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EFFECTS OF AN ORGANOCHLORINE AND PYRETHROID PESTICIDE FORMULATION ON SOIL'S CULTURABLE MICROBIAL POPULATION

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Abstract- Non-target effect of pesticide on soil organisms is an important area of ecotoxicology, due to their roles in ecosystem sustainability. Thus, the effects of an organochlorine (endosulfan) and a synthetic pyrethroid (cypermethrin) pesticide formulation on soils' culturable microbial population were investigated. The study was carried out on loamy sand soil sample types collected from Ondo State, Forest Reserve, Owena, Nigeria. Pesticide application rates in the range of half of the normal field rate (0.5FR), the normal field rate (FR), two times the normal field rate (2FR), four times the normal field rate (4FR) and eight times the normal field rate (8FR) were applied to the soil microcosm design in the laboratory; while the untreated soil serve as control. The experiment set up was completely randomized in three replicates and soil samples were collected from each of the set up at 5 days interval over 35 days period of exposure for analysis. The pour plate technique was used for the enumeration of bacterial and fungal colonies on nutrient agar and potato dextrose agar respectively. The results indicated that both pesticides had effects on the population of bacterial and fungal counts of the soil samples with significant ($p < 0.05$) adverse effect observed at the treatment rate above the normal field application rate. At lower application rates (0.5FR, FR and 2FR), temporary inhibitory effect on bacterial and fungal population were observed. The progressive increase in inhibitory effect with corresponding increase in concentration of endosulfan and cypermethrin were noticed.

Keywords: Organochlorine pesticide, Synthetic pyrethroid, Endosulfan, Cypermethrin.

1. INTRODUCTION

Agricultural system relies on the use of chemicals to optimize crop production in order to meet the challenges of increasing global population and higher demands for food. The use of these chemicals in modern farming is viewed as an integral part of the success of agricultural industry. The introduction of pesticide in agricultural system has some success in preventing, suppressing and eradicating the pest that reduced production quality and quantity in the sector. However, the increase and continuous application of pesticides may result in soil pollution. When pesticides are applied, the possibilities exist that these chemicals may exert certain effect on soil quality especially on non-target organisms [1].

Organochlorine pesticide formulations are derivative of chlorinated hydrocarbons [2]. They are well known environmental contaminants among the group of persistent organic pollutant. Endosulfan is among the representative compound of organochlorine. Others are hexachlorocyclohexane (HCH), dichlorodiphenyl trichloro ethane (DDT), lindane and chlordane. Worldwide, most of the organochlorine pesticides were banned due to their neurotoxic effects and tendency to persist in the environment for years [3].

Pyrethroids are group of synthetic organic pesticides that are similar in structure to natural insecticide produced by flower species of Chrysanthum plant [4]. They are axonic excitotoxins that damage the voltage dependent sodium channels of the cellular organisms. The pyrethroid group comprises of alpha cyano-group (e.g. fenvalerate, cypermethrin and cyphenothrin) and non-alpha cyano-group (e.g. alletrin and permethrin). Alpha-cyano pyrethroid are primarily used for agricultural purposes due to their medium toxicity properties; while non-alpha cyano group are utilised majorly for non-agricultural purposes [5].

Endosulfan and cypermethrin are insecticidal pesticides. Cypermethrin is among the approved pesticide formulation for agricultural used in Nigeria, while endosulfan on the order hand has been listed as part of the banned pesticide products [6]. Reports have indicated that there is every possibility that endosulfan is still being illegally used by some farmers in Nigeria [7]. Soil is a unique medium of bio-interactions. The indiscriminate use of pesticides could disturb the soil environment by affecting flora and fauna including microflora of soil system [8]. Soil microbiota are the key engine that drive the biotransformation processes in the soil system. The non-target effect of pesticide on soil organisms is an important area of ecotoxicology, due to vital roles played by these organisms in soils' ecosystem sustainability. Thus, the Food and Agriculture Organization (FAO) recommended that in registering new pesticide, the detailed data should be provided on the possible effects of such pesticide on non-target organisms [9].

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This is to ensure such pesticide did not have unacceptable effects on non-target organisms. Hence, the effects of an organochlorine (endosulfan) and synthetic pyrethroid (cypermethrin) pesticide formulation were assessed on soils' culturable microbial population.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Microbiology Laboratory, Kwara State University, Malete.

2.2 Soil Sample Collection

The loamy sand soils of Ondo State, Forest Reserve, Owena, Nigeria were used for this study [7]. Surface soils (0-15 cm layer) from this area were collected with soil auger. The soil samples were bulked, crumbled and mixed thoroughly to form composite samples and taken to the laboratory for further processing.

2.3 Analysis of Moisture Content of the Soil Sample

The moisture content of the soil sample was analyzed using the method described by Zain et al. [10]. Ten grams (10 g) of soil sample was placed in weighing glass beaker and initial weight was determined. This was then followed by oven drying at 70 ^{o}C for 24 h. The beaker was repeatedly weighed until the final stable weight of the soil sample obtained. The moisture content was expressed as percentage using the formula:

$$\text{Moisture content} = \frac{\text{Weight of moist soil} - \text{Weight of dried soil}}{\text{Weight of dried soil}} \text{ x } 100$$

2.4 Determination of Water Holding Capacity of the Soil Sample

Water holding capacity was determined by placing 3 g of soil sample on a piece of initially weighed Whatman filter paper, followed by oven-drying at 70 ^{o}C for 24 h. Oven-dried soil on the weighed Whatman filter paper was weighed before dipping into water until soil becomes saturated. The soil was then placed in a humid enclosure to drain off the water before weighing again, and expressed in percentage using the formula:

$$\text{Water Holding Capacity} = \frac{\text{Mass of water contained in saturated soil}}{\text{Mass of saturated soil}} \text{ x } 100$$

2.5 Soil Preparation for Pesticide Treatments

The study was carried out using microcosm design. An organochlorine (endosulfan) and a synthetic pyrethroid (cypermethrin) were respectively used for the treatment of soil samples.

The method described by El-Ghamry et al. (2000) was adopted for the treatment procedure with little modification. Five geometrical pesticide treatment rates were applied to the soil samples. The pesticide treatments rate range from half the normal field application rate (0.5FR), recommended field rate (FR), two times the field application rate (2FR), four times the field application rate (4FR) to eight times the field application rate (8FR). The corresponding concentration for each of the pesticide treatment rate ranged from 0.5FR, FR, 2FR, 4FR to 8FR, respectively were:

- Endosulfan (µg/kg): 2.75, 5.50, 11.00, 22.00, 44.00
- Cypermethrin (µg/kg): 3.13, 6.25, 12.50, 25.00, 50.00

The treatments rate were calculated using the formula as described by Zain et al. [10]:

$$\text{X (µg/kg) soil} = \frac{\text{Recommended field application rate (g a.i. / ha)}}{\text{Amount of a.i.in formulation (g a.i./ L) x 450 L/ha}} \text{ x } \frac{1000000\ \mu g}{1kg}$$

Where;

"g a.i" represent the gram of active ingredients present in pesticide formulation.

Fifty milliliters (50ml) volumes of pesticide formulation were applied to 500 g of soil samples by hand-spraying. The respective treated soils were replicated thrice and the remaining samples were treated with deionised water to serve as control. The treated soils were mixed thoroughly by constant shaking for 5 min. They were then incubated in the dark at room temperature. The moisture content of soil samples was maintained at 50 % of the maximum water holding capacity by adding deionised water as needed.

2.6 Effect of Pesticide on Soils Culturable Bacterial and Fungal Population

The pour plate technique was used for the enumeration of bacterial and fungal population respectively. Nutrient agar plate (NA) supplemented with 0.1 g/L cyclohexamide was used for bacterial cultivation; while potato dextrose agar plate (PDA) supplemented with 30 mg/L streptomycin sulphate was used for cultivation of fungi [11]. The nutrient agar medium and potato dextrose agar medium were prepared according to manufacturer's instruction. The impact of pesticide on culturable microbial population was assessed from the soil sample at 5, 10, 15, 20, 25, 30 and 35 days period of exposure. Five sub-soil samples from each microcosm were aseptically randomly collected with a sterile 10 mm cork borer. They were mixed together to form a composite sample. One gram of the soil was then used to make a serial dilution under aseptic condition. The dilution was made up to 10^{-7} fold and 0.1 ml of 10^{-4} and

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10^{-7} were inoculated on agar media plates in triplicates. The inoculated plates were inverted and incubated at room temperature (28 ± 2 ºC). The colonies that formed on the plates were counted with colony counter after 48 h and 7 days of incubation for bacteria and fungi respectively. The counted colonies data were expressed in colony forming unit (cfu/g) of dry weight of soil.

2.7 Statistical Analysis

The data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 21. The microbial counts of various pesticides treated soils were analyzed using analysis of variance (ANOVA) and further subjected to Duncan multiple range test (DMRT) to compare the mean values for significant difference. Differences were considered statistically significant at $P \leq 0.05$ [12].

3. RESULTS

3.1 Impact of Endosulfan on the Mean Culturable Bacterial Populations

The mean culturable bacterial counts in endosulfan-treated soils and untreated control soils over the thirty-five days period of exposure were represented in Fig. 3.1. The treatments were control (C), half of the field rate (0.5FR), field rate (FR), x2 of the field rate (2FR), x4 of the field rate (4FR) and x8 of the field rate (8FR).

In the first five (5) days of exposure period (DEP), the mean culturable bacterial counts ranged from 42.3 x 10^6 cfu/g, 52 x 10^6 cfu/g, 137.0 x 10^6 cfu/g, 153.7 x 10^6, 164.0 x 10^6 cfu/g to 197.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, 0.5FR, FR and control respectively (Fig. 1). Significant reductions ($p < 0.05$) in bacterial counts were observed in all the treated soils as compare to control.

The mean culturable bacterial counts recorded from soils at ten (10) DEP, ranged from 45.0 x 10^6 cfu/g, 53.7 x 10^6 cfu/g, 113.3 x 10^6 cfu/g, 131.7 x 10^6 cfu/g, 136.0 x 10^6 cfu/g to 162.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and control respectively (Fig. 3.1). The reductions in bacterial counts were significant ($P < 0.05$) only in the soil samples that received endosulfan treatment level higher than 2FR.

The mean culturable bacterial counts observed in fifteen (15) DEP ranged from 62.7 x 10^6 cfu/g, 73.0 x 10^6 cfu/g, 104.3 x 10^6 cfu/g, 109.3 x 10^6 cfu/g, 119.7 x 10^6 cfu/g to 122.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and C respectively (Fig. 1). The reductions in bacterial counts observed in the soil treated with endosulfan formulation at the concentration below 2FR were not significant ($p > 0.05$).

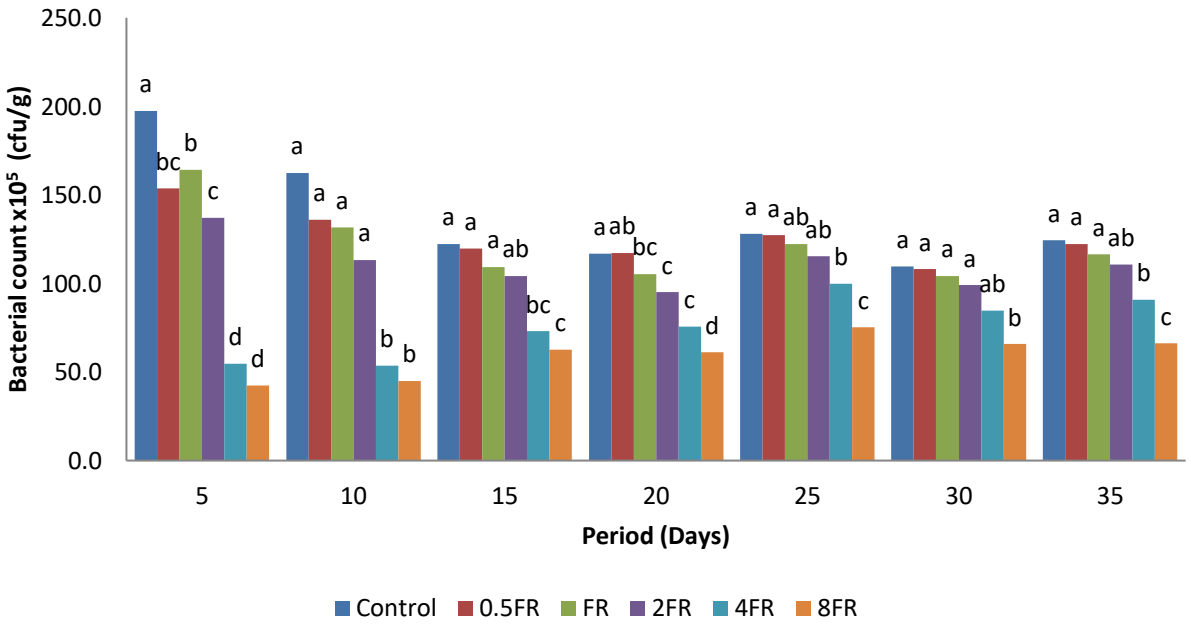


Fig. 3.1 Mean Culturable Bacterial Counts in Soil Treated with Endosulfan Pesticide

Keys: FR = Field rate; the significant effect ($P \leq 0.05$) of pesticide treatment at a particular period of exposure was indicated by different letters (i.e. a, b, c).

At the twenty (20) DEP, the mean culturable bacterial counts ranged from 61.0 x 10^6 cfu/g, 75.7 x 10^6 cfu/g, 95.0 x 10^6 cfu/g, 105.3 x 10^6 cfu/g, 116.7 x 10^6 cfu/g and 117.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively (Fig. 3.1). Significant reductions ($p < 0.05$) in bacterial counts were only observed in soil treated with 4FR and 8FR of endosulfan respectively.

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The mean culturable bacterial counts observed in twenty-five (25) DEP ranged from 75.3 x 10^6 cfu/g, 100.0 x 10^6 cfu/g, 115.3 x 10^6 cfu/g, 122.3 x 10^6 cfu/g, 127.3 x 10^6 cfu/g to 128.0 x 10^6 cfu/g for 8FR, 4FR, 2FR, FR, 0.5FR and C respectively. Significant reduction ($p < 0.05$) in the mean culturable bacterial counts was only observed in the soil treated with the highest concentration (8FR) of endosulfan.

The mean culturable bacterial counts observed in thirty (30) days of exposure ranged from 65.7 x 10^6 cfu/g, 82 x 10^6 cfu/g, 99.0 x 10^6 cfu/g, 104.3 x 10^6 cfu/g, 108.0 x 10^6 cfu/g to 109.7 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and C respectively. Statistical analysis showed that the reduction in bacterial counts as observed for all the treatments was only significant in the soil sample treated with the highest treatment rate (8FR).

At the thirty-five (35) days of exposure, the mean culturable bacterial counts ranged from 66.3 x 10^6 cfu/g, 90.7 x 10^6 cfu/g, 110.7 x 10^6 cfu/g, 116.3 x 10^6 cfu/g, 122.3 x 10^6 cfu/g to 124.3 x 10^6 cfu/g for 8FR, 4FR, 2FR, FR, 0.5FR and C respectively. The significant reduction in bacterial counts was only observed in the soil treated with 8FR of endosulfan ($p < 0.05$).

3.2 Degree of Impact of Endosulfan Treatments on Bacterial Population Relative to Control over the Period of Exposure

The degree of impact of endosulfan pesticide treatments on bacterial populations was presented as percentage response (inhibition or stimulation) and shown in Fig. 3.2.

On the soil samples treated with half of the field rate (0.5FR), the highest inhibitory effect (22.1%) on bacterial population was observed at 5 days of exposure period (DEP); while a slight stimulatory effect (0.6%) was observed at twenty DEP (Fig. 3.2).

The field rate (FR) treatments had the highest inhibitory effect (18.9%) on bacterial population at 10 DEP. However, stimulatory effect was not observed throughout the period of exposure.

For the soil treated with 2 times the field rate (2FR) of endosulfan, the highest inhibitory effect (30.6%) on bacterial population was observed at 25th DEP with the least inhibitory effect (9.8%) recorded at 25 DEP.

The highest inhibitory effects in the soil treated with 4 times and 8 times the field rate of endosulfan respectively (72.3% and 78.6% respectively) were observed at the 5th days of DEP, while the least inhibitory effect (21.9% and 40.1% respectively) were observed at 25 and 35 DEP.

The progressive decline in inhibitory effects on soil culturable bacterial population were observed over the period of exposure in all the treatment levels. The lower treatment rates showed slight recovery over the period of exposure.

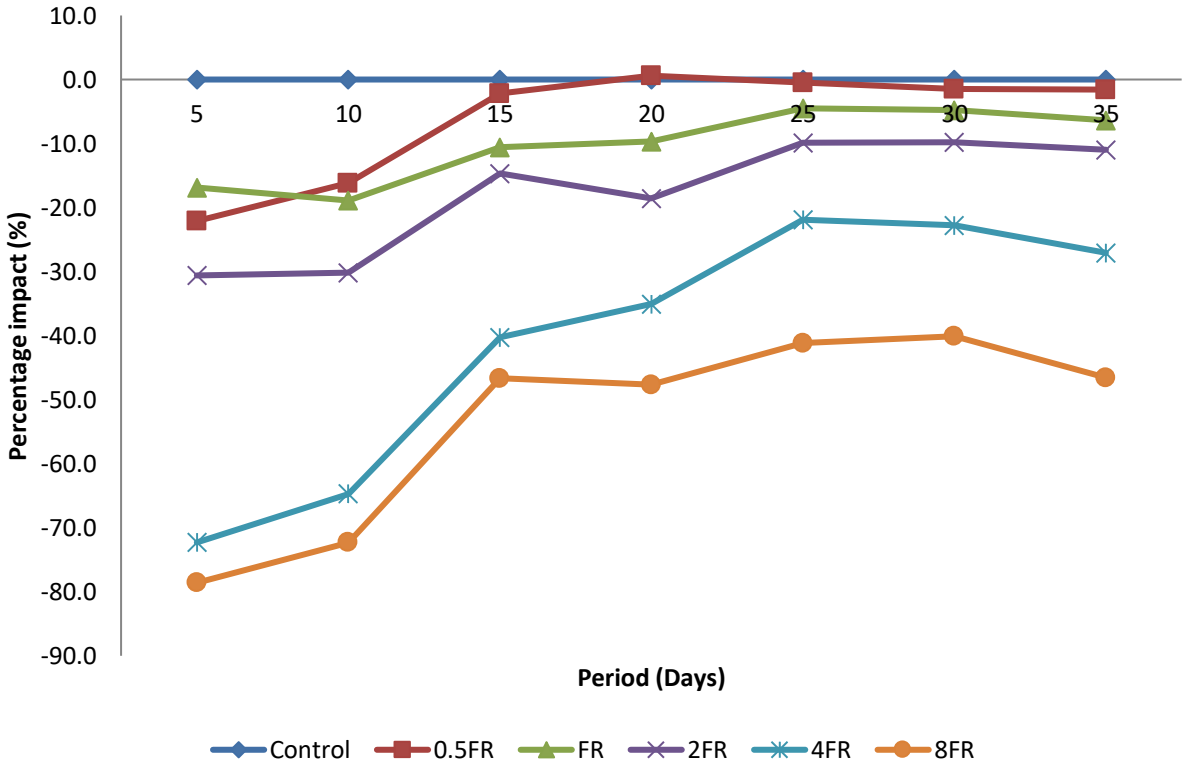


Fig. 3.2 Degree of Impact of Endosulfan Treatments on Bacterial Population Relative to Control over the Period of Exposure

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3.3 Impact of Cypermethrin Pesticide on the Mean Culturable Bacterial Populations of Soil

The mean culturable bacterial counts in treated soils and untreated control soils over the period of exposure were represented in Fig. 3.3.

In the first five (5) days of exposure period (DEP), the mean culturable bacterial counts ranged from 40.3 x 10^6 cfu/g, 52.3 x 10^6 cfu/g, 74.3 x 10^6 cfu/g, 80.3 x 10^6, 112.7 x 10^6 cfu/g to 129.7 x 10^6 cfu/g of soil samples for 8FR, 4FR, FR, 2FR, 0.5FR and control respectively (Fig. 3). Significant reductions ($p < 0.05$) in culturable bacterial counts were observed in the soil treated with cypermethrin formulation above the normal field application rate.

At the ten (10) days of exposure period, the mean culturable bacterial counts ranged from 53.7 x 10^6 cfu/g, 68.3 x 10^6 cfu/g, 72 x 10^6 cfu/g, 77.3 x 10^6 cfu/g, 100.3 x 10^6 cfu/g to 113.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, FR, 2FR, 0.5FR and control respectively (Fig. 3.3).

The mean culturable bacterial counts observed in fifteen (15) DEP ranged from 55.0 x 10^6 cfu/g, 72.3 x 10^6 cfu/g, 74.3 x 10^6 cfu/g, 78.0 x 10^6 cfu/g, 97.3 x 10^6 cfu/g to 103.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively (Fig. 3). The effect of cypermethrin treatments level on soil bacterial count followed the same trend as observed in 5th and 10th days of exposure.

At the twenty (20) days of exposure, the mean culturable bacterial counts ranged from 59.0 x 10^6 cfu/g, 81.7 x 10^6 cfu/g, 89.0 x 10^6 cfu/g, 93.3 x 10^6 cfu/g, 101.3 x 10^6 cfu/g to 104.3 x 10^6 cfu/g of soil samples for 8FR, 4FR, FR, 2FR, C and 0.5FR respectively (Fig. 3.3). Significant reduction ($p < 0.05$) in bacterial counts was only observed in soil treated with the highest cypermethrin formulation (8FR).

The mean culturable bacterial counts in twenty-five (25) days of exposure ranged from 74.3 x 10^6 cfu/g, 92.7 x 10^6 cfu/g, 98.3 x 10^6 cfu/g, 101.3 x 10^6 cfu/g, 103.7 x 10^6 cfu/g to 105.0 x 10^6 cfu/g for 8FR, 4FR, C, 0.5FR, FR and 2FR respectively.

The mean culturable bacterial counts as observed in thirty (30) days of exposure ranged from 68.3 x 10^6 cfu/g, 80.3 x 10^6 cfu/g, 97.0 x 10^6 cfu/g, 99.3 x 10^6 cfu/g, 102.0 x 10^6 cfu/g to 102.7 x 10^6 cfu/g of soil samples for 8FR, 4FR, C, 0.5FR, FR and 2FR respectively.

At the thirty-five (35) days of exposure, the mean culturable bacterial counts ranged from 69.0 x 10^6 cfu/g, 85.7 x 10^6 cfu/g, 97.3 x 10^6 cfu/g, 101.0 x 10^6 cfu/g, 103.7 x 10^6 cfu/g to 104.3 x 10^6 cfu/g for 8FR, 4FR, C, 2FR, 0.5FR and FR respectively. Result showed that there were no significant impacts ($p > 0.05$) of cypermethrin formulation on the mean culturable bacterial counts of treated soil samples.

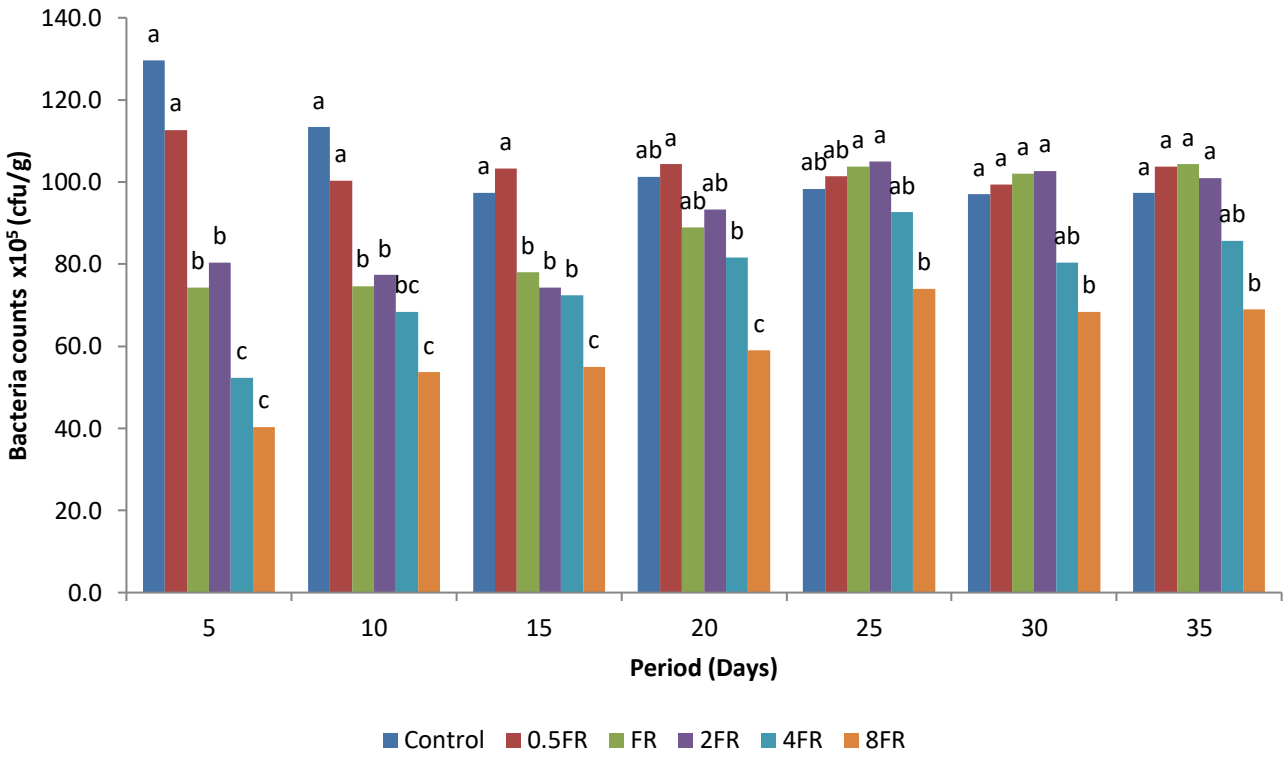


Fig. 3.3 Mean Culturable Bacterial Counts in Soil Treated with Cypermethrin Pesticide

Keys: FR = Field rate; the significant effect ($P \leq 0.05$) of pesticide treatment at a particular period of exposure was indicated by different letters (i.e. a, b, c).

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3.4 Degree of Impacts of Cypermethrin Treatments on Bacterial Population Relative to Control Over The Period of Exposure

The degree of impacts of cypermethrin pesticide treatments on bacterial populations were presented as percentage response (inhibition or stimulation) as shown in Fig. 3.4.

On the soil samples treated with half of the field rate (0.5FR), the highest inhibitory effects of cypermethrin were observed at 5 and 10 days of exposure period (DEP) with the highest inhibition (13.1%) on bacterial population recorded at the 5th DEP); while stimulatory effects (6.2%, 2.9%, 3.1%, 2.4% and 6.5%) were observed at 15, 20, 25, 30 and 35 DEP (Fig. 3.4).

The field rate (FR) treatments showed the inhibitory effect on bacterial population at 5, 10 and 15 DEP with the highest inhibition (42.7%) observed at 5 DEP; while slight stimulatory effects were observed at 25 (5.5%), 30 (5.2%), and 35 (7.2%) DEP.

Cypermethrin treatment concentration of 2 times the field rate (2FR) showed the inhibitory effects at 5, 10 and 15 DEP with highest inhibitory effect (38.1%) on bacterial population observed at 5 DEP. Slight stimulatory effects (6.8%, 5.9% and 3.8%) were observed at 25, 30 and 35 DEP respectively.

The 4FR and 8FR of cypermethrin treatment levels showed inhibitory effect on bacterial population of treated soils throughout the period of experiment (Fig. 3.4). The highest inhibitory effects of the soil treated with 4 and 8 times the field rate respectively (59.7% and 69.9%) on bacterial population were observed at the 5 DEP.

Generally, the inhibitory effect of cypermethrin on soil bacteria increases with increase in treatment rate. However, progressive decline in inhibitory effect of cypermethrin on soil bacterial population over the period of exposure were observed.

The marked recovery in bacterial population was observed at 15 day (0.5FR) and 25 day (FR and 2FR respectively) of experimental period.

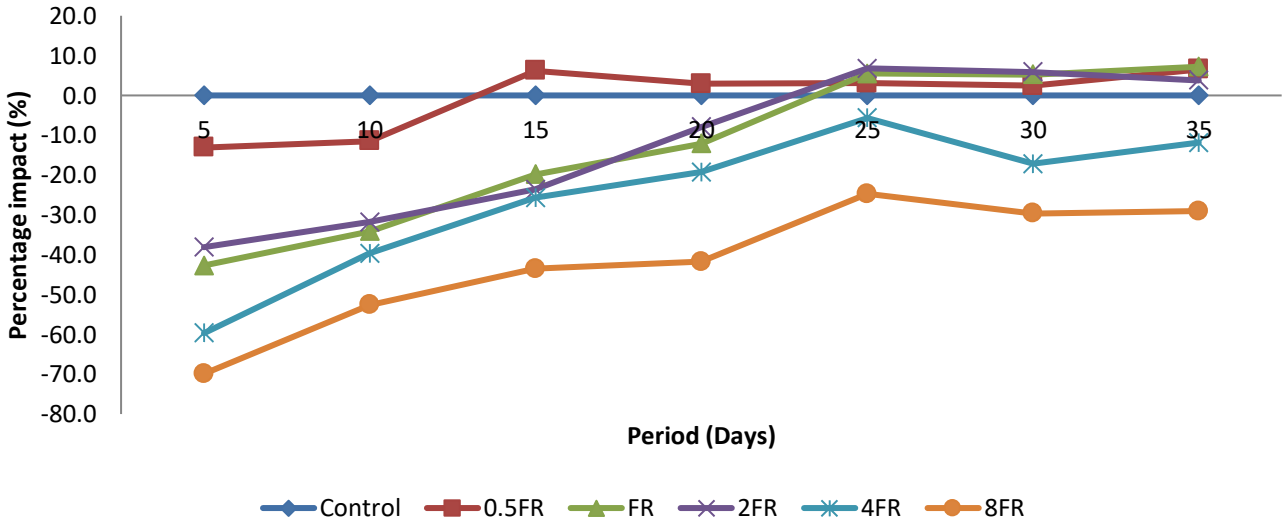


Fig. 3.4 Degree of Impact of Cypermethrin Treatments on Bacterial Population Relative to Control over the Period of Exposure

3.5 Impact of Endosulfan Pesticide on Mean Fungal Populations of Soil

The mean fungal counts in treated soils and untreated control soils over the period of exposure were represented in Fig. 3.5. The treatments were control (C), half of the field rate (0.5FR), field rate (FR), x2 of the field rate (2FR), x4 of the field rate (4FR) and x8 of the field rate (8FR).

In the first five (5) days of exposure period (DEP), the mean fungal counts ranged from 16.3 x 10^4 cfu/g, 22.0 x 10^4 cfu/g, 27.7 x 10^4 cfu/g, 30.7 x 10^4, 32.0 x 10^4 cfu/g to 32.3 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and control respectively (Fig. 5).

At the ten (10) days of exposure period, the mean fungal counts ranged from 16.0 x 10^4 cfu/g, 24.3 x 10^4 cfu/g, 29.3 x 10^4 cfu/g, 30.7 x 10^4 cfu/g, 31.0 x 10^4 cfu/g to 32.7 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, 0.5FR, C and FR (Fig. 3.5).

The mean fungal counts observed in fifteen (15) DEP ranged from 15.0 x 10^4 cfu/g, 21.7 x 10^4 cfu/g, 26.3 x 10^4 cfu/g, 29.0 x 10^4 cfu/g, 31.0 x 10^4 cfu/g to 31.7 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and control respectively (Fig. 3.5).

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At the twenty (20) days of exposure, the mean fungal counts ranged from 15.3 x 10^4 cfu/g, 22.7 x 10^4 cfu/g, 27.0 x 10^4 cfu/g, 29.3 x 10^4 cfu/g, 30.7 x 10^4 cfu/g to 31.3 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, 0.5FR and control respectively (Fig. 3.5).

The mean fungal counts in twenty-five (25) days of exposure ranged from 12.7 x 10^4 cfu/g, 24.0 x 10^4 cfu/g, 27.0 x 10^4 cfu/g, 30.3 x 10^4 cfu/g, 30.7 x 10^4 cfu/g to 32.0 x 10^4 cfu/g for 8FR, 4FR, 2FR, FR, 0.5FR and control respectively.

The mean fungal counts as observed in thirty (30) days of exposure ranged from 16.3 x 10^4 cfu/g, 20.7 x 10^4 cfu/g, 23.7 x 10^4 cfu/g, 29.7 x 10^4 cfu/g, 29.7 x 10^4 cfu/g to 30.3 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, C, FR and 0.5FR respectively.

At the thirty-five (35) days of exposure, the mean fungal counts ranged from 16.0 x 10^4 cfu/g, 22.7 x 10^4 cfu/g, 26.3 x 10^4 cfu/g, 28.0 x 10^4 cfu/g, 28.3 x 10^4 cfu/g to 29.0 x 10^4 cfu/g for 8FR, 4FR, 2FR, 0.5FR, FR and control respectively.

Generally, the decrease in fungal counts in endosulfan pesticide formulation treated soils across all the treatment levels over the period of exposure was only significant for highest treatment rate (8FR) as observed in the first five days of exposure to 30 DEP. However, there were no significant differences among the fungal counts of all the treated soils at 35 DEP.

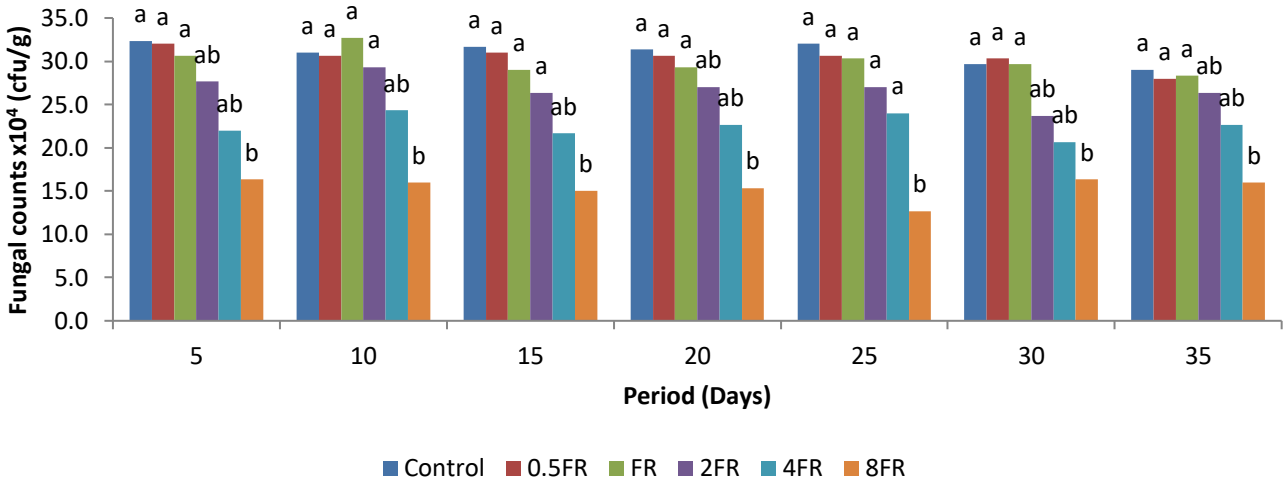


Fig. 3.5 Mean Fungal Counts in Soil Treated with Endosulfan Pesticide

Keys: FR = Field rate; the significant effect ($P \leq 0.05$) of pesticide treatment at a particular period of exposure was indicated by different letters (i.e. a, b, c).

3.6 Degree of Impacts of Endosulfan Treatments on Fungal Population Relative to Control over the Period of Exposure

The degree of impacts of endosulfan pesticide treatments on fungal populations were presented as percentage response (inhibition or stimulation) as shown in Fig. 3.6.

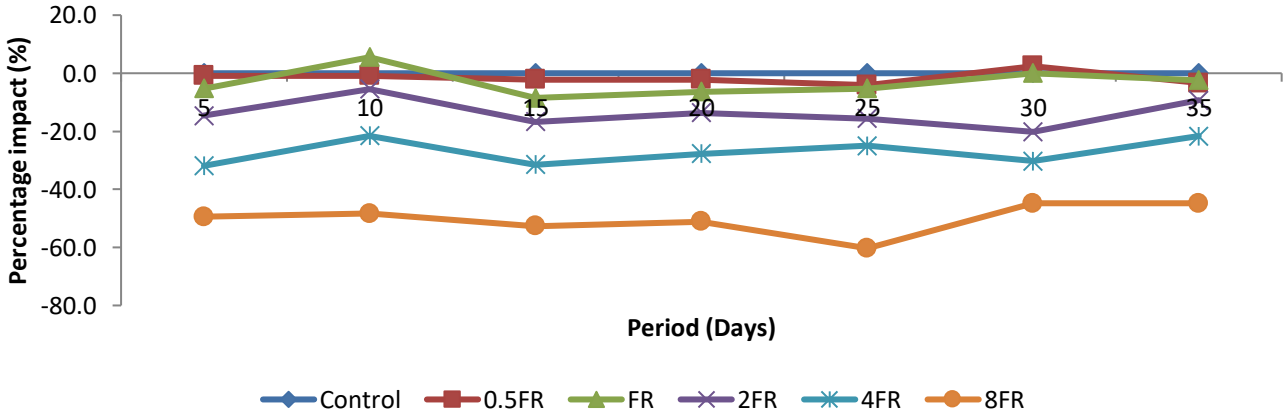


Fig. 3.6 Degree of Impact of Endosulfan Treatment Levels on Fungal Population Relative to Control over the Period of Exposure

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3.7 Impact of Cypermethrin Pesticide on the Mean Fungal Populations of Soil

The mean fungal counts in treated soils and untreated control soils over the period of exposure were represented in Fig. 3.7. The treatments were control (C), half of the field rate (0.5FR), field rate (FR), x2 of the field rate (2FR), x4 of the field rate (4FR) and x8 of the field rate (8FR).

In the first five (5) days of exposure period (DEP), the mean fungal counts ranged from 24.0 x 10^4 cfu/g, 27.0 x 10^4 cfu/g, 27.7 x 10^4 cfu/g, 30.0 x 10^4, 31.3 x 10^4 cfu/g to 32.7 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, 0.5FR, FR and control respectively (Fig. 3.7). Although, the decreases in fungal counts were observed across all the treatment levels; these decreases were not statistically significant ($p > 0.05$).

At the ten (10) days of exposure period, the mean fungal counts ranged from 22.3 x 10^4 cfu/g, 26.3 x 10^4 cfu/g, 27.3 x 10^4 cfu/g, 30.3 x 10^4 cfu/g, 30.7 x 10^4 cfu/g to 31.7 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR (Fig. 3.7). Cypermethrin pesticide treatments did not show any significant impact on fungal population of treated soil ($p > 0.05$).

The mean fungal counts observed in fifteen (15) DEP ranged from 21.0 x 10^4 cfu/g, 25.0 x 10^4 cfu/g, 29.3 x 10^4 cfu/g, 31.0 x 10^4 cfu/g, 31.3 x 10^4 cfu/g to 32.3 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, C, FR and 0.5FR respectively (Fig. 3.7). The effect of cypermethrin treatments level on soil fungal count followed the same trend as observed in 5th and 10th days of exposure. There were no significant differences among the fungal counts of all the treatment levels ($p > 0.05$).

At the twenty (20) days of exposure, the mean fungal counts ranged from 18.0 x 10^4 cfu/g, 26.0 x 10^4 cfu/g, 28.3 x 10^4 cfu/g, 30.0 x 10^4 cfu/g, 30.3 x 10^4 cfu/g to 31.7 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively (Fig. 3.7). No significant differences observed in fungal counts of all the treatment levels ($p > 0.05$).

The mean fungal counts in twenty-five (25) days of exposure ranged from 19.3 x 10^4 cfu/g, 23.7 x 10^4 cfu/g, 28.7 x 10^4 cfu/g, 29.0 x 10^4 cfu/g, 29.3 x 10^4 cfu/g to 30.0 x 10^4 cfu/g for 8FR, 4FR, 2FR, FR, C and 0.5FR respectively. The decreases in fungal counts of all the cypermethrin treated soils were not statistically significant ($p > 0.05$).

The mean fungal counts as observed in thirty (30) days of exposure ranged from 19.7 x 10^4 cfu/g, 25.0 x 10^4 cfu/g, 29.0 x 10^4 cfu/g, 29.7 x 10^4 cfu/g, 29.7 x 10^4 cfu/g to 30.7 x 10^4 cfu/g of soil samples for 8FR, 4FR, 2FR, C, 0.5FR and FR respectively. Statistical analysis showed that there were no significant differences in the mean fungal counts of all the experimental soil samples ($p < 0.05$).

At the thirty-five (35) days of exposure, the mean fungal counts ranged from 22.0 x 10^4 cfu/g, 26.3 x 10^4 cfu/g, 29.0 x 10^4 cfu/g, 29.7 x 10^4 cfu/g, 30.0 x 10^4 cfu/g to 30.7 x 10^4 cfu/g for 8FR, 4FR, 2FR, FR C and 0.5FR respectively. Result showed that there were no significant impacts of cypermethrin treatments on the mean fungal counts of soils samples ($p > 0.05$).

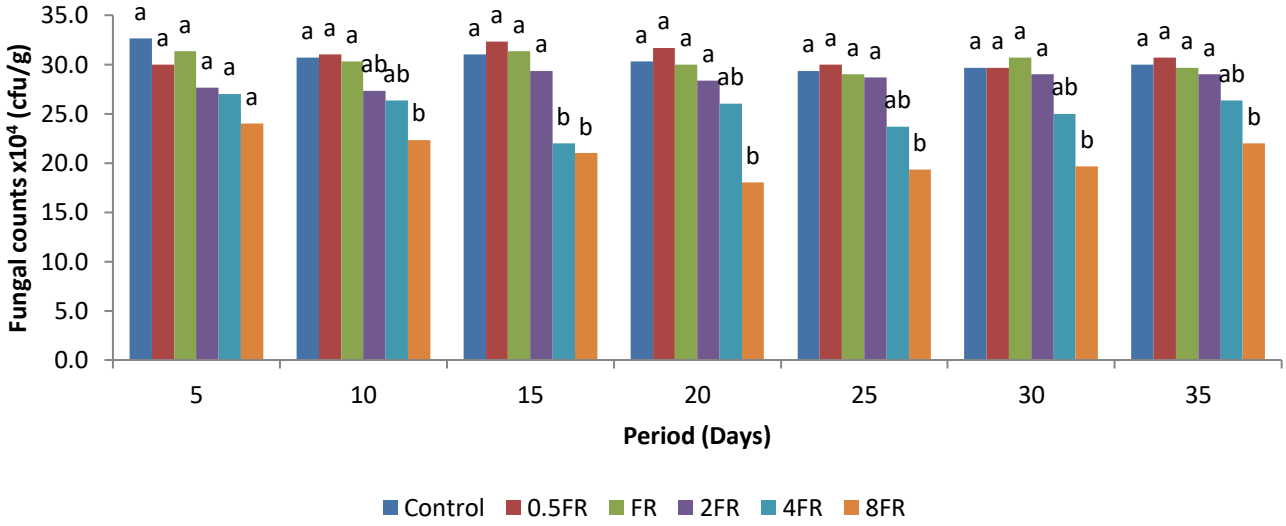


Fig. 3.7 Mean Fungal Counts in Soil Treated with Cypermethrin Pesticide

Keys: FR = Field rate; the significant effect ($P \leq 0.05$) of pesticide treatment at a particular period of exposure was indicated by different letters (i.e. a, b, c).

3.8 Degrees of Impacts of Cypermethrin Treatments on Fungal Population Relative to Control over the Period of Exposure

The degree of impacts of cypermethrin pesticide treatments on fungal populations were presented as percentage response (inhibition or stimulation) as shown in Fig. 3.8.

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On the soil samples treated with half of the field rate (0.5FR), the only slight highest inhibitory effects (8.3%) of cypermethrin were observed at 5 days of exposure period (DEP) with the marked recovery observed throughout the remaining period of experiment (Fig. 3.8).

The field rate (FR) treatments showed slight inhibitory effects on fungal population at 5 (3.9%), 10 (0.9%) and 15 (0.9%) DEP; while slight stimulatory effects were observed at 15 (0.9 %), 25 (1.0%), 30 (3.3%) and 35 (1.0%) DEP.

Cypermethrin treatment concentration of 2 times the field rate (2FR) showed the inhibitory effects at from 5 days after exposure to 30 DEP. However, a slight recovery was observed at 35 DEP.

The 4FR and 8FR of cypermethrin treatment levels showed inhibitory effect on fungal population of treated soils throughout the period of experiment (Fig. 8). The highest inhibitory effects of the soil treated with 4 and 8 times the field rate on fungal population (19.5% and 40.6%) were observed at the 25 and 20 DEP respectively.

Generally, the inhibitory effect of cypermethrin on soil fungi increases with increase in treatment rate. However, progressive decline in inhibitory effect of cypermethrin on soil fungal population over the period of exposure were observed.

The marked recovery in fungal population was observed at 15 day (0.5FR) and 25 day (FR and 2FR respectively) of experimental period.

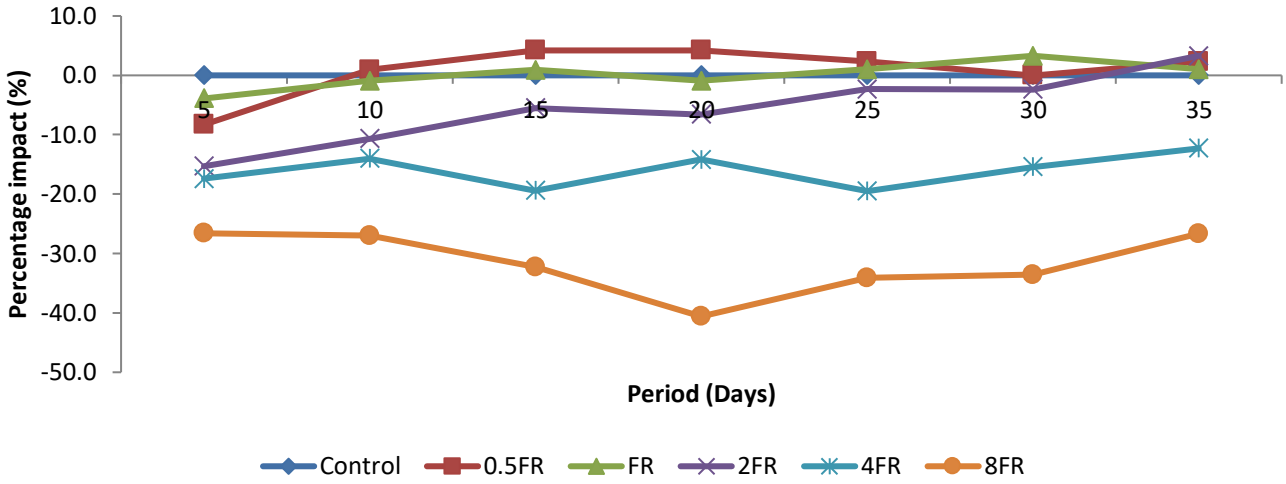


Fig. 3.8 Degree of Impact of Cypermethrin Ttreatment Levels on Fungal Population Relative to Control over the Period of Exposure

4. DISCUSSION

Worldwide, pesticide application has been an integral part of successful agricultural practices. However, indiscriminate use, coupled with the inherent properties of pesticide compounds and their possibility of having effects on non-target organisms has made them the pollutants of concern in the environment.

Organochlorine pesticide formulations are derivative of chlorinated hydrocarbons and they are among the well-known environmental contaminants belonging to the class of persistent organic pollutant. Endosulfan is one of the representative chemical compounds of organochlorine [2].

Pyrethroids are group of synthetic organic pesticides that are similar in structure to natural insecticide produced by flower species of Chrysanthum plant [4]. Cypermethrin belongs to alpha cyano-group and are primarily used for agricultural purposes.

Cypermethrin is among the approved pesticide formulation for agricultural used in Nigeria, while endosulfan on the order hand has been listed as part of the banned pesticide products [6].

Non-target effects of pesticide on soil organisms have been an important area of ecotoxicology, due to vital roles played by these organisms in soils' ecosystem sustainability. Thus, the Food and Agriculture Organization (FAO) recommended that in registering new pesticide, the detailed data should be provided on the possible effects of such pesticide on non-target organisms [9]. This is to ensure that such pesticide did not have unacceptable effects on non-target organisms.

The results of the present study revealed that these pesticides treatments had differential effects on soil organisms which were highly determined by the pesticide type, rate of application and length of exposure.

Endosulfan pesticide applied to the soil at concentration corresponding to half of normal field rate (0.5FR), normal field rate (FR) and two times the normal field rate (2FR) only caused significant reduction in soil bacterial counts at

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the first five day of exposure. Subsequently, no significant impact were observed in these treatment rates (0.5FR, FR and 2FR) throughout the remaining period of exposure. However, the higher endosulfan treatment rate (4FR and 8FR respectively) caused significant reduction in bacterial population and effect lingered throughout the experimental period. On the other hand, no significant differences were observed in the mean fungal counts of the soil samples treated with endosulfan at 0.5FR, FR, 2FR and 4FR throughout the period of exposure. Whereas, the highest endosulfan treatment rate (8FR) caused significant reduction in fungal population of the soil samples.

The negative impact of endosulfan pesticide on the population of bacteria and fungi at higher application rate was in agreement with the findings of Joseph et al. [13] and Dey et al. [14] who reported that endosulfan pesticide applied to the soil samples at recommended field rate caused a slight but non-significant short inhibitory effect on bacterial and fungal populations in the soil; while significant reduction in bacterial and fungal population was only observed in the soil samples treated with higher doses of endosulfan. Adebayo et al. [15] studied the effect of endosulfan insecticide (Karate® and Thiodan©) on the population dynamics of four different soil microorganisms and only observed a marked decrease in bacterial population of the soil samples treated with concentration above the recommended rate; whereas, the fungal population was significantly affected at the field application rate and two times the field application rate. In a recent paper by Lakshmi and Vinay [16], pesticides (including endosulfan) caused significant reduction in bacterial population of the treated soil at 1:10, 1:20, 1:40, 1:80 and 1:100 application rate. The non-significant impact of endosulfan pesticide at lower concentration might be explained by its high affinity features to adsorb to soil components thereby reduces its bioavailabity and bioactivity. The negative impact on soil bacteria and fungi at higher concentration as observed in this study could be attributed to the electrophilic reactivity features of carbon-chlorine bond present in all organochlorine (including endosulfan) that facilitate displacement of chlorine and subsequent attachment to a cell's bionucleophile, thereby causing irreversible damage to the cell. Cremlyn [17] reported that the biocidal properties of endosulfan could be attributed to the presence of chlorine molecule in its parent structure.

In contrary to this result, Nasreen et al. [18] reported that application of endosulfan to groundnut planted black clay soils at normal field rate significantly stimulated the bacterial and fungal populations of the soil samples. They observed that increases in treatment rate caused a significant increase in microbial population.

Cypermethrin pesticide applied to the soil at the normal field rate concentration (FR) and the higher treatment levels (2FR, 4FR and 8FR) were observed to cause significant reduction in bacterial population from the first five day of exposure period to the fifteen day of exposure period. However, marked recoveries in bacterial population from cypermethrin pesticide treatments were noticed from 20 days of exposure period to the last day of observation in all the treated soil samples except in soil samples with highest cypermethrin treatment rate (8FR) where significant decrease in bacterial population was observed throughout the period of exposure. The fungal population in the soil samples was only negatively affected at the highest treatment rate (8FR) of cypermethrin applied to the soil.

Inhibitory effect of cypermethrin at 125 parts per million (ppm), 250 ppm, 500 ppm and 1000 ppm application rate on soil bacteria counts that disappeared at 21 day after application under laboratory and field studies was reported by Ahmed and Ahmad [19]. Goswami et al. [20] studied the effect of cypermethrin on soil microbial biomass in an alluvial soil and showed that at normal field rate, cypermethrin pesticides caused transient toxic effect on soil microorganisms. The result was also corroborated by the findings of Sethi et al. [21] who observed an initial strong inhibitory effect of cypermethrin treatment at 1000 ppm rate on bacterial population and gradual recovery after the 12 day of exposure period. Tu [22] reported decreases in fungal population in soil samples treated with cypermethrin in the first two weeks of application but gradual recovery was observed after 3 weeks of application. Filimon et al. [23] reported a significant decreased in bacterial population in the soil sample treated with cypermethrin. However, Srinivasulu and Ortiz [24] observed that the application of cypermethrin at recommended field rate resulted in increased bacterial and fungal populations of tomato cultivated soils but a dramatic decrease in bacterial and fungal populations in the soil treated with concentration above the recommended field rate were reported.

The toxicity of cypermethrin to microorganisms could be attributed to its capability to inhibit ATPase enzyme which is involved in the movement of ions against a concentration gradient and regulate active transport in cellular organisms. ATPase enzyme system which is involved in transport of metal across the cell membrane had been reported in bacteria, archea and eukaryotes [25]. Vijverge and Bercken [26] pointed out that the major mechanism of cypermethrin action against a cellular organism is to alter ion permeability of the cell membrane, thereby causing damage to voltage dependent sodium channel of the cell. Generally, application of these pesticide formulations at normal field rate poses low or no risk to bacterial and fungal populations of the soil system as observed in this study. However, there were indications that the higher concentration of these pesticide formulations could have negative impact on microbial community of the soil sample.

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