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Activity of Acetylcholinesterase (AChE) in OxycarenusHyalinipennisCosta (Hemiptera: Lygaeidae, 1847) After Exposure to Chrysanthemum Indicum l. ExtractRupture

Muhammad Abubaker Hassan*, Shoaib Freed

Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahaudinzakariya University, Multan, Pakistan

Research Article

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***For Correspondence**

Muhammad Abubaker Hassan, Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahaudinzakariya university, Multan, Pakistan

E-mail: ahawan97@outlook.com

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ABSTRACT

Oxycarenus Hyalinipennis Costa (Hemiptera: Lygaeidae) is serious pest of *Gossypium Hirsutum L* which is commercially grown to generate foreign exchange reserves for Pakistan. Both adult and nymph causes reduction of oil content, weight loss of seeds, germination rate and stains cotton with pink color during ginning. The main objective of the study was to check the effect of *Chrysanthemum Indicum* flower extract on the activity of acetylcholinesterase (AChE) in *O. Hyalinipennis.* The adults of *O. Hyalinipennis* were exposed to different concentration (8, 10, 12 and 14 mg/20 ml) by leave dip method prior to biochemical analysis of surviving bug samples. The results demonstrated that *C. Indicum* extract affect the activity of AChE in *O. Hyalinipennis* adult. Maximum enzymatic activity was 0.544 α-Na µmol/min/ mg protein at day 1 in T3 (12 mg/20 ml) treatment while in control maximum activity was 0.160 α-Na µmol/min/ mg protein at day 4 whereas highest and lowest mortality was (66.66 and 0%) at 14 and 8 mg/20 ml concentrations respectively. The rise in activity of AChE is one of the main reasons to develop resistance in insect. This can be managed by adaptation of alternative controlmethods.

INTRODUCTION

*Gossypium Hirsutum L*is commercially grown as a source to generate foreign exchange reserves for Pakistan [1]. The average yield of cotton in Pakistan is lower as compared to other cotton growing countries [2]. About 30-40% yield loss is associated with insect pests which are also considered as vital limiting factors [3,4].

Oxycarenus Hyalinipennis Costa (Hemiptera: Lygaeidae) also known as Dusky cotton bug (DCB) is an economic pest of family malvaceae[5,6]. Both adult and nymph feeds on seeds and leaves which results in both quantitative and qualitative losses to various plants including cotton in most countries of the world [7-9]. *O. Hyalinipennis C*auses economical damage by decreasing oil contents, weight loss of seeds, germination rate and also stains cotton lint with pink color during the process of ginning due to crushing of bugs[8,10]. Resistance has been developed by *O. Hyalinipennis* against several insecticides like imidacloprid, spirotetramat, triazophos, fipronil and nitenpyram but resistance level varies from low to very high which is due to the misuse of insecticides in Pakistan [11-13]. The occurrence of *O. Hyalinipennis*is increasing in *Bt*Cotton genotype due to lesser number of insecticide applications for the management of bollworms. With the growing awareness about the damages caused by DCB the necessity for its management is gaining more attention [14]. Nowadays, the only solution available for the farmer is to use chemical insecticides and almost 90% of farmer use chemical insecticides to protect their crops and regular insecticide exposer over a long course of time lays the foundation of resistance. Insect develop resistance by changing target site, decrease infiltration and metabolic resistance arbitrate by detoxifying enzymes such as acetylcholinesterase (AChE), glutathione transferases (GST), and esterases (EST) support to improve resistance against pesticides[15].

Awareness about hazards related to use of synthetic pesticide resulted in need to discover alternate methods to control pest. Products of biological nature in their raw form or oil extract offers limitless opportunities as bio-pesticides. Ecofriendly nature of bio-pesticides make them best control method against agriculture pest[16]. Most plants used for medicinal purposes contains compounds which restrict the growth of insect and feeding activity or behave like repellents[17]. *Chrysanthemum Indicum L* has anti-inflammatory, immunomodulatory and anti-microbial compounds like 1,8-cineole, camphor, borneol and bornyl acetate [18]. Looking at the important cases of pesticide resistance and their ecological effects. The present experiment was conducted to evaluate the effect of acetone extract of *C. Indicum* flowers on activity of AChE in *O. Hyalinipennis.*

MATERIALS AND METHODS

Plant collection and extraction

Chrysanthemum Indicum flowers were collected from Bahauddin Zakariya University, Multan and its adjacent areas. The collected flowers were washed 3 times using tap water and rinsed by distilled water and shade dried. *C. Indicum* petals were milled using a domestic grinder and kept in air tight jar for future use. 50gram of *C. Indicum* powder was taken in a beaker with 100 ml of acetone solvent and stirred with magnetic stirrer and afterwards left for 24 hours.

Insect culture

O. Hyalinipennis nymphs and adults were collected from cotton fields of District Multan. The collected insect population were maintained in the laboratory condition with 27 ± 2°C of temperature and 60%-70% relative humidity within plastic jar and feed on fresh leaves of *Hibiscus rosa-sinensis* L. The insects from F_1generation were utilized for the examination

Bioassay

Botanical extract of *C. Indicum* was tested to ind out its lethal and sub-lethal effect against *O. Hyalinipennis* Different concentrations (8,10,12,14 ml/20 ml solution) were formed for *C. Indicum*. Fresh leaves of *H. rosa-sinensis* were used for the treatment by leaf dip-method and treated leaves were dried by placing them on tissue paper than shifted in polystyrene petri dish separately having five newly emerged adults of *O. Hyalinipennis.* For each treatment four replication were made. The leaves in control group were treated with distilled water. The mortality data was collected after every 24 hours for next 96 hours.

Enzyme assay

After treating *O. Hyalinipennis* with *C. Indicum*extract than activity of acetylcholinesterase was noticed following the procedure of SEREBROV with minor alterations[19]. The treated *O. Hyalinipennis* was crushed within 1.5 ml Eppendorf tubes containing 50 ml of NaCl (0.15 M). After crushing, the samples were centrifuged at 5000 rpm for 10 minutes in centrifuge machine and only supernatants wereused. Samples were made for four sequential days for treated adult swith four replications were used for each concentration of *C. Indicum* extract for AChE evaluation.

Assay of acetylcholinesterase

ELLAMAN method was used to check the activity of ACH by using acetylcholine iodide (0.075M) as substrate and changes in absorbance at 412nm wavelength were noticed at 20 sec interval[20].

Data analysis

Experimental data and values were applied to analysis variance using Microsoft Excel 2013 and Statistix 8.1 software were used for statistical analysis and making graphs to explain the detoxification activities of AChE enzyme.

RESULTS

Animals

Extract of C. Indicum lowers doses of 8, 10, 12 and14 mg per 20 ml were applied against adults of *Oxycarenus Hyalinipennis* Costa. Among these doses 10 ml give maximum mortality of *O. Hyalinipennis* followed by 8, 12, 14 ml, respectively **Figure 1**

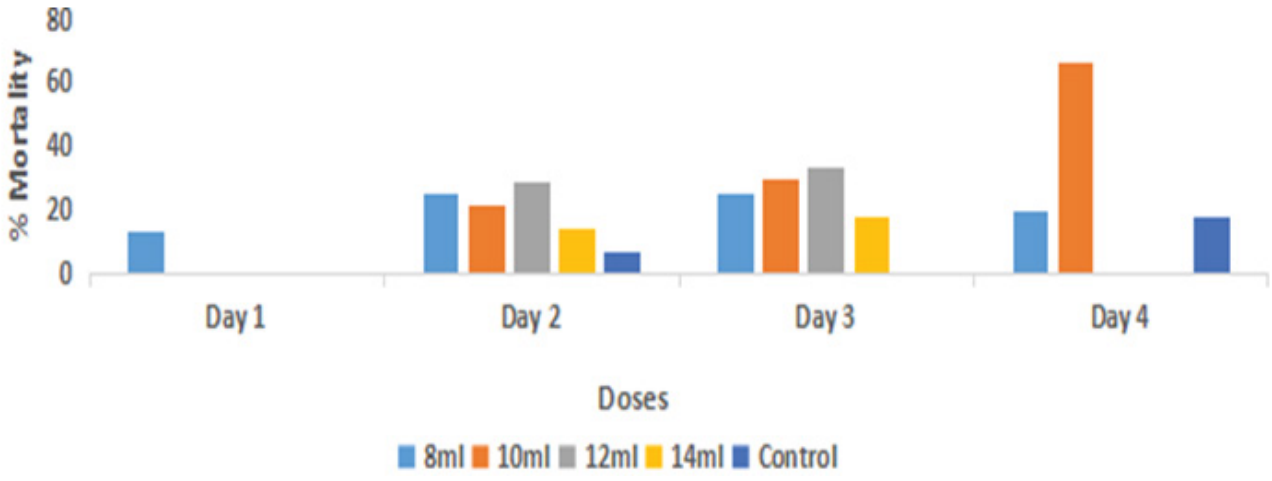

Figure 1. Percentage motility of *O. Hyalinipennis* exposed to different doses of *C. Indicum* flower extract.

The lowest mortality percentage (0%) was observed in T2, T3, T4 and control after 24 hours while the highest mortality percentage (66.66) was observed in T2 after 96 hours.

Activity of acetylcholinesterase (AChE)

After treating *O. hyalinipennis* with different doses of extract, activity of AChE (acetylcholinesterase) was observed. In treated samples, maximum enzymatic activity was 0.544153 α-Na µmol/min/ mg protein at day 1 in T3 (12 mg/20 ml) treatment while in control maximum activity was 0.160311 α-Na µmol/min/ mg protein at day 4 (**Figure 2 and Table 1**).

Table 1. AChE Activity (α-Na µmol/min/ mg protein) comparison at high, low and control doses on 4 consecutive days.

		Days			
		1	2	3	4
α-Na µmol/ min/protein	High dose	0.075066	0.045663	0.147424	0.864187
	Low dose	0.158137	0.13046	0.271435	0.098294
	Control	0.05309	0.08402	0.160311	0.163331

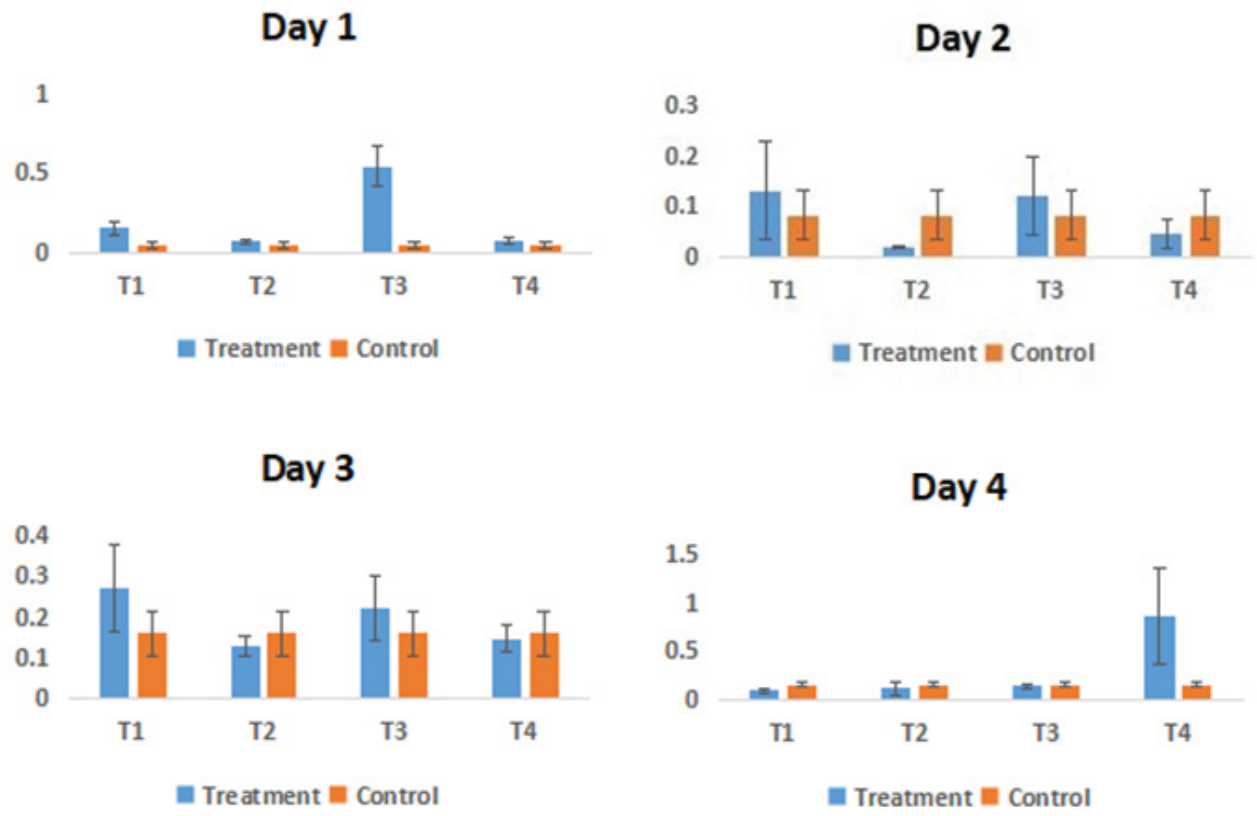

Figure 2. Acetylcholinesterase (AChE) activity in adult of O. Hyalinipennis after exposing with *C. indicum* flower extract on 4 consecutive days.

DISCUSSION

Biochemical and bioecological reasons are believed to be the source of insect resistance mechanisms against several insecticides[21]. Pyrethroids were widely used against most agricultural pests, including *O. Hyalinipennis*[22]. *Chrysanthemum Indicum* plant had insecticidal properties against many serious pests because of natural source of pyrethrin. Rajalakshmi justiied the anti-microbial activity against plant pathogen by *C. indicum l*eaf extract[23]. KAMARAJ observed highest toxic effects of acetone and methanol *C. Indicum l*eave extract against mosquitolarvae[24].Outcome of present analysis support results of previous analysis on detox enzymes of insects. When construction of detox enzyme increased in insect body they causedtheinsensitivity oftarget sites or by detoxification of foreign elements[25]. Insects detoxify insecticides by various enzymes like acetylcholinesterase (AChE), acid and alkaline phosphatases and excrete them from their body[26]. Acetylcholine (AChE) is a neurotransmitter which is present in the nervous system which is hydrolyzed by acetylcholinesterase (AChE) and high resistance was detected due to increase activity of AChE activity in *Tribolium Castaneum* after exposing with permethrin and dilubenzuron which shows direct relationship of resistance development with enzymes in insects [27,28]. In recent study similar results were observed after exposing *O. hyalinipennis* with various doses of *C. indicum* flower extract. Among all doses the maximum AChE activity was observed in 12 mg/20 ml sample of day 1 while lowest was 8 mg/20 ml sample of day 4.

CONCLUSION

The overall results suggest the potential of *C. Indicum*flower extract as a bio pesticide for crop protection against *O. Hyalinipennis* but further investigation is needed on behalf of active ingredients accountable for insecticidal properties to conclude some absolute recommendations.

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