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Effect of herbicides on physicochemical properties and microorganisms of soil in Ago-Iwoye, Ogun State, Nigeria

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Abstract

*Farmers in Nigeria are mainly concerned with targeted effects of pesticides without attention to their secondary effects on the soil microbiological and physicochemical properties, which are crucial to soil fertility. Hence, these chemicals are indiscriminately applied. This study therefore investigates the effects of paraquat (1,1'-*Dimethyl-4,4'-*bipyridinium dichloride) and glyphosate(N-(phosphonomethyl) glycine), at the manufacturer's recommended application rates and at doubled these concentrations, on soil physicochemical properties and microorganisms. Soil pH, organic carbon, nitrogen, phosphorus content and cation exchange capacity were determined. Microorganisms were isolated and enumerated using dilution plate method. The results obtained revealed no significant effect of the herbicides on soil physicochemical properties at P>0.05. Both herbicides significantly reduced microbial counts in the soil (as compared with the herbicide untreated soil).Fungal population reduced from 9.97±3.5 to 7.63±3.28 as compared to the bacterial population whose count showed almost no difference (1.67±1.41 to 1.65±1.32). Glyphosate had a higher inhibitory effect compared to paraquat. Bacillus, Pseudomonas, Aspergillus, Penicillium and Rhizopus were the most frequently isolated microbial genera. The study reveals that soil microorganisms may be adversely affected by the application of herbicides. This could be detrimental to the sustainability of soil fertility.*

Keywords: Herbicides; physicochemical; microorganisms; soil; fertility.

Introduction

Modern agriculture depends on synthetically produced pesticides which are used for the protection of plants, to produce crops of higher quality, to reduce the input of labour and energy into crop production and to optimize yields (Das and Mukherjee, 2000). However, it has been reported that 2-3% of applied chemical pesticides are effectively used for preventing, controlling and killing pests (Environmental Protection Agency, 2005). Most of the pesticides therefore reach the non-target parts of the agricultural ecosystem (Ayansina and Amushan, 2013) and compromise the quality of soils, air, continental and coastal water bodies (Surekha*et al.*, 2008).Many peasant farmers in some developing countries now use high concentrations of pesticides with the premise of enhancing their production (Ayansina and Amusan, 2013).

Soil microorganisms are the most abundant of all the biota in the soil (Vig*et al.*, 2006).They are the primary soil decomposers driving key ecosystem processes such as organic matter decomposition, nutrient cycling and, eventually plant productivity. The presence or absence of microorganisms in the soil can be used as indicator of soil fertility and sustainability (Pandey and Singh, 2004).Factors such as the soil physicochemical parameters are known to exert positive influence on the activities of soil microbes and the action of pesticides in soil. It has also been reported that indiscriminate use of pesticides do not only disturb the soil environment with respect to flora and fauna, but also has adverse effects on the physicochemical properties of soil such as pH, organic matter, salinity, alkalinity leading to soil nutrients depletions (Sarnik*et al*.,2006).

Pesticides have been widely used in Nigeria for controlling pests, of Cocoa, Cotton, Cowpea and many other crops and examples include Gamalin 20, atrazine, Paraquat and Glyphosate (Anon, 1999). As at August 2008, Nigeria had registered a total number of 354 pesticides and agrochemicals and it has also been estimated that about 125,000 - 130,000 metric tons of pesticides are applied every year in Nigeria (Asogwa and Dongo, 2009). More recently, owing to pressure on farmland and a reduction in farm lands due to rural-urban migration, the intensive use of land with increasing use of pesticides, particularly herbicides such as glyphosate (Roundup®) and paraquat(Grammoxone®) are gaining more recognition (Mbuk*et al.,* 2009). Herbicides account for over 50% of pesticides widely used in most agricultural production to prevent or inhibit the growth of weeds which in turn improve the yield and output of the farm products (Zimdahl, 2002). Effects of herbicides on microbial growth in soil may be stimulating or depressive depending on the chemical type and concentration used, microbial species and the environmental condition as at the time applied (Zain*et al*., 2013).

1,1’-Dimethyl-4,4’-bipyridinium dichloride, a fast-acting, non-selective contact herbicide that is absorbed by the foliage is one of the most widely used herbicides in the world, and in most countries where it is registered it can be used without restriction (Dinis-Oliveira *et al.*,2006). (N-(phosphonomethyl) glycine on the other hand is a systemic broad-spectrum herbicide used for the control of weeds. These two herbicides are the most commonly used to control weeds at concentrations above the recommended rates by local farmers in Nigeria (Ayansina and Oso, 2013) and since WHO (EPA, 2005) data shows that only 2-3% of applied chemical pesticides are effectively used for preventing and killing pests, while the rest persist in the soil therefore, the ultimate “sink” of the pesticides applied in agriculture and public health care is the soil with its attendant toxicity on the environment and non-target sectors of the agricultural ecosystem, the recognition of the risks associated with pesticides usage is very vital.

This study is therefore aimed at investigating the effects of paraquat(1,1’-Dimethyl-4,4’-bipyridinium dichloride) and glyphosate(N-(phosphonomethyl) glycine) applied at the manufacturer’s recommended field application rate and twice the recommended field rate on soil physicochemical properties and microorganisms under field conditions.

Materials and methods

Experimental design of study site

The study was conducted with four treatments replicated three times using a randomized complete block design. Each plot with mixed weeds (broad leaves and grasses) such as *Agreratum conyzoides, Alternan therapungens* and *Chomolaena odorata* measured 6m x 3m with 3m alley and polythene nylon used for separation between plots to prevent drift and interference was set up at the Olabisi Onabanjo University College of Agricultural Sciences Tree crop Nursery Development project site. The geographic coordinate of the study area lies between latitude 06^0 56^1 01.7^{11}N – 06^0 $56^1 31.5^{11}$N and longitude 003^0 $49^1 56.3^{11}$E – 06^0 $56^1 24.7^{11}$E.

Herbicides

The herbicides used were 1, 1-di methyl 1-4, 4-biyridilium chloride (paraquat, pH ,7.4) a contact herbicide belonging to the Bipyridylium family marketed as Gramoxone R (200 g/l) and N-(phosphonomethyl) glycine (glyphosate, pH 7.0), a systemic organophosphorus herbicide marketed as roundup at 360 g/l .

Treatments

The treatments were as follows: post-emergence application of 1,1’-Dimethyl-4,4’-bipyridinium dichloride and N-(phosphonomethyl) glycine at the field recommended application rates (P1, G1) at 4l/h (350 ml of P1 in 15L of water, 350 ml of G1 in 15L of water), twice the recommended application rates (P2, G2) at 8l/h (700ml of P2 in 15 L of water, 700ml of G2 in 15 L of water) respectively and the untreated control (C) plot sprayed with 15 L of water. Knapsack sprayer filtered with a 2.5 deflector nozzle was used for spraying.

Soil sampling

Top soil samples (0-15 cm deep) were randomly collected using soil auger from 8-10 places per plot before herbicides applications and subsequently at 1 week interval for a period of 8 weeks. The soil samples from different places for the same weed control treatment were bulked and representative composite samples for each treatment were taken to the laboratory for analysis

Analysis of soil physicochemical properties

The physicochemical properties of the soil samples: pH, organic carbon, total nitrogen, available phosphorus were analyzed using glass electrode pH meter (Orion, USA), Walkley and Black's rapid titration method, macro Kjeldahl method and Olsen's method respectively (Jackson, 1973). Cation exchange capacity in the soil was determined by the procedure described by Black., (1965).

Analysis of microbial population and identification of microorganisms

Enumeration of the populations of bacteria and fungi in the soil was performed by serial dilutions at 10^{-6} and 10^{-4} dilutions respectively, followed by pour plate methods. Nutrient agar (Oxoid UK®) was used for bacteria and incubated at 35°C for 24 - 48 h while Potato dextrose agar (Oxoid UK®) was used for fungi and incubated at 35°C for 72 h. Bacterial isolates were characterized to genus level based on morphological, cellular and biochemical characteristics as described by Bergey's manual of systematic bacteriology (1984), while fungi identification was carried out in accordance with descriptions in Barnett and Hunter, (1972) and Cappucino and Sherman, (2002).

Statistical analysis

Data generated from the study were subjected to One-way analysis of variance (ANOVA). Comparison of means was done with Duncan Multiple Range Test at p=0.05.

Results

The effect of 1,1'-Dimethyl-4,4'-bipyridinium dichloride and N-(phosphonomethyl) glycine applications on soil physicochemical properties is presented in Fig1. The results showed no significant impact of the herbicide usage on soil pH, total nitrogen, organic carbon, phosphorus and cation exchange capacity contents.

Applications of 1,1'-Dimethyl-4,4'-bipyridinium dichloride and (N-(phosphonomethyl) glycine at both recommended and twice the recommended rates resulted in significant reduction in bacterial population. Twice recommended rates exhibited higher reduction effects than the normal recommended rate**.** However, gradual increases in bacterial populations in the herbicides treated plots were observed at the fourth week of sampling. This continued till the end of the sampling period (Fig 2).

Fig. 3 shows that both herbicides exhibited adverse effects on the population of fungi in the soil at both concentrations with N-(phosphonomethyl) glycine having more adverse effect. The reduction of the fungal population continued till the end of the sampling period.

Mean total bacterial count in the control soil sample was significantly higher than those in the herbicides contaminated soils. The mean bacterial count in recommended field rate of 1,1'-Dimethyl-4,4'-bipyridinium dichloride soil sample was significantly higher than those of the twice the recommended rate, and on both rates of N-(phosphonomethyl) glycine soil samples.

Mean fungal count was significantly higher in the control soil samples compared to the herbicides contaminated soil samples while there was no significant difference in the mean counts of the herbicides soil samples (Table 1).

Table 2 shows that herbicides at both concentrations had noticeable reduction effects on the occurrence and distribution of all bacterial species in the soil samples. *Bacillus* spp. and *Pseudomonas* spp. were the dominant bacterial species in both the control and treated soil samples. The occurrence of all fungal species in the herbicides contaminated soils were reduced with recommended rate of glyphosate which contained the lowest number of isolates (Table 3).

Discussion

The results showed that there were no significant impact of the herbicides usage on soil pH, total nitrogen, organic carbon, phosphorus and cation exchange capacity contents. The non-significant effects of the herbicides on soil pH corroborates the results obtained by other researchers (Ayansina and Amusan, 2013;Stanley*et al.*,2013). 1,1'-Dimethyl-4,4'-bipyridinium dichloride and N-(phosphonomethyl) glycine had no significant impact on the nitrogen content of the soil which is in agreement with the work of Vig *et al.,* (2006) who reported that available nitrogen levels were similar in

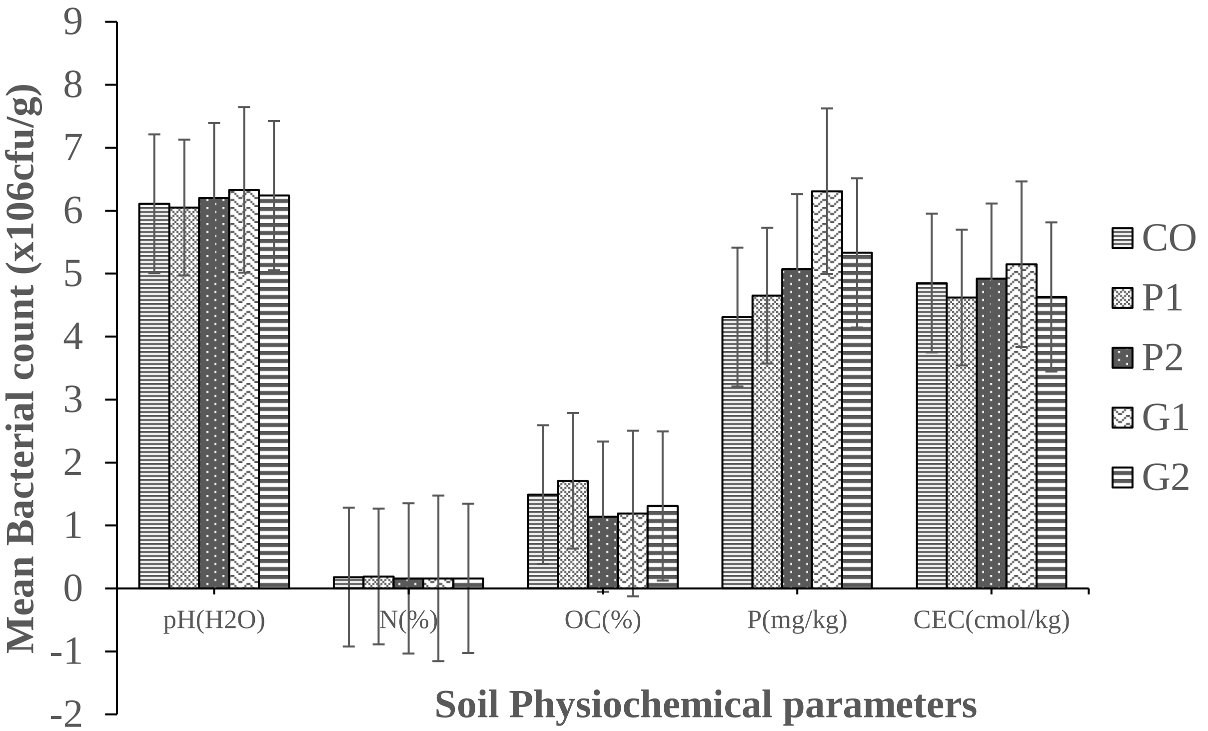

Fig 1: Mean values of Soil Physicochemical Parameters in Herbicides contaminated plots

Key: P1-Recommended rate of 1, 1’-Dimethyl-4,4’-bipyridinium dichloride,P2- Twice recommended rate of 1, 1’-Dimethyl-4,4’-bipyridinium dichloride, G1- Recommended rate of N (phosphonomethyl) glycine, G2-Twice recommended rate of N-(phosphonomethyl) glycine, CO-Control. Error bars represent differences in mean values.

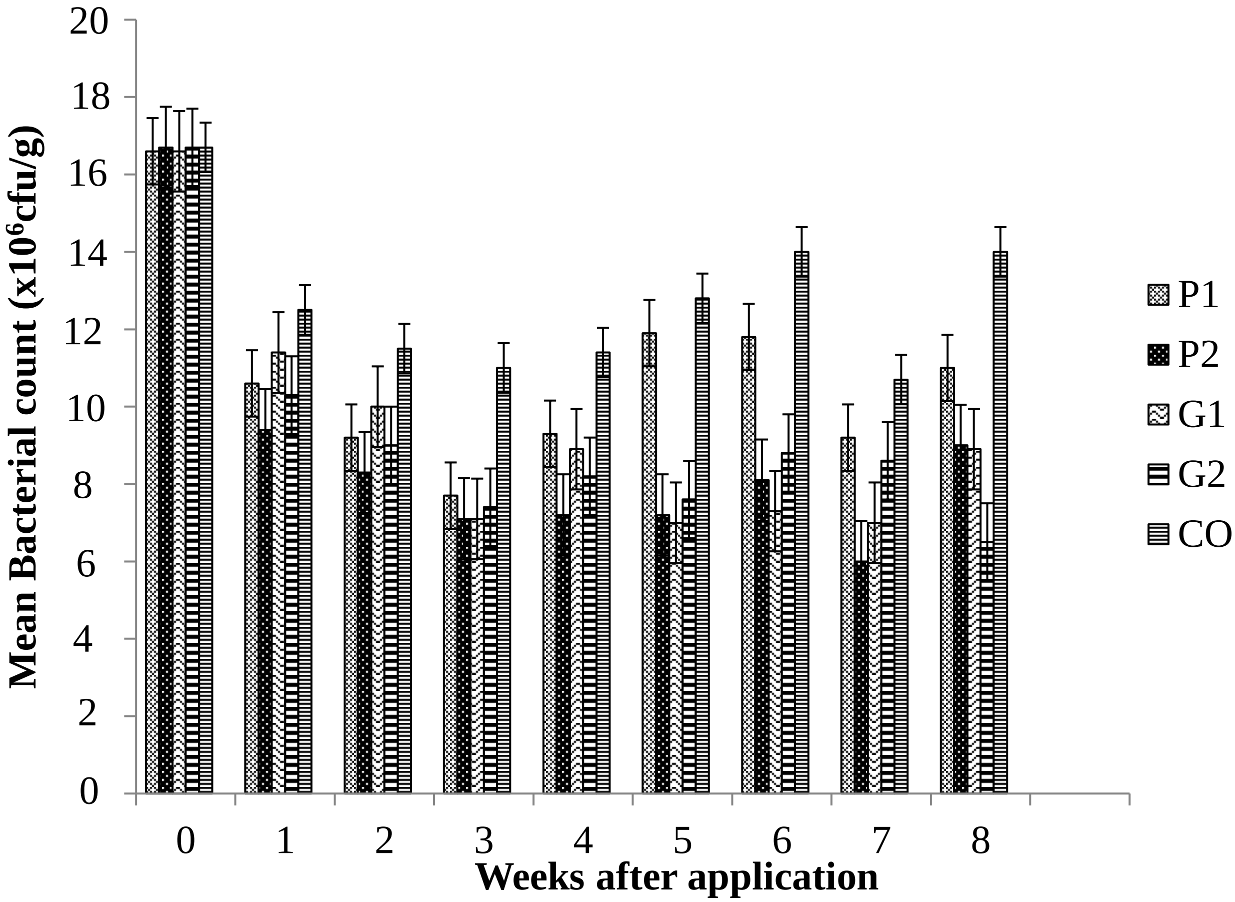

Fig 2: Effect of 1, 1'-Dimethyl-4,4'-bipyridinium dichloride and N (phosphonomethyl) glycine on the population of total bacteria

Key: P1-Recommended rate of 1, 1'-Dimethyl-4,4'-bipyridinium dichloride, P2- Twice recommended rate of 1, 1'-Dimethyl-4,4'-bipyridinium dichloride, G1- Recommended rate of N (phosphonomethyl) glycine, G2- Twice recommended rate of N-(phosphonomethyl) glycine, CO-Control. Error bars represent standard deviation

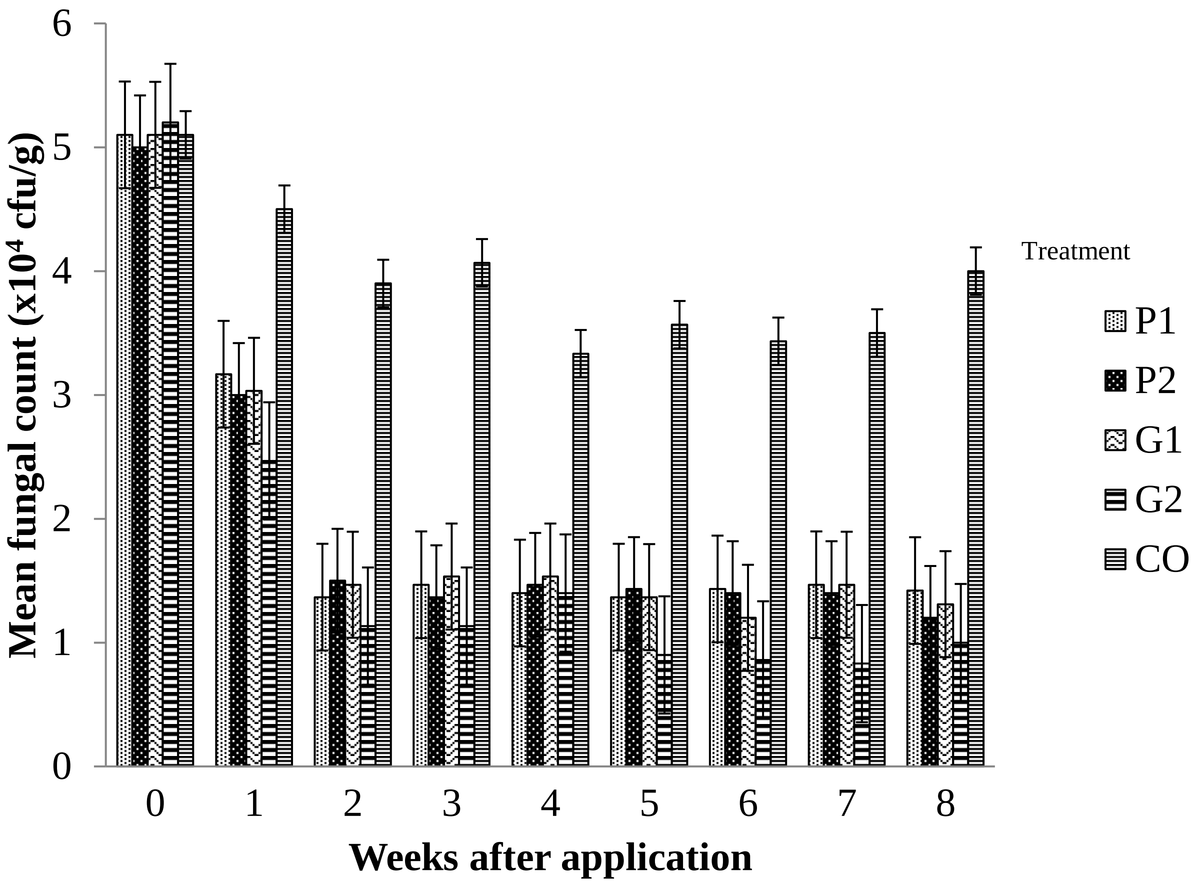

Fig 3: Effect of 1, 1'-Dimethyl-4,4'-bipyridinium dichloride and N (phosphonomethyl) glycine on the population of fungi

Key: P1-Recommended rate of 1, 1'-Dimethyl-4,4'-bipyridinium dichloride, P2- Twice Recommended rate of 1, 1'-Dimethyl-4,4'-bipyridinium dichloride, G1- Recommended rate of N (phosphonomethyl) glycine, G2-x2 Recommended rate of N-(phosphonomethyl) glycine, CO-Control.Error bars represent standard deviation

Table 1: Effects of herbicides on soil microbial counts

Microbial Group	Herbicide	Mean
Bacterial Count (x10^6cfu/g)	P1	9.97±3.55^b
	P2	7.63±3.28^a
	G1	8.42±4.35^a
	G2	8.58±2.19^a
	Control	11.98±3.02 ^{c}
Fungal Count (x10^4cfu/g)	P1	1.67±1.41^a
	P2	1.65±1.32^a
	G1	1.66±1.41^a
	G2	1.25±1.32^a
	Control	3.76±1.58^b

Values are means compared by Duncan’s Multiple Range Test at the 5% Level (P=0.05)
Means sharing similar letters within the column are not significantly different from one another

Key: P1-Recommended rate of 1, 1’-Dimethyl-4,4’-bipyridinium dichloride, P2- Twice Recommended rate of 1, 1’-Dimethyl-4,4’-bipyridinium dichloride, G1- Recommended rate of N (phosphonomethyl) glycine, G2-Twice the recommended rate of N-(phosphonomethyl) glycine, CO-Control

Table 2: Effect of herbicides on the occurrence and distribution of predominant bacteria in soil

Bacteria	Treatments				
	CO	P1	P2	G1	G2
	Number and frequencies	Number and frequencies	Number and frequencies	Number and frequencies	Number and frequencies
Bacillus spp.	22 (26.5%)	16 (19.3%)	13 (15.7%)	16 (19.3%)	16 (19.3%)
Pseudomonas spp.	16 (29.6%)	10 (18.5%)	9 (21.4%)	9 (16.7%)	10 (18.5%)
Micrococcus spp.	8 (28.6%)	7 (25.0%)	6 (21.4%)	7 (25.0%)	-
Staphylococcus spp.	7 (26.9%)	5 (19.2%)	6 (23.1%)	4 (15.4%)	4 (15.4%)
Streptococcus spp.	7 (33.3%)	4 (19.0%)	3 (14.3%)	4 (19.0%)	3 (14.3%)
Proteus spp.	5 (35.7%)	3 (21.4%)	4 (28.6%)	1 (7.1%)	1 (7.1%)
Corynebacterium spp.	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	1 (33.3%)
Klebsiella spp.	2 (2.9%)	1 (2.2%)	1 (2.4%)	1 (2.3%)	1 (2.8%)
Seriatia sp.	1 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	69	46	42	43	36

Key: P1-Recommended rate of 1, 1’-Dimethyl-4,4’-bipyridinium dichloride, P2- Twice the recommended rate of 1, 1’-Dimethyl-4,4’-bipyridinium dichloride, G1- Recommended rate of N (phosphonomethyl) glycine, G2-Twice the recommended rate of N-(phosphonomethyl) glycine, CO-Control

Table 3: Effect of herbicides on the occurrence and distribution of predominant fungi in soil

Fungi	Treatments				
	CO	P1	P2	G1	G2
	Number and frequencies	Number and frequencies	Number and frequencies	Number and frequencies	Number and frequencies
Aspergillus spp.	16 (38.1%)	9 (21.4%)	8 (19.0%)	5 (11.9%)	4 (9.5%)
Penicillum spp.	7 (33.3%)	5 (23.8%)	2 (9.5%)	4 (19.0%)	3 (14.3%)
Rhizopus spp.	4 (17.4%)	4 (17.4%)	3 (13.0%)	6 (26.1%)	6 (26.1%)
Mucor spp.	7 (43.8%)	3 (18.8%)	1 (6.3%)	3 (18.8%)	2 (1.3%)
Fusarium spp.	7 (46.7%)	1 (6.7%)	4 (26.7%)	2 (13.3%)	1 (6.7%)
Total	41	22	18	20	16

Key: P1-Recommended rate of 1, 1'-Dimethyl-4,4'-bipyridinium dichloride, P2- Twice the recommended rate of 1, 1'-Dimethyl-4,4'-bipyridinium dichloride, G1- Recommended rate of N (phosphonomethyl) glycine, G2-Twice the recommended rate of N-(phosphonomethyl) glycine, CO-Control both control and soil treated with insecticides on the field.

Comparative studies on the count of bacteria and fungi in soil treated with pesticides and an untreated carried out by Cyconand Piotrowska-Seget, (2007), using 1, 1'-Dimethyl-4,4'-bipyridinium dichloride and N-(phosphonomethyl) glycine resulted in significant reduction in bacterial and fungal populations which corroborated the findings of Tuand Bollen (2006) that1'-Dimethyl-4,4'-bipyridinium dichloride decreased both total mould and bacterial populations in Chehalis silt loam which is similar to the result obtained in this research. Moorman *et al*. (2001) and Stanley *et al*. (2013), observed suppressed microbial populations in soil as a result of herbicides treatments. However, this finding contradict the works of Zabaloy (2008) and Grenni *et al*. (2009) who observed increases in microbial populations after pesticides treatment. According to Grossbard and Atkinson (1985), soil microorganisms are an ideal community to evaluate non-target effects because they are affected both directly and indirectly by glyphosate.

Gradual increases in bacterial populations was observed at the fourth week of sampling which continued till the end of the sampling period while fungal population reductions continued till the end of the sampling period. This trend in bacterial population was also reported by Ayansina and Oso, (2006) and Ayansina and Amusan (2013) in soil treated with herbicides under laboratory conditions and Korpraditskul *et al*. (1988) in a field experiment. The fluctuation in bacterial populations observed could be attributed to environmental changes and resultant pollution from the use of herbicides on the field as reported by Korpraditskul *et al*. (1988) and an indication that the adverse effect may be temporary since increases were noticed after the initial decrease. Microorganisms take part in the degradation process of the herbicides and the concentrations of these herbicides coupled with their toxic effects gradually decrease after which the degraded organic herbicides provides the substrate with carbon, which leads to an increase of the soil micro flora observed.

Fungi population in this study was more adversely affected than the bacterial population by herbicides application which corroborate the work of Wilkinson and Lucas (2007) in which 1,1'-Dimethyl-4,4'-bipyridinium dichloride was found to be more fungal toxic than other herbicides to a range of organisms. Contrary to the finding of this study, Busse *et al*. (2001) found little evidence that repeated applications of N-(phosphonomethyl) glycine on the field is detrimental to microbial populations in soil.

Doubling the recommended rates of 1,1 '-Dimethyl-4,4'-bipyridinium dichloride and N-(phosphonomethyl) glycine resulted in significant decreases in total bacterial counts when compared with the recommended rates. Ayansina and Amusan (2013) also reported significant reductions in bacterial population following the use of higher concentrations of 1,1 '-Dimethyl-4,4'-bipyridinium dichloride and N-(phosphonomethyl) glycine in a laboratory experiment.

Comparatively, N-(phosphonomethyl) glycine had higher inhibitory effect on soil bacterial and fungi populations than 1,1 '-Dimethyl-4,4'-bipyridinium dichloride. This is at variance with the findings of Sebiomo*et al*.(2013) who reported that 1,1 '-Dimethyl-4,4'-bipyridinium dichloridecontaminated soil had lower bacterial population than that treated with N-(phosphonomethyl) glycine. The higher inhibitory effect of N-(phosphonomethyl) glycine could be attributed to the inhibition of amino acid synthesis via the shikimic acid pathway which is only found in microorganisms and higher plants (Grossbard and Atkinson, 1985).

Reduction of predominant bacterial and fungal genera as well as the elimination of some genera in the herbicides contaminated soils is an indication that some microorganisms were inhibited as a result of the toxic effects of the herbicides. This could be detrimental to sustaining soil fertility since beneficial soil bacteria like *Bacillus* and *Pseudomonas* species are useful in the biological controlof plant pests and pathogens or used asplant growth promoters while fungi like *Aspergillus, Mucor* and *Rhizopus* contribute to soil aggregation and fertility. Their continuous reduction or suppression in soil could be detrimental to plant productivity (Singh and Singh, 2005)

Conclusion

Modifications in the count and activity of microorganisms may lead to an imbalance in the biological equilibrium of soil, which in turn reduces its fertility. This study confirms that the use of high concentrations of herbicides as practiced by Nigerian farmers is detrimental to the sustainability of soil fertility. Farmers in developing counties need to be educated on the cautious and proper use of pesticides so as to curtail the dangers associated with indiscriminate use of these chemicals. Also, the use of cheaper, eco-friendly alternatives such as organic farming and bio-control agents that can enhance crop production and are less detrimental to the soil and microbial population should be encouraged..

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