growth of *H. oryzae,* among three *Trichoderma sp*., *T. hamatum* reduced up to 67.76% radial growth. *T. viride* caused 52.4% reduction of mycelial growth followed *Pseudomonas fluorescens* (51.8%reduction) against *Curvularia lunata.* Srinivas and Ramkrishan (2005) tested bio control effect of *T. viride* against seed borne pathogen of rice reduced radial growth of the pathogens.

Selvarraj and Anamalai (2015) used two species of *Trichoderma* against *Sarochladium oryzae* and found 96% inhibition of radial growth by *T. harzianum.* Halgearkar *et al.,* (2014) recorded significant suppression of mycelial growth of *Helminthosporium oryzae (D. oryzae)* by *T. viride* and *T. harzianum.* He also reported 97.77% inhibition by Bacillus *subtilis* against *C. lunata* fallowed *T. viride.* In the current study also *Bacillus subtilis* induced 42.41% growth inhibition.

Efficacy of various fungicides against the seed borne pathogens of rice

A total numbers of 12 different fungicides were tested against the radial growth of *F. fujikuroi, H. oryzae* and *C. lunata* for their efficacy *in-vitro* condition.

Effect on *F. fujikuroi*

It was evident from the data all the chemicals showed significantly different growth inhibition as compare to control.

Carbendazim 0.1%, propeconazole 0.1%, copper oxy chloride 0.3%, carbendazim + mancozeb 0.1% carboxin +thiram 0.2% and tebuconazole 0.1% completely inhibited the growth of *F. fujikuroi* exhibiting 100% growth inhibiting over control.

Thiophenate methyl 0.1% and hexaconazole 0.2% also reduced the growth of test pathogens to 94.4% and 89.04% respectively. Other fungicides were also good in comparison to control (Table 4 and Figure 3a).

Table.1 Effect of various plant extracts (10%) on the growth inhibition of rice seed mycoflora

Treatments			Radial growth (mm)			
	Fusarium	%	*H. oryzae*	%	*Curvulari*	%
	fujikuroi	inhibition		Inhibition	*a lunata*	inhibition
		over		Over		over
		control		Control		control
Datura	35.83	53.16	35.83	58.58	29.33	59.17
	(6.02)		(6.02)		(5.45)	
Garlic bulb	0.00	100	0.00	100	0.00	100
	(0.71)		(0.71)		(0.71)	
Mint	63.67	16.77	85.00	1.73	68	5.33
	(8.00)		(9.24)		(8.40)	
Neem leaves	73.00	4.58	71.67	17.14	70.0	2.55
	(8.57)		(8.49)		(8.40)	
Onion bulb	68.33	10.68	84.67	2.12	69.17	3.70
	(8.30)		(9.23)		(8.33)	
Karanj leaves	59.00	22.88	70.67	18.30	53.00	26.21
	(7.65)		(8.43)		(7.26)	
Neem oil	37.50	50.98	55.33	36.03	39.17	45.47
	(6.09)		(7.46)		(6.29)	
Turmeric	52.17	31.80	45.83	47.02	39.83	44.55
Rhizomes	(7.25)		(6.81)		(6.34)	
Garlic bulb+	0.00	100	0.00	100	0.00	100
neem + datura	(0.71)		(0.71)		(0.71)	
Control	76.50	-	86.50	-	71.83	-
	(8.77)		(9.33)		(8.49)	
SEm(±)	0.32		0.15		0.15	
CD (5%)	0.97		0.47		0.45	

*Figures in the parentheses indicates √ + 0.5 transform values

Table.2 Effect of various plant extracts (20%) on the growth inhibition of rice seed mycoflora

Treatments			0Radial growth (mm)			
	Fusarium fujikuroi	% inhibition over control	*H. oryzae*	% inhibition over control	*Curvulari a lunata*	% inhibition over control
Datura	27.17	64.79	27.33	61.69	21.00	71.94
	(5.25)		(5.24)		(4.63)	
Garlic bulb	0.00	100	0.00	100	0.00	100
	(0.71)		(0.71)		(0.71)	
Mint	61.33	20.53	63.17	11.44	67.67	9.57
	(7.86)		(7.98)		(8.25)	
Neem leaves	62.67	18.79	37.33	46.67	59.00	21.15
	(7.91)		(6.13)		(7.71)	
Onion bulb	27.83	63.94	27.83	60.98	39.67	46.99
	(5.30)		(5.31)		(6.31)	
Karanj leaves	14.67	80.99	23.67	66.82	49.83	33.41
	(3.87)		(4.89)		(7.09)	
Neem oil	33.33	56.81	31.67	55.60	27.33	63.48
	(5.79)		(5.65)		(5.26)	
Turmeric	44.67	42.11	31.17	56.30	21.50	71.27
Rhizomes	(6.69)		(5.62)		(4.69)	
Garlic bulb+	0.00	100	0.00	100	0.00	100
neem + datura	(0.71		(0.71)		(0.71)	
Control	77.17	-	71.33	-	74.83	-
	(8.81)		(8.47)		(8.68)	
SEm(±)	0.29		0.28		0.22	
CD (5%)	0.87		0.85		0.66	

*Figures in the parentheses indicates √ *+ 0.5* transform values

Table.3 Effect of various biocontrol agents on the growth inhibition of rice seed mycoflora

	Treatments			Radial growth (mm)			
		F. fujikuroi	*% inhibition*	*H. oryzae*	*% inhibition*	*C. lunata*	*% inhibition*
T1	Control	88.5	-	71.33	-	90.00	-
T2	*Trichoderma*	19.37	78.11	46.17	35.27	42.83	52.41
	Viride						
T3	*T. harzianum*	41.67	52.92	52.0	27.10	48.50	46.11
T4	*T. hamatum*	29.67	66.47	23.0	67.76	52.83	41.30
T5	*P. fluorescens*	39.00	55.93	18.50	74.06	43.33	51.86
T6	*Bacillus*	18.83	78.72	55.17	22.66	51.83	42.41
	Subtilis						

Table.4 Effect of various fungicides on the growth inhibition of rice seed mycoflora

Treatment	Dosage			Radial growth (mm)			
S		*F.*	%	*H. oryzae*	%	*C.*	% inhibition
		fujikuroi	Inhibition		inhibition	*lunata*	over control
			over control		over control		
*T1	0.3%	18.20	74.72	17.1	79.35	15.7	82.55
		(4.30)*		(4.19)*		(4.01)	
T2	0.2%	22.33	68.98	0.0	100	0.0	100
		(4.78)		(0.71)			
T3	0.1%	0.0	100	24.33	70.62	48.5	46.11
		(0.71)		(4.97)		(6.99)	
T4	0.1%	4.03	94.40	11.0	86.71	31.0	65.55
		(2.12)		(3.19)		(5.61)	
T5	0.1%	0.0	100	0.0	100	0.0	100
		(0.71)		(0.71)		(0.71)	
T6	0.2%	7.53	89.04	0.0	100	0.0	100
		(2.83)		(0.71)		(0.71)	
T7	0.3%	29.33	59.26	18.0	78.26	0.0	100
		(5.46)		(4.29)		(0.71)	
T8	0.15%	12.90	82.08	14.0	83.09	22.5	75
		(3.63)		(3.18)		(4.76)	
T9	0.3%	0.0	100	0.0	100	0.0	100
		(0.71)		(0.71)		(0.71)	
T10	(0.1%)	0.0	100	0.0	100	0.0	100
		(0.71)		(0.71)		(0.71)	
T11	(0.2%)	0.0	100	0.0	100	0.0	100
		(0.71)		(0.71)		(0.71)	
T12	(0.1%)	0.0	100	0.0	100	0.0	100
		(0.71)		(0.71)		(0.71)	
T13		72.0		82.83		90.0	
		(8.45)		(9.12)		(9.51)	
SEm(±)		0.24		0.25		0.15	
CD (5%)		0.72		0.74		0.45	

*Figures in the parentheses indicates √ *+ 0.5* transform values

*T1- Cymoxanil 8% + Mancozeb 64% (0.3%), T2- Propineb (0.2%), T3- Carbendazim (0.1%), T4- Thiophanate methyl 70% (0.1%),T5- Propiconazole 25% EC (0.1%), T6-Hexaconazole 5% EC (0.2%), T7- Azoxystrobin 23% SC (0.3%), T8- Difenconazole 25% EC (0.15%), T9- Copper oxy chloride 50%WP (0.3%), T10- Carbendazim + Mancozeb WP (0.1%), T11- Carboxin + Thiram (0.2%), T12- Tebuconazole (0.1%).

Fig.1a Efficacy of different plant extracts against radial growth of Seed mycoflora- a, b- *F. fujikuroi*

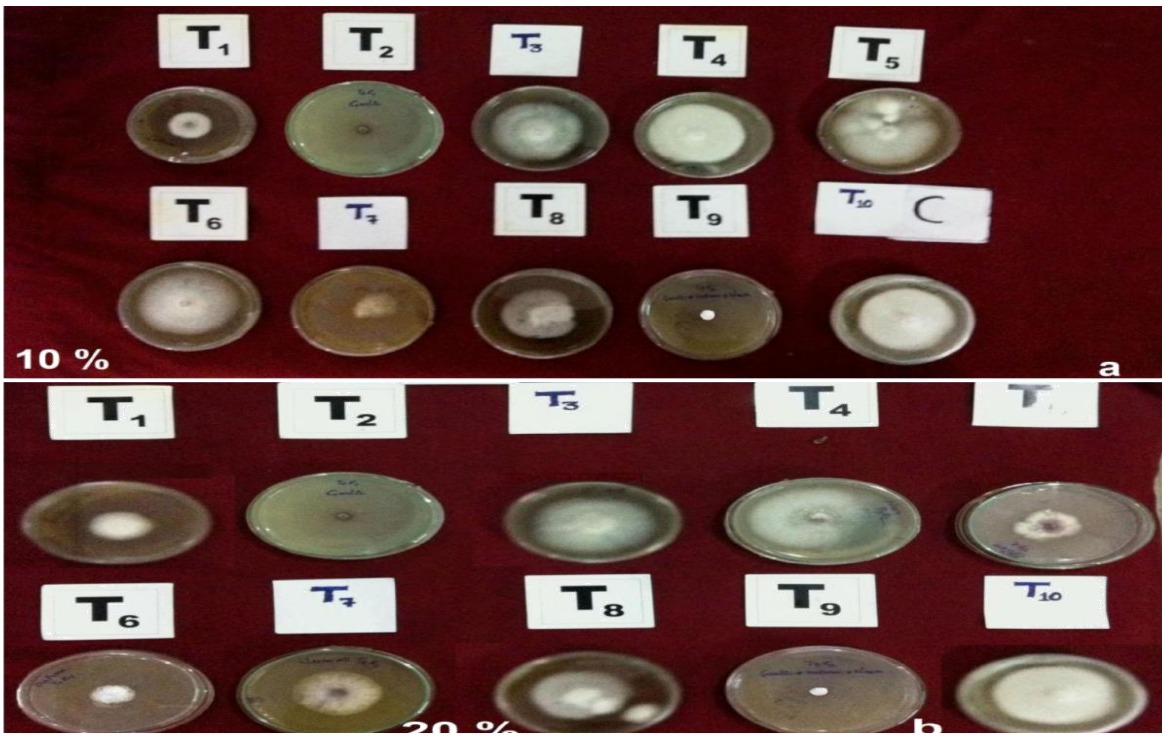

Fig.1b Efficacy of different plant extracts against radial growth of seed mycoflora c, d- *C. lunata*

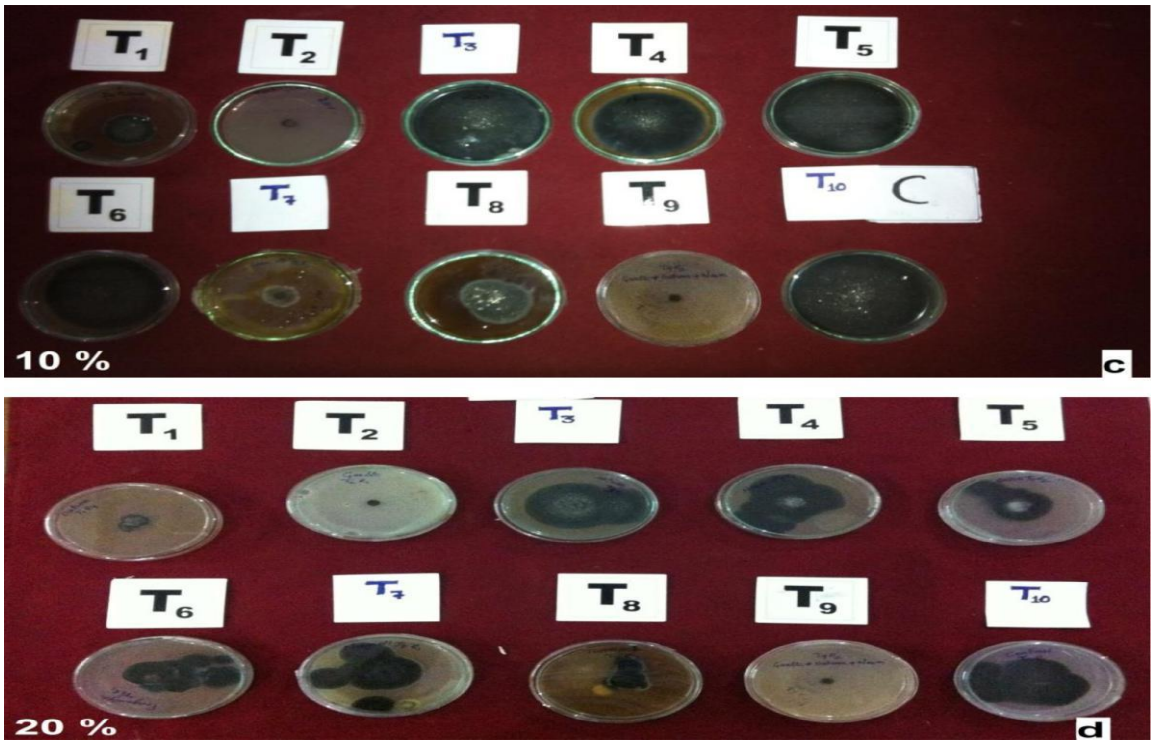

Fig.2 Efficacy of different plant bio-agents against radial growth of different seed mycoflora a- *F. fujikuroi*, b- *H. oryzae* c- *C. lunata*

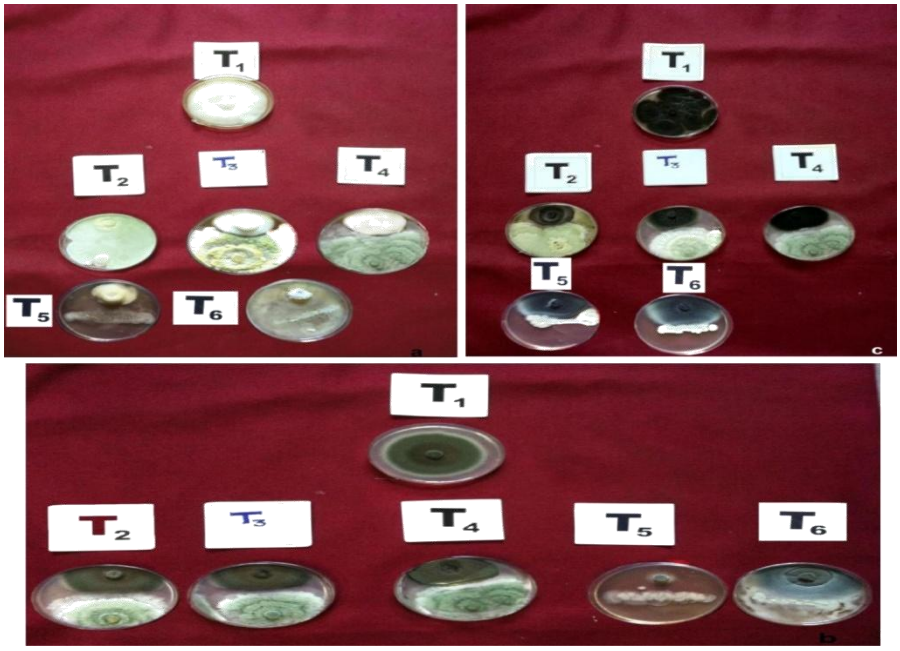

Fig.3 Effect of various chemicals on the radial growth of differnet seed mycoflora a= *F. fujikuroi*, b= *H. oryzae*, c= *C. lunata*

Effect on *H. oryzae*

All the chemicals exhibited significantly different reaction and inhibiting the radial growth of *H. oryzae* as compare to control.

Propineb 0.2 %, propinconazole 0.1%, hexaconazole 0.2%, copper oxy chloride 0.3%, carbendazim + mancozib 0.1%, carboxine + thiram 0.2% and tebuconazole 0.1% completely inhibited the growth of test pathogens. Thiophenate methyl 0.1% and difenoconazole 0.15% exhibited similar reaction against *H. oryzae* and so cymoxanil 8% + mancozeb 0.3% and azoxystrobin 0.3% showing at par reaction (Table 4 and Figure 3b).

Effect on *C. lunata*

Similar trends were also observed in *C. lunata* as in the above in case of *H. oryzae*. Here Azoxystrobin 0.3% also induce 100% growth inhibition of *C. lunata*. Cymoxanil + mancozib 0.3% was found to the next best fungicides against *C. lunata* showing 82% growth inhibition against control (Table 4 and Figure 3c).

A total number of twelve different fungicides were tested against *Fusarium fujikuroi, Helminthosporium oryzae and Curvularia lunata for their* efficacy. Carbendazim 0.1%, Tebuconazol 0.1%, copper oxychloride 0.3%, Carbendazim+Mancozeb 0.1% and Carboxin+Thiram 0.2% completely inhibited the growth of *F. fujikuroi*. Thiophanate methyl 0.1% and Hexaconazole 0.2% also reduced the growth of test pathogen to 94.4% and 89.4% respectively Propineb 0.2%, Propiconazol, Hexaconazol 0.2% copper oxychloride 0.3%, Carboxin+Thiram 0.2% Carbendazim+Mancozeb 0.1%, and Tebuconazol 0.1 % completely inhibited the growth of *H. oryzae* in *in vitro* conditions. Butt *et al.,* (2011) reported marked suppression of *Helminthosporium sp*. by 50% using Tabesinum, Mancozeb and *Curvularia sp*. using Topsin M and mancozeb. The growth of *C. lunata* was also completely inhibited by above fungicides along with Azoxystrobin 0.3% inducing 100 % growth inhibition, Propineb 0.2% was less effective against *F. fujikuroi* inducing 68.98%. Similarly Carbendazim was less effective in controlling *H. oryzae* and *C. lunata*. Copper oxychloride 0.3%, Carbendazim+Mancozeb 0.1% Propiconazol 0.1%, Carboxin+Thiram 0.2%, Tebuconazol 0.1 % were found to be best fungicides imparting 100% growth inhibition against all the three pathogens.

Nine plant extracts were tested against three pathogens i.e *F. fujikuroi*, *C. lunata* and *H. oryzae* both in 10 and 20 per cent concentration. Garlic bulb extract alone and in combination with datura and neem leaf extract completely inhibited the mycelial growth of all the three pathogens, in both concentration followed by datura leaf extract.

Three species of *Trichoderma* and bacterial biocontrol agents i.e. *Pseudomonas fluorescens*, and *Bacillus subtilis* were tested against *F. fujikuroi*, *H. oryzae* and *C. lunata* in dual culture method. *T. viride* and *B. subtillis* inhibited the growth of *F. fujikuroi* up to 78.11% and 78.7% respectively. *P. fluorescens* inhibited 74.06% radial growth of *H. oryzae* which was more than *B. subtilis* (22.66% inhibition). 52.4% growth reduction was found by *T. viride* against *C. lunata* followed by *P. fluorescens* (51.8% reduction).

Twelve different fungicides were tested against *F. fujikuroi*, *H. oryzae* and *C. lunata* in poison food technique. Propiconazol 0.1%, Carbendazim + Mancozeb 0.1%, Carboxin + Thiram 0.2% Tebuconazol 0.1% and copper oxychloride 0.3% completely inhibited the growth of all three pathogens. Thiophanate methyl 0.1% reduced the radial growth of *F. fujikuroi* and *H. oryzae* up to 94.4% and 86.71% respectively.

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