IN VITRO STUDIES ON THE REACTION OF FUNGI *Trichoderma* TO DIFFERENT HERBICIDES USED IN TEA PLANTATION

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ABSTRACT

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An experiment was conducted at the Plant Pathology laboratory of Bangladesh Tea Research Institute on September, 2006 paper investigated selected genus of *Trichoderma* and herbicides aimed at defining the effect of different concentrations on the development of mycelium of the isolates studied and spore germination. Five commonly used herbicides like Glyphosate 41SL (2022.66 PPM), Paraquat 20 W/W (746.66 PPM), Bimaster 240/120AS (1680.00 PPM), Kem- Amin 58SL (2165.33 PPM) and T5= Butachlor 5G (281.25 PPM) were considered as treatments. Concentrations (PPM) were calculated on the basis of their doses of applications recommended by BTRI. Herbicides were added with melted media separately maintaining the said concentrations of each. *Trichoderma* was isolated from tea soil by soil dilution method and maintained pure culture on PDA media. After preparation of spore suspension, it was plated with herbicides added PDA media. Every 24 hours observations revealed that Bimaster 240/120AS completely inhibited the growth of *Trichoderma*. Though the fungus structure was seen in the form of spot like sporulation on the PDA treated with Paraquat 20 W/W, Kem- Amin 58SL and Butachlor 5G. But further these could not produce mycelium. Only 21-30% mycelial growth was found in cultured treated with Glyphosate 41SL.

Keywords: Herbicidal effect, *Trichoderma*, spore germination

INTRODUCTION

Trichoderma spp. have been found as effective biocontrol agent of soil borne plant pathogenic fungi such as *Fusarium, Sclerotium, Rhizoctonia* etc (Chet and Inbar, 1994). The genus *Trichoderma* is not only one of the most common, isolated from various habitats, soil fungi but also known to be secreting to the environment various secondary metabolites of a wide spectrum of effects on various fungal groups, especially pathogenic fungi. Bissett (1984) characterized the genus *Trichoderma* as "rapidly growing colonies bearing tufted or pustulate, repeatedly branched conidiophores with lageniform phialids and hyaline or green conidia born in slimy heads". Biological control of plant pathogens have been considered as a potential control strategy in recent years. So that it may be used eco-friendly as biocontrol agent and nature as well as microorganisms will relatively be saved. *Trichoderma* species have gained considerable importance as a successful biocontrol agent for the control of soil borne diseases (Chet and Elad, 1982). *Trichoderma* has been exceptionally good model with which to study biocontrol because it is ubiquitous, easy to isolate and culture, grow rapidly on many substrates, affect a wide range of plant pathogens, is rarely pathogenic on higher plants, acts as a mycoparasite, competes well for food and site, produces antibiotics and has an enzyme system capable of attacking a wide range of plant pathogens.

The response of the microflora to pesticide treatments is apart from physico- chemical interactions between pesticides and the soil that influence the availability of the compounds. The response to biostatic action differs substantially from that to biocidal action of pesticides. Biostatic action only changes the composition of the microbial population when utilizable substrates enter the soil, such as residues of pesticides treated crops. However the fungi, just like all the live organisms, depend on the effects of external factors which can modify their morphological characteristics as well as physiological functions. Such factors can include e.g. soil contamination with heavy metals, fungicides and herbicides. Metals can also show a non-specific effect on many cellular structures and influence metabolic processes by enzymes blocking (Badura *et al*. 2000 and Mehra *et al*. 1991). Fungicidal mechanisms of fungicides are related with disturbing physiological functions of fungi. Also herbicides, besides their herbicidal effect, influence the species composition and interactions between fungal species (Moliszewska, 2001 and Wachowska, 1999).

Long term application of herbicides indirectly causes a decrease in activity because less nutrients become available for heterotrophs by the reduction of the vegetative cover (Voets *et al*. 1974, Kruglow *et al*. 1975, Anderson and Drew, 1976). An essential and relatively poorly researched is an effect of chemical compounds introduced into the environment on non-pathogenic soil microflora and also on interactions between antagonists and fungi pathogenic for plants, which makes the present research into the effect of abiotic factors on antagonistic fungi of *Trichoderma*

genus justifiable. The present paper investigated selected genus of *Trichoderma* and herbicides aimed at defining the effect of different concentrations on the development of mycelium of the isolates studied and spore germination.

MATERIALS AND METHODS

Preparation of culture media

Potato Dextrose Agar (PDA) media was prepared in the laboratory of Plant Pathology. Media and necessary glassware were sterilized in autoclave.

Isolation of Trichoderma

Soils were collected from 15 different marks of tea areas under main farm of Bangladesh Tea Research Institute at 0-9 inches. All collected soil samples were mixed thoroughly to make a composite sample. As working samples 1 gram (dry weight basis) soil was taken from composite sample. 1 gram 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. 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 five 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,* 2001). Sub culturing was performed and the culture of *Trichoderma* in pure form was maintained.

Concentration of herbicides

Five herbicides like T_0= Control (Blank), T_1= Glyphosate 41SL (2022.66 PPM), T_2= Paraquat 20 W/W (746.66 PPM), T_3= Bimaster 240/120AS (1680.00 PPM), T_4= Kem- Amin 58SL (2165.33 PPM) and T_5= Butachlor 5G (281.25 PPM) were used in this experiment. Concentrations (PPM) were calculated on the basis of their doses of application recommended by BTRI. Specific volume of herbicides was added with melted media separately maintaining the said concentrations of each.

Preparation of spore suspension

An inoculum of spores was placed in a tube of sterile water. A clean sterile pipette was used to transfer an aliquot, eg. 1 ml of this to a tube with 9 ml sterile water. This was continued up to decimal dilutions. In the final dilution, 1 ml spore suspension was added to melted agar that was treated with herbicides and then incubated at room temperature (Anonymous, 1983).

The experiment was laid out with 6 treatments along with one control having 4 replications of each. Data were recorded in terms of growth of *Trichoderma* on PDA media.

RESULT AND DISCUSSIONS

Observations were taken after 24 hours of plating and continued as daily observation. After 24 hours of plating the spore germination on PDA was observed in the form of spot like structures that were categorized as following scale-

Point		Number of Spot like structures was found in PDA
0	=	No structures was found
1	=	01-10 structures were found
2	=	11-20 structures were found
3	=	21-30 structures were found
4	=	31-40 structures were found
5	=	41-50 structures were found
6	=	> 50 structures were found

From the categorized data the experiment presented showed that the highest point (5.75 out of 6.00) was observed in control followed by T_1 (4.75 out of 6.00). Lowest (0.50) response was recorded in T_5. No growth change was noticed in T_4 (Table1). On the basis of highest grade point (6), 95.83% spot like structures were observed in T_0 while lowest 8.33% was in T_5 (Figure 1). No significant (0.05) different was observed in T_2 and T_3.

Table 1. Points against the number of structures

Treatments	Points against the number of structures (Mean of four replications)
T_0	5.75 a
T_1	4.75 b
T_2	2.25 c
T_3	1.75 c
T_4	00 d
T_5	0.5 d

Same letters followed by the figures are not statistically different at 5% level of significance by DMRT

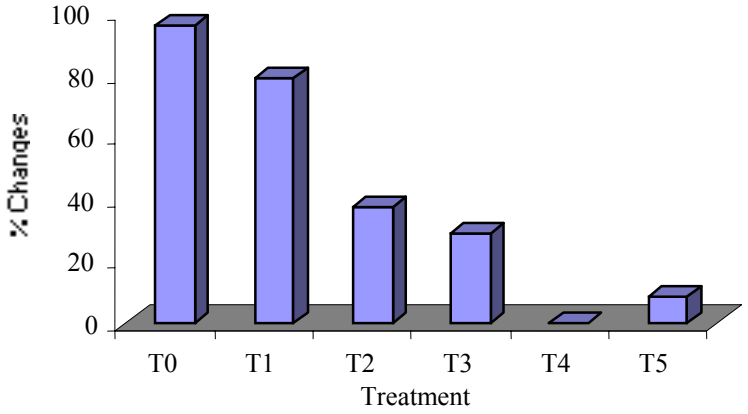

Figure 1. Percent Changes over highest point

After 48 hours of plating, spot like structures were in further observation. Mycelial growth was found on some spot like structures. These observations were categorized by the following scale-

Point	Changes of spot like structures was recorded
0	= No mycelium produce
1	= 1-10% of the structures produce white cottony like mycelia
2	= 11-20% of the structures produce white cottony like mycelia
3	= 21-30% of the structures produce white cottony like mycelia
4	= 31-40% of the structures produce white cottony like mycelia
5	= 41-50% of the structures produce white cottony like mycelia
6	= > 50% of the structures produce white cottony like mycelia

All spot like structures do not show growth of mycelia. In the plate containing melted agar in which no herbicides were mixed more than 50% spot like structures produced white cottony mycelia, while 20-30% white cottony mycelia produced in T_1 which was statistically similar to T_0. No mycelial growth was observed in T_2, T_3, T_4 and T_5 (Table 2). After 48 hours of plating, mycelial colony size was also recorded. From the four replications of T_0 and T_1, randomly 10 colony size of each were measured. As in T_2, T_3, T_4 and T_5 no mycelial growth was observed so that the measurement of colony size was ignored. The maximum size was found in T_0, Followed be T1 (Table 3).

Table 2. Points against the changes of spot like structures

Treatments	Points against the number of structures (Mean of four replications)
T_0	5.5 a
T_1	2.75 b
T_2	00 c
T_3	00 c
T_4	00 c
T_5	00 c

Same letters followed by the figures are not statistically different at 5% level of significance by DMRT

Table 3. Colony size of the mycelia

Treatments	Size of the mycelial colony (cm)										
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	Average
T_0	6.0	6.0	5.1	5.2	5.5	5.75	4.95	6.10	6.35	5.98	5.69
T_1	3.00	3.20	2.40	2.10	3.10	4.10	3.20	4.95	5.10	2.90	3.41
LSD at 5% level of significance											0.549

From the above, it is presented that, Bimaster 240/120AS completely inhibited mycelium growth, weakened sporulation and spore germination of *Trichoderma*. Though the fungus was seen in the form of spot growth in cultures treated with Paraquat 20 W/W, Kem- Amin 58SL and Butachlor 5G. But further these could not produce mycelium. Only 20-30% mycelial growth was noticed in cultured treated with Glyphosate 41SL.

Ciraj, 1996, Klimach *et al*, 1998 and Wachowska, 1999 reported that growth of saprophytic fungi of *Trichoderma* genus can be also limited by other herbicides like Gesard 500 SC, Roundup or preparations containing atrazine. Supporting this, the present experiment set the more or less similar information regarding the growth, sporulation, spore germination, development of mycelia of *Trichoderma* against different herbicides.

Further research work regarding this with more herbicides should be initiated to confirm the present findings both in vitro and in vivo with more details.

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