Journal of AgriSearch, 9 (2): 140-144
An Open Access International Peer Reviewed Quarterly

ISSN : 2348-8808 (Print), 2348-8867 (Online)
https://doi.org/10.21921/jas.v9i02.10119

Effect of Pesticides on Population Dynamics of Trichoderma sp. and Tomato Plant in Pot Culture Study

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ABSTRACT

A pot culture study was conducted to see the effect of different agrochemicals on the survival of Trichoderma in the soil in association with tomato plants during March to June 2018. Two fungicides (Copper oxychloride and Mancozeb), two insecticides (Monocrotophos and Chlorpyriphos), two herbicides (Metribuzin and Pendimethalin) were used to assess their effect in pot culture condition with tomato as a test plant. Effect of Trichoderma in presence of the chemicals under study on germination % of tomato seeds was non-significant. The treatments incorporated with chemicals (Trichoderma with chlorpyriphos, metribuzin and pendimethalin) showed an inhibitory effect on growth parameters (height, number of leaves and branches, leaf area and fresh weight) of the tomato plant. Trichoderma in presence of copper oxychloride recorded a plant height of 40.75 cm at 60 DAS at par with control (40.83 cm). Among the treatments applied with chemicals, at 15 DAS the population of Trichoderma was maximum in control i.e. Trichoderma alone (19.58×104 cfu g-1 of soil) which is statistically at par with Trichoderma+mancozeb 75% WP (19.08×104 cfu/g of soil) and Trichoderma+monocrotophos 36% SL (Soluble Liquid) (19.50×104 cfu g-1 of soil), while the lowest population of Trichoderma was observed in soil with the treatment of pendimethalin (10.49×104). The two herbicides metribuzin and pendimethalin and the insecticide chlorpyriphos had overall inhibitory effect on the biocontrol agent Trichoderma and the tomato plant in the present study.

Keywords: Trichoderma, mancozeb, copper oxychloride, monocrotophos, chlorpyriphos, metribuzin, pendimethalin.

ARTICLE INFO

Received on	:	03.11.2022
Accepted	:	09.05.2022
Published online	:	16.06.2022

INTRODUCTION

Trichoderma spp. have provided one of the first economical antagonistic control methods against soil-borne pathogen like *Fusarium, Sclerotium, Phytophthora* and *Pythium,* etc. (Kumar *et al.,* 2021). *Trichoderma* species induce various mechanisms to affect the growth of plant pathogens, control diseases and improve overall plant health and yield. The success of *Trichoderma* strain as BCAs is due to their high reproductive capacity, ability to survive under varied climatic conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms (Martinez-Medina *et al*., 2017). It also has the capacity to synthesize antibiotics, vitamins and hormones that also enhance their bio-control activity (Zeilinger *et al*., 2016), enhance seed germination of flowering plants, increase phosphorus uptake by plants (de Santiago *et al*., 2011) and induce systemic resistance in plants. Once *Trichoderma* comes into contact with roots, they colonize the root surface cortex, compete for nutrients or space, provide tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients and induced resistance (Li *et al*., 2015). Some strains of *Trichoderma* show compatibility with pesticides and have been successfully used in the IPM strategy (Ramanagouda and Naik, 2021). Factors affecting the growth of *Trichoderma* under laboratory conditions have been extensively studied, but the correlation between various growth responses in *in-situ* and *in-vivo* conditions is dubious. This limited knowledge on the ecology of these bio-control agents coupled with the variability in chemical environment limits the success of *Trichoderma* as a bio-control agent under field situations (Kang *et al*., 2021). For the effective use of *Trichoderma* spp. in bio-control of soil-borne plant pathogens, it is necessary that the antagonist is able to survive, grow and proliferate in the soil and rhizosphere. Generally, specific conditions must prevail for maximum activity (López -Bucio *et al*., 2015). Most of such studies have been conducted on agar, which gave presumptive results for antagonism Understanding the effect of soil factors on *Trichoderma* spp. may provide clues to which abiotic soil factor has the most influence on the bio-control activity of the fungus. Therefore, the present investigation was carried out to study the effect of some pesticides on the survival and proliferation of *Trichoderma* spp. in soil.

MATERIALS AND METHODS

The investigation was carried out during March to June, 2018 in the laboratory and experimental greenhouse of the Department of Plant Pathology, School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema Campus, Nagaland, India. The experimental site is located in the foothills of Nagaland and situated at 25°45'43" N latitude and 93°53'04"E longitudes at an elevation of 310 m above mean sea level.

Source of Trichoderma

Talc formulation of *Trichoderma* having an average cfu count of 13×10^4 was obtained from the Department of Plant Pathology,

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Culture media

Trichoderma selective media (TSM) [Magnesium sulphate ($MgSO_4$) 0.2g, Dipotassium hydrogen phosphate (K_2HPO_4) 0.9g, Potassium chloride (KCl) 0.15g, Ammonium nitrate (NH_4NO_3) 1.0g, Glucose 3.0g, Agar agar 20.0g, Chloramphenicol 0.25g, Metalaxyl 0.3g, Rose Bengal 0.15g, Captan 0.02g, Water 1000ml) was used for estimating *Trichoderma* population in the soil.

The nutrient ingredients such as $MgSO_4$, K_2HPO_4, KCl, NH_4NO_3 and glucose were added to 1000 ml boiling water and then 20 g agar agar was added to this boiling nutrient solution. One hundred ml of the medium was dispensed into 250 ml Erlenmeyer flasks and autoclaved. Before plating the medium, the remaining ingredients i.e. chloramphenicol, captan, metalaxyl and Rose Bengal were added to the medium, mixed thoroughly and then poured into the Petri plates.

Application of Trichoderma

Talc formulation of *Trichoderma* was applied at 10g pot^{-1} at the time of pot filling with a pot size of 22 cm diameter, 19 cm height and approximately containing 6 kg of soil.

Evaluation of different pesticides on survival of Trichoderma in soil

Most commonly used pesticides, two fungicides *viz.* copper oxychloride (Blitox 50WP, @0.4%), mancozeb (Indofil M-45 75WP, @0.2%); two insecticides *viz.* chlorpyriphos (Tricel 20%EC, @0.25%) and monocrotophos (Monocil 36%SL, @ 0.40%) and two herbicides *viz.* pendimethalin (Stomp 33%EC, @1.0 l ha^{-1}) and metribuzin (Sencor 70%WP, @ 1 kg ha^{-1}) were used to assess their effect on the test isolate of *Trichoderma*. The experiment was laid out in Completely Randomized Design (CRD) with seven treatments including a control consisting of *Trichoderma* treatment only and four replications each. Tomato crop of variety Samrudhi F1 hybrid was sown @ ten seeds pot^{-1} and finally keeping three plants pot^{-1} after germination and seedling stand. The *in vivo* pot culture experiment was conducted using pots of 22 cm diameter and 19 cm height containing approximately six kg of soil. The agrochemicals are applied 10 days after sowing of tomato plants at the recommended dose.

Estimation of Trichoderma population from treated soil

The estimation of *Trichoderma* population was done at 15 days interval by collecting the soil sample from the pots under study using the dilution plating technique. One gram of air-dried representative rhizosphere soil sample from each treatment having tomato crop was separately mixed in nine ml of sterile distilled water in culture tube making it 10^{-1} dilution. This soil suspension was serially diluted in sterile distilled water. Dilution of 10^{-4} was used for monitoring *Trichoderma* from inoculated treatments at 15, 30, 45 and 60 DAS.

One hundred µl of each diluted soil suspension was pipetted out with the help of a micropipette onto Petri plates containing TSM medium under aseptic conditions and spread with a sterile spreader. The inoculated plates were incubated at 28 ± 1°C for 4 days. Observations on the number of colony forming units (cfu) of *Trichoderma* were recorded.

Observations taken

The percent germination of tomato seeds was calculated using the formula-

$$\text{Percent germination} = \frac{\text{Number of seed germinated}}{\text{Number of seed planted}} \times 100$$

The observation was recorded on the 15th day after sowing. The observations of the number of leaves per plant, number of branches per plant and leaf area (cm^2) were recorded at 15 DAS and it was continued for a period of 2 months at 15 days intervals. The plant heights in (cm) were measured from the ground level to the base of the terminal leaf, commencing from 15 days after sowing, with the help of a linear scale. The observation was taken at 15 days interval for a period of 2 months. Freshly uprooted tomato plants were rinsed in running water and weighed (g) 60 DAS of the crop. The mean weight of three tomato plants pot^{-1} was taken for the fresh weight.

The data were subjected to analysis by Fisher's method of analysis of variance. The significance of variance among the data was calculated out by calculating the 'F' value and comparing it with the tabulated value of 'F' (Senedecor and Cochran, 1967). The treatment means were also compared among themselves by calculating Critical differences (CD) as follows:

$CD_{0.05} = S.Ed \times t_{0.05}$ for error degrees of freedom

The standard error of the difference of mean (S.Ed) was calculated by using the formula:

$$S.Ed = \sqrt{\frac{2 \text{ x error mean square}}{\text{number of replication}}}$$

Where S.Ed = standard error difference.

$T_{0.05}$ = Table value of student's't' obtained at 5% probability test.

The percentage value and population numbers were transformed into corresponding values by Arcsine transformation and square root transformation.

RESULTS AND DISCUSSION

Effect of Trichoderma and different agrochemicals on tomato plant

Germination percentage

The result of the effect of *Trichoderma* in presence of different agrochemicals on germination % of tomato recorded at 15 DAS is depicted in Table 1. It is evident from the table that all the agrochemicals applied in the present experiment do not affect the germination of tomato plants and *Trichoderma* was not affected by the chemicals applied to the soil. In terms of germination % value the highest (100%) was recorded in *Trichoderma*+Metribuzin 70% WP (100%) and *Trichoderma*+Pendimethalin 33% EC (100%). The germination % of tomato seeds in the study ranged from 92.50% to 100.00% and all the treatments were statistically at par. In other words, the agro-chemicals didn't lower the performance of *Trichoderma* in terms of seed germination of tomato.

Similar works have been reported that in chlorpyriphos amended soils, both in the presence or absence of gypsum, germination of chickpea was substantially higher and on par with that of pathogen uninoculated check perhaps due to the toxic effect of the insecticide on *S. rolfsii* alongside Th4. Dubey *et al.* (2012) reported that under field experiments, a combination of soil application of PBP 16G (*T. virens*) and seed treatment with either a combination of Pusa 5 SD (*T. virens*) and carboxin or Pusa 5 SD (*T. virens*) alone was superior in

Table 1: Effect of pesticides on Trichoderma population in soil and their combined effect on growth parameters of tomato plant

Treatments	Germination % of tomato at 15 DAS	Plant height (cm) at different DAS				Number of leaves at different DAS				Number of branches at different DAS			Leaf area (cm^2) at different DAS				*Trichoderma* population at different DAS [Average no. of cfu g^{-1} ($\times 10^4$)]			
		15	30	45	60	15	30	45	60	30	45	60	15	30	45	60	15	30	45	60
T_0 - *Trichoderma* (Control)	92.50	4.90	19.16	33.37	40.83	3.66 (2.03)	13.00 (3.67)	27.08 (5.23)	34.00 (5.84)	3.00 (1.87)	5.66 (2.47)	6.91 (2.72)	2.33	6.40	5.95	22.13	19.58	8.25	6.91	15.50
T_1 – *Trichoderma* +Copper oxychloride	92.50	5.87	14.75	28.83	40.75	3.91 (2.088)	9.33 (3.11)	23.58 (4.90)	37.75 (6.18)	2.00 (1.56)	5.58 (2.46)	7.00 (2.73)	2.20	5.75	7.46	19.72	13.75	6.58	8.41	15.08
T_2 – *Trichoderma* +Mancozeb	95.00	6.96	17.58	28.16	32.83	5.33 (2.41)	12.41 (3.59)	22.75 (4.80)	30.08 (5.52)	3.00 (1.87)	5.00 (2.33)	7.00 (2.73)	2.01	6.55	7.89	20.83	19.08	5.91	9.99	14.41
T_3 – *Trichoderma* +Monocrotophos	95.00	6.12	15.63	29.08	33.16	4.95 (2.32)	12.75 (3.63)	27.58 (5.27)	28.66 (5.40)	3.00 (1.87)	5.83 (2.51)	6.58 (2.66)	2.32	7.02	8.25	19.45	19.50	9.58	8.33	16.33
T_4 – *Trichoderma* + Chlorpyriphos	92.50	5.33	8.83	13.58	13.50	4.00 (2.10)	10.83 (3.49)	13.33 (3.71)	13.33 (3.71)	2.00 (1.58)	4.33 (2.19)	4.33 (1.68)	2.48	6.37	2.62	2.85	10.08	6.91	10.16	14.00
T_5 – *Trichoderma* +Metribuzin	100.00	5.07	8.88	11.75	11.75	3.75 (2.06)	10.22 (3.27)	12.75 3.63)	13.33 (3.71)	2.00 (1.58)	4.33 (2.19)	4.33 (1.68)	2.44	6.87	1.98	2.80	12.25	6.33	7.08	13.25
T_6 – *Trichoderma* +Pendimethalin	100.00	5.33	9.12	13.00	13.08	3.58 (2.01)	11.26 (3.42)	12.08 (3.54)	12.08 (3.58)	2.00 (1.51)	3.91 (2.05)	3.91 (2.10)	2.06	7.99	1.83	2.65	10.49	16.16	10.16	12.58
SEm ±	3.49	0.40	0.97	1.81	1.046	0.10	0.11	0.16	0.13	0.04	0.07	0.03	0.17	0.69	0.64	1.72	1.31	1.43	1.15	0.90
CD (p=0.05)	10.27 (NS)	1.17	2.86	5.34	3.07	0.29	0.32	0.49	0.39	0.14	0.21	0.09	0.51 (NS)	2.03	1.89	5.10	3.87	4.21	3.39 (NS)	2.67

* Mean weight of three plants/pot; Values in parentheses are square root transformed values

increasing the seed germination, shoot and root length in chickpea.

Plant height

The data pertaining to the effect of various treatments in relation to plant height at 15, 30, 45 and 60 DAS are presented in Table 1. The highest plant height at 60 DAS was recorded in control that is *Trichoderma* alone (40.83 cm) which is statistically at par with *Trichoderma*+Copper oxychloride 50% WP (40.75 cm).

Similar findings to the present investigation were reported by Ganesan *et al.* (2007) that *Trichoderma harzianum* increased the growth of the groundnut plant while managing the stem rot disease caused by *Sclerotium rolfsii*. Yaqub and Shahzad (2008) reported that maximum plant length was recorded in plants where seeds were pelleted with *T. harzianum* as compared to other treatments. There are several reports where *Trichoderma* spp. effectively increased plant growth and controlled plant diseases (Martinez-Medina *et al.*, 2017).

The lowest plant height was observed in *Trichoderma* + Metribuzin 70% WP treatment (11.75 cm) followed by *Trichoderma* + Pendimethalin 33% EC (13.08 cm) and *Trichoderma* + Chlorpyriphos 20% EC (13.58 cm) which were significantly lower than the rest of the treatment which are at par. The present findings are in line with the work done by Gafar *et al.* (2013) who reported the negative impact of insecticides Malathion and Sevin on the plant height of the cucumber plant. Grichar *et al.* (2001) reported the phytotoxicity of pendimethalin at 1.2 kg a.i. ha^{-1} which reduced sesame plant stands by 8 to 98% compared to the untreated check.

Number of leaves

The result of different agro-chemical treatments on the number of leaves of tomato plant at 15, 30, 45 and 60 DAS are presented in Table 1. The maximum number of leaves at 60 DAS was recorded in *Trichoderma*+Copper oxychloride 75% WP (37.75) followed by (Control) *Trichoderma* (34).

The results of the present study also are in close agreement with the findings of Uddin *et al.* (2018), that *T. harzianum* significantly increases plant growth parameters of tomato plant. *Trichoderma* inhibits growth of plant pathogens and produces higher plant height and a greater number of leaves (Topolovec-

Pintarić, 2019).

The lowest number of leaves was observed in *Trichoderma*+Pendimethalin 33% EC (12.08). Prohmchum (1985) stated post-transplanting application of Pendimethalin on tomatoes and cabbages inhibited growth and induced malformation of the growing shoot. Continuous treatment of Pendimethalin resulted in swelling of the hypocotyls.

Number of branches

The data pertaining to the effect of *Trichoderma* in presence of different agrochemicals on number of branches at 15, 30, 45 and 60 DAS is presented in Table 1. The maximum number of branches at 60 DAS was recorded at *Trichoderma*+Copper oxychloride (7) and *Trichoderma*+Mancozeb 75% WP (7) followed by (Control) *Trichoderma* (6.91), *Trichoderma* + Monocrotophos 36% SL (96.58) which were all statistically at par.

The results of the present study also are in close agreement with the findings of Uddin *et al.* (2018), that *T. harzianum* significantly increases the number of branches and fresh and dry weights of branches per plant in tomato.

The lowest number of branches was observed in *Trichoderma* + Pendimethalin (3.91). In the present study, applications of pendimethalin reduced the number of branches lower than the other treatments. The higher seedling phytotoxicity could be attributed to pendimethalin concentration in soil. This is also in line with the work done by Sondhia (2013) who reported that harvest residues of pendimethalin were found in tomato, cauliflower and radish crops. Smith (2004) reported that pendimethalin can cause phytotoxicity to Indian spinach (*Basella alba* L.) at higher dose that is required for better weed control resulting in reduced number of leaves and various deformations in leaf.

Leaf area (cm^2)

The result of different treatments on leaf area at 15, 30, 45 and 60 DAS are presented in Table 1. The maximum leaf area (cm^2) at 60 DAS was recorded in control *Trichoderma* (22.13), followed by *Trichoderma*+Mancozeb 75% WP (20.83) and *Trichoderma* + Copper oxychloride 50% WP (19.72). The present findings are also in line with the findings of Lo and Lin (2002) who reported that soil amended by *Trichoderma* sp. had a marked increase in leaf area. Our result also indicates that *Trichoderma* was compatible with and therefore, not inhibited by the fungicides mancozeb and copper oxychloride in pot soil.

The lowest leaf area was recorded in *Trichoderma*+ Pendimethalin 33% EC (2.65) followed by *Trichoderma*+ Metribuzin (2.80) and *Trichoderma* + Chlorpyriphos 20% EC (2.85). The present finding is also in line with Abdel-Gawad (2010) who reported that pesticides affected crop growth and reduces its yield and quality due to their phytotoxicity and also due to their effects on soil fertility and salinity as stated by Lang and Cai (2009). Severe crop phytotoxicity and damage symptoms reported in the literature range from reduced or inhibited germination, reduced root length, protein and nucleic acid contents of root tips, injured flowers, to complete crop failure and residual persistence of herbicides in crop and soil (Sinha *et al.*, 1996).

Fresh weight

The results pertaining to the effect of *Trichoderma* in presence of different agrochemicals on the fresh weight of tomato plant at 60 days after sowing (DAS) are presented in Table 2. The highest fresh weight was recorded in *Trichoderma* + Monocrotophos (15.68g) which is at par with *Trichoderma* + Mancozeb (13.62 g) and *Trichoderma*+Copper oxychloride (11.82g).

While the lowest was recorded in *Trichoderma*+Pendimethalin (1.38g), *Trichoderma*+Metribuzin (2.22g) and *Trichoderma* + Chlorpyriphos (3.18). The present findings are also in line with Abdel-Gawad (2010) who reported that pesticides affected crop growth and reduces its yield and quality due to their phytotoxicity and also due to their effects on soil fertility and salinity as stated by Lang and Cai (2009). Further, Gafar *et al.* (2013) reported similar effects of pesticides on plant growth and soil.

Table 2: Effect of *Trichoderma* in presence of different agrochemicals on fresh weight (g) of tomato plant

Treatments	Fresh Weight (g) * (60 DAS)
T_0 - *Trichoderma* (Control)	9.78
T_1 – *Trichoderma* + Copper oxychloride	11.82
T_2 – *Trichoderma* + Mancozeb	13.62
T_3 – *Trichoderma* + Monocrotophos	15.68
T_4 – *Trichoderma*+ Chlorpyriphos	3.18
T_5 – *Trichoderma* + Metribuzin	2.22
T_6 – *Trichoderma* + Pendimethalin	1.38
SEM ±	0.98
CD (p=0.05)	2.88

(* Mean weight of three plants/pot)

Effect of different agrochemicals on Trichoderma population

It is discernible from the data presented in Table 1 that there is a significant effect of pesticides on the *Trichoderma* population in soil (15, 30 and 60 DAS). The highest population of *Trichoderma* could be obtained from the soil with no pesticide (19.58×10^4 g^{-1}) that is at par with soil treatment of mancozeb (fungicide), and monocrotophos (insecticide). On the other hand, the lowest *Trichoderma* population was recorded from treatment with chlorpyriphos, an insecticide (10.08×10^4 g^{-1}) followed by pendimethalin, metribuzin (herbicides), and copper oxychloride (fungicide) that are at par with each other. The data also reveal that there is a reduction in the *Trichoderma* population in the soil of all treatments at 30 and 45 DAS and an increase in the population number per gram soil at 60 DAS.

It is observed from the review of past literature that the effect of pesticides on various species of *Trichoderma* is contradictory. Compatibility of *Trichoderma* with mancozeb was observed by Gampala and Pinnamaneni (2010). Saxena *et al.* (2014) studied that captaf, thiram and copper oxychloride were compatible with the test antagonist *T. viride* up to 100 μg a.i. ml^{-1}, while mancozeb up to 250 μg a.i. ml^{-1}, as these did not adversely affect the growth of the test antagonist. Shrivastava (2015) reported that fluchloralin and metribuzin slightly reduce the sporulation of bioagent whereas, it was slightly stimulated by pendimethalin.

CONCLUSION

Trichoderma in presence of pesticides copper oxychloride, mancozeb, monocrotophos, chlorpyriphos showed positive result on the growth parameters of tomato plants while the herbicides metribuzin and pendimethalin showed inhibitory effect on account of offsetting *Trichoderma* effect. Currently used pesticides are required further compatibility study in broader field condition for integration in the eco-friendly and sustainable management of plant diseases and improving plant productivity.

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Citation:
Lalremruatpuii A and Banik S. 2022. Effect of pesticides on population dynamics of trichoderma sp. and tomato plant in pot culture study. *Journal of AgriSearch* **9**(2):140-144