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EFFECT OF SELECTED PESTICIDES ON MICROBIAL COMMUNITY AND ENZYME ACTIVITY

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ABSTRACT

In modern agriculture, chemical pesticides are frequently used in agricultural fields to increase crop production. Besides combating insect pests, these insecticides also affect the activity and population of beneficial soil microbial communities. The laboratory studies were conducted to resolute the effect of Imidacloprid (Insecticides), Paraeforce (Herbicide), and ultramax plus (Fungicide) on microbial communities and enzyme activities using their recommended rates. The microbial populations were estimated using the standard Pour-plate technique. Urease and cellulase activities were determined using the respective protocols. The resolute showed that the insecticides (Imidacloprid) at 0.13g/g soil depressed bacterial population by 2 log orders of magnitude (from 20000000 to 200000 cells), paraeforce (herbicide) and Ultramax plus inhibited bacterial population by 2 log orders of magnitude respectively. Imidacloprid depressed fungal population from 2300000 to 40000 cells, paraeforce decreased fungal populations by 2 log orders of magnitude, whereas Ultramax plus inhibited fungal population from 2300000 cells to 10000 cells. Imidacloprid depressed the population of actinomycetes from 2500000 cells to 125000 cells. Whereas paraeforce completely wiped off actinomycetes in the soil. Ultramax plus inhibited actinomycetes by 2 log orders of magnitude. On the activity of enzymes in the soil, the three pesticides did not show any depressive effect on cellulase activity but rather stimulatory. The effect of the three pesticides on Urease activity was either stimulatory or to Urease activity. Thus, there is need to assess the effect of indiscriminate use of pesticides on soils microbes and enzyme activities to avoid destroying the beneficial ones.

Keyword: *Pesticides, Enzyme, Microbial community, Imidacloprid, Ultramax plus, paraforce.*

INTRODUCTION

Pesticides are extensively used in agriculture as a part of pest control strategies. Owing to their xenobiotic characteristics, Pesticides may adversely affect the proliferation of beneficial soil microorganisms and their associated transformation in the soil. The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions in addition to nature and concentration of the pesticides (Aurelia, 2009, Setfii and Gupta 2013). Microorganisms are capable of carrying out degradation and detoxification, metabolism, whereas they make use of the pesticide as carbon and energy sources. Shimde*et al*., (2015) reported that most pesticides work by poisoning pests, while the systemic pesticides move inside a plant following absorption by the plant, with insecticides and most fungicides, this movement is usually upward (through xylem tissue) and outward. Stoytcheva (2011) observed that the systemic insecticides which poison pollen and nectar in the flower may kill bees and other needed pollinators. Subclasses of pesticides include, herbicides, insecticides, fungicides, rodenticides, nematicides and biocides. Pesticides would pollute air, soil and water resources, contaminate the food chain and disrupt ecosystem balance for example high contamination of pesticides in soil may influence processes such as plant growth and the activity and diversityof biotic populations Continuous useof pesticides may accumulate in appreciable quantities as well as their degradation products in soil ecosystem. Hainda*et al*., (1999) in a study on pesticides residues, Significance and management observed that pesticides that affect the activities of soil microbes may ultimately affect soil nutrient quantity resulting in severe ecological consequences. Soil fauna (e,g earthworms, nematodes, micro arthropods and protozoa) are important in organic matter decomposition and soil structure formation and are useful bio-indicators to study xerobioticecotoxicity in soil but they are affected by the application of herbicides. It was reported that application of prosulfuron, an herbicide inhibited N_2O, and NO production by the bacteria (Kinney *et al*, 2005). Gupta (1994) also reported reduction of soil protozoa due to recommended rates of 2, 4 – D, Simazine, diuron, and Cotoron. The application of pesticides on soils not only affect soil microorganisms but affect soil enzymes also. Soil contains free enzymes, immobilized extracellular enzymes and enzymes within microbial cells (Mayanglambam*et al*., 2005). They are indicators of biological equilibrium (Frankenberger and Tabatabai 1991), fertility (Schuster and Schroder, 1990); Antonious, 2003) Quality (Dick, 1994; Bricket and Dick 1998), and changes in biological status of soil due to

pollution (Nannipieri*et al*. 1990). The roles of soil enzyme and their activities are defined by their relationships with soil and other environmental factors (e.g acid rain, heavy metals, pesticides reaching the soil may disturb local metabolism or enzymatic activities (Toppe*t al*., 1997). Negative impact of pesticides on soil enzymes like hydrolases, oxidoreductases, and hydrogenase activities has been widely reported in the literature (Perucci and Scarponi, 1998; Menon *et al*., 2005). Nitrogenase is the enzyme used by organisms to fix atmospheric nitrogen gas (N_2). Application of pesticides affects the efficiency and activity of nitrogenase enzyme. Singh and Wright (1999) observed a decrease in total nitrogenase activity (measured from pots sown with Pistumsativum plants) with the application of herbicides. Adverse effects of pesticides have also been reported on nitrogenase activities of N-fixing bacteria, methylotrophic bacteria and cyanobacteria (Chalame*t al*. 1997; Dunska, 2004). In contrary, reported applications of Pesticides significantly stimulated rhizosphere – associated nitrogenase activity (Kanungo*et al*., 1995). The present study was aimed to determine the effects of pesticides on soil microbial community and enzyme (Urease and Cellulase) activities in soils.

MATERIALS AND METHODS

Soil Sample Collection

Soil samples from top 15cm depth from the university of Uyo arboretum were collected using soil auger. Soil samples were collected from three different sampling points. The soil was sandy clay loam and had no history of pesticides treatment. A total of three samples were collected nto sterile polythene bags and brought to the laboratory and kept in the refrigerator at 4ºC to maintain the biological activity of the soil microbes and enzyme activities.

Application of Pesticides

The pesticides under study include: Imidacloprid2.5g/100g soil (insecticide), Paraforce 2.5 ml/100g soil (herbicide), Ultramax plus 5.3g/100g soil (fungicide). The calculated amount of each pesticide was measured into a plastic cup containing 100g of soil sample mixed thoroughly and moistened with sterile distilled water. The plastic cups thereafter were covered with sterile aluminum foil to prevent contamination and later incubated at 30ºC in an incubator for three days. The expected toxicity of the pesticides to the soil organisms is three days.

Microbial Isolation Techniques

Serial dilution:

Ten – fold serial dilution of the soil samples was made as described by Collins and Lyne (1876)

Inoculation and incubation

One milliliter of appropriate ten-fold serial dilutions of the soil sample were inoculated onto Nutriebt agar (Oxoid CM 319) Sabourad Dextrose Agar plates in triplicates using pour plate technique and were also used for the isolation of Actinomycetes using the Dextrose soil extract Agar. Inoculated plates were incubated at 28±2ºC for 18-24 hours and 48-72 hours for the enumeration of total heterotrophic bacteria, fungi and Actinomycetes respectively. Visible discrete colonies in inoculated plates were counted and expressed as colony forming units per gram (cfu/g) of soil sample.

Maintenance of pure culture

Discrete colonies were purified by repeated sub-culture unto appropriate agar media. Pure cultures were preserved on Nutrient agar slant and stored in the refrigerator (4ºC) and ambient temperature (28ºC ±2ºC) for further test.

Determination of Enzyme Activity

Assay of soil Urease

Urease activity was determined as described by Pancholy and Rice (1973). Briefly, one milliliter (1ml) toluene was thoroughly mixed with 10g field moist soil sample in a 100ml Erlenmeyer flask. After 15mins. 20ml of phosphate buffer (pH 6.7), and 20ml of 10% urea solution were added to the flask. The reactants were incubated at 37ºC for 24 hours followed by shaking for 15mins with 30mlKcl solution. The contents wasfiltered with WhatmanN0. 41 filter paper and the filtrate made up to 100ml with deionized water. Aliquot (5ml) was analysed for the NH_4-N per g soil.

Assay of Cellulase in Soil

Cellulase activity was determined as described by Pancholy and Rice (1973). Briefly, one milliliter (1ml) toluene was mixed with 10g of moist soil sample in a 100ml Erlenmeyer flask. After 15 mins, 20ml5M acetate buffer (pH 5.9) and 20ml freshly prepared 2% carboxymethylcellulase (CMC) were poured into the flask. The soil mixture was incubated at 37ºCfor 24 hours followed by centrifugation at 4000 rmp for 20 mins.

The mixture was then filtered through a WhatmanNo. 41 filter paper and aliquot was analysed for reducing sugar content using colorimeter and optical density measured at 578 nm. Cellulase activity was expressed as mg reducing sugars produced for g of soil.

Statistical Analysis:
Descriptive statistics such as mean, standard deviation and ANOVA were used.

RESULTS AND DISCUSSION

The insecticide (imidacloprid) at 0.13 g/100g soil inhibited bacterial population by 2 log orders of magnitude from 20000000 to 200000/g soil, whereas paraforce (herbicide), and Ultramaxplus (fungicide) suppressed bacterial density by 2 log orders of magnitude respectively, but were significantly ($P<0.05$) different from each other, that is, from 200000000 to 1050000/g soil in paraforce treated soil, and Ultamax depressed bacteria from 20000000 to 1800000 cells/ g soil. Imidacloprid at 0.13g/100 g soil significantly at ($P<0.05$) inhited fungi by 2 log order of magnitude, that is from 2300000 cells in the control to 40000 cells in the soil treated with imidacloprid (Table 1). Paraforce decreased fungi by 2log orders of magnitude in relative to the control. In the same vein, Ultramax plus depressed fungal population by 2 log orders of magnitude in relative to the control (Table 1) of the three pesticides, ultramax was the most inhibitory to fungi as shown by the reduction in population density from 2300000 cells to 10000 cells (Table 1). This observation was however expected as ultramax plusis fungi's target pesticide. Although this study was not directed at any specific genera of bacteria, fungi or actinomycetes but at the estimated total population of each of this group of soil microorganisms. Imidacloprid depressed actinomycetes by 1 logorder of magnitude from 2500000 cells to 125000 cells, while paraeforce showed the most inhibitory effect by completely wiping out the population of actinomycetes (Table 1). Paraeforce appeared to be a very potent biocidal compound as it completely eliminated all the actinomycetes propagules in the population of bacteria and fungi by 5.25% and 0.75% respectively. The reason for this observation might be due to high permeability of the herbicide (paraforce) on actinomycetes cells.

Ultramax plus depressed bacterial propagules by 9% and fungi by 0.43% respectively (Table 1). Ultramax plus inhibited actinomycetes by 2 log

orders of magnitude and by 2.5%. This observation corroborated with the report by Aggarval*et al*. (2005) that application of herbicide inhibited $N_2O$and NO production by the bacteria. Gupta (1994) reported reduction of soil protozoa due to application of herbicide.

Table 1: Effect of selected pesticides on soil microorganisms

Pesticides	Pesticides application rate (µg/g)	Bacteria (cfu/g soil)	Fungi (cfu/g soil)	Actinomycetes (cfu/ g soil)
Untreated soil	0	20000000	23000000	250000000
Imidacloprid	50	205000	40000	12500000
Paraforce	25	105000	150000	0
Ultramax plus	800	180000	10000	650000

Note: Data are mean of three replicates

Soil contains free enzymes, immobilized, exracellular enzymes, and enzymes within microbial cells (Mayamglambam*et al*. 2005). They are indicator of biological equilibrium, fertility, quality, and changes in the biological status of soil due to pollution (Trans – Cepeda*et al*. 2000). The role of soil enzymes and their activities are defined by their relationships with soil and other environmental factors (e.g. acid rain, heavy metals, pesticides and other industrial chemicals that affect their activities (Burns 1982). In this study, it was observed that soil treated withimidacloprid had an improved activity of cellulase enzyme significantly at ($P<0.05$) compared to the control. This result was in agreement with previous work by Mohiddin*et al*. (2010) reported that the activity of cellulase in terms of glucose released from cellulose was more pronounced at 0.5 kg/ha soil, under the influence of the insecticides imidacloprid and acephate. Similarly, application of soil with paraforce (herbicide) at 250 g/ha significantly increased cellulase activity more than the control. The fungicide (Ultamax plus) applied to soil also stimulated the activity of cellulase at 800 g/ha significant ($P<0.05$) different from that of the control. The fungicide (ultramax plus) applied to soil stimulated the urease activity at the concentration of 5.3 g/g which was significantly ($P<0.05$) higher than the control. The Insecticide (imidacloprid) and the herbicide (Paraeforce) applied to soil at the concentrations of 0.13g/100g soil and 2.5 ml/100g soil respectively was neither inhibitory or stimulatory to Urease activity. Ultramax plus and paraeforce depressed fungal cells density by 2 log orders of magnitude respectively. Imidacloprid and ultramax plus inhibited actinomycetes cells density 1 log order of magnitude, while the herbicide paraeforce completely wiped out all the actinomycetes

population. This report showed that paraeforce is the most potent pesticide as it completely eliminated the actinomycetes population.

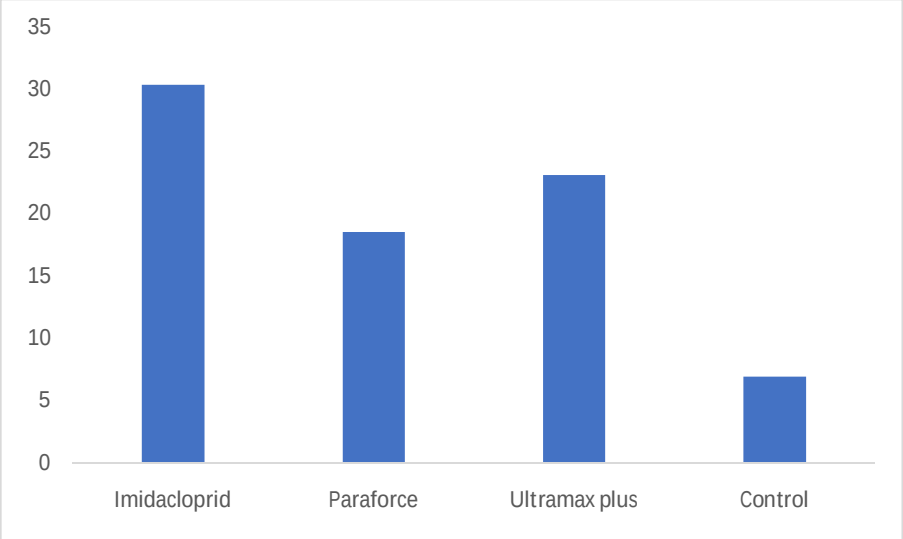

Fig 1: Effect of pesticides on cellulase activity

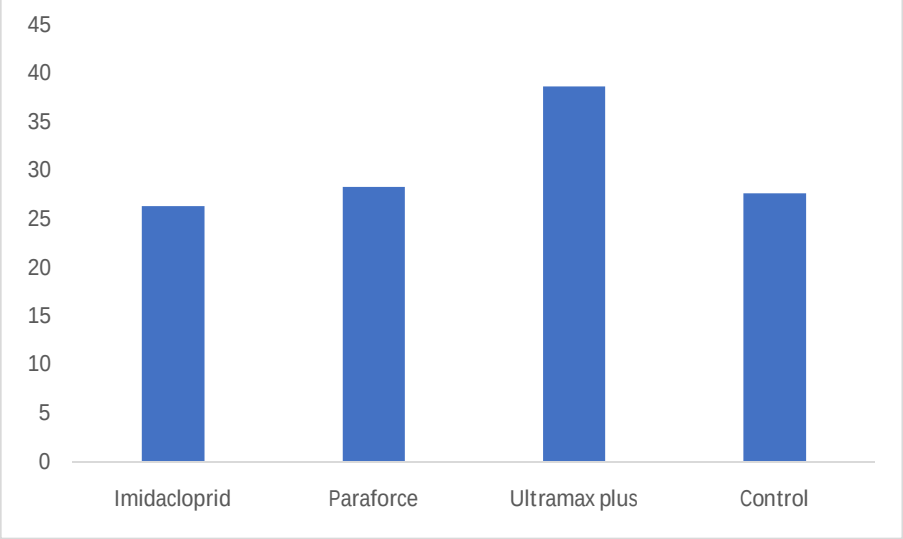

Fig 2: Effect off pesticides on Urease activity.

The non-toxicity or inhibitory effect of pesticides on soil enzymes may however be due to factors such as adsorption of pesticides on soil colloids and solubility of pesticides which are known to have a mitigating effect on toxicity. This observation confirms the reports Mohiddin*et al*. (2010)

reported that activity of cellulase in terms of glucose released from cellulose was more pronounced.

CONCLUSION: The effects of selected pesticides on microbial community and enzyme activities was demonstrated. It was observed that Imidacloprid (insecticide) decreased bacterial cells density by 2 log orders of magnitude, paraeforce (herbicide) and Ultramax plus inhibited bacterial cell density by 2 log orders at magnitude respectively.

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