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Biochemical effects of deodar oil in comparison to imidacloprid and carbosulfan on pupae of mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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Abstract

This study was conducted to evaluate the biochemical changes in the pupae of mealworm treated with LC_{50} concentration of Deodar oil in comparison to imidacloprid and carbosulfan insecticides. The larvae of mealworm beetle were treated with deodar oil (3.41 %), carbosulfan (0.086 %) and imidacloprid (0.023 %) through feeding method. The trerated larvae when able to reach the pupal stage successfully were crushed at the pupal stage for the biochemical analysis. Deodar oil and carbosulfan inhibited cholinesterase activity while imidacloprid enhanced this activity in the pupae of mealworm. ALP activity was inhibited with Deodar oil while enhanced when treated with imidacloprid and carbosulfan in the pupae of mealworm. It is concluded that the chronic effects of insecticides are carried out in the next developmental stage of mealworm pest from larvae to pupae. Deodar oil, a biopesticide has a strong biochemical effect on pupae of mealworm pest by altering their enzymatic activities. Deodar oil is environment friendly and can be effectively used as a substitute of synthetic insecticides.

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Introduction

Synthetic insecticides and pesticides have reduced the harmful insect pests, played a great role in agricultural sector, protected the crops from the attacks of insects and prevented humans from vector-borne diseases but the illegitimate usage of synthetic pesticides produce many problems as pollution, resistance, effects on beneficial insects, residues problems, animals and human health etc. these problems realized toxicologists and entomologists to introduce eco-friendly chemicals for the control of insect pests. About 1800 species of plants have been tested to possess insecticidal characteristics (Jacobson, 1982, Grainge *et al.,* 1985). The trees of *Cedrus deodara* are located in the northern regions Khyber Pakhtunkhwa, Pakistan. Its oil have been applied on the feet of infected horses, camels and cattles as insect repellent (Gamble, 1922) and also against house flies and pests of stored grains (Singh *et al.,* 1984, 1989, Singh and Rao, 1985, Singh and Agarwal, 1988).

Imidacloprid and carbosulfan were tested for comparison as standard compounds. Imidacloprid is a member of neonicotinoids while carbosulfan belongs to carbamate insecticides. Imidacloprid is used for the controlling of nematodes, mites and insect pest. It works on nicotinic acetyl choline receptors (Liu *et al.,* 2005) and hyper stimulate the nervous tissue which leads to the death of insect (Bloomquist, 2009). According to Cheng *et al.,* (2004) acetylcholinesterase (AChE) of *Liposcelis bostrychophila was more sensitive to carbosulfan insecticide.*

Phosphatase is a fat body enzyme in insect involve in the breakdown of phosphoric esters to alcohol and phosphate (Heong *et al.,* 2011). Alkaline phosphatases in insect are distributed in organs and tissues like salivary gland (saliva), haemolymph, reproductive system, intestine, Malpighian tubules, appendage and fat body. They may work in insect developmental process, hormone synthesis, nerve conduction, caste formation in case of social insects, substance metabolism and diapauses. They help insect to make them resistant to insecticides. Alkaline phosphatases may be targeted by some insecticides. In insects, the different reactions too many biocides might be connected with the low level in the activity of alkaline phosphatase and many changes in the activity of acid phosphatase (El-Mageed *et al.,* 2008).

Mealworms *Tenebrio molitor*, a stored grain pest, was selected as experimental insect. Its life cycle consist of egg, larva, pupa and adult. The pupa was selected for biochemical analysis.

In this study the response of AChE and ALP activity was evaluated in the indirectly treated pupae of mealworm with Deodar oil, imidacloprid and carbosulfan.

Materials and methods

Collection and rearing of insects

Adult mealworms were collected from Karachi city, Pakistan. Bran and potatoes were provided in large bowls to insects as food materials. For the rearing of mealworms, the methodology of Ramos *et al.,* (2011) was followed.

Tested chemicals

Deodar oil, imidacloprid and carbosulfan were the experimental chemicals. These chemicals were provided in the bran as bait. Each chemical which kills 50% larvae was considered as LC_{50} concentrarion and was calculated according to Finney, (1971) and the remaining 50% larvae were followed until reached to pupal stage.

Preparation of supernatant

The pupae of mealworms were crushed in deionized water and was centrifuged at 3500 rpm, thus supernatant was obtained and was transferred to cuvette. The enzymes activities were estimated in spectrophotometer (model 721-2000).

Kits used

Kit with Catalogue no. CE 190 was used for the estimation of acetylcholinesterase (AChE) activity while for the the determination of ALP activity, Alkaline phosphatase Kit (Global) was used by the methods of Kind and King, (1954).

Results

Cholinesterase activity

Cholinesterase percent inhibition, in *Tenebrio molitor* pupae was calculated as 20 and 20% at 60 seconds period treated with Deodar oil and carbosulfan respectively while 26.6% enhancement was noted in imidacloprid effected pupae (Table 1B) and mean activity is given in Table 1A and Fig. 1.

Alkaline phosphatase (ALP) activity

ALP percent enhancement, in *Tenebrio molitor* pupae treated with carbosulfan and imidacloprid, was calculated as 16.32 and 47.2%.

Respectively while 32.8% ALP inhibition was found in pupae of mealworm treated with Deodar oil (Table 2B) and mean activity is given (Table 2A, Fig. 2).

Table 1A. Effect of deodar, carbosulfan and imidacloprid on cholinesterase activity against mealworm *Tenebrio molitor* pupae.

Insecticides	Time (sec)	Mean activity(U/l)	SD	SE	Range at 95% Confident limit
Control	60	117.3	23.46	13.56	143.87 - 90.72
Check	60	125.12	13.54	7.82	140.46 - 109.77
Deodar	60	93.81	13.54	7.82	148.28 - 117.59
Imidacloprid	60	148.58	27.08	15.65	179.27 - 117.88
Carbosulfan	60	93.84	0	0	93.84 - 93.84

Legend: SD, Standard Deviation; SE, Standard Error.

Table 1B. Inhibition and enhancement of acetylcholinesterase enzyme in pupae of mealworm.

Insecticides	% Concentration of Compound	%Inhibition	%Enhancement
Control	00	00	00
Check	00	----	6.66
Deodar	3.41	20	---
Imidacloprid	0.023	----	26.6
Carbosulfan	0.086	20	---

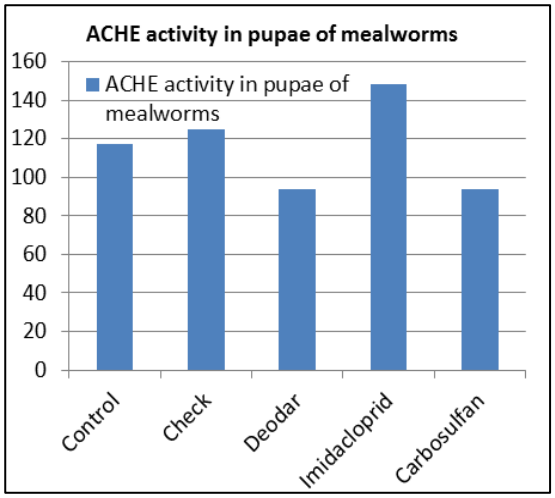

Fig. 1. Mean AChE activity in pupae of mealworms.

Table 2A. Effects of deodar, carbosulfan and imidacloprid on Alkaline phosphatase against mealworm *Tenebrio molitor* pupae.

Insecticides	Mean activity (u/L)	S.D	S.E	Range
Control	41.39	3.76	2.17	45.64 - 37.13
Check	42.31	2.92	1.68	45.62 - 39.00
Deodar	27.81	5.25	3.03	33.76 - 21.86
Imidaclporid	60.92	3.19	1.84	64.54 - 57.31
Carbosulfan	48.14	3.90	2.25	52.56 - 43.72

Table 2B. Inhibition or enhancement of Alkaline phosphatase in pupae of mealworm.

Insecticides	% Concentration of Compound	% Inhibition	% Enhancement
Control	00	00	00
Check	00	---	2.24
Deodar	3.41	32.8	---
Imidacloprid	0.023	---	47.2
Carbosulfan	0.086	---	16.32

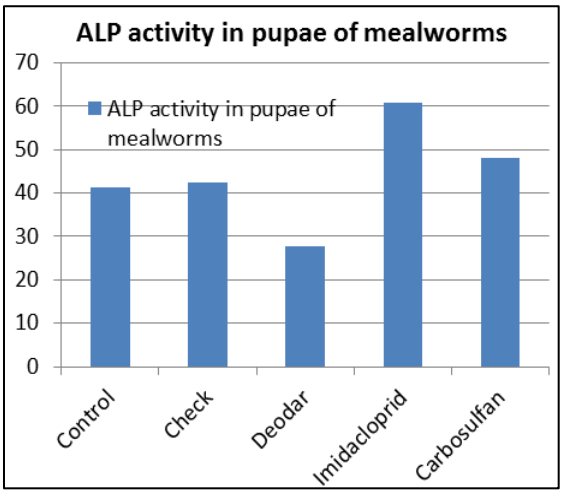

Fig. 2. Mean ALP activity in pupae of mealworms.

Discussion

The present study indicates that the chronic effects of insecticides may retain in the next developmental stage of insect. In recent work the larvae of mealworms were treated with insecticides and the biochemical effects were observes in the next stage i.e. pupa. Cholinesterase and Alkaline phosphatase were fluctuated in the pupae when the larval stage of *Tenebrio molitor* exposed to the LC_{50} dose of Deodar oil, imidacloprid and carbosulfan. Inhibited trend in the activity of Cholonesterase was noted in the supernatant of the pupae of mealworms when treated with Deodar oil and carbosulfan while the activity was enhance in imidacloprid treated pupae. ALP activity was noted to be increased in imidacloprid and carbosulfan affected pupae of mealworms while this activity was decreased in Deodar treated pupae, by comparing the results with that of the control pupae. For the accuracy of the results the check group was also kept, for solvent (methanol) changes in case of Deodar oil solubility, which shows negligible changes in the activity of enzymes. This indicates that the enzymes fluctuation was not due to methanol but was due to the toxic effect of Deodar oil. This report is considered to be the new as no cited investigations available in this regard.

Singh and Singh, (2003) reported the synergistic effect of *Cedrus deodara* with *Annona squamosa* and *Lawsonia inermis* on the activity of acetylcholinesterase in *Lymnaea acuminate* snail. They reported that the enzyme was inhibited 60% and 58.70% respectively. Similar investigations were presented by *Rao et al.,* (2003) who observed the activity of acetylcholinesterase in the brain of harmful snail *Achatina fulica exposed to* 40% and 80% of *LC_{50} of Cedrus deodara for 24h treatment. They calculated 23.08 % and 38.47 % inhibition of AChE when the snail was exposed to 40 and 80 % of cedrus deodara respectively. I*n the present report mealworm pupae were indirectly treated with plant origin oil i.e. Deodar oil due to which cholinesterase was inhibited 20 %. Our report is similar in case of inhibition of the enzyme although the species are different. Deodar oil should be mixed with other plant extract for its synergistic effect to extend the present work. Plant origin extracts other than Deodar may inhibit cholinesterase level in various organisms (Yousuf *et al.,* 2015, Rana *et al.,* 2015). Yadav *et al.,* (1998) observed maximum decreased level of Acetaylcholinesterase in the brain of *Ciarías batrachus* (teleost) exposed to carbofuran insecticide. According to Cheng *et al.,* (2004) acetylcholinesterase (AChE) of *Liposcelis bostrychophila was more sensitive to carbosulfan insecticide. These reports are in favor of the present investigations as Cholinesterase has been decreased in pupae of mealworms treated with carbosulfan. Equal inhibition (20 % in both cases) in Cholinesterase level was noted, due to Deodar oil and carbosulfan, in the recent study so Deodar oil can be used in place of synthetic chemicals to save the environment from pollution.*

Cholinesterase has been increased in response to indirectly treated Tenebrio molitor pupae with imidacloprid. The report is supported by the previous literatures. Zhu *et al.,* (2017) discovered comparatively greater Cholinesterase activity in imidacloprid (Advice) treated bees (*Apis mellifera*). Boily *et al.,* (2013) reported that imidacloprid is very toxic to honey bees and increase the activity of AChE in many invertebrate animals. They noticed the impact of imidacloprid on AChE activity of honey bee and documented enhanced level of AChE in honey beehives located nearer to different maize field coated with imidacloprid and in caged bees treated with sub-lethal concentration of imidacloprid. Morakchi *et al.,* (2005) they reported that AChE activity increases in German cockroach when exposed to the neonicotinoid i.e. acetamiprid. It is presumed that as neonicotinoids hold the binding-site of acetylcholine, this compound accumulate in the synapses and thus activating the action of AChE, in a typical substrate enzyme cellular response.

ALP activity has been inhibited 32.8% when mealworm pupae indirectly treated with Deodar oil. As per available literatures this report is also be considered to be the 1st. Plant origin extract have been described to inhibit ALP activity in other insects. Azab *et al.,* (2011) studied the comparative Alkaline

phosphatase changes in whitefly and aphid when they exposed to Azadirachtin, Clove oil etc. According to their report, ALP activity was reduced to 5. 5 and 2.16% in aphids and whitefly, respectively, treated with Azadirachtin. Similar reports were conducted by Megahed *et al.,* (2013) and Ayub *et al.,* (2005).

The indirect treatment to mealworm pupae with imidacloprid and carbosulfan cause enhanced trend in the activity of ALP. This thing was also observed by many researchers. Arafat *et al.,* (2014) evaluated biochemical changes caused by imidacloprid concerned with hepatotoxicity in male albino mice. They observed elevation in alkaline phosphatase when the mice were fed with high dose 15mg/kg/day of Imidacloprid. Phosphatase is involved in the detoxification of insecticides so in this study its activity was altered in response of Deodar oil, Imidacloprid and carbosulfan treated mealworms pupae.

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