Impact of Pesticides on Microbial Population in LASUSTECH Agricultural Field, Nigeria

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Abstract: Pesticides are used to increase crop production. Besides combating pests, pesticides affect the activity of beneficial soil microbial communities thereby affecting the nutritional quality of soils. This study investigated the impact of pesticides on the ecological activity of microorganisms in LASUSTECH agricultural field. Twenty milliliters of Fungicides (Copper (1) oxide metalyxl and 2,3-Dichlorovinyl dimethyl phosphate (DDVP), Insecticides (Chlorpyrifos, Cyperrmethrin, Deltamethrin and Dimethoate) and Herbicide (Glyphosphate) was separately added to 2000g of soil. The microbial counts were examined by serial dilution and plating methods weekly for four weeks. The results demonstrated a decrease in the microbial count in all the soils treated with pesticides and the controls but the percentage decrease in soils with pesticides is higher than the percentage decrease in the controls. The highest decrease in bacterial count was recorded in soil treated with Deltamethrin (from 20 to 7 x 10^5cfu/mL) and the lowest decrease was recorded in the soils treated with Cypermethrin (from 40.5 to 20.5 x 10^5 cfu mL), DDVP (from 43 to 23 x 10^5 cfu mL). For fungi, the highest decrease in population was recorded in the soil treated with DDVP (from 23 to 6 x 10^6 cfu mL) and the lowest decrease was observed in the soil treated with Chlopyrifos (from 7 to 3.5 x 10^6 cfu mL).In conclusion, this study has shown that the investigated pesticides significantly decreased the microbial population in soil. This confirms and reinforces the reports that prolong use of pesticides in soil destabilizes soil microbial activity and ecology.
Keywords: Fungicides, Herbicides, Insecticides, Microbial- ecology, Pesticides.

INTRODUCTION

The usage of various chemicals is very rampant with the currently practiced agriculture. Different pesticides are employed in combating the effects of different pests on crops; this enables farmers to have high yield of crop production and cater for the increasing demand for food supplies for the ever growing world population. (Zhu *et al*., 2004). Concern for pesticide contamination in the environment has assumed great importance and the fate of pesticides in the soil environment in respect of pest control efficacy, non-target organism exposure and offsite mobility has become a matter of environmental concern (Hafez and Thiemann, 2003). The fertility of the soil may be compromised by the adverse effects of pesticidal chemicals on soil microorganisms (Araujo *et al.,* 2003).

There are several kinds of pesticides available for different purposes, these include i) insecticides which could be organophosphate, synthetic pyrethroid, pyrethroid ester ii) fungicides which could be systemic and contact and iii) herbicides and these could be broad- spectrum systemic herbicide (Gupta *et al*., 2021). An ideal pesticide should be toxic only to the target organism, biodegradable and should not leach into ground water. Unfortunately, this is rarely the case and the widespread use of pesticides in modern agriculture is of great concern (Johnsen, 2001). Pesticides absorbed in the soil environment are subject to various degradative processes both physical and biological. Degradation processes which brings about total eradication of pesticides from soil include microbial degradation, chemical hydrolysis, photolysis, vitality, leaching, surface runoff and combination of two or more of these processes.(Beigel *et al*., 1999; Crouzet *et al.*, 2016). The level of contribution of each of these mechanisms to the total degradation of the pesticides in the soil is dependent on the physicochemical properties of the pesticides (e.g water solubility, sorptive affinity), characteristics of the soil (e.g pH, organic matter content, microbial biomass, redox status),environmental conditions(e.g. temperature, moisture etc) and management practices(e.g. application rate, formulation type) and within each of these variables there are also complex interactions and interdependence(Elliot *et al*., 1996; Aktar *et al*., 2009)). Thus, pesticides behaviour should be studied in different types of soils including problem soils.

A large quantity of un-degraded pesticides may remain in the soil due to constant use of pesticides and these may accumulate in the ecosystem causing serious environmental challenges to both man and animals (Hafez and Thiemann, 2003). By implication, it is important to investigate the residue and degradation pattern of pesticides in crops, soil and water accordingly for plant protection, public health and environmental safety. It is well known that pesticides affect non target microorganisms in soil and the degradation of pesticides in soil and their effect on microorganism should be studied so that their use can be properly regulated (Hafez and Thiemann, 2003). Hence this study sought to investigate the effect of various pesticides on the soil microbial population of LASUSTECH agricultural farmland.

Materials and Methods

Description of Pesticides used

The pesticides used include chlorpyrifos (an organophosphate insecticide, acaricide and miticide), cypermenthrin (a synthetic pyrethroid insecticide), deltamethrin (a pyrethroid ester insecticide), dimethoate (organophosphorus insecticide), 2,3-Dichlorovinyl dimethyl phosphate –DDVP (an organophosphate insecticide), Copper (i) oxide + Metalyxl (a combination of systematic and contact fungicide) and glyphosate (a broad-spectrum systemic herbicide).

Description of Study Area

This study was conducted in Lagos State University of Science and Technology(LASUSTECH) formerly (Lagos State Polytechnic) Ikorodu campus, located at about 26 km North-East of the city of Lagos, along Sagamu road between Latitude 6.6463^{o} N and Longitude 3.5179 ^{o} E.

Sample Collection

Loamy Soil sample was collected from a farmland in LASUSTECH at a surface depth 0-15 cm (top soil) and transported to the laboratory for treatment.

Soil Preparation and Treatment

The soil sample was air dried and sieved with a 2 mm mesh to ensure homogenous fine particle size. Two thousand grams (2000 g) of soil sample was weighed and placed in labelled pots of 5mm perforation. Eight pots were used for each pesticide and its controls. One pot each for dilutions10^{-5}& 10^{-8} for bacterial count, 10^{-6} & 10^{-8} for fungal count and one pot each for the dilution controls. Twenty millilitres of each pesticide was applied to the soil samples weekly for four weeks except for the control pots.

Enumeration of Total Heterotrophic Bacterial Count and Total Fungal Count

Twenty-eight grams (28.0g) each of nutrient agar and potato dextrose agar (PDA) was weighed and dissolved in 1000ml of distilled water in a beaker and autoclaved at 121^{o}1C for 20minutes. After cooling five drops of sterilized flagyl (Metronidazole B.P.400 mg) was added to the nutrient agar to prevent the growth of fungi. Ninety millilitres (90ml) of sterile saline water was measured into Mc Cartney bottle and 10g of the pesticides treated soil sample was added to it giving the stock solution. A serial dilution up to 10^{-8} was prepared by transferring 1ml of the soil suspension of the stock solution to the first test tube into the second dilution using a sterile pipette for each dilution. From the supernatant of the solution and aliquot of 0.1ml of the appropriate dilution was dispensed into labelled sterilized Petri dishes using sterile pipettes before adding 15mL of cooled agar. That was carefully rotated to mix properly and allowed to solidify. The plates were inoculated by each of the dilutions and incubated aerobically at 30^{o}C for 48hours. This was also done for the controls which are soil samples without pesticides. The total counts were counted manually after 48 hours of incubation. This represents a viable count, that is, plate count (CFU/mL) = (No of colonies on plate) x (dilution factor) (Obire and Anyanwu, 2009).

Monitoring and Sampling

One hundred millilitres of sterile water was sprinkled on the soil samples every two days to ensure adequate moisture/aeration within the soil. One gram of each soil sample were collected and analyzed weekly for the microbial load for twenty eight days.

Microbial Load of Pesticides-treated Soil Samples and their Controls

In this present investigation the number of bacterial colonies were observed and counted on nutrient agar (NA) containing antifungal agent, the fungal colonies were observed and counted on Potato dextrose agar (PDA). The controls which are soil samples without pesticides were also observed and counted weekly for four weeks and the results are shown in tables and charts.

The total microbial population of the bacteria, fungi and the controls was recorded weekly as dilutions 10^{-5}, 10^{-5} control, 10^{-8} and 10^{-8} control for bacterial count and 10^{-6}, 10^{-6} control, 10^{-8} and 10^{-8} control for fungal count.

Comparison of Effect of Various Pesticides on Microbial Load

In this present investigation the average number of bacterial/fungal colonies was calculated as;

$$\frac{\text{number of bacterial/fungal colonies for dilution } 10^{-5/-6} + \text{ dilution } 10^{-8}}{2}$$

These were observed and compared among the various pesticides used weekly for four weeks

Statistical Analysis

Data generated microbial enumeration was subjected to was expressed in graphs and tables. Comparison of effect of various pesticides on microbial load was subjected to analysis of variance (ANOVA).

RESULTS

This study investigated the impact of pesticides on the population of soil microorganisms isolated in LASUSTECH agricultural field. The results demonstrated a decrease in the microbial count in all the soils treated with pesticides and the controls but the percentage decrease in soils with pesticides is higher than the percentage decrease in the untreated soils (Controls).

The highest decrease in bacterial count was recorded in soil treated with Deltamethrin (from 20 to 7 x 10^5CFU/mL), followed by soil treated with Chlorpyrifos (from 15.5 to 5.5 x 10^5 CFU/mL), followed by soil treated with Glyphosate (from 16.5 to 7 x 10^5 CFU/mL), followed by soil treated with Cu_2O+M (from 17.5 to 10 x 10^5 CFU/mL), and the lowest decrease were recorded in the soils treated with cypermethrin(from 40.5 to 20.5 x 10^5 CFU/mL), 2,3-Dichlorovinyl dimethyl phosphate and Dimenthoate (from 43 x 10^5 to 23 x 10^5 CFU/mL).

The highest decrease in fungal population was recorded in the soils treated with DDVP (from 23 to 6 x 10^6 CFU/ mL), followed by Glyphosphate (from 13.5 to 3.5 x 10^6 CFU/mL) and Cypermethrin (from 19.5 to 9.5 x10^6 CFU/mL), followed by Dimethoate (from 21.5 to 12.5 x 10^6 CFU/mL), followed by Cu_2O+M (from 7 to 2 x 10 ^{6} CFU/mL) followed by Deltamethrin (from 10.5 to 6.5 x 10 ^{6} CFU/mL) and the lowest decrease was observed in the soil treated with Chlorpyrifos (from 7 to 3.5 x 10 ^{6} CFU/mL)

DISCUSSION

Table 1 showed the total bacterial and fungal count in chlorpyrifos-treated soil and controls for four weeks. Table 1 showed a considerable decrease in the microbial load within the four weeks of investigation but the decrease of microbial load in all the chlorpyrifos- treated soil samples are considerably more than the controls. Within the four weeks chlorpyrifos decreased bacteria count from 18 to 6 CFU mL for dilution 10^{-5} and from 13 to 5 CFU mL for dilution 10^{-8}, the fungal count also reduced from 9 to 5 CFU mL for dilution 10^{-6} and 5 to 2 CFU mL for dilution 10^{-8} at the end of four weeks. These results are consistent with the report of Walia *et al.*, (2018) who concluded that the addition of chlorpyrifos decreased the count of bacteria and fungi in all pesticides types and at all incubation periods.

Table 2 showed that the addition of Cu_2O+Metalyxl decreased the total count of bacteria and fungi within incubation period of four weeks. Table 2 showed that there was a reduction in bacterial count from 20 to 11 CFU mL for dilution 10^{-5} and from 15 to 9 CFU mL for dilution 10^{-8}, the fungal count also reduced from 8 to 3 cfu mL for dilution 10^{-6} and 6 to 1 CFU mL for dilution 10^{-8}. This agrees with former report that Most copper (Cu) based fungicides have a deleterious effect on the population of N-fixing bacteria and can decrease the frequency of some fungal species such as Aspergillus responsible for plant growth and development (Zwiekn *et al.,* 2003;Kei-Boahen *et al*., 2001; Smith *et al*., 2000; Kostov and Cleemput, 2001).

Table 3 shows the effect of cypermethrin on the microbial population of the LASUSTECH agricultural field. It revealed that there was decrease in microbial populations in all the soil samples including the soil samples without cypermethrin but the decrease in microbial populatios in cypermethrin treated soil samples are more. Table 3 showed that there was reduction in bacterial count from 23 to 7 CFU mL for dilution 10^{-5} and from 17 to 5 CFU mL for dilution 10^{-8}, the fungal count also reduced from 12 to 7 CFU mL for dilution 10^{-6} and 9 to 6 cfu mL for dilution 10^{-8}. Similar results were observed by (Goswami *et al*., 2013; Wesley *et al*., 2017), they all reported that reduction in the soil microbial population can be linked with the toxic effect of Cypermethrin on soil microbes. The addition of Cypermethrin adversely affected the metabolic process and significantly reduced ammonifying, nitrifying and denitrifying bacteria compared to the untreated sample (controls) (Nicoleta *et al*., 2015).

Table 4 shows the total bacterial and fungal count in deltamethrin-treated soil and controls for four weeks. The results showed a considerable decrease in the microbial load within the four weeks of investigation but the decrease of microbial load in all the deltamenthrin- treated soil samples is considerably more than the controls. Table 4 showed that within the four weeks deltamenthrin decreased bacteria count from 23 to 7 CFU mL for dilution 10^{-5} and from 17 to 5 CFU mL for dilution 10^{-8}, the fungal count also reduced from 12 to 7 CFU mL for dilution 10^{-6} and 9 to 6 CFU mL for dilution 10^{-8} at the end of four weeks. These results are consistent with Jokar *et al*. (2021) who concluded that the metagenomics analysis showed that the introduction of deltamethrin to the soil had a significant adverse effect on the soil normal flora and some dominant species in the control microcosm were completely destroyed by deltamethrin.

Table 5 showed that the addition of DDVP decreased the total count of bacteria and fungi within incubation period of four weeks. Table 5 showed that there was a reduction in bacterial count from 46 to 28 CFU mL for dilution 10^{-5} and from 40 to 18 CFU mL for dilution 10^{-8}, the fungal count also reduced from 15 to 7 CFU mL for dilution 10^{-6} and 11 to 5 CFU mL for dilution 10^{-8}. This agrees with former report that the population of bacteria and fungi in the sampled soils decreased as the week increased after exposure to DDVP (Adigun *et al*., 2022). It has also been reported that Dichlorvos have some adverse effects on soil microbial population (Kalyanee and Hemen, 2011).

Table 6 showed the effect of dimethoate on the microbial population of this agricultural field. It revealed that there was decrease in microbial populations in all the soil samples including the soil samples without dimethoate but the decrease in microbial populatios in dimethoate treated soil samples are more. Table 6 showed that there was a reduction in bacterial count from 46 to 28 CFU mL for dilution 10^{-5} and from 40 to 18 CFU mL for dilution 10^{-8}, the fungal count also reduced from 25 to 15 CFU mL for dilution 10^{-6} and 11 to 9 CFU mL for dilution10^{-8}. Similar results were observed by (Haleem *et al*., 2013) who reported that themalathion, diazinon and dimethoate were used separately to the soil at 50, 100 and 250 ppm. These three pesticides were investigated for their effects on microbial (bacteria, fungi and actinomycetes) population at 24, 48 and 72 hrs on the population of under lab conditions. The microbial population were investigated using the standard dilution plate technique. All the soil microorganisms were affected by the insecticides, the inhibition pattern were the same in all microorganisms which is depending on concentration and time of exposure.

Table 7 showed that the addition of Glyphosate decreased the total count of bacteria and fungi within the four weeks of investigation. Table 7 showed that there was decrease in bacterial count from 19 to 9 CFU mL for dilution 10^{-5} and from 14 to 5 CFU mL for dilution 10^{-8}, the fungal count also reduced from 10 to 3 cfu mL for dilution 10^{-6} and 17 to 4 cfu mL for dilution 10^{-8}. This results are consistent with (Newman *et al.,* 2016; Partoazar *et al.*, 2011) who reported that the introduction of glyphosate in the soil leads to the reduction in population of bacteria, microbial biomass and acidobacteria population.

Figure 1 showed a considerable decrease in the bacterial counts in all the pesticide-treated soil samples for the period of four weeks investigation. Table 8 showed that the highest decrease in bacterial count was recorded in soil treated with Deltamethrin (from 20 to 7 x 10^5CFU/mL), followed by soil treated with Chlorpyrifos (from 15.5 to 5.5 x 10^5 CFU/mL), followed by soil treated with Glyphosate (from 16.5 to 7 x 10^5 CFU/mL), followed by soil treated with Cu_2O+M (from 17.5 to 10 x 10^5 CFU/mL), and the lowest decrease were recorded in the soils treated with cypermethrin(from 40.5 to 20.5 x 10^5 CFU/mL), 2,3-Dichlorovinyl dimethyl phosphate and Dimenthoate (from 43 x 10^5 to 23 x 10^5 CFU/mL). This is consistent with the former report of Mehjin *et al.*(2019); Yousaf *et al.*(2013) that the addition of all pesticides; Herbicide (Glyset I.P.A, Glyphosate 48%) and two insecticides Miraj (Alphacypermethrin 10%) and Malathion (50% WP) pesticides applied at 0, 50, 100. 200 ppm levels decreased the count of bacteria at all incubation periods.

Figure 2 showed a considerable decrease in the total fungal count in all the pesticide-treated soil samples throughout the four weeks of investigation. Table 9 showed that the highest decrease in population was recorded in the soils treated with DDVP (from 23 to 6 x 10^6 CFU/ mL), followed by Glyphosphate (from 13.5 to 3.5 x 10^6 CFU/mL) and Cypermethrin (from 19.5 to 9.5 x10^6 CFU/mL), followed by Dimethoate (from 21.5 to 12.5 x 10^6 CFU/mL), followed by Cu_2O+M (from 7 to 2 x 10 ^{6} CFU/mL) followed by Deltamethrin (from 10.5 to 6.5 x 10 ^{6} CFU/mL) and the lowest decrease was observed in the soil treated with Chlorpyrifos (from 7 to 3.5 x 10 ^{6} CFU/mL). This is supported by a report from Goswami *et al.* (2013); Mehjin *et al.* (2019) that the presence of Miraj, cypermetnrin insecticide, herbicide (glyphosate) and malathion reduced the fungi count at all concentrations and period of incubations. The addition of Miraj at 50ppm, 100ppm and 200ppm, decreased the fungi count in the first week of incubation 60%, 61% and 63% respectively. While the reduction at 7th week was 48%, 50% and 62% respectively. Goswami *et al.,* 2013 concluded that these insecticides application had toxic effects on soil microorganisms.

CONCLUSION

It is well known that pesticides affect non target microorganisms in soil and the degradation of pesticides in soil and their effect on microorganisms should be studied so that their use can be properly regulated (Hafez and Thiemann, 2003).In conclusion, this study has shown that the investigated pesticides significantly decreased the population in soil. This confirms and reinforces the reports that wide and prolong use of pesticides creates pollution of the soil and destabilization of soil microbial activity and ecology, therefore proper monitoring and regulation of pesticides application is recommended.

Table 1: Total Bacterial and Fungal Count for Four weeks in Chlorpyrifos-treated Soil and Controls

WEEK	NUMBER OF COLONIES(cfu/ml)							
	BACTERIA				FUNGI			
	10^{-5} Dil	10^{-5} Cont	10^{-8} Dil	10^{-8} Cont	10^{-6} Dil	10^{-6} Cont	10^{-8} Dil	10^{-8} Cont
ONE	18	59	13	54	8	27	5	25
TWO	11	51	8	50	9	23	5	17
THREE	8	43	6	41	5	20	3	17
FOUR	6	38	5	32	5	16	2	15

Table 2: Total Bacterial and Fungal Count for Four weeks in Copper (i) oxide + Metalyxl- treated Soils and Controls

WEEK	NUMBER OF COLONIES (cfu/ml)							
	BACTERIA				FUNGI			
	10^{-5} Dil	10^{-5} Cont	10^{-8} Dil	10^{-8} Cont	10^{-6} Dil	10^{-6} Cont	10^{-8} Dil	10^{-8} Cont
ONE	20	59	15	54	8	32	6	25
TWO	16	57	12	51	6	28	5	20
THREE	12	55	11	43	5	23	3	17
FOUR	11	49	9	38	3	23	1	16

Table 3: Total Bacterial and Fungal Count for Four weeks in Cypermethrin-treated Soils and Controls

WEEK	NUMBER OF COLONIES (cfu/ml)							
	BACTERIA				FUNGI			
	10^{-5} Dil	10^{-5} Cont	10^{-8} Dil	10^{-8} Cont	10^{-6} Dil	10^{-6} Cont	10^{-8} Dil	10^{-8} Cont
ONE	47	59	34	54	23	26	10	20
TWO	40	57	24	51	20	27	13	17
THREE	31	43	23	31	16	23	9	15
FOUR	23	32	18	21	11	15	8	10

Table 4: Total Bacterial and Fungal Count for Four weeks in Deltamethrin-treated Soils and Controls

WEEK	NUMBER OF COLONIES (cfu/ml)							
	BACTERIA				FUNGI			
	10^{-5} Dil	10^{-5} Cont	10^{-8} Dil	10^{-8} Cont	10^{-6} Dil	10^{-6} Cont	10^{-8} Dil	10^{-8} Cont
ONE	23	59	17	43	12	27	9	25
TWO	13	50	12	38	12	23	8	17
THREE	9	41	9	34	9	20	6	17
FOUR	7	32	5	31	7	16	6	15

Key: Dil = Dilution, Contr = Control

Table 5: Total Bacterial and Fungal Count for Four weeks in DDVP-treated Soils and Controls

WEEK	NUMBER OF COLONIES (cfu/ml)							
	BACTERIA				FUNGI			
	10^{-5} Dil	10^{-5} Cont	10^{-8} Dil	10^{-8} Cont	10^{-6} Dil	10^{-6} Cont	10^{-8} Dil	10^{-8} Cont
ONE	46	59	40	54	15	27	11	25
TWO	35	50	27	51	15	23	11	20
THREE	31	41	22	43	9	17	8	17
FOUR	28	32	18	38	7	15	5	16

Table 6: Total Bacterial and Fungal Count for Four weeks in Dimethoate-treated Soils and Controls

WEEK	NUMBER OF COLONIES (cfu/ml)							
	BACTERIA				FUNGI			
	10^{-5} Dil	10^{-5} Cont	10^{-8} Dil	10^{-8} Cont	10^{-6} Dil	10^{-6} Cont	10^{-8} Dil	10^{-8} Cont
ONE	46	59	40	54	25	32	11	17
TWO	35	57	27	51	23	28	10	15
THREE	31	55	22	43	20	23	9	15
FOUR	28	49	18	38	15	16	9	10

Table 7: Total Bacterial and Fungal Count for Four weeks in Glyphosate-treated Soils and Controls

WEEK	NUMBER OF COLONIES (cfu/ml)							
	BACTERIA				FUNGI			
	10^{-5} Dil	10^{-5} Cont	10^{-8} Diln	10^{-8} Cont	10^{-6} Dil	10^{-6} Cont	10^{-8} Dil	10^{-8} Cont
ONE	19	59	14	54	10	23	17	27
TWO	14	50	11	51	9	21	12	25
THREE	11	41	7	43	6	17	8	20
FOUR	9	32	5	38	3	15	4	16

Table 8: Average Bacterial Count in Pesticides- treated Soil Samples for Four Weeks

WEEK	AVERAGE BACTERIAL COLONIES (cfu/ml)						
	Chlo	Cu_2O+M	Cyp	Delta	DDVP	Dimethoate	Glyphosate
ONE	15.5	17.5	40.5	20	43	43	16.5
TWO	9.5	14	32	12.5	31	31	12.5
THREE	7	11.5	27	9	26.5	26.5	9
FOUR	5.5	10	20.5	7	23	23	7

Key: Dil = Dilution, Cont = Control, Chlor = Chlorpyrifos, Cyp = Cypermethrin; Delta = Deltamethrin

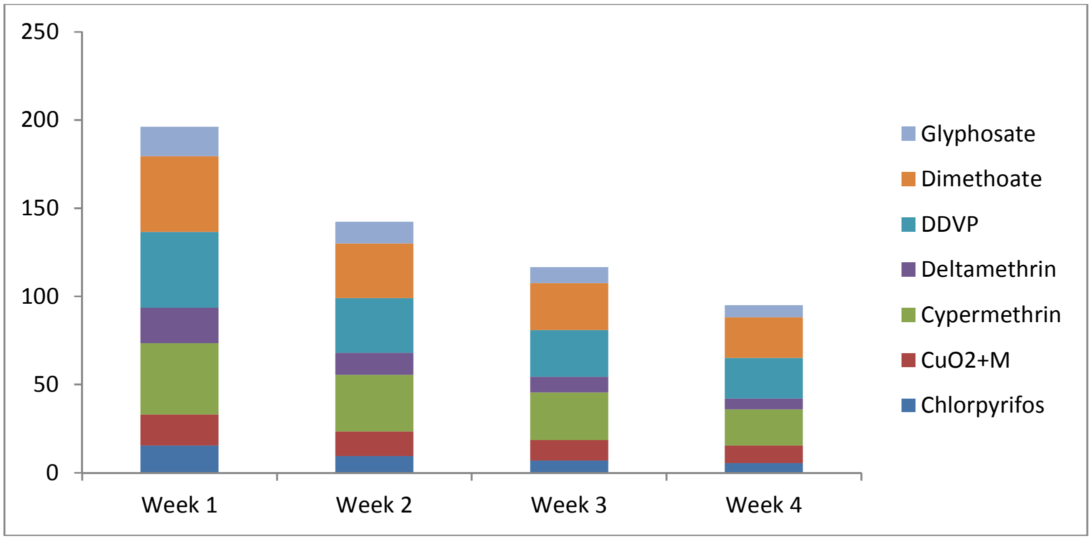

Figure 1: Comparison of Effect of Various Pesticides on Bacterial Count

Table 9: Average Fungal Count in Pesticides- treated Soil Samples for Four Weeks

WEEK	AVERAGE FUNGAL COLONIES (cfu/ml)						
	Chlor	**Cu_2O+M**	**Cyp**	**Delta**	**DDVP**	**Dimethoate**	**Glyphosate**
ONE	7	7	19.5	10.5	23	21.5	13.5
TWO	6.5	5.5	15	10	23	19	10.5
THREE	4	4	12.5	7.5	8.5	16	7
FOUR	3.5	2	9.5	6.5	6	12.5	3.5

Key: Chlor = Chlorpyrifos, Cyp = Cypermethrin; Delta = Deltamethrin

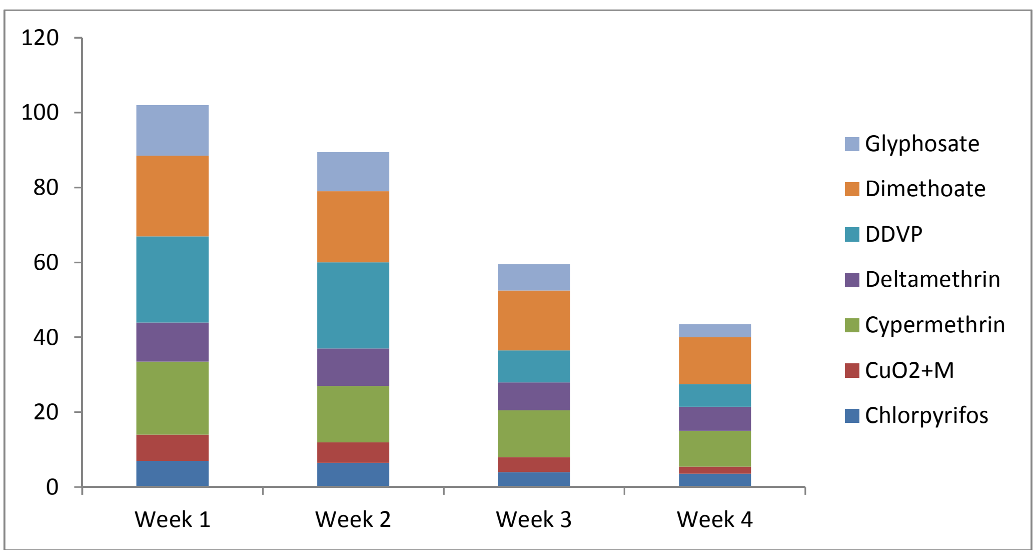

Figure 2: Comparison of Effect of Various Pesticides on Fungal Count

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