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Degradation of Oxyfluorfen Herbicide by Soil Microorganisms Biodegradation of Herbicides

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Abstract: Three laboratory experiments were carried out to study the biodegradation of Oxyfluorfen herbicide (Goal) in a soil with no history of pesticides application at two temperatures (28 and 40°C) with or without the addition of mineral fertilizers (NPK). Different concentrations of Oxyfluorfen were applied to the soil samples and the herbicide residue was determined using Gas Liquid Chromatography (GLC) at 0 time and then at 15 days intervals for 45 days. Various genera of microorganisms were isolated on different semi-selective media from soils treated with different concentrations of Oxyfluorfen. The ability of these isolates to utilize Oxyfluorfen as a sole source of carbon and energy was studied. Results indicated that biodegradation of Oxyfluorfen in soil incubated at 40°C after 45 days of incubation was better (55.2-78.3%) than in soil incubated at 28°C (17.5-36.6%). Addition of mineral fertilizers (NPK) increased the biodegradation of Oxyfluorfen in soils. Intensive degradation (27.8-55.5%) was observed in NPK fertilized soils incubated at 40°C after 15 days of incubation at all Oxyfluorfen concentrations. Ten potential Oxyfluorfen degraders were identified. Results obtained showed that within 21 days, *Bacillus* spp. had the ability to degrade 80-95.6% of Oxyfluorfen followed by *Pseudomonas* sp. (82.2%), *Arthrobacter* spp. (82.2%), *Aspergillus* sp. (77.8%), *Mycobacterium* sp. (75.6%), *Micrococcus* sp. (73.3%) and *Streptomyces* sp. (68.9%). It could be concluded that biodegradation of Oxyfluorfen in soils is significantly affected by temperature and the microbial species. These microorganisms are considered as potential candidates for use in any program aiming at decontamination of Oxyfluorfen polluted sites.

Key words: Biodegradation, oxyfluorfen, NPK, residue, goal

INTRODUCTION

Pesticides are toxic substances that control pests which may interfere directly with our health, production and protection of food and our prosperity. Agriculture and vector-control programs are the greatest users of pesticides; as significant amounts are used in animal, plant and public health pest control (Yadav, 2010).

However, the residues of an applied pesticide remain in the environment for variable periods of time (Jilani and Khan, 2004). The persistence of pesticide residues in soil may poses serious threats to the environment and indeed can lead to acute and chronic health effects such as cancer, central nervous system disorders, birth defects, genetic mutation, sterility and even death (Khalil, 2003; Silva *et al*., 2007). Moreover, the improper disposal of hazardous pesticides from manufacturing plants, surface runoff, leaching, accidental spills and other sources have also contaminated soil, ground water and surface water (Neumann *et al*., 2002; Sudo *et al*., 2002).

Preliminary reports (Abdelbagi *et al*., 2000, 2003) indicated that soil microorganisms are potential degraders cleaning highly polluted soils and dump sites; a process known as bioremediation (Nwachukwu and Osuji, 2007). Bioremediation of different pesticides have been tried by various investigators in different countries with variable success (Imran *et al*., 2004; Yu *et al*., 2005; Osman, 2006; Silva *et al*., 2007).

Introduction of pesticides in the Sudan probably goes back to 1907 when arsenite of soda was tested for control of locusts. In 1970 Goal (Oxyfluorfen); which is a contact herbicide, was used in most of irrigated schemes alone or as a mixture with Diuron in areas of cotton, ground nut, broad beans, fruits, vegetables and in non-crop areas. According to Banaga (1991), the area treated with Goal in Gezira scheme increased drastically from approximately 14700 hectares in 1976 to 105000 hectares in 1990. Oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene) is a diphenyl ether herbicide. It is a selective pre-and postemergent herbicide used to

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control certain annual broadleaf and grassy weeds. It acts by inhibiting protoporphyrinogen oxidase and causes disruption of cell membranes and may also act as electron transport inhibitors. Toxicity symptoms include leaf chlorosis and necrosis caused by the loss of cell membrane integrity (U.S.EPA/OPP, 2002).

Oxyfluorfen is considered to be highly resistant to degradation in soil environments since it is not hydrolyzed at pH 5, 7 or 9 (U.S. EPA, 1992). It is estimated that only 15% of the Oxyfluorfen applied to a soil surface is degraded within 28 days while laboratory studies indicated that its soil half-life was 6 months (U.S. EPA, 1984). Chakraborty *et al.* (2002) studied the degradation of Oxyfluorfen by *Azotobacter chroococcum* in sterilized mineral salt medium. This bacterium degraded more than 60% of the added Oxyfluorfen within 7 days, after which the Oxyfluorfen concentration was gradually reduced from 240 to 96.22 ppm and the bacterium utilized Oxyfluorfen as a sole carbon source.

The objective of this study was to isolate, characterize and identify microorganisms capable of degrading Oxyfluorfen herbicide in soil.

MATERIALS AND METHODS

Collection of soil samples: This study was conducted during June 2006-Oct. 2007. The samples were taken at 10 cm depths from Elsilait site, Khartoum State, Sudan, where there is no history of pesticides application.

Media: Six different media were used throughout this study as recommended by Tepper *et al.* (1993), Moubasher (1993) and Schmidt and Wolff (1997). These were: Meat Peptone Agar (MPA), Starch Ammonium Agar (SAA), Nitrate Agar (NA), Czapeks Dox Agar (CZA), Malt Extract Agar (MEA) and Mineral Salt (MS) medium.

Each medium was sterilized by autoclaving at 121°C under a pressure of 15 Ib/in^2 for 15 min.

Biodegradation of oxyfluorfen herbicide in soils: Oxyfluorfen 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 0.0, 96, 200, 400, 800 and 4000 mg kg^{-1} soil and mixed well. Two sets of beakers were then incubated in the dark, one set at 28 and the other at 40°C, for 45 days. In another experiment, soil samples were fertilized with Nitrogen, Phosphorus and Potassium (NPK fertilization) before the addition of Oxyfluorfen at different concentrations. Nitrogen in the form of urea, Potassium in the form of KCl and Phosphate in the form of super-phosphate were added at zero time at a rate of 375, 187.5, 178.5 mg per beaker, respectively. A control set of fertilized soil to which no pesticide was added has been included.

Isolation and maintenance of oxyfluorfen degraders: Serial dilution technique and the spread plate method (Tepper *et al.*, 1993) were used for isolation of Oxyfluorfen degrading microorganisms from 10 g Oxyfluorfen-wetted-soil at zero time and then at 15 days intervals for 45 days. At each interval, tentative Oxyfluorfen degraders were isolated on each of the recommended media (MPA, SAA, NA and CZA).

Determination of oxyfluorfen residues in 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. Injector port, oven and detector temperatures were 260, 215 and 290°C, respectively.

Microbial degradation of oxyfluorfen in mineral salt liquid media: The 50 mL of MS medium supplemented with 0.02% Oxyfluorfen was inoculated with a pure culture of each of the tentative Oxyfluorfen degraders and incubated at 28°C for 21 days. At the end of the incubation period, five ml of each culture was extracted in hexane (Budde, 1996) and analyzed for residual Oxyfluorfen by GLC as described above.

Identification of oxyfluorfen degraders: Bacterial Oxyfluorfen degraders were identified following recommended standard cultural, microscopical and biochemical characteristics (Collins *et al.*, 1995; Collee *et al.*, 1996).

Fungal degraders were identified by microscopical and cultural characteristics in MEA as well as in CZA media as suggested by Moubasher (1993) and Schmidt and Wolff (1997).

RESULTS

Biodegradation of Oxyfluorfen herbicide in soils incubated at 40°C: Table 1 shows that high degradation percentages ranging between 55.2-78.3% were recorded after 45 days for all concentrations tested. Intensive degradation (28.1, 62.5 and 42.8%) of Oxyfluorfen started after 15 days of incubation at concentrations of 96, 200 and 400 mg kg^{-1} soil, while at 800 mg kg^{-1} soil and at 4000 mg kg^{-1} soil obvious degradation (58.6 and 41.8%)

Table 1: Residues and degradation percentages of oxyfluorfen in soils incubated at 40°C

Days	Concentration of oxyfluorfen (mg kg^{-1} soil)	Residues of oxyfluorfen (mg kg^{-1} soil)	Degradation (%)
15	96	69	28.1
	200	75	62.5
	400	229	42.8
	800	745	6.9
	4000	3761	6.0
30			
	96	40	58.3
	200	75	62.5
	400	200	50.0
	800	331	58.6
	4000	2328	41.8
45			
	96	27	71.9
	200	50	75.0
	400	87	78.3
	800	183	77.0
	4000	1791	55.2

Table 2: Residues and degradation percentages of oxyfluorfen in soils incubated at 28°C

Days	Concentration of oxyfluorfen (mg kg^{-1} soil)	Residues of oxyfluorfen (mg kg^{-1} soil)	Degradation (%)
15	96	87	9.4
	200	179	10.5
	400	368	8.0
	800	588	26.5
	4000	3 441	14.0
30			
	96	87	9.4
	200	158	21.0
	400	352	12.0
	800	576	28.0
	4000	3365	15.9
45			
	96	61	36.6
	200	137	31.5
	400	256	36.0
	800	553	30.9
	4000	3302	17.5

was started after 30 days of incubation. After 15 days of incubation 62.5 and 42.8% of the herbicide present were degraded at 200 and 400 mg kg^{-1} soil, respectively. No further degradation of Oxyfluorfen was observed in soil treated with 200 mg kg^{-1} soil. However, at 800 and 4000 mg kg^{-1} soil a drastic increase in Oxyfluorfen degradation was observed after 30 days.

Biodegradation of oxyfluorfen herbicide in soils incubated at 28°C: At 28°C the degradation percentages of Oxyfluorfen were generally very low at all concentrations tested as compared to those observed at 40°C (Table 2). After 15 days of incubation only 9.4, 10.5, 8, 26.5 and 14% were degraded for concentrations (mg kg^{-1} soil) 96, 200, 400, 800 and 4000, respectively.

After 30 days of incubation, moderate variations were observed in the degradation percentages for most

Table 3: Residues and degradation percentages of oxyfluorfen in soils fertilized with NPK and incubated at 40°C

Days	Concentration of oxyfluorfen (mg kg^{-1} soil)	Residues of oxyfluorfen (mg kg^{-1} soil)	Degradation (%)
15	96	56	41.7
	200	89	55.5
	400	289	27.8
	800	561	29.9
	4000	1950	51.3
30			
	96	22	77.1
	200	59	70.5
	400	222	44.5
	800	322	59.8
	4000	260	93.5
45			
	96	17	82.3
	200	44	78.0
	400	74	81.5
	800	104	87.0
	4000	120	97.0

applications except for herbicide application at 200 mg kg^{-1} soil where it doubled. After 45 days of incubation, only 36.6, 31.5, 36, 30.9 and 17.5% were degraded at concentrations (mg kg^{-1} soil) of 96, 200, 400, 800 and 4000, respectively.

Biodegradation of oxyfluorfen herbicide in soil treated with NPK: The effect of mineral fertilizers (NPK) on microbial abilities to degrade Oxyfluorfen was studied. The results (Table 3) showed that intensive degradation of Oxyfluorfen started after 15 days of incubation for all of the herbicide concentrations tested. Percentages of Oxyfluorfen degradation (41.7 and 51.3%) were observed for soil applications of 96 and 4000 mg kg^{-1} soil, respectively. After 30 days of incubation, 77.1, 70.5, 44.5, 59.8 and 93.5% were degraded for the concentrations 96, 200, 400, 800 and 4000 mg kg^{-1} soil, respectively. However, after 45 days of incubation and at 4000 mg kg^{-1} soil concentration, 97% of Oxyfluorfen was degraded, while at 96 mg kg^{-1} soil, 82.3 degradation percentage was observed.

Biodegradation of oxyfluorfen in mineral salt liquid medium: Ten potential Oxyfluorfen degraders were identified based on their cultural, microscopical and physiological characteristics according to Bergey's Manual of Systematic Bacteriology (Garrity *et al*., 2001). The ability of these degraders to utilize Oxyfluorfen as a sole source of energy and carbon was assessed in mineral salt liquid medium. After 21 days of incubation, all of the isolates have displayed different degradation rates in the range of 35.6-95.6% (Table 4). Degradation with KSB 8 (*Bacillus* sp.), KSB 12 (*Bacillus* sp.), KSB 15

Table 4: Potentialities of different isolates to degrade oxyfluorfen in mineral salt liquid medium

Treatment (inoculation by the isolates)	Source	Residue of oxyfluorfen (mg mL^{-1}) after 21 days incubation	Degradation (%)	Identification
Control (liquid medium)	-	0.045*	00.0	-
KSB 8	Oxyfluorfen-treated soil (4000mg kg^{-1}) +NPK	0.002	95.6	*Bacillus* sp.
KSB 12	Oxyfluorfen-treated soil (200 mg kg^{-1}) +NPK	0.007	84.4	*Bacillus* sp.
KSB 15	Oxyfluorfen-treated soil (400 mg kg^{-1})	0.008	82.2	*Arthrobacter* sp.
KSB 2	Oxyfluorfen-treated soil (800 mg kg^{-1})	0.008	82.2	*Pseudmonas* sp.
KSB 6	Oxyfluorfen-treated soil (96 mg kg^{-1}) + NPK	0.009	80.0	*Bacillus* sp.
KSB 19	Oxyfluorfen-treated soil (800 mg kg^{-1})	0.010	77.8	*Aspergillusterreus*
KSB 9	Oxyfluorfen-treated soil (400 mg kg^{-1}) + NPK	0.011	75.6	*Mycobacterium*sp.
KSB 14	Oxyfluorfen-treated soil (4000mg kg^{-1})	0.012	73.3	*Micrococcus* sp.
KSB 18	Oxyfluorfen-treated soil (200 mg kg^{-1})	0.014	68.9	*Streptomyces* sp.
KSB 16	Oxyfluorfen-treated soil (800 mg kg^{-1})	0.029	35.6	*Arthrobacter* sp.

*The initial oxyfluorfen concentration

(*Arthrobacter* sp.), KSB 2 (*Pseudomonas* sp.) revealed high degradation percentages of 95.6, 84.4, 82.2 and 82.2, respectively.

Based on microscopical and cultural characteristics, KSB 19 is tentatively identified as *Aspergillus terreus* according to the guidelines given by Balajee *et al*. (2007).

DISCUSSION

Results obtained in this study demonstrate that degradation of Oxyfluorfen herbicide increases as the temperature increases. The degradation % was found to be better at 40°C than at 28°C at all concentrations tested. It is generally reported that the effect of temperature on pesticides degradation depends on the molecular structure of the pesticide (Buyuksonmez *et al*., 2000). The results presented in this study is in accordance with the results of Castillo and Torstensson (2007) who reported that temperature affects adsorption by altering the solubility and hydrolysis of pesticides in soil. Authors attributed their findings to the fact that adsorption of pesticides is reduced as a result of increasing solubility due to a general increase in temperature. Microbial activity is stimulated by an increase in temperature; the maximum growth and activity of microorganisms in soil occurs at 25-35°C (Hogg, 2005) and pesticide degradation is optimal at a mesophilic temperature range of around 25-40°C (Topp *et al*., 1997). Results obtained in this study is in accordance with the results of Osman (2006) who reported a high biodegradation rate of the fungicide Amistar at higher temperature. Similarly, Mandelbaum *et al*. (1993) reported much higher mineralization of Atrazine herbicide by *Pseudomonas* sp. at 40°C than at 15°C but is minimal at 7°C. Zhu *et al*. (2004) reported faster degradation of Fipronil insecticide at 35 than at 25°C in non-sterile clay-loamy soil.

Results displayed in Table 1 and 3 indicate that the degradation of Oxyfluorfen in soils fertilized with NPK was higher than in non-fertilized soil. The biodegradation of organic compounds is often slow because one or more of the inorganic nutrients needed for microbial growth are in low concentration in natural environments (Jean *et al*., 2008). It was also suggested that the addition of nitrogen and phosphorus may increase the biodegradation of organic compounds (Jean *et al*., 2008). Application of chemical fertilizers and manure to soil stimulates growth of microorganisms in the soil (Parham *et al*., 2003; Alamri, 2009). In the same way, Atagana (2004) and Jones and Alexander (1988) found that adding N and P to the soil enhances microbial growth and the biodegradation of p-cresol and p-nitrophenol. Similar results were obtained by McGhee and Burns (1995) who reported that NPK fertilizer significantly increased degradation of 2,4-D and MCPA by *Xanthomonas maltophilia*.

Biodegradation of diphenyl ether herbicides were studied by many researchers; Takase *et al*. (1986) isolated a *Pseudomonas cruciviae* strain that utilized diphenyl ether as a source of carbon and energy. Fortina *et al*. (1996) isolated four spore-forming bacteria that were able to degrade acifluorlen. They found that the degrading strains belonged to the species *Bacillus thuringiensis*, *Clostridium perfringens* and *Clostridium sphenoides*. Furthermore, Hiratsuka *et al*. (2001) reported the degradation of diphenyl ether herbicides by the lignin-degrading basidiomycete *Coriolus versicolor*.

Keum *et al*. (2008) reported that nitrodiphenyl ether herbicides, including Oxyfluorfen were rapidly degraded by the bacterial strain *Sphingomonas wittichii*. Similarly, Niki and Kuwatsuka (1976) found that *Bacillus subtilis* and *Bacillus megatherium* degraded about 24-79% of Oxyfluorfen in nutrient broth medium whereas *Pseudomonas* spp. degraded about 18-52% of Oxyfluorfen after 2 days of incubation under the same conditions.

Chakraborty *et al*. (2002) studied the degradation of Oxyfluorfen by *Azotobacter chroococcum* in mineral salt medium and reported that the bacterium degraded more than 60% of the added Oxyfluorfen within 7 days.

CONCLUSION

Present results showed that high degradation percentages (82.2-95.6%) of Oxyfluorfen herbicide were obtained by species of the genera *Bacillus*, *Arthrobacter* and *Pseudomonas*. We recommend the use of these microorganisms for the decontamination of heavily polluted sites or agricultural fields. Since the biodegradation of Oxyfluorfen was significantly better at 40°C it would be friendlier to the environment to use this herbicide with summer crops in Sudan and other tropical regions.

REFERENCES

Abdelbagi, A.O., M.A. Elmahi and D.G. Osman, 2000. Residues of some chlorinated hydrocarbon insecticides in soil of limited or no pesticide use in the Sudan. Arab J. Plant Prot., 18: 35-39.

Abdelbagi, A.O., A.M. Elmahi and D.G. Osman, 2003. Organochlorine insecticides residues in Sudanese soil of intensive pesticide use and in surface soil of Qurashi pesticide store. U. K. J. Agric. Sci., 11: 59-68.

Alamri, S.A., 2009. Use of microbiological and chemical methods for assessment of enhanced hydrocarbon bioremediation. J. Biol. Sci., 9: 37-43.

Atagana, H.I., 2004. Biodegradation of phenol, o-cresol, m-cresol and p-cresol by indigenous soil fungi in soil contaminated with creosote. World J. Microbiol. Biotechnol., 20: 845-849.

Balajee, S.A., J. Houbraken, P.E. Verweij, S.B. Hong, T. Yaghuchi, J. Varga and R. A. Samson, 2007. *Aspergillus* species identification in the clinical setting. Stud. Mycol., 59: 39-46.

Banaga, A., 1991. Pesticide Use in Gezira Workshop on Effects of Pesticides on Health. Wad Medani, Sudan.

Budde, W.L., 1996. Analysis of Organohalide Pesticides and Commercial Polychlorinated Biphenyl Products in Water by Micro-Extraction and Gas Chromatography. The Manual of Manuals: Laboratory Analytical Methods Manual. Environmental Protection Agency Environmental Monitoring Systems Laboratory

Buyuksonmez, F., R. Rynk, T.F. Hess and E. Bechinski, 2000. Occurrence, degradation and fate of pesticides during composting. Part II: Occurrence and fate of pesticides in compost and composting systems. Comp. Sci. Utiliz., 8: 61-81.

Castillo, M.P. and L. Torstensson, 2007. Effect of biobed composition, moisture and temperature on the degradation of pesticides. J. Agric. Food Chem., 55: 5725-5733.

Chakraborty, S.K., A. Bhattacharyya and A. Chowdhury, 2002. Degradation of oxyfluorfen by *Azotobacter chroococcum* (Beijerink). Bull. Environ. Contam. Toxicol., 69: 203-209.

Collee, J.G., A.G. Fraster, B.P. Mamion and A. Simmons, 1996. Mackie and McCartney Practical Medical Microbiology. 14th Edn., Longman, Singapore, New York. 978.

Collins, C.H., P.M. Lyne and J.M. Grange, 1995. Collins and Lyn's Microbiological Methods. 7th Edn., Butterworth-Heinemann Ltd, England. 493.

Fortina, M.G., A. Acquati and R. Ambrosoli, 1996. Identification of spore-forming strains involved in biodegradation of acifluorfen. Res. Microbiol., 147: 193-199.

Garrity, G.M., M. Winters and D.B. Searles, 2001. Taxonomic Outline of the Procaryotic Genera Bergey's Manual of Systematic Bacteriology. 2nd Edn., Bergey's Manual. New York. 1414.

Hiratsuka, N., H. Wariishi and H. Tanaka, 2001. Degradation of diphenyl ether herbicides by the lignin-degrading basidiomycete *Coriolus versicolor*. Applied Microbiol. Biotechnol., 57: 563-571.

Hogg, S., 2005. Essential Microbiology. Jon Wiely and Sons, Ltd., West Sussex, England. pp: 468.

Imran, H., K.M. Altaf and J.G. Kim, 2004. Malathion degradation by pseudomonas using activated sludge treatment system (Biosimulator). Biotechnology, 3: 82-89.

Jean, J.S., M.K. Lee, S.M. Wang, P. Chattopadhyay and J.P. Maity, 2008. Effects of inorganic nutrient levels on the biodegradation of benzene, toluene and xylene (BTX) by *Pseudomonas* spp. in a laboratory porous media sand aquifer model. Biores. Technol., 99: 7807-7815.

Jilani, S. and M.A. Khan, 2004. Isolation, characterization and growth response of pesticides degrading bacteria. J. Biological Sci., 4: 15-20.

Jones, S.H. and M. Alexander, 1988. Phosphorus enhancement of mineralization of low concentrations of p-nitrophenol by *Flavobacterium* sp. in lake water. FEMS Microbiol. Lett., 52: 121-126.

Keum, Y.S., Y.J. Lee and J.H. Kim, 2008. Metabolism of nitrodiphenyl ether herbicides by dioxin-degrading bacterium *Sphingomonas wittichii* RW1. J. Agric. Food Chem., 56: 9146-9151.

Khalil, A.B., 2003. Isolation and characterization of 2,4-dichlorophenoxyacetic acid degrading organisms from soil in Jordan valley. Biotechnology, 2: 73-85.

Mandelbaum, R.T., L.P. Wackett and D.L. Allan, 1993. Mineralization of the s-triazine ring of atrazine by stable bacterial mixed culture. Applied Environ. Microbiol., 59: 1695-1701.

McGhee, I. and R. Burns, 1995. Biodegradation of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) in contaminated soil. Applied Soil. Ecol., 2: 143-154.

Moubasher, A.H., 1993. Soil Fungi in Qatar and other Arab Countries. Center of Scientific and Applied Research, University of Qatar, Doha, Qatar, ISBN-13: 9992121025, pp: 566.

Neumann, M., R. Schulz, K. Schafer, W. Muller, W. Mannheller and M. Liess, 2002. The significance of entry routes as point and non-point sources of pesticides in small streams. Water Res., 36: 835-842.

Niki, Y. and S. Kuwatsuka, 1976. Degradation of diphenyl ether herbicides in soil. Soil Sci. Plant Nutr., 22: 223-232.

Nwachukwu, E.O. and J.O. Osuji, 2007. Bioremedial degradation of some herbicides by indigenous white rot fungus, *Lentinus subnudus*. J. Plant Sci., 2: 619-624.

Osman, A.G., 2006. Degradation of the fungicide azoxystrobin by soil microorganism. U. of K. Agric. Sci., 14: 124-134.

Parham, J.A., S.P. Deng, H.N. Da, H.Y. Sun and W.R. Raun, 2003. Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. Biol. Fertil. Soils, 38: 209-215.

Schmidt, A. and M.H. Wolff, 1997. Morphological characteristics of Aspergillus fumigates strains isolated from patient samples. Mycoses, 40: 347-351.

Silva, T.M., M.I. Stets, A.M. Mazzetto, F.D. Andrade and I.B. Carneiro, 2007. Degradation of 2,4-d herbicide by microorganisms isolated from Brazilian contaminated soil. Brazil. J. Microbiol., 38: 522-525.

Sudo, M., T. Kunimatsu and T. Okubo, 2002. Concentration and loading of pesticide residue in lake Biva basin(Japan). Water Res., 36: 315-329.

Takase, I., T. Omori and Y. Minoda, 1986. Microbial degradation products from biphenyl related compounds. Agric. Biol. Chem., 50: 681-686.

Tepper, E.Z., V.K. Shilnikova and G.I. Preverzeva, 1993. A Manual of Microbiology. 4th Edn., Kolas Publishers, Moscow, pp: 170.

Topp, E., T. Vallaeys and G. Soulas, 1997. Pesticides Microbial Degradation and Effects on Microorganisms. In: Modern Soil Microbiology, Van Elsas, J.D., J.T. Trevors and E.M.H. Wellington (Eds.). Mercel Dekker, Inc., New York, USA., pp: 547-575.

U.S. EPA, 1984. Environmental Effects Branch. Chemical Profile: Oxyfluorfen. US EPA, Washington, DC.

U.S. EPA, 1992. Pesticide Environmental Fate One Liner Summaries: Oxyfluorfen. Environmental Fate and Effects Division, Washington, DC.

U.S.EPA/OPP, 2002. Registration eligibility decision (Red) oxyfluorfen. Washington, DC., http://www.epa.gov/oppsrrd1/REDs/ oxyfluorfen_red.pdf

Yadav, S.K., 2010. Pesticide applications-threat to ecosystems. J. Hum. Ecol., 32: 37-45.

Yu, Y.L., X. Wang, Y.M. Luo, J.F. Yang, J.Q. Yu and D.F. Fan, 2005. Fungal degradation of metsulfuron-methyl in pure cultures and soil. Chemosphere, 60: 460-466.

Zhu, G., H. Wu, J. Guo and F.M.E. Kimaro, 2004. Microbial degradation of fipronil in clay loam soil. Water Air Soil Pollut., 153: 35-44.