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SOIL PHYSICO-CHEMICAL PROPERTIES AND MICROFLORA AS INFLUENCED BY PARAQUAT APPLICATIONS

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ABSTRACT
A field experiment was conducted to investigate the effects of paraquat (1, 1'-di methyl-4,4'-biyridinium dichloride) at the recommended and twice the recommended field application rates on soil physicochemical properties and microorganisms. The effects of the herbicide on soil pH, organic carbon, nitrogen, phosphorus content and cation exchange capacity were analyzed along with microbial populations and the growth and distribution of representative soil microorganisms were obtained using standard procedures. There was no significant effect of paraquat on soil physicochemical properties at $P>0.05$. Paraquat applications at both concentrations caused reduction in the bacterial and fungal populations with twice the recommended rate having more adverse effect when compared with the control while the fungal populations were more adversely affected than the bacteria populations by herbicide treatment. Reduced number of predominant bacteria and fungi genera as well as the elimination of some secondary genera as observed in paraquat treated soils could be detrimental to the sustainability of soil fertility. These results are discussed in line with the soil management practices for sustainable crop production.

Key words: *Paraquat, Soil, Bacteria, Fungi, Physicochemical properties*

INTRODUCTION

Many peasant farmers in many developing countries especially in rural areas which constitute the mainstay of economy now use high concentrations of pesticides with the premise of enhancing their crop production (Mattews, 1992; Ayansina and Amusan, 2013). This practice raises the question of the effects of the use of higher concentrations of pesticides above that recommended by the manufacturers since it has been reported that 2-3% of applied chemical pesticides are effectively used for preventing, controlling and killing pests, while the rest persist in the soil (EPA, 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 and continental and coastal water bodies (Surekha *et al.*, 2008).

Soil microorganisms are the most abundant of all the biota in the soil and are responsible for driving nutrient and organic cycling as well as soil fertility (Vig *et al.*, 2006). Comparative studies of the numbers of bacteria and fungi in soil treated with pesticides in comparison with untreated soil are one of the fundamental approaches commonly used for the assessment of microbial response to environmental stress (Cycon and Piotrowska-Seget, 2007). Modifications in the count and activity of microorganisms may lead to upsetting the biological equilibrium of soil, which in turn depresses its fertility. Persistence of pesticides in soil has also been found to depend on their doses as well as the physicochemical properties of the soil.

Paraquat (1,11-dimethyl-4,41-bipyridinium dichloride) also known as methyl viologen is an important member of bipyridylium family of non-selective herbicides. It is a fast acting non-selective compound which destroys tissues of green plants on contact and by translocation within the 7 days (Suntres, 2002). Although, Paraquat has been banned or severely restricted in most countries, its use continues in some developing countries. Murugesani *et al.*,2010) So widespread is its use in weed control that local farmers in Nigeria regard it as a household chemical and use concentrations above the recommended rate on their farms (Ayansina and Oso, 2006).

The effect of application of high concentrations of pesticides as practiced by local farmers on the field where there are interactions with other ecological factors in Nigeria on soil physicochemical properties and microorganisms has so far received little attention. This study was therefore undertaken to investigate the effect of paraquat applied at the manufacturer's recommended rate and twice the recommended rate on soil microorganisms and physicochemical properties.

MATERIALS AND METHODS

The study was conducted with two treatments replicated three times with a randomized complete block design. Each plot with mixed weeds (broad leaves and grasses) measured 6m x 3m with 3m alley and polythene nylon was 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 located at Ago-Iwoye, Ogun State .Nigeria. The area is located between latitudes $6^{0}55$ and 70^{0}N and between longitudes $3^{0}45$ and $40^{0}5$E.

The commercial formulation of paraquat marketed under the brand name Gramoxone, 200g/l (Syngenta crop protection) was purchased at Ogun State Agricultural Development Project (OGADEP) store at Ijebu-Ode, Ogun State.

The treatments were as follows: post-emergence application of paraquat at the field recommended application rate (P1) at 4l/h (350 ml in 15 L sprayer), twice the recommended application rate (P2) at 8l/h (700ml in 15 L sprayer), and the untreated control (C) plot sprayed with water. Knapsack sprayer filtered with a 2.5 deflector nozzle was used for spraying.

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

The physicochemical properties of the soil samples: pH, organic carbon, total nitrogen, available phosphorus were analyzed using glass electrode pH meter method, 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 (Black, 1965).

Analysis of Microbial population and Identification of Microorganisms

The enumeration of the populations of bacteria and fungi in the soil was done using serial dilution technique and pour plate method on Nutrient agar (Oxoid UK®) at 10-6 dilutions and Potato Dextrose Agar (Oxoid UK®) at 10-4 dilutions respectively. Incubation was done at 35°C for 24 - 48 h. for bacteria and 72h for fungi. Major Bacterial isolates were characterized based on cultural characteristics, staining reactions and biochemical reactions. Bacteria Identification was further made with reference to the Bergey's manual of Systematic Bacteriology (1984) while fungi Identification was carried out as described by Barnett and Hunter, (1972) and by using a Laboratory Manual (Cappucino and Sherman, 2002).

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

RESULTS AND DISCUSSION

The effect of paraquat on soil physicochemical properties is shown in Table 1. There were no significant impact of paraquat usage on soil pH, total nitrogen, organic carbon, phosphorus and cation exchange capacity contents. Komal (2001) also reported that there was no alteration in pHs of the fields treated with pesticides because in soil the clay and humus fraction acts as a buffer system. The changes or variations observed in the other soil chemical properties on the field which were not significant may have been due to climatic conditions or as a consequent of mineralization of the pesticides.

Paraquat at both the recommended and twice the recommended rates resulted in reduction in bacterial and fungal populations (Fig 1 and 2). Tu and Bollen (2006) also reported that paraquat decreased both total mould and bacterial populations in Chehalis silt loam. Gradual increases in bacterial populations was observed at the fourth week of sampling which continued till the end of the sampling period while fungal populations reductions continued till the end of the sampling period (Fig 1 and 2). 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 by Korpraditskul *et al.,*(1988) in a field experiment.

The initial decrease in bacterial counts observed in this study could be attributed to the toxic effects of the herbicides which are normally most severe immediately after application, when their concentrations in soil are the highest. Later on microorganisms take part in a degradation process and herbicide concentration and its toxic effect gradually decline up to half-life after which the degraded organic herbicide provides the substrate with carbon, which leads to an increase of the soil micro flora while the fluctuation in bacterial population may be attributed to nutritional and environmental changes as well as chemical pollution that takes on the field.

Fungi population in this study were more adversely affected than the bacterial population by paraquat treatment (Fig 2)which corroborates the work of Wilkinson and Lucas (2007) in which paraquat was found to be more fungi toxic than other herbicides to a range of organisms. Contrary to the above reports, Busse *et al.* (2001) found little evidence that repeated field applications of glyphosate is detrimental to microbial populations and processes in soil. The mean bacterial and fungal counts in the control soil were significantly ($P<0.05$) higher than those in the paraquat plot soils while mean bacterial count in recommended field rate of paraquat plot soil was significantly ($P<0.05$) higher than that of twice the recommended rate plot soil. There was however no significant differences in the mean fungal counts of both concentrations of paraquat treated soils (Table 2).

Twice the recommended rate of paraquat (P2) had higher reduction effect on soil bacterial and fungal populations (Table 2), Moorman *et al.,* (2001) and Ayansina *et al.*, (2013) had reported that higher herbicides concentrations applications resulted in significant reduction of bacterial counts. The indiscriminate use of high concentrations of pesticides with the hope to promote effective weed and insects control (Mathews, 1992) might work for some years but will lead to loss of beneficial microorganisms which will invariably lead to loss of fertility in soils.

Presented in Table 3 is the occurrence, distribution and frequency of isolation of the major bacteria genera isolated from the control and paraquat treated soils. The microorganisms isolated in this research are those common to the natural environment (Ayansina and Amusan 2013). The herbicide at both concentrations had marked reduction effects on the occurrence and distribution of all the bacterial species in the soil. *Bacillus* spp. and *Pseudomonas* spp. were the predominant and most frequently isolated bacterial species in both the control and paraquat plots soils. Both organisms have been reported to be dominant in pesticides treated soils (Bollag and Liu 1991; Taiwo and Oso, 1997; Das and Mukherjee, 2000; Ayansina and Amusan 2013). The herbicide at both concentrations used had reduction effects on the occurrence and distribution of all the bacterial species which corroborates the earlier findings of Sakata *et al*., (1992) and Ayansina and Amusan, (2013) and at both rates led to the elimination of Serratia and Corynebacterium spp. The predominant and most frequently isolated fungal species in both control and treated soils were *Aspergillus* and *Penicillium* species which corroborates the work of Ayansina and Amusan (2013) in which this fungus was dominant in soil treated with herbicides. Applications of paraquat at both concentrations had marked reduction effects on the occurrence and distribution of all the fungal species in the soil with twice the recommended rate having the lowest number of isolates (Table 4).

Bacillus and *Pseudomonas* species help to suppress pest and pathogens as well as promote plant growth, some of these strains have been developed into fungicides, insecticides or as generic plant growth while *Aspergillus* and *Mucor* and *Rhizopus* species are involve in soil aggregation and fertility hence their continuous reduction or suppression in soil could be detrimental to plant productivity in the future (Singh and Singh, 2005).

CONCLUSION

This study was able to confirm that the use of paraquat at field application recommended rate and twice the recommended rate had no adverse effect on soil pH, percentage nitrogen, organic carbon, organic matter and cation exchange capacity however the herbicide suppressed microbial populations with higher concentration leading to greater inhibition and suppression of soil bacteria and soil fungi. This is detrimental to the sustainability of soil fertility. Soil fertility is determined by the presence of sufficient nutrients and a sufficient number and diversity of soil microorganisms. A reduction in microbe's number may disturb a specific process in the food web performed by an individual or group, and disrupts the different components relying upon it. Farmers in Nigeria and other developing counties need to be educated on the cautious and proper use of pesticides so as to curtail the dangers associated with indiscriminate use Continuous use of pesticides needs be monitored constantly with respect to their persistence in soil, plants and effects on soil organisms in terms of ill effects and toxic residues. Agricultural practices that enhance soil sustainability and management such as the incorporation of carbon rich organic matter like compost and other organic amendments into the soil which can serve as sources of energy and nutrients for soil organisms, planting of cover crops and weed suppressive crops, mixed farming in which leguminous crops are incorporated should be encouraged

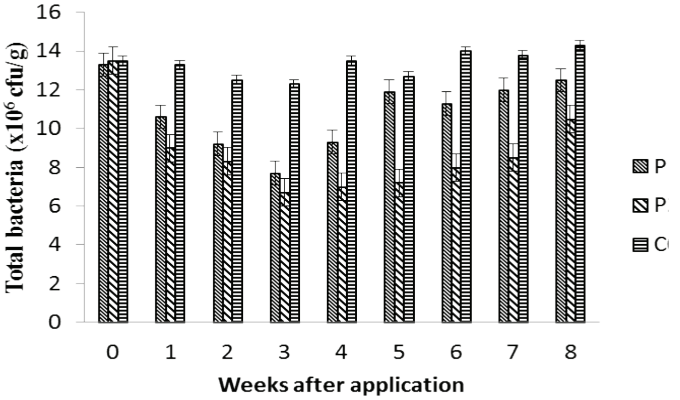

Fig 1: Effect of paraquat on the population of total bacteria

Key: P1-Recommended rate of Paraquat, P2-x2 Recommended rate of Paraquat, CO-Control

Error bars represent standard error

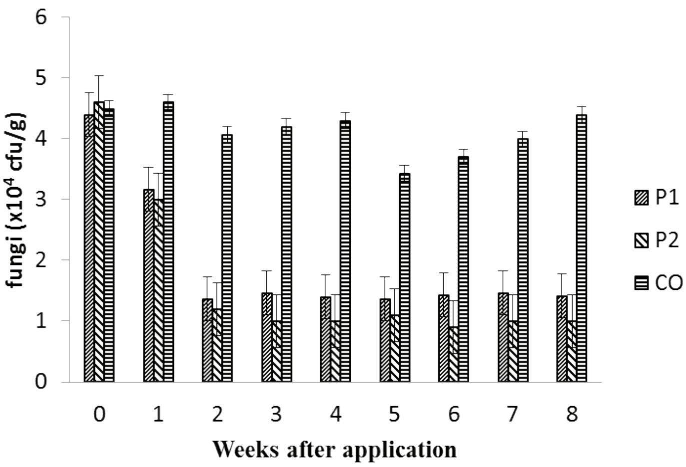

Fig 2: Effect of paraquat on the population of fungi

Key: P1-Recommended rate of Paraquat, P2-x2 Recommended rate of Paraquat, CO-ContError bars represent standard error

Table 1: Mean values of soil physicochemical parameters in control and paraquat treated soil (pooled data).

	Parameters				
Treatments	pH	N(%)	OC	P	CEC
CO	6.13±0.43a	0.18±0.19	1.49±1.81	4.31±2.14	4.85±1.76
P1	6.07±0.44	0.19±0.20	1.71±2.23	4.65±1.35	4.62±1.33
P2	6.02±0.58	0.16±0.15	1.14±1.32	5.07±2.59	4.92±1.81
Mean	6.07	0.18	1.45	4.68	4.80
$LSD_{0.05}$	n.s.	n.s.	n.s.	n.s.	n.s.

aMean±s.d.; n.s.: no significant.
Values are means compared by LSD at the 5% Level (P=0.05)
Key: P1- Recommended rate of Paraquat, P2- X2 Recommended rate of Paraquat, CO-Control

Table 2: Comparative effects of paraquat on bacterial and fungal populations

Microbial Group	Herbicide	Mean
BacterialCount(x106cfu/g)	P1	9.90±0.04b
	P2	7.60±0.03a
	Control	11.9±0.03c
	Mean	9.80
	$LSD_{0.05}$	S
Fungal Count (x104cfu/g)	P1	1.67±0.141a
	P2	1.65±0.13a
	Control	3.76±0.16b
	Mean	2.36
	$LSD_{0.05}$	S

aMean±s.d.; s: significant.
Values are mans compared by LSD at the 5% Level (P=0.05)
Key: P1- Recommended rate of Paraquat, P2- X2 Recommended rate of Paraquat, CO-Control

Table 3:Effect of paraquat on the occurrence, distribution and frequency of isolation predominant genera of bacteria in control and treated soils

Microorganism	No of Isolates Treatments		
	CO	P1	P2
Bacteria	Number and frequencies	Number and frequencies	Number and frequencies
Bacillus sp.	22 (31.9%)	16 (34.8%)	13 (31.0%)
Pseudomonas sp.	16 (23.2%)	10 (21.7%)	9 (21.4%)
Micrococcus sp.	8 (11.6%)	7 (15.2%)	6 (14.3%)
Staphylococcus sp.	7 (10.1%)	5 (10.9%)	6 (14.3%)
Streptococcus sp.	7 (10.1%)	4 (8.7%)	3 (7.1%)
Proteus sp.	5 (7.2%)	3 (6.5%)	4 (9.5%)
Corynebacterium sp.	1 (1.4%)	0 (0.0%)	0 (0.0%)
Klebsiella sp.	2 (2.9%)	1 (2.2%)	1 (2.4%)
Seriatia sp	1 (1.4%)	0 (0.0%)	0 (0.0%)
Total	69	46	42

Key: P1- Recommended rate of Paraquat, P2- X2 Recommended rate of Paraquat, G1- Recommended rate of Glyphosate, G2- X2 Recommended rate of Glyphosate, CO-Control

Table 4: Effect of paraquat on the occurrence, distribution and frequency of isolation predominant genera of fungi in control and treated soils

Microorganism	No of Isolates Treatments		
	CO	P1	P2
Fungi	Number and frequencies	Number and frequencies	Number and frequencies
Aspergillus sp	16 (39.0%)	9 (40.9%)	8 (44.4%)
Penicillum sp	7 (17.1%)	5 (22.7%)	2 (11.1%)
Rhizopus sp	4 (9.8%)	4 ((18.2%)	3 (16.7%)
Mucor sp	7 (17.1%)	3 (13.6%)	1 (5.6%)
Fusarium sp	7 (17.1%)	1 (4.5%)	4 (22.2%)
Total	41	22	18

Key: P1- Recommended rate of Paraquat, P2 -x2 Recommended rate of Paraquat, CO-Control

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