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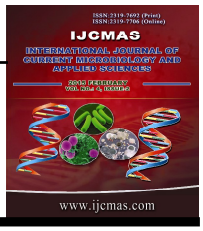

Original Research Article

Interaction Effects of Insecticides on Microbial Populations and Dehydrogenase Activity in Groundnut (*Arachis hypogeae l.*) Planted Black Clay Soil

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Endosulfan, Profenofos, Soil enzyme, Groundnut *(Arachis hypogeae L.)* soil

A B S T R A C T

A laboratory study was conducted to investigate the influence of endosulfan and profenofos on soil bacterial, fungal and actinomycetes populations and the activity of dehydrogenase (measured in terms of TPF formed from TTC) in black clay soil collected from fallow groundnut (*Arachis hypogeae L.*) fields of Kurnool district. Bacterial, fungal populations and dehydrogenase activity increased with increasing concentration of the pesticides up to 5.0 kg ha^{-1}, where as actinomycetes population increases up to 2.5 kg ha^{-1}. Higher rates of (7.5, 10.0 kg ha^{-1}) these pesticides were either toxic/innocuous to the urease activity or microbial population.

Introduction

Study of the effect of pesticides on soil micro flora and their beneficial activities forms an important part of the pesticides "risk assessment". The intensive use of these pesticides in agricultural soils, there may be an interaction with soil micro flora and their metabolic activities besides, controlling the different pest population (Baxter and Cummings, 2008). Therefore the behavior of the total micro flora and their biological activities under continuing pesticide input is an important aspect of research of the agricultural ecology (Li *et al.*, 2008). Soil microbes are the driving force behind many soil processes, including the transformation of organic matter, nutrient release and degradation of xenobiotics (Zabaloy *et al.*, 2008). Actinomycetes and fungi are important microbes for the degradation and utilization of a wide range of complex organic molecules (Lacey, 1983; Watson and Williams, 1974). Bacteria account for almost half of the soil microbial biomass (Alexander, 1997) and are responsible for further degradation. Microflora of soils are

of major concern because of their role in sustaining agricultural productivity through various biochemical reactions mediated by soil enzymes (Mahía *et al.*, 2008; Madakka and Rangaswamy, 2009). In spite of the maximum potential of soil enzymes in maintaining soil biodynamics, Profenofos (*0*-4-bromo-2-chlorophenyl-*O*-ethyl-*S* propylphosphorothioate), it is a non systemic insecticide and acaricide with contact and stomach action used against mites, leafhoppers, thrips, aphids, mealy bugs and cotton stainer.

Endosulfan is a chlorinated cyclodiene insecticide currently used throughout the world for the control of numerous insects in a wide variety of food and non food crops. Due to its high degree of toxicity it persists in soils. Although this pesticide have been restrictively used or even banned their persistence and bioaccumulation still be found in soils. Thus it is required to estimate soil biological responses to the pesticides. So from past 10 decades more specific prominence has been given to soil enzymes because these are indicators of biological equilibrium (Frankenberger and Tabatabai, 1991), fertility (Antonious, 2003), quality (Bucket and Dick, 1998) and changes in the biological status of soil due to pollution (Nannipieri *et al.*, 1990; Trasar-Cepeda, 2000).

Some of the microbial processes for assessing the effects of contaminants on soil health include dehydrogenase; an intracellular enzyme belonging to oxidoreductases present in all soil microorganisms used as a measure of total microbial activity in soil (Trevors, 1984). Dehydrogenase activities are very important for soil quality. Soil dehydrogenase is a specific kind of enzyme which plays a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors (Sebiomo *et al.*, 2011). Only limited studies were available on the influence of soil enzymes with Agrochemicals (Kalyani *et al.*, 2009). Hence, an attempt was made in this study to find out the interaction effects of these pesticides on dehydrogenase and microbial populations in the black clay soil of agricultural importance. Hence the present study has been undertaken to investigate the effect of endosulfan and profenofos on microbial population and dehydrogenase activity in groundnut cultivated black clay soil.

Materials and Methods

Soil

A black clay soil from different sites of groundnut cultivated fields of Kurnool district of Andhra Pradesh, India collected randomly near the rhizosphere zone using trowel at a depth of 0–12 centimeters and mixed thoroughly to prepare a homogeneous composite sample, air dried at room temperature samples were cleaned by removing plant material and other debris and passed through 2 millimeter sieve, stored at 4^{o}C prior to analysis. Mineral matter of soil samples was done by following the method (Jackson, 1971).

Soil pH was determined by using 1:1.25 soils to water ratio in systronic digital pH meter. Organic matter in soil samples was estimated by Walkley - Black oxidation, total nitrogen content in soil samples was determined by Micro- Kjeldahl method (Johnson and Ulrich, 1960). Electrical conductivity was measured by Conductivity Bridge and the contents of nitrite – nitrogen (Barnes and Folkard, 1951) contents of nitrate – nitrogen by Brucine method (Ranney and Bartlett, 1972). The important Physico-chemical properties of the black clay soil are presented in Table 1.

Insecticides

To determine the influence of selected insecticides on soil enzyme activities, endosulfan an organochlorine insecticide (35% emulsifying concentration) was obtained from the Hoechst Schering Agra euro (Ltd). Gujarat and profenofos an organophosphate (50% emulsifying concentration) was obtained from Sudarsha industries Ltd, Pune, India.

Soil incubation Studies

Dehydrogenase activity (E.C. 1.1.1.1)

To study the effect of endosulfan and profenofos on dehydrogenase, 5 g of dried black clay soil was taken separately in test tubes (12 × 125 mm) containing different concentrations of insecticides 10, 25, 50, 75, and 100 µg g^{-1} soil which are equal to 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1} of field application rates. In order to maintain 60% water holding capacity (WHC), about 2 ml of deionized water was added to test tubes containing black clay soil. Untreated soil samples served as controls. All the treatments, including controls were incubated in the dark at 28 ± 4°C for 1, 2, 3, 4, and 5 weeks. During the incubation period certain amount of distilled water was added to maintain the soil WHC. Triplicate soil samples were withdrawn for the enzyme assay.

Assay of dehydrogenase

The method employed for the assay of dehydrogenase was developed by (Casida *et al.*, 1964). This method is based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). Each soil sample was treated with 0.1 g of $CaCO_3$ and 1 ml of 0.18 mm aqueous solutions of TTC and incubated for 24 hours at 30°C. The TPF formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a Spectronic 20 D spectrophotometer (Milton Roy Co.).

Enumeration of Microbial Population

The effect of different concentrations of selected insecticides, endosulfan and profenofos on microbial populations in groundnut fields of black clay soil samples, in triplicates, were determined. Aliquots (0.05 ml) from stock solutions of the pesticides were applied to 5 g portions of soil contained in test tubes (15 × 150 mm). The final concentrations (w/w) of each pesticide included 10, 25, 50, 75 and 100 µg g-1 soil, which are equivalent to 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} (Anderson, 1978). The soil samples received only distilled water served as controls. Soil samples were then homogenized to distribute the pesticide, and enough distilled water was added to maintain at 60% water holding capacity (WHC) and incubated at room temperature (28 ± 4°C).

Seven days after incubation, duplicates of each treatment were withdrawn for estimation of the bacterial population. Aliquots were prepared from 10^{-1} to 10^{-7} from treated and untreated soil samples by serial dilution plate method on nutrient agar medium and subsequently incubated for 24 h in an incubator at 30°C (Shukla and Mishra, 1997). After incubation, bacterial colonies grown on nutrient agar medium were counted by the Quebec colony counter. Bacterial populations were enumerated and expressed as the number of colonies formed per gram of soil (dry weight basis) (Shetty and Magu, 2000). Soil plate method was used to assess fungal propagules developing on Rose Bengal agar medium and subsequently incubated for five days at 25°C (Shukla and Mishra, 1997). The population of actinomycetes was estimated by using Ken Knight's agar medium and

subsequently incubated for 3 days in the dark at 30°C (Balasubramanian and Sankaran, 2001).

Statistical analysis

The activity of dehydrogenase and microbial population was calculated on the basis of soil weight (oven dried). Data were analyzed using one-way ANOVA and the differences contrasted using Duncan's multiple range (DMR) test (Megharaj *et al.,* 1999; Gooty Jaffer Mohiddin *et al.,* 2011). All statistical analysis was performed at ($P \leq 0.05$) using the SPSS statistical software package.

Results and Discussion

Dehydrogenase activity

The black clay soil sample was treated with different concentrations (1.0, 2.5, 5.0, 7.5, and 10 kg ha^{-1}) of endosulfan and profenofos, were incubated for 7 days to determine the selective influence of the two insecticides, on the activity of dehydrogenase by exposing it to triphenyl tetrazolium chloride (TTC).

Dehydrogenase activity was enhanced by the application of endosulfan and profenofos at 5.0 kg ha^{-1} of graded levels in black clay soil, whereas in the concentration of pesticides at levels of 7.5 to 10.0 kg ha^{-1} was either toxic or innocuous to the enzyme activity. The two insecticides, endosulfan and profenofos at 50 ppm levels, individually caused increments of 19-93% and 14–74% in dehydrogenase activity over control, were recorded following 24 hrs of incubation respectively in black clay soil after 7 days (Table 2).

There was a progressive increase in accumulation of formazan was recorded with increase in period of incubation up to 3rd week (Fig. 1).

Effect of insecticides on soil microflora

Bacterial population

Bacterial populations were significantly higher in black clay soil treated with endosulfan and profenofos at 1.0, 2.5, 5.0 and 7.5 kg ha^{-1} than in the untreated control, after 7 days of incubation (Fig. 2). Bacterial population in black clay soil was enhanced with increasing concentrations (up to 7.5 kg ha^{-1}) of two insecticides used in the present study. Stimulation in bacterial populations in the range of 19–56% by endosulfan and 14–60% by profenofos at all three levels i.e., 10, 25 and 50 ppm for 7 days of incubation in black clay soil occurred (Fig. 2). The individual stimulatory effect of monocrotophos, quinalphos and cypermethrin at 5.0 kg ha^{-1} has been confirmed on nitrifiers, nitrogen-fixing organisms and the population of *Azospirillum* sp. in soils of groundnut fields (Rangaswamy, 1990) and similar observations were observed when pesticides profenofos, deltamethrin, difenoconazole, thiram and their combinations viz., profenofos + cypermethrin and deltamethrin + endosulfan on the populations of *Azospirillum* sp. in groundnut soils (Madakka and Rangaswamy, 1999). chlorpyrifos at 10–300 μg g^{-1} decreased the population of bacteria in loamy soil (Martinez-Toledo *et al.,* 1992b).

Whereas profenofos at the same levels increased the population of bacteria (Martinez-Toledo *et al.,* 1992a). In Chinese loamy soils, methamidophos at 0.5, 2.5, 5 and 10 μg g^{-1} inhibited the population of bacteria strongly throughout the incubation period (Xu *et al.,* 1997). No significant change in total viable count of bacteria was observed when treated with phorate, carbofuran, carbosulfan, thiamethoxam, imidacloprid, chlorpyrifos, monocrotophos

both at high and lower concentrations (Sarnaik *et al.,* 2006).

Adversely affected *Rhizobium* sp. The population was found by the application of herbicides, atrazine, isoproturon, metribuzin, and sulfosulfuron in chickpea grown soils in all concentrations (Khan *et al.,* 2006). Wang *et al.,* (2006) concluded that the effect of methamidophos and urea reduced microbial biomass and enhanced functional diversities of soil microbial communities. That is, some species of bacteria might be enriched in soils under methamidophos stress. Similar observations were found by Demanou *et al.,* (2006).

They investigated the effect of a combined application of copper and mefenoxam on the functional diversity of soil and found that microbial populations were increased. In other study benzene and heavy metals reduce the number or diversity of bands in bacterial DGGE gels, indicating toxicity responses (Trevors, 1984).

Sáez *et al.,* (2006) observed the effect of some pesticides on the growth and denitrifying activity of *Xanthobacter autotrophicus* CECT 7064. Chen *et al.,* (2007) and Lin *et al.,* (2007) who investigated the associated impact of inorganic fertilizers, heavy metals, and pesticides on microbial communities in soils.

Similarly, Madhaiyan *et al.,* (2006) who studied the effect of various pesticides on the growth and survival of Gluconacetobacter diazotrophicus strain PAL5. The monocrotophos, lindane and dichlorvos proved lethal to *Gluconacetobacter*, while in case of endosulfan, chlorpyrifos, and malathion effects were intermediate. Recently, Gundi *et al.,* (2005) investigated diverse effects of insecticides on two enzyme activities was in concomitant to populations of cellulolytic and amylolytic microbes in soils treated with insecticides and their combinations. Niewiadomska (2004) and Niewiadomska and Klama (2005) reported the adverse effects of carbendazime, thiram (fungicides), and imazetapir (herbicide) on nitrogenase activity of Rhizobium leguminosarum, Sinorhizobium meliloti, and Bradyrhizobium sp. Wang *et al.,* (2007) demonstrated that the addition of high concentration of butachlor applied in combination with Cd significantly affected the diversity of microbial community.

Fungal Populations

Endosulfan and profenofos were tested for their effects on fungal populations in black clay soil. The data obtained from these experiments were furnished in Figure 3. Fungal populations in black clay soil were increased with increasing concentrations (up to 5 kg ha^{-1}) of all the tested insecticides. Stimulation in fungal populations in the range of 52-148% by endosulfan and 25-100% by profenofos at all three levels i.e., 10, 25 and 50 ppm for 5 days incubation (Fig. 3).

In contrary, In contrary, phorate considerably stimulated a population of fungi in soil than fenvalerate under laboratory conditions (Das and Mukherjee, 1998a). In their study, both insecticides affected fungal composition and diversity in soil by stimulating relative proportion of *Penicillium* and reducing *Rhizophus*. In a similar study, even under field conditions, fenvalerate exerted a stimulatory effect on fungal populations (Das *et al.,* 1995). Sigler and Turco (2002) revealed that the chlorothalonil removed a number of bands from the fungus community DGGE profile of agricultural and turfgrass soils 2 weeks following application.

Actinomycetes Populations

Endosulfan and profenofos were tested for their effects on actinomycetes populations in black clay soil as described. The data obtained from these experiments were furnished in Figure 4.

The data presented in the Figure 4 reveal the impact of different concentrations of insecticides endosulfan and profenofos on actinomycetes population in black clay soil after 7 days of soil incubation. Stimulation in actinomycetes populations in the range of 50-85% by endosulfan and 55-83% by profenofos at all three levels i.e., 10, 25 and 50 ppm for 5 days incubation (Fig. 4). In contrary, Shetty and Magu (2000) reported that metalaxyl at 0.5 ppm, incubated for 4 and 8 weeks significantly stimulated the actinomycete population in a sandy loam soil. Current findings revealed that actinomycete population was inhibited at 10 kg ha^{-1} of the selected insecticide application in black clay soil (Fig. 4). Gundi *et al.,* (2005) studied the effect of three insecticides (monochrotophos, quinalphos, and cypermethrin) on microbial populations in a black clay soil. They observed synergistic effects at the lower level and adverse effects at the highest level of the insecticides. In contrary, toxic effects of pesticides (captan, deltamethrin, isoproturon, and pirimicarb) were observed on freshwater sediment microbial communities even at concentrations predicted to be environmentally safe (Widenfalk *et al.,* 2004). Wang *et al.,* (2007) investigated the combined effect of cadmium (Cd) and butachlor on microbial activity.

Table.1 Physicochemical Characteristics of the Soil

Properties	Black Clay soil
Sand (%)	61.7
Silt (%)	15.2
Clay (%)	23.8
pH[a]	7.4
Water holding capacity (ml g^{-1} soil)	0.31
Electrical conductivity (m.mhos)	260
Organic matter [b] (%)	1.078
Total nitrogen [c] (%)	0.046
NH_4^+ - N (µg g^{-1} soil) [d]	8.97
NO_2^- - N (µg g^{-1} soil) [e]	0.412
NO_3^- - N (µg g^{-1} soil) [f]	1.340

Where a = 1:1.25 = Soil: Water slurry, b = Walkley-Black Method (Jackson, 1971), c = Micro-Kjeldhal Method (Jackson, 1971), d = Nesslerization method (Jackson, 1971), e =Diazotization Method (Barnes and Folkard, 1951), f = Brucine Method (Ranney and Bartlett, 1972)

Table.2 Activity of Dehydrogenase* under the Impact of Different Concentrations of SElected Insectides in Black Clay Soil for 24 hours After 1 Week

Concentration of insecticides (kg ha^{-1})	Black clay soil	
	Endosulfan	Profenofos
	24 hours	24 hours
0.0	57 ± 1.732^{a} (100)	57 ± 1.732^{a} (100)
1.0	68 ± 1.154^{b} (119)	65 ± 2.886^{b} (114)
2.5	86 ± 2.309^{c} (151)	75 ± 2.886^{b} (131)
5.0	110 ± 5.773^{d} (193)	99 ± 0.577^{c} (174)
7.5	78 ± 1.154^{c} (137)	73 ± 1.732^{b} (128)
10.0	55 ± 2.886^{e} (96)	58 ± 1.732^{a} (102)

*µg formazan g^{-1} soil formed after 24 hours incubation with triphenyl tetrazolium chloride (TTC). Figures, in parentheses, indicate relative production percentages.

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Fig. 1 Effect of selected insecticides on dehydrogenase* in black clay soil at 5.0 kg ha^{-1}. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test. Bars in the figures represent means of three replicates

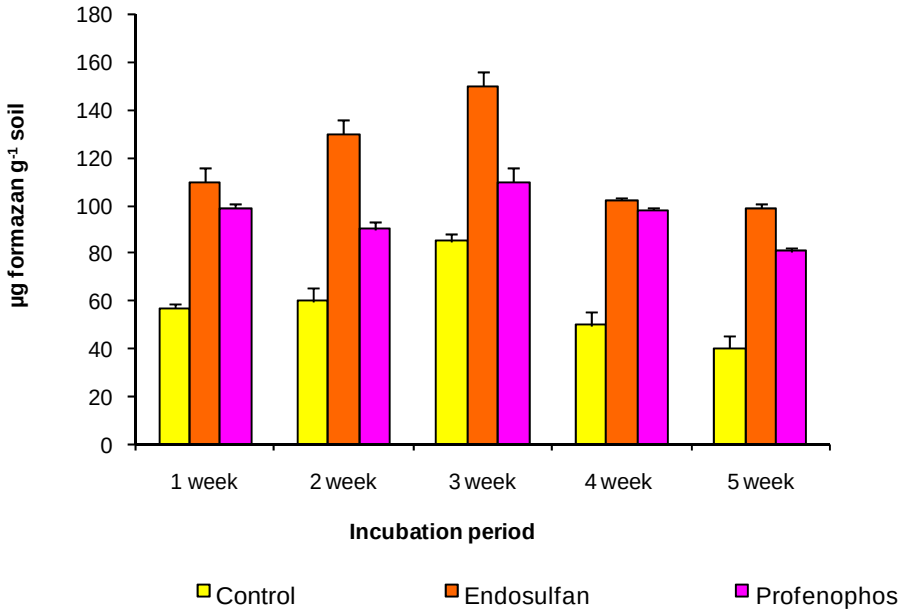
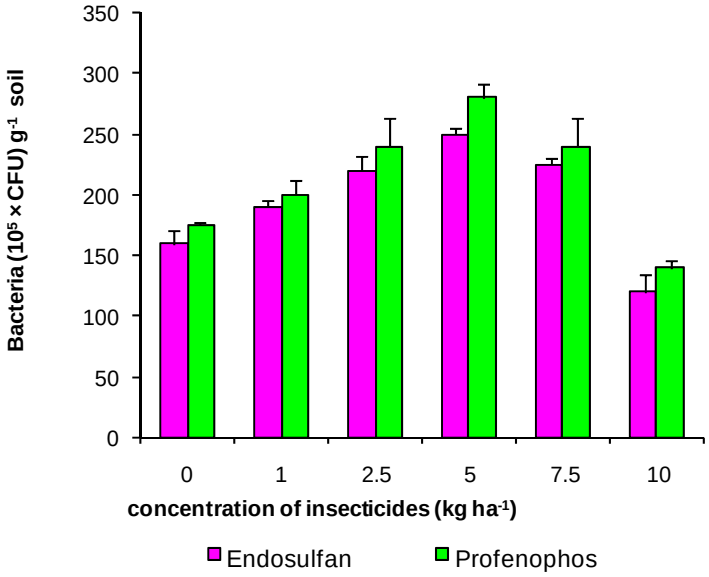
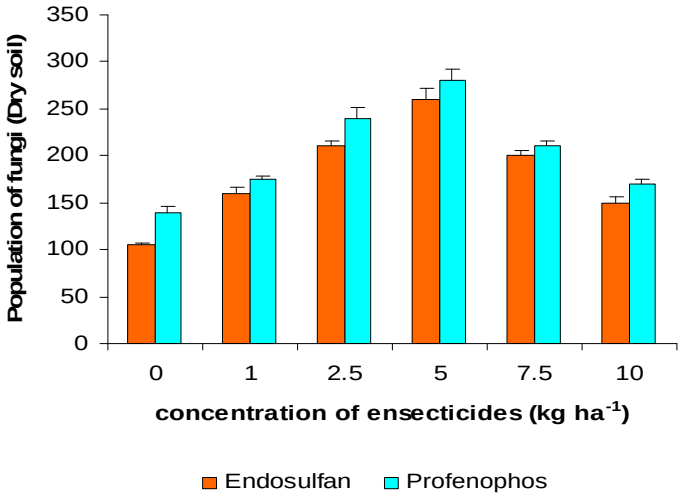


Fig.2 Figures, in parentheses, indicate relative production percentages. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test



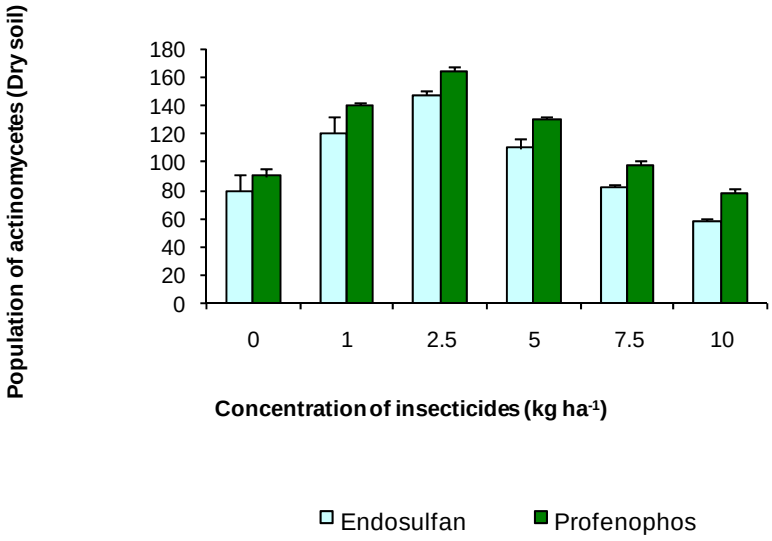
* Number of colonies per gram soil = No. of colonies X Dilution factor
Dry weight of soil

Fig.3 Figures, in parentheses, indicate relative production percentages. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test



* Number of colonies per gram soil = No. of colonies X Dilution factor
Dry weight of soil

Fig.4 Figures, in parentheses, indicate relative production percentages. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.



*Number of colonies per gram soil = No. of colonies X Dilution factor / Dry weight of soil

They demonstrated that the addition of the high concentration of butachlor applied in combination with Cd significantly affected the diversity of microbial communities. Almost similar results were reported by Chen *et al.,* (2007) and Lin *et al.,* (2007) who investigated the associated impact of inorganic fertilizers, heavy metals, and pesticides on microbial communities in soils.

This study has shown that the applications of endosulfan and profenofos to black clay soil increased the enzyme activity and microbial populations up to 5.0 kg ha^{-1} and decreased the activity when increased pesticide concentrations in black clay soil. The stimulation and pronounced activity of dehydrogenase by selective pesticides was at 20 day period of incubation. Prolonged incubation up to (40 days) of insecticides treated soils on the enzyme activity showed no effect. These results of the present study clearly indicate that these insecticides at field application rates enhance the activity of dehydrogenase and microbial populations in soils.

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References

Alexander, M. 1977. Introduction of soil microbiology, 2nd edn., John Wiley and Sons, New York. Pp. 126–167.

Anderson, J.R. 1978. Pesticide effects on non-target soil microorganisms. In:

Hill, I.R., Wright, S.J.L. (Eds), Pesticide microbiology. Academic Press, London, Pp. 313–533.

Antonious, G.F. 2003. Impact of soil management and two botanical insecticides on urease and invertase activity. *J. Environ. Sci. Health - Part B*, 38(4): 479–488.

Balasubramanian, K., Sankaran, S. 2001. Effect of pendimethalin on soil microorganisms. *Indian Agricult.,* 45: 93–98.

Barnes, H., Folkard, A.R. 1951. The determination of nitrites. *Analyst*, 76(907): 599–603.

Baxter, J., Cummings, S.P. 2008. The degradation of the herbicide bromoxynil and its impact on bacterial diversity in a top soil. *J. Appl. Microbiol.*, 104: 1605–1616.

Bucket, J.Z., Dick, R.P. 1998. Microbial and soil parameters in relation to N mineralization in soils of diverse genesis under differing management systems. *Biol. Fertil. Soils*, 27(4): 430–438.

Casida, L.E., Klein, D.H., Santoro, T. 1964. Soil dehydrogenase activity. *Soil Sci.*, 98: 371–376.

Chen, J., Xuliang, Z., Huiju, X., Zhihui, B., Hongyan, Q., Hongxun, Z. 2007. Associated impact of inorganic fertilizers and pesticides on microbial communities in soils. *World J. Microbiol. Biotechnol.*, 23: 23–29.

Das, A.C., Mukherjee, D. 1998a. Insecticidal effects on soil microorganisms and their biochemical processes related to soil fertility. *World J. Microbiol. Biotechnol.,* 14: 903–909.

Das, A.C., Chakravarty, A., Sukul, P., Mukherjee, D. 1995. Insecticides: Their effect on microorganisms and persistence in rice soil. *Microbiol. Res.,* 150: 187–194.

Demanou, J., Sharma, S., Weber, A., Berndt-Michae, W., Njine, T., Monkiedje, A., Munch, J.C., Schloter, M. 2006. Shifts in microbial community functions and nitrifying communities as a result of combined application of copper and mefenoxam. *FEMS Microbiol. Lett.*, 260: 55–62.

Frankenberger, Jr., W.T., Tabatabai, M.A. 1991. 2-glutaminase activity of soils. *Soil Biol. Biochem.,* 23: 869–874.

Gooty Jaffer Mohiddin, Mandala Srinivasulu, Mekapogu Madakka Venkatampalli Rangaswamy, 2011. Influence of selected insecticides on enzyme activities in groundnut (*Arachis hypogaea* L.) soils. *Dyn. Soil Dyn. Plant*, 5(1): 65–69.

Gundi, V.A.K.B., Narasimha, G., Reddy, B.R. 2005. Interaction effects of soil insecticides on microbial populations and dehydrogenase activity in a black clay soil. *J. Env. Sci. Heal.,* 40: 269–281.

Jackson, M.L. 1971. Soil chemical analysis. Prentice Hall, New Delhi, India.

Johnson, C.M., Ulrich, A. 1960. Determination of moisture in plant tissues. *Calif. Agricult. Bull.,* 766: 112–115.

Kalyani, S.S., Sharma, J., Singh, S., Dureja, P., Lata, 2009. Enrichment and isolation of endosulfan degrading microorganism from tropical acid soil. *J. Environ. Sci. Health B*, 44: 663–72.

Khan, M.S., Zaidi, A., Rizvi, P.Q. 2006. Biotoxic effects of herbicides on growth, nodulation, nitrogenise activity, and seed production in chickpeas, *Commun. Soil Sci. Plant Anal.,* 37: 1793.

Lacey, J. 1983. Actinomycetes in soils, compost and foders. In: Skiner, F.A., Sykes, G., (Eds), Actinomycetes: characteristics and practical importance. *Soc. Appl. Bacteriol.*

Symp., Series No. 2; Academic Press: London. Pp. 231–235.

Li, G., Wang, K., Liu, Y.H. 2008. Molecular cloning and characterization of a novel pyrethroid hydrolyzingesterase originating from the Metagenome. *Microb. Cell Factories*, 7: 38.

Lin, Q., Zhao, H.M., Chen, Y.X. 2007. Effects of 2,4-dichlorophenol, pentachlorophenol and vegetation on microbial characteristics in a heavy metal polluted soil. *J. Environ. Sci. Health B.,* 42: 551–557.

Madakka, M., Rangaswamy, V. 2009. Effect of pesticides and insecticide combinations on *Azosirillum* sps in groundnut soils. *Pollut. Res.,* 28: 105–109.

Madhaiyan, A., Poonguzhali, S., Hari, K., Saravanan, V.S., Sa, T., 2006. Influence of pesticides on the growth rate and plant-growth promoting traits of *Gluconacetobacter diazotrophicus. Pest. Biochem. Physiol.,* 84: 143–154.

Mahía, J., Cabaneiro, A., Carballas, T., Díaz-Raviña, M. 2008. Microbial biomass and C mineralization in agricultural soils as affected by atrazine addition. *Biol. Fertile soils,* 45: 99–105.

Martinez-Toledo, M.V., Salmeron, V., Gonzalez-Lopez, J. 1992b. Effect of the insecticides pyrimifos-methyl and chlorpyrifos on soil microflora in an agricultural loam. *Plant Soil,* 147: 25–30.

Martinez-Toledo, M.V., Salmeron, V., Gonzalez-Lopez, J. 1992a. Effect of an organophosphorus insecticide, profenofos, on agricultural soil microflora. *Chemosphere,* 24: 71–80.

Megharaj, M., Singleton, I., Kookana, R., Naidu, R. 1999. Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil. *Soil Biol. Biochem.,* 31(11): 1549–1553.

Nannipieri, P., Grego, S., Ceccanti, B. 1990. Ecological significance of the biological activity in soil. In: Bollag, J.-M., Stozky, G. (Eds), Soil biochemistry, Vol. 6. Marcel Dekker, New York, NY, USA. Pp. 293–355.

Niewiadomska, A. 2004. Effect of carbendazim, imazetapir and thiramon nitrogenase activity, the number of microorganisms in soil and yield of red clover (*Trifolium pretense L*). *Pollut. J. Environ. Stud.,* 13: 403–410.

Niewiadomska, A. Klama, J. 2005. Pesticide side effect on the symbiotic efficiency and nitrogenise activity of Rhizobiaceae bacteria family. *Pollt. J. Microbiol.,* 54: 43–48.

Rangaswamy, V. 1990. Interactions between insecticides and micro-organisms in soils. Ph.D. Thesis, Sri Krishnadevaraya University, Anantapur, India.

Ranney, T.A., Bartlett, R.J. 1972. Rapid field determination of nitrate in natural waters. *Commun. Soil Sci. Plant Anal.,* 3: 183–186.

Sáez, F., Pozo, C., Gómez, M.A., Martínez-Toledo, M.V., Rodelas, B., Gónzalez-López, J. 2006. Growth and denitrifying activity of *Xanthobacter autotrophicus* CECT 7064 in the presence of selected pesticides. *Appl. Microbiol. Biotechnol.,* 71: 563–567.

Sarnaik, S.S., Kanekar, P.P., Raut, V.M., Taware, S.P., Chavan, D.S., Bhadbhade, B.J. 2006. Effect of application of different pesticides to soybean on the soil microflora. *J. Environ. Biol.,* 27: 423–426.

Sebiomo, A., Ogundero, V.W., Bankole, S.A. 2011. Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *Afr. J. Biotechnol.,* 10(5): 770–778.

Shetty, P.K., Magu, S.P. 2000. Effect of metalaxyl on soil microbial population. *J. Tropic Agricult.,* 38: 63–65.

Shukla, P.K., Mishra, R.R. 1997. Influence of herbicides on microbial population and enzyme activities in potato (*Solanum tuberosum*) field soil. *J. Agricult. Sci.,* 67: 610–611.

Sigler, W.V., Turco, R.F. 2002. The impact of chlorothalonil application on soil bacterial and fungal populations as assessed by denaturing gradient electrophoresis. *Appl. Soil Ecol.,* 21: 107–118.

Trasar-Cepeda, C., Leir´os, M.C., Seoane, S., Gil-Sotres, F. 2000. Limitations of soil enzymes as indicators of soil pollution. *Soil Biol. Biochem.*, 32(13): 1867–1875.

Trevors, J.T. 1984. Dehydrogenase in soil: a comparison between the INT and TTC assay. *Soil Biol. Biochem.,* 16: 673–674.

Wang, J., Lu, Y., Shen, G. 2007. Combined effects of cadmium and butachlor on soil enzyme activities and microbial community structure. *Environ. Geol.*, 51: 1093–1284.

Wang, M.C., Gong, M., Zang, H.B., Hua, X.M., Yao, J., Pang, Y.J., Yang, Y.H. 2006. Effect of methamidophos and urea application on microbial communities in soils as determined by microbial biomass and community level physiological profiles. *J. Environ. Sci. Health* B. 41: 399–413.

Watson, E.T., Williams, S.T. 1974. Studies on the ecology of Actinomycetes in soil- VII: Actinomycetes in a coastal sand belt. *Soil Biol. Biochem.,* 6: 43–52.

Widenfalk, A., Svensson, J.M. Goedkoop, W. 2004. Effects of the pesticides captan, deltamethrin, isoproturon and pirimicarb on the microbial community of a freshwater sediment. *Environ. Toxicol. Chem.,* 23: 1920–1927.

Xu, B.J., Zhang, Y.X., Zhu, N.W., Ming, H., Zao, Y.H. 1997. Effects of methamidophos on soil microbial activity. Environmental behaviour of crop protection chemicals. *Proceedings of an International Symposium on the use of Nuclear and Related Techniques for Studying Environmental Behaviour of Crop Protection Chemicals*, Vienna, Australia, Pp. 489–494.

Zabaloy, M.C., Garland, J.L., Gomez, M.A. 2008. An integrated approach to evaluate the impacts of the herbicides glyphosate, 2,4-D and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina. *Appl. Soil Ecol.*, 40: 1–12.