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Influence of Endosulfan and Quinalphos on Biological Activities in Paddy (*Oryza sativa*) Soil

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ABSTRACT

Pesticides are widely used in India for protection of agricultural crops from pests. However, these pesticides pose various threats to organisms, including humans, and hamper soil microbial activity. Hence, they are a cause for concern, as a measure of soil fertility and health. A study was undertaken to assess the effect of endosulfan and quinalphos on dehydrogenase and phosphatase activities in paddy soil under laboratory conditions. The results indicated that a strong negative influence on dehydrogenase and phosphatase activities at higher concentrations (7.5 to 10.0 kg ha^{-1}). However the stimulatory concentration of both enzymes was at 5.0kg ha^{-1}. The dehydrogenase was significantly enhanced at 5.0 kg ha^{-1} level for endosulfan and quinalphos with individual increments of 61-100% and 92-96% in comparison to control. Whereas phosphatase also follows the similar trend for both pesticides with individual increments of 20-138% and 17-118%.

Keywords: Endosulfan, Quinalphos, Dehydrogenase, Phosphatase, Paddy rice (*Oryza sativa)* Soil.

Abbreviation: Triphenyltetrazolium chloride (TTC)

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1. INTRODUCTION

Pesticides:
These are substances meant for preventing, destroying or mitigating any pest. They are a class of biocide. The most common use of pesticides is as plant protection products (also known as crop protection products), which in general protect plants from damaging influences such as weeds, diseases or insects. This use of pesticides is so common that the term pesticide is often treated as synonymous with plant protection product, although it is in fact a broader term, as pesticides are also used for non-agricultural purposes.

Pesticides are extensively used in agriculture as a part of pest control strategies. These are recognized as a source of potential adverse environmental impacts and their persistence in soil and ground water has grown considerably (Tejada, 2009). When a pesticide is released deliberately or accidentally into the environment about 0.1% reaching the target organism while the remaining 0.99% reaches the soil causing not only trouble local metabolizing or enzymatic activities (Liu et al., 2008) but also disturb the soil ecosystem and thus, may affect human health by entering in the food chain, have raised considerable public concern. Among the monocot crops, Rice *Oryza sativa* (Asian rice) is one of the major, important, profitable crops grown throughout the year in India. In Andhrapradesh, the total area of Kurnool district is 17658 sqkms (6.4% of total state area) with a cultivable land of 11.34 lakh hectres (Anonymous. 2011). Rice is the major source of food for as much as 60% of the world's population (Mabbett, 1991) and is the predominant food crop in the tropical countries. In tropical countries rice has also been identified as one of the crops that is particularly susceptible to the negative impacts of pesticide use (Asian Development Bank. 1987). This is attributed due to indiscriminate and intensive use of pesticides associated with this crop. Several insect pests are reported to attack paddy crops at various stages of growth impending potential crisis for monocot. Among these, target pests such as, bacterial blight caused by *Xanthomonas oryzae,* bacterial Leaf Streak caused by *Xanthomonas oryzae,* Foot Rot caused by *Erwinia Chrysanthemi,* Downy mildew caused by *Sclerophthora macrospore*, Leaf Smut caused by *Entyloma oryzae*, Aggregate sheath spot caused by *the Ceratobasidium oryzae stove*. Indeed, the losses of the total world rice crop due to insects has been estimated to occur at a rate of 28%, which is four times greater than the average for other world cereal crops (Bunton, 1991). Anthropogenic soil or paddy rice soil or paddy soil is specifically referred to as a soil type developed on any parent material or soil through the oxidation reduction process arising from artificially periodically flooding in a paddy rice soil (Xi, 1998). It is well a known fact that a soil is an open but self regulating ecosystem with a large diversity of microbial populations (Kizilkaya et al., 2004) Soil microbes are a key component in soil ecosystems, dominating the cycling of nutrient elements and playing a

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Table 1
Physicochemical characteristics of the soils

Properties	Black soil
Sand (%)	65.1
Silt (%)	26.3
Clay (%)	11.1
pH[a]	7.4
Electrical conductivity (m.mhos)	234
Organic matter [b] (%)	1.32
Total nitrogen [c] (%)	0.072
NH_4^+ - N (µg g^{-1} soil) [d]	7.24
NO_2^- - N (µg g^{-1} soil) [e]	0.41
NO_3^- - N (µg g^{-1} soil) [f]	0.88

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)

major role in soil productivity (Lin et al., 2004a). Increasing use of pesticides in agriculture led to the development of soil microbial testing programs for examination of the side effects (Swaminathan et al., 2009). The testing programs include measurement of activities of soil enzymes, and physico-chemical properties of soil. In particular soil enzyme activities have been used as indicators of rates of soil nutrient cycling, involved in catalyzing reactions necessary for organic matter decomposition, energy transfer, environmental quality and crop productivity (Quian et al., 2009) due to the fact that relative assays are easily used and these measurements are sensitive to changes in soil management (Kandeler et al., 2006). It is of great importance to investigate the possible impacts of endosulfan and quinalphos on soil biological activities, particularly in flooded soil. 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). Endosulfan comprises two parent isomers alpha and beta endosulfan and the alpha to beta ratio of technical endosulfan is about 7:3 and both isomers are extremely toxic to aqueous organisms. Due to its high degree of toxicity it persists in soils, water and become an important group of contaminants. Although this pesticide have been restrictively used or even banned their persistence and bioaccumulation still be found in soils. Thus it is essential to estimate soil biological responses to the pesticides. To date, many efforts have been made to understand the effect of pesticides on soil enzyme activities, dehydrogenase and phosphatase but little is known about the effect of endosulfan and quinalphos. Quinalphos (O, O-diethyl-O-quinoxalin-2-yl phosphoro-thioate) due to its acaricidal and insecticidal properties is in large scale use in India. From an annual consumption of 300 metric tons during 1977, the use has risen to 1000 metric tons (Anonymous, 1979). The objective of the present study is to evaluate the effect of endosulfan and quinalphos applied at normal field and high concentrations in the laboratory. dehydrogenase activity is very important for soil quality (Wang et al., 2010). Soil acid phosphatase is sensitive to contamination (Liu et al., 2004) Hence dehydrogenase and phosphatase activities were selected because of their significance in soils.

Dehydrogenase:
A dehydrogenase (also called DHO in the literature) is an enzyme that oxidizes a substrate by a reduction reaction that transfers one or more hydrides (H−) to an electron acceptor, usually NAD+/NADP+ or a flavin coenzyme such as FAD or FMN.

Phosphatase:
A phosphatase is an enzyme that removes a phosphate group from its substrate by hydrolysing phosphoric acid monoesters into a phosphate ion and a molecule with a free hydroxyl group (seedephosphorylation). This action is directly opposite to that of phosphorylases and kinases, which attach phosphate groups to their substrates by using energetic molecules like ATP. A common phosphatase in many organisms is alkaline phosphatase. Another large group of proteins present in archaea, bacteria, and eukaryote exhibits deoxyribonucleotide and ribonucleotide phosphatase or pyrophosphatase activities that catalyze the decomposition of dNTP/NTP into dNDP/NDP and a free phosphate ion or dNMP/NMP and a free pyrophosphate ion.

2. SCOPE OF THE STUDY

The objective of the study is to determine the behavior endosulfan and quinalphos in both soils and to evaluate the responses of soil enzymes. This information will be useful for predicting the environmental fate of these widely used insecticides for continuous use and for understanding the potential adverse effects of intensive treatment with endosulfan and quinalphos on dehydrogenase and phosphatase.

2.1. Materials and methods

2.1.1. Soil used in the present study

Soil samples taken from paddy-cultivated fields of Kurnool district, Adhra Pradesh, India were chosen from a depth of 12 cm, air-dried and sieved through 2 mm sieve before usage. Physico-chemical characteristics of soil were analyzed by using standard methods and listed in Table 1.

2.1.2. Analytical methods for characterization of soil samples for physicochemical properties

Mineral matter of soil samples such as sand, silt, and clay contents were analyzed with use of different sizes of sieves by following the method of Alexander. Soil pH was measured at 1:1.25 soils to water ratio in systronics digital pH meter with calomel glass electrode assembly. Organic carbon content in soil samples was estimated by the Walkley -Black method and the organic matter were calculated by multiplying the values with 1.72 (Jackson, 1971). Electrical conductivity of soil samples after addition of 100 ml distilled water to 1 gram soil samples was measured by Conductivity Bridge. Total nitrogen content in soil samples was determined by the method of Micro-Kjeldhal method (Jackson, 1971). Content of inorganic ammonium-nitrogen in soil samples was determined by a Nesslerization method (Jackson, 1971) and the contents of nitrite-nitrogen (Barnes and Folkard, 1951) and contents of nitrate- nitrogen by Brucine method (Ranney and Bartlett, 1972) after extraction with water were determined respectively.

2.1.3. Insecticides used in the present study

To determine the influence of selected insecticides on soil enzyme activities, endosulfan an organochlorine insecticide (35 % emulsifying concentration) and quinalphos an organophosphate (25% emulsifying concentration) was obtained from Hoechsstschering Agro Ero (Ltd) and Sulphurmills Limited, Andheri (E), Mumbai. India respectively.

2.2. Microbial assays

2.2.1. Dehydrogenase activity in soils (E.C. 1.1.1.1)

To check the activity of dehydrogenase under the influence of two insecticides, at different concentrations in black soils. A series of standard solutions of 2, 3, 5-triphenyltetrazolium chloride (TTC) covering the concentrations of 100 – 1000 µg per ml was prepared for calibration. To study the effect of two insecticides on dehydrogenase, 5 g of dried black soil was taken separately in test tubes (12 × 125 mm) containing different concentrations of insecticides 10, 25, 50, 75, and 100 µg g^{-1} soils 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 soil, untreated soil samples served as controls. All the treatments, including controls were incubated in the dark at 28 ± 4°C. After a week triplicate soil samples were withdrawn for the assay of dehydrogenase activity. The method employed for the assay of dehydrogenase was developed by Casida et al., This method is based on the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). Each soil sample was treated with 0.2 ml toluene, 0.1 g of $CaCO_3$ and 1 ml of 0.18 mm aqueous solutions of TTC and incubated for 24 h at 30°C. The TPF formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a Spectronic spectrophotometer. Further, the experiment was repeated with the stimulatory concentrations of pesticides (i.e. at 5.0 kg ha^{-1}) for 2, 3, 4 and 5 weeks to estimate the dehydrognase activity.

2.2.2. Phosphatase activity in soils (E.C. 3.1.6.1)

To assay the activity of phosphatase under the influence of two insecticides, at different concentrations were determined in black soils. A series of standard solutions of *p*-Nitro

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Table 2
Activity of dehydrogenase* under the influence of different concentrations of selected pesticides in soil for 24 hours after a week days

Conc. of Pesticides (kg ha^{-1})	Endosulfan	Quinalphos
	24hrs	24hrs
0.0	98 ± 1.154 d (100)	98 ± 1.154 c (100)
1.0	86 ± 1.732 e (88)	72 ± 1.732 d (73)
2.5	158 ± 0.577 b (161)	188 ± 0.577 b (192)
5.0	196 ± 1.732 a (200)	192 ± 2.309 a (196)
7.5	116 ± 2.309 c (118)	68 ± 0.577 e (69)
10.0	36 ± 0.577 f (37)	24 ± 1.154 f (24)

*µg glucose per gram soil formed after 24 hours incubation with triphenyl tetrazolium Chloride (TTC). Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Table 3
Activity of phosphatase* under the impact of different concentrations of selected pesticides in soil after 10 days

Conc. of Pesticides (kg ha^{-1})	Endosulfan	Quinalphos
0.0	90.81± 1.154 d (100)	90.81 ± 1.154 e (100)
1.0	108.81± 1.154 c (120)	106.08 ± 1.732 d (117)
2.5	154.36 ± 0.577 b (170)	147.12 ± 1.154 b (162)
5.0	216.20 ± 2.309 a (238)	198.17 ± 2.309 a (218)
7.5	109.08 ± 0.577 c (120)	120.08 ± 0.577 c (132)
10.0	54.12 ± 2.309 e (59)	68.58 ± 1.154 f (76)

*µg *P* - Nitro Phenol (PNP)g^{-1} soil formed after 24 hours incubation with *p* – nitrophenyl phosphate (PNPP). Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test

Groundnut: The peanut, or groundnut (*Arachis hypogaea*), is a species in the legume or "bean" family (Fabaceae). The peanut was probably first domesticated and cultivated in the valleys of Paraguay. It is an annual herbaceous plant growing 30 to 50 cm (1.0 to 1.6 ft) tall. The leaves are opposite, pinnate with four leaflets (two opposite pairs; no terminal leaflet); each leaflet is 1 to 7 cm (⅜ to 2¾ in) long and 1 to 3 cm (⅜ to 1 inch) across. The flowers are a typical pea flower in shape, 2 to 4 cm (0.8 to 1.6 in) (¾ to 1½ in) across, yellow with reddish veining. *Hypogaea* means "under the earth"; after pollination, the flower stalk elongates causing it to bend until the ovary touches the ground. Continued stalk growth then pushes the ovary underground where the mature fruit develops into a legume pod, the peanut – a classical example of geocarpy. The pods are 3 to 7 cm (1.2 to 2.8 in) long, containing 1 to 4 seeds. Peanuts are known by many other local names such as earthnuts, ground nuts, goober peas, monkey nuts, pygmy nuts and pig nuts. Despite its name and appearance, the peanut is not a nut, but rather a legume.

phenyl phosphate (PNPP) covering the concentrations of 100 – 1000 µg µl^{-1} was prepared for calibration. Two-gram portions of each soil, in triplicates, were treated with the selected insecticides at 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha $^{-1}$ concentrations. Soil samples with-out insecticide treatment served as controls. Soil samples in test tubes with and without insecticide treatment were incubated at room temperature (28 ± 4^{o}C). After 10 days of incubation, soil extract was prepared in distilled water for the assay of phosphatases according to the method described by Tabatabai (1994); Srinivasulu et al., (2011). Soil samples were transferred to 100 ml Erlenmeyer flask, and 0.2 ml of toluene, 6 ml of 0.1 M maleate buffer (pH 6.5) and 2 ml of *p*-Nitro- phenyl phosphate disodium salt was added. The flasks were swirled for a few seconds to mix the contents, stoppered and incubated at 37^{o}C for 30 min. The reaction was stopped by adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH followed by swirling the flask, for a few seconds, and the soil suspension was filtered through a Whatman No.1 filter paper. The liberated *p*-Nitrophenol in the filtrate was determined at 410 nm in a Spectronic-20D spectrophotometer. Further, the experiment was repeated with the stimulatory concentrations of pesticides (i.e. at 2.5 or 5.0 kg ha^{-1}) for 20, 30 and 40 days to estimate the phosphatase activity.

2.3. Statistical Analysis

The activities of the dehydrogenase and phosphatase were 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 test (DMRT) (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.

4. RESULTS

4.1. Dehydrogenase activity

The effect of different application rates of endosulfan and quinalphos on dehydrogenase activity is presented in Table 2. After a week days of incubation enzyme activity increased in all the treatments (2.5, 5.0, 7.5, kg ha^{-1}) except at 1.0 and 10.0 kg ha^{-1} level. The maximum dehydrogenase activity was observed at 5.0 kg ha^{-1} (stimulatory) and lowest activity at 10.0 kg ha^{-1} level. The dehydrogenase activity was significantly enhanced at 5.0 kg ha^{-1} level in black soil for endosulfan and quinalphos showed individual increments of dehydrogenase activity ranged from a high increase 61 – 100%, 92 – 96% in comparison to control (Table 2). The stimulatory concentration of the above pesticides (5.0 kg ha^{-1}) induces the highest enzymatic activity after 2, 3, 4, 5 weeks of incubation in both soils (Fig 1). A further increase in the stimulatory concentration of insecticides decreased the rate of dehydrogenase activity after 3 weeks and then decline phase was started from 3 to 5 weeks of incubation (Figure 1).

4.2. Phosphatase activity

Phosphatase activity (Table 3) showed a variable pattern in response to different insecticide concentration after 10 days of incubation. Enzyme activity increased under all the treatments (1.0, 2.5, 5.0, 7.5, kg ha^{-1}) except 10 kg ha^{-1} level compared to the controls in black soils. The maximum enzyme activity was observed at 5.0 kg ha^{-1} (stimulatory) and lowest activity at 10.0 kg ha^{-1} level (Table 3). The activity of phosphatase was significantly increased at 5.0 kg ha^{-1} of endosulfan and quinalphos caused individual increments ranged from 20 – 138% and 17 -118% in comparison to control after 10 days of incubation of black soils (Table 3). The stimulatory concentration induces the highest enzymatic activity after 20, 30, 40 days of incubation in comparison with control in black soils (Figure 2). A further increase in the incubation periods for a prolong period (up to 40 days) the stimulatory concentration of fungicides decreased the rate of phosphatase activity after 30 days and then decline phase was started from 30 to 40 days of incubation (Figure 2).

5. DISCUSSION

In general black soil is having high organic matter than in red soil (Srinivasulu et al., 2011) and also black soil is having high WHC. Hence in the present study black soil is selected to study the effect of organochlorine and organophosphorous insecticides. Dehydrogenase activity is present only in viable cells and it is a useful indicator of overall microbial activity in the soil (Wei-Xiang et al., 2004). The increase in soil dehydrogenase activity possibly indicated active metabolism of the compound by microbes either for use as a nutrient source for the detoxification of this compound. Pesticide hydrolysis by microbial enzymes can serve as a detoxification mechanism that can govern pesticide resistance and in turn determine the rate of pesticide degradation in the environment. Gu et al., (2009) observed dehydrogenase activities always were higher in PI312777 soil than in Liaojing-9 soil during the whole growth stages, especially during late growth stages (from elongation to mature). Furthermore, allelopathic rice PI312777 released allelocheical 5,7,4'-trihydroxy-3'5'-dimethoxyflavone into paddy soil in their early growth stages (maximal level of 47.5 ± 7.2 µg g^{-1} soil at seedling stage) and then declined dramatically at elongation stage. While non-allelopathic Liaojing-9 released significantly low concentrations of the flavone during the whole growth stages. In vitro studies, soil was incubated with 5,7,4'-trihydroxy-3'5'-dimethoxyflavone or root exudates of allelopathic PI312777 and non-allelopathic Liaojing-9 under flooded and non-flooded

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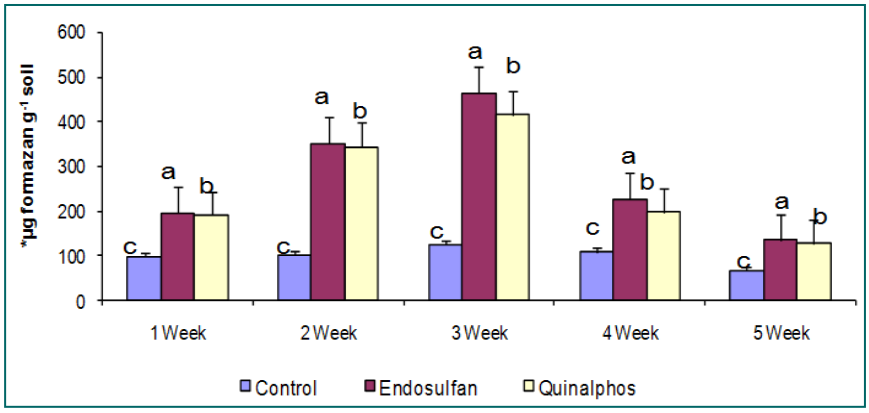

Figure 1

Influence of endosulfan and quinalphos at 5.0 kg ha^{-1} on dehydrogenase* activity in black soil *µg formazan g^{-1} soil formed after 24 h incubation with triphenyl tetrazolium chloride (TTC) after 1st, 2nd, 3rd, 4^{th,} 5th week. The values are the means ± S.E. for each incubation period, followed by the different letter which are significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

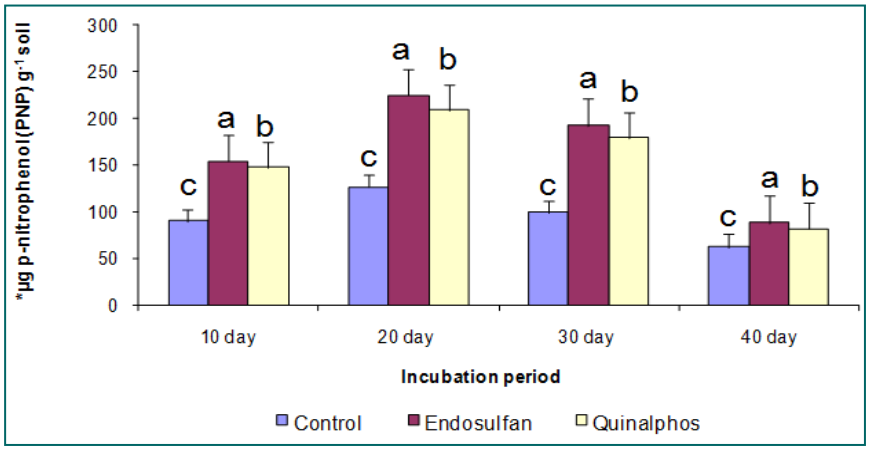

Figure 2

Influence of endosulfan and quinalphos at 5.0 kg ha^{-1} on phosphatase* activity in black soil *µg *P* - Nitro Phenol (PNP)g^{-1} soil formed after 24 hours incubation with *p* – nitrophenyl phosphate (PNPP) after 10, 20, 30 and 40 days. The values are the means ± S.E. for each incubation period, followed by the different letter which are significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

conditions, respectively. Both root exudates and the flavone increased dehydrogenase activities under both flooded and non-flooded conditions. Michel Amery Defo et al., (2011) noticed dehydrogenase activity was enhanced from 1 to 30 days of incubation in Mendong soil while in Minkoa-Meyos there was no measurable effect on endosulfann on dehydrogenase activity. Hang Min (2001) observed the activity of dehydrogenase increased gradually and reached up to the highest level on the 16th day after application and then decreased with butachlor with concentrations of 5.5, 11, 22 µg/ g soil . Surya kalyani et al., (2010) noticed in all the treatments (1ppm, 10ppm, 100ppm) the dehydrogenase activity significantly increased with the application of endosulfan up to the 3rd week of incubation further the decline in the enzyme activity follows. Similar type of results was noticed by Gooty Jaffer Mohiddin et al., (2011) with acephate and imidacloprid on dehydrogenase activity in groundnut soils. Rangaswamy et al., (1994) reported the significant enhancement in dehydrogenase activity by monocrotophos, quinalphos, cypermethrin and fenvalerate upto the concentration of 2.5 kg ha^{-1}. But higher rates of 5-12.5 kg ha^{-1} of these insecticides were either innocuous or toxic to dehydrogenase activity during 7 days incubation. At 2.5 kg ha^{-1}, these insecticides stimulated the dehydrogenase activity up to 21 days and then gradually declined later (Rangaswamy et al., 1994). This observation was well in conformity with the stimulatory response by the pesticides towards dehydrogenase at lower concentrations in soils recorded in the present study. Significant increase in dehydrogenase activity was noticed with permethrin (FMC 33297), FMC 45498, Shell WL41706, Shell WL43467 and Shell WL43775 in 0.5 and 5 µg g^{-1} after 3 weeks of incubation (Tu, 1980b). Methyl parathion at 15 kg ha^{-1} was reported to stimulate soil dehydrogenase activity (Naumann, 1970). Likewise, tefluthrin, DOWCO 429X and DPX 43898 at 10 mg kg^{-1} induced increase in dehydrogenase activity in a sandy loam soil during the 2 weeks while dehydrogenase activity was initially reduced by tefluthrin and unaffected by the other pesticides in an organic soil after 2 weeks (Tu, 1990). But dehydrogenase activity was unaffected by several pesticides (Chendrayan et al., 1980; Tu, 1981a). Similarly, additive, synergistic and antagonistic interactions were recorded towards dehydrogenase activity in soils by the combination of several pesticides (Dzantor and Felsot, 1991; Malkomes, 1982). The data presented in Table 2, revealed that a significant inhibition occurred at higher concentrations (10 kg ha$^{-1)}$ of endosulfan and quinalphos on dehydrogenase activity in black soils, collected from paddy fields. In a similar manner, Gowda (1973) reported inhibition of dehydrogenase activity in peptone-amended soil by benomyl at 100 to 10,000µg g^{-1} soil.

In general, the dehydrogenase activity was relatively less in the soil maintained under non-flooded conditions as reported by Chendrayan et al., (1980). This can be expected because dehydrogenase activity is significantly more pronounced in flooded soils, as most dehydrogenases are anaerobic origin (Chendrayan et al., 1980). There was a progressive increase in the accumulation of formazan with increasing period of incubation up to 21 days, which gradually decreased further. Hence the dehydrogenase activity was enhanced significantly more at 5.0 kg ha^{-1} of the two insecticides. In fact application of insecticides to soils led to an initial striking increase in dehydrogenase activity. Similarly carbaryl at 10, 50, and 100 ppm, Quinophos at 25 ppm, Carbofuran and quintazene at 100 ppm was known to inhibit dehydrogenase activity (Rangaswamy, 1989; Rangaswamy et al., 1994; Gundi et al., 2005).

Endosulfan, an organophosphorous pesticide with a reactive cyclic diester group, may be degraded by the action of esterases, phosphatases or sulphatases of a combination of these enzymes. Phosphatase catalyzes the hydrolysis of a variety of organic phosphomonoesterase that are widely implicated in the degradation of organophosphorous pesticides (Kanekar et al., 2004) the increase in the activity of these enzymes suggests the possible involvement of this group of enzymes in the degradation of endosulfan as well. Michel Amery Defo et al., (2011) noticed there was no significant change in the phosphatase activity in the Minkoa-Meyos soil was observed. However the addition of endosulfan on Mendong soil increased the phosphatase activity. After 60 days of incubation, the addition of endosulfan on soil inhibited the activity of these enzymes, except in Mendong soil, where the stimulation of this activity for concentrations ≤ 10 µg/g of dry soil was observed. Surya Kalyani et al., (2010) noticed in all the treatments the acid phosphatase activity was significantly increased with the application of endosulfan upto 45 days in all treatments (10ppm, 100ppm) except at 1ppm where the activity was higher at 14 days of incubation further increase in the incubation decline in the enzyme activity was found. Two

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organophosphorus insecticides, monocrotophos and quinalphos and two pyrethroids, cypermethrin and fenvalerate all at the concentrations of 1-5 kg ha^{-1}, significantly increased the phosphatase activity but above this concentration i.e. 7.5 kg ha^{-1}, these insecticides were inhibitory to the enzyme activity (Rangaswamy and Venkateswarlu, 1996). Chlorpyrifos, terbufos and fonofos increased activities of acid phosphatase in loam soil sites in the field (Sikora et al., 1990). In a similar way, phorate and fenvalerate had a stimulatory effect towards phosphorus-mobilization in soils under laboratory and field conditions (Das and Mukherjee, 1994, 1998a,b). On the contrary, tefluthrin, DOWCO 429X and DPX 43898, when applied at 10 mg kg^{-1}, induced a reduction of phosphatase activity in an organic soil, but stimulation in a sandy soil was reported by Tu (1990). Likewise, in an organic soil, parathion, triazophos, permethrin and fonofos at 5 mg kg^{-1} reduced the phosphatase activity by 2-fold (Tu, 1981b). But malathion and parathion at the same level stimulated the activity in a clay soil (Tu, 1989). On the other hand, the phosphatase activity was not affected by the application of pesticides to some other soils. In a clay loam soil, diazinon, chlorpyrifos, thionazin and trichloronate at 1 or 10 kg ha^{-1} were innocuous to phosphate mobilization (Tu, 1981a). Tu (1970) reported that the addition of Bay-37289, diazinon, Dursban and zinophos at 10 and 100 µg g^{-1} did not result in significant differences in phosphorus mineralization. In the field study, fenamiphos at 18.6 kg ha^{-1} had no effect on phosphatase activity (Ross et al., 1984). In a similar study under laboratory conditions, fenamiphos at 37 and 930 mg kg^{-1} had no deleterious effect on the activity of phosphatase (Ross and Speir, 1985).

6. CONCLUSION

The results obtained in the present study clearly indicate that the insecticides endosulfan and quinalphos profoundly enhanced the activities of both dehydrogenase and phosphatase at 5.0 kg ha^{-1}. Based on these results, it is concluded that the microbial activities (i.e., enzyme activities) were not affected by the insecticides applied at recommended levels in the agricultural system to control insect pests.

SUMMARY OF RESEARCH

1. This work, within the limit of available resource, has provided useful information as to what concentration of pesticide will not alter the enzyme activity.
2. It has availed scientists the opportunity to research more on the usefulness of the Interaction of pesticides on biological activities.

FUTURE ISSUES

From the findings, the highest concentration of pesticides i.e., 7.5 to 10.0 kg ha^{-1} is innocuous or toxic to the enzyme activity compared to 1.0 to 5.0 kg ha^{-1}, suggesting that 5.0 Kg ha^{-1} having a good stimulatory activity on enzyme which in turn a good indication for maintaining soil health.

DISCLOSURE STATEMENT

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CONFLICTS OF INTEREST

"The author(s) declare(s) that there is no conflict of interests regarding the publication of this article."

REFERENCES

1. Alexander M. Introduction to soil microbiology. *Wiley Estern Ltd*. New Delhi., 1961
2. Anonymous, Agriculture production plan for Kurnool District, Andhra Pradesh, India *Department of Agriculture*, Kurnool, Andhra Pradesh, India., 2011
3. Anonymous, Report of working group on pesticides Industry for the plan (1978-79 to 1983-84) Ministry of Petrolium, chemicals and fertilizers. *Government of India. India*., 1979
4. Asian Development Bank, Handbook on the use of pesticides in the Asia–Pacific region. Manila, Philippines, 1987, 146–149
5. Barnes H, Folkard BR. The determination of nitrite. *Analyst*., 1951, 76, 599-603
6. Bunton J, Pesticides to avert hunger. *Far Eastern Agric*.January/February., 1991, 8–10
7. Casida LE, Klein DH, Santoro T. Soil dehydrogenase activity. *Soil Sci.* 1964, 98, 371-376
8. Chendrayan K, Adhya TK. Sethunathan N. Dehydrogenase and invertase activities of flooded soils. *Soil Biol. Biochem*., 1980, 12, 271-273
9. Das AC, Mukherjee D. Effect of insecticides on the availability of nutrients, nitrogen fixation, and phosphate solubility in the rhizosphere soil of rice. *Biol. Fertil. Soils*, 1994, 18, 37-41
10. Das AC, Mukherjee D. Insecticidal effects on soil microorganisms and their biochemical processes related to soil fertility. *World J. Microbiol. Biotechnol*, 1998a, 14, 903-909
11. Das AC, Mukherjee D. Insecticidal effects on the activity and numbers of non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms and on some chemical properties of rice soil. *Microbiol. Res*., 1998b, 153, 355-361
12. Dzantor EK. Felsot AS. Microbial responses to large concentrations of herbicides in soil. *Environ. Tox. Chem*., 1991, 10, 649-655
13. Gooty Jaffer Mohiddin, Madala Srinivasulu, Mekapogu Madakka, Kondeti Subramanyam and Vengatampalli Rangaswamy. Influence of selected Insecticides on Enzyme Activities in Groundnut (*Arachis hypogea* L.) Soils. *Dynamic Soil, Dynamic Plant*. 2011, 5, 65 – 69
14. Gowda TKS. Studies on the effect of benlate on microflora and other biological properties of soils of Mysore State. MSc thesis, *University of Agricultural Science*, Banglore, India, 1973, 160
15. Gu Y, Wang P, Kong CH. Urease, invertase, dehydrogenase and polyphenoloxidase activities in paddy soil as influenced by allelopathic rice variety. *European Journal of Soil Biology,* 2009, 45, 436-441
16. Gundi VAKB, Narasimha G, Reddy BR. Interaction Effects of soil insecticides on microbial populations and dehydrogenase activity in a black clay soil. *J. Eni Sci Heal.* 2005, 40, 269-281
17. Hang Min, Yang-fang Ye, Zhong-Yun Chen, Wei-xiang Wu, Du Yufeng. Effect of butachlor and enzyme activities in paddy soil. *Journal of Environmental Science and Health B* 2001, 36(5), 581-595

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Influence of Endosulfan and Quinalphos on Biological Activities in Paddy (*Oryza sativa*) Soil,
Discovery Agriculture, 2013, 1(2), 67-72,

18. Jackson ML. Soil Chem. Anal. *Prentice Hall India*, New Delhi. 1971, 535-538
19. Johnson CM. Ulrich A. Determination of moisture in plant tissues. *Calif. Agri. Bull*. No. 766. In Soil and Plant analysis for tree culture (Ed., S.A. Wilde et al.,) *Obortage publishing Co. Oxford and Bombay*, 1960, 112-115
20. Kanekar PP, Bhadbhade BJ, Deshpande NM, Sarnaik SS. Biodegradation of organophosphorous pesticides. *Proc Indian Natl Sci Acad B* 2004, 70(1), 57-70
21. Kizilkaya R, Askin T, Bayrakli B, Salgam M. Microbiological characteristics of soils contaminated with heavy metals. *European Journal of Soil Biology* 2004, 40, 95-102
22. Lin L, Biyela PT, Puckree T, Bezuidenhout CC. A study of the water quality of the Mhlathuze River, KwaZulu-Natal (RSA): Microbial and physico-chemical factors. *Water SA* 2004a, 39(1), 16-22
23. Liu G, Xu D, Wang L, Li K, Lui W. Effect of organic and inorganic compounds on the enzymes in soil under acid rain stress. *J Environ Studies,* 2004, 6, 737-744
24. Liu J, Xie J, Chu Y, Sun C, Chen C, Wang Q. Combined effect of cypermethrin and copper on catalase activity in soil. *J. Soils Sed,* 2008, 8, 327–332
25. Mabbett T. Pest management in rice. *Far Eastern Agric*.January/February 1991. 9
26. Malkomes H-P. Effect of two pesticide treatment systems for sugar beets on dehydrogenase activity and straw decomposition in soil. *J. Plant Dis. Prot*., 1992, 89, 705-714
27. Megharaj M, Singleton I, Kookana R, Naidu R. Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil. *Soil Biology and Biochemistry,* 1999, 31, 1549-1553
28. Michel Amery Defo, Thomas Njine, Moise Nola, Florence Sidonie Beboua. Microcosom study of the long term effect of endosulfan on enzyme and microbial activities on two agricultural soils of Yaounde-Cameroon. *African Journal of Agricultural Research*, 2011, 6(9), 2039-2050
29. Narayana Rao VVS. Effect of pesticides on ammonification. *In*: pesticides *and nitrogen cycle.* (Ed. R. Lal). CRC Press, Florida. 1998, 2, 2-41
30. Naumann K, Zur Dynamik der Bodenmikroflora nach Anwendung von Pflanzenschutzmitteln. IV. Untersuchungen uber die Wirkung von Parathion-Methyl auf die Atmung und die Dehydrogenase-Aktivitat des Bodens. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg Abt II*. 1970, 125, 119-133
31. Quian K, Tang T, Shi T, Wang F, Li J. Cao Y. Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after deviation with 4 chloro-3,5-dinitrobenzotrifluride. *Analytica Chimica Acta,* 2009, 635, 222-226
32. Rangaswamy V, Reddy BR, Venkateswarlu K. Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin fenvalerate. *Agric. Ecosys. Environ*. 1994. 47, 319-326
33. Rangaswamy V, Charyulu PBBN, Venkateswarlu K. Effect of monocrotophos and quinalphos on soil population and nitrogen-fixing activity of *Azospirillum* sp. *Biomed. Environ. Sci.* 1989, 2, 305-314
34. Rangaswamy V, Venkateswarlu K. Phosphatase activity in groundnut soils as influenced by selected insecticides. *J. Environ. Biol.* 1996, 17(2), 115-119
35. Ranney T, Bartlett RJ. Rapid field determination of nitrate in natural waters. Comm. *Soil Sci. Pl. Anal.* 1972, 3, 183-186
36. Ross DJ, Speir TW. Changes in biochemical activities of soil incubated with the nematicides oxamyl and fenamiphos. *Soil Biol. Biochem*., 1985, 17, 123-125
37. Ross DJ, Speir TW, Cowling JC, Whale K.N. Influence of field applications of oxamyl and fenamiphos on biochemical activities of soil under pasture. *New Zealand. J. Sci.* 1984, 27, 247-254
38. Sebiomo A, Ogundero VW, Bankole SA. Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *African Journal of Biotechnology,* 2011, 10(5), 770-778
39. Sikora LJ, Kaufman DD, Horng LC. Enzyme activity in soils showing enhanced degradation of organophosphate insecticides. *Biol. Fertil. Soils*. 1990, 9, 14-18
40. Srinivasulu M, Jaffer Mohiddin G, Subramanyam K, Rangaswamy V. Effect of insecticides alone and in combination with fungicides on nitrification and phosphatase activity in two groundnut (*Arachis hypogeae* L.) soils. *Environ Geochem Health,* 2011. (DOI 10.1007/s10653-011-9399-x.)
41. Surya Kalyani S, Jitender Sharma, Prem Dureja, Surender Singh and Lata. Influence of Endosulfan on Microbial Biomass and Soil Enzymatic Activties of a Tropical Alfisol. *Bull Environ Contam Toxicol,* 2010, 84, 351-356
42. Swaminathan P, Prabaharan D, Uma L. Fate of few pesticides-metabolizing enzymes in the marine cyanobacterium phormidium valderianum BDU 20041 in perspective with chlorpyrifos exposure. *Pesticide Biochemistry and Physiology,* 2009, 94, 68-72
43. Tabatabai M.A. Soil enzymes, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis American Society of Agronomy, *Madison,* 1994, 775-833
44. Tejada M. Evolution of soil biological properties after addition of glyphosate, diflufencian and glyphosate + diflufencian herbicides. *Chemosphere,* 2009, 76, 365-373
45. Trevors JT. Dehydrogenase activity in soil, a comparison between INT and TTC assay. *Soil Biol Biochem*, 1984, 16, 673-674
46. Tu CM. Effect of four experimental insecticides on enzyme activities and levels of adenosine in mineral and organic soils. *J. Environ. Sci Health.* 1990, 25, 787-800
47. Tu CM. Effect of four organophosphorus insecticides on microbial activities in soil. *Appl. Microbiol*., 1970, 19, 479-484
48. Tu CM. Effect of pesticides on activity of enzymes and microorganisms in a clay loam soil. *J. Env. Sci. Health*. 1981a, 16, 179-181
49. Tu CM. Effect of some pesticides on enzyme activities in an organic soil. *Bull. Environ. Contam. Toxicol.* 1981b, 27, 109-114
50. Tu CM. Effects of some experimental insecticides on Microbial activities on mineral and organic soils. *J. Environ. Sci. Health B,* 1989, 24, 57-64.
51. Tu CM. Influence of pesticides and some of the oxidised analogues on microbial population, nitrification and respiration activities in soil. *Bull. Environ. Contam. Toxicol*, 1980b, 24, 13-19
52. Wang F, Yao J, Chen H-L, Chen K, Trebse P, Zaray G. Comparative toxicity of chlorpyrifos and its oxon derivatives to soil microbial activity by combined methods. *Chemosphere,* 2010, 78, 319-326
53. Wei-xiang W, Qing-fu Y, Hang M. Effect of straws from Bt transgenic rice on selected biological activities in water-flooded soil. *Eur. J Soil Biol,* 2004, 40:15-22
54. Xi CF. Chapter 15, Anthropogenic Soils. In: Xi, C.F. (Ed.), Soils of China. *China Agriculture Press*, Beijing, China, 1998, 704-737. In Chinese.
    Yun Long YU. Min Shan. Hua. Fang. Xiao. Wang. Xiao. Qiang. Chu. Responses of soil microorganisms and enzymes to repeated applications of chlorothalonil. *J. Agric. Food Chem*. 2006, 54, 10070-10075

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