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COMPATIBILITY OF TRICRODERMA VIRIDE AND ITS INTERACTION WITH DIFFERENT FUNGICIDES

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Abstract— **Triehoderma viride can thrive in diverse environmental conditions as aggressive colonizers of soil and the roots of plants and act as natural bioagent to protect plants from infection by soil-borne fungal pathogens. Laboratory experiments were conducted to test the possibility of combining fungicides with Triehoderma viride to work out their compatibility to devise a suitable integrated management of soil borne plant diseases. Six fungicides Blitox, Thiophenate methyl, Roxiltabucanazole, Ridomil, Bavistin and Captan were evaluated at different concentration. Present investigation suggests that compatible fungicides can be used with Triehoderma in an IDM package to control soil borne plant pathogens.**

Index terms- **Triehoderma viride, Fungicides, Plant diseases, Plant pathogens,**

I. INTRODUCTION

Decades of laboratory experiments on biological control has led to the development of potential antagonistic fungi Trichoderma spp. There are several reports available in the literature indicating successful biological control of Sclerotiumrolfsii, Sclerotlnia, Pythium Aphanidermatum, Rhizoctonia solani, R.bataticola, Sclerotinia sclerotiorumand Fusarium by Trichodenna and Gliocladium spp. under laboratory and field conditions. Besides controlling plant diseases, plant growth promotion activities have also been attributed by these biocontrol agents. Better exploitations of these biocontrol agents need technological advances in mass production of bioagents, their delivery system and standardization of shelf life of bio formulations. The ecofriendly approach to control plant disease includes use of cultural practices and biorational (bioagents) approach. Different cultural methods such as field sanitation, destruction of alternate host, incorporation of organic amendment can control the disease to great extent. In 1970, Kozaka (1) was reported that early sowing and transplanting encourage early development of disease due to suitable weather condition while encouragement of spread of disease was reported due to high seed rate and plant density.

Nowadays more emphasis is being given to reduce the use of chemical and switch over to non chemical means of plant disease control such as biological control. There are many reasons behind this, such as evidences of pesticide residues in food, developments of fungicide resistance etc. In this context, the Trichoderma spp. has been evoked as a potential biological control agent which can grows very fast and successfully competes with plant pathogens in soil or in seed surface. Several in vitro screening of Trichoderma spp. supported the great potential of Tricoderma spp. to inhibit the mycelial growth. R. solani (2), concluded that it is more important to develop a suitable method for mass production of inoculums, preparation of suitable formulations and method application for successful biological control. There are some reports available in the literature indicating successful biological control of sheath blight using fungal bioagents, applied as seed treatment, oil treatment and foliar spray. Soil-borne diseases are consequence from the reduction of biodiversity of soil antagonistic organisms. Fungicide applications to soil, kills important beneficial fungi and also weakens the natural antagonistic activity (3).

Trichoderma species as a potential biological control agent. In an IDM package, incorporation of natural products provides a viable solution to the environmental problems caused by synthetic pesticides. Identification of these compounds and their further testing may be an effective approach to minimise the use of hazardous chemicals (4).To develop an effective disease management programme, the compatibility of potential bioagents with fungicides. Combination of chemicals and compatible bioagents in an IDM strategy protects the seeds and seedlings from soil-borne and seed-borne inoculums (5). Integration of compatible bioagent with pesticides may enhance the effectiveness of disease control and provide better management of soil borne diseases (6). The combination of biological control agents with fungicides would provide similar disease suppression as achieved with higher fungicide use (7).

II. MATERIAL AND METHODOLOGY

The present investigation was carried out at Biotech lab. Department of Biotechnology Seth G.L. Bihani S.D. (P.G.) College, Sri Ganganagar. Pure culture of T. viride was collected from the Department of Pathology, Agriculture

Research Station Sri Ganganagar Rajasthan. Compatibility tests were conducted under in vitro condition to find out safer fungicides against Trichoderma. Six fungicides viz Blitox, Thiophenate methyl, Roxiltabucanazole, Ridomil, Bavistin and Captan.

A. Sub culturing of Bioagents

The pure culture of bioagent(T. viride) was studied for their morphological characters. Eight days old pure culture raised on 2 % PDA was used. Mycelium and fruiting bodies were stained in 1% aqueous cotton blue and mounted in Amman's mounting medium (Lactophenol) for observation and measurements. To confirm the identity of bioagents, morphological characters were compared with stock culture maintained in Plant Pathology laboratory of Agricultural station, Sri Ganganagar.

*B. Compatibility with in different species of Tricoderma was studied using dual cultural technique***.**

- *Inhibition by individual bioagent:*-To study inhibition by individual bioagent, 20 ml of PDA was poured in 9 cm culture plates. The agar medium in the culture plates was seeded with the potential antagonist (8 mm culture disc of 4 days old culture) opposite each other near the periphery of petriplates. Each treatment had 3 replications. The medium inoculated with the (T.viride) alone served as a control. The plates were then incubated in B.O.D. at 25 ± 2°C. After every 24 hours of inoculation, the diameter of mycelium growth of both antagonist was measured.The growth of these test fungi is compared with control. The percent inhibition of individual bioagent was calculated by using following formula:

colony diameter of T. hiazianum in treatment - Colony diameter of T. viride in I real met n

colony diameter of T. hiazianum in control

For calculating inhibition of T.viridethe formula was reversed by substituting value of T.harzianum with T. viride

- *Over growth:*- Over growth of one species on other was observed and recorded by sign of + and - comparative bases as under: No overgrowth (-) Average overgrowth (+) Good overgrowth (++) Very good over growth (+++)
- *Colony character*: - Colony character of each Trichoderma spp. was recorded by observing control plate of each spp. individually. Observations were recorded on colony character, growth pattern (floppy, airy, densed, compressed) and growth habit (even growth/undulation etc.)To study non target effect of different fungicides on the mycelial growth of Trichoderma viride.
- *Procedure***:-** These studies were conducted using poison food technique Different fungicides in different concentration (ppm) i.e. 500, 1000, 1500 and 2000 were mixed with PDA before sterilization to study the inhibitory effect against mycelial growth of T. harzianum using poision food technique (Dhingra&Sinclain, 1944). After cooling, the plates were incubated with bit of trichodrama in the center. Mycelial growth of Trichodermawas then recorded after 24 hr., 48 hr., 72 hr., 96 hr. of incubation at 25 ± 2°C. For this interaction study following fungicides were used: Blitox, Captan, Bavistin, Ridomil, Thiophenate methyl, Roxiltubecanezole.

III. RESULTS

A. Ten days old culture medium grown on Potato Dextrose Agar was studied for morphological characters.

- *Colony characters***:** Sterile hyphae, grow rapidly, green patches, cushioned aerial mycelium, floccousseptate mycelium
- *Conidiophores*: Hyaline, highly branched, primarily branching at regular intervals, paired or in whorls at three.
- *Conidia*: 1-celled, ovoid, born in small terminal cluster, rapid growth, green or brown.
- *Chlamydospores*: Fairly abundant, intercalary and terminal.
- *Phialides*: Ampuliform to subglobose, rise mostly in crowded and diverse walls.

B. Compatibility of different species of Trichoderma

Two local isolates of bioagents (T. viride) made available from stock culture of Pathological laboratory of Agriculture Research Station, Sriganganagar were evaluated for their compatibility by recording percent inhibition, inhibition zone and colony character of two bioagents using dual culture technique. Antagonistic properties of these two different bioagents are presented in Table 1(A) and 1 (B). The radial growth of both bioagents was recorded at 24 hour, intervals till 96 hour. Data presented in Table 1(A) and 1 (B) revealed higher percent inhibition of T.viride(30%) by T.harzianum. The percent inhibition of T. harzianum by T. viridewas 22.22%.

C. Interaction studies of bioagent with fungicides

Laboratory experiments were conducted to observe the compatibility of T. viride with fungicides. The results revealed that at the selected concentrations of fungicides. The percent compatibility decreased with an increase in the concentration of fungicide.

D. The following fungicides were studied for interaction studies.

- *Blitox:* The observations on this aspect of study are given in Table 2 Fig. 1(a). All the concentrations of this chemical inhibited mycelia growth in T. viride showed full growth at 500 ppm concentration while at 1000 and 1500 ppm it showed lesser growth as compared to control. No growth was observed at 2000 ppm.

- *Thiophenate methyl***:** The effect of various concentrations of this chemical on mycelial growth of T. viride are tabulated in Table 3 Fig. 1 (b). All the concentrations of this chemical inhibited mycelial growth of T. viride.
- *Roxiltabucanazole:* However, T.viride showed full growth at 500 ppm but after that the growth decreased drastically (Table 4).Complete inhibition was recorded at 1500 and 2000 ppm. (Fig No.1(c))
- *Ridomil:* Table 5, Fig. 7 show the effect of various concentrations of this chemical on mycelial growth of T. viride. All the concentrations except 500 ppm of this chemical showed lesser growth as compared to control. In case of T.viride, full growth was observed at 500ppm but at 2000 ppm concentration complete inhibition of mycelial growth was observed. Fig. 1(d)
- *Bavistin:* Observations with regard to the effect of various concentrations of Bavistin on mycelial growth of T. viride are tabulated in Table 6, Fig. 8.All the concentrations of this chemical showed lesser growth as compared to control. Bavistin when used at 2000 ppm concentration altogether inhibit the mycelial growth of T. viride. Fig. 1(e)
- *Captan:* The effect of various concentrations of captan on mycelial growth of T. viride is tabulated in Table 7, Fig. 9. While in case of T.virideit showed full growth at 500 ppm. The growth decrease drastically at 1000 ppm concentration. No growth was recorded at 1500 and 2000 ppm concentrations. Fig. 1(f)

IV. CONCLUSION

The present investigation was carried out at S.D. (P.G.) College, Sri Ganganagar. The pure culture of T. viride were studied for their morphological characters. Colonies of these bioagents were green in colour. Canidia were single celled and canidophorus were hyaline and branched. Phialides were ampuliform to subglobose. Maximum and inhibition of T.viride was recorded. However in both the cases the percent inhibition was below 30% which suggest that these bioagent may be used as a mixture for commercial bioagent formulation.

Present finding indicates that seed treatment or soil application of Trichoderma would be compatible with Thiophenet methyl, Roxil, Bavistin and Ridomil at lower concentration for the integrated management of soil borne diseases. T. viride can be combined with seed treatment fungicides like Ridomil, Bavistin and Roxil at lower concentrations. Our future studies are directed to determine the compatibility of Trichoderma and chemicals in managing soil borne diseases of various crops under greenhouse and field conditions. Long term goal is to develop an integrated disease management strategy by combing Trichoderma and chemicals so as to prevent pathogen from gaining resistance as well as in building up of Trichoderma population levels in the soil that will be effective on a long term basis.Interaction studies of bioagent(T. viride) with fungicides indicated non compatibility of Thiophenatemethyl with T.virideat all the concentrations used. Roxil was found at lower concentration (500 ppm) it was found compatible with T.viride. Blitox also showed incompatibility with T. virideit may be used at lower concentration. Similarly Bavistin and Ridomil are compatible with T. virideat lower concentration only. Captan was the only fungicide which showed with T.viridefull compatibility upto 500 ppm dose only. This study suggest limitation or otherwise of these chemicals in integrating with bioagents.

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Table 2: Effect of various conc. *Blitox* on mycelial growth of *T.viride*

Cone, in ppm.	Myeelial growth(cm) at different interval of time			
	24	48	72	96
Control	2.8	4.8	6	9
500	2.5	4.5	6.2	9
1000	NG	1.6	2.8	5
1500	NG	0.8	1	1.5
2000	NG	NG	NG	NG

Table 3: Effect of various cone, of *Thiophenate Methyl* on mycelial growth of *T.viride*

Cone, in ppm.	Mycelial growth(cm) at different interval of time			
	24	48	72	96
Control	3.1	5.0	6.2	9
500	NG	NG	NG	NG
1000	NG	NG	NG	NG
1500	NG	NG	NG	NG
2000	NG	NG	NG	NG

Table 4: Effect of various conc., *of Roxiltabucanazole*on mycelial growth of *T.viride*

Cone, in ppm.	Mycelial growth(cm) at different interval of time			
	24	48	72	96
Control	2.0	4.6	6.5	9
500	1.3	4.9	6.2	9
1000	NG	0.4	0.8	1
1500	NG	NG	NG	NG
2000	NG	NG	NG	NG

Table 5: Effect of various conc., of *Ridomil*on mycelial growth of *T.viride*

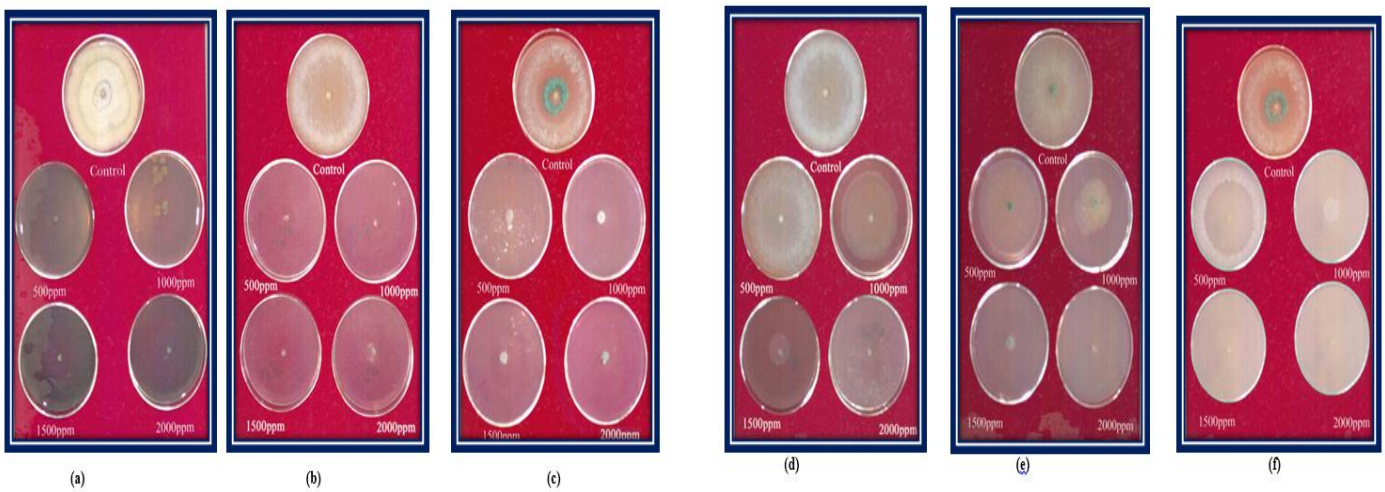
Cone, in ppm.	Mycelial growth(cm) at different interval of time			
	24	48	72	96
Control	2.2	4.2	6	9
500	2.0	3.9	6.2	9
1000	1.9	4.0	5.8	7
1500	NG	1.3	2.2	2.7
2000	NG	NG	NG	NG

Table 6: Effect of various conc., of *Bavistin*on mycelial growth of *T.viride*

Cone, in ppm.	Mycelial growth(cm) at different interval of time			
	24	48	72	96
Control	. 2	3.8	7.2	9
500	1	3.2	5.1	7.6
1000	NG	3.2	4.1	4.5
1500	NG	0.8	1.0	1.3
2000	NG	NG	NG	NG

Table 7: Effect of various conc., of *Captan*on mycelial growth of *T.viride*

Cone, in ppm.	Mycelial growth(cm) at different interval of time			
	24	48	72	96
Control	2.7	4.6	6.5	9
500	1.4	3.2	6.4	9
1000	0.8	1.5	2.0	2.7
1500	NG	NG	NG	NG
2000	NG	NG	NG	NG



Showing the effect of different concentration of fungicides on mycelial growth of *T. viride*
Figure 1. (a- f) (Balitox, Thiophenete methyl, Roxil, Ridomil, Bavistin & Captan)