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Research Paper

Toxicological Effect of some commonly used Pesticides (Herbicides, Insecticides and Fungicides) on Soil Fungi

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Pesticides are considered to be effective crop protection chemicals in modern agriculture. However, they can also disrupt microbial processes that are essential for continued soil fertility. The present study was carried out to evaluate the toxicological effect of three pesticides namely; an insecticide known as Lara force (lambd-cyhalothrin 2.5% EC)), an herbicide known as Amin seal (720g/l Di-methylamine salt as an aqueous solution) and a fungicide know as Ridomil Gold (66wp metanaxin). Soil samples were collected from the depth of about 5-20 cm into a sterile plastic container for laboratory analysis. Potato dextrose agar (PDP) was used for enumeration and isolation of fungi. Incubation was at 25°C for about 3 to 7 days. The soil pesticides were separately applied to soil samples in the following doses: Control (soil without pesticide), Normal dose recommended by the manufactures, double of the dose and half of the recommended dose. Serial single dilutions of the soil were prepared using one gram of the soil sample. After which the cultural characteristics and the microscopic features were used to identify the various fungal isolates. The result showed that, fungal isolates such as *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus nigricans*, *Aspergillus tamari*, *Cladosporium* sp., *Penicillium citrinum* were isolated from the soil samples. The different doses of the pesticides inhibited the growth of the fungal species at various degrees. Hence, the greatest changes were induced by the double dose with percentage frequency occurrence of fungal isolates ranging from 0 to 4% for soil treated with insecticide, 0 to 20 % for soil treated herbicide and 0 % was recorded for soil treated with fungicide. The results of the study also indicate that there were differences in the values of the population of the fungal species in the treated and untreated soil samples when they were subjected to different exposure periods with the control (Untreated soil) having a significantly larger population of fungal isolates ($p<0.05$) than those isolated from the untreated soil samples. The findings of the study show that the fungicide exhibited higher adverse ($p<0.05$) effect on the fungal species than the insecticides and herbicides at the same duration of exposure. While the trend of inhibition on growth of fungi was observed from the initial effects of the different pesticides until 10 DAT, rise in fugal population was observed at 15 DAT, The present study showed that the pesticides treatments significantly affected the percentage occurrence of the fungal species and their populations in the soil, and the degree of inhibition was closely related to doses and exposure periods of pesticides applications and also varied with the type of pesticides. Thus, the presence of pesticides residues in soil which could have direct impacts on soil fungi is a matter of great concern.

Keywords: Toxicological effect, pesticides, herbicides, insecticides, fungicides, soil, fungi

INTRODUCTION

Pesticides are chemical groups more widely used by man, both to kill and protect harmful organisms and to improved the growth of plants and for the control of vectors and pests of public health. Matthew, (2006) defined pesticides as one of the few toxic substances released deliberately into the environment to kill living organisms [e.g., weeds (herbicides), insects (insecticides), fungus (fungicides), and rodents (rodenticides), nematode (nematicides)]. Thus, the term pesticide refers insecticides, herbicides, fungicides and various other substances used to control pests (Mishra *et al*., 2001). Therefore, the purpose of pesticides is to destroy certain living organisms, as well as constituting a particular group of biocides that can reach a wide lethality. The need to produce a greater quantity and quality of food by pest control resulted in intensive use of pesticides over the last 50 years (Garcia, 1997; Chaverri *et al.,* 2000). Pesticides are chemicals used by man to control agricultural pests and their correct application is the most accepted and effective for maximum production and quality of crops (Bolognesi, 2003). The increasing use and improper handling of these substances have raised concerns about the risks and damage that could result in the economy, the environment and public health (Audus, 2008). Pesticides poisoning represent problems for handlers, implement or work with these products.

Pesticides are widely used against a range of pests infesting agricultural crops. Agriculture is considered as the largest consumer (around 85% of the world production) of the pesticides to chemically control various pests. The use of pesticides in agriculture has highly increased during the last 40 years to increase crop yields (Wassila *et al.,* 2014). Globally, about 3-109 kg of pesticides is applied annually with a purchase price of nearly $40 billion each year (Pan *et al.,* 2003). The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the soil environment. With the growing use of pesticides in contemporary agriculture, the issue of the impact of these chemicals on the composition of soil microorganisms and the processes they direct has received more attention (Andrea *et al.,* 2006). The applied pesticides may harm the indigenous microorganisms, disturb soil ecosystem, and thus, may affect human health by entering in the food chain. Adverse impacts of pesticides on soil microbial diversity and activities have been described by many researchers (Baxter *et al.,* 2006; Tarek *et al*., 2015). Similarly, pesticides also influences soil biochemical processes driven by microbial and enzymatic reactions. The microbial mineralization of organic compounds and associated bio-transformations such as nutrient dynamics and their bioavailability are also more or less adversely affected by the pesticides (Hussain *et al.,* 2009). There are also reports documenting the ability of soil microorganisms to degrade pesticides in the soil environment (Page and Thomson, 2013).

During the past four decades, a large number of herbicides have been introduced as pre and post-emergent weed killers in many countries of the world. In Nigeria, herbicides have since effectively been used to control weeds in agricultural systems (Adenikinju and Folarin, 1976). As farmers continue to realize the usefulness of herbicides, larger quantities are applied to the soil. But the fate of these compounds in the soils is becoming increasingly important since they could be leached, in which case ground water is contaminated or immobile and persist on the top soil (Ayansina *et al.,* 2003). These herbicides could then accumulate to toxic levels in the soil and becomes harmful to microorganisms, plants, wild life and man (Amakiri, 1982). There is an increasing concern that herbicides not only effect the target organism (weeds) but also the microbial communities present in soils, and those non-target effects man reduce the performance of important soil functions. These critical soil functions include organic matter degradation, the nitrogen cycle and methane oxidation (Hutsch, 2001).

Fungicides are specific types of pesticides or biocidal chemical compounds that kill parasitic fungi or their spores. Diseases caused by fungi can be adequately controlled by fungicides (Oliver and Thewith, 2014).They are extremely used in pharmaceutical industry, agriculture, in protection of seed during storage and prevention of the growth of fungi that produce toxins. Hence fungicide production is constantly increasing as a result of their great importance to agriculture. However, some fungicide affects humans and beneficial microorganisms, insects, birds and fish (Nooruddin, 2011; Hemanth *et al*., 2016)).

Insecticides are used in crops to protect plants against different harmful insects and increases crop yield (Marioara *et al*., 2015). Although insecticides are usually applied in low concentration once in the soil they can alter the chemical and biological properties of that soil and also affect soil microorganisms. A serious concern of the agricultural community is the increase of insecticides residues in the environment (Braide *et al*., 2017). The effects of insecticides on soil microorganisms consist in the decrease of the number of microorganisms, alterations in biochemical activity, quantitative and qualitative decrease of the microbial community (Digrak and Ozcelik, 2004; Pandy and Singh, 2004; Cycon *et al*., 2006).

According to Monkiedje *et al*. (2002) the effect of insecticides on microbial communities in soil is difficult to determine because many variables such as habit, soil structure, organic and inorganic composition, texture, pH and temperature. However the assessment of the effect of insecticides on the functionality of an ecosystem can be achieved based on metabolic activity and microbial biomass determinations (Nannipieri *et al*., 2003). An insecticide is a pesticide used against insects in all developmental forms. They include ovicides, larvicides and adulticides used against the eggs, larvae and adult respectively (McEwen and Stephenson, 1996). Insecticides have been used indecrimately resulting into severe environmental contamination and pollution.

The residual effects include carcinogenicity, mutagenicity, reproductive toxicity, respiratory and circulatory problems (Braide *et al*., 2017). The soil microorganisms like bacteria, fungi, algae and nematodes are known to play important role in soil nutrition through their role in decay of plant and other organic matter in soil. Hence anything that disrupts their activity could be expected to the nutritional quality of the soil and can cause serious consequences.

Also, microorganisms that live in soil can be killed not only by chemicals applied to the soil, but also by those that reach the soil in drift from aerial sprays of washed off foliage (Mishra *et al*., 2001).

Fungi are members of the group of eukaryotic organisms that includes microorganisms such as yeasts and moulds as well as the more familiar mushroom. Fungi are important part of microbial ecology. The majority of which are known to decompose the lignin and the hard-to-digest soil organic matter, but some fungi consume simple sugars.

Fungi generally dominate in low pH or slightly acid soils where soil tends to be undisturbed (Lavelle and Spain, 2005). Fungal biomass varies widely within and across biomes in relation to litter composition, root density, and nutrient availability.

The use of pesticides to protect crops may alter the soil biological ability either by direct or indirect action. To understand the effect of pesticides on soil mycoflora and their beneficial activities is an important part of the pesticide's risk assessment.

It is not easy to predict the relationship between the chemical structure of pesticide and its effect on the various groups of soil microorganisms. Ever since the discovery of insecticides, the exploitation and exploration has brought have brought about economic prosperity and helped in nation building (Braide *et al*., 2017).

Certain pesticides are known to stimulate the growth of microorganisms, while others have depressive effects or no effects on microorganisms when applied at different concentrations or rates. The soil being an important recipient and reservoir for the accumulation of pesticides, an obvious point of concern is what happens to the pesticides in the soil, do they have adverse effects on soil community or not and whether their effects are permanent or temporary?

The vast majority of investigations regarding the effects of pesticides on soil microorganisms were conducted in soils under temperate conditions. However, it is surprising that tropical countries that make substantial use of pesticides for control of agricultural and other pest do not give attention of the effect of pesticides on soil microbes.

The present study represents a first investigation of toxicological effect of commonly used pesticides like an insecticide [Lara force (lambd-cyhalothrin 2.5% EC)], an herbicide (Amin seal (720 g/l Di-methylamine salt as an aqueous solution) and a fungicide [Ridomil Gold (66 wp metanaxin)] at different concentrations on soil fungi under laboratory condition in Nigeria.

MATERIALS AND METHODS

Collection of samples

The soil samples with no prior pesticides treatment were collected from three different locations at Bauchi road Campus, University of Jos, Plateau State, Nigeria in the warmth and damp month of October 2017. Soil samples were collected from top soil of about 5-20 cm and placed into sterile plastic bags for laboratory analysis. Three pesticides used for experiment were purchased from a local agricultural dealership store in Jos metropolis. The pesticides included an insecticide known as [Lara force (lambd-cyhalothrin 2.5% EC)], an herbicide known as Amin seal (720g/l Di-methylamine salt as an aqueous solution) and a fungicide know as [Ridomil Gold (66wp metanaxin)]. They were used in different concentration to contaminate the soil sample as prescribed by Adomako and Akyeampong, 2016).

Preparation of potato dextrose agar

The PDA was prepared by weighing 200 g of freshly peeled and washed potato in the laboratory. It was then boiled, mashed and the pulp squeezed through a fine sieve. Twenty gram of agar was added and boiled to dissolve and again 20 g dextrose was added and boiled to dissolve and make up to one litre with water. The content was then sterilized at (15) psi for 20 min in an autoclave. It was then removed and allowed to cool to an ambient temperature before use.

Soil treatment

The soil samples were sieved through a 2.0 mm width mesh to remove stones and plant debris. The soil treated with different doses of the pesticides as follows, normal the recommendation rate (RFR), Doubled recommendation rate (RFR) and Halved recommended rate (RFR) over the interval of five (5) days for fifteen (15) days exposure period in addition to the control sample as described by Zain *et al*. (2013).

Preparation of various concentrations of pesticides with soil samples

Normal concentration

For each of the soil samples, 4mls of the insecticide and herbicide were measured, but for the fungicide, 4g was measured. The pesticides was added to 20g of sand sample (dilution) for each sample and allowed to stand for one hour to allow proper penetration of the pesticide after stirring with a glass rod.

Halved concentration

For the half concentration, 2 mls of each of the herbicide and insecticides and 2 g of the fungicide respectively were diluted with each of the samples then it was mixed properly with a glass rod for even distribution of the pesticides.

Double concentration

Here, 8 mls of the insecticide and herbicides were measured; while 8 g of the fungicide was also diluted respectively with each of the samples, then it was mixed properly with a glass rod for even distribution of the pesticide.

Treatment of soil samples with pesticides and isolation of fungal isolates.

Here, one gram each of sand already mixed with each pesticide and the control (soil without treatment) was separately mixed with 99mls of sterile distilled water to give 10-1 dilution. Further dilutions (10 folds) were made after thorough shaking of the soil. The mixture was diluted until 10-8 dilutions were obtained. An aliquots portion (1 ml) of 10^{-3} and 10^{-4} were inoculated into the petridishes to which freshly prepared PDA which has cold to ambient temperature was also added and allowed to solidify. The plates were incubated at 25°C for 3-7 days and observed for growth of fungi (Harrigan and McCance, 1990; Cheesebrough, 2000). This was done for both the normal, half and double concentrations in triplicates. Colonies were formed on the plates after incubation and the total heterotrophic fungal counts were made by dividing the petridish into four quadrants at the reverse surface. Total colony forming units per grams was expressed as (cfu/g).

Identification of soil fungi

Identification of the soil fungi was based on colonial characterization examination. Wet mount was also done for the microscopic examination of fungi. References were made to text written by Domsch *et al.* (1980).

Statistical analysis

The results obtained were subjected to statistical analysis using 2-way analysis of variance (ANOVA) and means were compared using Duncan's multiple range test (DMRT) at $p<0.05$ using statistical analysis system (SAS).

RESULTS

Table 1 reveals the colonial and microscopic characterization of the fungal species isolated from the soil samples. The results show that fungal species exhibited different cultural characteristics such as: the colours, conidial and mycelia types. The results in (Table 1) also indicate that six fungal species were isolated from the soil samples, which include: *Aspergillus niger, Aspergillus oryzae, Rhizopus nigricans, Aspergillus tamari, Cladosporium spp, Penicillium citrinum.*

Percentage frequency of occurrence of fungal species isolated from treated and untreated soil

The results in (Figures 1-3) indicate the percentage frequency of occurrence of fungal species isolated from soil samples treated with the three (3) pesticides and the untreated soil (control).The Soil samples treatment with various pesticides resulted in significant reduction in the percentage frequency of occurrence of the soil fungi when compared to the control. The different doses of the pesticides inhibited the growth of the fungal species at various degrees. Hence, the greatest inhibitions were induced by the double dose with percentage frequency occurrence of fungal isolates ranging from 0 to 4% for soil treated with insecticide, 0 to 20 % for soil treated herbicide and 0% was recorded for soil treated with fungicide. Among fungal species isolated from the soil samples treated with insecticides, *A. oryzae* had the highest percentage occurrence (33%) followed by *A. tamari* (29 %), while *P. citrinum* had the least percentage occurrence of (0%). Overall, the results from (Figures 1-3) show that the highest percentage occurrences of the fungi were obtained from the treated soil with halved recommended dose, having percentage occurrence ranging from 20-54% in herbicide treatment, from 11-47% for in fungicide treatment and from 0-33% in insecticide treatment.

Effects of the pesticides treatment on mean viable fungal counts with respect to the exposure period

The results of the effects of the pesticides treatment at normal rate on mean viable fungal counts with respect to the exposure period, the day after treatment (DAT) are shown in (Table 2). Result obtained from the fungal enumeration of pesticides treated at various exposure periods showed a significant reduction (<0.05) in fungal counts compared to the control (Untreated soil sample). However, there were rise in the population of fungal species with counts ranging from 1.20×10^5 to 1.77×10^5 cfu/g for soil treated with insecticide (1.63×10^5 to 2.25×10^5 cfu/g), for soil treated with herbicide (0.32×10^5 to 0.70×10^5cfu/g) for soil treated with fungicide. Fungicide treatment at recommended rates resulted in lower counts of the fungal species compared to those of insecticide and herbicides. The mean fungal counts obtained from the untreated soil ranged from (4.80×10^5 to 5.00×10^5cfu/g).

Table 1. Colonial and microscopic characterization of fungal species isolated from the soil.

Cultural characteristics	Conidial arrangement under the microscope	Identity of isolate
Dark brown to black conidiophore	Conidial heads radiates tending to lose columns conidiophores stripes.	*Aspergillus niger*
White and fluffy strands	Filamentous fungus with septate hyphae characterize around vesicle with extending conodial chains.	*Aspergillus oryzae*
White mycelium but dark at maturity	Rhizoids, hyphae without septa, with unbranched sporangiophore.	*Rhizopus nigricans*
Dark green	Conidiophore stalt, hyaline mostly conspicuous rough wall.	*Aspergillus tamari*
Black colonies with dark pigmented conidia	Conidia are formed in simple branching chains.	*Cladosporium species*
Colony has a central greyish turguioise to greyish-orange	Products septate,hyaline (clear, not pigmental) hyphae, smooth wall conidiophores stripes are rather longs.	*Pencillium citrinum*

Table 2. Effect of pesticides treatment on soil fungal population at three exposure periods in the soil at the normal recommended rat.

Treatment	Fungal population (x10 cfu/g ^{5}) at different exposure periods in soil		
	5 DAT	**10 DAT**	**15 DAT**
Insecticide	1.28^{a}	1.20^{a}	1.77^{a}
Herbicide	1.97^{b}	1.63^{b}	2.25^{b}
Fungicide	0.57^{c}	0.32^{c}	0.70^{c}
Control (Untreated soil)	5.00^{d}	5.50^{d}	4.80^{d}
Mean	2.20	2.16	2.38
SE	0.080		
LSD	0.26		

Means with same letter in the columns and rows are not significantly different at 5% level of significant using least significant.SE= Standard error, LSD= least significant difference.

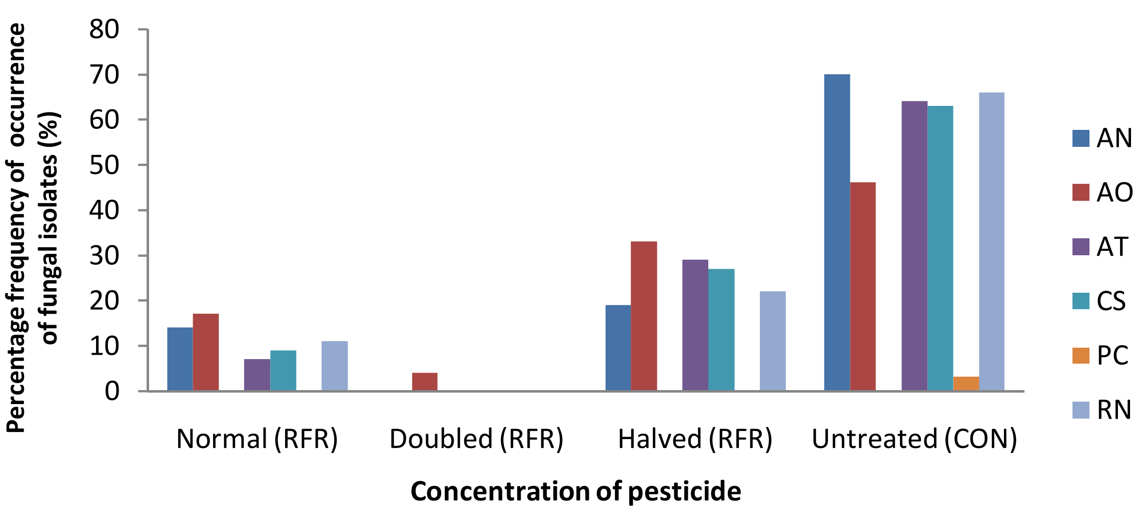

Figure 1. Percentage frequency of occurrence of fungal species isolated from soil samples treated with insecticide (Lara force) and the control (Untreated).

AN = *Aspergillus niger*, AO = *Aspergillus oryzae*, AT = *Aspergillus tamari*, RN = *Rhizopus nigricans*, CS = *Cladosporium* spp., PC= *Penicillium citrinum*, RFR =Recommended field rate, CON = Control.

Analysis of variance results shows that, only the exposure periods of the pesticides treatments had significant effect on the fungal counts ($p<0.05$), mean while interaction between exposure periods of the pesticides treatments and the fungal types had little effect on the fungal populations ($p>0.05$).

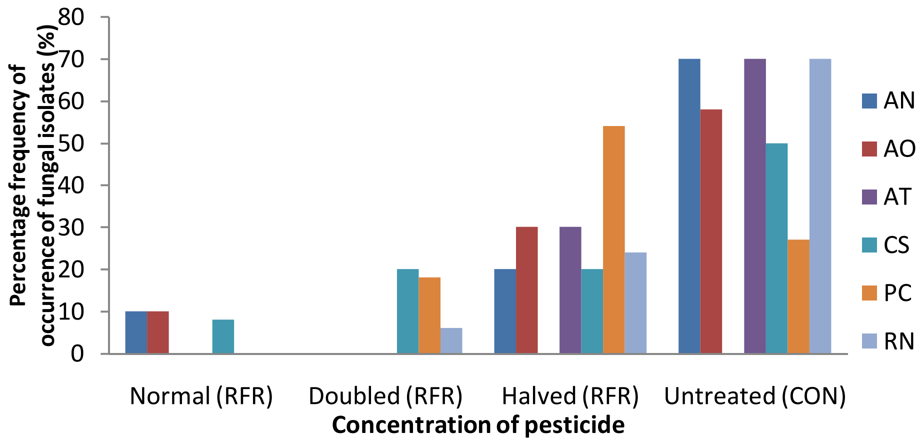

Figure 2. Percentage frequency of occurrence of fungal species isolated from soil samples treated with herbicide (Amin seal) and the control (Untreated).

AN = *Aspergillus niger*, AO = *Aspergillus oryzae*, AT = *Aspergillus tamari*, RN = *Rhizopus nigricans*, CS = *Cladosporium* spp., PC= *Penicillium citrinum*, RFR =Recommended field rate, CON = Control.

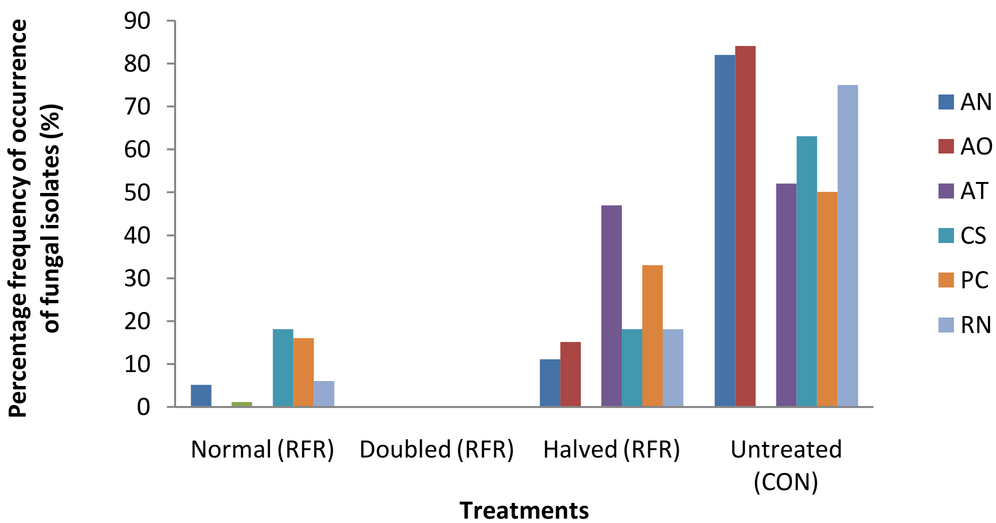

Figure 3. Percentage frequency of occurrence of fungal species isolated from soil samples treated with fungicide (Ridomil gold) and the control (Untreated).

AN = *Aspergillus niger*, AO= *Aspergillus oryzae*, AT= *Aspergillus tamari*, RN = *Rhizopus nigricans*, CS= *Cladosporium* spp., PC= *Penicillium citrinum*, RFR =Recommended field rate, CON = Control.

DISCUSSION

Pesticides are extensively used in agriculture as a part of pest control strategies in Nigeria. Nigeria is a densely populated country. About 80% of the people obtain their livelihood through agriculture or agricultural-based industries. Therefore to meet the demand of this ever-growing population, the agricultural production needs to be increased, to which the use of agrochemicals with an objective to effectively eradicate crop destroyers, becomes imperative (Braide *et al*., 2017). Owing to their xenobiotics characteristics, pesticides may adversely affect the proliferation of beneficial soil microorganisms particularly soil fungi population. The population of fungi isolated from the study was not much because the samples were collected on warm damp soil where fungi do not grow very well (Braide *et al*., 2017). The effect of pesticide treatment on soil fungi was determined based on the percentage frequency of occurrence of the fungal species before and after treatment at normal recommended field rate, double recommended field rate and halved recommended field rate and the growth inhibition of population of the organisms in the soil at normal recommended (which exhibited the highest inhibitory effect on the fungal isolates) at the three exposure periods of 5 DAT, 10 DAT and 15 DAT. The result obtained from the present study showed that the percentage frequency of occurrence of the fungal species in the untreated soil was higher than those of the treated soil. Taiwo and Oso, (1997) suggested that this decline in percentage could be due to the fact that the microorganisms that were treated with pesticides were susceptible to the products of the soil pesticides interaction, which could have possibly been fungicidal. In this study, the observed trends in the reduction of the frequency of occurrence of the fungal species when double doses of the pesticides were applied to the soil were similar to the observation made by Ayansina and Oso, (2006). These authors discovered that higher concentrations of herbicides resulted in much lower microbial counts when compared to soil treated with recommended doses. Braide *et al*. (2017) also reported that some of the insecticides employed in their study were toxic to microbial population at high concentrations. Hemanth *et al.* (2016) also reported that fungicide treated soil haboured less population of fungi in comparison to control. Other researchers also observed that at recommended field rate application of Glyphosate (an herbicide), the inhibition of fungal population were moderate. In this study, some of the fungal species were adversely affected by the pesticides than others in that their frequencies of occurrence were greatly reduced and in some other cases were completely inhibited. The present study agrees with the study by Tarek *et al*. (2015). The authors reported some fungi were tolerant to high concentration insecticide while others were found to be sensitive to it. According to Nada *et al*. (2002) most soil microorganisms are capable of decomposing pesticides such as herbicides, using them most frequently as source of biogenous elements. Fungal species isolated from this study, such as *Aspergillus* sp., *Rhizopus* sp. and *Penicillium* sp. have been found to be among the most intensive decomposers of pesticides. In general, pesticides affect soil microbes directly in which case they significantly affect microbial growth and multiplication as revealed in this study and other studies (McEwen and Stephenson, 1996; Digrak and Ozcelik, 2004; Pandy and Singh 2004; Zain *et al*. 2013). Experiments have shown that microbes may use pesticides as source of carbon (Radosevich *et al*., 1995). This may explain the increase in microbial populations obtained in the present study from 10DAT to 15 DAT of application of the pesticides. Some studies also reported increase populations of fungi after treatment with an herbicide known as glyphosate (Araujo *et al*., 2003) and increase soil microbial biomass (Hanley *et al*., 2002). The study also revealed that the highest inhibitions of the fungal population were observed among the soil treated with fungicide. However, the fungal populations were moderately inhibited when herbicide and insecticide were applied. This suggest that fungicide used in the study could inhibit a great number of cellulolytic mycoflora, while the lower inhibition of the fungal populations observed in the soil treated with insecticide and herbicide employed is an indication that they are less toxic to beneficial microbes in the soil. The study has shown that the presence of pesticides in soil residue could have direct impacts on soil microorganisms. At normal field recommended rates, pesticides are considered to have no major or long term effect on fungal population. It was reported that some of the fungal species isolated from the study have the ability to degrade pesticides thereby have the ability to grow and multiply in the presence of some of the pesticides used in this study. However, some of the fungal species were adversely affected depending on the application rates and types of pesticides used.

Conclusion

The findings of the study has shown that the effects of pesticides can either stimulate or depress the frequency of occurrence and population of fungal species depending on the chemicals (type and concentration) species and environmental condition. The present study has also revealed that the pesticides application to soil cause transient impacts on microbial population growth, when applies recommended field application rate. The results of the study, indicates that the presence of the pesticides used in the soil exerted considerable changes in the growth and development of soil microorganisms. The toxic effect of the pesticides was felt shortly after application on the fungal species. The pattern of change may vary as a result of differences in exposure period at concentration of the active ingredient in the formation and so many environmental factors. This is supported by the view that microbial flora response to pesticides manifests itself in a variety of ways depending on factors including the pesticides itself and inherent microorganism populations. In Nigeria, most of the farmers, especially those in the rural areas cannot read or understand pesticides label. This has resulted in the contamination of streams, rivers and ground water which is important natural resources. Also, this contamination also poses danger to non-target organisms as well as exposes human beings to many health implications. It is advised that farmers should be properly guided on the use of pesticides by the appropriate agencies to avoid killing the important organisms in the soil which break down organic compounds into simpler forms that are needed for plant growth.

REFERENCES

Adenikinju SA, Folarin JO (1976). Weed control in coffee in Nigeria with gramozone Proc. Six Annual Conf. Weed Sci. Soc. Nigeria. Pp. 1-8.

Adhikary P, Shil S, Patra, PS (2014). Effect of herbicides on soil microorganisms in transplanted chilli. Global Journal of Biology, Agriculture and health Sciences. 3(1): 236-238.

Adomako MO, Akeampong S (2016). Effect of some commonly used Herbicides on soil Microbial population. Journal of Environment and Earth Science, 6(1):30-38.

Amakiri MA (1982). Microbial degradation of soil applied herbicides. *Nigeria Journal of Microbiology* . 2:17-21.

Andrea S, Adl M, GuptaVS (2006). Protists in soil ecology and forest nutrient cycling. *Canadian Journal for Research,* 36: 1805–1817.

Araujo ASF, Monteiro RTR, Abakeli RB (2003). Effect of glyphosate on the microbial activity of two Brazilian soils. *Soil Biology and Chemistry*. 10:231-239.

Audus LJ (2008) . *Herbicides, Physiology, Biochemistry, Ecology,* Bedford College Edition. London p. 8.

Ayansina ADV, Ogunshe AAO, Fagade OE (2003). Environment impact assessment and microbiologist: An overview. Proc. Of 11th Annual National of Environment and Behaviour Association of Nigeria(EBAN), Pp. 26-27.

Ayansina ADV, Oso BA (2006). Effect of two commonly used herbicides on soil microflora at two different concentrations. *African. Journal of Biotechnol*, 5(2):129-132.

Baxter BJ, Horton T, Kretzer A, Bruns T (1999). Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytol*. 143, 409–418.

Bolognesi C (2003). Genotoxicity of pesticides: a review of human biomonitoring studies. *Mut Res*. 543:251-272.

Braide W, Agugwo GC, Adeleye SA, Ibegbulem CR, Azuike PA (2017). Effects of agrochemicals (insecticides) on microbial population in soil. EC Microbiology Research Article, 8(4):211-221.

Chaverri F, Soto L, Ramírez F, Bravo V (2000). Diagnóstico preliminary deluso de plaguicidas en los cultivos de arroz, banano, caña de azúcar, café, cebolla, melón, naranja, papa, piña, tomate, floresy plantasornamentales. IRET, Universidad Nacional, Heredia, Costa Rica. p. 47.

Cycon M, Piotrowska-Seget Z, Kaczynska A, Kozdo J (2006). Microbiological characteristics of a sandy loam soil exposed to tebuconazole and L-cyhalothrin under laboratory conditions. *Ecotoxicology*. 15:639-646.

Cheesebrough M (2000). *Media Sterilization. In: district laboratory manual for tropical countries* part 2. CambridgeUniversity Press. London. pp. 45-70.

Digrak M, Ozcelik S (2004). Effect of some pesticides in soil microorganisms. *Bulletin of Environmental Contamination and Toxicology*, 60(6): 916-922.

Domsch KH, Garm W, Anderson T (1980). *Compendium of soil fungi*. Acadamic Press Limited McGraw-Hill Publishers London, Pp.105-120.

Garcia J (1997). Introducción a los plaguicidas.Universidad Estatal a Distancia, San José, Costa Rica. pp. 450 .

Hanley RL, Senseman S, Hons FM (2006). Effect of the round –up ultra on microbial activity and biomass from selected soils. *Journal of Environmental Quality*. 31: 730-735.

Harrigan WF and McCance ME (1990). *Laboratory Methods in Food and Diary Microbiology*. Academic Press, London. p. 210.

Hemanth G, Ratna PK, Shiny PN, Samuel KK (2016). Fungicides effect on soil microflora in Tekkali Mandal, Srikakulam. International Journal of Research and development in pharmacy and life Sciences. 5(4):2245-2250.

Hussain S, Tariq S, Muhammad S, Muhammad A, Azeem Khalid (2009). Impact of pesticides on soil Microbial diversity, Enzymes and biochemical reactions. *Advances in Agronomy*, 102:159-200.

Hutsch BN (2001). Methan oxidation in non-flooded soil as affected by crop production. Invited paper of European Journal of Agronomy, 14:237-260.

Lavelle P, Spain AV (2005). *Soil Ecology*. Springer. New Delhi, Indian. p.357.

Marioara NF, Sorin, OV, Raxana P, Gabi D, Liliana PC, Mihai M, Daliborca CV (2015). The effect of some insecticides on some on soil microorganisms based on enzymatic and bacteriological analysis. *Romanian Biotechnology Letters*. 20(3): 10439-10446.

Matthew GA (2006). Pesticides: Health. Safety and Environment . Blackwell Publishing Oxford. UK. p. 20.

McEwen FL, Stephenson GR (1996). The use and significance of pesticides in the environment (5th edition). A Wiley,linter Science Publication, New York. Pp.155-157.

Mishra PC (2001). *Soil Population and Organisms*. Ashish Publicating House, Punjbi Bagh. New Delhi, Pp. 3-34.

Monkiedje A, Ilori MO, Spiteller M (2000). Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to sandy loamy soil. *Soil Biology and Biochemistry*, 34:263-276.

Nada AM, Mitar MG (2002). Effect of herbicides on microbiological properties of soil proceeding. *Novi Sad*, 102:5-21.

Nannipieri, P, Ascher J, Ceccherini MT, Landi L, Pierramellara G, Renella G (2003). Microbial diversity and soil function. *European Journal of Soil Science*, 54: 655-670.

Nooruddin T (2011). Fungicides beneficial and harmful aspects. Fungicides/ intechopen. https://wwwintechopen.com (retrieved 20th June 2018).

Oliver, RP, Thewith HG (2014). Fungicides in Crop Protection. 2nd Edition ABI Publishing, CAB International. p. 1.

Page BG, Thomson WT (2013). *The 2013 Newly Revised Insecticides, herbicide, Fungicide Quick Guide*. Kovak Books Bakers. Field, CA. p. 12

Pan CH, Chein YH, Hunter B (2003). The resistant to ammonia stress of *Penaeus monodon*, *Fabricius juvenile* fed diets supplemented with astaxanthin. *Journal of Experimental Marine Biology and Ecology*, 297 (1) 107-118.

Pandy S and Sindh DK, (2004). Total bacterial and fungal population after chloropyrifos and quinalphos treatment in groundnut soils. *Chemoshere*. 55(2): 283-290.

Radosevich M, Traina SJ, Hao YI, Touvinen OH (1995). Degradation and mineralization of atrazine by a soil bacterial isolate. *Environmental Microbial*, 61:279-302.

Taiwo LB, Oso BA (1997). The influence of some pesticided on soil microbial flora in related to changes in nutrient level, rock phosphate solution and P-release under laboratory conditions. *Journal of Agricultural Ecosystem and Environment* 65: 59-68.

Tarek MA, Ibrahim AM, Moustafa N (2015). Pesticide- fungi interaction and their impact on fungal population in agricultural soil. *International Journal of Microbiology and Allied Sciences*. 1(4):18-22.

Wassila R, Karine L, Laroche-Ajenberg E, Mougin, C, Latour X, Trinsoutrout-Gattin, I. (2014). Effect of pesticides on soil enzyme a review. *Environmental Chemistry Letters*. 12(2):257-273.

Zain, NMM, Rosil B, Kamaruzaman S, Morshed MM, Yahya A. (2013). Effected selected herbicides on soil microbial populations in oil palm plantation of Malasia. A microcosm experiment. *African Journal of Microbiology Research.* 7(5): 367-374.