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Effect of Endosulfan and Quinolphos on Enzyme Activities in Paddy Soil
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Abstract
This paper reports the effect of endosulfan and quinolphos at normal residue to elevated level (0-100 ppm) which is equivalent to field application rates of 0-10.0 kg ha⁻¹ on enzymatic activities (amylase and invertase) of a paddy soil. The results showed that activity of amylase and invertase declined significantly after the application of endosulfan and quinolphos at higher concentrations (7.5-10.0 kg ha⁻¹) while at lower concentrations (0-5 kg ha⁻¹) amylase activity showed an individual increments of 53-171, 45-139 and 34-192, 69-183% of increase at 24 and 72 hours of black paddy soil. Whereas invertase activity showed an individual increments of 15-29, 9-33 and 22-27, 20-23% of increase at 24 and 48 hours of black paddy soil respectively. The activity of amylase and invertase was decreased gradually on prolonged period of incubation up to 30 and 40 days. Overall the higher concentrations (7.5-10.0 kg ha⁻¹) of endosulfan and quinolphos were toxic or innocuous to amylase and invertase activities.

Keywords: Endosulfan, Quinolphos, Amylase, Invertase, Paddy rice (Oryza sativa) Soil.

Introduction
Among the monocot crops, Rice Oryza sativa (Asian rice) is one of the major, important, profitable crops grown throughout the year in India. In Andhra Pradesh, the total area of Kurnool district is 17658 sqkms (6.4% of total state area) with a cultivable land of 11.34 lakh hectares (Anonymous. 2011). Rice is the major source of food for as much as 60% of the world’s population (Mabbutt, 1991) and is the predominant food crop in the tropical countries. 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 Sclerotinia macrospore, Leaf Smut caused by Entyloma oryzae, Aggregate sheath spot caused by Ceratobasidium oryzae sativae. Indeed, 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). 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. 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). Pesticides are recognized as a source of potential adverse environmental impacts and their persistence in soil and ground water has grown considerably (Cox et al. 2001, Tejda, 2009). When a pesticide is released deliberately or accidentally in to the environment about 0.1% reaching the target organism while the remaining 0.99% reaches the soil causing not only trouble local metabolism or enzymatic activities (Carriger et al. 2006; Liu et al. 2008) but also disturb soil ecosystem and thus, may affect human health by entering in the food chain, have raised considerable public concern. Soil microbes are a key component in soil.
Soil enzymes are potential indicators and act as a biological catalysts of various important biochemical reactions to produce essential components and then plays an essential role in indicating the soil fertility (Pascual et al. 2000; Gracia et al. 2000; Benedetti and Dilly 2006; Bending et al. 2006; Quian et al. 2009). 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 (Dick 1994; Tabatabai 1994) due to the fact that relative assays are easily used and these measurements are sensitive to changes in soil management (Bending et al. 2004; 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.

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 has 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. To date, many efforts have been made to understand the effect of pesticides on soil enzyme activities, amylase and invertase but little is known about the effect of endosulfan and quinalphos.

**Materials and Methods:**

**Soil used in the present study**

Paddy black soil was used in the present study. Soil samples taken from paddy-cultivated fields of Kurnool district 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.

**Table 1:** Physicochemical characteristics of the soil

<table>
<thead>
<tr>
<th>Properties</th>
<th>Black soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>65.1</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>26.3</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>11.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
<tr>
<td>Water holding capacity (ml g⁻¹ soil)</td>
<td>0.47</td>
</tr>
<tr>
<td>Electrical conductivity (m mhos)</td>
<td>234</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>1.32</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td>0.072</td>
</tr>
<tr>
<td>NH₄⁺ - N (µg g⁻¹ soil)</td>
<td>7.24</td>
</tr>
<tr>
<td>NO₂⁻ - N (µg g⁻¹ soil)</td>
<td>0.41</td>
</tr>
<tr>
<td>NO₃⁻ - N (µg g⁻¹ soil)</td>
<td>0.88</td>
</tr>
</tbody>
</table>


**Analytical methods for characterization of soil samples for physicochemical properties**

Mineral matter of soil samples such as sand, silt, and clay contents were analysed with use of different sizes of sieves by following the method of Alexander (1961). Sent percent water holding capacity of soil samples was measured by finding amount of distilled water added to black paddy soil samples to get saturation point and then 60% water holding capacity of soil was calculated (Johnson and Ulrich, 1960). Soil pH was measured at 1:1.25 soil to water ratio in systronics digital pH meter with calomel glass electrode assembly. Organic carbon content in soil samples was estimated by the Walkley and Black method and the organic matter was 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 Nesslerization method (Jackson, 1971) and 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.

**Insecticides used in the present study**

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

**Enzymatic Assays**

**Invertase and Amylase activities**

Five gram portion of the soil samples were weighed and dispersed into sterile test tubes (25 x 150 mm). Stock solutions from selected insecticides were added at the rate of 10, 25, 50, 75 and 100 µg g⁻¹ soil equivalent to field application rates of 1.0, 2.5, 5.0, 7.5 and 100 kg ha⁻¹ respectively. Soil samples without insecticide treatment served as controls. Soil samples were mixed thoroughly for uniform distribution of insecticide added. Triplicates were maintained for each treatment at room temperature (28 ± 4°C) with 60% water holding capacity throughout the incubation period. After desired intervals of incubation, soil samples were extracted in distilled water for estimation of enzyme activities.

**Assay of Invertase (EC 3.2.1.26)**

For assay of invertase enzyme activity, the substrate sucrose (18 mM) was added to the soil samples and incubated for 24 and 48 hours. The amount of glucose liberated from sucrose was calculated according to the method employed for...
the assay of amylase was developed by Cole (1977) and followed by Tu (1981a and 1981b). The soil samples were transferred to 100 ml Erlenmeyer flasks and were treated with 1 ml of toluene to arrest the enzyme activity. After 15 minutes, 6ml of 0.2M of acetate phosphate buffer (5.5 pH) containing 2 % starch was added to each of the testing samples and closed with cotton plugs. After 24 hours and 72 hours of incubation the testing samples were made up to a volume of 50 ml with sterile distilled water and passed through Wattman No. 1 filter paper and the filtrate was assayed for amount of glucose by Nelson method (1944) in a Spectronic 20D Spectrophotometer (Milton Roy Company).

Assay of Amylase (EC 3.2.1.1)

The method employed for the assay of amylase was developed by Cole (1977) and followed by Tu (1981a and b).
Table 2. Activity of amylase* under the impact of different concentrations of selected pesticides endosulfan and quinalphos in black soil for 24 and 72 hours after 10 days.

<table>
<thead>
<tr>
<th>Conc. of Pesticides (kg ha⁻¹)</th>
<th>Endosulfan</th>
<th>Quinalphos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>72 hrs</td>
</tr>
<tr>
<td>0.0</td>
<td>194 ± 2.309 e (100)</td>
<td>220 ± 5.773 e (100)</td>
</tr>
<tr>
<td>1.0</td>
<td>304 ± 2.309 d (153)</td>
<td>294 ± 1.154 d (134)</td>
</tr>
<tr>
<td>2.5</td>
<td>321 ± 0.577 c (165)</td>
<td>475 ± 2.886 b (216)</td>
</tr>
<tr>
<td>5.0</td>
<td>525 ± 2.886 a (271)</td>
<td>642 ± 1.154 a (292)</td>
</tr>
<tr>
<td>7.5</td>
<td>348 ± 1.154 b (179)</td>
<td>396 ± 1.732 c (180)</td>
</tr>
<tr>
<td>10.0</td>
<td>162 ± 1.154 f (83)</td>
<td>196 ± 1.732 f (89)</td>
</tr>
</tbody>
</table>

*µg glucose per gram soil formed after 24 and 72 hours incubation with 2 % starch. Figures, in parentheses, indicate relative production Percentages. Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan’s multiple range (DMR) test.

Table 3. Activity of invertase* under the impact of different concentrations of selected pesticides in soils for 24 and 48 hours after 10 days.

<table>
<thead>
<tr>
<th>Conc. of Pesticides (kg ha⁻¹)</th>
<th>Endosulfan</th>
<th>Quinalphos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24hrs</td>
<td>48hrs</td>
</tr>
<tr>
<td>0.0</td>
<td>652 ± 1.154 d (100)</td>
<td>784 ± 2.886 b (100)</td>
</tr>
<tr>
<td>1.0</td>
<td>752 ± 1.154 c (115)</td>
<td>996 ± 1.732 a (127)</td>
</tr>
<tr>
<td>2.5</td>
<td>840 ± 5.773 a (112)</td>
<td>974 ± 2.309 c (122)</td>
</tr>
<tr>
<td>5.0</td>
<td>825 ± 2.886 b (126)</td>
<td>970 ± 5.773 c (124)</td>
</tr>
<tr>
<td>7.5</td>
<td>526 ± 5.773 e (81)</td>
<td>956 ± 2.309 d (122)</td>
</tr>
<tr>
<td>10.0</td>
<td>162 ± 1.154 f (25)</td>
<td>861 ± 0.577 e (33)</td>
</tr>
</tbody>
</table>

*µg glucose per gram soil formed after 24 and 72 hours incubation with 2 % starch. Figures, in parentheses, indicate relative production Percentages. Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan’s multiple range (DMR) test.

The soil samples were transferred to 100 ml Erlenmeyer flasks and were treated with 1 ml of toluene to arrest the enzyme activity. After 15 minutes, 6ml of 0.2M of acetate phosphate buffer (pH 5.5) containing 2% starch was added to each of the testing samples and closed with cotton plugs.

After 24 hours and 72 hours of incubation the testing samples were made up to a volume of 50 ml with sterile distilled water and passed through Wattman No. 1 filter paper and the filtrate was assayed for amount of glucose by Nelson’s method (1944) followed by Jaffer Mohiddin et al. (2010) in a
Spectronic 20-D Spectrophotometer.

Statistics Analysis
The activities of the invertase and amylase 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 test (DMRT) (Megharaj et al. 1999; Gooty Jaffer Mohiddin et al. 2011). All statistical analysis was performed at (P ≤ 0.05) using SPSS statistical software package.

Results and Discussion
The black soils were predominantly used for the cultivation of paddy (Oryza sativa) (Asian rice) in the Kurnool district of Andhra Pradesh, India. Due to the fact black soil has highest organic matter content than red soil (Srinivasulu et al. 2011) and also having high water holding capacity. For this reason black soil has been using predominantly for the cultivation of crops. Hence black soil was selected for our investigation under the influence of pesticides. Persistence of pesticide residues in the soil may have a significant impact on soil microbial communities and their functions such as the activity of enzymes, which are directly related to soil health and fertility and also to the removal of contaminants (Ingham et al. 1991; Beare et al. 1992).

Amylase activity showed a variable pattern in response to different insecticide concentration after 10 days of incubation (Table 2). 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 controls in black soil. The endosulfan and quinolphos have shown maximum enhancement in cellulase activity at 2.5 and 5.0 kg ha$^{-1}$ respectively and the activity was significantly decreased at higher concentration of 10.0 kg ha$^{-1}$ in black soil (Table 2). The insecticides, endosulfan and quinolophos showed individual increments in amylase activity were 53-171, 45-139 and 34-192, 69-183% of increase in comparison to control after 24 and 72 hrs at 2.5 and 5.0 kg ha$^{-1}$ black paddy soil respectively (Table 2). The stimulatory concentrations (2.5 to 5.0 kg ha$^{-1}$) of both endosulfon and quinolphos have shown the highest enzymatic activity after 20 days, and then decline phase was started and exhibited lowest activity after 30 and 40 days of incubation in black soil (Fig 1).

Similar to amylase, invertase also follow the same trend, stimulatory effect of endosulfan and quinolphos was observed at 10-25 ppm concentrations with an individual increment of two insecticidal treatments, over control were 15-29, 9-33 and 22-27, 20-23% of increase at 24 and 48 hours of black paddy soils incubated for 10-days (Table 3). Invertase activity was constantly higher in endosulfan and quinolphos treated soils than in the control (Fig 2), this trend follows up to 20 days of incubation. Furthermore, invertase activity of all treatments showed a decreasing tendency with prolong period of incubation upto 20 to 40 days. The relatively low activity of invertase might result from toxic effect of endosulfan and quinolphos on soil microorganisms which inturn produce invertase. Our results appeared to be consistent with previous reports, in which it is demonstrated that pesticides stimulated invertase activity of soils. (Srinivasulu and Rangaswamy, 2006; Gooty Jaffer Mohiddin et al., 2010). But in contrast several researchers showed that activity of invertase was significantly inhibited by chlorothalonil up to 37.7%, 13.9 and 34.2% respectively (Yun et al. 2006). Hu et al. (2011) observed that invertase activity at (8.0 mg kg$^{-1}$) of carbendazim and chloramphenicol shows inhibitory effect on invertase activity. A negative correlation was observed between the napropamide on invertase activity (Guo et al.2009).

Conclusion
The results of the present study thus, clearly indicated that the insecticides, endosulfan and quinolphos were profoundly enhanced the activities of both amylase and invertase, at 1.0-5.0 kg ha$^{-1}$. Based on the above results, it is concluded that the amylase and invertase were not affected, by the insecticides applied at recommended levels in agricultural system. A very few reports are available on the influence of endosulfan and quinolphos on amylase and invertase activities in paddy rice soils. Hence, further research is needed to evaluate the effect of these insecticides on soil enzyme activities.

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References


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