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SOIL PHOSPHATASE AND PROTEASE ACTIVITY IN RESPONSE TO APPLICATION OF TRIAZOPHOS-AN ORGANOPHOSPHORUS INSECTICIDE

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ABSTACT

The aim of the present study is to evaluate the effect of triazophos (an organophosphorus insecticide) on soil phosphatase and protease activity in paddy soil of Srikalahasti, Chittoor district, Andhra Pradesh. All the concentrations of the triazophos resulted in the significant inecrease in phosphatase and protease activity till second and third week of treatment. In comparison to untreated control, the activity of phosphatase and protease decreased in presence of all concentrations (10, 25, 50, 75 and 100 ppm) of triazophos. The maximum inhibition in soil Phosphatase and Protease activity was noticed in presence of 100 ppm after 28th day of treatment.

KEY WORDS: Triazophos, organophosphorus insecticide, phosphatase, protease.

INTRODUCTION

Pesticides are common, non point source environmental pollutants of soil. The use of pesticides to prevent or treat crop diseases has been an essential part of agricultural practices world-wide for last few decades. When a synthetic pesticide is released into the environment, about 0.1% is reaching the target organism, while the remaining 0.99% interferes local metabolism or activity of enzymes, and also affects human health by entering into the food chain which has raised considerable public concern.[1] The issue of an impact of these chemicals on the composition of soil microorganisms and the processes they direct have

received more attention and their background levels in the environment have increased greatly.[2][3]

Paddy (*Oriza sativa*) is a major crop in SriKalahasti, Chittoor district of Rayalaseema region, Andhra Pradesh, India. Insecticide like Triazophos is commonly used for insect control in paddy crop and other horticulture crops nowadays in Srikalahasti surrounding areas. Triazophos is a Thiophosphoric and is the active ingredient of the insecticide Hostathion. It is susceptible to formation of highly toxic and flammable phosphine gas in the presence of strong reducing agents such as hydrides.

Soil contains microorganisms (bacteria, fungi, actinomycetes, algae and protozoans,) and excretes a variety of enzymes (ureases, dehydrogenases, invertases, cellulases, amylases , proteases and phosphatases) soil enzymatic measurements can be used to provide a "biological index" of soil fertility and as an indicator for many soil biological processes. The activity of enzymes is affected by abiotic conditions (*e.g.*, temperature, moisture, oxygen content and soil pH) by the chemical structure of the organic matter and by its location in the soil strata.[4][5]

Phosphatases find widely in mammals to bacteria, and indicate their importance in fundamental biochemical processes.[6] Phosphatases represent a broad range of intracellular as well as soil-accumulated activities that catalyse the hydrolysis of both the esters and anhydrides of phosphoric acid.[7] Phosphatases play a crucial role in the phosphorous acquisition of microorganisms and plants, and thus in the cycling of it within the soil.[8]

Proteases occur naturally in all organisms. Bacteria also secrete proteases to hydrolyse (digest) the peptide bonds in proteins and therefore break the proteins down into their constituent monomers.[9] It has been shown that proteases in soil can hydrolyse not only added but also native soil proteins and peptides.[10]

The objective of this work was partly to study soil microbial enzymatic activities in the soil, in the presence of excessive quantities of triazophos in soil. Approach method was setting in a greenhouse environment to observe phosphatase and protease and soil induce respiration that is the keys for input parameters. The result was meaningful to reference as representative work of rehabilitation in agriculture soil.

METHODOLOGY

Soil collection

The soil samples were collected from Srikalahasti, Chittoor district a semi-arid region in Andhra Pradesh, India, to a depth of 10-15 cm were air dried and sieved through a 2mm sieve before use.

Stock preparation

Stock solution was prepared by triazophos mixed with methanol.

Phosphatase activity

One gram sample of soil in test tubes (15×150mm) were incubated with various concentrations of triazophos (10, 25, 50, 75 and 100ppm) . After incubation at room temperature (28 ±4˚C) for a period of 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th}day, triplicate soil samples were withdrawn to determine Phosphatase activity.

Assay of Soil Phosphatase

The assay of phosphatase activity was based on that of Tabatabai and Bremner (1969)[11] and adapted by Joanna Lemanowicz (2011).[12] Soil samples were transferred to 100 ml Erlenmeyer flasks. 0.2ml of toluene, 3ml of modified universal buffer (MUB) (PH 6.5) followed by 2ml of 0.03M p-nitrophenyl phosphate disodium salt was added. The flasks were swirled for a few seconds to mix the contents, stoppered and incubated at 37˚C for 30 min. The reaction was stopped by adding 1ml of 0.5 M $CaCl_2$ and 4ml of 0.5 M NaOH, followed by swirling of the flask for a few seconds, and the soil suspension was filtered through a Whatmann's No.1 filter paper. The p-nitrophenol produced in the filtrate was read at 410 nm in Spectronic 20D Spectrophotometer by referring to standard calibration curve prepared with the known amounts of the reaction product p-nitro phenol.

Protease activity

For determination of protease activity, two gram portions of soil was distributed separately into test tubes (25×200mm) and treated with triazophos (10, 25, 50, 75 and 100ppm).After incubation at 28±40C for a period of 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day, triplicate soil samples were withdrawn to determine the protease activity according to the method of Ladd and Butler (1972)[13] and adapted by Subramanyam *et al.* (2011).[14]

Assay of Soil Protease

Both the pesticide amended and non amended soil samples were incubated with 10ml of 0.1 M Tris (2- amino-2 (hydroxymethyl) propane-1:3diol) at P^H 7.5 containing sodium caseinate for 24 hours at 30˚C. An aqueous solution of trichloro acetic acid (4ml) was then added and the mixture was centrifuged. The supernatant liquid in suitable aliquots was treated with 3ml of 1.4 M Na_2CO_3, and 1ml of Folin Ciocalteu reagent (33.3% v/v) with rapid swirling. The blue color, thus formed after 30 minutes, was read at 700 nm in a Spectronic 20D Spectrophotometer. Tyrosine equivalents in soil extracts were estimated by referring to calibration curve prepared with known concentration of tyrosine.

Statistical analysis

The concentration of the Phosphatase and Protease enzyme was calculated on a soil weight (oven dried) basis. The insecticide treatments were contrasted with untreated controls and the significant differences between the values of incubation period and insecticide concentration were performed by using repeated measures ANNOVA with mixed factors and Duncan's Multiple Range (DMR) test.

RESULTS AND DISCUSSION

Phosphatase Activity

Phosphatase activity in paddy, *Oryza sativa* cultivated soil showed a variable pattern in response to various concentrations of triazophos after 1st, 7th , 14th, 21st and 28thdays of incubation periods. Enzyme activity significantly decreased at all the concentrations of triazophos and there was much difference in the activity compared to untreated control. In this study, the phosphatase activity was markedly more pronounced upto 14 days of incubation and then declined progressively with further incubation periods. The data obtained from these experiments are presented in the Figure 1. Similar inhibition in Phosphatase activity was also reported by Tu. (1995)[15] and Ismail *et al*.(1996)[16] with imidacloprid, and cyfluthrin. Similar results were also observed by Xie et al. (2004)[17] with triazophos, bensulfuron methyl, chlobenthiazone increasing concentrations decreased the Phosphatase enzyme activity in paddy soil. Two organophosphorus insecticides, quinalphos and monocrotophos and two pyrethroids, fenvalerate and cypermethrin all at the lower concentrations of 1-5 kg ha-1 was significantly stimulatory to the Phosphatase activity but these insecticides were inhibitory to the activity at higher concentrations i.e. 7.5 kg ha-1 in four experimental soils.[18] Similar stimulatory effect by cholorpyrifos on phosphatase

activity in the field conditions was noticed Sikora LJ. *et al*. (1990)[19]. In a similar way, phorate and fenvalerate had a stimulatory effect towards phosphorus mobilization in soils under laboratory and field conditions.[20][21][22] Pesticide, fenitrothion had no effect on the activity of phosphatase in soil.[23] In the same way, Tu. (1970)[24] reported that the addition of Bay-37289, diazinon, dursban and zinophos at 10 and 100µg g^{-1} showed insignificant difference in phosphorus mineralization. In a similar study, fenamiphos at 37 and 930 mg kg^{-1} had no deleterious effect on the activity of phosphatase.[25]

Table 1: Descriptive Statistics of phosphatase activity under the impact of different concentrations of triazophos in soils.

Concentration	1 day	7 days	14 days	21 days	28 days
0 ppm	104.38 ±5.6	115.8 ± 6.2	135.67 ± 7.2	112.87 ±8.8	94.61 ± 5
10 ppm	97.95 ± 7.4	107.98 ±5.5	118.43 ± 3.9	104.64 ±5.5	78.64 ± 7
25 ppm	85.51 ± 4.4	96.56 ± 3.4	109.91 ± 2.4	89.76 ± 4.5	68.01 ± 2.6
50 ppm	78.96 ± 3.2	87.63 ± 3.4	96.09 ± 4.4	77.16 ± 5.1	57.45 ± 4.1
75 ppm	69.3 ± 6.7	82.32 ± 4.8	86.85 ± 4.1	66.67 ± 3.3	48.56 ± 2.4
100 ppm	65.82 ± 6.4	73.21 ± 4.3	76.66 ± 3.6	58.22 ± 4.3	37.12 ± 3
Total	83.65 ±15.3	93.92 ±15.6	103.94 ± 20.7	84.89 ±20.7	64.07 ±19.9

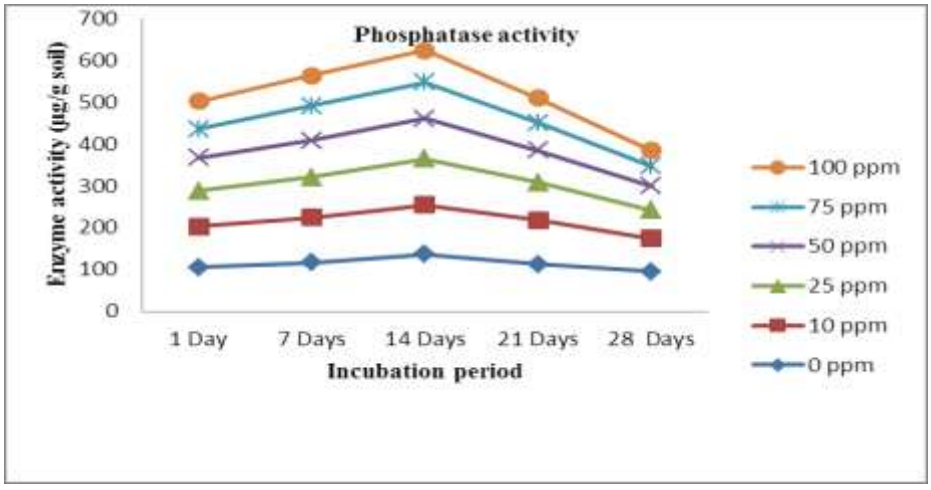

Figure 1: Effect of triazophos on phosphatase activity at different concentrations

Protease Activity

The hydrolysis of proteins, first phase in mineralization of soil organic nitrogen is dependent on enzymes, which are synthesized by plants and soil microorganisms. Protease plays a key role in this step. The present study reveals that the impact of different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha-1) of triazophos on protease activity has been studied in 24 hours, 7^{th} , 14^{th}, 21^{st} and 28^{th}days supplemented with 1% casein. Interestingly, Enzyme activity significantly decreased at all the concentrations of triazophos and there was much difference

in the activity compared to untreated control. In this study, the protease activity was markedly more pronounced up to 14 days of incubation and then declined progressively with further incubation periods. The data obtained from these experiments are represented in the Table 2 and Figure 2.In a study of Rangaswamy *et al*, (1994)[26] insecticides, quinalphos and monocrotophos of organophosphates and fenvalerate and cypermethrin of pyrethroid within a range of 2.5 kg ha-1 significantly stimulated the protease activity in a soil but these insecticides at higher concentration were toxic to the protease activity. Protease activity drastically decreased at higher concentrations (5.0, 7.5, 10.0 kg ha-1) of endosulfan and profenophos treated soils than the untreated controls throughout the experiment, suggesting that the enzyme is rather sensitive to profenophos and endosulfan. Interestingly, a stimulatory effect was observed at 10-25 ppm concentrations with individual increments of two insecticidal treatments, than the control, they are as follows: 13-47% and 2-15% in black clay soil after10 days of incubation period. This trend follows up to 20 days of incubation, when further prolonged in the period of incubation up to 40 days; a decline in enzyme activity was observed.[27]

Table 2: Descriptive Statistics of protease activity under the impact of different concentrations of triazophos in soils.

Concentration	1 day	7 days	14 days	21 days	28 days
0 ppm	260.67 ± 5.5	286 ± 5.6	319.67 ± 6.8	280.33 ± 7.8	255 ± 9.5
10 ppm	247 ± 11.1	270 ± 5.6	295 ± 6	258.33 ± 5	220 ± 6
25 ppm	228 ± 7	254.33 ± 4.7	272 ± 8.2	234.33 ± 7	203.67 ± 5
50 ppm	206.33 ± 8	241.67 ± 5.5	256.67 ± 4.5	214.67 ± 6.1	186.33 ± 4.7
75 ppm	179.67 ± 8	223 ± 4	244.67 ± 4.5	195 ± 6	168.33 ± 6.1
100 ppm	160.67 ± 7.8	192.67 ± 3.5	206.33 ± 4.7	180.67 ± 9.1	147.67 ± 8
Total	213.72 ± 37.1	244.61 ± 31.8	265.72 ± 37.6	227.22 ± 36.1	196.83 ± 36.4

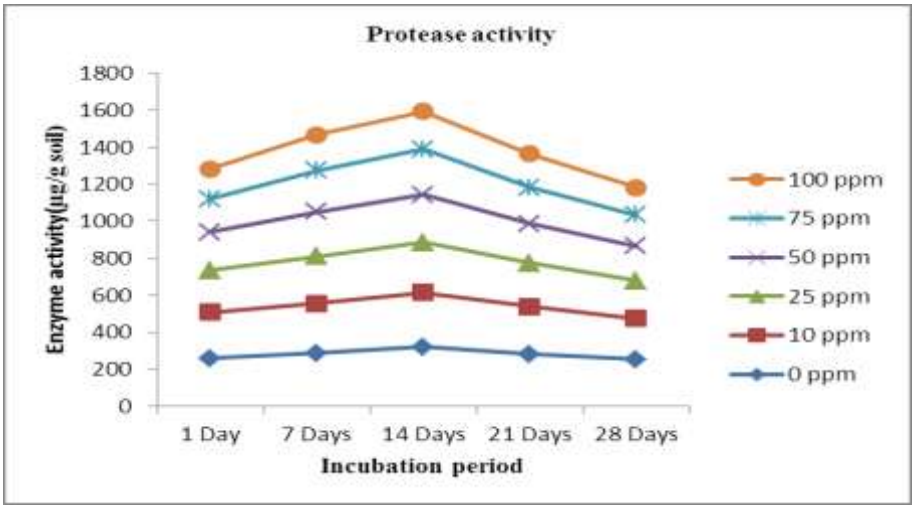

Figure 2: Effect of triazophos on protease activity at differen concentrations

CONCLUSIONS

Results revealed that the Soil Phosphatase and Protease were affected by the application of triazophos at all concentrations (10, 25, 50, 75 and 100 ppm). Overall, triazophos at a normal field dose would pose a threat to the Phosphatase and Protease activity whenever the triazophos concentration was increased. Based on the above results, it is concluded that the biological (Phosphatase and Protease) activities were affected by the triazophos applied at field application rate (3.5 kg h^{-1}) in the agricultural system to control insect pests.

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