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In vitro studies were carried out to evaluate the effect of *T. asperellum* and fungicides against important plant pathogens. Dual culture of *T. asperellum* against four plant pathogens viz. *Fusarium oxysporum* f. sp. *lycopersici*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Alternaria solani* revealed maximum inhibition of 77.76% was recorded on *A. solani*. Following poisoned food technique of fungicides against the pathogens at 0.1%, 0.2% and 0.3% concentrations, 100% inhibition of *S. rolfsii* was observed in all the concentration of Mancozeb and Carbemdazim @ 0.3%. Propiconazole at all concentration against *A. solani* showed 100% inhibition. Kitazin and Chlorothalonil at all concentration showed 100% inhibition against *R. solani.* Copper oxychloride was recorded to be more effective than Thiophanate methyl at all concentration against *F. oxysporum* f. sp. *lycopersici.* In case of compatibility test of *T. asperellum* with the fungicides, the results revealed that Mancozeb @ 0.1% and 0.2% was fully compatible whereas Azoxystrobin, Chlorothalonil, Mancozeb and Copper oxychloride @ 0.1% and 0.2% were found to be comparatively compatible. It has also been found out that progressive increase in concentration of fungicides was found to inhibit the mycelial growth of *T. asperellum* resulting to be non- compatible.

Keywords: Fungicides, plant pathogens, *Trichoderma asperellum*

INTRODUCTION

A major loss faced by agricultural sector is due to plant diseases (Kaur and Sharma, 2021). Crop losses due to diseases have been estimated between 20% to 40% despite crop protection measures (Savary *et al.* 2019) which will hinder the efforts for meeting the 60% increase in demand of food by 2050 (Van Esse *et al*., 2020). A soil inhabitant fungus, *Trichoderma* is considered as the most important fungal Bio Control Agent – BCA (Asad, 2022). Today, more than 25 isolates of *Trichoderma* species have been thoroughly studied and have shown great potential for significantly inhibiting phytopathogen growth (Poveda, 2021). Along with the fungicidal action it is reported to act as plant growth promoter (Bora *et al*. 2020). To reduce the losses, chemical fungicides cannot be neglected in spite of their adverse effect on the environment; fungicides still play an important role in the Integrated Disease Management (IDM). To reduce the environmental pollution, human health hazards cause by fungicides and for efficient plant disease management, compatibility of potential biocontrol agent with fungicides is essential (Thoudam and Dutta, 2014). To reduce the yield loss while improving food quality and, at the same time reducing the environmental population, the present study deals with the effect of antagonistic activity of *Trichoderma asperellum* against important plant pathogen and also identifying the most compatible fungicide with *Trichoderma asperellum*.

MATERIALS AND METHODS

The plant pathogens *Alternaria solani* (Early blight of tomato), *Rhizoctonia solani* (Fruit rot of tomato), *Fusarium oxysporum* f.sp. *lycopersici* (Wilt of tomato) and *Sclerotium rolfsii* (Root rot of bean) had been selected as these are important plant pathogens in Nagaland. Pure culture of *Trichoderma asperellum* and plant pathogens was obtained from the Department of

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Biocontrol activities of *T. asperellum* against the aforementioned pathogens were performed to test its antagonistic potential following Dual culture technique (Dennis and Webster, 1971) on PDA medium.

Effect of activity of fungicides against important plant pathogens

The efficacy of fungicides Carbendazim 50% WP and Mancozeb 75% WP against *Sclerotium rolfsii*; Chlorothalonil 78.12% WW and Kitazin 48% EC against *Rhizoctonia solani*; Copper oxychloride 50% WP and Thiophanate methyl 70% WP against *Fusarium oxysporum* f. sp.

lycopersici; Azoxystrobin 23% EC and Propiconazole 25% EC against *Alternaria solani* were evaluated following Poisoned Food Technique (Nene and Thapliyal, 1993) at three different concentrations 0.1%, 0.2% and 0.3%. Biocontrol activity of *Trichoderma asperellum* against the afore mentioned pathogens were performed to test its antagonistic potential following dual culture technique (Dennis and Webster, 1971) on PDA medium. The percent inhibition (PI) zone of pathogen was calculated using the following formula given by Vincent (1947).

$$PI = \frac{\text{Growth in control plates (C)-Growth in treated plates (T)}}{\text{Growth in control plates (C)}} \times 100$$

Investigation of compatibility of *Trichoderma asperellum* with fungicides

The afore mentioned fungicides were tested for their compatibility with *Trichoderma asperellum* at 0.1%, 0.2%, and 0.3% along with an untreated control. The per cent inhibition of *T. asperellum* was calculated based on the diameter of growth of the colony by using the formula given by Vincent (1947).

RESULTS AND DISCUSSION

Antagonistic activity of Trichoderma asperellum against plant pathogens

The results revealed that *T. asperellum* was effective against all the tested plant pathogens. Maximum inhibition of 77.76 % was recorded from *A. solani* which was found to be statistically at par with *R. solani* (73.26%). Per cent inhibition of *F. oxysporum* f. sp. *lycopersici* was 62.93% and was statistically at par with *S. rolfsii* (57.7%) after 4 days of *T. asperellum* inoculation (Table 1).

The present experiment on antagonistic activity of *T. asperellum* against plant pathogens was in agreement with the work of Manjunath *et al.* (2017) who reported that *T. asperellum* was effective against the plant pathogens *S. rolfsii* Sr1, *S. rolfsii* Sr3, *F. oxysporum* f. sp. *lycopersici* and *A. solani*. Wu *et al.* (2017) concluded that *T. asperellum* competes with the pathogens for limited space and nutrients, which results in poor growth of pathogens. As reported by Silva *et al.* (2011), *T. asperellum* utilizes chitin as carbon source and produce N- acetylglucosaminidase, â- 1, 3-glucanase, chitinase and protease which might have played a role in the process of antagonizing the fungal pathogens.

Effect of fungicides on different plant pathogens

For the present investigation, four plant pathogens were selected *viz*. *Alternaria solani, Sclerotium rolfsii, Fusarium oxysporum* f. sp. *lycopersici and Rhizoctonia solani* which commonly occur in Nagaland (Fig. 1). Two selected fungicides for each pathogen at concentrations *viz*. 0.1%, 0.2% and 0.3% were tested and evaluated. Results are presented in Table 2. The results obtained after poisoned food technique revealed that complete inhibition of *S. rolfsii* was observed with Mancozeb at all the concentration and also Carbendazim @ 0.3%. Carbendazim @ 0.1% and 0.2% was found to be less effective against *S. rolfsii*. The present investigation on the effect of fungicides on different plant pathogens found corroborate with the work done by Siddique *et al.* (2016) who reported that Bavistin gave the highest inhibition percentage of S. *rolfsii*. A similar observation against *S. rolfsii* was reported by Banyal and Singh (2007) and also observed that Carbendazim managed *Sclerotinia sclerotiorum* up to 70.8%, thus increasing the yield of bean.

In case of *R. solani* under *in vitro* test, Kitazin and Chlorothalonil at all the concentrations showed 100% inhibition against *R. solani.* These results was also found in agreement with findings of Monga and Raj 2016 who reported that Kitazin 1000 ppm gives 100% inhibition when tested against *R. solani* while Rajput *et al.* (2016) and Kumar *et al.* (2018) concluded that Chlorothalonil was highly effective against *R. solani.*

The growth of fungal pathogen *F. oxysporum* f. sp. *lycopersici* was found to be inhibited completely by Copper oxychloride at all concentration whereas the inhibition percentage by Thiophanate methyl was found to be 83.33%, 73.67% and 83.33% @ 0.1% 0.2% and 0.3% respectively. These findings corroborate the work done by Hedge *et al.* 2017 concluded that Thiophanate methyl and Copper oxychloride were effective in controlling *F. oxysporum.* However, these findings contradict the work done by several workers *viz.* Rajput *et al*. (2006), Sumana *et al.* (2012), Maitlo *et al*. (2014) who reported that Thiophanate methyl significantly reduced the mycelial growth of *F. oxysporum.*

Propiconazole at all concentration showed 100% inhibition and Azoxystrobin @ 0.3% showed highest inhibition of 89.2% against *A. solani*. Hence, Propiconazole was recorded to be more effective than Azoxystrobin against *A. solani* under *in vitro*. The present observation was also in accordance with the findings of Singh *et al.* (2018) who reported that Propiconazole gives cent percent inhibition of *A. solani* at 50, 100, 150 and 250 ppm. Arunakumar (2006) also reported that Azoxystrobin gives maximum inhibition of 78.60 % @ 0.1% concentration when tested against *A. solani.*

Compatibility Test of Trichoderma asperellum with different fungicides

Compatibility test of *Trichoderma asperellum* was evaluated against five systemic fungicides and three non-systemic fungicides at three different concentrations (0.1%, 0.2% and 0.3%). The percent inhibition over control was calculated seven days after inoculation, when the control plate was fully grown (Fig. 2; Table 3). Carbendazim even at the lowest concentration completely inhibited the growth of *T. asperellum* suggesting non- compatibility. Similar observations were recorded with Propiconazole, Thiophanate methyl and Kitazin. Azoxystrobin, Chlorothalonil, Mancozeb and Copper oxychloride at lower concentration (0.1% and 0.2%) did not completely inhibit the growth of the *T. asperellum* and were found to be comparatively compatible. But progressive increase in concentration of fungicides was found to inhibit the mycelial growth of *T. asperellum* resulting to be non- compatible.

The present investigation results on compatibility Test of *Trichoderma asperellum* with different fungicides were found in agreement with the work of Kumar *et al*. (2019) who concluded that Carbendazim 50% WP and Propiconazole 25% EC showed 100% inhibition followed by Thiophanate methyl 70% WP which showed 100% inhibition at higher concentration (500 and 1000ppm) WP and Mancozeb 75% WP was compatible at lower concentration when tested against *Trichoderma* isolates at 50, 100, 250, 500, 1000 ppm concentrations. Shashi kumar *et al*. (2019) also found Azoxystrobin 23% SC, and Mancozeb 75% WP compatible while Carbendazim 50% WP and Chlorothalonil 75%

Table 1: Antagonistic activity of *T. asperellum* against important plant pathogens

Treatment	Plant pathogens	Percent inhibition of mycelial growth*
T_{01}(control)	*Sclerotium rolfsii*	0
T_1	*Sclerotium rolfsii*	62.93 (52.49)
T_{02}(control)	*Rhizoctonia solani*	0
T_2	*Rhizoctonia solani*	57.7 (49.47)
T_{03}(control)	*Fusarium oxysporum* f.sp. *lycopersici*	0
T_3	*Fusarium oxysporum* f.sp. *lycopersici*	73.26 (58.88)
T_{04}(control)	*Alternaria solani*	0
T_4	*Alternaria solani*	77.76 (61.98)
SEm±		2.37
CD (p=0.05)		7.11

Values in parenthesis are angular transformed values
*Mean of three observations

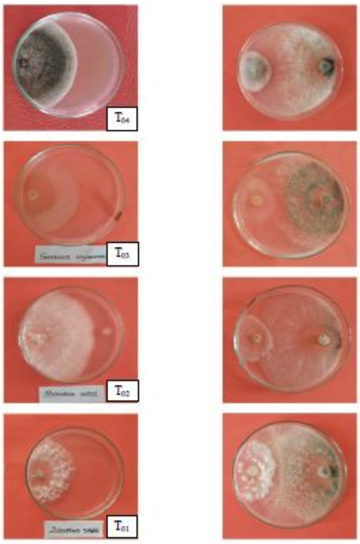

Fig 1: Effect of antagonistic activity of *T asperellum* against important Plant Pathogens

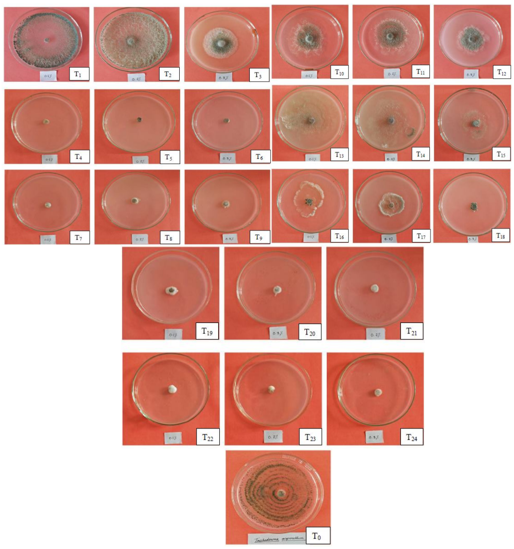

Fig 2: Compatibility Test of *Trichoderma asperellum* with different fungicides

Table 2: Effect of fungicides on different plant pathogens

Pathogen	Fungicides	Concentration	Inhibition percentage*
Sclerotium rolfsii	Control		0
	Carbendazim 50% WP	0.1%	26.23 (30.57)
		0.2%	58.1 (49.67)
		0.3%	100 (90.00)
	Mancozeb 75% WP	0.1%	100 (90.00)
		0.2%	100 (90.00)
		0.3%	100 (90.00)
SEm±			2.17
CD (p=0.05)			6.61
Rhizoctonia solani	Control		0
	Chlorothalonil 78.12% WP	0.1%	100 (90)
		0.2%	100 (90)
		0.3%	100 (90)
	Kitazin 48% EC	0.1%	100 (90)
		0.2%	100 (90)
		0.3%	100 (90)
SEm±			0
CD (p=0.05)			0
Fusarium oxysporum f. sp. *lycopersici*	Control		0
	Copper oxychloride 50% WP	0.1%	100 (90)
		0.2%	100 (90)
		0.3%	100 (90)
	Thiophanate methyl 70% WP	0.1%	83.3 (65.88)
		0.2%	73.67 (59.12)
		0.3%	83.3 (65.90)
SEm±			0.55
CD (p=0.05)			1.68
Alternaria solani	Control		0
	Azoxystrobin 23% EC	0.1%	85.86 (67.92)
		0.2%	88.06 (69.79)
		0.3%	89.23 (70.84)
	Propiconazole 25% EC	0.1%	100 (90)
		0.2%	100 (90)
		0.3%	100 (90)
SEm±			0.34
CD (p=0.05)			1.03

Values in parenthesis are angular transformed values
*Mean of three observations

Table 3: Compatibility Test of *Trichoderma asperellum* with different fungicides

Treatment	Concentration	Mean diameter of inhibition zone (cm)	Inhibition percentage*
T_0 - Control		9	0 (0)
T_1 - Mancozeb 75% WP	0.1%	9	0 (0)
T_2 - Mancozeb 75% WP	0.2%	9	0 (0)
T_3 - Mancozeb 75% WP	0.3%	5.5	42 (40.39)
T_4 - Carbendazim 50% WP	0.1%	0	100 (90)
T_5 - Carbendazim 50% WP	0.2%	0	100 (90)
T_6 - Carbendazim 50% WP	0.3%	0	100 (90)
T_7 - Thiophanate methyl 70% WP	0.1%	1.3	85.13 (67.34)
T_8 - Thiophanate methyl 70% WP	0.2%	1.2	86.96 (68.88)
T_9 - Thiophanate methyl 70% WP	0.3%	1.8	81.43 (64.53)
T_{10}- Azoxystrobin 25% SC	0.1%	6.63	26.26 (30.80)
T_{11} - Azoxystrobin 25% SC	0.2%	5.53	38.5 (38.34)
T_{12} - Azoxystrobin 25% SC	0.3%	4.71	47.4 (43.50)
T_{13} - Copper oxychloride 50% WP	0.1%	6.05	32.86 (34.95)
T_{14} - Copper oxychloride 50% WP	0.2%	5.61	37.53 (37.59)
T_{15} - Copper oxychloride 50% WP	0.3%	4.11	54.2 (47.41)
T_{16} - Chlorothalonil Tech. 78.12% WW	0.1%	2.9	58.66 (50.01)
T_{17} - Chlorothalonil Tech. 78.12% WW	0.2%	3.73	66.9 (54.97)
T_{18} - Chlorothalonil Tech. 78.12% WW	0.3%	1.58	82.46 (65.29)
T_{19} – Kitazina.i 48% EC	0.1%	1.71	80.9 (64.16)
T_{20} – Kitazina.i 48% EC	0.2%	1.18	87.03 (68.90)
T_{21} – Kitazina.i 48% EC	0.3%	1.25	86.2 (68.21)
T_{22} - Propiconazole 25% EC	0.1%	1.08	88.13 (69.86)
T_{23} - Propiconazole 25% EC	0.2%	1	88.9 (70.54)
T_{24} - Propiconazole 25% EC)	0.3%	0.7	89 (70.64)
SEm±			1.28
CD (p=0.05)			3.65

Values in parenthesis are angular transformed values*Mean of three observations

WP were found to inhibit the growth of *Trichoderma* spp. Manjunath *et al.* (2017) also reported that, *T. asperellum* showed 100% compatibility with Azoxystrobin and Mancozeb @ 100, 200, and 300 ppm. The compatible fungicides are assured to play an important role in the IDM strategy as to improve plant health and reduce environmental health hazards.

CONCLUSION

The bioagent *Trichoderma asperellum* was effective against the growth of soil and seed borne plant pathogens. The fungicides tested for each plant pathogen was also found effective. This can be incorporated in Integrated Plant Disease Management Strategy as it inhibits the growth of plant pathogens. Correct dose of application of fungicides helps to prevent pathogen resistance problem, human health hazards, environmental pollution and thus, effective for long term basis and better results.

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