Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(2):856-860

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Impact of Cartap hydrochloride on soil enzyme activities

P. Lizy Sravanthi*, T. Madhuri and P. Suvarnalatha Devi

Department of Applied Microbiology, Sri Padmavathi Mahila Visvavidyalayam (Women's University), Tirupathi, A. P., India

---

ABSTRACT

Soil enzymes play a vital role in catalyzing several important reactions necessary for life processes of microorganisms in soil. Soil enzymes play an important role in catalyzing reactions for the decomposition of organic matter and nutrient recycling in ecosystems. Microorganisms are the producers of enzyme activities in soil. Cartaphydrochloride, thiocarbamate insecticide, was assessed for its effect on Protease and Urease enzymes in soil at different concentrations of pesticide for definite period of incubation. Protease activity in soil samples treated with low concentrations (5, 10 and 25 ppm) of Cartap hydrochloride showed a tendency of recovery and gradually decreased with incubation period. Urease activity in treated and control soil samples during different days of incubation period was observed to be less than that of control throughout the incubation period.

Key words: Cartap hydrochloride, Protease activity, Urease activity, pesticide.

INTRODUCTION

In modern agricultural practices, pesticides are frequently used in the field to increase the crop production. Pesticides are the chemical substances that kill pests and herbicides are the chemicals that kill weeds. An ideal pesticide should have the ability to destroy target pest quickly and should be able to degrade non-toxic substances as quickly as possible. Pesticides are of primary importance because of their continuous entry into the soil. Pesticides enter the soil either by direct or indirect application. Enzymes contribute total biological activity of the soil and plant environment [1].Their efficiency may be influenced by the composition of surroundings in which they act as catalysts. So pesticides are expected to affect the behavior of enzymes [2]. Cartap hydrochloride is a member of Thiocarbamate family and is widely used against a relatively broad spectrum of insects, e.g., Lepidoptera, Coleoptera, Diptera and Hemiptera. It is especially effective against Lepidopterans such as the rice stem borer, diamond-back moth and common cabbage worm and Coleoptera such as the Colorado potato beetle, Mexican bean beetle etc.; These are used to kill boring insects and pests attacking crops like sugarcane, maize, vegetables and ornamental plants. Soil enzyme activities may predict the potent of soil to perform the biological changes. Enzymes in the soil are involved in many different aspects of the metabolism of soil organic matter. Thus the present investigation was aimed to focus on effect of Cartap hydrochloride on Protease and Urease activities.

EXPERIMENTAL SECTION

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).

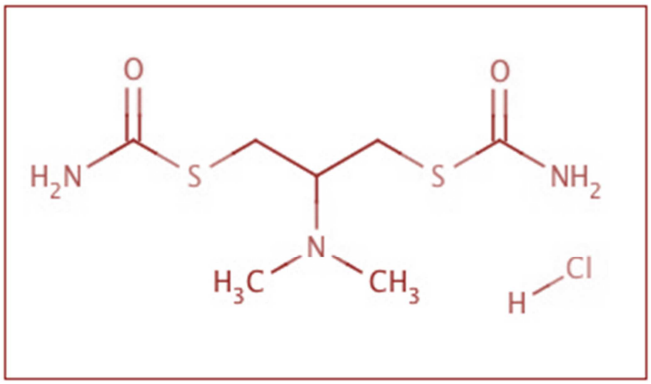

Figure 1: Chemical structure of Cartap Hydrochloride

Soil enzyme assays

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.

Assay of Protease

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 protease activity following the method [3].Soil samples including control were incubated with 10 ml of 0.1 M Tris (2-amino-2-(hydroxymethyl)- propane- 1,3-diol at pH 7.5) containing sodium caseinate (2% w/v) and incubated for 24 hrs at 30°C. To this, 4 ml of aqueous trichloro acetic acid (17.5% w/v) was added and the mixture was centrifuged. Suitable aliquots of the supernatant were further treated with 3 ml of 1.4 M Na_2CO_3 and 1 ml of Folin-Cicalteau reagent (33.3%, v/v).Blue colour formed was read after 30 mins at 700 nm. Tyrosine equivalents in soil extracts were estimated by referring to the calibration curve prepared with known concentration of tyrosine.

Assay of urease

Triplicate samples of soil were withdrawn during 1st, 7th, 14th, 21st and 28th day of incubation to determine the changes in urease activity according to Rangaswamy and Venkateswarlu[4].For determination of soil urease activity, soil samples were mixed with 4 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 1 ml of 1 M urea solution and incubated for 30 minutes at 37°C and suspensions were shaken for every 5 min. After incubation, 10 ml of 2 M KCl was added and the mixture was kept at 4°C for 10 min to stop the enzymatic reaction. Suspensions were centrifuged for 5 min. Estimation of NH^+_4 ion concentration of the supernatant was done by phenol hypochlorite method [5]. Supernatant (2 ml) was mixed with 5 ml of phenol- sodium nitroprusside solution and 5 ml of 0.02 M sodium hypochlorite. The mixture was for 30 mins in dark, and the blue colour so formed was measured at 630 nm in a UV-Visible spectrophotometer.

Statistical analysis

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 [6].

RESULTS AND DISCUSSION

The influence of Cartap hydrochloride on soil enzyme activities like Protease and Urease was studied as the activities of soil microorganisms are important in global cycling of carbon, nitrogen, phosphorus and sulphur, etc., because many substances cannot be degraded by organisms other than microbes[7].The biochemical activity of enzymes for certain reactions has been estimated to be important than that of microbial cells[8].

Protease activity

The variation in protease activity in control and Cartap hydrochloride treated soil samples during different days of incubation period was studied and summarized in (Fig 1).From 7th day onwards, protease activity in soil samples treated with 5, 10 and 25 ppm concentrations of Cartap hydrochloride slightly decreased and significant reduction was observed in the protease activity of soil samples treated with Cartap hydrochloride at higher concentrations 50 and 100 ppm throughout the period of incubation.In the present study, protease activity gradually decreased with increase in the concentrations of pesticide. The analysis of variance revealed that a significant difference in protease activity in soil samples treated at different concentrations of pesticide (F value 1759.248, p value 0.00). The insecticides were found to be toxic to protease activity in soils at levels higher than 25 ppm.The results obtained in the present study are in conformity with the observations made by Gita Rani and Behera[9].Soil protease activity, known to be of importance in soil nitrogen cycling[10]was stimulated up to 21 days of incubation, followed by a sharp decline after 28 days. However, decrease of protease activity in a native soil was reported after treatment with linuron at 10 mg kg-1, whereas, cartap-HCl at 100-1000 mg kg-1 inhibited the enzyme activity without any recovery during a period of 60 days[11].According to Satpathy and Behera[12].Protease activity showed a decline in metsulfuron-methyl-treated soil samples from the very first day after treatment. From the analysis of variance, it was evident that the variation in protease activity with respect to different concentrations of Cartap hydrochloride.

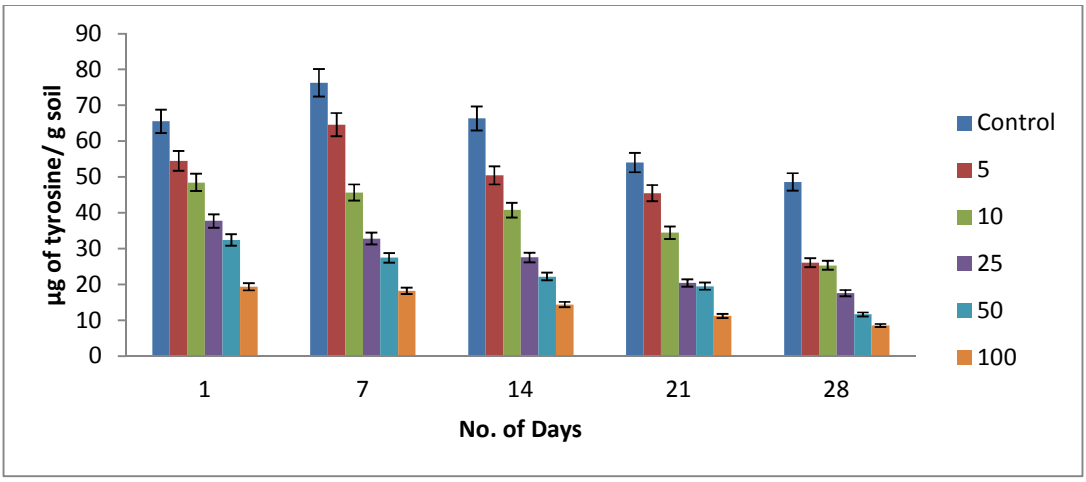

Figure 1: Effect of different concentrations of Cartap hydrochloride on protease activity with respect to different days of incubation periods

Urease activity

Urea is an organic chemical used as a nitrogenous fertilizer in agriculture. Conversion of organic nitrogen to inorganic nitrogen through hydrolysis of urea to ammonia and carbon dioxide is due to activity of Urease enzyme secreted by certain microorganism and plants. This enzyme is responsible for supply of nitrogen demand to growing crop. Since Urease has also been used in the evaluation of changes in soil quality, Urease activity in treated and control soil samples during different days of incubation period was significantly decreased throughout the incubation period (Fig 2).Urease activity was continued up to 21 days of incubation and then decreased after 21 days. This enzyme activity was continued up to 21 days of incubation and then decline in urease activity was observed. The analysis of variance revealed the difference in urease activity in soil samples treated at different concentrations (F value 674.374, p value 0.00). The inhibition of urease activity could be due the presence of Mn and Zn ions in the pesticide, as reported by Tabatabai[13].Urease has been studied more extensively relative to other soil enzymes because of its involvement in the breakdown of urea, a commonly used fertilizer[14].N100 treatment of Mancozeb brought about inhibition of urease activity. The urease activity was not affected by the low

concentration (0.1 µg/g) of metsulfuron-methyl. However, at concentrations above 0.5 µg/g in soils, there was lower urease activity than in controls throughout the incubation period. The urease activity did not show recovery during the entire incubation period. Other herbicides such as chlorbromuron and EPTC have shown stimulatory effects on urease activity in sandy loam soil[15]. Satpathy and Behera[12]reported that the insecticide malathion had an inhibitory effect on urease activity. The presence of metolachlor at 10 µg/g caused a reduction in urease activities for the entire 4-week study [16].Tu [17] reported that thiram at 10 ppm level decreased urease activity in both sandy and organic soil after 7 days of incubation and in contrast, 0.5 to 5µg levels of pyrethroids, permethrin, FMC 45498, Shell WL41706, WL43367, WL43775 were found to have no effect on urease activity in a sandy loam soil [18].

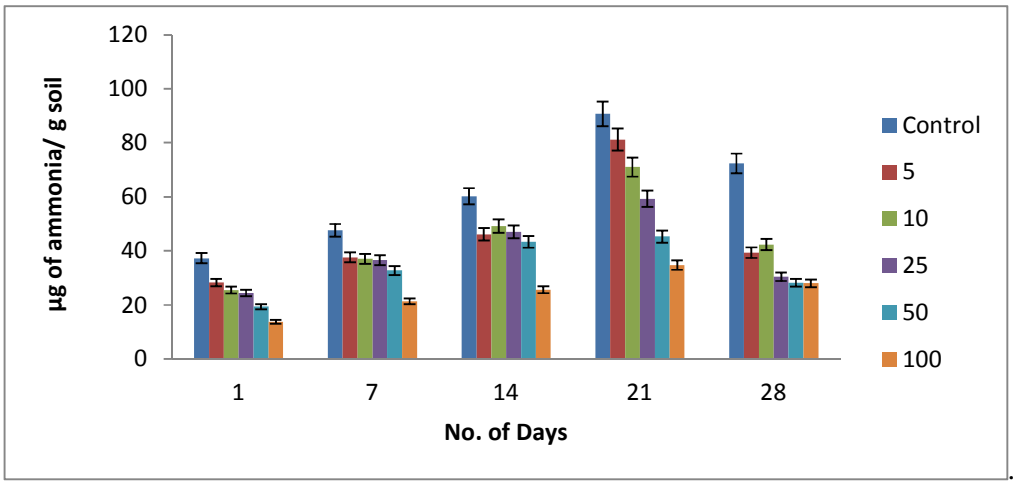

Figure 2: Effect of different concentrations of Cartap hydrochloride on urease activity with respect to different days of incubation periods

CONCLUSION

The effect of pesticides decrease with the increasing incubation period. Protease activity in soil samples treated with low concentrations (5, 10 and 25 ppm) of Cartap hydrochloride showed a tendency of recovery and gradually decreased with incubation period. Urease activity in treated and control soil samples during different days of incubation period was observed to be less than that of control throughout the incubation period. It was concluded that the enzyme activities were not harmed at the recommended field rates.

Acknowledgement
This work is supported by Dept. Applied Microbiology, Sri Padmavathi Women's University, Tirupati, Andhra Pradesh, India.

REFERENCES

[1] RPDick. Soil enzyme activities as integrative indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VVSR (Eds.). Biological indicators of soil health, CAB International, Wellingford., **1997**, 121- 156.
[2] JMBollag; SY Liu. *Soil Science Soc. America*, Madison, WI, USA. **1990**, 169- 211.
[3] TWSpeir;DJRoss. *New ZealandJ. Scie*., **1975**, 18: 231-237.
[4] VRangaswamy;KVenkateswarlu. *J. Environ. Ecol*., **1992b**, 10: 525-530.
[5] JKFawcett; JEScott. *J. Clin. Path.*, **1960**, 13:156-159.
[6] M Megharaj; KKookana. Singleton S. *Soil. Biol. Soil. Biochem*. **1999, 39**: 1549-1553.
[7] MAlexander, Introduction to Soil Microbiology. **1977**, 2nd edn. Wiley, New York.
[8] RG Burns, *Soil Biol.Soil Bioche..*, **1982,** 14: 423-427.
[9] SGitarani; NBehara. *J. Environ. Microbiol*., **1993,** 14(4): 301- 310.
[10] YKamimura;KHayano. *Soil Biology and Fertility of Soils*., **2000,** 30: 351–355.
[11] T Endo; KSakai.TNobutsura;S Michihiko..*J. Pest. Scie*., **1982,** 7: 101-110.
[12] G Satpathy;NJBehera. *J. Environ. Biol.*, **1993**, 4: 301-10.

[13] MATabatabai, *Soil. Bio. Soil. Biochem.*, **1977**, 9: 9- 13.
[14] DAMartens; JMBremner. *Pest.Scie*., **1997**,49: 352- 433.
[15] CMTu, *Bull. Environ. Conta.Toxico*., **1993**, 51: 30-9.
[16] BSIsmail; IDavid, Omar O. *J. Environ. Scien.Heal*.,**1996**, 31: 1267-78.
[17]CMTu, *J. Environ. Scie. Health*.,**1990**, 25:787-800.
[18] CMTu, *Bulle. Environ. Contamin.Toxico*., **1981b**, 27: 109-114.