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Effects of Oxyfluorfen Herbicide on Microorganisms in Loam and Silt Loam Soils

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ABSTRACT

This study was conducted under laboratory conditions to investigate the effect of different concentrations of oxyfluorfen herbicide on the numbers and diversity of the main groups of soil microflora in two soil types. The numbers of microorganisms in oxyfluorfen treated soils were determined at zero time and then at 15 days interval for 45 days. Oxyfluorfen residue in soils was determined by Gas Liquid Chromatography (GLC). Results showed that the numbers of organic nitrogen users were inhibited at 15 days with maximum inhibition percentage (65.2%) observed in Elsilait soil treated with 4000 mg kg^{-1}. The highest increment percentage (97.8%) was observed in Gerif soil sample treated with 400 mg kg^{-1} after 30 days. Maximum inhibition percentage in inorganic nitrogen users was 42.4% which was recorded for soil treated with 96 mg kg^{-1} herbicide. Numbers of *Mycobacterium* spp. which were absolutely dominant in the nitrate agar medium decreased as from the beginning of the incubation period up to 45 days. The highest increment percent (180.1%) in fungal population was observed in Elsilait soil treated with 4000 mg kg^{-1}. At low concentrations of the herbicide, degradation started after 15 days of incubation while at higher levels, a noticeable degradation was observed after 30 days of incubation in Elsilait loam soil. In Gerif silt loam soil the degradation percentages of the herbicide were generally very low at all concentrations tested compared to those observed in Elsilait loam soil. It could be concluded that the effect of oxyfluorfen on soil microorganisms depends on the group of microorganisms and soil type. While the growth of fungi, organic and inorganic nitrogen users, is enhanced, at least in low oxyfluorfen concentrations, the growth of *Mycobacterium* was inhibited at all concentrations tested. Higher degradation percentages were recorded in Elsilait soil (55.2-78.3%) compared to Gerif soil (6.0-9.5%).

Key words: Herbicides, oxyfluorfen, soil microflora, degradation

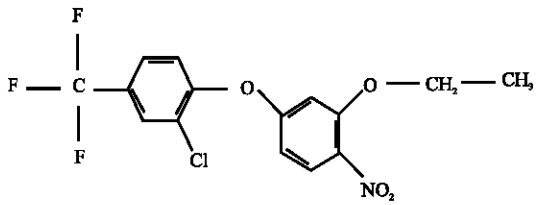
INTRODUCTION

The use of pesticide is still an integral essential part of agricultural production. Soil represents a major environmental compartment on which most of applied pesticides are finally deposited (Primental and Levifan, 1986). Heavy use of pesticides in agriculture is associated with significant undesirable side effects. An estimated three million reported cases of pesticide-associated acute poisoning occurs annually, resulting in 220,000 deaths; 99% of these occur in the developing world

(WHO, 2004) and most synthetic pesticides are accumulating in soil and ground water where they threaten the health of entire ecosystem (IDRC, 1993). Although pesticides are intended to protect crops, they may affect microorganisms and contaminate soil environment resulting in alterations in the equilibrium of soil processes for shorter or longer periods (Cycon and Piotrowska-Seget, 2007). The processes that govern the fate and transport of pesticides can be grouped into those that affect mobility (sorption, volatilization, plant uptake, wind erosion, runoff, leaching) and those that affect persistence (photodegradation, chemical degradation, microbial degradation) (Kerle *et al.*, 1996; Logan, 1999; Mackay *et al.*, 1997).

Sudan is considered as one of the main consumers of pesticides in Africa and the Arab world. Introduction of pesticides in the Sudan probably goes back to 1907 when arsenate of soda was tested for control of locusts. The period from the early sixties to the late seventies witnessed progressive intensification and expansion of the cropped areas with a subsequent increase in pest complexity and damage (Elsaid *et al.*, 2010). According to Banaga (1991), the area treated with oxyfluorfen only in Gezira Scheme-Sudan increased drastically from approximately 14164 ha in 1976 to 101172 ha in 1990.

Oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene) is a diphenyl ether herbicide.



Oxyfluorfen is a selective pre- and post-emergent contact herbicide used to control certain annual broadleaf and grassy weeds. It acts by inhibiting protoporphyrinogen oxidase, causes disruption of cell membranes and may also act as electron transport inhibitor (U.S.EPA/OPP, 2002).

Soil microorganisms are the first biota that are affected directly or indirectly by the toxic substances introduced to any soil. Soil microflora are suitable to act as "biomarkers" to evaluate ecotoxicological influence of chemicals, such as pesticides, on soil system (Doran and Zeiss, 2000; Cycon and Piotrowska-Seget, 2007).

Therefore, the objective of this study was to detect the effect of oxyfluorfen herbicide, on the numbers and diversity of the main groups of soil microflora and its degradation by naturally occurring microorganisms of two soil types. Results of this study will throw some light on the mutual interaction which occur in soil between oxyfluorfen herbicide and soil microflora.

MATERIALS AND METHODS

Soil samples were taken from two sites in Khartoum State, Sudan where there is no history of pesticides application. The two sites were Elsilait Scheme in the East of Khartoum and agricultural fields at the Blue Nile bank (Gerif) in Khartoum North. From each site, 11 kg soil sample was collected as follows: Fifteen auger samples were randomly taken at 15 cm depth from different parts in an area of one hectare. Large clods or particles were crushed to a uniform reasonable size and the samples were mixed thoroughly to make a composite sample. The physical and chemical characteristics of each soil were recorded in Table 1. Elsilait soil is (Entisol, Fluvents, Torrifluvents, Typic, Isohyperthermic) while Gerif soil is (Entisol, Torriorthents Typic Isohyperthermic). Elsilait Loam soil is more alkaline and have high N, P and OM compared to Gerif silt loam soil.

Table 1: Chemical and physical properties of soils

	Soil samples	
Soil properties	Elsilait loam soil	Gerif silt loam soil
pH	8.20	7.86
Electrical conductivity (mS cm^{-1})	2.00	3.17
Sodium absorption ratio	0.69555	0.35938
Nitrogen (%)	0.0336	0.0294
Organic carbon (%)	3.842	3.202
Organic matter (%)	6.623	5.520
Ca (meq L^{-1})	8.6	19.7
Mg (meq L^{-1})	2.4	4.1
Na (meq L^{-1})	1.6312	1.2397
K (ppm)	1.38	5.52
Phosphorus (ppm)	30.0	25.0
Sand (%)	46.88	34.24
Clay (%)	26.0	26.0
Silt (%)	27.12	39.76

Four different semi-selective media were used for isolation of microorganisms throughout this study as recommended by Tepper *et al.* (1993), Moubasher (1993) and Schmidt and Wolff (1997). These were: Meat Peptone Agar (MPA) for enumeration of organic nitrogen-using bacteria, Starch Ammonium Agar (SAA) to count inorganic nitrogen-using bacteria and actinomycetes, Nitrate Agar (NA) which was used for counting of microorganisms capable of growing onto poor media and the viable count of fungi was performed on Czapek Dox Agar (CZA) medium.

Effect of oxyfluorfen herbicide on soil microorganisms: Oxyfluorfen (240 g L^{-1} active ingredient) was obtained from the Central Trading Company, Khartoum, Sudan. Soil samples were divided into 600 g lots, each in a 1000 mL beaker and wetted to 60% field capacity. Oxyfluorfen was added to the soils, separately, at concentrations of 96, 200, 400, 800 and 4000 mg kg^{-1} and mixed well. In addition, control sets where no herbicide has been applied were prepared for comparison. Beakers were then incubated in the dark at 40°C for 45 days.

The numbers of Colony Forming Units (CFU) were determined by means of the serial dilution technique and the spread plate method (Tepper *et al.*, 1993) at zero time and then at 15 days intervals for 45 days of oxyfluorfen application. Counting was performed in four replications in a completely randomized design.

Determination of oxyfluorfen residues in two different soils: Residual oxyfluorfen was extracted in hexane at zero time and at 15 days intervals up to 45 days. Oxyfluorfen was analysed using GLC equipped with an Electron Capture Detector (ECD) and 3% OV-1-100-120 mesh glass column. Hydrogen gas at a flow rate of 42 mL min^{-1} was used as a carrier. Temperatures of injector port, oven and detector were 260, 215 and 290°C, respectively.

RESULTS

Effect of oxyfluorfen on soil microorganisms: All concentrations of oxyfluorfen used inhibited the growth of organic nitrogen users after 15 days of incubation. Maximum inhibition percentage (65.2%) was observed in Elsilait soil treated with 4000 mg oxyfluorfen/kg soil (Fig. 1). After 30 days of incubation, all herbicide concentrations used stimulated the growth of organic

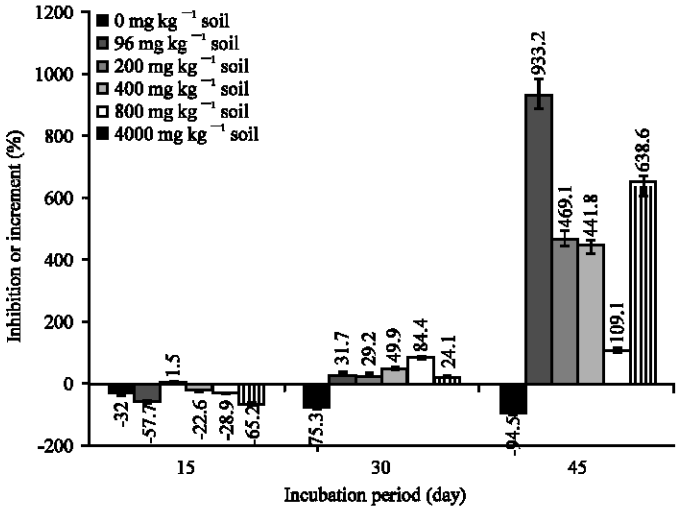


Fig. 1: Inhibition or increment percentages of organic nitrogen using bacteria by various concentrations of oxyfluorfen in Elsilait soil

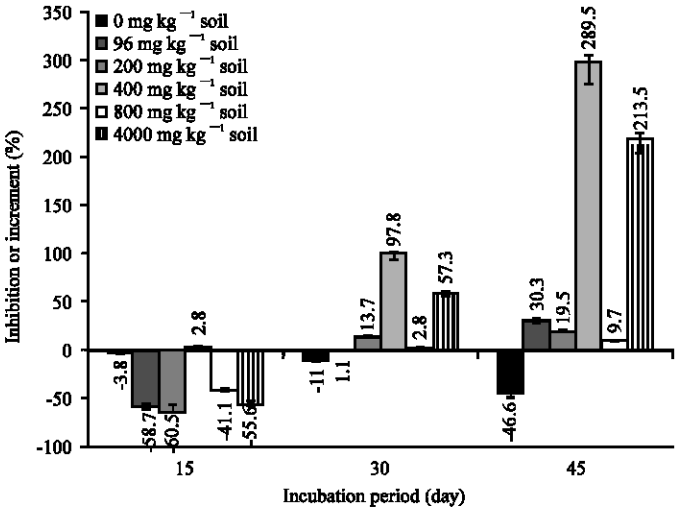


Fig. 2: Inhibition or increment percentages of organic nitrogen using bacteria by various concentrations of oxyfluorfen in Gerif soil

nitrogen users with the highest increment percentages of 97.8% observed in Gerif soil samples treated with 400 mg kg^{-1} herbicide (Fig. 2) and 84.4% in Elsilait soil samples treated with 800 mg kg^{-1} herbicide. In Elsilait soil, a strong stimulatory effect of the herbicide on organic nitrogen users was also observed after 45 days of incubation; for all treatments the herbicide increased the total number of microorganisms at all concentrations tested with a maximum increment percent at 96 mg kg^{-1} dose. Whereas, in Gerif soil, the highest increment percentage after 45 days was observed when 400 mg herbicide per kg soil was used.

The total number of inorganic nitrogen users decreased drastically in untreated soil samples collected from both sites during the incubation period. In Elsilait treated soils, the numbers of inorganic nitrogen users decreased after 15 days of incubation at the 96 and 200 mg kg^{-1} soil where the inhibition percentages were 42.4 and 23.9%, respectively. At higher concentrations (400, 800

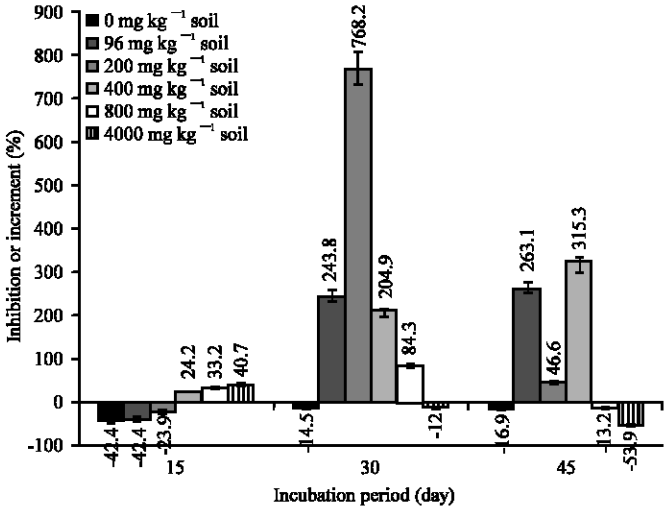


Fig. 3: Inhibition or increment of total number of inorganic nitrogen users by different concentrations of oxyfluorfen in Elsilait soil

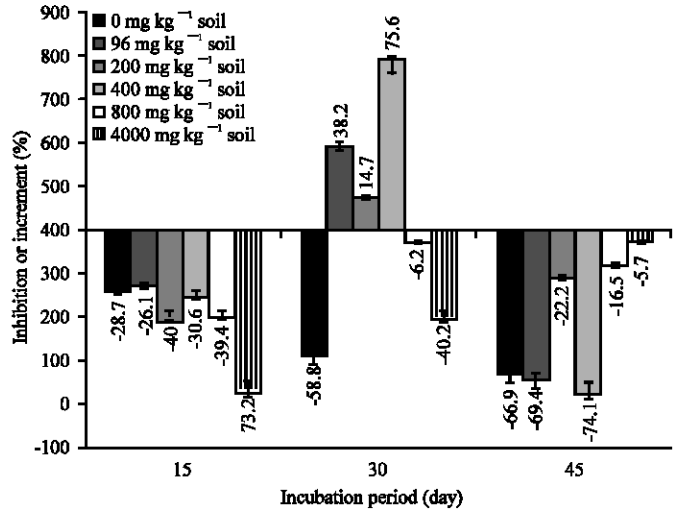


Fig. 4: Inhibition or increment of total number of inorganic nitrogen users by different concentrations of oxyfluorfen in Gerif soil

and 4000 mg kg^{-1}), the numbers of microorganisms increased as oxyfluorfen concentration increases with the highest increment percentage (40.7%) observed at 4000 mg kg^{-1} soil (Fig. 3). After 30 days incubation, the increment in the microorganisms' numbers was observed at all concentrations of the herbicide except at 4000 mg kg^{-1} where the number decreased drastically. The highest increment percentage was observed at 200 mg kg^{-1} soil. After 45 days, the numbers increased in soils treated with 96,200 and 400 mg kg^{-1} soil but sharply decreased at higher concentrations (800 and 4000 mg kg^{-1}).

In Gerif soil, a stimulatory effect of the herbicide on inorganic nitrogen users was observed at 30 days of incubation at oxyfluorfen applications of 96, 200 and 400 mg kg^{-1} where the increment percentages were 38.2, 14.7 and 75.6%, respectively. Otherwise, there was a clear inhibition for these microorganisms at 15 and 45 days of incubation (Fig. 4).

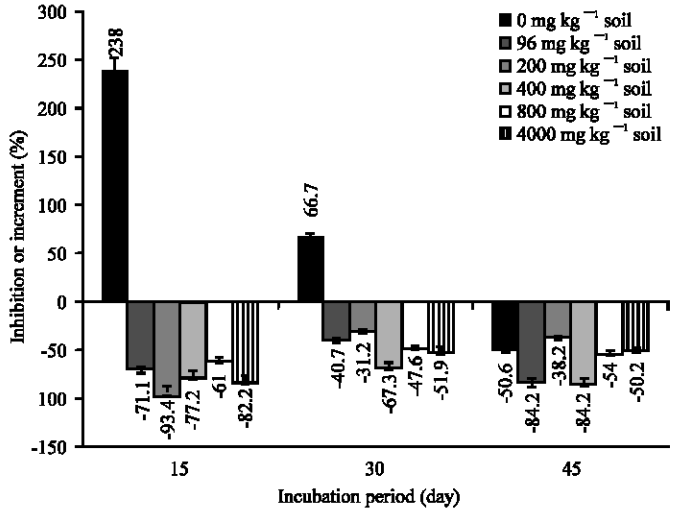


Fig. 5: Inhibition or increment of total number of microorganisms capable of growing on poor media by different concentrations of oxyfluorfen in Elsilait soil

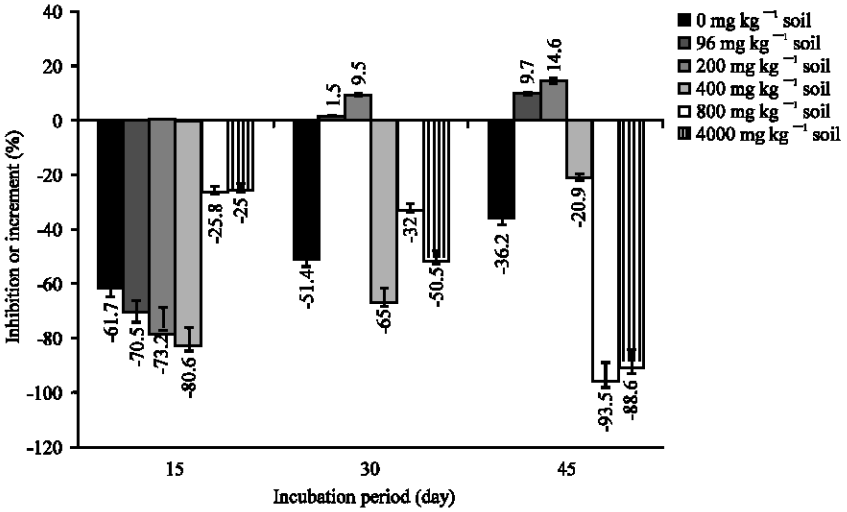


Fig. 6: Inhibition or increment of total number of microorganisms capable of growing on poor media by different concentrations of oxyfluorfen in Gerif soil

Results presented in Fig. 5 shows that the number of *Mycobacterium* sp. which were absolutely dominant in the NA medium decreased remarkably in Elsilait soil wetted with oxyfluorfen at all concentrations used except for 200 mg kg^{-1} soil.

In Gerif soil, a noticeable reduction in *Mycobacterium* spp. followed by high increment was observed after 45 days at concentrations 96, 200 and 400 mg kg^{-1} soil. As the herbicide concentration increased, the inhibitory effect of the herbicide increased steadily (Fig. 6).

Oxyfluorfen concentrations of 96, 200 and 400 mg kg^{-1} applied to Elsilait soil decreased the number of the mycoflora after 15 days of incubation while, Oxyfluorfen concentrations of 800 and 4000 mg kg^{-1} soil increased the number at the same incubation period by 156.1 and 173.2%, respectively (Fig 7). After 30 days, the highest increment percent (180.1%) in fungal population

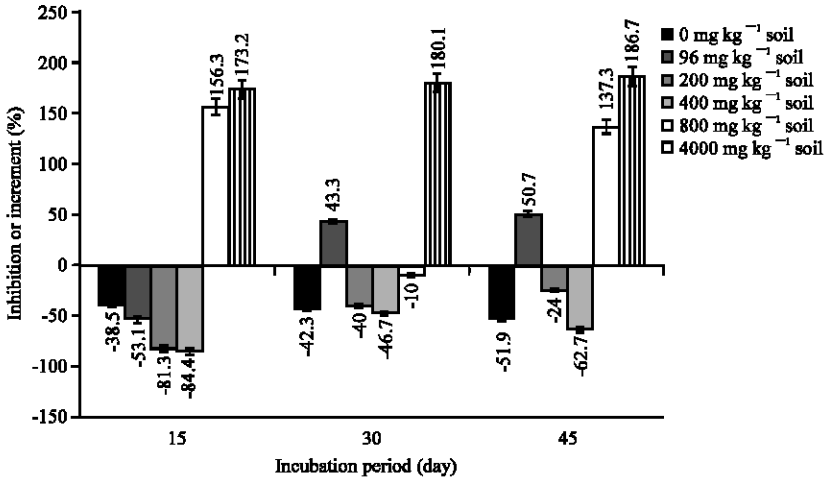


Fig. 7: Inhibition or increment percentages of fungi by various concentrations of oxyfluorfen in Elsilait soil

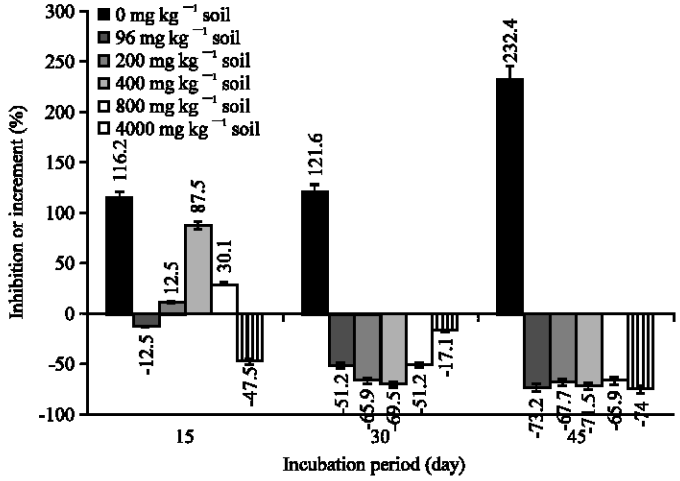


Fig. 8: Inhibition or increment percentages of fungi by various concentrations of oxyfluorfen in Gerif soil

was observed in soil treated with 4000 mg kg^{-1} soil. After 45 days of incubation, the number of fungal population increased. The highest percent of increment was observed at 4000 mg herbicide per kg soil.

The fungal population in Gerif soil treated with different concentrations of oxyfluorfen decreased steadily throughout the incubation period except at the oxyfluorfen concentration of 4000 mg kg^{-1} soil where no definite trend was observed (Fig. 8).

Biodegradation of oxyfluorfen in soils: Results in Fig. 9 shows that high degradation percentages (55.2-78.3%) were recorded for all concentrations tested by the end of the incubation period in Elsilait soil. Oxyfluorfen degradation started after 15 days of incubation at concentrations of 96, 200 and 400 mg kg^{-1} soil where 28.1, 62.5 and 42.8 degradation percentages were obtained. The lowest degradation percentages of 6.9 and 6.0 were recorded for Oxyfluorfen concentrations of 800 and 4000 mg kg^{-1} soil after 15 days of incubation, respectively. In comparison, higher

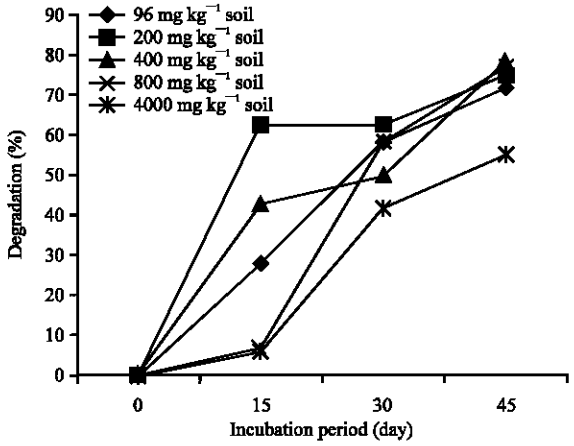


Fig. 9: Degradation percentages of oxyfluorfen in Elsilait soil

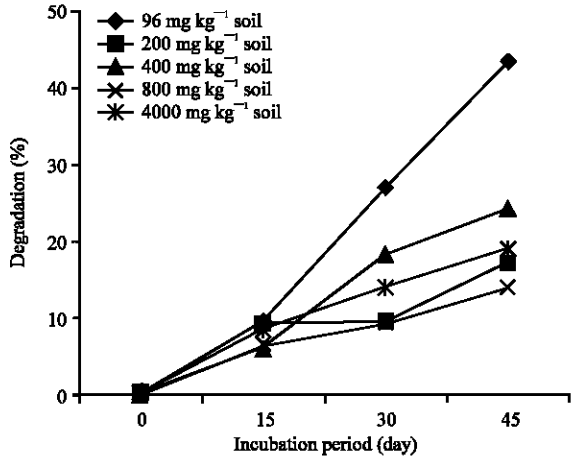


Fig. 10: Degradation percentages of oxyfluorfen in Gerif soil

degradation (58.6 and 41.8%) was recorded after 30 days at 800 and 4000 mg herbicide kg^{-1} soil. At 45 days, the degradation percentages recorded for all oxyfluorfen concentrations exceeded 70% except for the concentration 4000 mg kg^{-1} soil for which 55% degradation was recorded.

In Gerif soil the degradation percentages of oxyfluorfen were generally very low at all concentrations tested as compared to those observed in Elsilait soil (Fig. 10). After 15 days of incubation only 9.4, 9.5, 6.0, 6.3 and 8.5% were degraded for concentrations (mg kg^{-1} soil) 96, 200, 400, 800 and 4000, respectively. After 30 days of incubation the degradation percentages ranged between 9.4-27.1% while after 45 days the degradation percentages were in the range of 14-43.5%.

DISCUSSION

The results obtained showed that the effect of the pesticide on microbial populations depends on its concentration and the soil type. It has been noticed that soil properties like organic matter, soil texture, inorganic nutrients and pH affect soil microbial populations and persistence of pesticides. It has been generally reported that moisture, temperature, inorganic nutrients and plant cover affect pesticide degradation through their effect on diffusion, leaching and/or microbial

growth and cellular metabolism (Pal *et al.*, 2006; Irvin and Wilderer, 1988; Chin *et al.*, 1997; Somasundaram *et al.*, 1991). Initial application of pesticides may decrease the activity of microorganisms due to their toxicity but later the degraded pesticide would be used by the organisms that need organic nitrogen and carbon for cell proliferation (Chowdhury *et al.*, 2008). The extent of the change in microbial set-up due to pesticide application depends on the chemical structure of the pesticide and the conditions where microbes live. However, regaining this set-up will be affected quickly by stopping pesticide application (Galiulin *et al.*, 2001).

In this study, the application of Oxyfluorfen in low concentrations has increased the number of both organic and inorganic nitrogen users in the two soil types; although the numbers were higher in Elsilait than in Gerif soil. This may be due to higher content of N, P and OM in Elsilait soil. Similar results were obtained by Das and Debnath (2006), who reported the stimulatory effect of oxyfluorfen on the growth of non-symbiotic N_2 fixing bacteria in clay soil. This sustains the earlier reports of Das *et al.* (2003) who observed that application of oxyfluorfen stimulated microorganisms in the rhizosphere soils.

Our results are comparable to the results of Elsaid *et al.* (2010) who found that application of Endosulfan insecticide with urea and phosphate fertilizers increased the total number of inorganic nitrogen using bacteria after 45 days of incubation. A disagreement was reported by Osman *et al.* (2005). The authors did not report any conspicuous effect of Amistar fungicide on organic nitrogen users. Reductions on the total number of bacteria involved in nitrogen transformation in sandy loam soil treated with linuron herbicide, diazinon insecticide or mancozeb and dimethomorph fungicides were reported by Cycon and Piotrowska-Seget (2007). Fungal population, has increased in Elsilait oxyfluorfen-treated soil at all concentrations tested. However, Cycon and Piotrowska-Seget (2007) reported a similar stimulatory effect of Linuron herbicide on soil fungi at 22°C. Similarly, Das *et al.* (2005) reported a marked increase in the number of fungi in soil treated with the insecticide phorate. Out of fifteen pesticides studied only endosulfan has been found to depress soil fungal population (Iqbal *et al.*, 2001).

Although the numbers of *Mycobacterium* spp. counted in both soils after the addition of the herbicide were very low, there is a little increment observed in Gerif soil only when low oxyfluorfen concentrations were used. In the same way, results obtained by Taiwo and Oso (1997) indicated a marked reduction in the numbers of *Mycobacterium* spp. in sandy loam soil treated with atrazine and pyrethrin herbicides. These results are in contradiction to the results of Osman *et al.* (2005) who studied the effect of azoxystrobin fungicide on soil microorganisms capable of growing on poor media at 40°C. They found that, the fungicide stimulated the growth of *Arthrobacter* spp., *Mycobacterium* spp. and *Nocardia* spp.

Somasundaram *et al.* (1991) found that soil type has a profound influence on the persistence of pesticides and their transformation products. Similarly, Yen *et al.* (2003) indicated that the half life of oxyfluorfen ranged from 72 to 160 days depending on soil conditions. Serrano *et al.* (2010) found that oxyfluorfen and pendimethalin were degraded to a greater extent in the Biosolarization than in the Solarization treatment. Results presented in this study claim that oxyfluorfen degradation was higher in Elsilait soil than in Gerif soil. This could be possibly attributed to the availability of organic matter, soil pH, soil texture and nutrients content. The degradation of pesticides in soils well correlates with increase in organic matter content (Liu *et al.*, 2006). These organic carbonaceous materials were preferably utilized by the microorganisms for their growth and metabolism (Das *et al.*, 2003). Similar justifications were also reported by Pal *et al.* (2005) using pencycuron pesticide. pH plays an important role in the degradation

of organochlorine insecticides which persist longer in acidic than in alkaline soil (Matsumura, 1985). The mobility of acidic herbicides, Organophosphorus pesticides and atrazine herbicide were found to be higher in soils with higher pH (Somasundaram *et al.*, 1991). Soil particles can absorb pesticides, regulate their bioavailability and influence their persistence (Pal *et al.*, 2006). Both adsorption and absorption depends on the concentration and solubility of herbicides in soil solution, ion exchange capacity, organic matter content, pH, moisture and temperature of soil. Soils with heavy mechanical composition have a higher pesticide-absorbing capacity than light (sandy) soil (Barcelo and Hennion, 2003).

Addition of chemical fertilizers alters pesticides persistence because these nutrients increase microbial activity leading to pH change and causing pesticides hydrolysis (Mohamed *et al.*, 2011; Galiulin *et al.*, 2001).

CONCLUSIONS

Application of oxyfluorfen in low concentrations increased the numbers of both organic and inorganic nitrogen users in the two soil types, although the numbers were higher in Elsilait loam soil than in Gerif silt loam soil. Fungal population has increased in Elsilait loam soil at all oxyfluorfen concentrations. *Mycobacterium* was inhibited from the beginning up to the end of the incubation period (45 days). Although started after 15 days, high degradation percentages of oxyfluorfen were recorded at all concentrations tested. However, degradation was higher in Elsilait loam soil compared to Gerif silt loam soil.

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