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Effects of organophosphate insecticides on soil dynamics and enzymatic activities in alluvial soil of West Bengal, India



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ABSTRACT

The study investigated the impact of two organophosphate insecticides, monocrotophos, and profenofos, on microorganisms and enzymatic activities of soil. This was conducted in a laboratory using alluvial soil from Bidhan Chandra Krishi Viswavidyalaya, the experiment applied these insecticides at recommended rates (750 g and 1000 g a.i. ha⁻¹) both individually and in combination over 60 days. Soil samples were collected from each pot after 0 (after 1 h), 20, 40, and 60 days of incubation from these treatments-T1: Control (No insecticide applied), T2: Monocrotophos (36% EC) applied @ 750 g a.i. ha⁻¹, T3: Profenofos (50% EC) applied @ 1000 g a.i. ha⁻¹, T4: Monocrotophos (36% EC) and profenofos (50% EC) applied @ 375 and 500 g a.i. ha⁻¹, respectively. Monocrotophos (11%) generally stimulated bacterial growth more than profenofos (7.3 %), with a significant increase in bacterial populations observed initially, though numbers declined by the study's end. Actinomycetes responded notably to the T4 (45.4 %), while fungal populations were highest in T2 (23.3%) followed by T4 (3.9 %) and T4 (0.6 %). Regarding enzyme activities, phosphatase and dehydrogenase activities were enhanced in treatment T4 showing the greatest effect. Urease activity peaked with monocrotophos at 40 days. The results indicate that the effects of organophosphate insecticides on soil microbial communities and enzymatic activities are complex and vary over time, with combined applications often having more pronounced effects. This research highlights the need for careful consideration of insecticide impacts on soil health in pest management strategies. This study faced several challenges in terms of environmental control, microbial variability, and pesticide residue effects. It made significant contributions to our understanding of the complex interactions between pesticides and soil microbial communities. These insights are essential for minimizing harm to soil health.

Keywords: Organophosphates, Pesticides, Soil microorganisms, Soil microbial activity, Environmental pollution, Soil enzymes, Enzymatic activity, Pesticide degradation, phosphatase activity, Dehydrogenase activity, Urease activity, pesticide impact, Bacterial population.

Introduction

India's agriculture is central to its economy, with 60-70% of the population relying on farming. Pesticides, including organophosphates, are extensively used to protect crops from pests. However, only about 0.1% of applied pesticides reach their target organisms, while most contaminate the soil (Carriger et al., 2006; Pimentel, 1995).

Organophosphates, such as monocrotophos and profenofos, are widely used to enhance agricultural productivity by controlling pests. Despite their effectiveness, these pesticides can disrupt soil microbial activity and fertility, leading to environmental pollution and potentially impacting soil productivity and agricultural sustainability. Monocrotophos and profenofos are approved for controlling soil pests like larval corn rootworms and cutworms in corn.

These pesticides are known to be non-persistent, meaning their effects on soil microorganisms can be significant, as their degradation is influenced by soil microbes.

Soil is a dynamic system inhabited by diverse microorganisms, including bacteria, actinomycetes, and fungi, which play vital roles in nutrient cycling and the degradation of organic matter. (El-Shahaat et al., 1987; Bhuyan et al., 1993). These microorganisms are important for soil health and productivity, and any disruption in their activities can affect soil functions and global nutrient cycles. Pesticides, which can persist in the environment, may disturb the balance of soil microbial communities, impacting processes like mineralization and nutrient recycling.

Studies indicate that non-target organisms can be harmed by pesticides, leading to disturbances in soil microbial communities, which in turn disrupt soil balance and impact microbial diversity and activity (Tortella et al., 2013; Pimentel, 1995). The existing equilibrium of the soil ecosystem is being disrupted by this (Chen et al., 2015). The strength, range, and duration of the parent chemicals or their metabolites determine the changes in soil activity that are observed (Margni et al., 2002).

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Pesticides may have an impact on microorganisms by lowering their quantity, variety, biochemical activity, and altering the organization of the microbial community (Smith *et al.* 2000; Chen *et al.* 2003). The first biota to be directly and indirectly impacted by harmful compounds added to soil is the soil microflora. Because soil microorganisms react quickly to pollutants, they are valuable indicators for determining how pesticides are affecting the environment (Doran and Zeiss, 2000; Edwards *et al.*, 1996).

The objective of the study is to examine the impact of monocrotophos and profenofos, two commonly used organophosphate insecticides, individually or in combination on soil microorganisms and enzymatic activities. The study will specifically focus on evaluating the growth of bacteria, actinomycetes, and fungi, as well as the functions of soil enzymes such as acid phosphatase, alkaline phosphatase, dehydrogenase, and urease in an alluvial soil. This research aims to comprehend the influence of these insecticides on soil health and productivity, addressing an important knowledge gap.

Material and Methods

The study utilized alluvial soil (Typic Haplustepts) collected from Barajaguli Farm, BCKV, Nadia, West Bengal, with latitude +22.9452° N and longitude +88.5336° E. Later, It was conducted under laboratory conditions at, BCKV, Nadia. The soil was initially analyzed to assess its microbiological and physicochemical characteristics.

For microbiological study, the soil analysis was done with the fresh soil samples to enumerate the proliferation of bacteria, actinomycetes, fungi, and different enzymatic activities in soil. The soil undergone air drying in a shaded area, grinding, and sieving through a 2 mm sieve for the physicochemical analysis.

Treatments-T1:Control (No insecticide applied), T2: Monocrotophos (36% EC) applied @ 750 g a.i. ha⁻¹, T3: Profenofos (50% EC) applied @ 1000 g a.i. ha⁻¹, T4: Monocrotophos (36% EC) and profenofos (50% EC) applied @ 375 and 500 g a.i. ha⁻¹, respectively. The effects of two organophosphate insecticides, monocrotophos and profenofos, either alone or in combination at their recommended field application rates on the proliferation of soil microorganisms (total bacteria, actinomycetes, fungi), as well as enzymatic activity (dehydrogenase activity, acid, and alkaline phosphatase activity, and urease activity) in alluvial soil, were investigated in a pot culture experiment performed in a laboratory environment. After 0 (after 1 hour), 20, 40, and 60 days of incubation, soil samples were taken from each pot using the procedure described by Das and Mukherjee (2000b). By examining the sub-samples, microbial populations, and metabolic alterations were rapidly determined.

To monitor the changes in the soil's nutritional condition during the pot culture experiment, the chemical characteristics of the soil as a result of microbial transformations were recorded every 20 days. Standard techniques were used to identify the general features of the soil, and Table 1 presents the findings.

Table 1. General characteristics of the soil

| Soil characteristics | Results |
|------------------------------|-------------------|
| Taxonomic class (USDA, 1975) | Typic Haplustepts |
| Soil texture class | Silty clay |

| | |
|--|-------|
| Soil texture | |
| Sand (%) | 16.88 |
| Silt (%) | 44.00 |
| Clay (%) | 39.12 |
| Water holding capacity (%) | 55.3 |
| Particle density (g cm ⁻³) | 3.10 |
| Bulk density (g cm ⁻³) | 1.24 |
| Electrical conductance (dS m ⁻¹) | 0.13 |
| pH (1 : 2.5 Suspension) | 6.71 |
| Oxidizable OC (g kg ⁻¹) | 6.10 |
| CEC [cmol (p ⁺) kg ⁻¹] | 19.16 |
| Total bacteria (CFU × 10 ⁵ g ⁻¹) | 46.3 |
| Total actinomycetes (CFU × 10 ⁵ g ⁻¹) | 18.0 |
| Total Fungi (CFU × 10 ⁴ g ⁻¹) | 21.1 |
| Acid phosphatase (μg pNP g ⁻¹ h ⁻¹) | 394.3 |
| Alkaline phosphatase (μg pNP g ⁻¹ h ⁻¹) | 66.8 |
| Dehydrogenase (μg TPF g ⁻¹ h ⁻¹) | 17.4 |
| Urease (μg g ⁻¹ 2h ⁻¹) | 18.9 |

The soil's pH and EC were measured using a soil water suspension (1:2.5) that was made by periodically shaking the mixture for 30 minutes (Jackson, 1973). Organic carbon was estimated by the Walkley-Black method.

For microbiological analysis, Soil samples were assessed to count the colony forming units (CFU) of total bacteria, actinomycetes, fungi using the serial dilution technique and pour plate method. (Salle, 1973). The agar plates were placed in an incubator at a temperature of 30 ± 1°C for 7 days, and the colony-forming units on each plate were enumerated. To determine the total population of bacteria in the soil, Thornton agar medium with a pH of 7.4 was employed. For counting the total population of actinomycetes in the soil, Jensen's agar medium with a pH ranging from 6.5 to 6.6, as described by Jensen in 1930, was utilized. Martin's Rose Bengal medium was employed for enumerating the fungal population in the soil.

The technique for Acid and alkaline phosphatase activity is based on measuring the amount of p-nitrophenol (pNP) that is released into the soil following an hour at 37°C incubation with p-nitrophenol phosphate (pNPP) (Tabatabai and Bremner, 1969, Eivazi and Tabatabai, 1977). To assess the activity of soil dehydrogenase, 2,3,5-triphenyltetrazolium-chloride extracts were reduced. A spectrophotometer was used to measure the extracts' absorbance at 485 nm after they had been filtered. The amount of TPF produced g⁻¹ h⁻¹ was used to quantify the ensuing dehydrogenase activity. The technique for determining of urease activity of soil is based on detecting the amount of ammonia generated after soil samples are incubated in a urea solution for two hours at 37°C (Tabatabai and Bremner, 1972).

Results and Discussion

The use of organophosphate insecticides has been increased because of their less persistence in soil as compared to other chemical compounds like chlorinated hydrocarbon insecticides which are very stable and persistent in the environment for a long time. The current study aims to examine the impact of two organophosphate insecticides (viz., monocrotophos and profenofos and their combination) at their field level recommended dose on the proliferation of major microorganisms such as total bacteria, actinomycetes, and fungi, some enzymatic activities related to microbial performances such as acid phosphatase, alkaline phosphatase activities, dehydrogenase activity, and urease activity.

4.1. The impact of organophosphate insecticides on the growth of microorganisms

Table 2. Effect of organophosphate insecticides on the growth of microorganisms

| Treatments | Proliferation of total bacteria (CFU×10 ⁵ g ⁻¹) in soil | | | | | | Proliferation of actinomycetes (CFU×10 ⁵ g ⁻¹) in soil | | | | | | Proliferation of fungi (CFU×10 ⁴ g ⁻¹) in soil | | | | | |
|------------|--|---------|---------|---------|------|-------|---|---------|---------|---------|------|-------|---|---------|---------|---------|------|-------|
| | 0 (1h) | 20 days | 40 days | 60 days | Mean | SD | 0 (1h) | 20 days | 40 days | 60 days | Mean | SD | 0 (1h) | 20 days | 40 days | 60 days | Mean | SD |
| T1 | 46.3 | 156.9 | 81.8 | 71.4 | 89.1 | 47.59 | 18 | 59.2 | 42.9 | 36.8 | 39.2 | 17.02 | 21.1 | 78.1 | 22.5 | 20.8 | 35.6 | 28.33 |
| T2 | 46.3 | 185.8 | 82.7 | 80.3 | 98.8 | 60.35 | 18 | 73.3 | 36.4 | 43.4 | 42.8 | 22.99 | 21.1 | 76 | 33.8 | 44.7 | 43.9 | 23.47 |
| T3 | 46.3 | 171.9 | 83.1 | 70.1 | 92.9 | 54.86 | 18 | 103.6 | 54.1 | 41.9 | 54.4 | 36.06 | 21.1 | 61.8 | 31.2 | 29.2 | 35.8 | 17.86 |
| T4 | 46.3 | 164.9 | 90 | 81 | 95.6 | 49.92 | 18 | 112.3 | 43.3 | 54.5 | 57 | 39.89 | 21.1 | 61.8 | 33.8 | 31.2 | 37 | 17.43 |
| Mean | 46.3 | 169.9 | 84.4 | 75.7 | - | | 18 | 87.1 | 44.2 | 44.2 | - | | 21.1 | 69.4 | 30.3 | 30.9 | - | |
| p-value | 0.995 | | | | | | 0.807 | | | | | | 0.944 | | | | | |

(CFU: Colony Forming Unit)

4.1.1. Effect of organophosphate insecticides on the growth of total bacteria

The total bacteria (Fig. 1) was increased by 11 %, 7.3%, and 4.3% in T2, T4, and T3 treatment respectively over the control treatment. Similar observations with different types of organophosphate insecticides were also recorded earlier by different workers (Ivashkin, 1987; Das and Mukherjee, 1998; Sultan, 2010). It was also revealed that the application of different types of organophosphate insecticides did not exert a uniform effect on the growth and activities of total bacteria throughout the experimental period. On average, the total bacterial growth was highest on the 20th day of sampling when compared with the initial day of the experiment. Though, the growth of total bacteria was decreased on the 40th day followed by the end of the experiment.

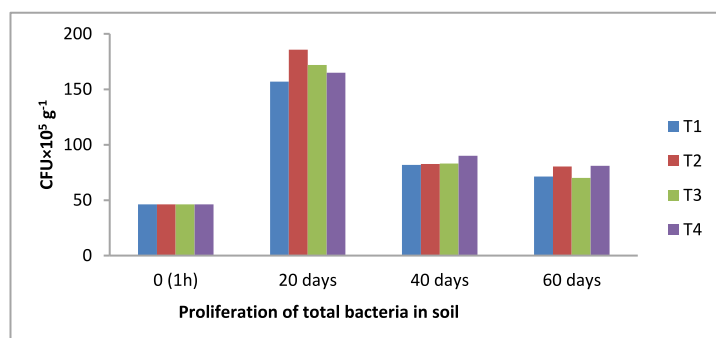


Fig 1: Effect of organophosphate insecticides on total bacteria in soil

4.1.2. Effect of organophosphate insecticides on the proliferation of actinomycetes in soil

The proliferation of actinomycetes responded differentially to the application of different organophosphate insecticides in soil (Table 2). The proliferation of actinomycetes was increased by 45.4%, 38.8%, and 9.2% with the application of combination of monocrotophos and profenofos, single use of profenofos and monocrotophos, respectively. Similar stimulatory effects of organophosphate insecticides on actinomycetes were also recorded earlier by different workers (Shukla *et al.*, 1990; Vig *et al.*, 2008). It was also revealed that single application of profenofos responded better than that of monocrotophos. It was also observed that, the growth and proliferation of actinomycetes were highly increased on the 20th day of incubation when compared with the initial sampling day. Thereafter, the growth and activities of actinomycetes were decreased on the 40th day of incubation, followed by an increase on the 60th day of incubation. From the experimental data, it was also observed that profenofos and degraded products supported the population of actinomycetes only up to the 20th day of application followed by a gradual decline up to the end of the experiment.

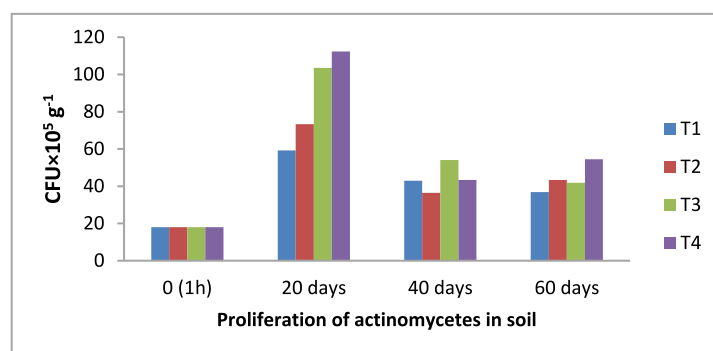


Fig 2: Effect of organophosphate insecticides on actinomycetes in soil

4.1.3. Effect of organophosphate insecticides on the proliferation of fungi in soil

The proliferation of fungal propagules was found to be maximum (Fig. 3), which was increased by 23.3% with the single application of monocrotophos as compared to the untreated control soil. The proliferation of fungal propagules was also found to be increased in treatment T4 and T3 by 3.9% and 0.6% respectively, as compared to untreated control. However the fungal population was decreased on the 20th day of incubation in case of single use of profenofos and combination of insecticides as compared to untreated control soil. Similar reports with organophosphate insecticides by different soil fungi were also reported earlier by different workers (Omar *et al.*, 1993; Hasan, 1999; Cycon *et al.*, 2009; Harish, 2013).

A stimulatory effect of organophosphate insecticides on fungal population was also previously recorded by several workers (Das and Mukherjee, 1998; Min, 2006).

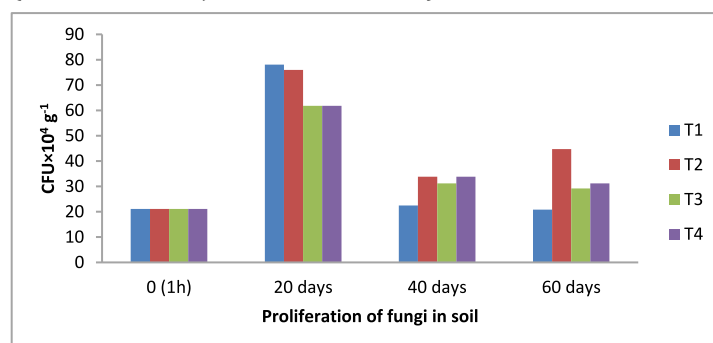


Fig 3: Effect of organophosphate insecticides on fungi in soil

4.2. Effect of organophosphate insecticides on the activities of soil enzymes

4.2.1. Effect of organophosphate insecticides on acid phosphatase activity in soil

On an average 15.2% increase was observed in T4 followed by T3 and T2, which were 7.7% and 5.2%, respectively (Table 3). This indicated that monocrotophos and profenofos as well as their degraded products were beneficial to the acid phosphatase activity in soil.

On an average, the activity of acid phosphatase was increased on all other sampling days, as compared to the initial sampling. The stimulation was highest on the 40th day of incubation followed by the 60th, and 20th days, respectively. Stimulations of acid phosphatase by different organophosphate insecticides were also reported earlier by different researchers (Rangaswamy and Venkateswarlu, 1996; Madhuri and Rangaswamy, 2002; Jastrzębska, 2011). From the results *p*NP: *p*-nitrophenol it was also revealed that application of different types of insecticides and their combination responded almost similarly towards the activity of acid phosphatase in soil during the incubation period of 60 days.

Table 3. Effect of organophosphate insecticides on the activities of soil enzymes in soil

| Treatments | Activity of acid phosphatase ($\mu\text{g pNP g}^{-1} \text{ h}^{-1}$) in soil | | | | | | Activity of alkaline phosphatase enzyme ($\mu\text{g pNP g}^{-1} \text{ h}^{-1}$) in soil | | | | | | Dehydrogenase activity ($\mu\text{g TPF g}^{-1} \text{ h}^{-1}$) in soil | | | | | |
|------------|--|---------|---------|---------|-------|-------|---|---------|---------|---------|-------|-------|--|---------|---------|---------|------|------|
| | 0 (1h) | 20 days | 40 days | 60 days | Mean | SD | 0 (1h) | 20 days | 40 days | 60 days | Mean | SD | 0 (1h) | 20 days | 40 days | 60 days | Mean | SD |
| T1 | 400.2 | 437.1 | 487 | 409.8 | 433.5 | 38.93 | 178.1 | 193.3 | 173.7 | 182.5 | 181.9 | 8.41 | 18.9 | 29.6 | 26.8 | 30.8 | 26.5 | 5.38 |
| T2 | 400.2 | 463.6 | 469.3 | 491.5 | 456.2 | 39.19 | 178.1 | 178.9 | 184.7 | 195.4 | 184.3 | 7.98 | 18.9 | 30.8 | 37.3 | 34.5 | 30.4 | 8.10 |
| T3 | 400.2 | 442.1 | 509 | 515.7 | 466.8 | 55.43 | 178.1 | 198.2 | 191.4 | 222.9 | 197.7 | 18.79 | 18.9 | 33.3 | 31.7 | 32.9 | 29.2 | 6.90 |
| T4 | 400.2 | 513.3 | 531.1 | 553.1 | 499.4 | 68.12 | 178.1 | 218.3 | 205.7 | 217.8 | 205 | 18.84 | 18.9 | 35.4 | 30.8 | 31.7 | 29.2 | 7.15 |
| Mean | 400.2 | 464 | 499.1 | 492.5 | - | | 178.1 | 197.2 | 188.9 | 204.7 | - | | 18.9 | 32.3 | 31.7 | 32.5 | - | |
| P- value | 0.381 | | | | | | 0.130 | | | | | | 0.880 | | | | | |

TPF: 2,3,5,- triphenylformazan

4.2.2. Effect of organophosphate insecticides on alkaline phosphatase activity in soil

This indicated that the degree of the beneficial effect on the alkaline phosphatase activity was in the order of T2 < T3 < T4. The alkaline phosphatase activity was increased by 12.7%, 8.7%, and 1.3% in Treatment T4, T3, and T2 respectively over the control treatment. Among the insecticides, no one exerted a detrimental effect on the activity of alkaline phosphatase in soil. This pointed out that the pesticides and their degraded products did not affect the functions of the enzyme. An increase in the activity of the enzyme by different organophosphate insecticides was reported earlier also by different workers (Mayanglambam *et al.*, 2005; Singh and Singh, 2008; Pandey and Singh, 2006). On an average, the activity of alkaline phosphatase was highest on the 60th day of incubation when compared with the initial day of experiment.

4.2.3. Effect of organophosphate insecticides on the activity of dehydrogenase in soil

The dehydrogenase activity was increased by 36 %, 30.4 %, and 12.6 % in Treatment T4, T3, and T2 respectively over the control treatment.

During the incubation period of 60 days, T4 resulted maximum increase in dehydrogenase activity on 60th day of incubation, whereas T2 resulted lowest increase on the 40th day of incubation. It was investigated that the single use of monocrotophos showed an alternate increase and decrease in the activity of dehydrogenase enzyme. Dehydrogenase activity was increased on the 20th day of incubation, followed by a decrease on the 40th day of incubation, and reached to the maximum on the 60th day of incubation. Single use of profenofos also showed a similar response as monocrotophos.

4.2.4. Effect of organophosphate insecticides on the activity of urease in soil

From the present investigation it was found that the urease activity was the highest in T2 followed by T3 and T4 compared to untreated control soil. This was also observed that the urease enzyme activity increased in case of all the treated soil in all the three sampling days as compared to untreated soil. The urease activity was increased by 14.7%, 10.2% and 10.2% when monocrotophos applied in single, profenofos used in single and both the insecticides used in combination, respectively as compared to untreated control.

Conclusion

Application of both the insecticides, either alone or in combination, in general, had a stimulatory effect on the growth and proliferation of microorganisms in the soil. As compared to untreated control, a higher rise in the population of total bacteria and fungi was recorded with monocrotophos followed by the combined application of both the insecticides in soil, while the proliferation of actinomycetes was highly induced when both the insecticides were applied in combination followed by profenofos and monocrotophos, respectively. During the incubation period of 60 days, in general, the microbial populations were remarkably augmented after 20 days of incubation days followed by a gradual decrease up to the end of the experiment. As compared to untreated control, the highest proliferation of bacteria was recorded after 20 days of sampling under monocrotophos followed by profenofos and their combined application, respectively, while a reverse trend was recorded with the actinomycete population. On the other hand, the fungal population was somewhat repressed due to the application of organophosphate insecticides after 20 days of sampling. The ANOVA results indicate that there are no statistically significant differences between the treatments (T1, T2, T3, T4) for the proliferation of bacteria, actinomycetes, and fungi over time, as the p-values are much higher than the typical significance level of 0.05.

The effect of organophosphate insecticides on the activities of the studied soil enzymes was more or less uniform. In general, the phosphatase and dehydrogenase activities were highly induced when the soils were treated with monocrotophos and profenofos in combination followed by the single application of profenofos and monocrotophos, respectively while the urease activity of the soil was the highest due to the incorporation of the monocrotophos. It was also revealed that the activity of acid phosphatase, in general, was more pronounced than that of alkaline phosphatase in soil. Considering the incubation period of 60 days, it was revealed that the enzyme activities did not follow a uniform trend. For example, the highest stimulation on the activities of acid phosphatase was recorded after 40 days of sampling, while that of alkaline phosphatase and dehydrogenase was recorded during the later stages of the experimental period. Urease activity, on the other hand, was more or less steadily increased during the experimental period of 60 days. Considering the individual insecticide treatment, it was revealed that the highest augmentation was recorded for acid phosphatase and dehydrogenase with the combined application of the insecticides after 60 days, for alkaline

phosphatase with profenofos, and for urease activity with monocrotophos after 40 days of sampling. The ANOVA results indicate that there are no statistically significant differences between the treatments (T1, T2, T3, T4) for the activities of acid phosphatase, alkaline phosphatase, and dehydrogenase enzymes, as the p-values are higher than the typical significance level of 0.05.

Future scope

This article finds the following gaps:

- This study would extend the research to long-term field trial. This would help to assess the real-world impact of organophosphate insecticide on soil health over multiple growing season, taking into account factors like weather pattern, soil type variation, and agricultural practices.
- Research could further examine the broader ecological effects of these insecticides on non-target organisms in the soil ecosystem.
- Future research could focus on how different combinations of insecticides and their concentrations interact on soil microbial communities and enzymatic activities.
- This study could help to develop bioremediation strategies to mitigate the negative impact of pesticide use on soil health.

Conflict of interest

The author declare that the research article was made with absence of any kind of financial as well as commercial relationships that could be produced as a conflict of interest.

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