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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(2): 152-159

www.thepharmajournal.com
Received: 01-12-2019
Accepted: 05-01-2020

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Impact of chlorpyriphos pesticide on microbial populations and enzymatic activities in cotton planted soil

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DOI: https://doi.org/10.22271/tpi.2020.v9.i2d.4365

Abstract
Evaluation of impact of an organophosphate pesticide, chlorpyriphos on soil quality indicators such as microbial populations and enzymatic activities has been done in the present study. Soil collected from cotton cultivated field of Bawani Khera, Bhiwani (Haryana, India) was administered with different concentrations of chlorpyriphos i.e. 0.1 ppm, 1 ppm, 10 ppm, 100 ppm. Control was also kept with no treatment with pesticide. Populations of bacteria, actinomycetes, fungi and activities of enzymes such as amylase, invertase, alkaline phosphatase and acidic phosphatase were assessed at 1st, 7th, 14th and 21st day of incubation. Lower concentrations (0.1ppm and 1ppm) were found to be beneficial but higher concentrations (10ppm and 100ppm) lead to reduction in microbial populations and enzymatic activities. Bacterial, actinomycetal and fungal populations were found to decrease by 31%, 20% and 32% with the concentration of 10 ppm and 70%, 61% and 40% with the 100 ppm respectively. Similar trend of reduction was also observed in the enzymatic activities. Amylase, invertase, alkaline phosphatase and acidic phosphatase activities showed overall reduction of 11%, 16%, 38% and 18% with the application of 10 ppm and 60%, 66%, 67% and 54% with the application of 100 ppm of the chlorpyriphos respectively.

Keywords: Pesticide, chlorpyriphos, microbial population, amylase, invertase, alkaline phosphatase, acidic phosphatase

1. Introduction
Soil is vital alive system that contains almost all kinds of living organisms. Soil maintains their lives efficiently healthy and well sustained. In return to it, trillions or billions of bacteria, fungi, and other kind of microbes execute interactions (positive or negative) with each other as well as with their non-living counterpart existing in the soil and make it lively, well growing medium. They use dead organic matter of soil as food and carry out physical and biochemical changes in them and ultimately transforming them to simpler molecules. The whole process adds- up nutrients to the soil and in turn maintains the soil health.

Agriculture solely depends upon healthy soil. India is an agriculture based country. According to Agricultural Indian Census- 2011, 61.5% of the Indian rural population depends upon agriculture. In Haryana state, out of every 100 hectares 3/4 is cultivated. The production of food grains per hectare is much higher in Haryana than in any other state of the country. Thus, economy of the state is dominated by agriculture. Therefore, it is an essential aspect to maintain the soil health in the state. There are several possible threats to the soil health such as soil compaction, soil erosion, soil contamination with agrochemicals, water logging, desalinization, desertification, loss of soil biodiversity etc. These all threats are hazardous and can damage the productivity of the soil. Among these threats over use of agrochemicals especially pesticides adversely affects the biological composition of soil and its fertility. These also alter the functionality of soil in terms of enzyme activities (Bending *et al*. 2007; Xia *et al*. 2011; Punitha *et al*. 2012; Walia *et al*., 2014) [6, 38, 28, 12].

Pesticides are the chemicals that are used to get rid of crop damaging and other household pests. No doubt these are controlling pests and hereby facilitating better growth of crops. But it has also been analyzed and well documented that out of tremendous amounts of pesticides used, only 0.1% reaches the target pest and more than 99.7% persists and adversely affects the environment (Pimentel, 1995; Andrea *et al*., 2000; Carriger *et al*., 2006; Baxter and Cummings, 2008) [26, 18, 8, 5]. Approximately 80,000 tones- of pesticides are produced annually in India (Devi *et al*., 2017) [10] and pesticide market is growing at the upsetting rate of 12-13 per cent per annum.

States wise highest consumption of pesticides is in Maharashtra, followed by Uttar Pradesh, Punjab and Haryana (Subash *et al*., 2017). Among crops maximum pesticides of around 50% are used in cotton that covers only 5 percent of the cropped area. This consumption has led to the exhaustion of soil fertility and a reduction in sustainable crop production (Sharma *et al*., 2003) [32]. Among pesticides their come a wide range of chemical compounds including insecticides, fungicides, herbicides, plant growth regulators and others (Gevao *et al*. 2000) [13]. Present study is mainly concentrated on an organophosphate pesticide named Chlorpyriphos which is an organophosphate insecticide, most widely used against various insects, termites, and beetles throughout the globe. In 1965, it was introduced by Dow chemicals. It acts on the nervous system of insects by inhibiting acetyl cholinesterase enzyme. The crops in which this pesticide is used are cotton, corn, almonds and fruit trees including oranges, apple, bananas etc. The half-life of this is from 10 to 120 days. The extensive use of the pesticide has resulted in environmental contamination. Its residues have also been detected in food items. Negative impacts of several pesticides on soil microbial diversity and enzyme activities like hydrolases, oxidoreductases, dehydrogenases, proteases, acid and alkaline phosphatases, amylases, cellulases, invertases has been evaluated earlier by many researchers (Monkiedje *et al*. 2002; Tejada. 2009; Rasool and Reshi., 2010; Sumit 2011; Jastrzebska. 2011; Defo *et al*. 2011; Sebiomo *et al*. 2012) [25, 35, 29, 34, 16, 9]. Enzyme activities in the soil environment are considered to be a major contributor to overall soil microbial activity and soil quality (Visser, S. and Parkinson, D. 1992; Dick *et al*., 1994) [36, 11]. Therefore it is necessary to examine the effects of pesticides on soil micro flora and their activities as a part of the pesticide's threat evaluation. This study is designed to determine the impact of Chlorpyriphos on soil quality, which have been evaluated throughout, by the analysis of microbial counts and selected soil enzyme activities after the application of different doses of chlorpyriphos in soil procured from cotton planted field.

2. Materials and Methods

2.1 Chemicals

Chlorpyriphos (20EC), Crop Chemicals Ltd. was purchased from local pesticide supplier. All other chemicals used were of AR grade from Hi-Media, laboratories.

2.2 Media

Media such as Rose Bengal Chloramphenicol Agar, Ken-Knight and Munarier's Medium and Nutrient Agar Medium were prepared by dissolving the ingredients in distilled water and sterilized at 15 psi (121 ^{0}C) pressure for 20 min after adjusting the pH. 2.3 Soil samples Soil samples were collected from rhizospheric region at a depth of 0-10 cm from cotton planted field of Bawani Khera village of Bhiwani (Haryana, India). These soil samples were mixed properly and partially air dried overnight and then sieved through 2mm mesh sieve.

2.4 Physiochemical analysis of soil

Soil pH was determined by pH meter. Water holding capacity was determined by filter paper method. Soil texture and micronutrients content were determined by Hi-media test kits.

2.5 Treatments

After sieving, soil was kept in petri-plates (50gm soil in each petri-plate) in the laboratory and was treated with the different concentrations of chlorpyriphos such as 0.1 ppm (T1), 1 ppm (T2), 10 ppm (T3) and 100 ppm (T4) respectively. Control was also kept that was without the treatment of chlorpyriphos. Acetone was used as a solvent for preparing stock solution of chlorpyriphos. The control soil samples were given only distilled water. After treatment soil samples were homogenized to distribute the chlorpyriphos, and enough distilled water was added to maintain at 50-60% water holding capacity (WHC) and incubated at 30°C. Sterilized distilled water was added after every two days of incubation to compensate for the loss of water by evaporation.

2.6 Effect of different concentrations of chlorpyriphos on microbial populations

The effect of different concentrations of chlorpyriphos was determined on microbial populations in the soil, in triplicates at 1st, 7th, 14th, 21st day after treatment with chlorpyriphos by using standard dilution technique. Bacterial, fungal and actinomycetes colonies were counted by plating 0.1 ml of suitable dilution on separate plate using spread plate method and incubated at 30 °C for 24 hrs. for bacterial colonies on nutrient agar media, at 30 °C for 3days for actinomycetes colonies on Ken Knight's agar medium and at 25 °C for five days for fungal colonies on Rose Bengal agar medium. Counts were expressed as the number of colonies formed per gram of soil (dry weight basis).

2.7 Preparation of Buffers

a. 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. pH was adjusted to 9.0.
b. 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. pH was adjusted to 5.0.
c. 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 4.0 ml of 0.2M dibasic sodium phosphate and total volume was made 100 ml by adding distilled water. pH was adjusted to 5.8.

2.8 Effect of different concentrations of chlorpyriphos on enzymatic activities

For estimation of the enzyme activities, duplicates of each treatment were withdrawn at 1st, 7th, 14th, 21st day after treatment with chlorpyriphos 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 used: Dinitrosalicylic acid (DSA) colour reagent*, Toluene, 1% Starch, 5% Sucrose, Phosphate buffer of pH 5.8.

*Solution A was prepared by adding 30 g sodium potassium tartarate in 50 ml of distilled water and solution B was prepared by adding 1 g of 3, 5-dinitrosalicylic acid (DSA) in 20 ml 2N NaOH. Solution

A and B were mixed properly and warmed slightly till dissolved completely. Then the volume of the solution was made to 100 ml by proper mixing.

2.9 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 hrs of incubation in dark(wrapped with aluminium foil) at 300 C. After incubation, the samples were centrifuged at 2000 rpm for 30 minutes. 1 ml of supernatant was mixed 2 ml of colour reagent and kept in water bath at 900C for 5 minutes. Left for cooling to room temperature and then 2 ml of distilled water was added. The absorbance was noted down at 540 nm. 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 (Para nitrophenyl phosphate) colorimetric method Reagents used: Para nitrophenyl 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)

2.10 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 para nitrophenyl 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 420nm 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.

3. Results and Discussion

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

3.1 Physicochemical properties of the soil

Physicochemical properties of the soil have been represented in the Table 1 as follows:

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

Name of the Property	Value
Clay (≤ 2.00mm) (%)	10
Silt (≤ 2.00mm) (%)	40
Sand (≤ 2.00mm) (%)	50
Soil Textural class	Loamy soil
Soil pH	5.2
Water holding capacity (%)	60
Iron(Fe)(ppm)	3.0-6.0
Manganese(Mn) (ppm)	0.2-2.0
Copper(Cu) (ppm)	0.0-0.5
Molybdenum(Mo) (ppm)	0.0-0.1
Zinc(Zn) (ppm)	0.5-2.0
Boron(B) (ppm)	1.0-2.0

3.2 Effect of chlorpyriphos on microbial populations

A. Bacterial population

Bacterial counts increased significantly up to 1ppm of chlorpyriphos concentration and decreased thereafter at higher concentrations i.e.10 ppm and 100 ppm by 31% and 70% respectively (Table 2. and Fig.1a.). The counts were also observed to decrease with the increase in number of days of incubation. Chlorpyriphos with concentration of 100 ppm (T4) showed lowest bacterial count (17.29 CFUx107/gm soil) followed by 10 ppm (T3) concentration of chlorpyriphos (39.86 CFUx107/gm soil). Soil treated with 0.1 ppm (T1) and 1 ppm (T2) increases the count of bacteria upto 59.06 CFUx107/gm soil and 64.61 CFUx107/gm soil, respectively. Interaction studies also revealed statistically significant effect on the bacterial counts (CD= 3.23; P=0.05). At the end of 1, 2 and 3 weeks of incubation bacterial counts were observed to increase significantly at 1ppm and decrease significantly at 10 and 100 ppm respectively. The adverse effect of higher concentrations may be due to toxic effect of chlorpyriphos or its degradation products such as TCP. Similar kind of effects has been noticed earlier by many researchers (Martinez-Toledo *et al*., 1992; Hindumathy and Gayathri., 2013; Walia *et al*., 2018) [24, 15 12]. Contrarily, non-toxic or favourable effects on microbial growth have also been observed (Sarnaik *et al*., 2006) [30]. Rani and his coworkers also in 2007 observed non- inhibitory effects of high concentrations of chlorpyriphos i.e. from 400 – 700 ppm. These may be due to degradation capabilities of bacteria involved in their study. Nasreen and his co-workers in 2015 found that bacterial populations, fungal populations and dehydrogenase activity increased with increasing concentration of the pesticides up to 5.0 kg ha-1, whereas actinomycetes population increases up to 2.5 kg ha-1. Higher rates of (7.5, 10.0 kg ha-1) pesticides were either toxic/innocuous to the urease activity or microbial population. Martinez-Toledo *et al*., 1992 [24] found decrease in the population of bacteria with the application of chlorpyrifos at 10- 300 µg g-1 in loamy soil. In Chinese loamy soils, methamidophos at 0.5, 2.5, 5 and 10µgg-1 inhibited the population of bacteria strongly throughout the incubation period (Xu *et al*., 1997) [39].

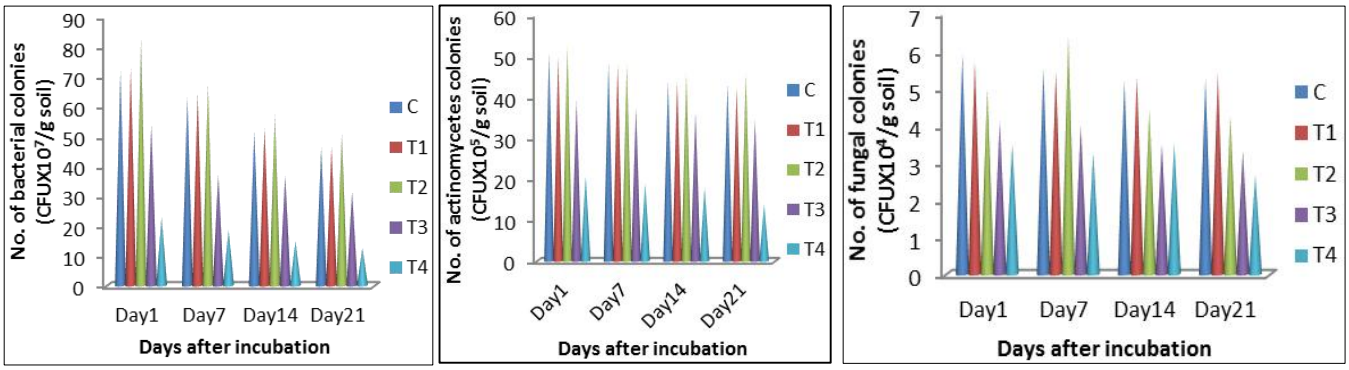
B. Actinomycetes population

Similar trend was observed in the results on varying concentrations of chlorpyriphos (0-100ppm) on actinomycetes count (Table 3 and Fig.1b.). Increase was observed up to 1ppm and thereafter, at high concentrations drastic decrease in the counts of 28% and 65% was observed at 10 and 100 ppm respectively. Actinomycetes count was observed to be

minimum with concentration of 100 ppm (T4) i.e.17.78 CFUx105/gm soil) followed by 10 ppm (T3) concentration of chlorpyriphos (36.85 CFUx105/gm soil). Soil treated with 1 ppm (T2) increased the count of bacteria upto 47.91 CFUx105/gm soil. Interaction between treatments and days of incubation showed maximum decrease in the counts in the 2nd week of incubation. Rajesh and his coworkers in 2015 [1] studied that application of carbosulfan at150, 200, 250 and 300 g a.i ha-1 and chlorpyrifos at 250 and 375 g a.i ha-1 in rice field did not show any noticeable adverse effects on soil bacterial and fungal count. Carbosulfan at 300 g ai ha-1 showed moderate toxic effect on the population of actinomycetes in rice field. Chlorpyrifos at 250 and 375 g a.i ha-1 and carbosulfan at 150, 200 and 250 g a.i ha-1 did not show any adverse effects on the soil actinomycetes count in rice field. Thus, microbes behave differently with the type and concentration of pesticides. Higher concentrations generally exert negative impact on microbes. Gundi and her co-workers in 2005 studied the effect of three insecticides (monochrotophos, quinalphos, and cypermethrin) on microbial populations in a black clay soil. They observed synergistic effects at the lower level and adverse effects at the highest level of the insecticides.

C. Fungal population

The results of effect of chlorpyriphos on fungal count showed slight decrease in the counts 1 ppm and thereafter drastic decrease in the counts by 62% and 54% at 10 ppm and 100 ppm respectively as compared to control (Table 4; Fig.1c.). Chlorpyriphos with concentration of 100 ppm (T4) showed lowest fungal count (3.24 CFUx104/gm soil) followed by 3.71 CFUx104/gm soil with the 10 ppm (T3) concentration of chlorpyriphos. Soil treated with 0.1 ppm (T1) showed almost similar count with control whereas 1 ppm (T2) showed slight decrease in the fungal count as compared to control. The interaction between treatments and days also showed statistically significant effects on counts (CD= 0.24; P=0.05). The counts increased in the 1st week and decreased in the 2nd and 3rd week of incubation. Supreeth and his coworkers in 2016 also observed the inhibitory effect of chlorpyrifos of two different concentrations i.e. 100 and 200 ppm on soil fungi after one day of incubation and further incubation for one and two weeks although increased the counts. They observed the overall decrease in the fungal diversity and dominated by only one species of actinomycetes. Bisht and his co-workers in 2014 observed that with the application of endosulfan in soil at lower doses (1-25 ppm), increased the fungal count from 17.67±1.15 to 23.00±2.00×103 CFU g soil-1. Thus, lower concentrations of endosulfan caused a stimulatory effect on fungal population as they probably utilized it as energy or other nutrient source. However, higher concentrations (50-500 ppm) of endosulfan caused inhibitory effect on the total fungal population.



a. Bacterial Population b. Actinomycetes Population c. Fungal Population

Fig 1: Effect of chlorpyriphos on microbial population's a. Bacterial population b. Actinomycetes population c. Fungal population

Table 1: Effect of chlorpyriphos on bacterial count* per gram (dry weight basis) of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day 1	Day 7	Day 14	Day 21	
C	71.94	63.01	51.57	46.31	58.21
T1	72.81	64.15	52.84	46.43	59.06
T2	82.59	67.41	57.19	51.26	64.61
T3	53.99	37.19	36.87	31.38	39.86
T4	22.99	18.57	14.92	12.69	17.29
Mean	60.86	50.06	42.68	37.61	

Factors	C.D.	SE(d)	SE(m)
Treatments	1.62	0.79	0.56
Days	1.45	0.71	0.50
Treatment x Days	3.23	1.59	1.12

*Number of colonies per gram soil = $\frac{\text{Colony forming units x dilution factor}}{\text{Dry weight of soil}}$

Table 2: Effect of chlorpyriphos on actinomycetes count per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day 1	Day 7	Day 14	Day 21	
C	50.64	48.22	43.78	43.09	46.43
T1	49.66	47.96	43.81	42.21	45.91
T2	52.35	47.99	45.62	45.67	47.91
T3	39.34	37.27	36.29	34.52	36.85
T4	20.76	18.84	17.84	13.68	17.78
Mean	42.55	40.06	37.46	35.83	

Factors	C.D.	SE(d)	SE(m)
Treatments	0.49	0.99	0.35
Days	0.44	0.89	0.31
Treatment x Days	0.98	1.98	0.69

*Number of colonies per gram soil = $\frac{\text{Colony forming units x dilution factor}}{\text{Dry weight of soil}}$

Table 3: Effect of chlorpyriphos on fungal count* per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day1	Day7	Day 14	Day 21	
C	5.90	5.53	5.21	5.24	5.46
T1	5.70	5.44	5.31	5.36	5.45
T2	4.97	6.40	4.41	4.24	5.01
T3	4.09	3.97	3.47	3.31	3.71
T4	3.50	3.27	3.53	2.66	3.24
Mean	4.83	4.92	4.38	4.16	

Factors	C.D.	SE(d)	SE(m)
Treatments	0.12	0.06	0.04
Days	0.11	0.05	0.04
Treatments x Days	0.24	0.12	0.09

$$*\text{Number of colonies per gram soil} = \frac{\text{Colony forming units x dilution factor}}{\text{Dry weight of soil}}$$

3.3 Effect of different concentrations of chlorpyriphos on enzyme activities

A. Amylase activity

The results of effect of varying concentrations of chlorpyriphos on amylase activity showed significant increase in the activity up to 1ppm and thereafter, at higher concentrations drastic decrease in the value was observed. At 10 ppm and 100 ppm the percentage decrease in the activity was 11% and 69% respectively (Table 5; Fig.2a.). Interaction between treatments and days of incubation was also found to be statistically significant (CD=0.70; p=0.05). The incubation period showed subsequent decrease in the activity from 1st to 3rd week. Similarly, Deborah and his coworkers in 2013 [3] observed stimulatory effect of imidacloprid and criadimefon, singly as well as in combination on the amylase activity at lower concentration whereas the enzyme activity decreased at a higher concentration. Nasreen and his coworkers in 2012 also observed enhancing effect of lower concentration of insecticides (2.5 kg/ha) on amylase activities, in black soil. But higher concentrations of insecticides (7.5 to 10.0 kg/ha) were found to be inhibitory.

B. Invertase activity

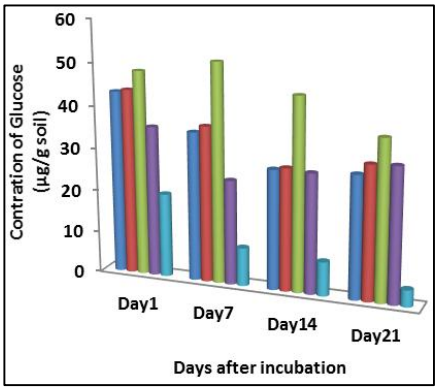
Similar trend was observed in the invertase activity (Table 6 and Fig. 2b.). At 10 ppm and 100 ppm the percentage decrease in the invertase activity were 16% and 66% respectively. Interaction between treatments and days of incubation was also found to be statistically significant (CD=0.64; P=0.05). The activity decreased subsequently from 1st to 3rd week of incubation. Recently, Ataikiru and his co-workers in 2019 also observed reduction in invertase activity in response to paraquat and carbofuran application in the soil at higher concentrations.

C. Alkaline phosphatase activity

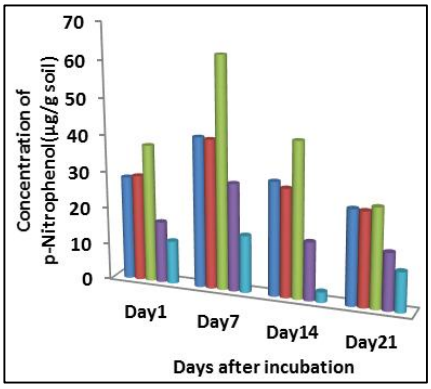
Effect on alkaline phosphatase activity (Table 7and Fig. 2c.) showed significant increase at 1ppm as compared to control. Thereafter, at higher concentrations (10-100ppm) drastic decrease in the value was observed. At 10 ppm and 100ppm the percentage decrease in the activities were 38% and 67% respectively. Interaction between treatments and days was also observed statistically significant (CD=0.98; P=0.05). The effect of incubation period showed that significant increase up to 1st week and thereafter decreased in the 2nd and 3rd week of incubation respectively. Similar trend was observed by Mahanta in 2016. Higher concentration of malathion inhibited the alkaline phosphatase activity as compared to lower concentration. Activity was also decreased with the time of incubation. Recently, Satapute and his coworkers in 2019 also concluded that phosphatase activity affected less at lower propiconazole (triazole pesticide) concentrations up to 2 weeks but thereafter activities were relentlessly reduced at higher concentrations and a long incubation period after 2 to 4 weeks.

D. Acidic phosphatase activity

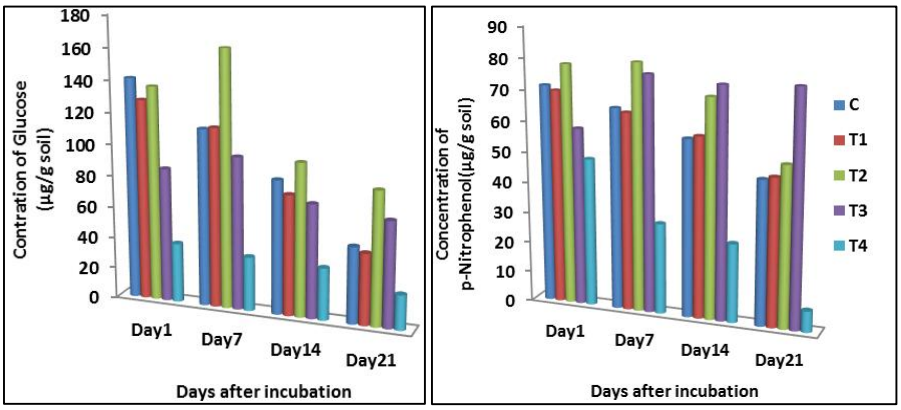
Effect of chlorpyriphos on acidic phosphatase activity (Table 8 and Fig. 2d.) showed significant increase at 1ppm and 10ppm of 16% and 18% respectively. Thereafter, at higher concentration (100ppm) drastic decrease (54%) in the value was observed. Interaction between treatments and days was also observed statistically significant. The activities decreased significantly with increase in the weeks of incubation i.e. from 1st week to 3rd week. Studies carried out by other researchers on effect of insecticides on phosphatase activity revealed variable effects i.e. sometimes inhibitory and sometimes stimulatory depending upon the concentrations and days of incubation. (Defo *et al*. 2011, Jastrzebska 2011) [9, 16]. Ataikiru and his coworkers in 2019 [2] found significant effect of Paraquat and Carbofuran on phosphatase activity w.r.t days of incubation in soil. Phosphatase activity decreased upto 3rd week and thereafter increased due to application of paraquat. Baćmaga *et al*. (2012) [4] reported the stimulatory effect of carfentrazone-ethyl on acid phosphatase and alkaline phosphatase. Phosphatase activities were also found to be decreased significantly by Filimon and his coworkers in 2015 due to application of cypermethrine and thiamethoxam at recommended rates in the soil.



a. Amylase Activity



b. Invertase Activity



c. Alkaline Phosphatase Activity d. Acidic Phosphatase Activity

Fig 2: Effect of chlorpyriphos on enzyme activities

Table 4: Effect of chlorpyriphos on amylase activity per gram of control and treated soil at different days of incubation

Treatments	Days of Incubation				Mean
	Day 1	Day 7	Day 14	Day 21	
C	43.16	35.18	28.22	28.91	33.85
T1	43.74	36.73	28.79	31.44	35.17
T2	48.28	51.55	45.34	37.58	45.69
T3	35.52	24.66	28.22	31.67	30.01
T4	19.83	9.02	8.04	3.96	10.21
Mean	38.11	31.42	27.72	26.71	

Factors	C.D.	SE(d)	SE(m)
Treatments	0.35	0.17	0.12
Days	0.31	0.15	0.11
Treatments x Days	0.70	0.34	0.24

Table 6: Effect of chlorpyriphos on invertase activity per gram of control and treated soil a different days of incubation

Treatments	Days of Incubation				Mean
	Day 1	Day 7	Day 14	Day 21	
C	140.86	112.70	85.29	48.85	96.92
T1	127.76	114.02	76.78	45.52	91.02
T2	136.55	162.81	97.30	85.00	120.42
T3	85.46	97.18	72.58	67.30	80.63
T4	37.93	34.60	33.27	22.35	32.04
Mean	105.71	104.26	73.04	53.80	

Factors	C.D.	SE(d)	SE(m)
Treatments	0.32	0.16	0.11
Days	0.28	0.14	0.1
Treatments x Days	0.64	0.32	0.22

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

Treatments	Days of Incubation				Mean
	Day 1	Day 7	Day 14	Day 21	
C	28.31	40.99	31.10	26.03	31.61
T1	28.94	40.65	29.58	25.77	31.24
T2	37.56	62.91	42.34	27.13	42.48
T3	16.77	29.45	15.96	15.62	19.45
T4	11.75	15.67	2.83	10.98	10.31
Mean	24.67	37.93	24.36	21.11	

Factors	C.D. SE(d)	SE(m)
Treatments	0.49 0.24	0.17
Days	0.44 0.21	0.15
Treatments x Days	0.98 0.48	0.34

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

Treatments	Days of Incubation				Mean
	Day 1	Day 7	Day 14	Day 21	
C	71.26	65.62	57.76	47.11	60.44
T1	69.81	64.42	58.83	48.12	60.30
T2	78.40	80.34	71.24	52.32	70.57
T3	58.10	76.90	75.20	76.26	71.61
T4	48.49	29.68	25.82	6.94	27.73
Mean	65.21	63.39	57.77	46.15	

Factors	C.D.	SE(d)	SE(m)
Treatments	0.42	0.21	0.15
Days	0.38	0.18	0.13
Treatments x Days	0.85	0.42	0.29

4. Conclusion

This study has come out with the conclusion that chlorpyriphos at lower concentration is beneficial for the microbial populations and enzymatic activities in soil but higher concentration i.e equal to or higher than 10ppm produce deleterious effects. These concentrations significantly decreased the counts and enzymatic activities at every week of sampling with a total duration of 21 days. Thus, higher concentrations were found untolerable in the soil for even a period of one week. These findings suggest that pesticides should be applied only at recommended rates. In our study at concentration 1ppm stimulatory effect was noticed. Although, that concentration is slightly higher than recommended dose of chlorpyriphos. Stimulatory effect may be due to presence of pesticide degrading microbes existing in the soil. It is suggested that these finding should be validated by caring out long term studies using new advanced technologies.

5. Acknowledgment: The authors thank the Department of Biotechnology, Chaudhary Bansi Lal University, Bhiwani for providing funds to carry out this work.

6. Conflicts of interest: There is no conflict of interest regarding publication of this work.

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