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Effect of atrazine and butachlor on soil microflora in agricultural farm in Anyigba, Nigeria

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ABSTRACT

The effect of two herbicides (atrazine and butachlor) on soil microbial populations, activities was assessed every fourteen days over a period of seventy (70) days. Soil samples from Kogi State University farm were treated with herbicides at company recommended rates, concentrations above and below the recommended rates. The observed effect of atrazine on bacterial counts at lowest concentration, highest concentration and recommended rates were4.42±0.68, 1.20±0.29 and 3.04±0.36 (x10 ^{7}cfu/g) respectively. Corresponding effect of butachlor were 4.38±0.89, 1.52 ±0.32, 3.34±0.35 (x10 ^{7}cfu/g) respectively. The effects of atrazine on fungal counts at lowest concentration, highest concentration and recommended rates were 16.0±2.3, 1.72±0.41, 9.82±0.54 (x10 ^{5}cfu/g) respectively while corresponding butachlor effects on fungal counts (x10^{5}cfu/g) were 17.2±2.9, 5.06±0.46, 13.4±0.4 respectively. Herbicide treatments at recommended and higher rates resulted in decreases in microbial counts. Pseudomonas spp., Bacillus spp. and Flavo bacterium spp. were the most frequently isolated bacteria while Aspergillus niger, Aspergillus flavus, Penicillium spp., Trichoderma spp., Mucor spp. And Fusarium spp. were the most frequently isolated fungi. From this study, butachlor is more microbial and environmental friendly than atrazine. Atrazine was more toxic to microflora.

Key words: herbicides, microflora, atrazine, butachlor, concentrations

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INTRODUCTION

Recognition that a weed is plant species growing where it is not desired, or plant out of place, or plant that is more detrimental than beneficial, is a basic principle of weed control [1]. The global drive for sustainable agriculture systems involves optimizing agricultural resources to satisfy human needs and at the same time maintaining the quality of the environment and sustaining natural resources [2]. In achieving this optimization, herbicide use is of great importance. Herbicides are substances or cultured biological organisms used to kill or suppress the growth of unwanted plants and vegetation[3]. During the past four decades, a large number of herbicides have been introduced as pre or post – emergent weed killers in many countries of the world [4].

In Nigeria, herbicides have since been effectively used to control weeds in agricultural systems [5]. As farmers continue to realize the usefulness of herbicides, larger quantities would be applied to the soil. But, the fate of these compounds in the soil is becoming increasingly important since they could be leached down in which case groundwater is contaminated or if immobile, they would persist on the top soil [6]. These herbicides could then

accumulate to toxic levels in the soil and become harmful to microorganisms, plants, wildlife and man [7]. Microorganisms play important roles in soil processes, among which are the recycling of essential plant nutrients, humus formation, and pesticide detoxification [8]. In agriculture, a major concern over the usage of herbicides is the possible harmful effects exerted on the soil micro flora, which contribute to soil fertility [9].

Atrazine powder (2-chloro-4 (ethylamino) –6-isopropylamino-1,3-5-triazine) is a widely used 5-triazine herbicide. The percentage purity of the technical grade atrazine is 97%. The impurities include dichlorotriazine,,tris(alkylamino) triazines and hydroxytriazines [10]. It is used as a selective pre- emergence herbicide in the control of broadleaf and grassy weeds in a variety of commercial crops as well as road side and fallow fields [11]. The recommended rate of atrazine powder is 3%w/v per kg of soil.Butachlor liquid (N-(butoxy methyl 2-chloro-N-(2,6-diethylphenyl) acetamide) is a selective herbicide that controls most annual and perennial plants. It is a pre-emergence herbicide. It has a percentage purity of 94% [12]. The recommended rate of butachlor liquid is 15%v/v per kg of soil. In recent years, there has been an increase demand for food to meet the ever increasing population. To meet the demand for food there is need to increase food production. To have more yield farmersapply herbicides to control weeds. In doing this the farmers in most cases in rural areas either apply it indiscriminately or above the recommended rate thereby affecting other living organism in the soil. Data on effect of atrazine and butachlor on agricultural soils in Kogi State is scratchy. This work investigates the effect of atrazine and butachlor on soil microflora in agricutural farm in Anyigba,Kogi State north central Nigeria. The data generated could serve as a baseline study in this area and to advice relevant government agency on the use of herbicides in farmland.

MATERIALS AND METHODS

2.1 Collection of Samples

Topsoil (0-15cm depth) was sampled from Kogi State University agricultural demonstration farm, which has been under continuous cultivation of maize and cassava without any history of herbicide application. The soil samples were sieved through a 2.0mm dish mesh to remove stones and plant debris:

2.2 Herbicides used

The herbicides used were purchased from a local dealer on herbicides in Anyigba. The herbicides were atrazine (designated as “A”) and butachlor (designated as “B”). The recommended rate of atrazine powder is 3%w/v per kg of soil. The recommended rate of butachlor liquid is 15%v/v per kg of soil.

2.3 Soil Treatments with Herbicides

Atrazine “A”

The recommended rate of 3%w/v concentration of the herbicide was mixed thoroughly with 1kg of soil sample. The other four concentrations above the recommended rate were 4.5%w/v, 6.8%w/v, 10.1%w/v, and 15.2%w/v. Concentrations below the recommended rate were 1%w/v and 2%w/v.

Butachlor “B”

The recommended rate of 15%v/v concentration of the butachlor was mixed with 1kg of soil sample. The other four concentrations above the recommended rate were 22.5%v/v, 33.8%v/v, 50.6%v/v, and 75.9%v/v. Concentrations below the recommended rate were 5%v/v and 10%v/v. One hundred ml (100ml) of each concentration was mixed thoroughly with 1kg of the soil sample. The set – ups were done in duplicates and incubated for 70 days. Samples were taken every 14 days and analyzed for microbial load.

2.4 Physicochemical Analysis

Soil: Water ratio of 1:1 was used to determine soil pH of herbicide-treated soils using pH meter (Mettler Toledo 420 model).Percentage organic matter was determined by the method described by Jackson [13] and particle size distribution was determined by Bouyoucos hydrometer method [14].

2.5 Microbial Enumeration and Identification

Nutrient agar was used for the enumeration of total heterotrophic bacteria by the pour plate method. Incubation was at 37^0C for 24h. Potato dextrose agar (PDA) was used for enumeration and isolation of fungi. Incubation was at 30^0C for 72h.

Bacterial isolates were characterized based on cultural characteristics, staining reactions and biochemical reactions. Identification was thereafter made with reference to Bergey's manual of Systematic Bacteriology (1984). Fungal isolates were characterized as described by Barnett and Hunter (1972).

Statistical analysis

Data generated from the study were subjected to analysis of variance (ANOVA) and the student's statistical t-test.

RESULTS AND DISCUSION

Table 1: Physicochemical Properties of the Soil

Soil Properties	Values
Ph	7.8
Moisture content	4.4
Textural class	Clay-sandy soil
Sand%	15.0
Silt%	10.0
Clay%	75.0
Organic carbon	17.0
Organic matter	30.1
Cation exchange capacity	23±22

Table 2: Mean viable bacterial counts on atrazine treated soil sample ($X10^7$cfu/g)

Days	Concentrations							
	Control	1%w/v	2%w/v	3%w/v	4.5%w/v	6.8%w/v	10.1%w/v	15.2%w/v
0	3.2	3.3	3.2	3.1	3.1	2.8	2.5	2.1
14	3.0	3.3	3.1	2.9	2.4	2.1	2.0	1.6
28	4.5	4.3	4.2	3.2	2.7	2.5	1.8	1.3
42	5.2	5.0	4.7	3.6	3.1	2.8	1.5	1.2
56	5.0	4.9	4.9	2.8	2.6	2.3	1.5	1.1
70	4.8	4.6	4.4	2.7	2.2	1.9	1.4	0.8
Mean±SD	4.50±0.88	4.42±0.68	4.26±0.07	3.04±0.36	2.60±0.34	2.32±0.35	1.64±0.25	1.20±0.29

Table 3: Mean viable bacteria counts of butachlor-treated samples ($X10^7$cfu/g)

Days	Concentrations							
	Control	5%v/v	10%v/v	15%v/v	22.5%v/v	33.8%v/v	50.6%v/v	75.9%v/v
0	3.2	2.8	2.7	2.6	2.5	2.0	2.0	1.7
14	3.0	2.9	2.7	2.8	2.6	2.3	2.1	1.9
28	4.5	4.2	3.9	3.5	2.8	2.6	2.2	1.7
42	5.2	4.8	4.6	3.7	3.1	2.9	1.8	1.6
56	5.0	4.9	4.9	3.5	3.0	2.9	1.5	1.3
70	4.8	5.1	4.4	3.2	2.7	2.5	1.3	1.1
Mean±SD	4.50±0.88	4.38±0.89	4.1±0.9	3.34±0.35	2.84±0.21	2.64±0.26	1.78±0.38	1.52±0.32

Table 4: Mean viable fungal counts on atrazine-treated soil samples ($X10^5$cfu/g)

Days	Concentrations							
	Control	1%w/v	2%w/v	3%w/v	4.5%w/v	6.8%w/v	10.1%w/v	15.2%w/v
0	13.1	12.7	12.7	8.9	6.6	5.1	3.4	1.9
14	13.1	12.9	12.7	9.1	6.6	5.3	3.7	2.2
28	16.4	16.5	16.2	10.3	6.9	5.5	3.3	2.0
42	18.5	18.7	18.0	10.4	7.0	5.2	3.1	1.8
56	17.0	17.5	17.2	9.8	6.7	5.0	3.1	1.4
70	16.7	14.4	16.5	9.5	6.4	4.7	2.9	1.2
Mean±SD	16.3±1.9	16.0±2.3	16.1±2.0	9.82±0.54	6.72±0.24	5.14±0.30	3.22±0.30	1.72±0.141

Table 5: Mean viable fungal counts of butachlor-treated soil samples (X10^{5}cfu/g)

Days	Concentrations							
	Control	5%v/v	10%v/v	15%v/v	22.5%v/v	33.8%v/v	50.6%v/v	75.9%v/v
0	13.1	12.3	12.3	12.8	11.4	10.1	7.8	5.2
14	13.1	12.5	12.3	12.9	11.8	10.5	8.1	5.7
28	16.4	16.6	16.9	13.5	12.2	10.9	8.5	5.3
42	18.5	18.9	18.3	13.9	12.9	11.6	8.9	5.0
56	17.0	19.2	16.9	13.7	12.9	11.3	8.3	4.8
70	16.7	19.0	16.6	13.2	12.6	11.1	7.9	4.5
Mean±SD	16.3±.9	17.2	16.2±2.3	13.4±0.4	12.5±.5	11.1±0.4	8.34±0.38	5.06±0.46

Table 6: Bacteria Isolated from Herbicides Treated Soil Samples

Control soil	Atrazine-treated soil	Butachlor-treated soil
Bacillus cereus	*Bacillus cereus*	*Bacillus cereus*
Flavobacterium sp.	*Pseudomonas sp*	*Pseudomonas sp.*
Actinomycetes sp.		*Flavobacterium sp.*
Proteus sp.		
Staphylococcus aureus		
Leuconostoc sp.		
Pseudomonas sp.		

Table 7: Fungi Isolated from Herbicides Treated Soil Sample

Control soil	A-treated soil	B-treated soil
Aspergillusflavus	*A.flavus*	*Fusarium sp.*
Aspergillusniger	*A. Niger*	*Aspergillusniger*
Fusarium sp.	*Penicillium sp.*	*Penicillium sp.*
Penicillium sp.		*Mucor sp.*
Trichoderma sp.		*Trichoderma sp.*
Rhizopus sp.		
Mucor sp.		

DISCUSSION

In this study, the concentration of herbicides influenced the microbial population. Similar observation on the effect of some herbicides concentration on microbial population has been reported [15]. Ayansina and Osohad reported that higher concentrations of herbicides treatments resulted in much lower microbial counts compared to soils treated with recommended doses [16]. Experiments have shown that microbes may use herbicides as a source of carbon [17]. This probably explains the increase in microbial populations obtained at the third week. Some studies report increased populations of fungi after treatments with butachlor [18] and increased soil microbial biomass [19] while in some cases no long-term change in microbial populations [20]. In this study, treatments of soil samples at concentrations higher than the recommended rates resulted in significantly lower bacterial and fungal counts compared to soils treated at recommended rates. Also, treatments at concentrations lower than the recommended rates had no significant effects on the soil bacterial counts. Results obtained showed that there was significant difference ($P<0.05$) in the effect of herbicides at concentrations higher than the recommended rate.

The atrazine herbicide at recommended and higher rates had significant effect on the mean viable bacterial counts as shown in Table 2 compared to butachlor-treated soils (Table 3). At concentrations lower than the recommended rates, the mean viable bacterial counts with butachlor (Table 3) were lower compared to atrazine (Table 2). The results indicated that butachlor had more effect on bacterial counts than atrazine at lower concentrations.

The atrazine herbicide had significant effect on the mean viable fungal counts at recommended and higher rates (3%w/v -15.2%w/v) as shown in Table 4, compared to butachlor treated soils shown in Table 5. At concentration lower than the recommended rate, (1%w/v and 2%w/v), fungal counts at day 14 were higher than the counts on 5%v/v and 10%v/v at day 14. But, in subsequent weeks, fungal counts at 5%v/v and 10%v/v were higher than that at 1% w/v and 2 %w/v (Tables 4 and 5). Herbicide treatments also resulted in the elimination of some microbial species. *Pseudomonas sp*. *Bacillus sp*. and *Flavobacterium sp*. were the most frequently isolated bacteria from herbicide treated soils. Bacteria eliminated by the herbicides were: *Actinomycetes sp., Proteus sp., Staphylococcus*

aureus, Leuconostoc sp., While *A. niger, A.Flavus, Penicilliumsp., Trichodermasp., Mucor sp., Fusarium sp.* were the most frequently isolated fungi fromherbicide treated soils.Fungus eliminated by the herbicides was*Rhizopus sp.*

Therefore, it could be deduced that, generally, atrazine had a more significant effect on fungal counts than butachlor because it caused more reduction in mean viable fungal counts than butachlor and the reduction is significan.
An initial, general rise in microbial counts was observed. This could be due to the fact that the soil microfloras were able to temporarily mineralize and use the herbicides as energy sources [2]. The initial rise in microbial counts was followed by a general decline in microbial counts. Cork and Krueger suggested that the decline in microbial counts (after each peak) must have been due to the fact that microbial populations that were tolerant of treated herbicide were susceptible to the products of soil – herbicide interactions [3], which could have possibly been bactericidal or fungicidal [3]. Atrazine, at an increased rate above the recommended rate, had tremendous effect on the microbial count. The effect of butachlor was not as significant as the atrazine herbicide. Generally, as the herbicide concentrations increased, the microbial counts reduced. However, mean viable bacterial counts on both herbicide treated soils were higher than that of fungal counts.

CONCLUSION

Overall, higher concentrations of both herbicide treated soil samples showed significant effects on the mean viable microbial counts than the counts at the recommended rates.Generally, microorganisms were not able to survive at higher concentrations herbicide treated soil samples. At lower concentrations, treatments of soil samples do not have significant effect on the microorganisms.

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