EFFECT OF AGROCHEMICALS ON THE GROWTH OF DIFFERENT TRICHODERMA ISOLATES

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INTRODUCTION

Biological control of plant pathogens through antagonists involves the use of beneficial microorganisms to attack and control plant pathogens, and the diseases they cause. It is an ecofriendly and potential approach under sustainable agriculture, apart them being a promising alternative to the use of chemicals (Patel et al., 2014). Biocontrol fungi (BCF) are beneficial organisms that reduce the negative effects of plant pathogens and promote positive responses in the plant. In agriculture, these fungi, improves plant growth and development, has biological control activity against other fungi and nematodes (brunner et al., 2005, Hanson and Howell, 2004, Hoyos et al., 2009). Application of Trichoderma spp. is such an example of biocontrol agent with plant growth promoting ability coupled with antagonistic effect in phytopathogens (Kumar et al., 2012). It has been found that the persistent use of fungicides could weak the natural antagonistic activity (Lenteren and Woets, 1988). There are Trichoderma tolerant strains that can survive field concentrations of chemical fungicides. Now we have several approaches that can be used to obtain Trichoderma strains resistant to chemical fungicides.

Within the several complex plant protection strategies, one may need to combine biocontrol agents with chemicals to achieve the targets (Kredics et al., 2003). The combined use of biocontrol agents and chemical pesticides has attracted much attention as a way to obtain synergistic or additive effects in the control of soil-borne pathogens (Locke et al., 1985). The effect of certain fungicides and herbicides on Trichoderma spp. was reported earlier with an emphasis on practical applications (Kredics et al., 2003).

However, no information is available on the compatibility of these commonly used plant protection chemicals or fertilizers with Trichoderma sp., the biocontrol agent. Hence a study has been undertaken to evaluate the in vitro effects of certain fungicides, insecticides, and herbicides commonly used to determine their influence on Trichoderma strains.

MATERIALS AND METHODS

Isolation of Trichoderma strains

The experiment was carried out at the Plant Pathology laboratory, College of Agriculture, Indore. Potato Dextrose Agar (PDA) medium was prepared in the laboratory. Medium and necessary glassware were sterilized in autoclave (Islam et al., 2008). Soils were collected from eight districts of Malwa and Nimad regions of west M.P. region, India. One gram (dry weight basis) soil was mixed into 9 ml of sterile distilled water. Then 1 ml of suspension was taken into another tube containing 9 ml of sterile distilled water to make 1:10 solution. This serial dilution technique was continued up to 1:10,000. From the final dilution (1: 10,000), 1 ml suspension was transferred to each of the three petridishes. 20 ml of melted agar medium was poured in each plate and mixed with the suspension by giving a gentle whirling motion to the plate and allowed them to incubate in room temperature (Islam et al., 2008). All the 16 isolates of Trichoderma were identified based on phenotypic characters like colony colour and growth.

ABSTRACT

In this study we aimed to assess the compatibility of heavily spraying insecticides, pesticides and herbicides in M. P. Region with Trichoderma strains by using Poison food technique. Four commonly used fungicides, four insecticides and four herbicides were tested at two different concentrations, 500 and 1000ppm. The growth of Trichoderma strains was mostly inhibited by the carboxin+thiram, thiophanate methyl, quizalofop ethyl and chlorpyriphos. While, Trichoderma strains could grow easily with the Pyraclostrbin+metiram, pyraclostrobin, butachlor, pendimethalin, imizathpyer, malathion and dimethoate. A progressive increase in percent inhibition of radial growth in the fungus was observed as the concentrations of the agrochemical increased. The present results will help delineate the possibility of combining Trichoderma strains, biocontrol agents and agrochemicals for use in an integrated pest management approach.

KEYWORDS
Biological control
Trichoderma strains
Fungicides
Insecticides
Herbicides

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Evaluation of pesticides on the mycelial growth of *Trichoderma* isolates

Tolerance to fungicides of *Trichoderma* species was evaluated using poisoned food method (Nene and Thapliyal, 1982). In this study, fungicides viz., Pyraclostrobin (20%WG), mixture of pyraclostrobin + metiram (18.7%WG), thiophanate methyl (70%WG) and mixture of carboxin (37.5%) + thiram (37.5%)WS, four commonly used herbicides viz., butachlor (60%EC), pendimethalin (30%EC), imizathryper(10%SL) and quizalofop ethyl (25%EC) and four commonly used insecticides such as malathion(50%EC), dimethoate(30%EC), imidacloprid (17.8%SL) and chlorpyriphos (20%EC) were added to PDA medium. The two concentrations of the agrochemicals selected were 500 and 1000ppm. PDA medium without fungicides served as control. A 5mm inoculum disc of *Trichoderma* species was cut from the margin of actively growing colony and placed in centre of each Petri plate. Petri plates were incubated at room temperature. Three petri plates were used for each treatment. Radial growth of the colony was measured after 72h and calculated the percent growth inhibition using the Sundar et al.’s formula

\[
\text{Percent Inhibition} = \left(\frac{X - Y}{X}\right) \times 100
\]

Where X is growth in control plate and Y is growth in treated plate.

Above experiment was designed according to the factorial complete randomized design (CRD) with three replications. All the data was subjected to analysis of variance (ANOVA) and significance of variance was presented at 5% level. The value in percentage was transformed by angular transformation.

**RESULTS**

**Isolation of Trichoderma**

Total sixteen strains of *Trichoderma* were isolated from diverse districts of west M.P., India from rhizospheric soil zone of 8 different crops. The isolates were Ts1, Ts2, Ts3, Ts4, Ts5, Ts6, Ts7, Ts8, Ts9, Ts10, Ts11, Ts12, Ts13, Ts14, Ts15, Ts16. These sixteen strains were isolated from diverse districts of west M.P., India from rhizospheric soil zone of 8 different crops. The isolates were Ts1, Ts2, Ts3, Ts4, Ts5, Ts6, Ts7, Ts8, Ts9, Ts10, Ts11, Ts12, Ts13, Ts14, Ts15, Ts16.

**Effect of fungicides on Trichoderma growth**

In the in vitro bioassay, the growth of *Trichoderma* strains was recorded after 72h. The results showed significant difference in the growth among the various treatments. The effect of the fungicides on the growth of *Trichoderma* was presented in Table 1. Among the four fungicides tested, mixture of carboxin + thiram was found highly inhibitory to the growth of *Trichoderma* strains, followed by thiophanate methyl, pyraclostrobin + metiram and pyraclostrobin at the 500ppm and 1000ppm tested in vitro. At the 500ppm and 1000ppm concentration highest growth inhibition caused by mixture of carboxin + thiram followed by thiophanate methyl, pyraclostrobin + metiram and pyraclostrobin amended medium respectively.

The strain Ts8 was found to be tolerant against Pyraclostrobin 20%WG and Pyraclostrobin + metiram 18.7%WG and the strain Ts5 against thiophanate methyl 70%WG, Whereas, Ts5 was found tolerant to Carboxin (37.5%) + Thiram (37.5%) WS up to a concentration of 500ppm. Although the compound pyraclostrobin was limited inhibitory in action, the compound pyraclostrobin + metiram was found complete inhibitory in action. This indicates that metiram compound was inhibitory in action among both. A progressive increase in percent inhibition of radial growth in *Trichoderma* strains was observed as the concentration of all the fungicides increased. The result of the present screening would help in the selection of biocontrol agents, which can be used, with reduced dose of selected fungicides for the control of plant pathogenic fungi.

**Effect of herbicides on Trichoderma growth**

The effect of the herbicides on the growth of *Trichoderma* found in Table 2. The herbicides tested in the experiment exhibited varying levels of inhibition on the growth of the *Trichoderma* strains. At the end of this study, results revealed that three insecticides out of four were compatible with the growth of *Trichoderma* strains at the recommended dosage under in-vitro circumstance. As the table 2 shows that the higher inhibition percentage was recorded against quizalofop ethyl On the other hand, lower sensitivity of *Trichoderma* strains to the other herbicides was observed.

At the observed concentratons of 500 ppm and 1000 ppm the higher percent inhibition exhibited by Quizalofop ethyi followed by butachlor, pendimethalin and imizathryper. Although the compound quizalofop ethyl was inhibitory in action, the strain Ts9 at 500 ppm concentration showed maximum tolerance of about 68.33 (57.36%) and Ts6 showed tolerance of about 78.15 (62.16%) at 1000 ppm concentration. The herbicides butachlor, pendimethalin and imizathryper showed lesser degree of toxicity towards *Trichoderma* strains, which indicated their compatibility with the test fungus.

**Effect of insecticides on Trichoderma growth**

The effect of the insecticides on the growth of *Trichoderma* were presented in Table 3. In terms of the insecticides tested in the present study, chlorpyriphos exhibited highest toxicity, followed by malathion, dimethoate and imidacloprid at both the concentrations 500ppm and 1000ppm. Although a gradual increase in inhibition was observed as the concentration of insecticides increased. None of the chemicals completely suppressed the growth of *Trichoderma* strains, even at the highest concentration. Although the compound chlorpyriphos was inhibitory in action, the strain Ts9 showed minimum percent inhibition of about 45.74 (42.42%) and 28.89 (32.53%) at 500 ppm and 1000 ppm respectively. Except chlorpyriphos, other insecticides viz. malathion, dimethoate and imidacloprid, exhibited a lesser degree of toxicity towards *Trichoderma* strains, which indicates their compatibility with the test fungus.

**DISCUSSION**

A total of 106 soil samples were collected from rhizosphere of 7 different crops and from 8 districts of west M.P. Out of these sixteen strains of *Trichoderma* were isolated through serial dilution technique. The result indicated that *Trichoderma* spp. could grow and survive in various kinds of soil conditions. This evidence agrees with the report of Harman et al. (2004).

Among the four fungicides tested, mixture of carboxin + thiram was most toxic to the growth of *Trichoderma* strains, followed by thiophanate methyl, pyraclostrobin + metiram and
pyraclostrobin at the 500ppm and 1000ppm tested in-vitro (Table 1). Most of the time, fungicides produce undesirable effects on non-targeting organisms, so the use of microorganisms that antagonize plant pathogenic fungi should be risk free (Benitez et al., 2004).

Although the compound pyraclostrobin was limited inhibitory in action, the compound pyraclostrobin + metiram was found inhibitory in action. This indicates that metiram compound was inhibitory in action. Although the carboxin + thiram mixture was completely inhibitory in action, the strain Ts3 at 500 ppm concentration showed maximum tolerance of about 66.67 (54.76%). A progressive increase in percent inhibition of radial growth in Trichoderma strains was observed as the concentration of all the fungicides increased.

This can be explained in terms of the variation in sensitivity of the test fungus to the fungicides (Nene and Thapliyal, 1993). Earlier reports suggest that biocontrol agents that can tolerate a certain level of fungicides were mixed with agrochemicals, resulting in eradication of diseases (De Cal et al., 1994).

Anderson (1978) opined that soil fungi and actinomycetes are not as susceptible to herbicides and insecticides as they are to fungicides. In this study also, Trichoderma spp. showed greater inhibitory effect was observed with fungicides than with herbicides and insecticides.

The herbicides tested in the present study exhibited varying levels of inhibition on the growth of the Trichoderma strains. The results indicated that quizalofop ethyl was incompatible with Trichoderma strains and the remaining 3 herbicides are not as susceptible to herbicides and insecticides as they are to fungicides.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pyraclostrobin 20%WG</th>
<th>Pyraclostrobin + metiram 18.7%WG</th>
<th>Thiphanate methyl 70%WG</th>
<th>Carboxin (37.5%)+ Thiram (37.5%)/WS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ts4</td>
<td>35.62 (36.62)</td>
<td>72.96 (58.70)</td>
<td>52.59 (46.51)</td>
<td>82.22 (65.10)</td>
</tr>
<tr>
<td>Ts3</td>
<td>25.56 (30.38)</td>
<td>25.56 (30.38)</td>
<td>63.33 (52.76)</td>
<td>86.67 (68.62)</td>
</tr>
<tr>
<td>Ts5</td>
<td>18.78 (22.60)</td>
<td>19.26 (22.60)</td>
<td>63.33 (52.76)</td>
<td>86.67 (68.62)</td>
</tr>
<tr>
<td>Ts6</td>
<td>25.56 (30.38)</td>
<td>25.56 (30.38)</td>
<td>63.33 (52.76)</td>
<td>86.67 (68.62)</td>
</tr>
</tbody>
</table>

Table 2:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Butachlor 60%EC</th>
<th>Pendimethalin 30%EC</th>
<th>Imazethapyr 10%SL</th>
<th>Quizalofop ethyl 25%EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ts4</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
</tr>
<tr>
<td>Ts3</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
</tr>
<tr>
<td>Ts5</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
</tr>
<tr>
<td>Ts6</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
<td>0.00 (0.41)</td>
</tr>
</tbody>
</table>

(Figures in parentheses are angular transformed values)
butachlor, pendimethalin and imazithyper showed lesser degree of toxicity towards *Trichoderma* strains, which indicated their compatibility with the test fungus. Although the compound quizaflorop ethyl was inhibitory in action, the strain *T*. *s* showed maximum tolerance to quizaflorop ethyl.

Sunil and Kulkarni (2004) reported that following agrochemicals were highly inhibitory to *T. harzianum* alachlor, carbadenzam, chlorpyriphos, glyphosate, organomercurial, thiram and trifluralin. Inhibitory effects increased with increase in concentrations from 500 to 2000 ppm

A significant variation was observed on the effect of herbicides on the growth of *Trichoderma* isolates. The variation was significant between the strains of *Trichoderma*. Among the insecticides tested chlorpyriphos has greater inhibitory effect on *Trichoderma* isolates growth. The strain *T*. *s* growth was less affected by chlorpyriphos. There was a visible morphological distortion and uneven growth pattern in the agar plates treated with insecticides (Table 3).

The result obtained in this experiment was supported by Tewari et al. (2014) who reported that among fungicides Thiophanate methyl was found incompatible with the test antagonist even at 25 1/4 g.a.i./ml and among the herbicides, butachlor and pendimethalin were found compatible with the test antagonist even at higher concentration (250 1/4 g.a.i./ml).

The efficacy of the agrochemicals on the inhibition of the growth of *Trichoderma* showed that the growth and multiplication was reduced with the increasing concentration of the pesticides which was supported by Mohammadi and Amini (2013) who conducted experiments on the effects of several pesticides on *Trichoderma harzianum* by using the growth rate and spore germination test methods.

**REFERENCES**


Mohammadi, A. and Amini, Y. 2015. The Influence of Pesticides and...


