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Full Length Research Paper

Effect of two commonly used herbicides on soil microflora at two different concentrations

Ayansina A. D. V. [1]* and Oso, B. A.[2]

[1]Department of Biological Sciences, Bowen University, P. M. B. 284, Iwo, Osun State, Nigeria.
[2]Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria.

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The effect of two commonly used herbicides (atrazine and atrazine + metolachlor) on non-target soil microflora was investigated over a period of 8 weeks. One kilogram soil samples each from maize farm were treated with the herbicides separately at company recommended and one and half (X1.5) recommended rates. Effects of the herbicides on soil pH and percentage organic matter were also investigated. Significant changes in soil pH and percentage organic matter were observed only in atrazine treated soils ($P < 0.05$). Herbicide treatments at both recommended and X1.5 recommended rates resulted in decreases in microbial counts. Higher concentrations of herbicides treatments resulted in much lower microbial counts compared to soils treated with recommended herbicide does. Herbicide treatments also resulted in the elimination of some microbial species. *Pseudomonas* sp. and *Bacillus* sp. were the most frequently isolated bacteria from herbicide treated soils. While A. *niger*, A. *Flavus, Penicillium* sp and *Trichoderma* sp were the most frequently isolated fungi from herbicide treated soils.

Key words: herbicides, microflora, atrazine, metolachor, concentrations.

INTRODUCTION

The global drive for sustainable agricultural systems involves optimizing agricultural resources to satisfy human needs and at the same time maintaining the quality of the environment and sustaining natural resources (FAO, 1989). In achieving this optimization, herbicide use is of great importance. Herbicides are substances or cultured biological organism used to kill or suppress the growth of unwanted plants and vegetations (Cork and Krueger, 1992).

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. In Nigeria, herbicides have since been effectively used to control weeds in agricultural systems (Adenikinju and Folarin, 1976) . 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 (Ayansina et al., 2003). These herbicides could then accumulate to toxic levels in the soil and become harmful to microorganisms, plants, wildlife and man (Amakiri, 1982) . Taiwo and Oso (1997) demonstrated the effect of some commonly used herbicides in Nigeria on the soil microflora and changes in nutrient levels under laboratory conditions.

Atrazine {2- chloro- 4-(ethylamino)-6-isopropylamino-1,3-5-triazine} is a widely used 5- triazine herbicide. It is used as pre-emergence herbicide in the control of broadleaf and grassy weeds in a variety of commercial crop as well as roadside and fallow fields (Munier-Lamy et al., 2002). Early studies on the environmental fate of atrazine have shown that it is transformed slowly by fungi (Kaufman and Kearney, 1970). Bacteria capable of metabolizing the 5-triazine herbicides to carbon dioxide were not reported during the first 30 years of application to agricultural fields.

Metolachlor [2-chloro-N-(ethy-6-methy(phenyl)-N-(2-methoxyl-1-methylethyl acetamide] is a selective herbicide used in the control of grassy weeds in the cultivation of corn, soybeans, peanuts, cotton and other crops. Metolachlor is often used in combination with other

*Corresponding author. E-mail: ayandvt2@pop.skannet.com.

broadleaved herbicides (e.g. atrazine, metobromuron and propazine) to extend the sprectrum of activity.

This work was carried out to know the effect of herbicide applications at different concentrations on the microflora of a tropical agricultural soil.

MATERIALS AND METHODS

Soil sampling

Top soil samples were collected from a maize farm at Ajibode area of Ibadan. The site has been under continuous cultivation of maize and cassava for at least ten years without any recent history of herbicide application. The soil samples were sieved through a 2.0 mm width mesh to remove stones and plant debris.

Herbicides

The herbicides used were purchased from a local agricultural delearship store in Ibadan. The herbicides are atrazine (designated "At" in this report) 800 g/kg ai powder and primextra (a combination of atrazine- 770 g/kg and metolachlor –330 g/kg – designated as "AtM"). The two herbicides are marketed by Ciba-Geigy Inc.

Physicochemical analysis

A Soil: water ratio of 1:1 was used for the determination of soil pH of herbicide treated soils using Jenway pH meter model 3150. Percentage organic matter was determined by the method described by Jackson (1958).

Soil treatments

The herbicides were first applied at company recommended rates to know their effect on the soil microflora. Thereafter, the herbicides were applied at one and half time (X1.5) of the recommended rates. Generally, the use of X1.5 recommended rates were taken to correspond approximately to the practices of the local farmers (Mathrews, 1992) . Recommended rate of atrazine (At) was 3.0 mg powder in 100 ml of deionised water mixed thoroughly with 1.0 kg of soil sample. The X1.5 recommended rate contains 50% more of atrazine powder.

Recommended rate of atrazine + metolachlor (AtM) was 150 ml in one litre of deionized water from which 100 ml were thoroughly mixed with 1.0 kg of soil sample. To obtain X1.5 recommended rate, 225 ml of AtM were mixed with one litre of deionized water from which 100 ml were thoroughly mixed with 1.0 kg of soil sample. Soil treatments were replicated.

Microbial enumeration and identification

Plate count agar (oxiod) was used for the enumeration of total heterotrophic bacteria by the pour plate method. Incubation was at 30°C for 24 – 48 h. Potato dextrose agar (PDA-oxoid) was used for enumeration and isolation of fungi. Incubation was at 25°C for 48 h.

Bacterial isolates were characterized based on cultural characteristics, staining reactions and biochemical reactions. Identification was thereafter made with reference to the 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 students statistical t –test.

Table 1. Effect of herbicides treatment on soil pH.

Soil Treatment	Weeks				Mean
	2	4	6	8	
At	5.2	5.2	5.0	5.2	5.15*
AtM	5.8	5.6	5.5	5.5	5.6**
Control	5.7	5.7	5.5	5.4	5.58

*Significant at $P < 0.05$.
**Insignificant.

Table 2. Percentage organic matter measurements in herbicide treated soils.

Soil Treatment	Weeks				Mean
	2	4	6	8	
A t	2.65	2.80	2.75	2.75	2.73*
AtM	1.75	1.43	1.79	1.77	1.68**
Control	2.01	2.05	2.01	1.98	2.01

*Significant at $P < 0.05$.
**Insignificant.

RESULTS AND DISCUSSION

The effect of herbicide treatments on soil pH in presented in Table 1 with the statistical test of significance. Atrazine (At) treatment resulted in significant reductions in soil pH while treatment with atrazine + metolachlor (AtM) did not resulted in any significant changes in soil pH ($P<0.05$). Soil treatment with atrazine also resulted in significant changes in percentage organic matter measurements as presented in Table 2. Atrazine + metolachlor treatment on the other hand did not resulted in any significant changes ($P<0.05$). Ali (1990) had shown that the fate of pesticides in the soil in greatly affected by the presence of organic matter in the soil by aiding their disappearance.

Results obtained from microbial enumeration of herbicide treated soils at both recommended and X1.5 recommended rates showed a reduction in microbial counts compared to the control. The effect of herbicides treatments on mean viable bacterial counts in presented in Table 3. At recommended rates, maximum bacterial counts were observed at week 3 in the two herbicide treated soils; At, 3.5 X 10^7 cfu/ml and AtM, 3.4 X 10^7 cfu/ml. These two counts were lower compared to the control soil of 5.1 X10^7 cfu/ml. Treatments at X1.5 recommended rates resulted in much lower bacterial counts compared to soils treated at recommended rates. A similar tread was reported by Taiwo and Oso (1997).

Effect of herbicide treatments an fungal counts in the

Table 3. Mean Viable bacterial courts in herbicide treated soils.

Weeks	Control ($X 10^7$ cfu/ml)	At ($X 10^7$ cfu/ml)		AtM ($X 10^7$ cfu/ml)	
		Recommended rate	X1.5 recommended rate	Recommended rate	X1.5 recommended rate
1	2.8^{ab}	2.8^{ab}	2.7^{b}	2.6^{ab}	2.6^{b}
2	5.2^{a}	3.3^{bc}	3.1^{d}	3.2^{bc}	3.0^{d}
3	5.1^{a}	3.5^{c}	3.3^{e}	3.4^{c}	3.2^{e}
4	5.4^{a}	3.4^{c}	3.1^{d}	3.2^{cd}	2.6^{d}
5	4.3^{a}	3.0^{b}	2.8^{d}	2.8^{b}	2.3^{d}
6	4.0^{a}	2.8^{b}	2.6^{c}	2.7^{b}	2.4^{c}
7	3.5^{a}	2.5^{b}	2.4^{c}	2.5^{b}	2.3^{d}
8	3.1^{a}	2.3^{b}	2.4^{c}	2.3^{b}	2.3^{d}

Means with the same letters are not significantly different.

Table 4. Mean Viable fungal courts in herbicide treated soils .

Weeks	Control $x10^3$cfu/ml	At-$x10^3$ cfu/ml		AtM-$x10^3$ cfu/ml	
		recom. rate	X1.5 recom. rate	recom. rate	X1.5 recom. rate
1	11.0^{a}	8.9^{b}	5.5^{d}	8.9^{b}	5.5^{c}
2	15.5^{a}	9.1^{b}	8.0^{c}	9.3^{b}	7.5^{c}
3	17.75^{a}	9.5^{b}	10.5^{d}	9.4^{bc}	7.5^{c}
4	18.75^{a}	9.5^{c}	10.5^{c}	9.5^{c}	11.0^{c}
5	18.0^{a}	9.3^{c}	8.5^{d}	8.9^{cd}	8.0^{d}
6	17.75^{a}	8.9^{c}	8.5^{d}	8.9^{c}	7.0^{e}
7	17.0^{a}	8.9^{bc}	5.5^{cd}	8.7^{cd}	5.5^{cd}
8	16.5^{a}	8.0^{c}	5.5^{cd}	8.7^{c}	5.8^{cd}

NB: Means with the same letters are not significantly different

soil in presented in Table 4. Herbicide treatments at recommended rates resulted in lower fungal counts compared to the control soil. After a week of treatment, mean fungal counts obtained was 8.9 X 10^4 cfu/ml compared to 11.0 X 10^4 cfu/ml in control soil. Herbicide treatments at X1.5 recommended rates also resulted in further reduction in fungal counts. By week 2 of herbicide treatments mean fungal counts in "At" treated soil at recommended was 9.1 X 10^4 cfu/ml while at X1.5 recommended rate, mean fungal counts was 8.0 X 10^4 cfu/ml.

Generally, results from Tables 3 and 4 showed that mixed pesticide combinations (AtM) resulted in reduced microbial compare to soils treated with single herbicide (At). Herbicides are used in combinations to broaden the spectrum of weeds controlled by a given herbicide application and to reduce the dose of each components of the mixture applied per unit area i.e. low concentrations of the herbicides can be used (Akobundu, 1987).

An initial general rise in microbial counts reaching a maximum between week 3 and 4 was observed. Taiwo and Oso (1997) had shown a general increase in bacterial

Table 5. Bacteria isolated from control and herbicide treated soils.

Control Soil	At- treated soil	AtM treated soil
Bacillus sp. *Pseudomonas* sp. *Flavobaterium* sp. *Proteus* sp. *Actinomycetes*	*Bacillus* sp. *Pseudomonas* sp.	*Bacillus* sp. *Pseudomonas* sp.

Table 6. Fungi isolated from control and herbicide treated soils.

Control Soil	At- treated soils	AtM treated soils
A *niger* A *flavus* A. *ochracuis* *Rhizoctonia* sp. *Fusarium* sp. *Trichoderma* sp. *Penicillium* sp.	A. niger A *flavus* *Penicillium* sp.	A. *niger* *Trichodermas* sp.

and actinomycetes counts attaining a peak at week 3 after herbicide treatment, while fungal counts reached a peak at week 4 after treatment. The bacteria and fungi isolated from control and herbicide treated soils are presented in Tables 5 and 6, respectively.

The initial rise in microbial counts observed in soil treatments could be due to the fact that the soil microflora are able to temporarily mineralize and use the herbicides as energy sources (Kunc et al., 1985).

The initial rise in microbial counts was followed by a general decline in microbial counts. Taiwo and Oso (1997) have also suggested that this decline in microbial counts (after each peaks) must have been due to the fact that microbial populations that were tolerant of treated pesticides were susceptible to the products of soil-pesticide interactions, which could have possibly been bactericidal or fungicidal.

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