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Research Article

Synergestic Effect of Chlorpyrifos and Deltamethrin on Enzyme Activities in *Atractomorpha creulata*

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Abstract

The effect of pesticides chlorpyrifos and deltamethrin alone and in combination were evaluated against *Atractomorpha crenulata* nymph to determine carboxylesterase and acetylcholinesterase activities. The combied dose of Chlorpyrifos and deltamethrin showed the lowest carboxylesterase activity when compared to individual pesticides tested at concentrations of 200 ppm/cm^2 with 0.26 mg protein. Similar inhibitory effect was also showed with acetylcholinesterase activity when tested at concentrations of 200 ppm/cm2 with 0.01 mg protein. Hence, they could potentially substitute broad-spectrum synthetic toxins for *A. crenulata* control.

Keywords: *A. crenulata*, chlorpyrifos, deltamethrin, carboxylesterase and acetylcholinesterase.

Introduction

Acridoidea, as a superfamily under the order Orthoptera, has been subdivided into fourteen families (Dirsh, 1961). In India, only three families namely Eumastacidae, Pyrgomorphidae and Acrididae have so far been identified. These families include both generalists and specialists causing considerable damage to food crops and thereby to economy. Atractomorpha crenulata (Pyrgomorphidae is a highly polyphagous insect forming the dominant group amoung Acridoids in agroecosystem of Tamil Nadu. It is a highly polyphagous pest with a very wide range of host plants both cultivated as well as wild. Nymphs and adults nibble leaf lamina causing irregular holes. In case of severe attack, dust with 4% carbaryl or endosulfan.

Carboxylesterases are a large group of hydrolyzing enzymes that cleave ester bonds (Oakeshott *et al*., 2005). These detoxification enzymes play an important role in insecticide resistance and have been associated with resistance to several insecticide classes in many insects (Ranson *et al*., 2003 and Li *et al*., 2007). Due to its role in insecticide metabolism, the measurement of carboxylesterase activity is used as a biochemical indicator of insecticide resistance in many insect species. Acetylcholinesterase is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine in the nervous system. Organophosphorous insecticides, such as diazinon, target AChE and irreversibly inhibit the enzyme by phosphorylating a serine hydroxyl group within the enzyme active site

(Wang *et al*., 2004). This study was initiated to understand the kinetics of carboxylesterase and Acetylcholinesterase inhibition by chlorpyrifos and deltamethrin on *Atractomorpha creulata*.

Materials and Methods

Stock colonies of *Atractomorpha creulata* were generated with nymphs collected from a Rice field, Chengalpet, Tamil Nadu, India in 2010. The nymphs were reared on fresh *R. communis* leaves were provided with cut ends immersed in knob's solution. The nymph of *A. crenulata* were maintained at room temperature 25-30^{0}C and 70% relative humidity, with a photoperiod of 14:10 (L:D). After several generations, insects from the stock colonies were used for the tests.

The efficacy of chlorpyrifos, deltamethrin and its combination against the *A. creulata* was determined using the small glass tubes (~6mm x 40mm). Four different concentrations of pesticides (25, 50, 100 and 200 ppm) were used. All the concentrations were diluted with acetone. Circular discs (140 cm^2) were cut from the leaves of *Ricinus communis* (L.) and 2 ml of the test compounds viz., chlorpyrifos, deltamethrin and combination of both in acetone (at 25, 50, 100 and 200 ppm concentrations/cm^2 area of leaf) was spread using a fine pipette on the leaf. The discs were left to dry, and then weighed. Twenty nymphs were placed in the center of each Petri dish. The bioassay was conducted with the nymphs that had eaten approximately 50% of the control discs or after 10 h.

Midguts of 10 mid fifth instar larvae were used after the removal of the gut contents, and homogenized in ice cold 20mM phosphate buffer (pH 8.0) (100 mg tissue/1ml buffer) using Potter-Elvehjam homogenizer. The homogenate was centrifuged at 10,000g for 20 mts at 4^o C. The supernatant was used directly as the enzyme source for quantitative estimation of carboxylesterase activity.

The carboxylesterase activity was assayed by the technique of Manikandan and Ravishankar, 1998. A standard reaction mixture (1.5ml) consisting of 100 µl of enzyme, 250 µl of α - naphthyl acetate solution (1mM) dissolved in acetone, and 1.15 ml of 20mM phosphate buffer (pH 8.0) was incubated at room temperature for 30 minutes. The reaction was stopped by adding 250µl of coupling reagent (3% Fast Blue B salt in 3.5% sodium dodecyl sulfate). The strong blue colour developed was measured at 605nm, with a U-3210 Hitachi spectrophotometer against a blank in which the volume of enzyme supernatant was replaced by the same volume of buffer. The concentration of substrate hydrolyzed was determined from a standard curve of α - naphthol. The specific activity was expressed as n-moles of α-naphthol released /min/mg protein.

Acetyl choline esterase activity was determined by the method based on the hydrolysis of acetyl choline by the action of cholinesterase (Kenedal and Boettiger 1967). Aliquot of 0.25ml of freshly prepared homogenate was mixed with 0.25 ml of acetylcholine solution (Reagent C) were pipetted in to 18% 150mm standard Pyrex test tubes and then volume was made upto 2ml by adding distilled water. After that 4ml of alkaline hydroxyl amine (Reagent F were added to each test tubes and shaken vigorously. After 2 mins 2ml of HCL solution (Reagent G) were added to the samples followed by 2ml of 0.37 M ferric chloride solution (Reagent H), with through mixing after each addition. The samples were filtered through whatmann filter paper and transferred into cuvettes. Optical density of the brown colour developed, was determined at 540 nm by Hitachi U 3210 UV spectrophotometer. The OD values were plotted against micromoles of acetylcholine chloride.

Results and Discussion

Carboxylesterase activity of midgut tissue of Fourth instar larvae of *A. creulata* fed on chlorpyrifos, deltamethrin and its combinations plus *R. communis* treated leaves showed significantly lower activity when compared with the control (0.36 mg tissue) (Fig. 1). The activity decreased with the increasing concentration of the test compound. Lowest activity was observed at 200 ppm/cm^2 (0.26 mg protein) for combination of chlorpyrifos and deltamethrin followed by deltamethrin (0.27 mg protein) and chlorpyrifos (0.29 mg protein). Similarly Healy *et al*., 1991 showed that the midgut had the highest activity of esterases in insects.

Figure 1. Carboxylesterase activity of Fourth instar larvae of *A. crenulata*

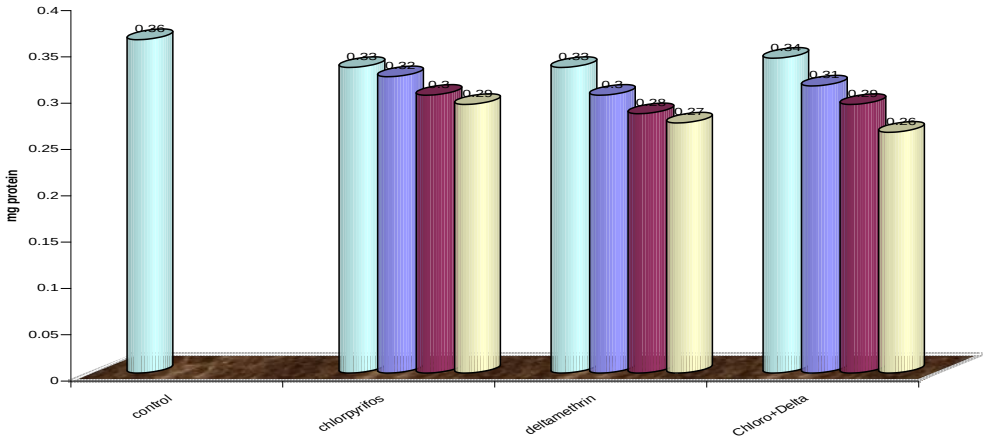
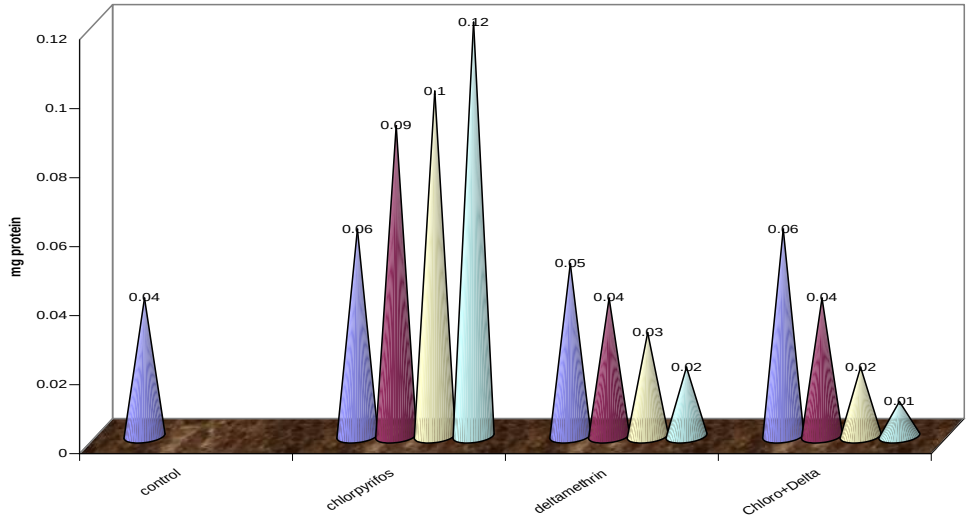


Figure 2. Acetyl cholinestrase activity of Fourth instar larvae of *A. crenulata*



Ghumare *et al*., (1993) observed that the carboxylesterase activity was the maximum in *S. litura* fed on mint when compared to the other hosts like cabbage, cotton, tomato, castor and concluded that the allelochemicals present in mint definitely influence the enzyme activity.

Cholinestrase activity of midgut tissue of Fourth instar larvae of *A. creulata* fed on chlorpyrifos, deltamethrin and its combinations plus *R. communis* treated leaves showed significantly lower activity when compared with the control (0.04 mg protein) except chlorpyrifos treated nymph (Fig. 2). The activity decreased with the increasing concentration of the test compound. Lowest activity was observed at 200 ppm/cm^2 (0.01 mg protein) for combination of chlorpyrifos and deltamethrin followed by deltamethrin (0.02 OD mg protein). But chlorpyrifos treated nymph gave the higher activity of enzyme when compared to control. Kojima *et al*., 1970 demonstrated that esterases and phosphatases exhibit a greater degree of polymorphism because they act on a class of molecules that directly come from external environment. Therefore, the substrates of esterases are qualitatively and quantitatively highly variable. The present investigation indicated that the pesticides chlorpyrifos and deltamethrin is a toxin to *A. creulata*.

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