EFFICACY OF PROFENOFOS ON MICROBIAL POPULATIONS AND ENZYMATIC ACTIVITIES IN VEGETABLE PLANTED SOIL

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***Abstract*:** Pesticides are chemical substances that are used for the protection of crop and vegetables from innumerable insect pests and to improve crop yield and quality. Repeated applications of pesticides contaminate the soil and disturb the soil environment by affecting soil micro flora and various physicochemical properties of soil. In view of above problem, the present study examines the effect of different concentrations of pesticides profenofos (0.1, 1, 10 and 100 ppm) were used to show their impact on soil quality indicators like micro flora and enzyme activities of soil collected from vegetable planted field, Bhiwani (Haryana, India). Soil sample without pesticide served as control. These were withdrawn for evaluation after every seven days with a total period of 21 days. Lower concentrations (0.1 and 1.0 ppm) were found to be beneficial but higher concentrations (10 and 100 ppm) lead to reduction in bacterial counts and enzymatic activities of soil. Bacterial and actinomycetes populations were found to be reduced by 12.43 % and 16.56% with the concentration of 10 ppm and the reduction was 29.73% and 51.10% at 100 ppm concentration, respectively. Analogous trend of reduction was also observed in enzymatic activities like amylase, invertase, alkaline phosphatase and acidic phosphatase showed overall drop of 3.53%, 36.79%, 19.83% and 16.35% with the application of 10 ppm and 3.74%, 76.88%, 46.19% and 28.21% at the concentration of 100 ppm profenofos, respectively. Low concentration is beneficial for P-solubilizing bacteria but at higher concentration showed deleterious effect on the alkaline and acidic phosphatase enzyme. These results concluded that profenofos has considerably deleterious impact on soil micro flora, which may be results in harmful effect on nutrients uptake and plant growth. These research areas enhanced future research based on molecular technique, contrary to traditional approach, which are used for quantification of net impact on soil biology.

Keywords: actinomycetes; bacteria; enzyme activities; profenofos.

I. INTRODUCTION

Soil is a complex dynamic ecosystem that consisting of organic and inorganic molecules, minerals, nutrients, moisture and diversity of flora and fauna **(Pandey and Singh, 2004).** It upholds the balance between its physical, chemical and biological aspects **(Dorans and Safley, 1997)** and also play vital role for sustaining environmental quality at different levels**.** Healthy soil is inevitability for agriculture and food production. Thus, it is essential to maintain soil health to ensure proper agricultural production. Soil possesses many active sites (polar, non-polar and ionic) that are capable of retaining pesticides and other residues. Soil contaminations pose threat to micro flora and micro fauna of soil as well as their environment **(Braschi *et al*., 2011).**

Pesticides are used to control pests but some of these reflect negative consequences along with controlling pests. They remain accumulated in the environment for long duration and affect the health of soil, plants, animals and even human beings. A number of studies have been described to show their adverse impact on soil quality. They interact with soil organisms and their metabolic activities **(Ingram *et al*., 2005; Wang *et al*., 2006).** Due to application of pesticides there is the formation of pesticide residues in the soil, concluding with the immersion of these xenobiotic compounds and they also destroy useful non-target

organisms of soil which are responsible for increasing the soil fertility along with target organisms in agricultural crops **(Anuradha *et al.*, 2015).** They also influence soil enzymes, which are essential catalysts ruling the quality of soil life. In particular, the activity of soil enzymes control nutrient cycles, and, in turn, fertilization **(Riah *et al.*, 2014).**

Pesticides are used habitually in agriculture that includes diverse groups of inorganic and organic chemicals. They have the collection of broad range of chemicals such as insecticides, herbicides, fungicides, nematocides, rodenticides, plant growth regulators, defoliants, soil fumigants, fruit sensible agents etc. **(Gevao *et al.*, 2000).** These chemicals have been characterized on the basis of varying criteria such as target pest, chemical composition, soil persistence or half-life, spectrum of activity, mode of action, available formulation, toxicity, volatilization behavior, solubility etc.**.** The main chemical groups are organochlorine, organophosphate, carbamate, pyrethroids, triazine and sulfonylurea **(Afify *et al.*, 2010).** Most of these have been found to be toxic when used in large amount than recommended. These affects directly as well as indirectly to soil productivity and agro ecosystem quality **(Imfeld and Vuilleumier, 2012).** When pesticides are applied in the environment, they undergo transformation processes by various mechanisms like physical, chemical and biological agents where microorganisms play a vital role. The transformation mechanism includes oxidation, hydrolysis, reduction, etc. catalyzed by various types of enzymes. They are indicators of biological equilibrium, fertility (**Antonious, 2003)** and changes in the biological status due to soil pollution **(Bending *et al.*, 2004)**. The chemical and physical properties of pesticide are an important factor in degradation process *i.e.* associated with abiotic as well as biotic components; the latter has received much attention **(Hafez and Theimann, 2003)**. Therefore, microbial community in soil is important in degrading the pesticides as well as maintaining soil health by carrying out various functions.

Microflora of soil is of major concern because of their role in sustaining agricultural productivity through innumerable biochemical reactions facilitated by soil enzymes **(Madakka and Rangaswamy, 2009).** Major pesticides produced in India are Mancozeb, 2-4-D, Acephate, Profenofos, etc. (**Subash *et al.*, 2017**). Profenofos (o-4-bromo-2-chloro-pheny-o-ethyl-s-prophyl-phosphorothioate) is an organophosphate insecticide that was first registered in United States in 1982. It is a non-systemic insecticide and acaricide with contact and stomach action used against insects, termites, beetles, leaf hopper, aphids, bugs, mites, thrips, cotton stainer etc. in variety of crops such as cotton, corn, almond, maize, potato, soyabean, sugarbeet etc. and decreases their population by inhibiting acetyl cholinesterase enzyme of nerve impulse. This study is mainly concerted on evaluation of impact of different concentration of Profenofos in soil against microbial diversity and enzyme activities.

Microbial communities in soil ecosystems provide various important functions like decomposition of organic material, recycling of nutrients, nitrogen and carbon cycle, storage and release of nutrients, plant growth promotion by providing a major food source at the base of food webs. They are also capable of degrading the soil-associated organic pollutants and thus contribute to remediation of contaminated ecosystems which reduces the effect of pollution. Thus, many microbial functions are critical to crop production, soil sustainability and environmental quality and the impact of pesticides on the diversity of soil microbial communities and enzymatic activities become vigorous (**Gupta *et al.*, 2013**).

This study has been done to determine the potential of soil health indicator, which will be evaluated throughout, by the analysis of microbial counts and selected soil enzyme activities after the application of different doses of Profenofos in soil procured from vegetable planted field. Enzymes chosen for study are important due to their critical role in C (cellulase, amylase), N (urease), P (acid and alkaline phosphatase) cycles and nutrient mineralization processes.

I. MATERIALS AND METHODS

2.1 CHEMICALS

Profenofos (50EC, Celeron) Excel Crop Care Limited was purchased from local pesticide supplier. All other chemicals used were of AR grade from Hi-Media, laboratories.

2.2 Media

All media like Ken Knight, Munarier's Medium and Nutrient Agar were prepared by dissolving the ingredients in distilled water and sterilized at 15 psi (121^0C) pressure for 20 min after adjusting the pH. The compositions of different media used for isolation are given below;

2.3 Soil samples

Soil samples were collected from vegetable (Ridged gourd; *Luffa acutangula*) planted field of Bhiwani (Haryana, India) at a depth of 0-10 cm. These soil samples were partially air dried overnight and then sieved through 2mm mesh sieve.

2.4 Physiochemical properties of soil

After sieving physiochemical properties of soil such as soil texture, organic and micronutrients content was determined by using Hi-media test kits**.** Soil pH was determined by pH meter. Water holding capacity was determined by filter paper method.

2.5 Treatments/ Experiments

Sieved soil was kept in 20 Petri-plates (50 gm soil in each Petri-plate) in the laboratory and was treated with the different concentrations of Profenofos like 0.1 ppm (T1), 1 ppm (T2), 10 ppm (T3) and 100 ppm (T4), respectively and control was kept without treatment. The control soil samples were given only distilled water. After treatment soil samples were homogenized to distribute the profenofos pesticides, and enough distilled water was added to maintain at 50-60% water holding capacity (WHC) and incubated at 30°C.

2.6 Effect of different concentrations of Profenofos on microbial population

The effect of different concentrations of Profenofos was determined on microbial populations in the soil, in triplicates at 1st, 7th, 14th, 21st day after treatment with Profenofos.

a.) For estimation of the bacterial population, duplicates of each treatment were withdrawn for serial dilution and plating on nutrient agar medium and subsequently incubated for 24 h in an incubator at 30°C. After incubation, bacterial colonies grown on nutrient agar medium were counted and expressed as the number of colonies formed per gram of soil (dry weight basis).

b.) For estimation of the population of actinomycetes plating was done on Ken Knight's agar medium and subsequent incubation was done for 3 days in the dark at 30°C.

2.7 Preparation of Buffers

i.) Tris-HCl buffer of pH 9.0:- 0.2M Tris (hydroxymethyl) Aminoethane and 0.2N HCl were prepared separately 100 ml each. Thereafter, 50ml of 0.2M Tris (hydroxymethyl) Aminoethane was mixed with 5.0ml of 0.2N HCl and total volume was made 200ml by adding distilled water.

ii.) Citrate buffer of pH 5.0:- 0.1M Citric acid and 0.1M Sodium Citrate were prepared separately 100 ml each. Thereafter, 20.5 ml of 0.1M Citric acid was mixed with 29.5ml of 0.1M Sodium Citrate and total volume was made 100ml by adding distilled water.

iii.) Phosphate buffer of pH 5.8:- 0.2M dibasic sodium phosphate and 0.2M monobasic sodium phosphate were prepared separately 100 ml each. Thereafter, 46.0ml of 0.2M monobasic sodium phosphate was mixed with 0.4 ml of 0.2M dibasic sodium phosphate and total volume was 100 ml by adding distilled water.

2.8 Effect of different concentrations of Profenofos on enzymatic activities

For estimation of the enzyme activities, duplicates of each treatment were withdrawn at 1st, 7th, 14th and 21st day after treatment with Profenofos and enzymatic activities were determined in triplicates using the following methods:

a.) **Estimation of Amylase and Invertase activities was done by Dinitrosalicylic acid (DNS) Colorimetric Method**

Reagents Required:- Toluene, 1% Starch, 5% Sucrose, Phosphate buffer of pH 5.8 and Dinitrosalicylic acid (DSA) Colour Reagent: 1 g of 3, 5- dinitrosalicyclic acid (DSA) was added with 20 ml 2N NaOH. 30 g sodium potassium tartarate was taken with 50 ml of distilled water. These two solutions were mixed properly and warmed slightly till dissolved completely. Then the volume of the solution was made to 100 ml by proper mixing.

Method of estimation:

To the three gram soil taken in test tube 0.2 ml of toluene was added, mixed and left for 15 minutes. Thereafter 6 ml of Phosphate buffer (pH 5.8) and 6 ml of substrate (1% soluble starch for amylase and 5% sucrose for invertase) were added to the test tubes, mixed well and left for 24 h of incubation in dark (wrapped with aluminum foil) at 30^{0}C. After incubation, the samples were centrifuged at 2000 rpm for 30 minutes. 1 ml of supernatant was mixed 2 ml of color reagent and kept in water bath at 90^{0}C for 5 minutes. Left for cooling at room temperature and then 2 ml of distilled water was added. The absorbance was noted at 540 nm with the help of UV-Vis Spectrophotometer. Standard curve was prepared by taking glucose as standard. In Blank 6 ml distilled water was added in place of substrate and rests of the steps were kept same.

b. Estimation of Acid and Alkaline Phosphatase activities by using PNPP (Paranitrophenyl phosphate) colorimetric method

Reagents required: Paranitrophenyl phosphate, 0.1N NaOH, Tris-HCl buffer of pH 9.0 (for Alkaline Phosphatase activity), Citrate buffer of pH 5.0 (for Acidic Phosphatase activity)

Estimation of Phosphatase Activity:

5gm of soil was taken from each set i.e. control and treated soils in test tubes in triplicate. Thereafter, added 20 ml of paranitrophenyl phosphate (10µg/ml) in these tubes and incubated for two hours, except for the blank sample, and then centrifuged at 10,000 rpm for 5 minutes. The blank sample was mixed with PNPP and immediately centrifuged. Now from each centrifuged tube 1ml supernatant was taken in labelled test tubes and 2ml of 0.1N NaOH is added. The absorbance of each sample is then estimated at 420 nm by using a UV-visible spectrophotometer. The standard curves were prepared by taking different concentrations of p-nitrophenol in buffers (acidic and alkaline). Enzyme activities were expressed in terms of concentration of p-nitrophenol in µg/g of soil.

II. RESULTS AND DISCUSSION

In the present study, the effect of different concentrations of Profenofos pesticide was evaluated on microbial counts and enzymatic activities in the soil at different days of incubation.

3.1 Bacterial and actinomycetes population in soil

The evaluation of the adverse effect of pesticide on the microbial count and the soil enzyme activity were evidenced in soil amended with Profenofos. The soil samples were amended with 0.1, 01, 10 and 100 ppm of Profenofos and moisture content was maintained regularly and appropriate samples were withdrawn at regular intervals of 00, 07, 14 and 21 days. The populations of bacterial and fungal isolates in terms of colony forming units (CFUs) were determined using viable plate count technique using N-agar and RBS-agar plates. All the plating were performed in triplicates and represented as mean values.

3.2 Physiochemical properties of the soil

Physiochemical properties of soil were analyzed by using Hi-media test kits that are represented in the Table 1.

Table 1: physiochemical properties of the soil used in the study

Sr. No.	Name of the Property	Value
1.	Clay (<=2.00mm) (%)	10
2.	Silt (<=2.00mm) (%)	30
3.	Sand (<=2.00mm) (%)	60
4.	Soil Textural class	Sandy loam
5.	Soil pH	6
6.	Water holding capacity	60%
7.	Iron (Fe) (ppm)	3.0 - 6.0
8.	Manganese (Mn) (ppm)	0.2 - 2.0
9.	Copper (Cu) (ppm)	More than 2.0
10.	Molybdenum (Mo) (ppm)	0.0 - 0.1
11.	Zinc (Zn) (ppm)	0.0-0.5

3.3 Activity of profenofos on bacterial count of soil (control and treated) at different days of incubation

Effect of Profenofos on bacterial count/gm of soil (control and treated) at different days of incubation was determined in **Table 2 and Fig. 1(a).** During investigation it was observed that the lower concentration of Profenofos (1 ppm) increased the bacterial counts by 25.95% but the higher concentrations (10 ppm and 100 ppm) reduced the bacterial count by 12.43% and 29.73%, respectively. **Mall *et al.* (2013)** investigated that the applications of profenofos at recommended dose did not caused any significant change on soil microbial population. The continued use of Profenofos in the soil showed the adverse effect on the microbial count **(Tejada *et al.* 2001)**. **Kochhar (2017)** in their studies determined that profenofos 50 EC up to the concentration of 20 ppm did not show any adverse effect on soil microbial activity. Profenofos with concentration of 100 ppm (T_4) showed lowest bacterial count (13.00 CFUx10^7/gm soil). It was followed by Profenofos with concentration of 10 ppm (T_3) and 1 ppm (T_2) treated soil in which bacterial count 16.20 CFUx10^7/gm soil and 23.30 CFUx10^7/gm soil, respectively was recorded. Profenofos with concentration of 0.1 ppm (T_1) resulted in (18.60 CFUx10^7/gm soil) which was statistically at par with control (18.50 CFUx10^7/gm soil) (Table 2). Interaction between the treatments and days of incubation was found to be statically significant (CD= 0.09; p=0.05). The effect of incubation period revealed that bacterial counts were found to be increased upto 14th days of incubation and thereafter decrease was observed at 21st days of incubation.

Table 2: effect of different concentrations of profenofos on bacterial counts per gram of soil (control and treated) at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day14	Day21	
Control (Without Treatment)	19.40	21.20	18.00	16.00	**18.50[a]**
T_1 (0.1 ppm Profenofos)	19.00	20.00	18.00	17.40	**18.60[a] (-0.54)**
T_2 (01 ppm Profenofos)	22.00	23.00	23.40	24.60	**23.30 (-25.95)**
T_3 (10 ppm Profenofos)	14.30	13.00	17.50	20.00	**16.20 (12.43)**
T_4 (100 ppm Profenofos)	12.00	10.00	14.00	16.00	**13.00 (29.73)**
Mean	**77.10**	**79.20**	**90.90**	**79.70**	

C.D. for Tretments (T) = 0.26, SE(m) = 0.52 **C.D. for Days (D) =0.12, SE(m) = 0.25**
C.D. for T×D= 0.09, SE(m)=0.18 **Values with the same superscript do not differ significantly**

* Number of colonies per gm soil= Colony forming units x dilution factor / Dry weight of soil

3.4 Effect of different concentration of profenofos on actinomycetes count per gram of soil (control and treated) at different days of incubation

Impact of organophosphate insecticides, Profenofos was assessed on actinomycetes populations of soil (control and treated) at different days of incubation was represented in the **Table 3 and Fig. 1(b).** Actinomycetes are important microbes for the degradation and utilization of a wide range of complex organic molecules (**Watson and Williams, 1974**). The degradation characteristics of this insecticide at different concentrations and incubation periods were investigated. The results on varying concentrations of profenofos (0 ppm-100 ppm) on actinomycetes counts showed that concentration upto 1 ppm favoured the growth of actinomycetes which increases upto 33.53% at the 1ppm concentration of Profenofos. Pesticides may act as a source of energy and nutrients to multiply for some groups of microorganisms. According to **Nasreen *et al.* (2015**) when we applied the recommended doses, some pesticides may be increases the microbial population, beyond that, there is no adverse effect on microbial population. They observed the increase in actinomycetes population increases up to utilization of 2.5 kg of profenofos per hectare. At higher concentrations (10ppm-100ppm), actinomycetes count was decrease upto 16.56% and 51.10%, respectively during investigation. Interaction between counts and days of incubation was also found statically significant (CD= 1.51; p=0.05). Counts were found to be increased at every week upto observation.

Profenofos with concentration of 100 ppm (T_4) showed lowest actinomycetes count (26.08 CFUx10^5/gm soil) followed by 10 ppm (T_3) concentration of Profenofos. Soil treated with 0.1 ppm (T_2) and 1 ppm (T_2) increases the count of actinomycetes upto 54.17 CFUx10^5/gm soil and 71.21 CFUx10^5/gm soil, respectively (Table 3). Higher rates of (7.5, 10.0 kg/ha) the pesticides was either toxic or innocuous to the urease activity of microbial population. In contrary, **Nasreen *et al.* (2015)** observed the stimulation in actinomycetes populations in the range of 55-83% by profenofos at 10, 25 and 50 ppm concentration for 5 days incubation. Current findings revealed that actinomycetes population was inhibited at 10 and 100 ppm of the selected insecticide application in vegetable planted soil **(Fig. 2)**. **Gundi *et al.*, (2007)** studied the effect of three insecticides (monochrotophos, quinalphos, and cypermethrin) on microbial populations and observed synergistic effects at the lower level and adverse effects at the highest level of the insecticides.

Table 3: effect of profenofos on actinomycetes count per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day14	Day21	
Control (Without Treatment)	52.83	60.67	51.50	48.33	**53.33**
T_1 (0.1 ppm Profenofos)	54.33	61.83	52.83	47.67	**54.17 (-1.58)**
T_2 (1 ppm Profenofos)	61.83	72.33	77.33	73.33	**71.21 (-33.53)**
T_3 (10 ppm Profenofos)	23.67	42.00	50.67	61.67	**44.50 (16.56)**
T_4 (100 ppm Profenofos)	9.33	20.33	31.33	43.33	**26.08 (51.10)**
Mean	**40.40**	**51.43**	**52.73**	**54.87**	

C.D. for Tretments (T) = 0.76, SE(m) = 0.26 **C.D. for Days (D) =0.68, SE(m) = 0.24**
C.D. for T×D= 1.51, SE(m)= 0.53 **Values with the same superscript do not differ significantly**
* Number of colonies per gram soil = Colony forming units x dilution factor

Dry weight of soil

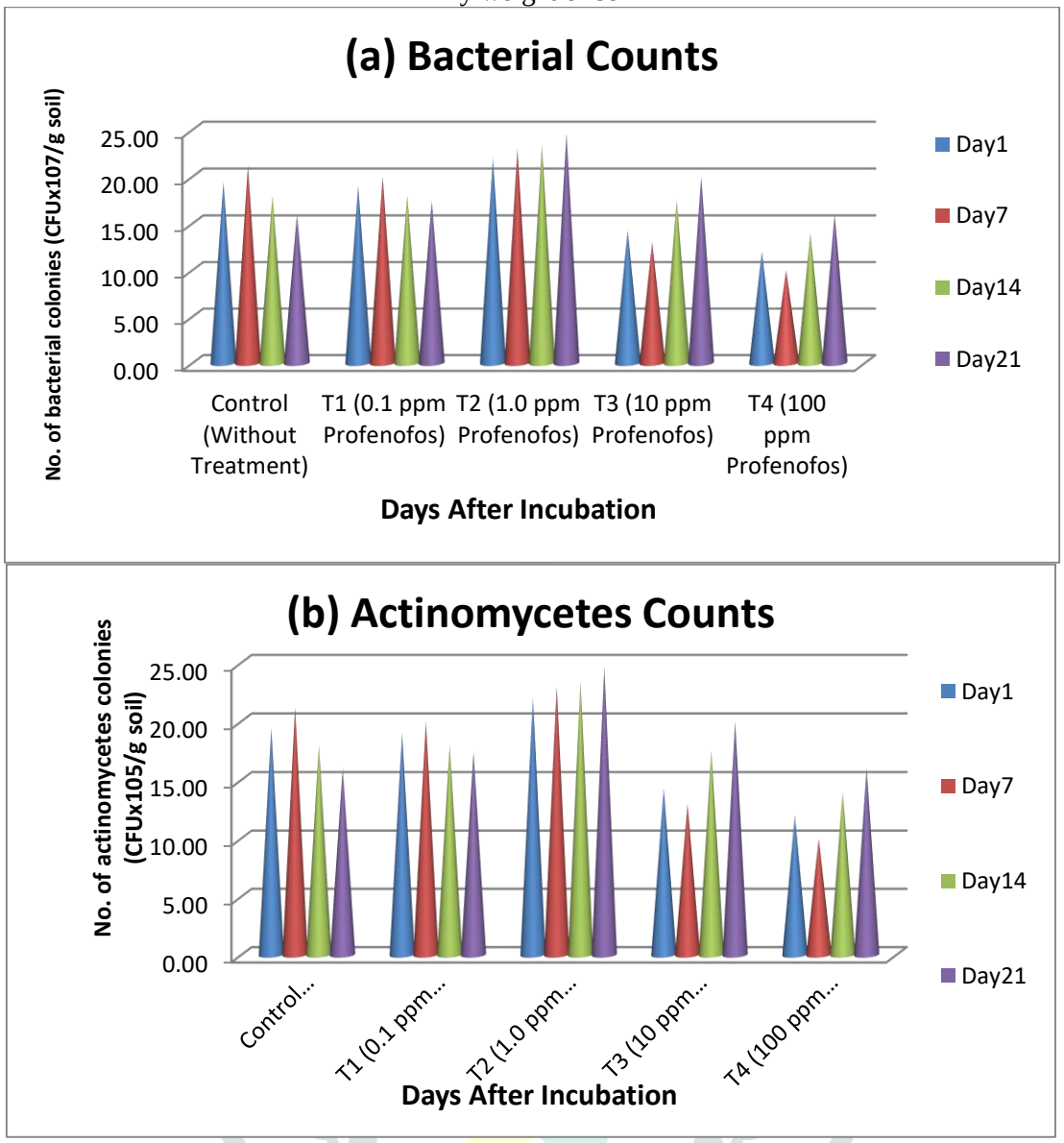

Fig. 1: effect of profenofos on bacterial and actinomycetes counts per gram of soil (control and treated) at different days of incubation

Soil Enzyme Activity

Soil enzymes play a key role in the energy transfer through decomposition of soil organic matter and nutrient cycling, and hence play an important role in agriculture. These enzymes catalyze many vital reactions necessary for the life processes of soil microorganisms and also help in stabilization of soil structure. Although microorganisms are the primary source of soil enzymes, plants and animals also contribute to the soil enzyme pool. Soil enzymes respond rapidly to any changes in soil management practices and environmental conditions. Their activities are closely related to soil organic matter (SOM), soil physical properties, microbial activity, biomass etc. Hence, soil enzymes are used as sensors for soil microbial status, for soil physio-chemical conditions, and for the influence of soil treatments or climatic factors on soil fertility. Soil enzymes are necessary catalysts for decomposition of soil organic matter (SOM) and nutrient cycling and, strongly influence energy transformation, environmental quality, and agronomic productivity.

In general, enzymatic activity decreases with an increase in soil depth. Further, soil enzymes activities are sensitive indicators of soil quality/health because they respond quickly to either environmental stress or soil management practice changes (**Sravanthi *et al.* 2015**). Moreover, availability of well-documented assays for a large number of soil enzyme activities makes them the preferred tool for assessing soil health and managing productivity of an ecosystem.

Major Soil Enzymes and their Functions

Major soil enzymes used as soil function indicators are presented in **Table 4**. The activities of these soil enzymes can be used for a meaningful assessment of reaction rates for important soil processes, soil productivity, microbial activity, inhibiting effects of pollutants, etc. **(Nare *et al.*, 2014).**

Table: 4 major soil enzymes and their functions

Enzyme	Source	Reaction catalyzed	End product	Soil function indicated	Factors influencing enzyme activity
α-Amylase	Plants, animals and microorganisms	Starch hydrolysis	Glucose and/or oligosaccharides	C-cycling	Management practices, type of vegetation, environment, and soil types
β-Amylase	Mainly plants	Starch hydrolysis	Maltose		
Invertase	bacteria, plants, animals, microorganisms and soils	Sucrose hydrolysis	Glucose and fructose	C-cycling	Management practices, type of vegetation, environment, and soil types
Alkaline phosphatase	Mainly bacteria	Hydrolysis of esters and anhydrides of phosphoric acid	Phosphate (PO_4)	P-cycling	Organic matter content, pH, management practices, pollution, crop species, and varieties
Acid phosphatase	Plants, fungi, and bacteria				

3.5 Effect of different concentrations of profenofos on enzyme activity per gram of soil (control and treated) at different days of incubation

3.5.1 Amylase activity

Effect of different concentration of profenofos on amylase activity per gram of soil (control and treated) at different days of incubation was determined and has been represented in the **Table 5 and Fig.2 (a).** The results of amylase activity showed a variable pattern in response to different concentration (0 ppm, 1 ppm, 10 ppm and 100 ppm) after 21 days of incubation (Table 4). The insecticides, profenofos showed individual increments in amylase activity were 17-21%. At higher concentrations (10 ppm - 100 ppm) showed reduction of about 3 to 4% respectively. Interaction between amylase activity and days of incubation was also found to be statically significant. Amylase activity increased significantly upto 7days and thereafter reduction was observed upto 21days of incubation. **Nasreen *et al.*, (2012)** observed amylase activities, significantly enhanced at 2.5 kg/ha in black soil after 10 days of incubation. Furthermore increase in concentration of insecticides decreased the rate of enzyme activity. This effect was continued up to 20 days of incubation in black soil. Whereas, the decline phase was started after 20 days and the minimum enzyme activities were noticed at the end of 40 days of incubation. But higher concentrations of insecticides at the level of 7.5 to 10.0 kg/ha were either toxic or innocuous to amylase activity in black soil.

3.5.2 Invertase activity

Invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) is the enzyme that catalyzes the hydrolysis of sucrose and yields glucose and fructose, is widely distributed in bacteria, plants, animals, microorganisms and soils. The activity of this enzyme in soils deserves special recognition because its substrate, sucrose, is one of the most abundant soluble sugars in plants. Invertase is partially responsible for the transformation/ decomposition of plant litter/organic matter in soils. Invertase is ubiquitous enzyme that occurs in plant tissues and soil organisms (**Frankenberger and Johanson, 1983).** During present investigation the activity of invertase at different days of incubation was determined with different concentration of Profenofos and has been represented in the **Table 6 and Fig. 2(b).** The results showed significant increase in the activity at 1ppm of about 12.39%. Thereafter, increase in concentration (10ppm-100ppm) resulted in the significant decrease of invertase activity around 36.79% and 76.88%, respectively. Interaction between treatments and days of incubation also showed statically significant (CD=2.90; p=0.05).

3.5.3 Phosphatase Activity

Phosphatases are a group of hydrolases that catalyze the hydrolysis of ester-phosphate bonds, leading to the formation of phosphate. These enzymes can be a good indicator of the organic phosphorus mineralization potential and biological activity of soils and strongly control the biotic pathways of phosphorus (P) *i.e.* an essential element for life, which is often limiting in terrestrial ecosystems. Phosphatase enzymes are also used by soil microorganisms to access organically bound phosphate nutrients

(**Mahanta, 2016**). Acid and alkaline phosphatases are very sensitive and respond very well to changes in the soil management practices. The present investigation provides information about the non-target effects of profenofos toward activities of acid and alkaline phosphatases of soil at different days of incubation by using "OPSTAT" and "ANOVA" statistics analysis. There was a gradual increase in the enzyme activity upto 7th days and after that decreased upto 21st day. Increase in concentration of an insecticide from 0.1 to 1.0 ppm/gm soil increased the enzyme activity, and concentrations of 10.0 and 100 ppm/gm soil resulted in significant decrease in the activities.

3.5.4 Alkaline phosphatase activity

Effect of profenofos on alkaline phosphatase activity per gram of control and treated soil at different days of incubation was determined and has been represented in the **Table 7 and Fig. 2(c).** The results of varying concentrations of profenofos (0ppm-100ppm) on alkaline phosphatase showed that slight increase in the activity at 1ppm. Thereafter at higher concentrations (10 ppm-100 ppm) decrease of about 19% and 46% was observed. Interaction between the treatments and days of incubation showed that initial increase in the activities up to 7days then decrease up to 14 days and further increase was observed between 14 to 21days.

3.5.5 Acidic Phosphatase activity

Effect of profenofos on acidic phosphatase activity per gram of control and treated soil at different days of incubation was determined and has been represented in the **Table 8 and Fig. 2(d).** The results showed that slight increase in the acidic phosphatase activity upto 1ppm. Thereafter, at higher concentrations (10 ppm - 100 ppm) decrease was observed of about 16% and 28% respectively. Interaction between the treatments and days of incubation showed that upto 7days not much affect in the activity was observed but after 7days, significant decrease in the activity was observed.

The enzymatic activities of acid and alkaline phosphatase respond differently to insecticides. Indeed, the same insecticide may inhibit acid phosphatase and stimulate alkaline phosphatase activity, and vice versa (**Cycon´ *et al.*, 2010**; **Defo *et al.*, 2011**; **Jastrzebska 2011**). The difference in behaviour of both acid and alkaline phosphatases toward pesticides can be attributed to the structure of soil microbial communities and their sensitivity to pesticides applications (**Klose *et al.*, 2006**). Insecticides had inhibitory effects on phosphatases (**Madhuri and Rangaswamy 2002**; **Yao *et al.*, 2006**). Overall, pesticides appear to have an inhibitory effect on the enzymatic activities involved in the phosphorus cycle.

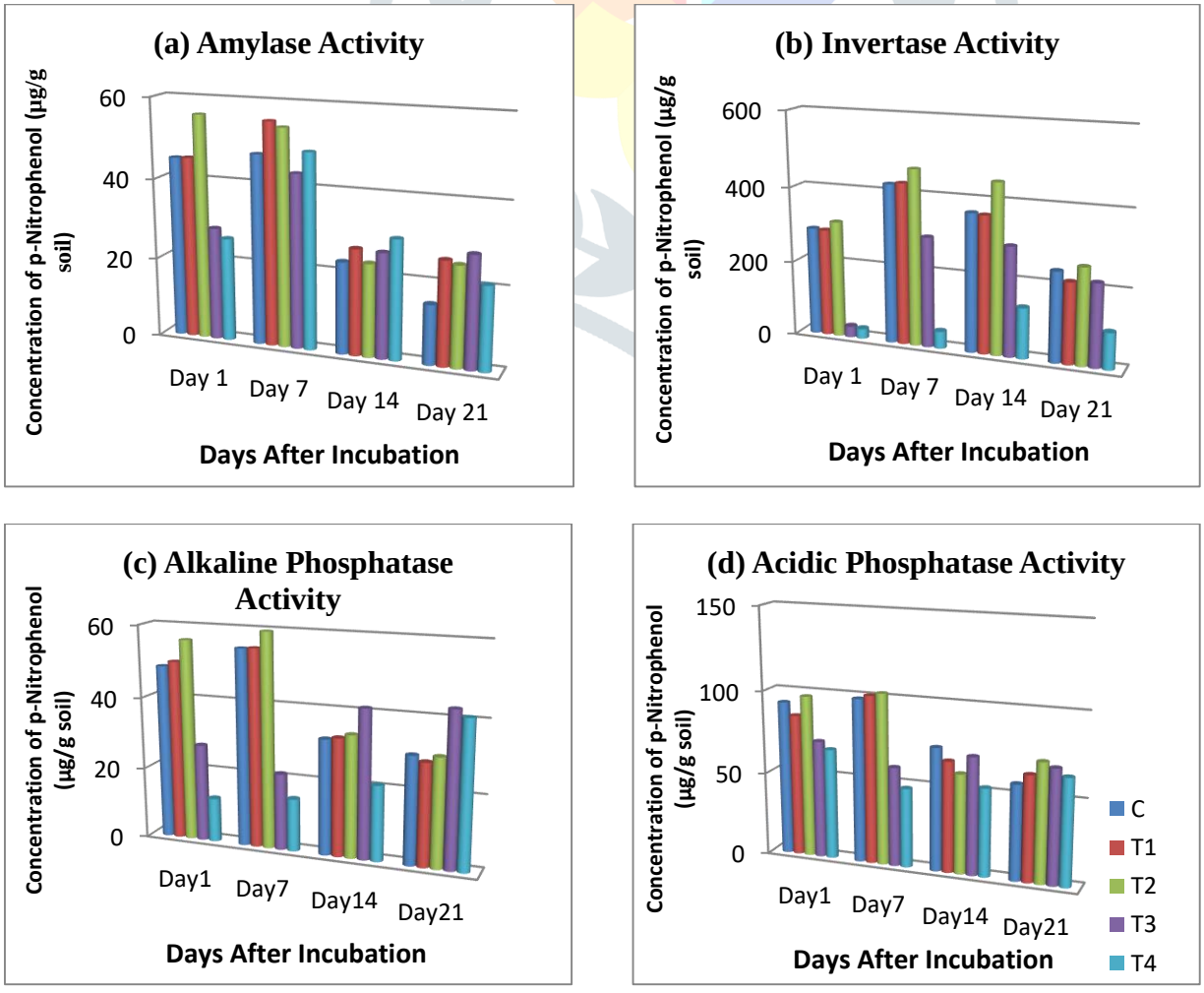

Fig.2: effect of profenofos on enzyme activities

Table 5: effect of different concentrations of profenofos on amylase activity per gram of control and treated soil microbes at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day14	Day21	
Control (Without Treatment)	45.12	47.36	23.05	14.95	**32.62**
T_1 (0.1 ppm Profenofos)	45.29	55.41	26.56	25.98	**38.31 (17.44)**
T_2 (1 ppm Profenofos)	55.98	54.08	23.28	25.12	**39.62 (21.46)**
T_3 (10 ppm Profenofos)	28.22	43.39	26.27	27.99	**31.47 (3.53)**
T_4 (100 ppm Profenofos)	25.92	48.74	29.89	21.04	**31.40 (3.74)**
Mean	**40.11**	**49.80**	**25.81**	**23.01**	

C.D. for Tretments (T) = 0.28, SE(m) = 0.10 **C.D. for Days (D) =0.25, SE(m) = 0.08**
C.D. for T×D= 0.57, SE(m)= 0.19 **Values with the same superscript do not differ significantly**

Table 6: effect of profenofos on invertase activity per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day14	Day21	
Control (Without Treatment)	288.62	422.88	366.44	238.91	**329.21**
T_1 (0.1 ppm Profenofos)	286.61	427.59	363.22	214.54	**322.99 (1.89)**
T_2 (1 ppm Profenofos)	311.04	465.92	448.85	256.10	**370.48 (12.39)**
T_3 (10 ppm Profenofos)	29.03	293.28	290.75	219.26	**208.08 (36.79)**
T_4 (100 ppm Profenofos)	26.04	45.87	135.06	97.47	**76.11 (76.88)**
Mean	**188.27**	**331.11**	**320.87**	**205.26**	

C.D. for Tretments (T) = 1.45, SE(m) = 0.51 **C.D. for Days (D) =1.30, SE(m) = 0.45**
C.D. for T×D= 2.90, SE(m)= 1.01 **Values with the same superscript do not differ significantly**

Table 7: effect of profenofos on alkaline phosphatase activity per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day14	Day21	
Control (Without Treatment)	48.56	54.76	32.12	29.96	**41.35[a]**
T_1 (0.1 ppm Profenofos)	49.99	55.02	32.74	28.45	**41.55[a] (0.48)**
T_2 (1 ppm Profenofos)	56.11	59.59	33.89	30.19	**44.95 (8.71)**
T_3 (10 ppm Profenofos)	27.20	21.33	41.24	42.85	**33.15 (19.83)**
T_4 (100 ppm Profenofos)	12.30	14.70	21.04	40.95	**22.25 (46.19)**
Mean	**38.83**	**41.08**	**32.21**	**34.48**	

C.D. for Tretments (T) = 0.38, SE(m) = 0.13 **C.D. for Days (D) =0.34, SE(m) = 0.12**
C.D. for T×D= 0.77, SE(m)= 0.27 **Values with the same superscript do not differ significantly**

Table 8: effect of profenofos on acidic phosphatase activity per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day14	Day21	
Control (Without Treatment)	92.80	98.44	73.88	57.27	**80.60**
T_1 (0.1 ppm Profenofos)	85.28	100.77	66.60	63.35	**79.00 (1.99)**
T_2 (1 ppm Profenofos)	97.32	102.65	59.78	71.52	**82.82 (2.75)**
T_3 (10 ppm Profenofos)	70.87	59.59	70.62	68.61	**67.42 (16.35)**
T_4 (100 ppm Profenofos)	66.55	47.75	53.01	64.13	**57.86 (28.21)**
Mean	**82.56**	**81.84**	**64.78**	**64.98**	

C.D. for Tretments (T) = 0.28, SE(m) = 0.10 **C.D. for Days (D) =0.25, SE(m) = 0.09**
C.D. for T×D= 0.56, SE(m)= 0.20 **Values with the same superscript do not differ significantly**

Relationships between pesticide mechanisms of action and enzymatic responses

The understanding and interpretation of enzymatic responses after pesticides' addition are very difficult. Indeed, the observed responses are the resultant of numerous factors. There are direct/or indirect interactions of pesticides with soil enzymes (**Gianfreda and Rao 2008**). Among them, it can be cited the binding of pesticide with the active site of the enzyme which affect their catalytic activities (**Tabatabai, 1994**) or the use of pesticides as a nutriment source by the microorganisms which may shift not only the balance between the communities but more directly the biosynthesis of enzymes by induction or repression phenomena (**Zabaloy *et al.*, 2012; Chishti *et al.*, 2013**).

The direct phenomena must also be added the indirect impacts of pesticides on microbial community structure which lead to changes in soil enzymatic activities (**Lo, 2010**). These impacts are strongly related to functional redundancy of the target activity (**Griffiths and Philippot, 2013**) and the intrinsic properties of soil, pH, humus, clay content or organic matter that influence the accessibility of pesticides (**Defo *et al.*, 2011; Mun˜oz-Leoz *et al.*, 2013**). At present, we lack the necessary information on how these different phenomena interact in order to predict a general response for a given enzyme.

Insecticides that altered the movement of ions across the nerve cell membranes induce rather a positive response of soil enzymatic activities while insecticides inhibiting the enzyme acetyl cholinesterase of nerve impulses caused rather a negative response (**Riah *et al.*, 2014**).

III. CONCLUSION

The intensive use of pesticides in agriculture resulted of those chemical into soil, air and water ecosystems. Pesticides applied to soil at planting persist throughout the development of plant roots. Therefore, some of the chemical interacts with micro-organisms in soil and rhizosphere. The appliance of pesticides in minimum doses is effective and also helpful however inexcusable uses cause their harmful effects have become manifold and far reaching. Sadly, chemical pesticides still continue to be used in massive proportions in several elements of the planet that interfere with the soil-microbes and soil-enzyme interactions, ultimately resulting in environmental degradation. As such, it's hoped that this work and its results are going to be helpful to the agricultural communities and thereby encourage them to modify to less toxic or biological alternatives for our own prosperity.

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