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Full Length Research Paper

Effect of Cartap Hydrochloride on Amylase and Cellulase Enzyme Activities in Agricultural Soil

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Abstract

The effect of Cartap hydrochloride on soil enzyme activities was carried out at different concentrations of pesticide on the activities like Amylase and Cellulase. Initial suppression of amylase activity in treated soil over the control was observed during incubation period and enzyme showed recovery with increasing incubation period. Cellulase activity varied during different days of incubation and recovery was observed from 14th day onwards in the soil samples treated with Cartap hydrochloride. Significant depression in glucose production was observed in Cartap hydrochloride treated soils over the control from the first day of treatment.

Key words: *Cartap hydrochloride, Pesticide, Amylase activity, Cellulase activity*

Introduction

The goal of pesticide use is to apply products that will remain in the target area long enough to control the specific pests and then degrade into harmless compounds in the soil, air and water without contaminating the environment. In India, the challenge in improving agricultural productivity still remains the curtailment of crop losses due to pests, estimated at about 50% of the total food production. According to (Abhilash and Nandita, 2009) two million tons of pesticides were consumed per year throughout the world. Among the Asian countries, pesticide consumption was more in China followed by Korea, Japan and India. Soil enzymes mediate many processes occurring in soil. These are derived from micro organisms, plant roots and soil animals. They play an important role in the organic matter turnover and degradation of xenobiotics. The insecticide reaches the soil after spraying or after washing by rainfall. Soil enzyme activities predict the potential of the soil in performing some biological processes responsible for the fertility of soil. Amylase is a starch hydrolyzing enzyme, synthesized by plants, animals, microorganisms. It is widely distributed in the soil. So it plays a vital role in the breakdown of starch. It catalyzes the hydrolytic depolymerization of polysaccharides. Cellulase is the abundant organic compound found in the biosphere. Cellulases catalyze the degradation of cellulose and polysaccharides. Activities of cellulase are primary indication of some of the physicochemical properties of the soil. Studies have shown that activities of amylase and cellulase in agricultural soils are affected by several factors. Thus the present investigation was aimed to focus on effect of Cartap hydrochloride on amylase and Cellulase activities.

Materials and Methods

Pesticide

Cartap hydrochloride is one of the main insecticides used in India particularly for the crops of Rice and Sugarcane to control weevil and caterpillars. It acts at very low concentration and its efficacy is very prolonged. It controls all stages of the insect life cycle. Its basic chemical structure is *S,S*-[2-(dimethylamino)-1,3-propanediyl] dicarbamothioate and is normally used as the hydrochloride (Cartap hydrochloride). Its molecular Weight is 273.80 and the molecular Formula is $C_7H_{15}N_3O_2S_2$ HCl (Fig 1).

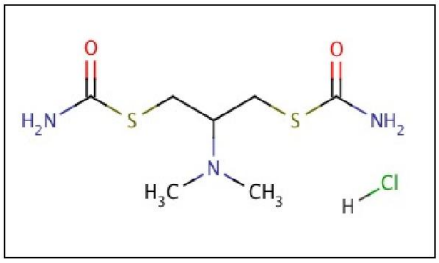

Figure 1. Chemical structure of Cartap Hydrochloride

Study of soil enzyme assays

Amylase activity

Five gram portions of 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 soil equivalent to field application rates of 1, 2.5, 5.0, 7.5 and 10 kg ha^{-1} respectively. Soil samples without insecticide treatment served as controls. Soil samples were mixed thoroughly for uniform distribution of insecticide added. Duplicates 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. Triplicate samples of soil were withdrawn during 1st, 7th, 14th, 21st and 28th day of incubation to determine the changes in amylase activity. The method employed for the assay of amylase activity was the same followed by (Tu 1981a and1981b and Singaram and Kamala Kumari, 2000).

Assay of amylase

Soil samples were transferred to 100 ml Erlenmeyer flasks and 1 ml of toluene was added. After 15 minutes, 6 ml of 0.2 M acetate phosphate buffer (pH 5.5) containing 2% starch was added to the soil samples and the flasks were stoppered and kept for 24 hrs at 37°C. Soil extracts were passed through Whatman No.1 filter paper and glucose content in the filtrate was assayed according to Nelson Somagyii method (1944).

Cellulase activity

After incubation at 28 ± 4°C, triplicates of control and treated soil samples were withdrawn at 1st, 7th, 14th, 21st and 28th day of incubation for determination of cellulase activity following the method of (Pancholy and Rice, 1973).

Assay of cellulase

The soil samples were transferred into 100 ml Erlenmeyer flasks and 1 ml of toluene was added to stop the enzyme reaction. The contents in the flasks were mixed thoroughly and after 15 min 10 ml of acetate buffer (pH 5.9) was added followed by 10 ml of 1% Carboxy methylcellulose (CMC). The flasks were then incubated for 24 hrs at 37°C. At the end of the incubation period 50 ml of distilled water was added. The suspension was filtered through Whatman No.1 filter and the volume of the filtrate was made up to 100 ml with distilled water. The reducing sugar content in the filtrate was determined by Nelson Somagyii method (1944). Suitable aliquots of filtrate were taken in test tubes and 1 ml of alkaline copper reagent was added and covered with marbles and placed in boiling water bath for 20 min. the tubes were then cooled under running tap water and 1 ml of arseno molybdate reagent was added. The final volume in tubes was made up to 5 ml with distilled water and bluish green colour was read at 500 nm in a UV- Visible spectrophotometer. The amount of glucose was calculated by referring to a calibration curve.

Statistical analysis

All the data were expressed on an air-dry soil basis and were averages of two or three replicate determinations. The data of Cartap hydrochloride impact on microbial populations and soil enzymes were interpreted by using Two- way ANOVA; means were compared by least significant difference test (LSD).Data was analyzed for significant differences ($P \leq 0.01$) between pesticide treated soil and untreated soils using Duncan's Multiple Range (DMR) test (Megharaj et al., 1999).

Results and Discussion

Enzymes are catalysts produced by both soil microbes and plants. Soil contains free or immobilized extracellular enzymes, and enzymes within microbial cells. Enzymes are compound specific and accelerate biochemical reactions like nutrient cycling. When the enzymes react with their specific compounds, they remain unaltered when the products formed. Soil microbes and enzymes are responsible for maintaining the soil fertility through degradation and mineralization of organic matter including plant and animal residues. Soil enzyme activities are suggested to be sensitive indicators of soil quality as they respond quickly to either environmental stress or management practice changes. The present study focused on impact of different concentrations of Cartap hydrochloride on different soil enzyme activities like amylase and cellulase.

Amylase activity

Amylase activity in control and Cartap hydrochloride treated soil samples were studied during different days of incubation and the results were summarized in Table and Fig. The results showed an initial suppression of amylase activity in treated soil over the control. During incubation period, the production of glucose (µg/g soil) from starch was increased steadily in duration depending manner and highest productivity was recorded after 7th day of incubation period at 5, 10 and 25 ppm concentrations of Cartap hydrochloride. The amylase activity has significantly decreased with the increasing concentrations of Cartap hydrochloride, i.e., 50 and 100 ppm. However, enzyme slightly increased on 21 and 28 days of incubation. The effect of different concentrations of Cartap hydrochloride on soil enzyme activities was studied. The amylase activity has significantly decreased with the increasing concentrations of Cartap hydrochloride. The analysis of variance revealed that difference in amylase activity in soil samples treated at different concentrations during different incubation periods were observed as significant. (F value 354.372, p value 0.00). Similar result was obtained by (Rangaswamy and Venkateswarlu, 1992) a marked suppression of amylase activity in groundnut soil treated by

insecticides as monocrotophos, quinalphos, cypermethrin, and fenvarelate. Similarly, Tu (1993) reported that 11 herbicides used in his study inhibited amylase activities after 1 day of incubation. On the other hand, (Tu ,1988) reported that malathion, carbofuran, and permethrin at a high level after 3 days were stimulatory in the formation of glucose from added starch.

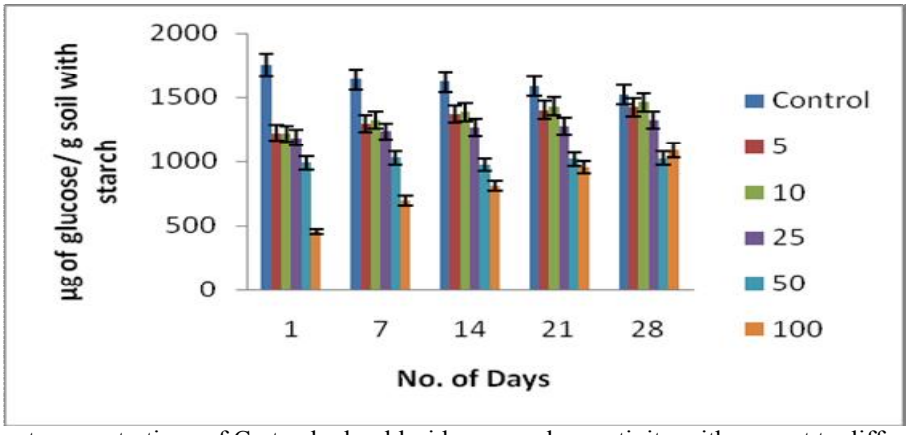

Figure 2. Effect of different concentrations of Cartap hydrochloride on amylase activity with respect to different days of incubation periods

Cellulase activity

Cellulase activity in control and Cartap hydrochloride treated soil samples varied during different days of incubation as illustrated in Table and Fig. Depression in glucose production was observed in Cartap hydrochloride treated soils over the control from the first day of treatment. Slight increase was observed at 50 ppm of the soil samples treated with Cartap hydrochloride and then decreased at 100 ppm concentration. The celullase activity has decreased with the increasing concentrations of Cartap hydrochloride. The method developed and used for the assay of cellulase activity in soils is based on colorimetric determination of reducing sugars in soil extracts formed from the carboxy methylcellulose in the presence of soil cellulase. Reduction in glucose production was observed in Cartap hydrochloride treated soils over the control from the first day of treatment. Cellulase activity was significantly decreased from 14th day onwards in the soil samples treated with Cartap hydrochloride. Cellulase activity in Cartap hydrochloride treated samples was significantly ($P<0.01$) comparable with control sample from 7 to 28 days of incubation period. The analysis of variance revealed that difference in cellulase activity in soil samples treated at different concentrations during different days of incubation period were observed as significant. (F value 1243270.758, p value 0.00). The same result was observed by Ramudu et al., 2011, showed the activity of cellulase under influence of different concentrations (1.0, 2.5, 5.0, 7.5, and 10.05kg/ha^{-1} of fungicides after 10 days. Rangaswamy and Venkateswarlu 1992 noticed that the insecticides at higher concentrations of 7.5 and 10.05kg/ha^{-1} were toxic to cellulase activity. Tu 1982, 1988 and Jayamadhuri and Rangaswamy 2005 observed similar trend of cellulase activity. Captafol at 10 parts/10^{6} was significantly inhibited mineralization of cellulase in a sandy loam soil. A distinct depression was observed with chlorothalonil, under all conditions tested, that is, at the usual dose, in both flooded and nonflooded soil. Similarly, trichlamide at 10 times recommended field rate (i.e., 40mg/kg) incubated for 4 weeks under flooded soil conditions, inhibited the cellulolytic activity. According to Arinze and Yubedee 2000 benlate, calixin and captan inhibited the activity of cellulase in *Fusarium monoliforme* isolates. Impact of pesticides on soil enzymes show considerable diversity depending upon the type of insecticide used, rate and mode of application, climatic factors and composition of soil organic matter etc.(Tsirkov, 1970).

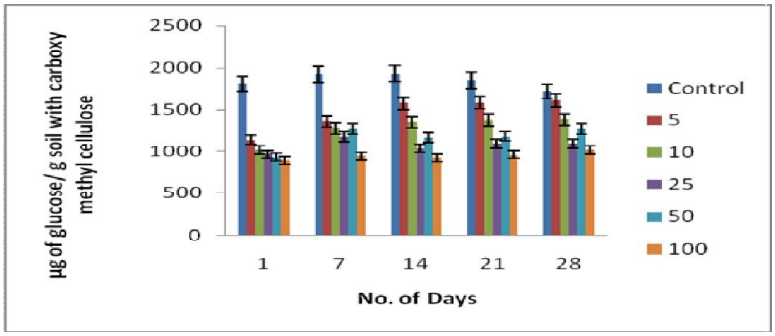

Figure 3. Effect of different concentrations of Cartap hydrochloride on cellulase activity with respect to different days of incubation periods

Values are the means of triplicates± SD.

***Significant át 1% level.*

Conclusion

Generally, the effect of pesticides decreases with the increase in the incubation period. The production of glucose (µg/g soil) from starch was increased steadily in duration depending manner. The amylase activity has significantly decreased with the increasing concentrations of Cartap hydrochloride. However, enzyme showed recovery with increasing incubation period. Cellulase activity varied during different days of incubation. Significant depression in glucose production was observed in Cartap hydrochloride treated soils over the control from the first day of treatment. Recovery was observed from 14th day onwards in the soil samples treated with Cartap hydrochloride. It was concluded that the enzyme activities were not harmed at the recommended field rates.

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