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Impact of triazophos insecticide on paddy soil environment

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Abstract: A laboratory incubation study was carried out to elucidate the dynamic response of insecticide (triazophos) on a paddy field soil health under controlled moisture (flooded soil) and temperature (25℃). The insecticide was applied at five levels that were 0.0 (control), 0.5 field rate (FR), 1.0 FR, 5.0 FR, and 10.0 FR, where FR was 1500 ml/hm^2, and the parameters were studied at 1, 4, 7, 14, and 21 days after treatments' addition. The electron transport system (ETS)/dehydrogenase activity exhibited a negative correlation with insecticide concentrations, and the activity affected adversely as the concentration increased. The higher doses of 5 and 10 field rates significantly reduced the ETS activity, while lower rates failed to produce any significant inhibiting effect against the control. The toxicity of insecticide decreased towards decreasing the ETS activity with the advancement of incubation period. The insecticide caused an improvement in the soil phenol content and it increased with increasing concentration of insecticide. The insecticide incorporation applied at various concentrations did not produce any significant change in soil protein content and it remained stable throughout the incubation period of 21 – days. The response of biomass phospholipid content was nearly similar to ETS activity. The phospholipid content was decreased with the addition of insecticide and the toxicity was in the order: 10 FR (field rate) > 5 FR > 1.0 FR > 0.5 FR > control and it also decreased with incubation period.

Keywords: electron transport system (ETS) activity; phenol; phospholipids; protein; insecticide; anoxic conditions

Introduction

Rice is the staple diet around 30% of the world population and around 60% of the Asian population. This crop is preferentially or generally cultivated under submerged soil conditions for reasons of better yields and topographical situations (Reichardt, 1997). The importance of soil microorganisms affecting soil fertility has been known for many years (Russell, 1905), while their influence on soil quality has been emphasized more recently (Visser, 1992). Soil microbial biomass comprises 1% to 6% of the total organic N (Anderson, 1989; Jenkinson, 1988) in soil. They fix atmospheric nitrogen, decompose organic matter, and perform other biochemical transformations like ammonification, nitrification etc. responsible for the release of plant nutrients (Alexander, 1977). They improve physical properties such as structure, porosity, aeration, and water infiltration by forming and stabilizing soil aggregates (Gupta, 1988). Because of its rapid turnover, microbial biomass is a sensitive indicator of changes in climate (Insam, 1990), tillage systems (Lynch, 1980), crop rotations (Campbell, 1991), and pollutant toxicity (Chander, 1993; Subhani, 2000). Productivity of irrigated rice crops is declining over years of continuous, intensive cultivation and appears to be associated with a decreasing N supply capacity of the soil (Olk, 1998), which in turn may be attributed due to the enrichment of phenolic compounds in these soils (Olk, 1996). Most studies of soil enzymes have been confined to arable agricultural and forest soils. But a flooded rice soil is predominantly anaerobic and as a result differs from a nonflooded soil in several physical, chemical and biological characteristics. Despite extensive studies on the chemistry (Ponnamperuma, 1972) and microbiology (Yoshida, 1975) of flooded soil, our knowledge of their enzyme activities is limited, especially under different management practices.

Modern agriculture and industry depend on a wide variety of synthetically produced chemicals, including insecticides, fungicides, herbicides and other pesticides. Continued widespread use and release of such synthetics has become an everyday occurrence, resulting in environmental pollution. In this context, the influence of pesticides on the microbial activity of soil microorganisms has been studied by some investigators both in pure culture (Sanchez, 1994) and in mixed populations (Nayak, 1982).

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Recurring application of pesticides may have deleterious effects on the non-target organisms and their activities in the soil ecosystem(Tu, 1976). Pesticides generally appear to have no adverse effects on the population of total bacteria in soil except at concentrations exceeding recommended rates(Lal, 1988). Evidence suggests that a distinct shift to the dominance of pesticide-degrading aerobes after intensive use of a pesticide(diazinon and HCH isomers) in flooded soil occurs due to the ability of the aerobes to utilize the pesticide for growth as an energy source vis-a-vis cometabolism during anaerobic transformation(Sahu, 1990).

Microbial activities important to effects on crop productivity and nutrient cycling can be altered by agricultural management practices(Doran, 1987). Management practices have been used primarily in the past to improve productivity(Lovell, 1995). The microbial biomass is a sensitive indicator of changes resulting from agronomic practices and other perturbations of the soil ecosystem(Gunapala, 1998). Free phenolic acids exist in the soil solution comprising $< 0.01\%$ of the total soil organic matter(Vaughan, 1980) and both toxic or stimulatory effects on plant and microbial growth have been documented(Wang, 1967; Anderson, 1974). These phenolic acids can be readily utilized by soil microbes(Sparling, 1981).

The present study was designed to investigate the dynamic response of insecticide applied at various concentrations on soil microbiological and biochemical properties in a paddy field soil.

1 Materials and methods

1.1 Soil

A laboratory incubation experiment was conducted to see the dynamic response of insecticide (triazophos) on a paddy field soil under flooded soil condition at constant temperature of 25℃. The soil used was sampled from the surface layer (0-15 cm) of an agricultural field used for rice cultivation near Jinhua City, Zhejiang Province, China, after removal of surface water. The soil was dried passed through a 2 mm sieve and stored at 4℃ prior to analyses. A subsample of the soil was taken, air-dried, ground, and analyzed for various physico-chemical characteristics(Anderson, 1993; Table 1).

Table 1 Characteristics of the soil used

Soil texture	Silt loam	CEC, meq/100g soil	7.33
Sand, %	28	Water-holding capacity, %	51
Silt, %	56	Total organic carbon, g/kg	15.25
Clay, %	16	Available N, mg/kg	106.40
pH	4.74	Available P, mg/kg	13.34

1.2 Soil treatments and incubation conditions

The pretreated soil in portions equivalent to 150g oven-dry weight was placed into 250 ml glass beakers. A total number of 75 beakers (5 treatments × 3 replications × 5 stages) were prepared to accommodate various treatments. The soil samples were first adjusted to 40% of water-holding capacity (WHC) by adding distilled water and then pre-incubated at 25℃ for 7 days (conditioning period). Appropriate quantities of an insecticide (triazophos) were added as 1.0 ml volume maintaining the concentrations of 0 (control), 0.5 FR, 1 FR, 5 FR, and 10 FR. The 1 field rate(i.e., 1 FR) used for insecticide was 1500 ml/hm^2. All the soils including control were adjusted at the same moisture level of flooded condition (the water level was 2 cm above the soil water interface). Then all the beakers were covered with plastic sheets having small holes and incubated at 25℃ in the dark for 21 days. At the expiry of a particular incubation period of 1, 4, 7, 14, and 21 day after application(DAA) of insecticide samples were taken out and analyzed for various parameters. All results are the mean of three replicate determinations and are expressed on an oven-dry soil weight basis (105℃, 24h). The moisture was maintained constant by adding distilled water at regular intervals throughout the incubation period.

1.3 Soil assay

Electron transport system(ETS)/dehydrogenase activity was measured using the reduction of 2-(*p*-

iodophenyl-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride(INT) to iodonitrotetrazolium formazan(INT-formazan/INTF)(Benefield, 1977). The absorbance values obtained photometrically were converted to nmoles INT-formazan/(min·g)(dry soil) using a standard curve of INT-formazan(INTF). The total soluble phenol compounds of the soil organic matter were determined with Folin Ciocalteau's phenol reagent as described by Box(Box, 1983) and total protein also by reaction of Folin Ciocalteau reagent with amino acids/proteins containing phenolic hydroxyl groups(Lerch, 1993). The total phospholipids were determined by means of its phosphate content. Inorganic *o*-phosphate is released by digestion of the lipid extract with potassium persulfate, and color is developed by reaction of phosphate with ammonium molybdate and malachite green(Frostegard, 1991). Data were examined by analysis of variance using Statistix 4.1 software.

2 Results

2.1 Dehydrogenase/ETS activity dynamics

Measurements of ETS activity dynamics in the control and insecticide-treated soil are presented in Fig.1. There was a consistent decrease in the ETS activity with increasing insecticide concentration irrespective of the incubation stage(Fig.1) during first week of incubation, while later on it increased. The ETS activity in the control soil after 1 day of incubation was 725 nmol INTF/(min·g) soil, but thereafter it declined during first week of incubation and then during the 2nd and 3rd week it increased more than that of the 1 day. Similar trend in ETS activity was observed in insecticide-treated soils but their increase was not so prominent as was in the control soil and also these enhancements were negatively effected with the insecticide concentrations. The addition of insecticide at various levels markedly reduced the ETS activity as compared to the control. The application of insecticide at 0.5 FR did not exercise any significant change in ETS activity in all incubation stages. However, insecticide spiked at 1.0 FR, 5 FR, and 10 FR, after 1 day of incubation, produced marked reductions by 3.9%, 6.6%, and 10.7% in the ETS activity, respectively, as against the control. During the 1st week of incubation, the insecticide addition caused sharp reductions, especially at higher application rates, relative to the control. Later, during the 2nd and 3rd week a rapid increase in ETS activity was observed in all insecticide concentrations, though this increase was more prominent in control soil and this increase decreased as the concentration increased (Fig. 1).

2.2 Soil phenol content dynamics

The dynamics of soil phenol contents in the control and insecticide-treated soils is illustrated in Fig. 2. A consistent increase in the soil phenol content was recorded with increasing insecticide concentrations irrespective of the incubation stage during the first week of incubation, which decreased later on. In most of the incubation stages (especially during the first seven days) insecticide did not cause any significant increments in soil phenol contents at 0.5 FR and 1.0 FR additions as compared to the control. However, 5 FR and 10 FR applications resulted in the significant enhancements in soil phenol contents in all incubation stages as relative to the control. It is further evident from the data that during the 1st week of incubation, there was a significant increase in soil phenol status with the advancement of incubation period/time as compared to the 1st day measurements. Later on, after 14 days of incubation slight declines were observed and these depressions further increased after 21 days of incubations (Fig. 2). The results further revealed that insecticide used at 0.5 FR, 1.0 FR, 5 FR, and 10 FR increased the soil phenol content by 0.1%, 0.

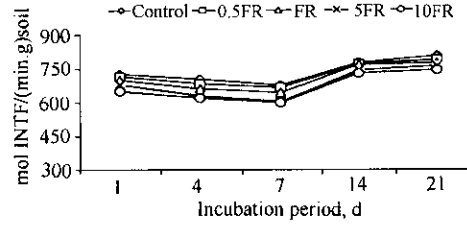

Fig. 1 Effect of insecticide concentration and incubation periods on ETS activity dynamics

6%, 1.5%, and 2.7% after 1st day of incubation, respectively, relative to the control. The corresponding values after 4th and 7th days of incubation were 0.1%, 0.7%, 1.8%, 2.9% and 0.1%, 0.1%, 2.0%, 3.2%, respectively. While, the corresponding values for 14th and 21st days were 0.4%, 0.9%, 2.0%, 3.1% and 1.1%, 1.4%, 2.8%, 3.2%, respectively, as against the control. The increase in soil phenol content produced by various insecticide concentrations was found to be in the order of 10 FR > 5 FR > 1.0 FR > 0.5 FR > control.

2.3 Soil protein content dynamics

The measurements of soil protein content dynamics in response to the insecticide treatments are exhibited in the Fig.3. There was a slight but a consistent(nonsignificant) increase in the soil protein content was observed with the increased insecticide concentration irrespective of the incubation stage. However, a consistent but again non-significant(very slight) depletion in the soil protein content was noticed with the advancement of incubation period irrespective of the insecticide concentration used. On overall basis, no significant or marked changes in soil protein content were observed in the individual application rate or incubation period. As for as application rates are concerned, the minimum increase in soil protein status was produced by 0.5 FR(0.2%) and maximum increase achieved in 10 FR was 1.3% at 1st day of incubation, which was found almost constant throughout the incubation period. Similarly, negligible depletions in soil protein levels were recorded with the expiry of time, which were also found to be negligible(< 1.0 %) in all the cases(insecticide concentrations) during the whole incubation period(21 days).

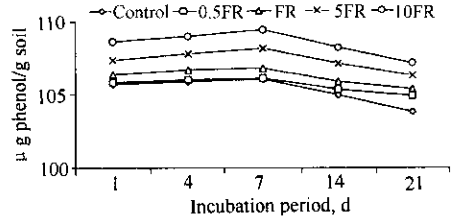

Fig. 2 Effect of insecticide concentration and incubation periods on soil phenol dynamics

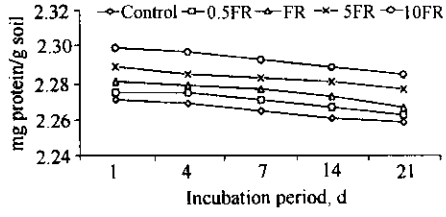

Fig. 3 Effect of insecticide concentrations and incubation periods on soil protein dynamics

2.4 Microbial biomass phospholipid content dynamics

The data regarding the changes in the biomass phospholipid content in the control and insecticide-treated soils over the entire incubation period of 21 days is presented in Fig.4. The application of insecticide to soil slightly reduced the phospholipid content with the advancement of incubation period irrespective of the application levels and it (phospholipid content) also decreased consistently with increasing insecticide concentrations. The minimum reduction in the phospholipid content was observed with 0.5 FR and the maximum by 10 FR in all incubation stages. The 0.5 FR and 1.0 FR did not exert any significant change in the phospholipid content, however, significant (though slight) declines were noticed at 5 FR and 10 FR of insecticide additions at all incubation stages, relative to the control. The decrease in phospholipid content at different concentrations was ranged between 0.2% - 2.0% for all measurements, compared to the control. While the reduction in phospholipid content was improved with the passage of incubation time from a minimum of 0.1% to a maximum of 0.9% during the whole incubation period, as estimated against the 1st day measurements. No significant changes were recorded in the control, 0.5 FR and 1.0 FR soil at different incubation

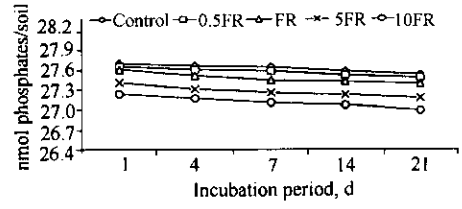

Fig. 4 Effect of insecticide concentrations and incubation periods on phospholipid dynamics

periods. However, at 5 FR and 10 FR additions of insecticides a significant depletion was evident only at 21st of incubation when compared to the 1st day phospholipid content measurements, while the other incubation stages within respective application rate did not produce any significant depletions.

3 Discussion

The results of the present study revealed that ETS activity displayed a negative correlation with insecticide doses, and the activity affected adversely as the concentration of the insecticide increased. The higher concentrations(5 and 10 field rates) significantly inhibited ETS activity, while the lower rates(0.5 FR and 1.0 FR) did not exert any significant reducing effect. This might be explained as the half-life of the insecticide used is about 1 month or so and after 21 days of incubation this response is understandable, as 0.5 FR and 1.0 FR did not produce any significant inhibition of ETS activity and microorganisms might have used it as an energy source(El-Ghamry, 2000). However, higher doses resulted in high reductions. Anderson(Anderson, 1981) and Lal(Lal, 1988) have reported similar observations. Ready recovery of the affected microorganisms as a result of fast degradation or immobilization of the insecticide under the experimental conditions might explain the reversible effects of the insecticide on the oxidoreductive and cellulolytic activities. On the other hand, it is possible that the impairment of one segment of the microbial community was compensated by increased activity of other genotypes with a resulting unchanged overall microbial activity(Atlas, 1978). Chendrayan and Sethunathan(Chendrayan, 1980) studied the effects of hexachloro-cyclohexane(HCH), carbaryl, benomyl, and atrazine on dehydrogenase activity in an alluvial soil under flooding conditions. They found that at 1 mg/kg, no inhibition occurred with any of the pesticides, but at a 10-fold rate dehydrogenase activity dropped temporarily to about 30% but recovered after 20 days. An even more drastic reduction was observed with benomyl at 10 mg/kg, with only 10% residual activity detected initially and still less than 50% after 20 days of incubation. The insecticide addition exhibited a consistent decrease in the ETS activity with increasing insecticide concentration irrespective of the incubation stage during the first week of incubation, while later on it increased. This might be due to toxicity of insecticide which was more pronounced at higher rates during the 1st week of incubation which (toxicity) declined later on due to its degradation and dilution in water with the passage of time(El-Ghamry, 2000). The present results are quite consistent with these findings.

An inverse relationship was observed between dehydrogenase/ETS activity and soil phenol contents. It is established that soil microorganisms can both produce and decompose phenolic compounds (Stevenson, 1994). There are many soil fungi capable of utilizing phenolic acids as their sole source of carbon (Henderson, 1955). Olk (Olk, 1996) also reported an accumulation of phenolic compounds in the soil organic matter, which he suggested that it is a characteristic of the anaerobic, or nearly anaerobic, soil conditions that exist at the initial stages of soil organic matter formation in submerged irrigated rice soils. It was found that phenolic acids can be readily utilized by soil microbes (Sparling, 1981) as they occur as the free acids in the soil solution and hence should be readily available for microbial degradation(Wang, 1967). The results of present study demonstrated a marked increase in soil phenol content with the higher doses of insecticide addition and it(phenol content) also increased with increasing pesticide concentration. This phenomenon might be due to decreased microbial activity with pesticide addition, particularly at higher rate of application, which might have resulted in lowering decomposition and utilization of phenol contents by soil microbial biomass(Sparling, 1981). The increase in phenol contents during 1st week, particularly at higher rates of insecticide, was probably due to toxicity effect of insecticide that reduced activity and later on with advancement of time as the toxicity reduced better utilization/decomposition

resulted in slightly reduced phenol content(Wingfield, 1977).

The addition of insecticide did not cause any marked change in the soil protein content and it was found nearly stable. The application of insecticide resulted in a slight(non-significant) increase in protein content compared to control, which might be due to relatively more mineralization of organic N in control soils than treated soil as insecticide addition might have reduced microbial population and activities(El-Ghamry, 2000). Microbial biomass C, N, and P gradually decreased as the soils became more reduced (anaerobic). This was probably explained by the reduced energy captured by microorganisms using alternate electron acceptors. Consequently, as the microbial biomass decreased there was a decrease in the amount of enzyme activity and decreased organic C, N, and P mineralization(McLarchey, 1998). The high resistance (stability) of organic N complexes in soil to microbial attack is of considerably significance to the N balance of the soil and several explanations are often given to explain this phenomenon. For instance, proteinaceous constituents(e.g., amino acids, peptides, proteins) are stabilized through their reaction with other organic constituents, such as lignins, tannins, quinones, and reducing sugars and biologically resistant complexes are formed in soil by chemical reactions involving NH_3 or NO_2^- with lignins or humic substances. The complexes thus formed have been shown to be highly resistant to mineralization by soil microorganisms (Stevenson, 1994). The increased phenol contents with increasing insecticide concentrations in this study might be a strong reason for increased protein contents(Olk, 1996). However, a slight non-significant depletion with advancement of incubation time was also noticed. This might be due to increased activity (N-mineralization) with the passage of time due to decreased toxicity(Wingfield, 1977).

The incorporation of insecticide caused marked changes in phospholipid content, especially at their higher rates of application, compared to the control. Phospholipids occur in the cell membranes of all living cells and are not used as storage products(Petersen, 1991). It has been reported that soil under aerobic condition contains larger amounts of phospholipids than under anaerobic (flooded) conditions (Reichardt, 1997). Inubushi (Inubushi, 1991) also reported similar findings. Frostegard (Frostegard, 1991) evaluated the use of total lipid phosphate ($L\text{-}PO_4$) as a measure of microbial biomass in soils with different organic matter content and suggested that the soil samples typically contained $\approx 30-50$ nmol $L\text{-}PO_4$ after persulfate digestion, a range that was suitable for the method used. Slightly lower values in present study might be due to anaerobic conditions as anoxic conditions caused reduction in(aerobic) microbial biomass (Inubushi, 1991). The lower quantities of phospholipids observed in insecticide treated soils than control soil, might be due to its toxicity as explained by Wingfield(Wingfield, 1977) and Soderstrom (Soderstrom, 1983). The present dynamic response study also demonstrated a decrease in phospholipid content with increased insecticide rates and with advancement of different incubation stages. Reichardt(Reichardt, 1997) also noticed a decline in phospholipid content, due to flooding, in continuously cropped, irrigated rice fields. So, results of these studies are quite consistent with the earlier findings.

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