

Studies on the effects of xenobiotic on radial growth and dry weight biomass of the soil fungus *Trichoderma* sp.

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Plant growth and development are greatly influenced by soil edaphic factor and microbial community present in the rhizosphere. Although some microorganisms are important, some can cause disease in plants and lead to the death of the plant. *Trichoderma* sp. is an important soil fungus which has the potential to control other soil-borne plant pathogens. To measure the bio-efficacy of *Trichoderma* sp. in integrated pest management, its compatibility along with other pesticides has to be assessed. GLV-71 (nitrosoglyphosate) and Elpida (emamectin benzoate) are the two commonly used herbicides and insecticides worldwide. We investigated the application of these two pesticides with their effect on the growth of *Trichoderma* sp. Different concentrations (half dose, field recommended dose and double dose) of the two pesticides were tested on the growth of the selected fungal species *in vitro*. The result shows that nitrosoglyphosate and emamectin benzoate have concentration dependent effect on the growth of *Trichoderma* sp. Between the two pesticides, GLV-71 shows greater effect on the growth of the microorganism. This experiment shows that Elpida exhibit more compatibility along with *Trichoderma* sp. as a potential bio-control agent rather than GLV-71.

Keywords: *Trichoderma* sp., bioefficacy, nitrosoglyphosate, emamectin benzoate, integrated pest management.

INTRODUCTION

Xenobiotics (from Greek *Xenos* meaning strange or foreigner) are compounds that do not occur in nature and are chemically synthesized. Such compounds are of crucial concern in the soil environment as they can influence many biological and biochemical reactions in soils. They may be grouped as antioxidants, carcinogens, drugs, environmental pollutants, food additives, hydrocarbons, and pesticides. Pesticides are chemicals used to eliminate or control a variety of agricultural pests that can damage crops and livestock and reduce farm productivity but they also have potential to affect non-target organisms and soil biochemical processes. The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides.

Trichoderma is a fungal genus described in 1794. It includes anamorphic fungi isolated primarily from soil and decomposing organic matter (Persoon, 1794). Strains within this genus include a wide spectrum of evolutionary solutions that range from very effective soil coloniz-

ers with high biodegradation potential, to non-strict plant symbionts that colonize the rhizosphere. They are facultative saprobes, and present in almost every soil type. They are known to produce an important enzyme like the cellulolytic enzymes (cellulase and hemicellulase), β -glucosidase, xylanase and chitinase. They are also an important biocontrol agent, a mycoparasites, used for controlling soil-borne fungal plant diseases (Papavizas, 1985). Mechanisms of their invasion include antibiosis, parasitism, inducing host-plant resistance, and competition. Most biocontrol agents are from the species *T. harzianum*, *T. viride* and *T. hamatum*. They form white, yellow-green or bright green colonies in pure culture. The conidia are borne on a short phialides on the conidiphores. This antagonistic potential is the base for effective applications of different *Trichoderma* strains as an alternative to the chemical control against a wide set of fungal plant pathogens (Chet, 1987; Harman and Bjorkman, 1998), and the biodegradative capacity is a source of useful enzymes in different industrial sectors (Harman and Kubicek, 1998).

Species of *Trichoderma* are being used either as seed

treatment or soil application. In both the cases, the antagonist has been continuously exposed to different insecticides applied to the field either in soil or as foliar sprays. Insecticides sprayed aerially reaches the soil by means of air currents or are washed off the plant surface due to rain and is likely to influence the efficacy of native or applied biocontrol agents like *Trichoderma*. Hence, it is necessary to assess *Trichoderma* compatibility to insecticides in order to use in the integrated disease management systems.

MATERIALS AND METHODS

The experiment is designed to show the effect of two pesticides which are commonly employed in Mizoram, viz. Elpida (emamectin benzoate) and GLV-71 (glyphosate), on *Trichoderma* sp. Three different concentrations of the pesticide, viz. field recommended dose, half dose and double dose, are prepared for this experiment. *Trichoderma* sp. is isolated from forest soil by serial dilution method which is cultured on Rose Bengal agar medium and Czapek Dox medium. Czapek Dox medium is used for stock solution.

Inhibition percentage of radial growth was measured based on control plate colony diameter using the formula (Waksman *et al.*, 1992):

$$\text{Mycelial growth inhibition (\%)} = \frac{A-X}{X} \times 100$$

Where,

X = growth of mycelial colony in control set after incubation period subtracting the diameter of inoculum disc.

A = growth of mycelial colony in treatment set after incubation period subtracting the diameter of inoculum disc. The average diameter of the fungal colonies was measured on the 3rd, 4th, and 5th day of incubation and percentage of mycelial growth inhibition was calculated.

Study was carried out by poisoned food technique using different concentrations of pesticides against *Trichoderma* (Jaworski, 1972). The field recommended doses of GLV-71 and Elpida are 0.437 g/100 ml distilled H₂O and 0.050 g/100 ml distilled H₂O, respectively. By using these, half and double dose of both the pesticides are also prepared and desired volume are added to solid and liquid cultures of *Trichoderma*.

Enumeration of fungal population

Serial dilution and plating method (Waksman *et al.*, 1992) was used for the isolation of fungal populations. 10 g of soil sample was added to 100 ml distilled water in a 100 ml conical flask to give 1:10 dilution. The flask was swirled for 15 minutes to get a homogenous solution. Then, 10 ml aliquot solution was transferred to another conical flask with 90 ml of distilled water to get 1:100 dilutions. Finally, 10 ml of this solution was again added to 90 ml distilled water in another flask to get 1:1000

dilutions.

Serial dilution technique:

10 g of soil + 100 ml H₂O (1:10)

10 ml of above solution + 90 ml H₂O (1:100)

10 ml of above solution + 90 ml H₂O (1:1000)

Rose Bengal agar medium (Martin, 1950) was used for the culture medium. Fungi were inoculated into the growth medium by adding 1ml of the aqueous solution (1:1000) to a plate containing the culture medium. The plates are gently rotated and the inoculums dispersed with a spreader during the addition of the aqueous solution. The plates were incubated for 7 days at 25±1°C in a BOD incubator.

Isolation and identification of the fungal species

The required fungal species was isolated and identified with the help of compound microscope. A thin thread of mycelia was taken out with the help of needle or pin from the mixed colony of the culture medium and it was then kept on a glass slide. The specimen is stained with cotton blue stain and it was then covered with a cover slip. The slide was then observed under compound microscope. The required fungal species *Trichoderma* sp. was identified and prepared for its pure culture. A portion of the colony was transferred to Czapek Dox agar medium for pure culture. After few days, the mycelium from the pure culture colony was transferred to Czapek Dox broth medium for stock culture, which was incubated for several days. *Trichoderma* sp. from this stock culture was used for further experiment.

Preparation of different solution of pesticides

Three different solutions of the pesticides are prepared by using the recommended rate as per labeled on the package. Based on this field recommended dose, a double and half dose of the pesticides are also prepared.

GLV-71

Half - 0.218 g/100 ml distilled H₂O

Recommended - 0.437 g/100 ml distilled H₂O

Double - 0.875 g/100 ml distilled H₂O

Elpida

Half - 0.025 g/100 ml distilled H₂O

Recommended - 0.050 g/100 ml distilled H₂O

Double - 0.100 g/100 ml distilled H₂O

Treatment of solid medium with pesticides

The above calculated weights of different doses of the pesticides were added to 100 ml of Czapek Dox Agar medium in six different conical flasks before it is autoclaved. The medium was then poured into Petri-plates, maintaining three replicates. Medium without pesticides treatment was also prepared for control. After the medium gets solidified, three holes are made in each plate using a cork borer. Inoculum from the stock *Trichoderma*

culture is then added to the medium inside the holes with a volume of 0.4ml. The plates are then incubated for in an incubator at $25\pm 1^\circ\text{C}$. These will be used for the determination of the growth rate by measuring the size of diameter of the colony. The radial growth of *Trichoderma* was measured on the 3rd, 4th and 5th day from incubation. The radial growth is measured by the total diameter minus the diameter of the cork borer.

$$R = \frac{d_2 - d_1}{2}$$

Where,

d_2 = diameter of colony

d_1 = diameter of cork borer.

Treatment of broth medium with pesticides

The above calculated weights of different doses of the pesticides are added to 100 ml of Czapek Dox Broth medium in six different conical flasks before it is autoclaved. The medium is then poured into test tubes, 15 ml each, maintaining three replicates for each dose. Medium without pesticides treatment are also prepared for control. Inoculums from the stock *Trichoderma* culture is then added to the test tubes with a volume of 1ml each. The test tubes are then incubated for 7 days in an incubator at $25\pm 1^\circ\text{C}$. These will be used for the determination of the growth by measuring the dry weight of the biomass. After 7 days, the biomass was filtered through Whatman filter paper no. 1. The initial weight and the final weight of the filter were compared.

Dry weight biomass is calculated by using the formula:

$$\text{Dry weight (gm)} = W_2 - W_1$$

Where,

W_2 = final weight of filter paper

W_1 = initial weight of filter paper

RESULTS

Effect of pesticides on the radial growth of *Trichoderma*

The radial growth of *Trichoderma* from four different plates treated with different dose of the pesticides (control, half, recommended and double) were recorded on the 3rd, 4th and 5th day from inoculation. Both the pesticides amended medium show inhibitory effect on the radial growth. GLV 71 showed more inhibitory effect on the radial growth than ELPIDA. The area of inhibition increased with the increase in the concentration of the pesticides. Maximum size of colony is found in control plate with 4.22 ± 0.061 cm after 3 days, 8.07 ± 0.130 cm after 4 days and 12.97 ± 0.063 cm after 5 days of incubation. Among the plate treated with GLV-71 - 80.52%, 95.78% and 100% inhibition are shown in half, recommended and double dose plate respectively after 3 days of incubation; 75.24%, 88.72% and 97.38% inhibition in

half, recommended and double dosages after 4 days; 70.53%, 82.95% and 93.23% inhibition in half, recommended and double plate after 5 days. Among the plate treated with ELPIDA - 54.99%, 74.1%, 84.21% inhibition in half, recommended, double plates after 3 days; 62.44%, 68.22%, 80.05% inhibition in half, recommended, double after 4 days; 66.34%, 71.56%, 77.38% inhibition in half, recommended, double dose after 5 days of incubation. The one-way ANOVA result shows a significant variation in the different size of the colony as shown in Figures 1 & 2.

Effect of pesticides on the dry weight biomass of *Trichoderma* sp.

Mycelium dry weight of *Trichoderma* sp. was measured from four different treatments, viz. control, half, recommended and double dose of GLV-71 and Elpida respectively. Control culture has maximum dry weight of 0.388 ± 0.016 g after 3 days of incubation. Among the cultures treated with GLV-71, dry weight of 0.182 ± 0.019 g is found in culture mixed with half dose, 0.100 ± 0.023 g in culture mixed with recommended dose and 0.040 ± 0.019 g in culture with double dose. In cultures treated with Elpida, dry weight of 0.205 ± 0.027 g is found in culture mixed with half dose, 0.138 ± 0.007 g in culture treated with recommended dose and 0.059 ± 0.030 g in culture treated with double dose. The one-way ANOVA results show a significant variation in the different size of the colony as shown in Figure 3.

DISCUSSION

The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides. The use of pesticides on agricultural field has also shown effect on non-target microorganisms. Such microorganisms are of great importance for the plants in many ways. Many pesticides have been used to study their effect on the population of soil micro biota.

In this experiment, two pesticides GLV-71 (herbicide) and Elpida (insecticide) were used to study their effect on *Trichoderma* sp. The present study reveals that these pesticides affect the growth rate of *Trichoderma* sp. and reduce production of spores with the increasing concentration of the pesticides.

Glyphosate is the active ingredient of several broad-spectrum commercial herbicides, which are translocated from leaves to roots and act by blocking the shikimic acid pathway in plants (Johal and Rahe, 1984; Steinruncken and Amrhein, 1980; Boocock and Coggins, 1983). Unlike the effect of herbicides on plants, very little is known about the mode of action of glyphosate on fungi. Glyphosate blocks the shikimic acid pathway in *Neurospora crassa* and presumably, could have the same effect in

Figure 1: Effect of GLV-71 on radial growth *Trichoderma* sp.

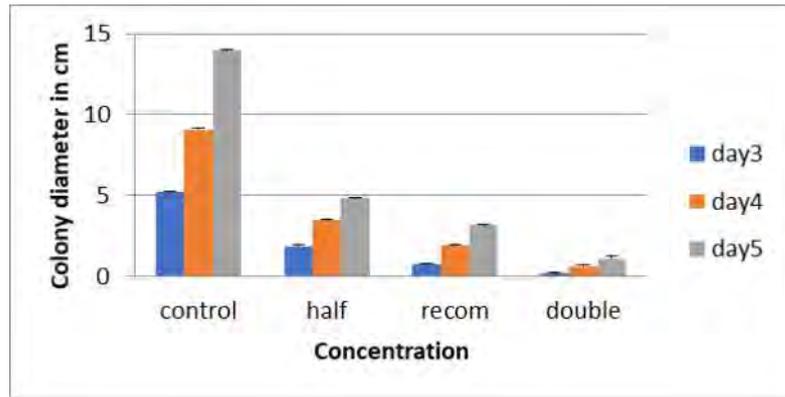


Figure 2: Effect of Elpida on radial growth of *Trichoderma* sp.

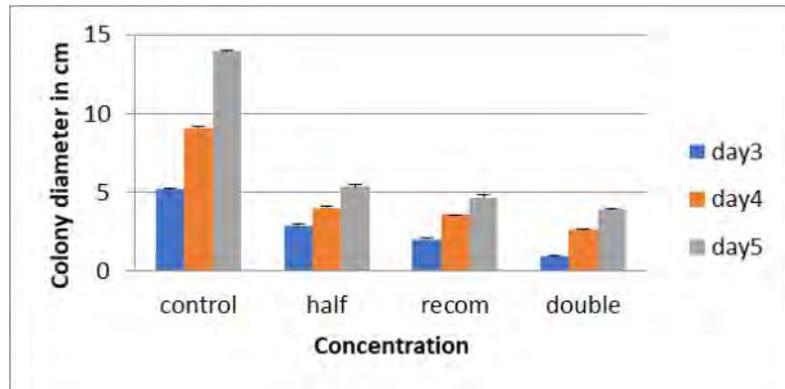
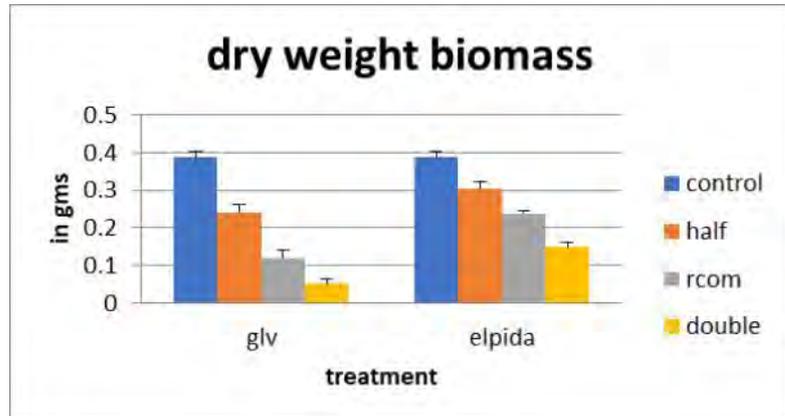


Figure 3: Effect of GLV-71 and Elpida on dry weight biomass of *Trichoderma* sp.



other fungal species (Ranganathswamy *et al.*, 2011).

Observations made on the radial growth of *Trichoderma* sp. indicated significant variation in the sensitivity of *Trichoderma* sp. isolate to pesticides or toxicity of pesticides towards *Trichoderma* isolates. When observations were recorded on radial growth for three consecutive days (3rd, 4th, and 5th day) in pesticides amended plates, the growth was significantly inhibited on the recorded three days of incubation. GLV-71 shows more inhibition on the growth of *Trichoderma* sp. than Elpida. Change in the concentration of commercial formulation of the pesticides also shows significant effect on the growth.

This finding suggests that *Trichoderma* sp. is more resistant to Elpida than GLV-71. This agrees with the find-

ings that new generation insecticides, *viz.* spinosad, emamectin benzoate, thiamethoxam and indoxacarb were highly compatible with both the *Trichoderma* isolates with less toxic effect on the radial growth. Thus, the potential biocontrol agent *Trichoderma* shows more compatibility with Elpida than GLV-71.

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