Effect of Sulphur, Lambda-Cyhalothrin *(pyrethroid)* and Pendimethalin *(dinitroaniline)* Pesticides on Agricultural Soil Bacteria

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Abstract

Application of synthetic pesticides is the need of modern era to attain food production and food demands of the increasing population. Pesticides kill, destroy or repel the target organism which includes pests and unwanted growth that affects the agricultural productivity but the knowledge of their effect on non target organisms including soil bacteria is important to control environmental disturbance. To check the effect of pesticides on soil bacteria a laboratory experiment was conducted where top soil samples collected from agricultural fields of Kasur district were sprayed with pesticides (Sulphur, Lambda Cyhalothrin and Pendimethalin), at recommended reference doses (RfD), 2RfD, 4RfD and 8RfD under normal environmental conditions. Afterwards, Microbial analysis was done at the end of each subsequent week for up to four weeks. Serially diluted soil samples were plated on Nutrient Agar media and incubated at 37 ⁰C for 24 hours. Number of bacteria was calculated by standard plate count method and results were statistically analyzed. The results revealed that Sulphur pesticides enhanced the bacterial population as compared to Lambda Cyhalothrin and Pendimethalin ones that suppressed the bacterial population when applied at RfD for a given period of time. It was concluded from the current study that the usage of synthetic agricultural pesticides should be minimized, moreover, when required only RfD of certain pesticide should be applied.

Keywords: *RfD Pesticide, agriculture, bio-engineer, bio-fixation, bio-fertilization.*

Introduction

The Green revolution or the third agricultural revolution demands the surplus quantity and improved quality of food production to fulfill the needs of increasing population, but to achieve that pesticide application is markedly increased especially

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in the agricultural sector (Tarfeen et al., 2022). Generally, it is accepted that application of synthetic or organic pesticides reduces the agricultural product loss and helps to improve the food quality (Strassemeyer et al., 2017). However, the relocation of applied pesticides from the target sites into the environmental media via volatilization, spray drift, leaching and surface runoff consequences in the disturbance the soil biota and soil health (Ruomeng et al., 2023).

Soil microorganisms (bacteria, fungi, actinomycetes and soil viruses) are the miniature biotic part of soil ecosystem. As the soil is enriched with bacterial population which act as bio-engineer of soil ecosystem, they perform a lot of vital functions such as solubilization and mineralization of inorganic nutrients (sulphur, zinc and phosphate etc), bio-fixation (fixation of nitrogen, carbon sequestration), siderphore production, hydrolytic enzyme production, bio-transformation and bioremediation etc (Osadebe & George, 2022).

Numerous scientific studies clearly revealed that the responses of bacterial species greatly vary from pesticides to pesticides. Application of Diclofop and Haloxyfop showed to disturb the sulphur cycling which is associated with bacteria (Darine et al., 2015). Whereas, application of Sulfonylurase, imidazolinones and triazolpyrimidines pesticides affect the bacterial community structure (Qian et al., 2018). Also, Glyphosate containing herbicide markedly disturbed the microbial diversity and microbial activity such as Phosphorous sorption (Chávez-Ortiz et al., 2022; Isa et al., 2021). Moreover, 2,4,D, aminopyralid and dicamba herbicide application resulted in lethal affects on the bacterial diversity (Aguiar et al., 2020) (Aguiar et al., 2020). Similarly, Bipyridinum and dipphenyl ether herbicides resulted in the disturbance of nitrogen fixing bacteria (Mohamed et al., 2021) and Lambda Cyhalothrin and mixture of Cypermethrin and Dimethoate caused elimination of some dominant soil bacteria and fungal genera (Ilusanya et al., 2020).

Another study revealed that imazethapyr produced inhibitory effects on bacterial population (Singh & Singh, 2020). Exceedingly high use of pesticides (glyphosate, atrazine, kitazin, metalyxal, fipronil, hexaconazol, monocrotophos, quizalofop and imidacloprid) significantly disrupt the structure, inhibit the growth, reduced the permeability and antioxidant production of *Mesorhizobiumciceri* (Shahid et al., 2021). Since limited information is available on the impact of commonly used pesticides in Pakistan and their impact on soil microbial community, therefore this study specifically focused on post application impact of Lambda Cyhalothrin, Pendimethalin and Sulphur containing pesticides on soil bacterial population.

Lambda Cyhalothrin is mostly used as insecticides on a broad spectrum of crops (mango, rice, cotton and brinjal), to kill leaf and plant hopper, mite, gall midge, hispa (Naveen et al., 2023). Moreover, Pendimethalin is used as pre-emergent selective herbicide to destroy the cultivation of annual grasses in crops cereals, cotton, sorghum, rice, potato and tobacco (Yadav et al., 2017)..

Material And Methods

Sample Collection and Preparation

The moist soil of the field was collected from district Kasur, one kilometer near Ganda Singh Boarder area. The topsoil (0-5cm) was collected and brought to study site where almost equal amount of soil was poured in four pots with three technical replicates. To check the effect of pesticides on soil microbes, three most commonly used pesticides (Sulphur (Bikko), Lambda Cyhalothrin (NADA) and Pendimethalin (Kick On)) were selected and prepared according to the manufacturer's instruction. Soil was then sprayed with pesticides as mentioned in Table 1. As, calculated quantity of pesticides were in fewer amount thus, they were further diluted in 20ml of water. All components were mixed homogenously with the aid of glass rod. All experimental treatments were run in three technical replicates, whereas blank which contained only water was used in the control pots. All experimental work was performed in the Laboratory of School of Zoology, Minhaj University Lahore.

Table 1: *Calculated Quantity of Pesticide Doses (μL) for Soil Treatment*

Experimental Groups	Pesticides	RfD (μL)	2RfD (μL)	4RfD (μL)	8RfD (μL)
1st set pot area (240.52 cm^2)	Lambda Cyhalothrin	601.3	1202.6	2405.2	4810.4
2nd set pot area (132.73 cm^2)	Pendimethalin	331.8	663	1327.3	2654.6
3rd set pot area (122.71 cm^2)	Sulphur	301.4	602.9	1205.87	2411.2

Bacterial Analysis and Identification

The pesticides treatment were given at the start of the experiment and bacterial analysis was done for four subsequent weeks. For bacterial growth estimation nutrient agar method was used while, to access bacterial population density standard plate count method was employed. Moreover, one gram of each soil sample was collected and serially diluted by dilution factor of 10^{-5}, and then 50μl of each serially diluted sample was spread on nutrient agar plates and incubated at 37ºC for 24 hrs. The Colony Forming Units (CFU) were counted and calculated as:

- **CFU=(Number of colonies × dilution factor)/ volume to be spread on culture plate**

For characterization and identification of the most common bacterial genera present in soil, five common bacterial colonies were selected and isolated by separately streaking and re-streaking method. Furthermore, Gram's staining, catalase, indole, sulphur reduction and motility test were performed for identification and characterization of the selected soil bacteria .

Graphical Representation and Statistical Analysis

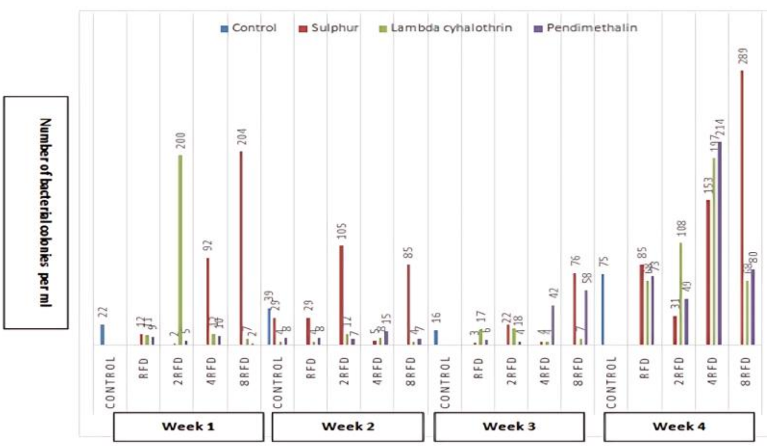
To analyze the effect of each dose of pesticides on selected bacterial population with respect to control, data was represented graphically. However, to compare the effect of pesticides with respect to each other two way ANOVA was performed by using SPSS 22.

Results

Effect of pesticides doses on bacterial count

Effect of the individual doses (RfD, 2RfD, 4RfD and 8RfD) of three selected pesticides (Sulphur, Lambda Cyhalothrin and Pendimethalin) on bacterial population is shown in figure 1.

Figure 1: *Effect of Pesticides Doses (RfD, 2RfD, 4RfD and 8RfD) on Bacterial Count in Subsequent Weeks*



Results of the experimental data indicated that during 1st week, Sulphur containing pesticides reduced the bacterial population at lower doses (RfD, 2RfD), while increased the density of bacterial population at higher doses (4RfD, 8RfD). Similarly, during 2nd week RfD and 4RfD reduced the bacterial population followed by the same effect in 3rd week. Moreover, all applied doses of Sulphur (RfD, 2RfD, 4RfD and 8RfD) during 4th week markedly enhanced the bacterial population as compared to the control group.

In case of Lambda Cyhalothrin, all applied doses reduced the density of bacterial population for two weeks, except 2RfD dose which showed an increased hike with respect to control ones. During the 3rd week bacterial population was almost similar to that of control group, whereas, 4th week data showed that doses of 2RfD, 4RfD enhanced the bacterial population in comparison to RfD, 8RfD , that was close to control group.

For up to two weeks, all doses of Pendimethalin reduced the density of bacterial population with respect to the control group. While in 3rd week, inhibitory effect remained at lower doses (RfD, 2RfD). However, during the 4th week almost all doses aid to enhance the density of bacterial population in the field soil.

Comparison of Pesticidal Effect on Bacterial Count

Overall effect of pesticides with respect to each other through weeks is represented in Table 2.

Table 2: *Overall Effect of Pesticides on Percentage Increase and Decrease of Bacterial Count Over the Weeks (Week x Pesticide interaction mean±SE)*

Week	Pesticide			Mean
	Sulphur	**Lambda Cyhalothrin**	**Pendimethalin**	
W1	252.27±212.4a	161.37±215.9ab	-70.46±08.40b	114.39±100.1A
W2	43.59±60.01ab	-82.05±04.91b	-76.28±04.95b	-38.25±25.23A
W3	64.06±107.1ab	-28.13±22.02ab	71.88±83.76ab	35.94±43.74A
W4	86.00±74.31ab	47.00±40.55ab	38.67±49.68ab	57.22±30.24A
Mean	111.48±61.17A	24.55±54.73A	-9.05±27.64A	

Note: Mean values that shared similar letter in a row or in a column were statistically non-significant (P>0.05). The Small letters represent comparison among interaction means and capital letters are used for overall mean. –ve values indicate percentage decrease in bacterial count and +ve values indicate percentage increase in bacterial count as compared to control.

Table 3: *Overall Effect of Pesticides Doses on Percentage Increase and Decrease of Bacterial Count (RfD x Pesticide interaction mean±SE)*

Week	**Pesticide**			**Mean**
	Sulphur	**Lambda cyhalothrin**	**Pendimethalin**	
RFD	-34.75±19.73b	-35.71±21.56b	-50.94±16.70b	-40.47±10.39B
2RFD	14.29±58.41b	199.09±204.73ab	-67.25±10.96b	48.71±72.53AB
4RFD	65.00±95.04b	-9.32±57.82b	57.94±67.14b	37.87±40.46AB
8RFD	401.39±151.62a	-55.88±16.99b	24.05±82.48b	123.19±79.68A
Mean	111.48±61.17A	24.55±54.73A	-9.05±27.64A	

Note: Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. . –ve values indicate percentage decrease in bacterial count and +ve values indicate percentage increase in bacterial count as compared to control.

Table 2 explains the trends of percentage increase and decrease of bacterial count through the weeks under the pesticides effect. Suplhur containing pesticide showed no negative impact on soil bacteria instead an overall percentage increase was observed in soil bacteria sprayed with sulphur over the weeks. Lambda Cyhalothrin sprayed soil had an increased bacterial count in week 1 as compared to control. In subsequent weeks (week 2 and 3) a percentage decrease in bacterial count was observed. As week 4 approached an increase in bacterial count was observed. In case of Pendimethalin sprayed soil, bacterial count was suppressed in week 1 and 2 as indicated by –ve values, however in week 3 and 4 bacterial count was significantly increased. The results indicated that Sulphur containing pesticides did not decrease the bacterial count whereas Pendimethalin had a negative impact on bacterial population in first two weeks however the effect wore off in later weeks. The data clearly indicated that overall Sulphur and Lambda Cyhalothrin containing pesticides markedly increased the bacterial population in field soil while, Pendimethalin resulted in decreased bacterial population.

Table 3 indicated that sulphur containing pesticides inhibited bacterial growth at RFD (-34.75±19.73), but as the dose was increased (2X, 4X and 8X) a consistent increase in bacterial count was observed. On the other hand Pendimethalin decreased the bacterial count at RfD and 2RfD (-50.94±16.70 and -67.25±10.96). Higher Pendimethalin doses supported bacterial growth as the bacterial count was high. Lambda Cyhalothhrin decreased bacterial count at RfD, 4RfD and 8RfD. There was significant difference among the effect of doses of pesticides on bacterial count. Overall effect of doses indicated that the bacterial count was significantly decreased at RfD and significantly increased at 8RfD.

Bacterial Identification and Characterization

Morphological Characterization

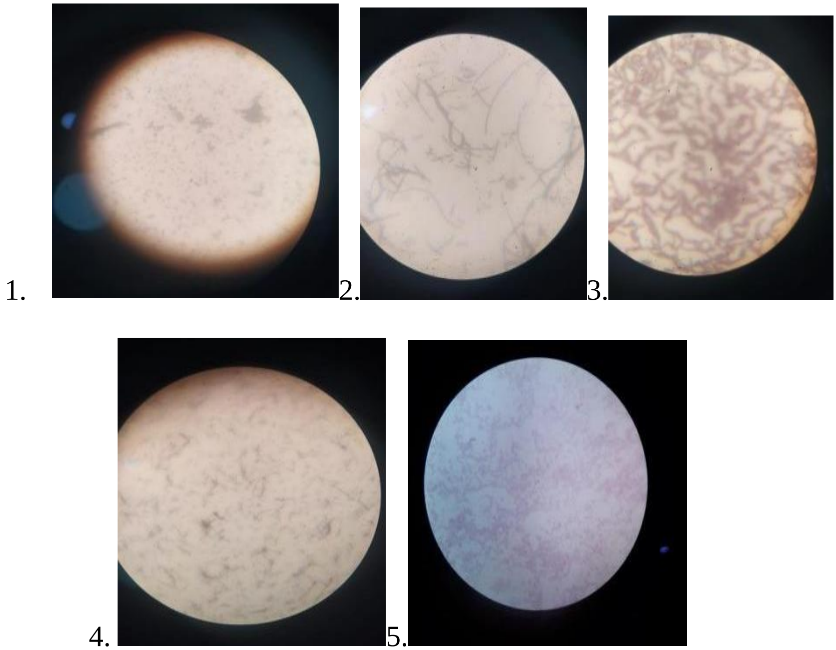
Five most commonly present bacterial strains (S1-S5) isolated from soil samples were characterized based on colony morphology and microscopic features. Results are noted in Table 4. Colony morphology analysis revealed that colonies of S1 strain were white, large and irregular. S2 strain had small, yellow and circular appearance. S3 strain colonies appeared white, small and circular in morphology, colonies of S4 strain were offwhite and medium whereas S5 colonies appeared colorless, small and circular on surface of Agar media. S1, S2 and S3 colonies were opaque in appearance whereas S4 and S5 were transparent. Microscopic characterization via gram staining demonstrated that strains S1, S2, S3 and S4 were gram positive whereas S5 strain was gram negative (figure 2). These morphological and staining properties provide preliminary insights into the taxonomic diversity of the isolated soil microbiota.

Table 4: *Morphological and Microscopic Characterization of Bacterial Strains Isolated form Field Soil*

Strain type	Color	Size	Shape	Margins	Elevation	Light Transparency	Microscopic identification
S1	White	Large	Irregular	Lobate	Flat with concentric layers	Opaque	Gram+ve, Coccus
S2	Yellow	Small	Circular	Entire	Raised	Opaque	Gram +ve, Rods in chain
S3	White	Small	Circular	Entire	Flat with concentric layers	Opaque	Gram +ve, Rods in curved shape
S4	Off white	Medium	Oval	Entire	Flat	Translucent	Gram +ve, Rods in clusters
S5	colorless	Small	Circular	Entire	Convexed	Transparent	Gram -ve, Rods

Note: -ve: Negative, +ve: positive

Figure 2: *Microscopic Representation of Isolated Bacterial Strains, Strain 1,2, 3,4(100X) and Strain 5(40X) from Left to Right*



Biochemical Characterization of the Field Soil Microbes

All strains showed negative indole test, negative Sulphur reduction test and negative motility test except strain 4 whereas, all strains showed positive catalase test.

Discussion

The results of this study revealed that during the first three weeks of post pesticides application, Sulphur containing pesticides showed a bactericidal effect at recommended field dose (RfD) while, higher doses enhanced the density of bacterial population. All its doses showed increased number in the bacterial population during the fourth week, while highest number was observed at 8RfD. According to previous studies, application of fungicides (Carbendazim, Metalaxyl-Mancozeb), dramatically changed the soil bacterial composition i.e. enhanced the population of *Pesudomonas, Microbacterium* and *Sphingobacterium* (Liao et al., 2022). Further studies revealed that soil microorganism use Sulphur as a nutrient and coenzyme source in their biological system (Thera et al., 2017). Among bacterial taxa Spingomonas and

Arthrobacter have approved ability to utilized fungicides and other toxic substance (Vasilchenko et al., 2023).

Lambda Cyhalothrin showed inhibitory effect for up to two weeks however, in third week bacterial population tried to maintain its original number. Inhibitory effect of Lambda Cyhalothrin was diminished during the fourth week. The possible reason of initial inhibition may be that average half-life of Lambda Cyhalothrin is 14 days. afterwards it is degraded by the soil microbes (Karpun et al., 2021). Moreover, inhibitory effects of Lambda Cyhalothrin on soil microbes were observed at higher doses. Study carried out by Filimon et al., (2015) revealed that this pesticide had negative impact on eco-physiological group of bacteria.

Furthermore, Pendimethalin showed a continuous decrease in the bacterial population for up to two to three weeks, except at higher doses where bacteria showed an increased multiplication rate. In fourth week significant increase in bacterial population was observed at higher doses of (4RfD, 8RfD), the possible reason might be that average half-life of Pendimethalin is 24.4 to 34.5 days (4 weeks approximately) and by the fourth week effect of pesticide has wore off (Kočárek et al., 2016). Other studies also indicated that pesticides (herbicides) had non-target effect at recommended field dose (RfD) for short span of time (Dennis et al., 2023). The pattern of negative effect of pesticides on mean bacterial population decreased as follow: Pendimethalin>Lambda Cyhalothrin>Sulphur. Mean bacterial population was higher in first week which was decreased in the second and third week with an increased trend in fourth week. Multiple environmental factors and pesticide soil relationship are responsible for such effects (Ćwieląg-Piasecka, 2023). Although overall comparative effect of pesticides on mean bacterial population and week wise effect on mean bacterial population was non-significant (Table 2).

Statistical analysis showed that highest effect of pesticides (low bacterial count) was at RfD while lowest effect (high bacterial count) on mean bacterial population was observed at higher dose (8RfD). On the contrary a study carried out by Mehajin revealed that higher doses of pesticides decreased the microbial population (Kurnianto et al., 2022).

It has been previously reported that initial inhibition of bacterial population by certain pesticides is due to the toxic effect of pesticides on selective bacterial species reducing the overall bacterial abundance and diversity. Over the time pesticide resistant or tolerant bacterial species survive and thrive as they have the ability to degrade and utilize the pesticides as a carbon source. This leads to selective increase in pesticide tolerant bacteria in later weeks. Moreover higher pesticide doses or concentrations enforce stronger selective pressure on pesticide sensitive bacterial species and support the proliferation of pesticide tolerant bacteria (Patyka et al., 2016; Shahid & Khan, 2022). Apart from that the presence of vegetation on the soil also mitigates the negative effect of pesticides on soil bacteria. Plants release organic compounds that support proliferation and degradative properties of pesticide resistant bacterial species. However, the degree of mitigation depends on type of vegetation, pesticide persistence and agricultural practices (Onwona-Kwakye et al., 2020; Walder et al., 2022).

Based on microscopic and biochemical characterization, isolated bacterial strains S2 and S3 were gram positive rods (*Streptobacilli*) S1 bacterial strain were gram positive coccus (*Streptococci*), S4 bacterial strain was gram positive rods in clusters (*Staphylobacillus)*, whereas one of the bacteria isolate (S5) were gram negative rods in cluster. All of the bacterial strains (S1, S2, S3 and S5) except S4 were catalase positive, indole negative, sulphur reduction negative and Non-motile. Krishnasamy et al., supported that *Streptococcus, Streptobacillus* and *Stephylobacillus* are the most common soil bacteria (Krishnasamy et al., 2019).

Conclusion

It is concluded from this study that pesticides (Lambda Cyhalothrin and Pendimethalin) decreased the bacterial population for up to two weeks after application. Instead, Sulphur containing pesticide showed bactericidal effect at the recommended field dose (RfD) only, while higher doses enhanced the bacterial population. Moreover, at fourth week of analysis there was significant increase in bacterial population in all doses. Half life of pesticides, leaching, adsorption, persistant nature of organic pesticides and change in composition of bacterial population could be the possible reasons of decreased bacterial population for up to two weeks. After that period of time microorganisms utilized the pesticides as source of carbon and biodegrade them. It is concluded that pesticides suppress bacterial population for sometime therefore their usage should be minimized as far as possible and only recommended field doses should be applied on soil when there is need. *Streptococcus, Streptobacillus* and *Staphylobacillus* were the most common soil bacteria according to this study.

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