Saudi Journal of Biological Sciences 26 (2019) 281–285

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

A comparative toxic effect of *Cedrus deodara* oil on larval protein contents and its behavioral effect on larvae of mealworm beetle (*Tenebrio molitor*) (Coleoptera: Tenebrionidae)

Islam Dad Buneri [a], Masarrat Yousuf [a], Mohammad Attaullah [b], Saifullah Afridi [c], Syed Ishtiaq Anjum [d,*], Habibullah Rana [e], Naveed Ahmad [f], Muhammad Amin [a], Muhammad Tahir [g], Mohammad Javed Ansari [h]

[a] *Toxicology Laboratory, Department of Zoology, University of Karachi, Karachi, Pakistan*
[b] *Department of Zoology, Shaheed BB University, Sheringal, Upper Dir, Pakistan*
[c] *Centre for Advanced Drug Research, COMSAT Institute of Information Technology Abbotabad, Abbotabad 22060, Pakistan*
[d] *Department of Zoology, Kohat University of Science and Technology, Kohat, Pakistan*
[e] *BRC, University of Karachi, Karachi, Pakistan*
[f] *Centre of Excellence in Marine Biology, University of Karachi, Karachi, Pakistan*
[g] *Faculty of Marine Science, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Balochistan, Pakistan*
[h] *Bee Research Chair, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia*

ARTICLE INFO

Article history:
Received 27 April 2017
Revised 1 June 2017
Accepted 5 June 2017
Available online 10 June 2017

Keywords:
Tenebrio
Mealworms
Cedrus deodara
Imidacloprid
Carbosulfan
Protein level
Toxicity

ABSTRACT

Cedrus deodara (deodar) is practically used, as insect repellent, in the northern areas of Pakistan but no data available therefore this study was conducted to evaluate the effectiveness of deodar oil as an alternate of conventional insecticides against the larval pest stage of mealworm beetle (*Tenebrio molitor*), by feeding method. The aim of the study was to investigate the effectiveness of deodar oil as an alternate of conventional insecticides against the larval pest stage of mealworm beetle (*Tenebrio molitor*), by feeding method. All tested chemicals showed efficacy against the pests. The LC_{50} was determined by probit analysis and was found to be 3.41, 0.086 and 0.023% of larvae treated with deodar oil, Carbosulfan and Imidacloprid respectively The LC_{50} treated larvae were subjected to the evaluation of protein activity, qualitatively and quantitatively. The protein level in tested insects was enhanced when treated with Imidacloprid, Carbosulfan and deodar oil. The electrophoretic profile of treated insects showed more bands in insects treated with *Cedrus deodara* oil. This electrophoretic profile appeared in 4, 5, 7 and 8 bands for tested chemicals including control. Antifeedant activity was observed for *C. deodara* as larvae were deterred to feed on the food found in the container.

1. Introduction

Synthetic insecticides have been found effective against stored product pests but proved to be hazardous to men and domestic animals. The over reliance and illegitimate use of synthetic pesticides especially insecticides since last four decades have led to a wide spectrum of pest problems like pest resistance to chemicals, resurgence of pests, residues in food and soil and risks to human and animal health. Entomologists of the world are in struggle to introduce alternatives of synthetics pesticides. They started work on plant products which possess biologically active components. About 1800 plants species have been described to contain properties against insects (Jacobson, 1982; Grainge et al., 1984). Different compounds have been prepared from various parts of plants which affect different types of insects (Naqvi et al., 1994). Many plants possess activities against stored grain pests. In the present study, Deodar oil was used for the first time in Pakistan against Mealworms to evaluate their toxic effects. *Cedrus deodara* belongs to the family Pinaceae, commonly called Deodar. It is found abundantly in the northern areas of Khyber Pakhtunkhwa, Pakistan and also in the Mediterranean and in the Western Himalayas. Cedrus oils are extracted from *Cedrus deodara* plants. The oil is

* Corresponding author at: Department of Zoology, Kohat University of Science and Technology, Kohat, Pakistan.
E-mail addresses: ishtiaq11@gmail.com, ishtiaq@kust.edu.pk (S.I. Anjum).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2017.06.005
1319-562X/

given a local name as Ranzra in KPK. The residents of KPK use this oil as insect repellent in the infected livestock. Different parts of the plant have been used as medicines in the area for a long time. The essential oil is distilled from the inner wood which is aromatic and is used on the feet of camels, cattle and horses as insect repellant (Gamble, 1922) and against houseflies and stored pests (Singh et al., 1984, 1989; Singh and Rao, 1985; Singh and Agarwal, 1988). Imidacloprid and Carbosulfan were used in this study as standard insecticides. Mealworm was used as experimental insect in the study. Carbosulfan belongs to Carbamate family of insecticides and is used to control insects, mites and nematodes. Carbosulfan inhibit cholinesterase activities in the target insects. The second tested insecticide in the present study was Imidacloprid, a member of Neonicotinoids insecticides, which belongs to compounds prepared from tobacco plants (Kim, 2006). Imidacloprid acts on nicotinic acetylcholine receptor i.e. produce harmful effects on the nervous system of the insects (Liu et al., 2004). This action overstimulate the nervous system and as a result of continue movements of muscles, paralysis and death may occur in the target insects (Bloomquist, 2009). Chaudhary et al. (2011) determined the toxicity of essential oil of *Cedrus deodara* against diamondback moth, *Plutella xylostella* L. but did not give its biochemical findings. In the present work, toxicity assessment and biochemical analysis of *Cedrus deodara* oil along with two chemicals was carried out in larvae of stored grain pest, *Tenebrio molitor*.

2. Materials and methods

Adult mealworms (*Tenebrio molitor)* were collected from different regions of Keamari Town Karachi, Pakistan. Karachi Port Trust (KPT) storage areas and different stored grain Shops were visited. Hand picking method was applied during collection. The insects were then brought to the insectary of the Department of Zoology, University of Karachi. Methodology of Morales-Ramos et al. (2011) was followed for the rearing of *Tenebrio molitor*. About 1Kg of bran and 1 kg of potato was taken inside large, deep, opened mouth bowl. For maintaining moisture, some amount of water was sprayed onto the bran and potatoes were embedded in the center of bran. Insects were reared at room temperature of about 25-30C° and about 70% humidity. *Tenebrio molitor* were released into plastic bowls and were protected from the attacks of insectivores and other enemies. The mouth of the bowl was closed with the help of black muslin cloth for maintaining darkness and rubber band. The beetles were kept undisturbed for about 15 days during which egg laying and hatching process occurred. Fresh bran was provided to larvae and after 6–7 days, the larvae were subjected to experimental analysis. A stock solution of 12% for Deodar oil and 1% solution each for Imidacloprid and Carbosulfan was prepared by using Charles's formula. After preliminary trials, five different concentrations from stock solution were prepared for each chemical. About 2 mL of specific concentration each compound was mixed /10 gram of bran in a small plastic bowl and then 20 larvae were released into it. The mouth of each bowl was closed with cloth. Non-treated insects i.e. Control and check was also maintained for environmental effects. Mortality was noted after 48 h of treatment. LC_{50} was calculated according to Finney (1971) method and Biostat software. The live 1 g of LC_{50} treated insects were kept in freezer. When the larval activities were completely diminished, then the larvae were crushed in 5 mL de ionized water with the aid of mortar pestle. The whole crushed sample was taken in a test tube and subjected to homogenizer for homogenization at 1000 rpm for 10 min. The homogenates was then centrifuged for 30 min at speed of 4000 rpm. The supernatants were used for estimation of total protein and electrophoresis while the lower layer was discarded. For the estimation of total protein contents, Ecoline® Kit #123119966314 and spectrophotometer model 721-2000 were used. Proteins were separated by sodium dodecyl sulphate (SDS) page gel electrophoresis according to the method of Laemmli (1970). Samples were boiled for 3–5 min at 93 °C in SDS sample buffer containing and then processed on 10% SDS gel. From each sample, 10 μL was loaded per slot of a pre cast 10% polyacrylamide gel. For the determination of protein mass, recombinant proteins (Bio-Rad) markers were used as molecular weight standards.

3. Results

Five different concentrations (0.75, 1.5, 3, 6 and 12%) of deodar oil were used and mortality was noted after preliminary trials as 17, 33, 49, 64, and 87% respectively. LC_{50} of *Cedrus deodara* was determined by Probit method and found to be 3.4% (Tables 1, 3 and Fig. 1). Lowest mortality was 17% caused by 0.75% oil while highest mortality was 87% due to 12% oil. The LC_{50} of Carbosulfan was 0.086% (Table 1, 5 and Fig. 2). Five concentrations i.e. 0.5, 0.25, 0.125, 0.0625 and 0.03125% caused 92, 75, 60, 42 and 26 percent mortality respectively. Minimum and maximum mortality was 26 and 92 percent respectively. The LC_{50} of Imidacloprid was 0.023% (Table 1 and Fig. 3). Five concentrations i.e. 0.00781, 0.0156, 0.0313, 0.0625 and 0.125% caused 22%, 44%, 61%, 74% and 96% mortality respectively. The lowest concentration (0.023%) caused 50% mortality which shows effectiveness of Imidacloprid against *Tenebrio molitor larvae* Table 4. By comparing the toxicity of three insecticides, the order of effectiveness was found as *Imidacloprid > Carbosulfan > Deodara.* Total protein contents were found to be 2.80, 2.68 and 4.14 mg/mL in larvae treated with

Table 1
Dose stimulus percentile *Tenebrio molitor* larvae treated with Insecticides.

Deodar dose stimulus	Carbosulfan dose stimulus	Imidacloprid dose stimulus	Insects killed percent
0.123291	0.003010474	0.001167389	1
0.326156	0.008064255	0.002802075	5
0.54795	0.013638875	0.004469656	10
0.825727	0.020661675	0.006465	16
1.027203	0.025775398	0.007868843	20
1.304193	0.03282654	0.009755126	25
1.615995	0.040786918	0.011831108	30
2.379413	0.060354905	0.016759464	40
3.413957	0.086999728	0.02319406	50
4.89831	0.125407418	0.032099141	60
7.212339	0.185573051	0.045470332	70
8.936638	0.230574186	0.055146846	75
11.34645	0.293650274	0.068366394	80
14.11497	0.366328121	0.083211822	84
21.27039	0.554954348	0.120359243	90
35.73477	0.938580555	0.191987903	95
94.53329	2.514206188	0.460826953	99
LC_{50} → 3.413957	LC_{50} → 0.086999728	LC_{50} → 0.02319406	

Table 2
Protein bands and their intensity.

Bands	Control	Imidacloprid	Carbosulfan	Deodar
1	+++	+++	+++	+++
2	+++	+++	—	+
3	—	—	—	+
4	—	–	+	+
5	++	+++	+	+++
6	–	–	+++	+++
7	–	–	+	+
8	+	++	++	++

Table 3
Toxicity assessment of deodar against *Tenebrio molitor* larvae.

% Concentration	Mean mortality	Median	S.D.	S.E.	Range
12	87	85	5.700877	2.549587	91.99719104—82.00280896
6	64	65	4.1833	1.870886	67.66693572—60.33306428
3	49	50	4.1833	1.870886	52.66693572—45.33306428
1.5	33	35	2.738613	1.224782	35.40057293—30.59942707
0.75	17	15	2.738613	1.224782	19.40057293—14.59942707
Control	1	0	2.236068	1.00003	2.960059587—0.960059587
Check	2	0	2.738613	1.224782	4.400572926—0.400572926

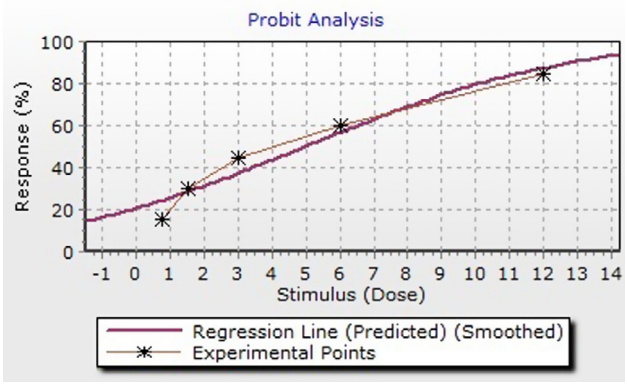

Fig. 1. Larvae treated with deodar. Response (0–100): Number of insects died stimulus (−1 to 14): Specific concentration of deodar extract.

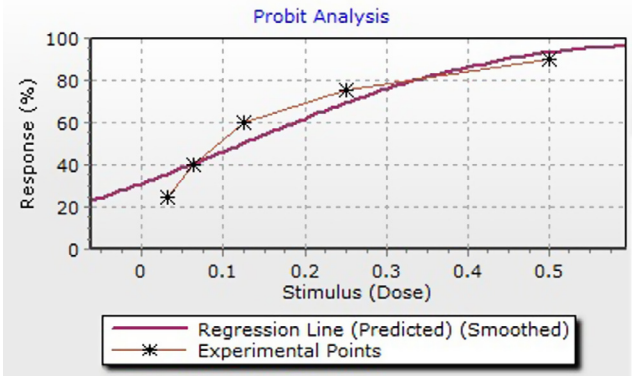

Fig. 2. Larvae treated with Carbosulfan. Response (0–100): Number of insects died. Stimulus (0–0.5): Specific concentration of Carbosulfan insecticide.

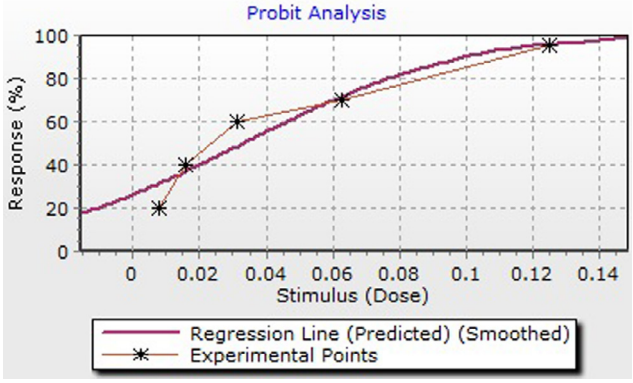

Fig. 3. Larvae treated with Imidacloprid. Response (0–100): Number of insects died. Stimulus (0–0.14): Specific concentration of Imidacloprid insecticide.

Carbosulfan, Imidacloprid and deodar oil respectively Fig. 4. While in control and check, it was found as 2.30 and 2.12 mg/mL respectively Fig. 4. The total protein amount in LC_{50} treated insects was enhanced as compared to the control while it was slightly decreased (7%) in the check sample (Fig. 3). Enhancement in the protein contents by the three chemicals was in the order of *Deodar > Carbosulfan > Imidacloprid.* Repellency test showed that the diet of larvae treated with Deodar oil repelled the insects. The larvae moved to the side of the container and avoid feeding (Fig. 6). The insect sample was subjected to electrophoresis. A maximum of eight and minimum of four bands of protein were appeared during electrophoresis (Fig. 5). Protein bands 1, 5 and 8 were observed and were common in all test samples. Band 2 was noted in control, Imidacloprid and deodar while missed in Carbosulfan treated samples. Band 3 was noted in deodar sample only and was absent in the remaining samples. Band 4 was missed in control and Imidacloprid samples while appeared in case of Carbosulfan and deodar treated samples. Likewise, bands 6–7 were disappeared in control and Imidacloprid while appeared in Carbosulfan and deodar treated samples. Deodar showed a maximum of eight bands, Carbosulfan five bands, Imidacloprid 3 bands and control showed 3 bands (Table 2). It was observed that total numbers of protein bands were increased when *Tenebrio molitor* larvae exposed to Carbosulfan, Imidacloprid and deodar oil (see Fig. 5 and Table 2).

4. Discussion

Mortality of mealworms (*Tenebrio molitor*) and impact on protein contents were the two main studied parameters in the present research work. The toxic effects of chemicals seem to have affected the protein contents in the treated samples. The more protein bands in the treated insects may indicate that the individual protein was broken into many abnormal fragments.

The essential oils of *Cedrus deodara* plant was used against second instars of the diamond back moth (*Plutella xylostella* L.) as effective larvicides (LC_{50} = 425 µg/mL) (Chaudhary et al., 2011). In the present research crude oils of *Cedrus deodar* was used against last larval instar of *Tenebrio molitor* and LC_{50} was found as 3.4%.It is known that the affectability of various insects' pests species could be vary for a similar substance. The different in LC_{50}value may be due to the difference in species and method of application. Makhaik et al. (2005)used essential oil of *Cedrus deodara* against mosquito species, *Aedes aegypti* and *Culex quinquefasciatus* and calculated LC_{50} as 2.5% against *Culex quinquefasciatus* after one hour of exposure. These results are comparable in their effectiveness in repelling different insect's species. In the present investigation, crude oil was used and the LC_{50} was found as 3.4%. The high LC_{50} in the present investigation may be due to the differences in cuticle of insect species as coleopterous species have hard and thick cuticle than the integument of mosquitoes, stage of development of insect, method of application of oil and duration of treatment. *Tenebrio* absorbed more tested chemical that resulted in slight high LC_{50} values. *Cedrus deodara* has been found effective against a variety of insects (Raguraman and Singh, 1997;Rahuman et al., 2009;

Table 4
Toxicity assessment of Imidacloprid against *Tenebrio molitor* larvae.

% Concentration	Mean mortality	Median	S.D.	S.E.	Range
0.125	96	95	4.1833	1.870886	99.66694–92.33306
0.0625	74	75	4.1833	1.870886	77.66694–70.33306
0.03125	61	60	4.1833	1.870886	64.66694–57.33306
0.015625	44	45	4.1833	1.870886	47.66694–40.33306
0.007813	22	20	2.738613	1.224782	24.40057–19.59943
Control	00	00	00	00	00–00

Table 5
Toxicity assessment of Carbosulfan against *Tenebrio molitor* larvae.

% Concentration	Mean mortality	Median	S.D.	S.E.	Range
0.5	92	90	2.738612788	1.224782105	94.40057293–89.59942707
0.25	75	75	3.535533906	1.581186899	78.09912632–71.90087368
0.125	60	60	3.535533906	1.581186899	63.09912632–56.90087368
0.0625	42	45	4.472135955	2.000060803	45.92011917–38.07988083
0.03125	26	25	4.183300133	1.870885569	29.66693572–22.33306428
Control	00	00	00	00	00–00

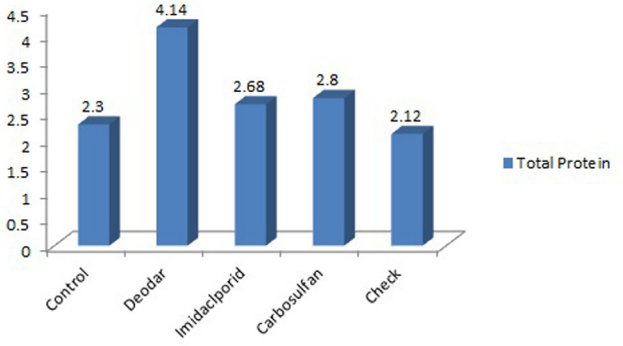

Fig. 4. Shows total protein (mg/mL) in treated, Control and Check mealworms larvae.

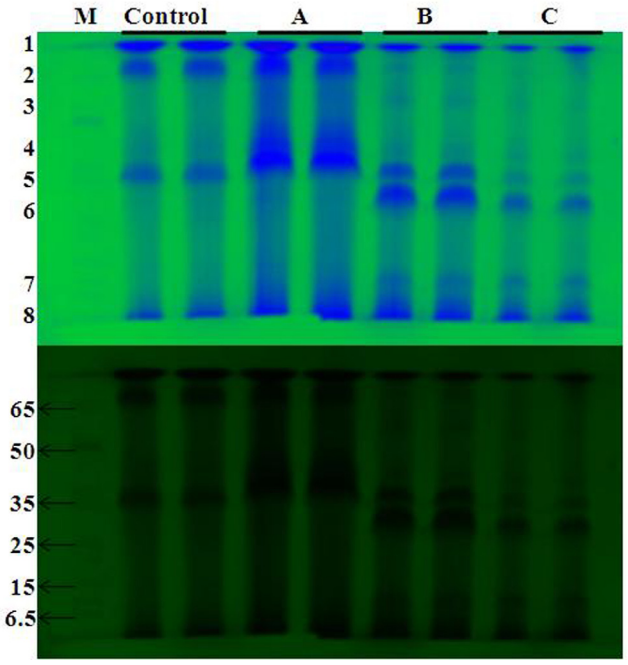

Fig. 5. Electrophoretic profile of *Tenebrio molitor* larvae treated with insecticides. 10% SDS-PAGE (*Left to right*) represents, Lane 1(M): 6.5–200 kD Protein Marker; Lane 2–3: Control (duplicates), Lane 4–5 (A); Imidacloprid (duplicates). Lane 6–7 (B): *Cedrus deodara* (duplicates); Lane 8–9(C): Carbosulfan (duplicates) respectively. Upper panel (*Top to bottom*) numbers represents the measureable protein bands in test samples (reduced): Lower panel in Lane 1(M): represent marker band mass in KD (kilo Dalton) respectively.

Rao and Singh, 2002;Delbeke et al., 1997). The findings suggesting that formulation chemistry can investigate the longevity of repellent activity and should be developed into commercial repellent products.

Gregory et al. (1994) assessed the effects of bran treated with Carbaryl, Dimethoate and Chlorpyrifos against adult and larval stage of *Tenebrio molitor*. According to them, Chlorpyrifos was most effective in terms of mortality of adult and larvae of *Tenebrio* respectively followed by Dimethoate and Carbaryl. By increasing the amount of bran bait of Carbaryl and Dimethoate, the insect showed increased response. After 1–5 days of treatment, they calculated ED_{50} for adults and larvae as 0.128 and 0.550 respectively. In the present investigation, Imidacloprid was found more effective followed by Carbosulfan and deodar oil. Ezz and Fahmy (2009) used mineral oil i.e. Alboleum as well as an IGR, Admiral (pyriproxyfen) on adult female mealybug *Ferrisia virgata* (Cockerel) and determined that total protein was enhanced by Admiral up to 29.2%. The amount of protein enhanced in the present study was 21.7% (2.80 mg/mL), 16% (2.68 mg/mL) and 80% (4.14 mg/mL) in larvae treated with Carbosulfan, Imidacloprid and Deodar oil respectively is similar to the previous study as in both, the amount of protein was increased. Marron et al. (2003) reported that facing stressful state like starvation, organisms develop two compensatory mechanisms whether to store extra energy or by reducing their metabolic rate. This study is very close to the present demonstration as protein contents enhanced in all treated insects so the mechanism of the increased protein in treated group may be due to starvation, and it is evident from the behavior of the larvae which move to the side of the container with deodar containing bran. Such type of protein sparing strategy was usually present in all insects during stress (McCue et al., 2015). Additionally, upon exposure of insects and other animals to various stressors, the production of most proteins declines, but heat shock proteins (HSPs) increase (King and MacRae, 2015; Chapuis et al., 2011). However, increased levels of protein oxidation have been connected with different biological outcomes including convulsion in many other animal species as well (Zhang et al., 2013). Saeed et al. (2010) studied the pattern of protein bands of *Culex pipiens* treated with *Allium satvium*, *Citrus limon* and *Bacillus thuringiensis* by comparing with

Fig. 6. Repellency test shows that bran mixed with Deodar extract deterred *Tenebrio molitor* larvae.

Albino mice. According to them, 18 protein bands were found in insect treated with 10ul of *Allium satvium* while in control batch there were 13 bands after 12 h of treatment. Of these two bands were common. After 24 and 48 h, the numbers of protein bands were reduced to 17 and 15 respectively. According to our finding, 8 protein bands appeared in the sample of *Tenebrio molitor* after 48 h of treatment while control sample showed only 4 bands. Our study confirm the previous reports as it is cleared from both that treated insects showed more protein bands and hence high protein levels as compared to control. This may be due to defense, resistance mechanism or stressful condition due to which *Tenebrio molitor* larvae increase their protein levels.

Deodar oil in concentrated form can be used as insect repellents on infected livestock and an environment friendly bio-pesticide in different dilutions, as well. Deodar extract may take the place of intramuscular injection used for tick's infestation. Imidacloprid, Carbosulfan and deodar oil cause increase in protein levels and are also toxic against *Tenebrio molitor* larvae.

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