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REPELLENCY OF RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* CAUSED BY LEAVE EXTRACT FRACTIONS OF HILL TOON, *CEDRELA SERRATA*

[a]**Farzana Parveen***, [b]**Anzela Khan**, [c]**Sumaira Zaib**
[a] *Department of Zoology, Shaheed Benazir Bhutto University, Main Campus, Sheringal, Khyber Pakhtunkhwa, Pakistan.*
[b] *Beaconhouse School System, Margala Campus, Islamabad, Pakistan.*
[c] *Hazara University, Garden Campus, Mansehra, Pakistan.*

A B S T R A C T

The plant origin pesticides have recently got great importance than the synthetic pesticides, against stored grains pests because of their lesser hazards to the environment and other related biomass. The present research was conducted to evaluate the repellency of 5th instars of the red flour beetle, *Tribolium castaneum* (Herbst.) caused by 5 doses of 4 fractions of the hill toon, *Cedrela serrata* Royle leaves extract after consecutive 1 h interval for 5 h by 2 methods. *Tribolium castaneum* was reared under controlled laboratory conditions at 30±1 °C, 60±2% RH and a 16:8 h (L:D). In method "A", at the highest dose, i.e., 40.8 µl/cm^2, the fractions of leaves extract showed repellencies belong to the classes: n-butanol fraction (repellency: 92.2±6.2; class: V) > ethyl acetate fraction (repellency: 74.8±8.3; class: IV) > methanolic extract (repellency: 67.2±8.6; class: IV) > aqueous fraction (repellency: 46.4± 8.3; class: III). In method "B", at the same dose, the same fractions showed repellencies belong to the classes: n-butanol fraction (repellency: 22.0±10.7; class: II) > ethyl acetate fraction (repellency: 20.4±7.3; class: II) > methanolic extract (repellency: 19.0±11.0; class: I) > aqueous fraction (repellency: 6.0±22.5; class: I). Repellency increased with increase of doses, however, it decreased with increase of duration of time. Method "A" was more significantly effective than method "B". The n-butanol fraction was the most effective than other fractions. Further research should be conducted on *C. serrata* to isolate its active compound and to use it on commercial bases against *T. castaneum*.

Keywords: Aqueous fraction (AQF), *Cedrela serrata*, ethyl acetate fraction (EAF), methanolic extract (ME), n-butanol fraction (NBF).

INTRODUCTION

The global post-harvest grain losses caused by insect damage ranged from 10-40% (Raja *et al*., 2001). The damage caused by red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenibrionidae) to various stored and food commodities like grain, flour and dried fruits is recorded to be 15-20% which is capable of measuring losses worth millions of rupees every year in a developing country like Pakistan (Khattak and Shafique, 1986). It produced significantly higher weight losses in wheat than other cereals (Lohar, 1997). A positive correlation was observed among damage proteins and fats of wheat, whereas, negative correlation was found in carbohydrate (Wakil *et al*., 2003).

In Karachi, *T. castaneum* is found damaging more wheat in Dockyard areas probably due to damp climate, which soften the hard pericarp of wheat grain. Control of these pests are prime important in order to meet the demands of increasing population. The outbreaks of these pests could be avoided either by protect ion and/or by treatment of the stored commodities with chemicals. Protection includes all the prophylactic measures and disinfection of stores, bins, bags and grains by using benzene hexachloride (BHC), baythion, diazinon, gardonaa and malathion etc. These chemicals were applied before the grains being stored in order to eliminate chances of future infestation of the pests (Naqvi and Perveen, 1991). Treatment of grains, on the other hand, has to be carried out with fumigants when infestation of the pests appears during the storage (Perveen *et al*., 2010b). Various companies recommended hydrogen cyanide (HCN), acrylonitrile, chloropicrin, ethylene dibromide, methyl bromide, ethylene oxide, ethylene dichloride, carbon

* Corresponding Author:
Email: farzana_san@hotmail.com

tetrachloride, petrol and dichlorovinyl dimethyl phosphate (DDVP) etc for fumigation against insect pests of stored products (Naqvi and Perveen, 1993).

Fumigants such as phosphine and methyl bromide were used quick and effective tools for insect control in food commodities. Despite their significance in assuring quality, several fumigants have been withdrawn or discontinued on grounds of environmental safety, cost, carcinogenicity, ozone depletion, insect resistance, toxic residues and other factors on stored grain (Ribeiro *et al*., 2003; Shaaya and Kostyukovsky, 2006; Perveen and Khan, 2012).

Plant-derived substances, natural plant products and bio-insecticides have recently become of great interest owing to their versatile applications (Baris *et al*., 2006), for protection of agricultural commodities due to their low mammalian and vertebrate's toxicity and low persistence, no undesirable effects on animals and human beings (Raja *et al*., 2001). Therefore, development of bio-insecticides has been focused as a viable pest control strategy in recent years (Hashim and Devi, 2003; Meena *et al*., 2006). The development of environmentally friendly insecticides, having specificity to insects has captured worldwide attention of scientists (Ishaaya and Degheele, 1998). Plants provide potential alternatives to currently used insect control agents. In the past, few indigenous plants of Pakistan were studied for repellent and feeding deterrent effects on *T. castaneum* (Jilani *et al*., 1993) and lesser grain borer, *Rhyzopertha dominica* (F.) (Jilani and Saxena, 1990). Essential oils having compounds monoterpenoids, offer promising alternatives to classical fumigants (Huang *et al*., 2000; Papachristos and Stamopolos, 2002, 2003), contact insecticides (Ndungu *et al*., 1995), antifeedants (Chiam *et al*., 1999) and may also affect some biological parameters such as growth rate, life span and reproduction (Villalobos, 1996).

Gosh (1947) reported that repellents are substances which as stimuli elicited "avoiding reactions" the insects. The physical repellents, visual stimulus, may be the surface texture of the substances on which insects perch. The chemical repellents cause insects to make oriented movement away from the source. Such stimuli cause a kinetic or dispersion mechanism from the sources more rapidly than if the area does not contain the stimulus. They inhibit feeding or oviposition when present in a place where insects would feed or oviposit. The response due to the stimulation of the insects chemoreceptors. They trigger a variety of important behavior patterns, including feeding, selection of habitat, hosts parasite responses and integrating caste functions among social insects.

Hill toon, *Cedrela serrata* (Royle) (Sapindales : Meliaceae) is comprising about 50 genera and 1400 species. The genus *Cedrela* is included in the tribe Cedreleae of the sub-family Swietenioideae, as is the genus Toona. All the old world species of *Cedrela* have been transferred to Toona. *Cedrela* differs from the latter by its prominent androgynophore with petals and filaments adnate to it, the cuplike calyx, the bigger and woodier capsule, and seedlings having entire leaflets. The specific name, '*serrata*', comes from 'serra' (a saw), referring to the toothed leaf-margins (Ram *et al*., 2000) (Figure 1). The objective of the present research was to determine repellency *T. castaneum* caused by leave extract fractions of *C. serrate*

MATERIALS AND METHODS

Insects rearing: The rearing method was adopted according to Naqvi and Perveen (1991 and 1993) with some modifications. Adults of red flour beetle, *Tribolium castaneum* (Herbst.) were collected from local godowns of Mansehra, Pakistan and reared under controlled temerature at 30±1 °C, RH 60±2% and a 16:8 h (L:D). The dark period was set from 06:00-14:00 h to facilitate observations in daytime. Ten pairs of adults of *T. castaneum* were taken in plastic bottles (height: 14 cm; dm: 8 cm) containing 300 g of wheat flour media (fine flour: wheat bran: Brewer's yeast; 7: 2: 1) and were tied with muslin cloth placed on a stand in laboratory to protect them from pests. They have incubation period 4-6 d (days), 6-7 larval instars and completed their life cycle in 22-25 d. When pure culture (after 4-5 generations) and sufficient population of uniform age and size was available, the experiments were started.

Preparation of extract of *Cedrela serrate:* The hill toon, *Cedrela serrata* leaves (Figure 1) were collected from Balakot, Mansehra, Pakistan and identified by the experts. They were rinsed and dried at 30±1 °C under shed in laboratory. Then were ground to fine powder using an electric blender, and concentrated in 80% methanol to obtain dark green gummy residues (30 g) (Perveen *et al*., 2010b) dissolved in distilled water on the bases of increasing polarity. Fractionations were made according to Rashid *et al*. (2009) with some modification (Perveen *et al*., 2012c). Firstly, a methanol extract was obtained and then used respective solvents to obtained n-butanol fraction (NBF), ethyl acetate fraction (EAF) and aqueous fraction (AQF). They were investigated for repellency of 5th instars of *T. castaneum.*

Insecticidal assays: For preparation of stock solution of each fraction, 5 g of each above-mentioned extract was dissolved in 35 ml of their respective solvents. Further, 5 doses 10, 7.5, 5.0, 2.5 and 1.0 ml with final volume was made up to 100 ml by addition of each of their respective solvents to make the doses 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm^2 for method "A" respectively, for repellency of 5th instars of *T. castaneum*. For method "B", 0.71, 1.07, 1.42, 2.14 and 2.85 µl/cm^2 doses were used. These doses were selected after preliminary experiments.

Repellency assays: For determining repellency of 5th instars of *T. castaneum* by *C. serrata* leaves extract in 2 ways: The method "A" was conducted according to McDonald *et al*. (1970) with little modifications. According to this method, filter papers (9 cm) were cut into two halves. Each one-half was treated with 0.5 ml of each dose of all fractions and their respective solvents by a micropipette uniformly. Then they were air-dried and each treated half-disc was attached lengthwise, edge-to-edge, to untreated (control: C_s) half disc with cello-tape in such a way that attachment did not interfere the free movement of insects and placed in petri dishes, separately. Ten pairs of 5th instars of *T. castaneum* were released at the center of each filter paper and covered. For each dose of all fractions, 3 replications were set for 5 times [n=5(3)]. Insects settled on each half of the filter paper disc were counted at 1 h interval for 5 h (Figure 2). The method "B" was conducted according to Talukder and Howse (1994) with little modifications. The procedure was the same as mentioned above, except their respective solvents were applied (50 µl) on untreated halves (Figure 2).

Figure 1. The hill toon, *Cedrela serrata* Royle (Sapindales: Meliaceae) was obtained from natural habitat, Balakot, Kaghan Road, Mansehra, Pakistan. Its leaves were used for the present research for determination of toxicity and residual effects.

Figure 2 Method "A": Repellency of the red flour beetle, *Tribolium castaneum* (Herbst.) by the hill toon, *Cedrela serrata* Royle leaves extract when filters papers were cut into two halves placed in petri dishes.

One halves of each of them treated with doses i.e., 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm^2, respectively, and other halves were kept as control (untreated: C_s). Ten pairs of fresh 5th instars of *T. castaneum* were released at the centre of each petri dish on filter paper then covered it. Experiments were set for 4 fractions, i.e., methanolic extract (ME, n-butanol fraction (NBF), ethyl acetate fraction (EAF) and aqueous fraction (AQF) [n=5(3)]. Insects settled on each half of the filter paper disc were counted at 1 h interval for 5 h (McDonald *et al*., 1970). Method "B": the procedure was the same as mentioned above, except their respective solvents (50 µl/cm^2) were applied on untreated halves and 0.71, 1.07, 1.42, 2.14 and 2.85 µl/cm^2 doses were used (Talukder and Howse, 1994).

Data analysis: Data were expressed as percent repulsion (%R) using the following formula for method **"A"** (McDonald *et al*., 1970): %R = (Nc–50)×2; where Nc: % of insects present in the control half; and for method **"B"** (Talukder and Howse, 1994): %R = A–B/total no of insects released×100; where A: number of insects on untreated half; B: number of insects on treated half. Positive values expressed repellency and negative values attractancy. The data was analyzed using analysis of variance after arcsine transformation. The average values were then categorized according to the scale (Table 1; McDonald *et al*., 1970; Perveen and Hussain, 2012).

Table 1. Repellency classes according to McDonald *et al*. (1970).

S. No.	Class	& R[1]
1	O	>0.01-0.1
2	I	0.1-20
3	II	20.1-40
4	III	40.1-60
5	IV	60.1-80
6	V	80.1-100

[1]%R: Percentage of repellency rate.

RESULTS

Method A: In the method "A", the control (untreated) half of filter paper was used. In general, it was observed that there is positive correlation between repellency and doses, i.e., increased with increase of doses, however, a negative correlation was found between repellency and time, i.e., decreased with increase of duration of time. Repellency in descending order of 4 fractions of *C. serrata* leaves extract was: NBF>EAF>ME>AQF (Figure 3).

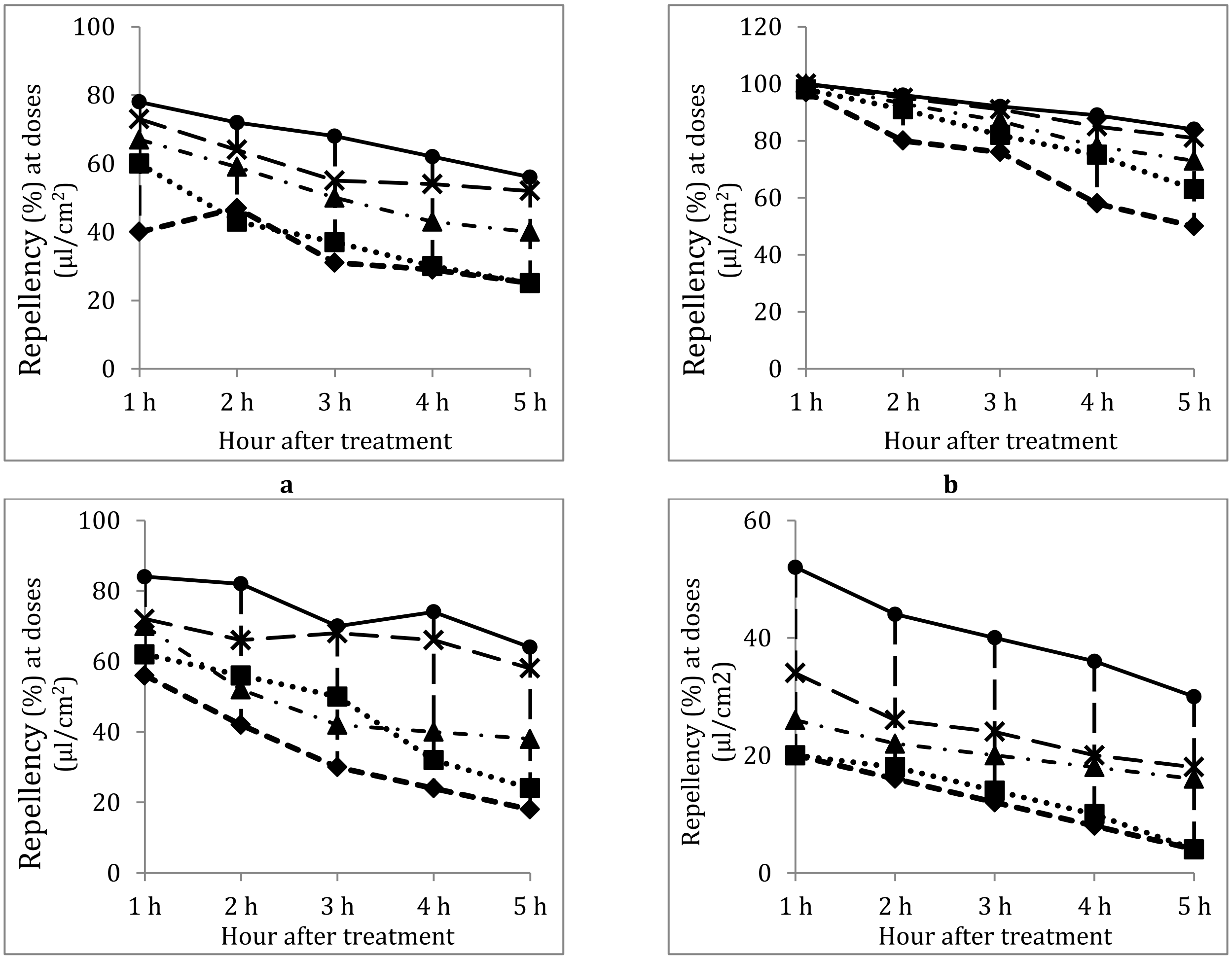

Figure 3 Repellency of the red flour beetle, *Tribolium castaneum* (Herbst.) caused by the hill toon, *Cedrela serrata* Royle leaves extract by untreated half filter paper (method "A") when 5th instars were used; 5 different doses ♦: 20.4 µl/cm²; ■: 25.5 µl/cm²; ▲: 30.6 µl/cm²; ×: 35.7 µl/cm²; ●: 40.8 µl/cm² of 4 different fractions, a: methanolic extract (ME); b: n-butanol fraction (NBF); c: ethyl acetate fraction (EAF); and d: aqueous fraction (AQF) were tested; h: hour after treatment; data were analyzed by using analysis of variance after arcsine transformation; 3 replications were set for 5 times for each dose of each fraction.

In case of ME, repellency (Mean) of solvent treated C_s was 19.2 which represents class I, whereas for 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm² doses mean repellency was 34.4, 39.0, 51.8, 60.4 and 67.2 represent classes II, II, III, IV and IV, respectively (Table 2). In case of NBF, after consecutive 1 h interval for 5 h of treatment, mean repellency of C_s was 55.8 which represents class III, whereas for 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm² doses, mean repellency was 72.2 belongs to class IV and; 81.8, 86.2, 90.4 and 92.2 represent class V (Table

2). In case of EAF, after consecutive 1 h interval for 5 h of treatment, mean repellency of C_s was 20.4 which represents class II, whereas for 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm² doses mean repellency was 34.0, 44.8, 48.4, 66.0 and 74.8 represent classes II, III, III, IV and IV, respectively (Table 2).

In case of AQF, after consecutive 1 h interval for 5 h of treatment, mean repellency of C_s was 5.2 which represents class I, whereas for 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm² doses mean repellency was 12.0, 13.2, 20.4, 24.4 and 46.4 represent classes I, I, II, II and III, respectively (Table 2).

Table 2 Fractions of the hill toon, *Cedrela serrata* Royle leaves extract were tested for repellency of 5th instars of the red flour beetle, *Tribolium castaneum* (Herbst.) by untreated half filter paper (method "A").

Sr. No.	Doses (µl/cm²)[1]	n[1]	ME[2]		NBF[2]		EAF[2]		AQF[2]	
			M±SD[3]	RC[1]	M±SD[3]	RC[1]	M±SD[3]	RC[1]	M±SD[3]	RC[1]
1	C_s	5(3)	19.2±8.4	I	55.8±20.8	III	20.4±11.2	II	5.2±4.2	I
2	20.4	5(3)	34.4±8.9	II	72.2±18.6	IV	34.0±15.7	II	12.0± 6.3	I
3	25.5	5(3)	39.0±13.6	II	81.8±13.7	V	44.8±16.7	III	13.2 ±6.4	I
4	30.6	5(3)	51.8±11.2	III	6.2±10.9	V	48.4±13.2	III	20.4±3.8	II
5	35.7	5(3)	60.4±8.4	IV	90.4±7.6	V	66.0±5.1	IV	24.4±6.2	II
6	40.8	5(3)	67.2±8.6	IV	92.2±6.2	V	74.8±8.3	IV	46.4± 8.3	III

[1]n: 3 replications were set for 5 times for each dose of each fractions; RC: Repellency classes according to McDonald *et al*. (1970; Table 1).
[2]ME: methanolic fraction; NBF: n-butanol fraction; EAF: ethyl acetate fraction; AQF: aqueous fractions of leaves extract of *C. serrata* with 5 different doses were tested.
[3]Data were analyzed by using analysis of variance after arcsine transformation.

Method "B"

In the method "B", respective solvents were applied on untreated halves. The same correlations were observed as found in method "A" between repellency and doses, and repellency and duration of time. In method "B" Repellency was the same sequence as in method "A", i.e., NBF>EAF>ME>AQF (Figure 4).

Mean repellency of ME of *C. serrata* against *T. castaneum* after consecutive 1 h interval for 5 h of treatment, for 0.71, 1.07, 1.42, 2.14 and 2.85 µl/cm² doses, mean repellency was 8.6, 10.8, 13.2, 16.0 and 19.0, respectively. All doses shows repellency class I (Table 3). Mean repellency of NBF of *C. serrata* against *T. castaneum* after consecutive 1 h interval for 5 h of treatment, for 0.71, 1.07, 1.42, 2.14 and 2.85 µl/cm² doses, mean repellency was 11.6, 13.8, 16.8, 19.4 and 22.0, respectively. The highest dose shows class II repellency, while all other doses show class I repellency (Table 3). Mean repellency of EAF of *C. serrata* against *T. castaneum* after consecutive 1 h interval for 5 h of treatment, for 0.71, 1.07, 1.42, 2.14 and 2.85 µl/cm² doses, mean repellency was 3.8, 11.8, 13.6, 17.8 and 20.4, respectively. The highest dose shows class II repellency, while all other doses show class I repellency (Table 3). Mean repellency of AQF of *C. serrata* against *T. castaneum castaneum* after consecutive 1 h interval for 5 h of treatment, for 0.71, 1.07, 1.42, 2.14 and 2.85 µl/cm² doses, mean repellency was 2.0, 3.2, 3.6, 5.2 and 6.0, respectively. All other doses show other doses show repellency class I (Table 3).

Table 3 Fractions of the hill toon, *Cedrela serrata* Royle leaves extract were tested for repellency of 5th instars of the red flour beetle, *Tribolium castaneum* (Herbst.) by solvent treated half filter paper (method "B").

Sr. No.	Doses (µl/cm²)[1]	n[1]	ME[2]		NBF[2]		EAF[2]		AQF[2]	
			M±SD[3]	RC[1]	M±SD[3]	RC[1]	M±SD[3]	RC[1]	M±SD[3]	RC[1]
1	0.71	5(3)	8.6±6.2	I	11.6±6.0	I	3.8±3.0	I	2.0±8.7	I
2	1.07	5(3)	10.8±6.9	I	13.8 ±6.4	I	1.8±4.5	I	3.2±13.1	I
3	1.42	5(3)	13.2±8.4	I	16.8±6.8	I	13.6±5.4	I	3.6±15.9	I
4	2.14	5(3)	16.0±9.2	I	19.4±9.6	I	17.8±6.3	I	5.2±19.3	I
5	2.85	5(3)	19.0±11.0	I	22.0±10.7	II	20.4±7.3	II	6.0±22.5	I

[1]n: 3 replications were set for 5 times for each dose of each fractions; RC: Repellency classes according to McDonald *et al*. (1970; Table 1).
[2]ME: methanolic fraction; NBF: n-butanol fraction; EAF: ethyl acetate fraction; AQF: aqueous fractions of leaves extract of *C. serrata* with 5 different doses were tested.
[3]Data were analyzed by using analysis of variance after arcsine transformation.

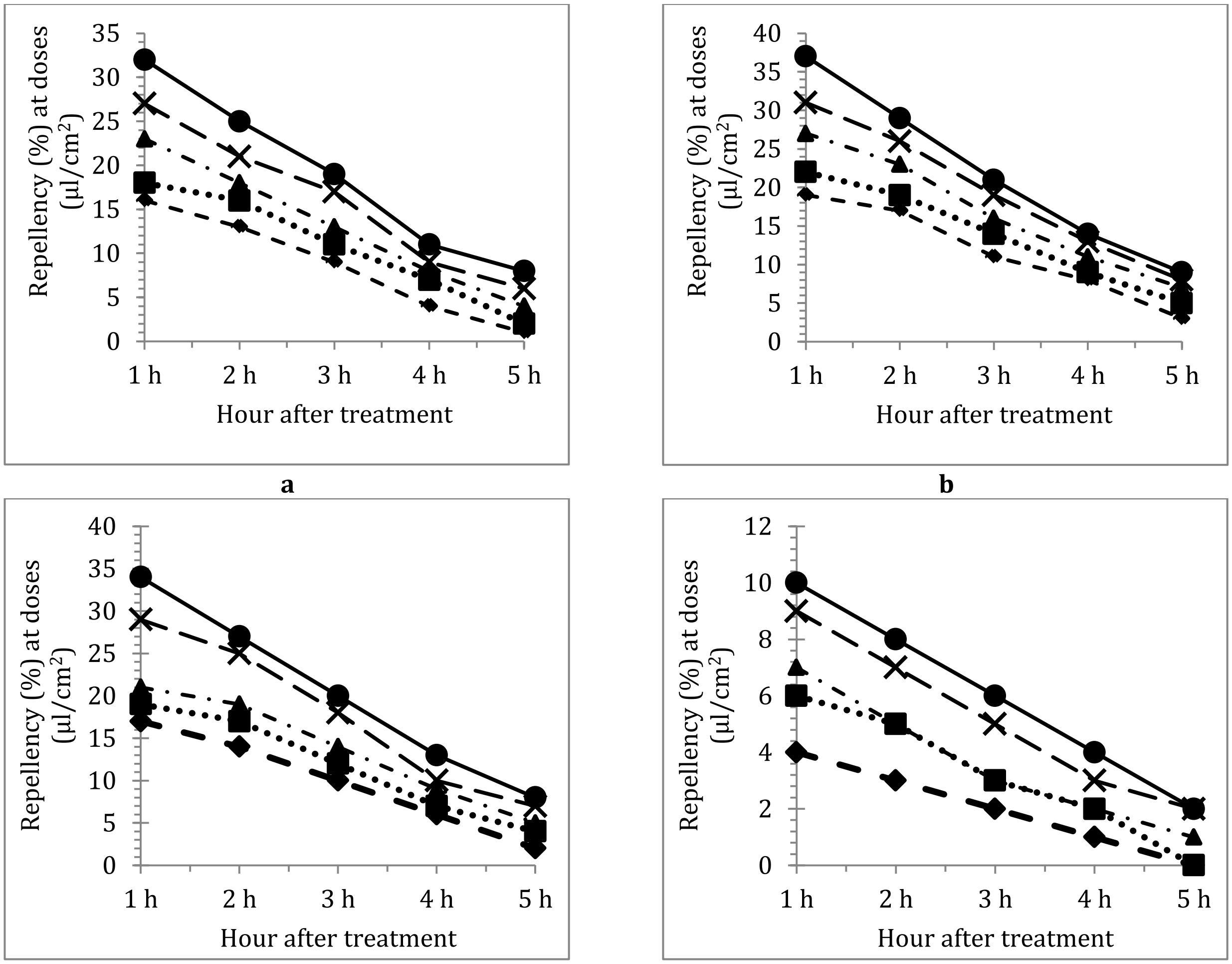

Figure 4 Repellency of the red flour beetle, *Tribolium castaneum* (Herbst.) caused by the hill toon, *Cedrela serrata* Royle leaves extract by solvent treated half filter paper (method "B") when 5th instars were used; 5 different doses ♦: 0.71 µl/cm²; ■: 1.07 µl/cm²; ▲: 1.42 µl/cm²; ×: 2.14 µl/cm²; ●: 2.85 µl/cm² of 4 different fractions, a: methanolic extract (ME); b: n-butanol fraction (NBF); c: ethyl acetate fraction (EAF); and d: aqueous fraction (AQF) were tested; h: hour after treatment; data were analyzed by using analysis of variance after arcsine transformation; 3 replications were set for 5 times for each dose of each fraction.

DISCUSSION

The bio-products are more significant for controlling the stored grains and agricultural pests due to their lesser harmful effects compared to chemical pesticides. Methanol was used as solvent for leaves extract of the hill toon, *Cedrela serrata*. The NBF, EAF and AQF of *C. serrata* leaves extract were studied for antioxidant and DNA protection activities (Perveen *et al*., 2012c) as well as for possible insecticidal assays like toxicity and residual effects (unpublished), for repellency of red flour beetle, *Tribolium castaneum* (at the present) and for phytochemical and spectrophotometric properties of active biological component(s) (unpublished). However, toxicity and residual effects of methanolic leaves extract of the yellow-berried nightshade, *Solanum surrattense* Burm. were determined against *T. castaneum* (Perveen *et al*., 2010b; Perveen and Khan, 2012). Therefore, the trend to use plant origin products has been increased recently for controlling the stored grains and agricultural pests.

In method "A" when filter paper half was untreated and other half was treated with lower to higher doses of ME, the repellency classes increased from I to IV. In the same way, for the same sequence of doses for NBF, the repellency classes increased from III to V, moreover, for EAF, they were II to IV and for AQF, they were I to III. Therefore, repellency was increased with increase of doses, hence, there is positive correlation between repellency and doses of different fractions of *C. serrata*.

In second method, when respective solvents were used as untreated half and other half was treated with lower to higher doses, repellency for ME and AQF laid to class

I with lower as well as higher doses whereas in case of n-butanol and EAF, with lower doses repellency class I but with highest dose, repellency class II was obtained. Thus, it was seen that when control was used as untreated filter paper, extracts showed good repellent (method "A"), whereas by using solvent treated half as control, extract showed low repellency and as second half contain solvent which also showed some repellency of 5th instars of *T. castaneum* (method "A"). Therefore, Method "A" was more significant than method "B".

Khanam *et al*. (2006) reported that the product (lignin) obtained from sugarcane bagasse showed strong repellent action against *T. castaneum* (76%) and confused flour beetle, *T. confusum* Jacquelin (88%) with the dose of 628.76 μg/cm². The results indicated that the repellent response of *T. confusum* was higher than that of *T. castaneum*. The present result showed 39.0, 81.8, 44.8 and 13.2% repellency with dose 25.5 μg/cm² by ME, NBF, EAF and AQF of *C. serrata*, respectively, against *T. castaneum*. The difference may be due to different plants used or due to difference in doses.

Farhana *et al*. (2006) tested three extracts of Coriander, *Coriandrum sativa* L.; bishop's flower, *Ammi majus* L. and Fenugreek, *Trigonella foenum-graecum* L. and showed repellent activity of adult beetles of *T. castaneum*. The intensity of repellent activity had been arranged in a descending order: *T. foenum-graecum* > *C. sativum* > *T. ammi.* In the present research, 4 fractions of *C. serrata* were used in 5 different doses against *T. castaneum* and the intensity of repellent activity had been arranged in a descending order of NBF > EAF > ME > AQF. The difference in result was due to different plants and stages of *T. castaneum* used or due to difference in doses but in both studies, plants extracts/fractions showed significant repellency in *T. castaneum*.

Rahman *et al*. (2007) studied the repellency of melgota, *Macaranga postulata* L. against rice weevil, *Sitophilus oryzae* (L.) and reported that among the fruit extracts 1, 2 and 4% of *M. postulata* showed 9.84, 12.76 and 22.43%, repellency, respectively, in *S. oryzae*. This showed that the highest repellency was observed with 4% extract and the repellent action increased with increase in doses of the extract applied. In the present research, leaves extract of *C. serrata* was used to check repellency against *T. castaneum* in 4 different fractions. When the filter paper half was untreated and other half was treated with 20.4, 25.5, 30.6, 35.7 or 40.8 μl/cm² doses of ME of *C. serrata,* the repellency (mean) of the same insect was 34.4, 39.0, 51.8, 60.4 or 67.2%. The same trend was observed in other fractions. Therefore, repellency increased with increase of doses and it was also proved that NBF was the most repellent fraction among all fractions even when used in lower doses. Moreover, both studies showed somewhat the same trend of results.

Othira *et al*. (2009) investigated the use of marubio, *Hyptis spicigera* Lam. preparations for pests' control. Studies were conducted in order to assess the insecticidal potency of *H. spicigera* extracts on maize weevil, *Sitophilus zeamais* (Motschulsky) and *T. castaneum.* They performed repellent effects of *H. spicigera* powder and oil against *S. zeamais* and *T. castaneum* by a choice bioassay system and showed that whole plant extracts and essential oils described could be useful for managing field populations of *S. zeamais* and *T. castaneum*. In the present research, all 4 fractions of the used extract showed repellent behavior against *T. castaneum* by all doses. Both the studies introduced an innovative approach to the use of traditional plant-based pesticides for grain protection as both gave good repellent for *T. castaneum.* Therefore, the fractions of leaves extract of *C. serrata* have great tendency of repellent activity for *T. castaneum*.

Moreover, if a bio-product should be marketed for the use of public or farmers, it is very important to study its different mode of action, biochemical analysis and other aspects for awareness and to prevent other hazards. As it was studied that sublethal doses of chlorfluazuron (IGR) were effects on reproductivity and viability (Perveen, 2000a and 2009c), ovarian development and oogenesis (Perveen and Miyata, 2000), ovarioles (Perveen, 2011d), ovarian biochemical constituents (Perveen, 2011b), on pupal-testis (Perveen *et al*., 2011), on spermatogenesis of adult (Perveen, 2011a), the testicular biochemical constituents (Perveen, 2011c), on the biochemical constituents of the eggs (Perveen, 2011e), in egg hatch (Perveen, 2006), on haemolymph-borne oviposition-stimulating factors (Perveen, 2009b) biochemical analyses of action of chlorfluazuron (Perveen, 2009a). If *C. serrata* leaves extract fractions would be marketed for stored grains and agricultural purposes, therefore, their all aspects would be studied as chlorfluazuron was studied.

CONCLUSION

At the highest dose of *C. serrata*, NBF showed the highest repellency belong to classes V and II for methods A and B, respectively, therefore, it was significant repellent compared with other fractions. Repellency was directly proportional with doses, however, it was inversely proportional with duration. Method "A" was more successful compared to method "B" for repellency. It is concluded through the results that *C. serrata* leaves extract's fractions showed significant repellent activity for *T. castaneum*.

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