EFFECT OF DIAZINON APPLICATION ON SOIL PROPERTIES AND SOIL MICROFLORA

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ABSTRACT

Rhizosphere (Rh) and Subsurface (Ss) soil samples were treated with diazinon for 30 days in the laboratory to determine the effect of the chemical on the soil physico-chemical properties and microbial populations. The pH, organic carbon, nitrogen, and phosphorus of both rhizosphere and subsurface soil increased from the 10th day to 30thday of treatment with a highest increment of 10- fold recorded in Phosphorus parameter of Subsurface soil sample and 6- fold increment in organic carbon content of Subsurface all at 20th day of diazinon treatment. There was an initial rise in bacterial population in the two treated soil samples thereafter a decline, while fungi population decreased with days of diazinon application. Marked reductions in microbial load were recorded in the treated soils as compared to control soil samples. Ten bacterial isolates and five fungal species were identified in the control rhizosphere soil, while in the treated rhizosphere soil only eight bacterial isolates representing 60% and two fungal species representing 40% survived diazinon treatment. Also six bacterial isolates and five fungal species were identified in the control Subsurface soil samples, while in the treated Subsurface soil sample only four bacterial isolates representing 60% and two fungal species representing 40% survived the diazinon treatment. In Rh &Ss, P*enicillium sp, Rhizopus sp., and Fusarium sp., Mucor sp., Rhizobium sp., and Nitrobacter sp.* isolated in all the control soil samples were inhibited in all the treated Rh and Ss soil samples.

Keywords: Diazinon, Soil microflora, and Soil chemical properties

INTRODUCTION

Diazinon 0,0-diethyl 0-2-isopropyl-4-methyl-6-pyrimidyl phosphorothioate is an organophosphate insecticide used as foliage spray to control agricultural pests and can also be used as plant growth regulator (Mateen *et. al.*, 1994). Other organophosphate insecticides are malathion, phorate, chlorpyriphos, bromophos, methylparathion and fenitrothion (Kenneth, 1990). Organophosphate insecticides are esters of organic salts of phosphoric acid and its derivatives (Kenneth, 1990). The chemical is highly toxic to man and animals and the residues are persistent in the environment (Chapalamadugu and Chaudhry, 1992).The degradation of these chemicals differ with different soil types (Kennenth, 1990).

Diazinon has an average half life of 30 days when applied to soil. Soils though differ in their physico-chemical properties; their fertility depends on physico-chemical parameters and activities of microorganisms. Factors influencing biodegradation of organic compounds in the soil had been reported to include pH, water, aeration, redox – potential, temperature and types of microflora present as well as the soil nutrient status (Holden and Firestone, 1997; Leahy and Colwell, 1990). Microorganisms in the soil can breakdown organic compounds thereby leading to their mineralization and availability of nutrients to plants. Microorganisms can also utilize these compounds for their metabolic activities. Hence, the objective of this work is to assess the effect of diazinon on physical, chemical properties and microbial populations of rhizosphere and subsurface soil samples.

MATERIALS AND METHODS

The soil samples were collected at different depth with the soil auger from the College of Environmental Resources and Management farm, University of Agriculture, Abeokuta Nigeria. The Rhizosphere (Rh) sample was the soil that came up with the root of the uprooted plant. The soil thereof was shaken into a sterile beaker.

The depth of the root was about 30cm, while the subsurface (Ss) sample was collected from the same point where the root was taken from 30-120cm depth.

Each soil sample comprised nine composite sub samples randomly collected. The soil samples were air dried and passed through a 2mm sieve. Soil organic carbon was determined by the Walkey-Black method (Walkey and Black, 1934). The soil pH was determined with a 1:1 soil to water ratio (IITA, 1979). Particle size distribution was measured by the hydrometer method (Gee and Baucler,1986). Available phosphorus was extracted with Bray P-1 extractant (Bray and Kurtz,1945) and measured on the spectronic – 20 electrophotometer at 660nm wavelength. Total nitrogen was determined using the macro-kjeldahl method according to Bremner (1960). Soil moisture was determined by weighing 100g soil (moistened) and oven dried at 105°C percentage moisture content. Soil temperature was taken insitu by soil thermometer.

Populations of bacteria in the soil were determined after serial dilution and plating on nutrient agar (Fawole and Oso, 1993). Identification of bacteria isolate was carried out according to Bergy's manual of determinative Bacteriology (Buchanan and Gibbons, 1974) following routine microbiological techniques described by Olutiola et.al., (1991) for colonial morphological and biochemical characteristics.

Fungal population was determined using potato dextrose agar and their identification was carried out according to the scheme of Barnett and Hunter (1972) after the isolates had been examined macroscopically using the needle mount method.

Treatment with diazinon.

Diazinon was added at a concentration of 160mg/kg of dry soil and diluted with 500ml of water. The pesticide was mixed separately with Rhizosphere and Subsurface samples. Each experimental treatment was 1kg soil in 4litre flask. The control soil was treated with distilled water only. The experiment was carried out in triplicates. The flasks were incubated for 30 days at ambient temperature.

For physico-chemical properties, samples were analysed before application and at 30th day of diazinon application, while for microbiological analyses, samples were withdrawn every 5 days. Microorganisms that survived the treatment were also monitored at the end of the incubation.

RESULTS AND DISCUSSION

The results of soil analysis prior to diazinon application are shown in Table 1.The soil textural class was sandy loam. The soil samples Rhizosphere (Rh) and Subsurface (Ss) differ in their physico-chemical properties. The pH values indicated that the Rhizosphere soil samples were slightly acidic while the subsurface soil samples tended more towards neutrality pH 7. Rh witnessed an initial change in pH to about 11.6% by the end of the 10th day and thereafter became stable throughout the duration of the experiment. However a gradual but none steady increase in pH was observed in Ss throughout the period of the experiment (Table 2).This may have implication for presence of other plant nutrients in the soil solution which was in line with the work done by Agboola and Odeyemi (1972) and Owoeye and Agboola (1993) that a slight change in pH has influence on plant nutrients in soil solution.

Table 1: Physico-chemical properties of Rhizosphere and Subsurface soil samples before diazinon treatment.

Soil Parameter	Rhizosphere (Rh)	Subsurface (Ss)
Temperature	26.6°C	28.6°C
Moisture content	9.8%	12.63%
Ph	5.5	6.1
Organic matter	3.0 %	1.9 %
Organic carbon C	1.7 %	1.1 %
Phosphorus P	17.1mg/kg.	12.1mg/kg
Nitrogen N	0.15 %	0.10 %
Clay	6.4%	10.0%
Silt	13.6%	12.4%
Sand	80.0%	77.6%
Bacteria count	22×10^5	16×10^5
Fungi count	8×10^5	6×10^5

Table 2: Percentage increase in chemical properties of soil samples (Rh&Ss) after treatment with diazinon between 0- 10days,10-20days,and 20-30 days.

Parameters	Days 0-10 %	10-20 %	20-30 %
pH	Rh 11,6	0.0	0.0
	Ss 0.0	1.63	1.61
Org.Carbon C %	Rh 11.8	10.52	57.14
	Ss 9.1	50.00	77.77
Phosphorus $mgPkg^{-1}$	Rh 1.17	5.20	6.59
	Ss 0.8	7.37	8.39
Nitrogen %	Rh 20	16.66	38.1
	Ss 50	26.66	47.4

*Rh-Rhizosphere soil ; *Ss-Subsurface soil

It was also revealed in Table-1 that the organic matter of rhizosphere (Rh) double the Subsurface (Ss) which was indicated by high percentage carbon content in the sample. It shows that the rhizosphere was richer in plants nutrient and other decayed animal and plant debris hence it could be used as a parameters to measure the extent of life support a plant could derive from it. This ascertion was further buttressed by the level of P, N and other microorganisms that were recorded in higher percentages in rhizosphere soil sample than subsurface soil sample. The moisture content of subsurface soil samples was found to be 13% higher than the rhizosphere. At 10th day of application of diazinon into Rh and Ss samples, a change in the micro-environment was observed, the pH of Rh had been ameliorated above 10% but afterwards remained fairly stable. In the subsurface soil a change was observed between 20th – 30th day of diazinon application but not as much as that recorded between 10th – 20th day of the experiment (Table 2).There was an increase in the organic carbon content in both Rh and Ss as the days of contact with diazinon was increased. A greater organic carbon accumulation was observed in the Ss than Rh at the end of 30th day of diazinon application (Table 3). In the Rh, the organic carbon build up at the end of 20th day, doubled that recorded at the end of 10th day of experiment while 30th day was 4-folds that recorded at 20th day of diazinon application. It shows that there was an increment in organic carbon builds up at Rh throughout the duration of diazinon application.

In the Ss, the organic carbon build up at 20th day was 6- fold more than the 10th day while the subsequent build up in between 20th-30th was at a declining rate of a 3-fold increase was recorded. The organic carbon increase in Rh was on the increasing side while that of Ss was also increasing but at a lower rate.The changes in Rh and Ss organic carbon content could be due to the chemical composition of the diazinon at one hand in Ss while at the Rh the build up may be due to combined actions of both the chemical and the microbial load recorded at this soil sample. It shows that diazinon indirectly may serve as a primer to adjust positively the organic carbon content of soils to the benefit of plant when applied to control insect on the field.

The percentage variation of Phosphorus content of Ss was more than Rh. A sharp build up of P content was noticed at 20th day of diazinon application at the Ss sample (Table 3). It was 10-fold more than what was recorded at 10th day while at Rh soil it was 5-fold more than what was recorded at 10th day of treatment. The subsequent build up between the 20th-30th day for both Ss and Rh soil was increasing at a lower rate (Tables 2&3). The ability of diazinon and that of other co-factors in adjusting P content thus reached the peak at the end of 20th day of the chemical application (Table 3). The changes in the P content in both Rh and Ss could be traced to both the contribution of the organophosphate chemical being investigated and microbial activities in the soil samples. The chemical may therefore serve as an advantage on a field low in P content but needing diazinon chemical compound to kill the pest infestation on it.

Table 3: Percentage cumulative increase in chemical properties of Rhizosphere (Rh) and Subsurface (Ss) samples over 30-days

Parameters		Days 0-10	0-20	0-30
pH	Rh	11.6	11.6	11.6
	Ss	0.0	1.6	3.3
Org.Carbon %	Rh	11.8	23	94
	Ss	9.1	63	190
Phosphorus $mgPkg^{-1}$	Rh	1.2	6.4	13.5
	Ss	0.8	8.5	17.4
Nitrogen %	Rh	20	40	93.3
	Ss	50	90	180

Table 4: Effect of prolong application of diazinon on physico-chemical properties of Rhizosphere and Subsurface samples.

Parameters	Days	pH	Org.carbon C %	Phosphorus $mgPkg^{-1}$	Nitrogen N %	Clay %	Silt %	Sand %
Rh	0-10	4.36a*	2.45c	2.45c	2.60b	6.4	13.6	80.0
	10-20	0.00b	3.46b	6.97a	2.60b			
	20-30	0.00b	7.86a	6.27b	6.93a			
Ss	0-10	0.00c	0.70c	0.86c	3.27b	10.0	12.4	77.6
	10-20	1.73c	5.20b	8.85b	1.85c			
	20-30	0.57c	12.12a	10.12a	4.93a			

*Each figure is the mean of three replicates. Means followed by the same letter within a soil sample parameters and chemical properties are not significantly different at P= 0.05 according to Duncan's multiple range test

The nitrogen build up is systematic in both Ss and Rh soil sample and was fairly uniform throughout the duration of the experiment (Table 3). A general decrease in N content in both Ss and Rh was observed at 10th and 20th day (Table 2) but later increased before termination of the experiment. The initial decrease recorded in N content may be due to some re-adjustment and adaptation of microorganism to the strange environment created by diazinon while the late increase may be due to some organisms that were able to adapt to this readjustment in the environment and later participated in degradation of the organic compound whose cumulative effect was translated to N content increase before the 30th day of the experiment.
At the end of 30th day of application of diazinon,the percentage distribution of soil particles(clay, silt and sand) in the Rh and Ss samples remained the same.

The changes in organic carbon C, nitrogen N and available phosphorus P of rhizosphere and subsurface soils show a significant difference ($P<0.05$) as the days of application of diazinon increased from 10th day (Table 4). The percentage cumulative increase overtime (Table 3) shows that diazinon application has more profound effect on subsurface soil samples than rhizosphere samples.The total observed changes in the chemical properties of the soil samples may be attributed to the diazinon composition 0,0-diethyl 0-2-isopropyl-4-methy-6-pyrimidyl phosphorothioate as well as death of pest and insects which are capable of increasing the organic matter status of the soil (Mateen et al 1994)

There were more bacteria population than fungal population in the two soil sample (Rh and Ss) Table 1. The microbial isolates from the samples show that rhizosphere has higher load of both bacteria and fungi 16% and 14% respectively than subsurface soil samples. This could be traced to better combination of factors such as temperature, pH and organic matter content of the Rhizosphere soil samples.

The temperature of subsurface soil was 2°C higher than rhizosphere soil sample (Table1).The variation in temperature between this samples may be responsible for the microbial distribution in the soil profile (Duran 1980).Also the pH of Rhizosphere was slightly acidic. High pH does not favour fungal growth while slightly acidic soil favours bacteria growth (Hans1997). The bacteria population in rhizosphere soil was 7% higher than subsurface soil sample. This maybe as a result of organic matter status in the rhizosphere which served as food reservoir for the reproduction and multiplication of the organisms.

Figs 1a & b show that the bacterial load for both Rh and Ss soil samples after application of diazinon increased to peak at 20th day, thereafter, a gradual decline while the fungi population nose-dived with days of diazinon application (Figs 2a & 2b). This gradual decline caused by the death of some soil organisms (Table 5) suggested that while there may be an increase in nutrient pool in the soil to the plant some of the microbial population that were still tolerant such as those above the critical point may be responsible for the degradation of the pesticide through their bactericidal and fungicidal action which was similar to the observation made by Smith and Mayfield (1977) when paraquat (1,1-dimethyl-4-bipyridinium) was used in sandy soil. However in leguminous field using diazinon may be disadvantageous because of elimination of bacterial like Nitrobacter spp and Rhizobium spp which will impair both nitrification process and nitrogen fixation respectively.

Table 5: Microbial isolates from control soils and Diazinon treated rhizosphere and sub surface soils at 30th day of treatment.

RHIZOSPHERE		SUB SURFACE	
Control soil	Treated soil	Control soil	Treated soil
Pseudomonas sp.	+++	*Pseudomonas sp.*	+++
Achromobacter sp.	+++	*Bacillus sp.*	+++
Flavobacterium sp.	+++	*Achromobacter sp.*	+++
Bacillus sp.	+++	*Micrococcus sp.*	+
Proteus sp.	+	*Flavobacterium sp.*	+++
Nitrobacter sp.	-	*Serratia sp.*	+
Serratia sp.	++	*A. niger*	++
Enterobacter sp.	++	*A. flavus*	++
Rhizobium sp.	-	*Fusarium sp.*	–
Micrococcus sp.	+	*Mucor sp.*	–
A. flavus	++	*Rhizopus sp.*	–
A. niger	++		
Penicillium sp.	-		
Rhizopus sp.	-		
Fusarium sp.	-		

+++ = Abundant; ++ = Moderately Present (critical point); + = Slightly Present; - = absent

The bacteria isolates were identified as *Bacillus subtilis, Micrococcus luteus Pseudomonas aeruginosa, Chromobacter lividum, Proteus morganii Flavobacterium sp., Enterobacter aerogenes* and *Serratia marcensis, while* fungi strains were identified as Aspergillus *niger, Aspergillus flavus, Penicillum notatum, Fusarium sp.*, and *Rhizopus sp.* (Table5). There was a marked reduction in species of bacteria and fungi in both soil samples after treatment with diazinon, yet some were effective in degrading the chemical. If moderately present is to be taken as a critical point below which no strong meaningful degrading action of both bacterial and fungi were felt, it therefore means that 60% of the bacterial spp and 40% of the fungi spp that were isolated after 30th day of the treatment with diazinon in both soil samples tolerated and utilized the chemical for both growth and development up to this stage.

In conclusion, diazinon application in this study does not change the textural class of the soil samples (Table 4), but cause anincrease in the chemical parameters of the rhizosphere and subsurface soil samples.The

Total viable count of bacteria before and after 30 days application of Diazinon

Fig 1a

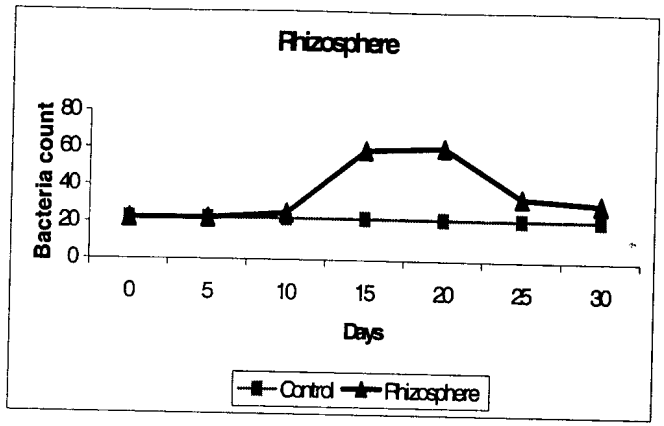
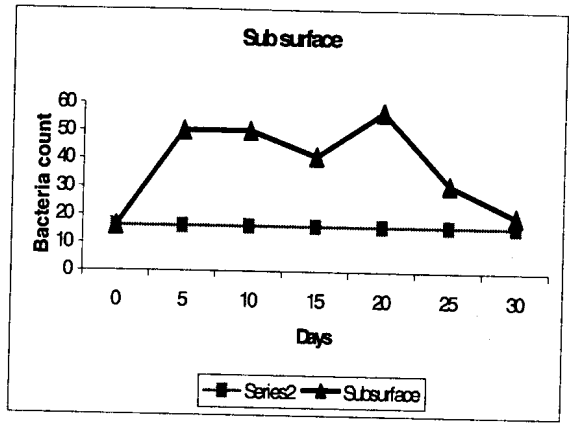


Fig. 1b



Total viable count of fungi before and after 30 days application of Diazinon to Rhizosphere and Subsurface

Fig 2a

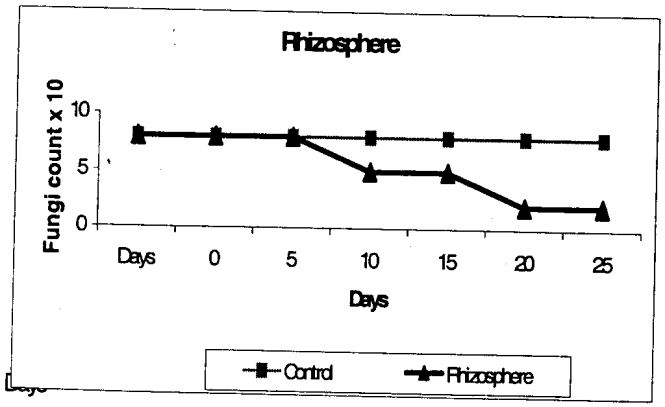
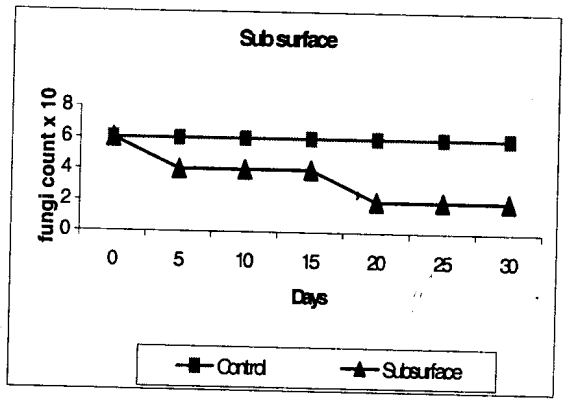


Fig. 2b



effect of diazinon on microorganisms varied with the bacteria and fungi isolated from the soil. While diazinon supported the growth of some bacteria till 20th day , it has toxic effect on others.

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