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Research Article

Effect of Pesticides on the Agricultural Essential Soil Microorganisms

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Abstract

In light of the rapidly increasing human population, extensive use of pesticides has been employed to maximise crop production. This has become a major environmental concern. To assess the influence of commonly used pesticides on soil microorganisms, a factorial experiment was conducted. The effects of organochlorine and organophosphate pesticides on soil microorganisms were examined using seven treatments. Soil samples were collected from the microcosm experiment after pesticide treatments. Bacterial and fungal populations were analysed at intervals of 1, 7, 14, and 21 days of incubation, using standard procedures. The total bacterial counts (BC) ranged from 1.98×10^5 cfu/g to 9.5×10^5 cfu/g, the fungal counts (FC) ranged from 1.43×10^3 cfu/g to 7.34×10^3 cfu/g, and the actinomycetes count (AC) ranged from 2.63×10^3 cfu/g to 13.97×10^3 cfu/g. The findings demonstrated that the impacts of organochlorine and organophosphate pesticides resulted in a reduction of soil microorganisms in contaminated soil compared to uncontaminated soil. In comparison to organochlorine pesticides, organophosphate pesticides caused the greatest reduction in microbial populations. This study highlights the pesticide's impact on soil microbes.

Keywords: Bacteria; Fungi; Microorganisms; Organochlorine; Organophosphate; Pesticides

Introduction

The Indian economy is primarily agriculture-based, but in recent years, the industrial sector has gained significant prominence. With the rapid development of industrialisation and urbanisation, contaminant accumulation in agroecosystems is rapidly increasing in developing countries. Globally, agriculture is one of the most significant human activities that impacts ecosystems. Human development, mitigating food crises, and addressing global hunger have been achieved substantially through this profession. Practitioners often have a mindset of maximising yield by using pesticides, fertilisers, and other chemicals, which are responsible for the high yield of crops. Pesticides are vital to agricultural productivity. Therefore, farmers have utilised them to manage weeds and insects, and they have been shown to enhance productivity in agriculture significantly [32,35]. Soil microorganisms interact differently with various types of chemical pesticides used in agricultural soils, depending on parameters such as pesticide type, soil quality, and existing microbial groups. The total quantity of bacteria, fungi, protozoa, and algae may rise or decrease depending on the pesticide's toxicity and effectiveness as a nutrition or energy source (Figure 1).

Pesticides can change the variety of soil microbial communities. It's externally applied and may affect the function

Figure 1: Harmful effects of pesticides on beneficial soil biota.

of microorganisms. Bacteria and arbuscular mycorrhizal fungi create symbiotic relationships with plants, promoting growth and development during stressed and normal conditions [25,26]. Chemical pesticides can reduce the population size of sensitive communities while increasing the number of pesticide-resistant microbes. This is due to the utilisation of organic compounds from dead microbial cells or the pesticide itself as an energy or carbon source, as well as reduced competition [7,8,13]. Pesticide use often leads to increased microbial biomass but decreased functional diversity [19,37]. Chemical pesticides can lead to the dominance of a few functional groups in soil, impacting community structure and biological activities. Hence, they may not affect soil microbial biomass or diversity, but they can alter the functional architecture of soil bacteria [18].

The effect and activity of pesticides on soil microorganisms are determined by the type, quantity, and soil conditions of the pesticide [33]. Malathion treatment reduced the population of actinomycetes in the soil [3]. They reported that during the first week of incubation, the addition of malathion at 50ppm, 100ppm, and 200ppm reduced the actinomycetes population by 34%, 36%, and 40%, respectively. During the seventh week, the population of actinomycetes decreased by 37%, 42%, and 50%. These findings indicate that the biggest negative effect was observed in soil treated with malathion, particularly at 200ppm [3]. A similar reduction in actinomycete population was observed by [11,36], who found that malathion treatment reduced the population of actinomycetes. They observed that the application of pesticides, including cypermethrin, chlorpyrifos, alone or in combination with mancozeb and carbendazim, in tomato cultivation at field application rates (7.5 and 10 kg/ha), significantly reduced the fungal population in soil [30]. Also, insecticides in combination with fungicides showed a negative effect on fungal populations. Similarly, studies have shown that the presence of malathion insecticide decreased fungal population [29,36]. They also reported that the most adverse effect on fungal population was seen in soil treated with malathion, especially at 200ppm [3]. Thus, the objective of this study is to assess the influence of two usually used pesticides on soil microorganisms.

Material and Method

Study area

The present study was conducted over two years during the Rabi season of 2019-20, 2020-21, and 2021-22 at the experimental dome (244×732 cm) of Panjab University, Chandigarh, India, which is located at 30.7601° N latitude and 76.7663° E longitude (Figure 2). The experimental region has a humid subtropical environment with maximum temperatures ranging from 16 to 25 °C and minimum temperatures ranging from 9 to 18 °C. The yearly rainfall averages around 1100 mm. A total of 27 plots were used during the whole study. The experimental design was established with a randomised block design (RBD).

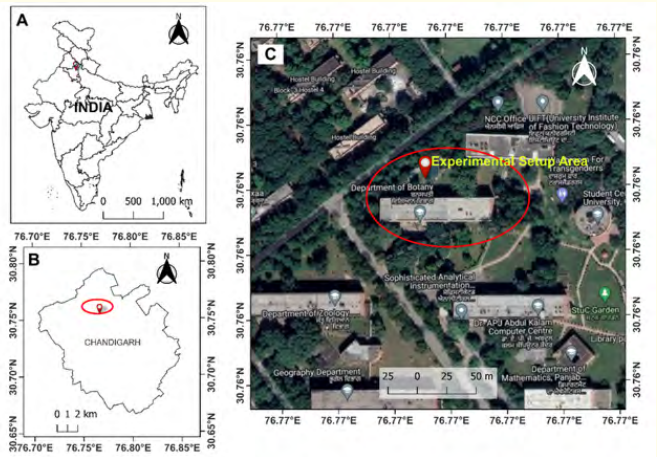

Figure 2: Map showing the location of the microcosm experiment set up in the Department of Botany, Panjab University campus, Chandigarh, India.

Physicochemical properties of experimental field soil

The present study focused on understanding the impact of pesticides on the microbial population in soil. Although certain basic physicochemical data were established throughout cropping seasons, the present study also focused on comprehensive soil parameters. Six samples were collected from various soil depths ranging from 0 to 15 cm in the experimental field. These soil samples from various locations were thoroughly combined to generate composite samples, which were then evaluated for basic physicochemical properties. The soil in the experimental plots was sandy loam in texture with a pH of 7.58, electrical conductivity was 0.06 dS/m at the time of seed planting, water holding capacity (WHC) was 38.7%, and bulk density (BD) was 1.13 g/cm^3. The chemical properties of the experimental soil were total soil organic C (0.63%), total N (39 mg/kg), and total P (43.85 kg/ha).

Treatment

To study the impact of pesticides on soil microorganisms, we selected two pesticides; one was Aldrin, which belongs to organochlorine pesticides (OCP), and for this, we gave seed treatment before sowing. Another was phorate, a type of organophosphate pesticide (OPP), for which we provided soil treatment before sowing (Table 1). During this study, pesticide treatments were applied under the permissible limit.

According to the experiment design, seven treatments were prepared, such as

- Control (T1),
- Low dose OPP, i.e., Phorate (T2),
- Medium dose OPP, i.e., Phorate (T3),
- High dose OPP, i.e., Phorate (T4),
- Low dose OCP, i.e., Aldrin (T5),
- Medium dose OCP, i.e., Aldrin (T6)
- High dose OCP, i.e., Aldrin (T7).

Pesticide	Experimental dose		
	Low	Medium	High
Phorate	0.156 g per m^2	0.312 g per m^2	0.625 g per m^2
Aldrin	0.625 µl/ml for 100 g	1.25 µl/ml for 100 g	2.5 µl/ml for 100 g

Table 1: Experimental doses of pesticides were utilised throughout the study.

Soil samples preparation and analysis

The soil samples were first air-dried in the laboratory and then crushed using a mortar and pestle. They were sieved through a 2-mm sieve to eliminate stone fragments, roots, and other unwanted impurities. Standard plate count methods were employed to examine microbial populations, including nutritional agar (NA) for bacteria, potato dextrose agar (PDA) for fungi, and starch casein nitrate (SCN) agar for actinomycetes. To analyse soil samples, 1 g was measured into a test tube with 9 mL of sterile distilled water and serially diluted to a dilution factor of 10^5. 1 mL of the relevant dilution was pipetted into a sterile plate with a suitable medium and incubated at 30°C. All plates were incubated inverted. Microbial counts were performed at 48 hours for NA, 72 hours for PDA, and 6 days for SCN [2,31]. The experiment was conducted at the Soil Ecosystem and Restoration Ecology Lab, Department of Botany, Panjab University, Chandigarh.

Statistical analysis

The data were statistically analysed using Microsoft Excel and SPSS. Statistical analyses were performed using SPSS-PC (IBM, version 25.0) for all required computations.

Result

Soil fertility depends not only on soil texture but also on its biological factors (beneficial microorganisms). Pesticide use may alter the diversity of microorganisms, thereby affecting soil fertility. Therefore, soil microorganisms play a crucial role in maintaining soil fertility. The results of the microbial counts for the experimental soil contaminated with OPP and OCP at different concentrations, as well as the control (T1) samples, are presented in Tables 1, 2, and 3. The total bacterial counts (BC) ranged from 1.98×10^5 cfu/g to 9.5×10^5 cfu/g, the fungal counts (FC) ranged from 1.43×10^3 cfu/g to 7.34×10^3 cfu/g, and the actinomycetes count (AC) ranged from 2.63×10^3 cfu/g to 13.97×10^3 cfu/g.

Bacteria count

On Day 1, there was a decrease in bacterial count (BC) under various pesticide treatments: T2 at 17.7%, T3 at 25.2%, T4 at 45%, T5 at 7.96%, T6 at 19.2%, and T7 at 32.7% compared to T1. On the 7th day, the bacterial count was reduced by 19.2% in T5 and 54.65% in T4. The presence of the pesticide decreased the bacterial count. On the 14th day of incubation, the maximum Coefficient of Variance (CV) was recorded in T7, i.e., 8.44 %, indicating high variability. On the 21st day, the maximum reduction in BC was recorded in T4,

i.e., 73.6%. At the same time, T2 and T3 had observed a similar trend in BC reduction, i.e., 52.5% and 53.25%, respectively. BC was decreased in T5, T6, and T7 also with 30.6%, 46.81%, and 62.06% respectively. Compared to T1, the minimum reduction in BC was observed in T5. A significant reduction in BC was recorded in all pesticide treatments compared to the control (Figures 3, 4, 5, and 6) (Table 2). A summary of multivariate ANOVA revealed a significant variation in BC under the influence of treatments and day intervals of incubation ($p < 0.000$) (Table 5).

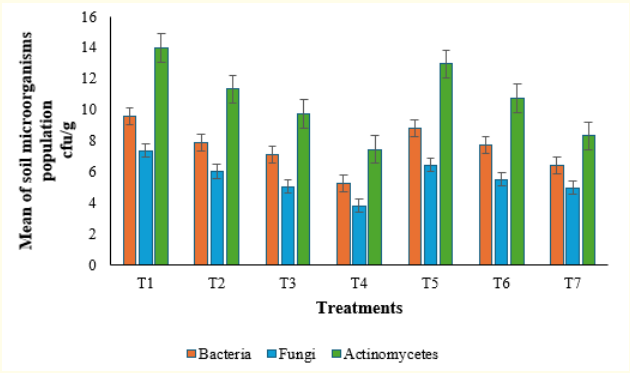

Figure 3: Effect of various pesticide treatments on the soil microorganism population (Bacteria×105, Fungi×10^3, and Actinomycetes×10^3) on the very 1st day.

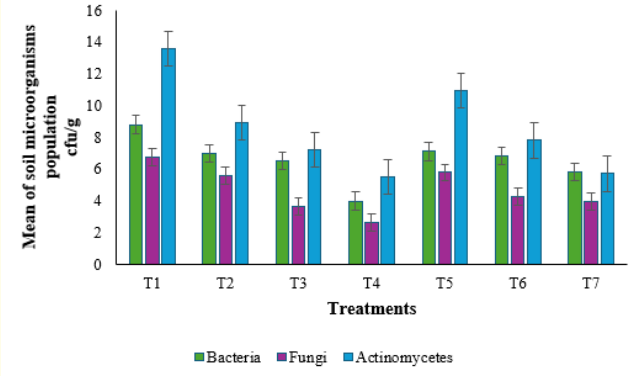

Figure 4: Effect of various pesticide treatments on the soil microorganism population (Bacteria×10^5, Fungi×10^3, and Actinomycetes×10^3) on the 7th day.

Treatments	DAY 1	DAY 7	DAY 14	DAY 21
T1	9.54 ± 0.06	8.8 ± 0.067	8.04 ± 0.069	7.50 ± 0.06
T2	7.85 ± 0.061	6.99 ± 0.075	5.8 ± 0.065	4.72 ± 0.11
T3	7.13 ± 0.062	6.53 ± 0.13	4.69 ± 0.132	3.54 ± 0.12
T4	5.26 ± 0.11	3.99 ± 0.063	3.56 ± 0.036	1.98 ± 0.036
T5	8.78 ± 0.099	7.11 ± 0.051	5.98 ± 0.057	5.23 ± 0.075
T6	7.71 ± 0.061	6.8 ± 0.11	5.32 ± 0.127	4.01 ± 0.061
T7	6.42 ± 0.11	5.83 ± 0.08	4.87 ± 0.15	2.86 0.18

Table 2: Effect of different pesticide concentrations on the bacterial population (Bacteria Count ×10^5, cfu/g) at various time intervals.

Fungal count

On Day 1st, the maximum fungal count (FC) was observed in T1 with 7.34 ×10^3 cfu/g, followed by T5 with 6.433×10^3 cfu/g, T2 with 6.02×10^3 cfu/g, T6 with 5.53×10^3 cfu/g, T3 with 5.04×10^3 cfu/g, and T7 with 4.98×10^3 cfu/g. The minimum FC was recorded in T4 at 3.81 × 10^3 cfu/g. A similar trend of FC was observed during 7, 14, and 21 days of incubation. On the 21st day of incubation, the highest reduction in FC was observed in T with 74.95% compared to T1. T5 exhibited a minimum reduction of 44.65%. A significant reduction in FC was recorded at 1, 7, 14, and 21 days of incubation (Figures 3, 4, 5, and 6) (Table 3). A summary of ANOVA revealed a significant variation in FC under the influence of pesticide treatments and day interval of incubation ($p < 0.00$) (Table 5).

Actinomycetes count

On the 1st day of incubation, the maximum actinomycetes count (AC) was observed in T1 with 13.97×10^3 cfu/g, followed by T5 with 12.96×10^3 cfu/g, T2 with 11.34×10^3 cfu/g, T6 with 10.73×10^3 cfu/g, T3 with 9.72×10^3 cfu/g, and T7 with 8.32×10^3 cfu/g. The minimum AC was recorded in T4 at 7.43 × 10^3 cfu/g. A similar trend of FC was observed during the 7th, 14th, and 21st days of incubation. On the 21st day of incubation, a maximum reduction

Treatments	DAY 1	DAY 7	DAY 14	DAY 21
T1	7.34 ± 0.04	6.72 ± 0.062	6.17 ± 0.048	5.71 ± 0.041
T2	6.02 ± 0.07	5.6 ± 0.07	4.08 ± 0.083	3.03 ± 0.08
T3	5.04 ± 0.13	3.67 ± 0.1	2.51 ± 0.08	1.93 ± 0.04
T4	3.81 ± 0.07	2.62 ± 0.05	1.61 ± 0.05	1.43 ± 0.047
T5	6.433 ± 0.073	5.78 ± 0.09	4.52 ± 0.06	3.16 ± 0.05
T6	5.53 ± 0.07	4.24 ± 0.084	3.122 ± 0.064	2.5 ± 0.073
T7	4.98 ± 0.093	3.97 ± 0.073	1.8 ± 0.07	2.27 0.11

Table 3: Effect of different pesticide concentrations on the Fungal Count ×10^3, cfu/g at various time intervals.

in FC was observed in T with 71.47% compared to T1. T5 exhibited the minimum reduction of 23.40%. On the 21st day of incubation, the highest CV was recorded in T4, i.e., 9.81%, indicating high variability. A significant reduction in AC was recorded during 1, 7,14, and 21 days of incubation (Figures 3, 4, 5, and 6) (Table 4). A summary of ANOVA revealed a significant variation in AC under the influence of pesticide treatments and day interval of incubation ($p < 0.00$) (Table 5).

Treatments	DAY 1	DAY 7	DAY 14	DAY 21
T1	13.97 ± 0.067	13.58 ± 0.09	11.37 ± 0.064	9.23 ± 0.12
T2	11.34 ± 0.15	8.922 ± 0.13	6.98 ± 0.11	5.93 ± 0.16
T3	9.72 ± 0.055	7.21 ± 0.153	5.91 ± 0.06	4.66 ± 0.22
T4	7.43 ± 0.13	5.53 ± 0.075	4.18 ± 0.09	2.633 ± 0.09
T5	12.96 ± 0.067	10.94 ± 0.154	7.93 ± 0.112	7.01 ± 0.054
T6	10.73 ± 0.175	7.8 ± 0.155	6.167 ± 0.082	5.91 ± 0.08
T7	8.32 ± 0.16	5.71 ± 0.089	5.07 ± 0.1	4.34 ± 0.13

Table 4: Effect of different pesticide concentrations on the Actinomycetes population (Actinomycetes Count ×10^3, cfu/g) at various time intervals.

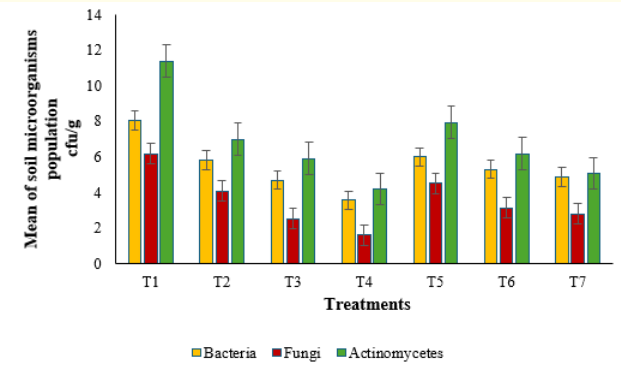

Figure 5: Effect of various pesticide treatments on the soil microorganism population (Bacteria×10^5, Fungi×10^3, and Actinomycetes×10^3) on the 14th day.

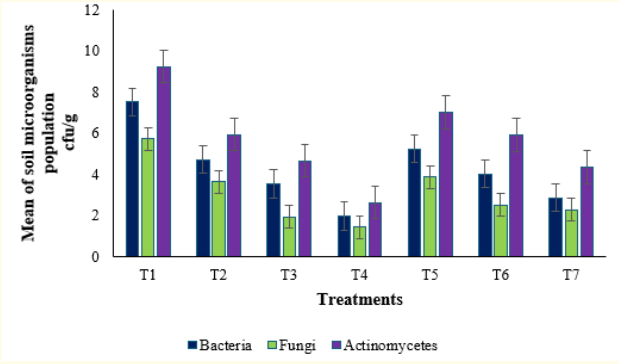

Figure 6: Effect of various pesticide treatments on the soil microorganism population (Bacteria×10^5, Fungi×10^3, and Actinomycetes×10^3) on the 21st day.

Tests of Between-Subjects Effects						
Source	**Dependent Variable**	**Type III Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**
TREATMENT	Bacteria	482.944	6	80.491	1028.679	.000
	Fungi	407.277	6	67.880	1404.381	.000
	Actinomycetes	1243.671	6	207.279	1626.456	.000
DAYS	Bacteria	376.042	3	125.347	1601.954	.000
	Fungi	246.335	3	82.112	1698.837	.000
	Actinomycetes	885.988	3	295.329	2317.365	.000
TREATMENT * DAYS	Bacteria	21.503	18	1.195	15.267	.000
	Fungi	11.070	18	.615	12.724	.000
	Actinomycetes	52.121	18	2.896	22.721	.000
Error	Bacteria	17.527	224	.078		
	Fungi	10.827	224	.048		
	Actinomycetes	28.547	224	.127		

Table 5: Summary of multivariate ANOVA for soil microorganisms under seven treatments at different time intervals.

Discussion

The extensive use of agrochemicals, including inorganic fertilisers and pesticides, is a cornerstone of modern agriculture. However, their overuse and indiscriminate application *(*especially over prolonged periods*)* have been shown to adversely affect soil ecology, often disrupting or diminishing populations of beneficial soil microorganisms [15,40]. Microbial biomass is an important indication of microbial activity because it gives a direct assessment of the relationship between microbial activities, nutrient transformation, and other ecological processes [27]. In general, a decrease in soil respiration indicates a reduction in microbial biomass [16] or an increase in respiration [12]. Some microbial groups can replicate utilising sprayed pesticides as a source of energy and nutrients; the pesticide may be harmful to other species [14].

In this study, the impact of pesticide application on the soil microbial community was assessed by measuring the colony-forming units (CFU/g soil) of bacteria, fungi, and actinomycetes on selective media. A significant difference in total bacterial count was observed across incubation periods of 1 to 21 days, due to differences in pest management practices. The microbial population dynamics were notably altered, with the highest bacterial count (9.54×10^5 CFU/g soil) recorded in T1, followed by T5 (8.78×10^5 CFU/g soil). This increase may be attributed to inter-culturing operations, which enhance soil pulverisation and aeration, thereby supporting microbial proliferation [6].

Bacterial count (BC) was severely impacted in treatments involving phorate, with a more pronounced decline than in those receiving aldrin. On Day 1, the reduction in BC relative to T1 was recorded as follows: T2 (17.7%), T3 (25.2%), T4 (45%), T5 (7.96%), T6 (19.2%), and T7 (32.7%). By Day 21, the maximum reduction was noted in T4 (73.6%), followed by T7 (62.06%), T3 (53.25%), T2 (52.5%), T6 (46.81%), and T5 (30.6%). The least reduction was consistently observed in T5, indicating a comparatively lower microbial toxicity of the pesticide used in this treatment. A significant reduction in BC was recorded in all pesticide treatments compared to the control. These observations are consistent with previous findings by [5,21], who reported that glyphosate application led to reduced microbial biomass and diversity. The reduction is believed to stem from the inhibition of amino acid synthesis via the shikimic acid pathway [10]. However, contradictory findings have also been reported, such as those by [24,38], who noted increased bacterial populations after glyphosate application, indicating variability depending on the environmental context and microbial composition. Similar results were observed by [9,39], who reported that the decrease in soil

microbial count and biomass can be associated with the toxic effect of cypermethrin on soil microorganisms. The presence of cypermethrin and thiamethoxam inhibited the metabolic process and significantly decreased the abundance of ammonifying, nitrifying, and denitrifying bacteria compared to the untreated sample [22].

The fungal count (FC) followed a similar trend. On Day 1, the highest FC was observed in T1 (7.34×10^3 CFU/g), followed by T5, T2, T6, T3, and T7. T4 exhibited the most substantial reduction in FC by Day 21 (74.95%), while T5 showed the least reduction (44.65%). These results are in agreement with those of [29,36], who also found that malathion significantly reduced fungal populations. Similarly, glyphosate was reported to suppress fungal growth [34]. Some researchers concluded that cypermethrin application had toxic effects on soil microorganisms [9]. Fungal populations were significantly reduced when the applied pesticide concentration increased above recommendation dose. Some researchers also reported that the presence of glyphosate decreased FC in all pesticide treatments. However, the reduction in FC was just significant at 200ppm. The addition of glyset 200 ppm decreased FC by 20% and 13% at the first week of the incubation period and the 7th week, respectively [3].

For actinomycetes count (AC), the highest was recorded in T1 (13.97×10^3 CFU/g), followed by T5, T2, T6, T3, and T7. T4 showed the lowest AC on Day 1 (7.43×10^3 CFU/g) and the minimum AC on Day 21 (2.63×10^3 CFU/g), along with the highest coefficient of variation (CV= 9.81%), indicating high variability in response. These findings align with those of [11,36], who reported a decline in actinomycetes following malathion exposure. The persistence of pesticides in soil, particularly due to repeated applications and the absence of microbial degradation mechanisms, contributes to their accumulation and to long-term impacts on microbial diversity [20]. Similar concerns were raised by [17], who studied the behaviour of persistent organochlorine pesticides (OCPs) in agricultural regions and emphasised their potential risk to the food web, given that soil microorganisms are integral to the first trophic levels.

Furthermore, [3,4] reported that the addition of certain pesticides significantly reduced microbial biomass and activity across multiple microbial groups, affirming the toxic effects observed in this study. Their findings reinforce that the degree of microbial suppression depends on the type, concentration, and duration of exposure to the applied pesticide. Pesticide applications used to protect crops can directly or indirectly impact soil microbial activity. However, the knowledge of predicting the impact of a pesticide on various soil microbial communities is still limited. Certain pesticides may promote the growth of microorganisms, while others inhibit or have no noticeable effect when applied at the recommended dose. Furthermore, because only around 1% of soil bacteria can be cultivated in a lab setting, conventional microbial characterisation techniques like standard plate count methods sometimes lack accuracy. Moreover, a viable substitute is provided by molecular methods for identifying soil microbial populations [1]. For example, the effect of pesticides on soil microbial populations can be assessed by monitoring carbon utilisation and nitrification. The nitrification test is expected to be reliable since soil fungi do not engage in the process, and only a few bacterial species can carry it out. Recently, studies have reported using functional genes such as amoA and amoB, which are involved in nitrification, to detect the effects of pesticides on bacteria [23]. Furthermore, certain mRNA quantification investigations have revealed that the nitrification process is particularly vulnerable to specific herbicides. Other molecular sequencing investigations have found that pesticides alter the organisation of microbial communities [28].

Thus, the enhanced and indiscriminate use of chemical pesticides has led to the depletion of soil fertility, microbial populations, and a reduction in crop production. Generally, up to 5% of the total organic carbon and N in soil is contained in the microbial biomass at any one time. The value of the microbial biomass content of soil, the activity of the biomass, and selected microbial nutrient processes act as indicators of soil health. Soil microorganisms play a vital role in maintaining soil health, ecosystem functions, and crop productivity. A complex matrix of organic and inorganic constituents of soil, particularly the rhizosphere, creates a unique and dynamic environment for the microorganisms, which affects plants and other associated microorganisms. A certain group of pesticides and the overdose of some pesticides have a deleterious effect on soil biota.

Conclusion

A significant reduction in microbial population is observed during the present study. Bacterial, fungal, and actinomycete populations

were significantly reduced when the pesticide concentration increased. The highest reduction in microbial population was observed under phorate treatments compared to aldrin. The findings of this study demonstrated that the impacts of OCP and OPP resulted in a decrease in the number of soil microorganisms present in the contaminated soil as compared to those in the uncontaminated soil. This might be because pesticides contain harmful ingredients. These insecticides can cause high death rates. Therefore, it is essential to limit the use of conventional pesticides while increasing the usage of biopesticides. The current findings underscore the need for judicious pesticide use and support the integration of biological alternatives and microbial biofertilisers in crop management strategies. Continuous monitoring of microbial dynamics after pesticide application is crucial for preserving soil health and maintaining ecosystem services essential to sustainable agriculture.

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