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EFFECTS OF COMMON HERBICIDES ON SOIL FUNGI IN A MAIZE (*Zea mays*) FARM

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ABSTRACT

The increasing use of pesticides in Agriculture have raised concerns over the unintended consequences, especially multiple applications of various forms of pesticides on the same farm land during a particular production period. This field trial was conducted to evaluate the effect of common farmland application of herbicides i.e. atrazine, xtravest , 2,4-D, on the species and population of soil fungi. Soil samples were collected thrice at a three month interval (July, September and December 2010) from a herbicide trial site located in Iddo town, Iddo Local Government area of Oyo State, Nigeria.The herbicides were applied at recommended rates either singly or in combination over the 2009 / 2010 growing seasons on a maize field. Their effects were tested on the fungal species present and their abundance using the soil dilution method. Data collected were subjected to descriptive and inferential statistics.The results showed the presence of different fungal genera on all the treated plots including the control. The fungi populations observed include *Aspergillus niger, Lasidiodiplodia theobromae, Nattrassia mangiferae, Trichoderma* spp*, Fusarium verticillum, Fusarium oxysporum, Rhizopus stolonifer, Macrophomina phaseolina and Colletotrichum gleosporioides*. *Lasidiodiplodia theobromae* occured the most (15/16) times among the treatments, across the sampling periods while *Rhizopus stolonifer* occured the least (1/16) number of times among the treatments through the sampling periods.Although, there were reductions in the number of isolated fungi across all treatment periods, however, the reductions were not consistent. In conclusion, the application of herbicide(s) may not exclude the use of other forms of pesticides in the management of soil microorganisms on the farm.

Keywords: Herbicide, Soil fungi, Treatment, Maize

INTRODUCTION

Crop production is constrained by a number of factors which include pests, diseases and weeds. These factors can act either singly or in combination to limit productivity (FiBL, 2011). Herbicides are chemicals used to kill or inhibit weed growth and they may be specie-specific or non-specific. Herbicides can be classified into a number of ways. These include mode of action, method of application, differential

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response among plants, pathway of herbicide movement in plant and others (DiTomaso, 2002; Varshney and Sondhia, 2010;Pennsylvania State University Extension, 2015). Herbicides commonly used on maize farms include 2, 4-D, atrazine, glyphosate, paraquat, xtravest, and a host of others.

Herbicides Researchers and Scientists have raised concerns over the unintended consequences of herbicides on non-target organisms in the soil including soil microorganisms (Karthikeyan *et al*., 2003; Prado *et al*., 2009). Most soil organisms proliferate rapidly in the soil and their diversity is largely due to the limitless variety of food and wide range of habitat found in the soil environment (FAO, 2002). Soil microorganisms can either be of beneficial or harmful effects to plants (Domergues *et al*., 1980). Specialized fungi can be pathogenic on the plant tissues while others form mutually beneficial relationships (mycorrhizae) with plants and assist in direct nutrient supply to the plants (Domergues *et al*., 1980).

Fungal hyphae, push their way between soil particles, roots, and rocks and are usually only several thousandths of an inch (a few micrometers) in diameter. A single hypha can span in length from a few cells to many yards when a few fungi, such as yeast, are single celled. Their number in soil varies from a few thousand to a few million per gram (Hoorman, 2011). They cannot synthesize their own food and they are dependent on complex organic substances (Hoorman, 2011). Fungi are dominant in acid soils because acidic environments are not conducive and or suitable for the existence of bacteria and actinomycetes (Bhoopander *et al*., 2005). Fungi require an optimum pH range of 4.5 – 6.5. They are also present in neutral and alkaline soils and some can even tolerate a pH of up to 8.0 (Wood, 1989 ; Bhoopander *et al*., 2005). Soil fungi are part of the extremely diverse group of microorganisms. Their biodiversity has been confirmed in soils (Hoorman, 2011).

Fungi are vital in the degradation and decomposition of cellulose, starch, pectin, lignin and hemi-cellulose in the organic matter added to the soil. They also serve as food for bacteria. They play an important role in soil aggregation and in the formation of humus. Some fungi form a mycorrhizal association with the roots of higher plants (symbiotic) and help in the mobilization of phosphorus and nitrogen ,while some are parasitic in nature and cause a number of plant diseases as wilts, rots, seedling blights, and so on and so forth. (NSW Department of Primary Industries, 2005). Fungi can be classified into three general functional groups based on how they get their energy. They can either be decomposers, mutualists or pathogens / parasites. Pathogens cause reduced production or death when they colonize the roots and other organisms. Root-pathogenic fungi as *Rhizoctonia* cause major economic losses in agriculture each year. Fungal diseases often reduce crop yield and lower crop quality by producing toxins which are harmful to human health (Placinta *et al*., 1999 and NSWDPI, 2005). Some of the most commonly observed fungi in the soil are *Alternaria*, *Aspergillus*, *Cephalosporium*,

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Mucor, *Gliocladium*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Botrytis*, *Monilia*, *Chaetomium*, *Pythium,Cladosporium*, and so on and so forth (Brady *et al.* (1999).

Research has been carried out on the effect of herbicides on soil microbiota and some researchers (Milicic, 1987; Schuster and Schroder, 1990;Radosevich *et al*., 1995;Milosevic *et al*., 2002) suggested that the rate of herbicide decomposition in the soil is influenced by several factors likethe properties of the herbicide applied, its dose as well as by the physical and chemical soil properties, soil moisture and temperature, plant cover, soil cultivation method and thetypes of microorganisms present. Some other researchers also pointed out that the toxicity level of an organic pollutant will depend on the soil characteristics such as the organic matter and clay content of the soil (Bossio *et al*., 1998 and Labud *et al*., 2007). This means that soil microorga-nisms in different environments will react differently to different herbicide applications. The intensive use of these herbicides has caused environmental concern, especially unforeseen consequences onsoil microbes (Karthikeyan *et al*., 2003). Since herbicides can potentially be toxic to organisms other than their intended target. Determining their impact on non-target organisms (such as microbes) in the soil has been of considerable interest (Bending *et al*., 2007 and Nur Masirah *et al*., 2013). Also, knowledge on the effects of on non-target organisms may cause a reduced application of other pesticides.

This study was therefore set up to assess the effect of common herbicide treatments on soil fungi population dynamics and diversity.

MATERIALS AND METHODS

The experiment was conducted on a maize farm at Iddo, Iddo Local Government Area of Oyo State. The farm used for the experiment lies on latitude 7.3964Ú North and longitude 3.9167Ú East. Soil samples collected from the herbicide trial site were first analyzed for their routine physical and chemical properties as presented in Table 1. Selection of the herbicides used were based on the extent of their use in Agriculture (Table 2). Soil samples were collected every three months (July, September and December 2010). The experimental design was a randomized complete block design (RCBD) with six treatments. Samples were collected randomly from three spots within each plot using a 10 cm soil auger. The samples collected from each plot were mixed thoroughly for uniformity, labelled and kept in individual bags for further analysis.

Sterilization of materials

All glass ware (Petri dishes, beakers, pipette, conical flasks, test tubes) used for the experiments were washed using detergent, rinsed and allowed to dry. The glass ware were afterwards sterilized in a hot air oven(Gallenkamp Hotbox Oven, Gallenkamp, UK) at 200^{o}C for one hour. Conical flasks were filled with water and plugged with cotton

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wool at the top to avoid contamination. They were then sterilized in the oven (Gallenkamp Hotbox Oven, Gallenkamp, UK) at 200^{o}C for one hour. Inoculating needles, spatula, cock borers were sterilized by dipping them in 70% ethanol and flaming them until red hot. The work benches were sterilized by swabbing with cotton wool soaked in 70% ethanol.

Preparation of growth media

Potato dextrose agar (PDA) was used as a medium for the growth and maintenance of the fungal isolates all through the experiment. The preparation of the PDA was done according to the manufacturer's recommendation. Exactly 39g of dehydrated PDA (Difco Laboratories, Michigan, USA) was dissolved in 1 litre of distilled water in a 1000 ml conical flask. 15ml of bacterial agar was also added for quick solidification of the PDA. The solution was autoclaved at 121^{o}C (1.05kg/cm^{2}) for 15 minutes and allowed to cool to 45-50^{o}C. The molten PDA was poured into sterile Petri dishes, 9 cm in diameter in such a way that the PDA filled the whole bottom of the Petri dishes. The mouth of the conical flask was flamed every time before and after pouring the PDA into the Petri dishes. The PDA was slowed to solidify and preserved in the refrigerator.

Isolation and identification of fungi from soil samples

The soil dilution method also known as the serial dilution method was used for the isolation of the different fungal species in the different soil treatments. One gram of each soil sample was weighed into sterile test tubes and 9 ml of sterile distilled water was added to each of the test tubes. This tube was thoroughly mixed to give a stock suspension. Fungal cultures were plated into freshly prepared PDA using 1:10^{4}, 1:10^{5}, 1:10^{6}, 1:10^{7} with the aid of individual sterile pipettes. A sterile glass rod was used to gently spread the suspension across the face of the solidified PDA. Inoculated Petri dishes were covered and sealed with paper tape. The dishes were turned upside down to avoid accumulation of condensation. They were incubated at 27^{o}C. After 72 hours, plates were examined daily for the development of fungal mycelium. Fungi growing on PDA were counted and each fungal specie found on each plate were isolated and sub-cultured. Flame sterilized needles were used to transfer the fungi isolates into a new sterile plate containing fresh PDA so as to obtain a pure culture for each of the fungal species. The Petri dishes were also sealed with paper tape to prevent any contamination and were incubated at 27^{o}C for 72 hours to further examine the fungal growth on the PDA. The various fungal isolates from each sample were sub-cultured again onto a fresh PDA to obtain a pure culture for identification. The identification was done in the laboratory of the Department of Crop Production, Soil and Environmental Management of Bowen University, Iwo and confirmation through assistance of Advanced Phytopathology Laboratory of International Institute of Tropical Agriculture (IITA) Ibadan Nigeria.A

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compound microscope was used in the identification and all guides laid down by the International mycological Institute were adhered to.

Statistical analysis

Data collected were subjected to both descriptive and inferential statistical analysis using SAS (2001). The descriptive statistics include frequency count and tables. Frequency of occurrence of each fungal isolate were recorded and expressed as a percentage of the total isolation made from each plot.

RESULTS AND DISCUSSION

Isolation and identification of fungi from soil samples.

The results showed that varying genera and population of fungi were observed in the plots treated with different herbicides as well as the untreated plot (control) through out the sample period. *Lasidiodiplodia theobromae* had the highest frequency of occurrence (%) through all the treatments.

Treatment with atrazine and 2, 4-D mix (Treatment E) had the most restraining effect on the fungal community as no single genera of fungi was isolated at the end of the third sampling period. A similar experiment was conducted by Namita and Deep (2008) and it showed that 2,4-D had the most toxic effect on soil mycoflora. Plots treated with xtravest (Treatment D) also had just one genera of fungi (*L.theobromae*) isolated at the end of the third sampling period (Table 3c). Despite atrazine's (Treatment B) persistence in the soil for long periods as reported by several scientists, plots treated with atrazine for four planting seasons through 2009 and 2010 had the largest number of fungi genera at the end of the third sampling period. This finding agrees with the report of Rogers *et al*. (1994) that pesticides applied at recommended levels and intervals seldomly have marked effects on soil microorganisms.Radosevich *et al.* (1995) reported that microbes may use atrazine as a source of carbon. Cook and Hutter (1981) also pointed out that herbicides may be a source of nutrition for microbes in which case they significantly affectmicrobial growth and multiplication as in when they are used as a source of nitrogen. Smith *et al*. (1976) reported similar findings. *Aspergillus* spp and *Peniculium* spp. were dominant in soil treated with paraquat. Ceballos *et al*. (2006) reported that (4-Chloro-2-methylphenoxy) acetic acid increased the severity of *Fusarium* root rot in red clover seedlings. Ali (1990) had shown that the fate of pesticides in soils is greatly affected by the presence of organic matter in the soil by aiding their disappearance.Ceballos *et al*.

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Table 1. Soil physical and chemical properties of the experimental site.

Soil property	Value obtained
pH (H_2O)	6.4
Organic carbon (g/kg)	2.18
Total N (g/kg)	0.53
Available P (mg/kg)	6.63
Ex. Bases (c/mol/kg)	
Ca	1.37
Mg	2.42
Na	0.90
K	0.62
Ex. Acidity	0.4
CEC (mol/kg)	5.71
Base sat. (c/mol/kg)	93.0
Sand (g/kg)	678
Silt (g/kg)	174
Clay (g/kg)	148

Table 2. Herbicide treatments

Treatments	Treatment description			
	First season 2009	Second season 2009	First season 2010	Second season 2010
A	Atrazine	Atrazine	2,4-D	2,4-D
B	Atrazine	Atrazine	Atrazine	Atrazine
C	Xtravest	Xtravest	Atrazine + 2,4-D (Mix)	Atrazine + 2,4-D (Mix)
D	Xtravest	Xtravest	Xtravest	Xtravest
E	Atrazine + 2,4-D (Mix)	Atrazine + 2,4-D (Mix)	Atrazine + 2,4-D (Mix)	Atrazine + 2,4-D (Mix)
F	No herbicide	No herbicide	No herbicide	No herbicide

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Table 3a. Frequency of isolation (%) of fungal pathogen from herbicide treated soil and untreated at three seasons

First sampling

Treatments	Fungus	Frequency of occurrence	Frequency of isolation Mean ± Standard deviation
A	*Aspergillus niger*	4	20.000 ± 12.94
	Lasidiodiplodia theobromae	31	
	Natrassia mangiferae	35	
	Rhizopus stolonifer	12	
B	*Fusarium oxysporum*	18	
	Macrophomina phaseolina	13	14.429 ± 6.68
	Natrassia mangiferae	20	
	Trichoderma spp	26	
	Fusarium oxysporum	11	
	Aspergillus niger	8	
	Lasidiodiplodia theobromae	11	
	Fusarium verticillum	11	
C	*Natrassia mangiferae*	20	25.000 ± 14.47
	Aspergillus niger	30	
	Fusarium oxysporum	42	
	Trichoderma spp	8	
D	*Lasidiodiplodia theobromae*	50	33.333 ± 14.43
	Trichoderma spp	25	
	Collectotrichum gleosporioides	25	
E	*Fusarium oxysporum*	25	33.333 ± 14.43
	Lasidiodiplodia theobromae	50	
F	*Trichoderma spp*	25	
	Fusarium verticillum	17	16.667 ± 11.67
	Natrassia mangiferae	17	
	Lasidiodiplodia theobromae	39	
	Trichoderma spp	11	
	Macrophomina phaseolina	8	
	Fusarium oxysporum	8	

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Table 3b. Frequency of isolation (%) of fungal pathogen from herbicide treated soil and untreated at three seasons

Second sampling

Treatments	Fungus	Frequency of occurrence	Frequency of isolation Mean ± Standard deviation
A	*Lasidiodiplodia theobromae*	61	33.333 ± 25.42
	Natrassia mangiferae	28	
B	*Aspergillus niger*	11	
	Lasidiodiplodia theobromae	35	20.000 ± 10.61
	Macrophomina phaseolina	25	
	Fusarium moniloforme	10	
	Trichoderma spp	30	
	Fusarium oxysporum	20	
C	*Lasidiodiplodia theobromae*	42	20.000 ± 12.79
	Fusarium verticillum	81	
	Collectotrichum gleosporioides	71	
	Aspergillus niger	71	
	Trichoderma spp	6	
D	*Aspergillis niger*	33	33.333 ± 0.58
	Trichoderma spp	33	
	Lasidiodiplodia theobromae	34	
E	*Fusaruim oxysporum*	33	33.333 ± 16.50
	Lasidiodiplodia theobromae	50	
F	*Trichoderma spp*	17	
	Aspergillus niger	31	25.000 ± 9.09
	Fusarium oxysporum	23	
	Trichoderma spp	13	
	Lasidiodiplodia theobromae	33	

(2011) again reported stimulating effects on fungus growth of haloxyfop-methyl. In another experiment, 4-(4-Chloro-2-methylphenoxy) butanoic acid, Bentazon, 4-(4-Chloro-2-methylphenoxy) butanoic acid + Fluozifop-p-butyl.,Bentazon+Flu-ozifop-p-butyl,Metribuzin, Flouzifop-pbutyl+Metribuzin, Cycloxydin, and Sethoxydin were shown to increase the population of soil fungi, 4 to 10

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Table 3c. Frequency of isolation (%) of fungal pathogen from herbicide treated soil and untreated at three seasons

Third sampling

Treatments	Fungus	Frequency of occurrence	Frequency of isolation Mean ± Standard deviation
A	*Aspergillus niger*	33	33.333 ± 0.58
	Lasidiodiplodia theobromae	34	
B	*Fusarium verticillum*	33	
	Aspergillus niger	22	20.000 ± 8.00
	Fusarium oxysporum	11	
	Lasidiodiplodia theobromae	32	
	Aspergillus flavus	11	
	Trichoderma spp	3	
	Natrassia mangiferae	21	
C	*Aspergillus flavus*	29	20.000 ± 8.22
	Trichoderma spp	14	
	Lasidiodiplodia theobromae	29	
	Fusarium verticillum	14	
	Aspergillus niger	14	
D	*Lasidiodiplodia theobromae*	100	100.020 ± 0.01
E	Nil *	-	-
F	*Aspergillus niger*	17	50.000 ± 46.67
	Lasidiodiplodia theobromae	83	

***Fungi not isolated**

times compared with the control (Ali *et al*., 2014). Although Nur Masirah *et al.* (2013) reported growth inhibitions of soil microrganisms treated with common herbicides in oil palm plantations in Malaysia. Herbicides have been found to affect microbes physiologically by changing their biosynthetic mechanism (a change in the level of protein biosynthesis is reflected on the ratio of extracellular and intracellular enzymes), by affecting protein biosynthesis (induction or repressionof synthesis of certain enzymes), by affecting the cellular membranes(changes in transport and excretion processes), by affecting plantgrowth regulators (transport of indolacetic acid, gibberellin

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synthesis and ethylene level) orapplication in high doses which may kill the organisms (Milosevic *et al*., 2002). Even though the xtravest treatment (Treatment D) gave the second least number of genera of fungi at the end of the third quarter, it had the highest mean frequency of occurrence across all the treatments. It is pertinent to note that the control plot (Treatment F) had the second least number of fungi genera isolated at the end of the third sampling period.

CONCLUSION

In conclusion, the application of tested herbicides may not exclude the use of other forms of pesticides in the management of soil microorganisms on the farm. However, indiscriminate use of chemical pesticides should be avoided because of the long term effects on the environment. Soil microorganisms should be effectively managed using an integrated pest management approach to reduce their adverse effects on the different stages of crop production.

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