International Journal of Agricultural Research and Review, Vol. 2(1) pp 018-026, January, 2014
Copyright © 2014 Spring Journals

Full Length Research Paper

Toxicity and effects of the hill toon, *Cedrela serrata* methanolic leaves extract and its fractions against 5th instar of the red flour beetle, *Tribolium castaneum*

Perveen F*[1] and Khan A[2]

[1]Department of Zoology, Shaheed Benazir Bhutto University (SBBU), Main Campus, Sheringal, Khyber Pakhtunkhwa, Pakistan
[2]Beaconhouse School System, Margalla Campus, H-8, Islamabad
*Corresponding Author's Email: farzana_san@hotmail.com

Accepted 18th January, 2014

The research was conducted to evaluate the toxicity and residual effects of the hill toon, *Cedrela serrata* Royle methanolic leaves extract and its fractions against 5th instar of the red flour beetle, *Tribolium castaneum* (Herbst). Insects were reared under controlled laboratory conditions at 30±2 °C, 60±10% RH and a 16:8 h (L:D). The toxicity result revealed that LD_{50} of methanolic extract (ME), n-butanol fraction (NBF), ethyl acetate fraction (EAF) and aqueous fraction (AQF) was 143, 18, 70 and 384 µl/cm^2, respectively. The most toxic fraction was NBF as its doses 20, 51, 102, 153 and 204 µl/cm^2 revealed 73, 79, 85, 93 and 99% mortality, respectively, against 5th instars of *T. castaneum*. The residual effect, during 1st day at the highest dose of ME, NBF and EAF showed 100% mortality while, AQF showed 32%. However, during 7th day, it was 10, 26, 11 and 0%, respectively, for the same dose of ME, NBF, EAF and AQF. It was concluded that *C. serrata* extracts and their fractions showed significant toxicity and residual effects against 5th instars of *T. castaneum*. It is suggested that further research on *C. serrata* should be conducted to find out its active biological component(s).

Keywords: *Cedrela serrata*, lethal dose, residual effects, toxicity, *Tribolium castaneum*.

INTRODUCTION

The global post-harvest grain losses caused by insect damage were 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; Perveen et al., 2013). It produced significantly higher weight losses in wheat than other cereals (Lohar, 1997). A positive correlation was observed among damage protein and fat of wheat, whereas, negative correlation was found in carbohydrate (Wakil et al., 2003; Perveen and Zaib, 2013).

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 is 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 are applied before the grains are 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 tetrachloride, petrol and dichlorovinyl dimethyl phosphate (DDVP) etc for fumigation against insect pests of stored products (Naqvi and Perveen, 1993).

Plant-derivatives have recently become of the great interest owing to their versatile applications (Baris et al., 2006). Natural plant products are biodegradable may provide structure lead for pesticidal discovery (Duke et al., 2000). Akhtar et al. (2004) demonstrated repellent and growth inhibiting effects of cobra lily, *Acorus indica* Schott; turmeric, *Curcuma longa* L. and sweet flag, *A.*

Figure 1. Rearing of the red flour beetle, *Tribolium castaneum* (Herbst) in bottles were kept in stand and box using light source in the Molecular Biology Laboratory, Department of Biochemistry, Hazara University, Mansehra, Pakistan

calamus L. Among the petroleum ether, acetone and ethanol extracts of *C. longa*, acetone extracts was the best repellent inhibitor against peach fruit fly, *Bactrocera zonata* Saunders (Siddiqui et al., 2006). Plant extracts containing compounds such as terpene, steroid, alkaloid, phenolic and cardiac glycosides (Duke, 1990) are known to affect insect behaviour and can function as deterrent to insect pests (Blaney et al., 1988; Ge and Weston, 1995; Mordue et al., 1998; Mancebo et al., 2000).

Fumigants such as phosphine and methyl bromide were used quick and effective tools for insect pests 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 pests resistance, toxic residues and other factors on stored grains (Ribeiro et al., 2003; Shaaya and Kostyukovsky, 2006; Perveen and Khan, 2012).

There has been growing interest in the use of natural plant products, bio-insecticides for protection of agricultural commodities due to their low mammalian and vertebrate's toxicity and low persistence in the environment, no undesirable effects on animals and human beings (Raja et al., 2001). Therefore, development of bio-insecticides has been focused as a viable pests control strategy in recent years (Hashim and Devi, 2003; Meena et al., 2006). The development of environmentally friendly insecticides, having specificity to insects along has captured worldwide attention of scientists (Ishaaya and Degheele, 1998). Plants provide potential alternatives to currently used insect pests control agents. In the past, few indigenous plants of Pakistan were studied for repellent effects on *T. castaneum* (Jilani et al., 1993) and their repellent and feeding deterrent effects against 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).

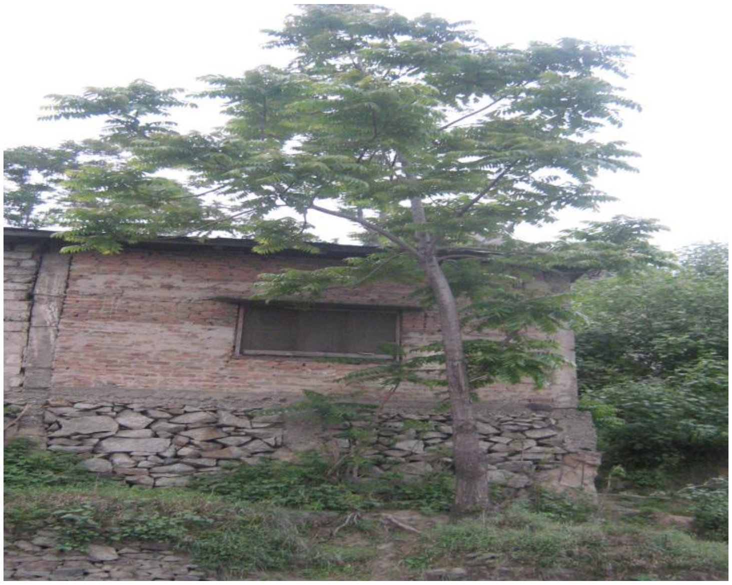
Hill toon, *Cedrela serrate* (Royle) (Sapindales : Meliaceae) is comprising about 50 genera and 1400 species, forms a large botanical family of mostly pantropical distribution. The trees are valued for their quality wood and resistance to attack of several insect pests (Banerji and Nigam, 1984). Therefore, its toxicity and residual effects were determined against 5^{th} instars of *T. castaneum* in this paper.

MATERIALS AND METHODS

The present research was carried out in the Molecular Biology Laboratory, Department of Biochemistry, Hazara University, Mansehra and Quaid-i-Azam University, Islamabad, Pakistan (Perveen et al., 2013; Perveen and Zaib, 2013) .

Insects rearing

Adults of red flour beetle, *Tribolium castaneum* were collected from local go downs of Mansehra, Pakistan and reared under controlled laboratory conditions at 30±2 °C, 60±10% RH and a 16:8 h (L:D) photoperiod. To facilitate observations, the dark period was set from 06:00-14:00 h. Adults of *T. castaneum* (10 pairs) 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 (Figure 1). The incubation period was 4-6 d (days), 6-7 larval instars and life cycle was completed in 22-25 d. The experiments were started when pure culture (after 4-5 generations) and sufficient population of uniform age and size was available (Perveen et al., 2013; Perveen and Zaib, 2013).



a

b

Figure 2. a) The hill toon, *Cedrela serrata* Royle (Sapindales: Meliaceae) was obtained from its natural habitat, Balakot, Kaghan Road, Mansehra, Pakistan. Its leaves were used for the present research for determination of toxicity and residual effects; **b)** The red floor beetle, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenibrionidae) has been used as test organism for the present research

Preparation of extract of *Cedrela serrata*

Leaves of the hill toon, *Cedrela serrate* (Figure 2) were collected from Balakot, Kaghan Road, Mansehra, Pakistan and was authenticated by Dr Habib Ahmed, Chairman and Incharge of the herbarium of the Hazara University, Mansehra, Pakistan, where voucher specimen was deposited (Perveen et al., 2012c). They were rinsed, and dried at laboratory temperature 25-27 °C under shed. Then were ground to fine powder using an electric blender, and concentrated in 80% methanol to obtain dark green gummy residue (30 g) (Naqvi and Perveen, 1991; Perveen et al., 2010b). Fractionations were made according to Perveen et al. (2012c) with some modification that the residues were dissolved in distilled water on the basis of increasing polarity to were selected after preliminary test for both toxicity and residual effects experiments.

Toxicity

The experiments were conducted by contact (filter feeding) method using 5th instars of *T. castaneum* (Naqvi obtain methanolic extract (ME), n-butanol fraction (NBF), ethyl acetate fraction (EAF) and aqueous fraction (AQF). They were investigated for different biological activities, i.e., toxicity and residual effects.

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 20.40, 51.02, 102.04, 153.06 and 204.08 µl/cm^2 for toxicity and 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm2 for residual effect, respectively. These doses and Perveen, 1993) to find out the effective range of extracts. One ml of above mentioned doses were applied on filter paper (whatman-41) (7 cm dm) placed in the petri dish and air dried. For determination of environmental effects, a control (C_E) and for solvent effect another control C_S (filter papers treated with solvent only) were kept. Five pairs of 5th instars of *T.*

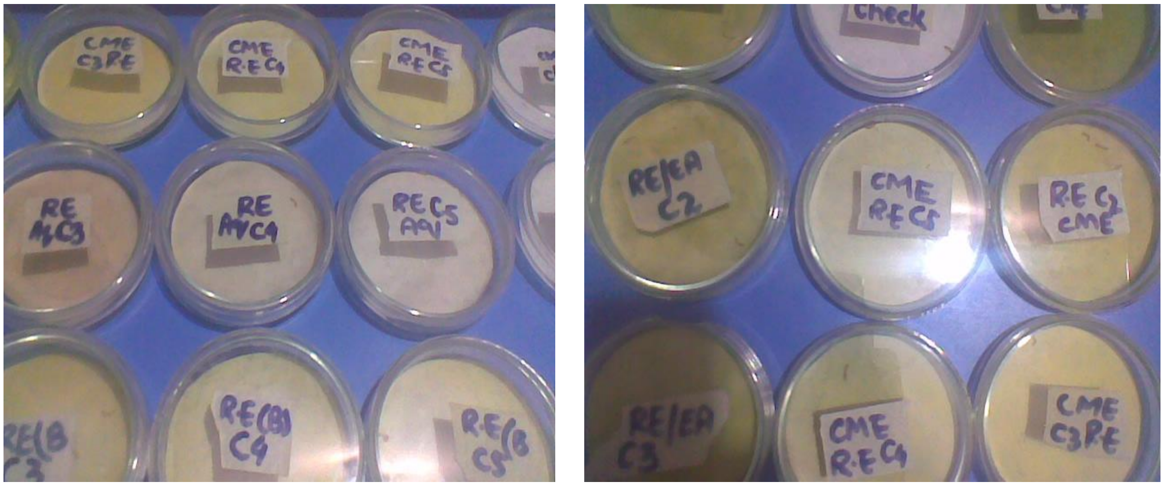


Figure 3. The residual effects of the hill toon, *Cedrela serrata* Royle extracts' 4 fractions were determined when 5 doses of each, i.e., 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm^2, viz., C_1, C_2, C_3, C_4 and C_5, respectively, were applied by contact (filter feeding) method and 5 pairs of fresh 5th instar of the red flour beetle, *Tribolium castaneum* (Herbst) were released daily up to 7 d in petri dishes, without changing filter paper and mortality % was observed after each 24 h.

castaneum were released in each petri dish. All batches were kept without food. The mortality counts were recorded after each 24 h. The experiments were discarded, if the mortality was more than 10% in C_E and $C_{S.}$ Results were shown in percentage (%) for each fraction and dose. Three replications were set for 4 fractions of 5 doses and performed for consecutive 7 d.

Residual effects

The residual effect of the same leaves extract was determined against 5th instars of *T. castaneum* (Naqvi and Perveen, 1991). The same doses and application method were used as in toxicity determination except fresh 5 pairs of 5th instars of *T. castaneum* were released daily up to 7 d in petri dishes without changing filter paper. Results were shown in % for each fraction and dose. Three replications were set for 4 fractions of 5 doses and performed for consecutive 7 weeks (Figure 3).

Data analysis

For toxicity and residual effects results observed were calculated using Abbott's formula: mortality % = (test mortality%-C1mortality%)/100–C1mortality×100(Perveen and Hussain, 2012).

RESULTS

The methanolic leaves extract and fractions of *C. serrata* were tested for the following biological activities against *T. castaneum*:

Toxicity

The toxicity results for ME of *C. serrata* against 5th instars of *T. castaneum* revealed minimum and maximum mortalities 10 and 59.3% at the lowest and highest doses, i.e., 20.4 and 204.1 µl/cm^2, respectively, whereas LD_{50} was 143.0 µl/cm^2. The same results for NBF against the same insect showed that minimum and maximum mortalities 73 and 99% at the lowest and highest doses, respectively, whereas LD_{50} was 17.9 µl/cm^2. The toxicity results for EAF exhibited minimum and maximum mortalities 35.5 and 79% at the lowest and highest doses, respectively, whereas LD_{50} was 69.8 µl/cm^2. The same for AQF revealed that minimum and maximum mortalities 5.3 and 17% at the lowest and highest doses, respectively, whereas LD_{50} was 384.0 µl/cm^2 (Table 1).

Residual effects

During 1st day residual effects of ME of C. serrata with all doses showed 100% mortality against T. castaneum. The C_s showed 57% mortality whereas mortality for C_E was non-significant at the same day. On 2nd day, C_s and 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm^2 showed 26, 30, 38, 49, 61 and 72% mortality, respectively. On 3rd day, mortality was 14, 19, 26, 32, 44 and 56%, respectively. On 4th day, mortality was 8, 12, 23, 35, 35 and 40%, respectively. On 5th day, mortality was 10, 10, 19, 20, 27 and 29%, respectively. On 6th day, mortality was 2, 5, 8, 11, 19 and 21%, respectively. On 7th day, mortality was 0, 2, 3, 5, 8 and 10%, respectively (Figure 4a).

Table 1. Toxicity of the hill toon, *Cedrela serrata* methanolic leaves extract and its fractions against 5th instar of the red flour beetle, *Tribolium castaneum* (Herbst)

SNo	Extracts[1] Doses (µl/cm^2)	ME[2] Mortality %	LD_{50}[3] (µl/cm^2)	NBF[2] Mortality %	LD_{30}[3] (µl/cm^2)	EAF[2] Mortality %	LD_{30}[3] (µl/cm^2)	AQF[2] Mortality %	LD_{30}[3] (µl/cm^2)
1.	C_E[3]	2.0		1.0		2.0		1.0	
2.	C_S[3]	8.4		67.4		27.8		4.5	
3.	20.4	10.0		73.0		35.5		5.3	
4.	51.0	18.0	143.0	79.3	17.9	46.0	69.8	8.0	384.0
5.	102.0	35.3		85.3		59.3		12.6	
6.	153.1	51.7		92.6		67.3		14.3	
7.	204.1	59.3		99.0		79.0		17.0	

[1]Extracts: 5 different concentrations of 4 different fractions of *C. serrata* were tested for toxicity against 5th instars of *T. castaneum*
[2]ME: methanolic extract; NBF: n-butanol fraction; EAF: ethyl acetate fraction; AQF: aqueous fraction
[3]LD_{50}: lethal dose at 50% mortality; C_E: untreated filter paper (control); C_S: treated with solvent (environmental factors control); %: percentage of 3 replications for each fraction and dose

The NBF of *C. serrata* showed 100% mortality against *T. castaneum* with all doses during 1st and 2nd day of experiment, while C_s showed 71 and 46%, respectively. Mortality for C_E was almost non-significant. On 3rd day, C_s and 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm^2 showed 26, 42, 51, 61, 73 and 81% mortality, respectively. On 4th day, mortality was 21, 30, 44, 49, 59 and 67%, respectively. On 5th day, mortality was 12, 23, 29, 28, 45 and 51%, respectively. On 6th day, mortality was 7, 10, 14, 19, 23 and 32%, respectively. On 7th day, mortality was 2, 6, 8, 10, 14 and 26%, respectively (Figure 4b).

The EAF of *C. serrata* showed 100% mortality against *T. castaneum* with all doses used while C_s showed 60%, during 1st day of experiment. Mortality for C_E was almost non-significant. On 2nd day, C_s and 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm^2 showed 40, 80, 89, 93, 100 and 100% mortality, respectively. On 3rd day, mortality was 28, 57, 61, 62, 68 and 74%, respectively. On 4th day, mortality was 17, 29, 26, 31, 39 and 45%, respectively. On 5th day, mortality was 9, 2, 6, 17, 24 and 30%, respectively. On 6th day, mortality was 6, 4, 3, 8, 13 and 19%, respectively. On 7th day, mortality was 0, 1, 2, 5, 7 and 11%, respectively (Figure 4c).

The AQF of *C. serrata* unlike other fractions did not show 100% mortality against 5th instars of *T. castaneum* with all doses used as well as C_s, during 1st day of experiment rather it showed 3, 10, 14, 21, 29 and 32 for C_s and 20.4, 25.5, 30.6, 35.7 and 40.8 µl/cm^2, respectively. Mortality for C_E was almost non-significant. On 2nd day, mortality was 1, 4, 10, 15, 19 and 23%, respectively. On 3rd day, mortality was 0, 2, 7, 7, 8 and 12%, respectively. On 4th day, mortality was 0, 0, 1, 3, 5 and 9%, respectively. On 5th day, mortality was 0, 0, 0, 1, 2 and 5%, respectively. On 6th day, mortality was 0, 0, 0, 0, 0 and 2%, respectively. On 7th day, 0% mortality was observed by C_s and all doses (Figure 4d).

DISCUSSION

The natural products may be used for pests control due to their less harmful effects as compared to synthetic pesticides. Methanol was used as solvent for preparation of ME of the hill toon, *Cedrela serrata*. The fractions of *C. serrata* leaves extract was studied for possible insecticidal assays like toxicity and residual effects. However, the same fractions of the same plant extract were used as antioxidant and DNA protection activities in a previous study (Perveen et al., 2012c).

The LD_{50} of ME was 143.0, NBF was 17.9, EAF was 69.8 and AQF of *C. serrata* was 384.0 µl/cm^2, respectively. This indicates that plant has significant toxicity against the red flour beetle, *Tribolium castaneum* which is apparent from their LD_{50} values. No similar work has been reported on *C. serrata* so far. However, Rashid et al. (2009) reported that crude dichloromethane extract of spiral grass, *Salvia cabulica* Benth exhibited significant (80%) insecticidal activity against *T. castaneum*. In present studies, the methanolic extract of *C. serrata* exhibited a significant (59.3%) insecticidal activity against *T. castaneum*. The difference may be due to different plant species or solvent used.

It was reported that the highest mortality 100% was observed at the maximum dose 16.8 µl/cm^2 after 24 h; LD_{50} was found 8.02 µl/cm^2; during 1st day, the highest mortality was 91.6% at maximum dose 11.8 µl/cm^2; and during 7th day, the highest mortality was 60.5% at dose 11.8 µl/cm^2 of the yellow-berried nightshade, *Solanum surrattense* Burm methanolic leaves extract against *T. castaneum* (Perveen et al., 2010b; Perveen and Khan, 2012). In another study, reduction in fertility was observed by the Indian oleander, *Nerium indicum* Mill leaves extract in adults *T. castaneum* compared with coopex (bioallethrin: permethrin) (Perveen and Shah, 2012). At the present, the highest mortality 99% was observed at the maximum

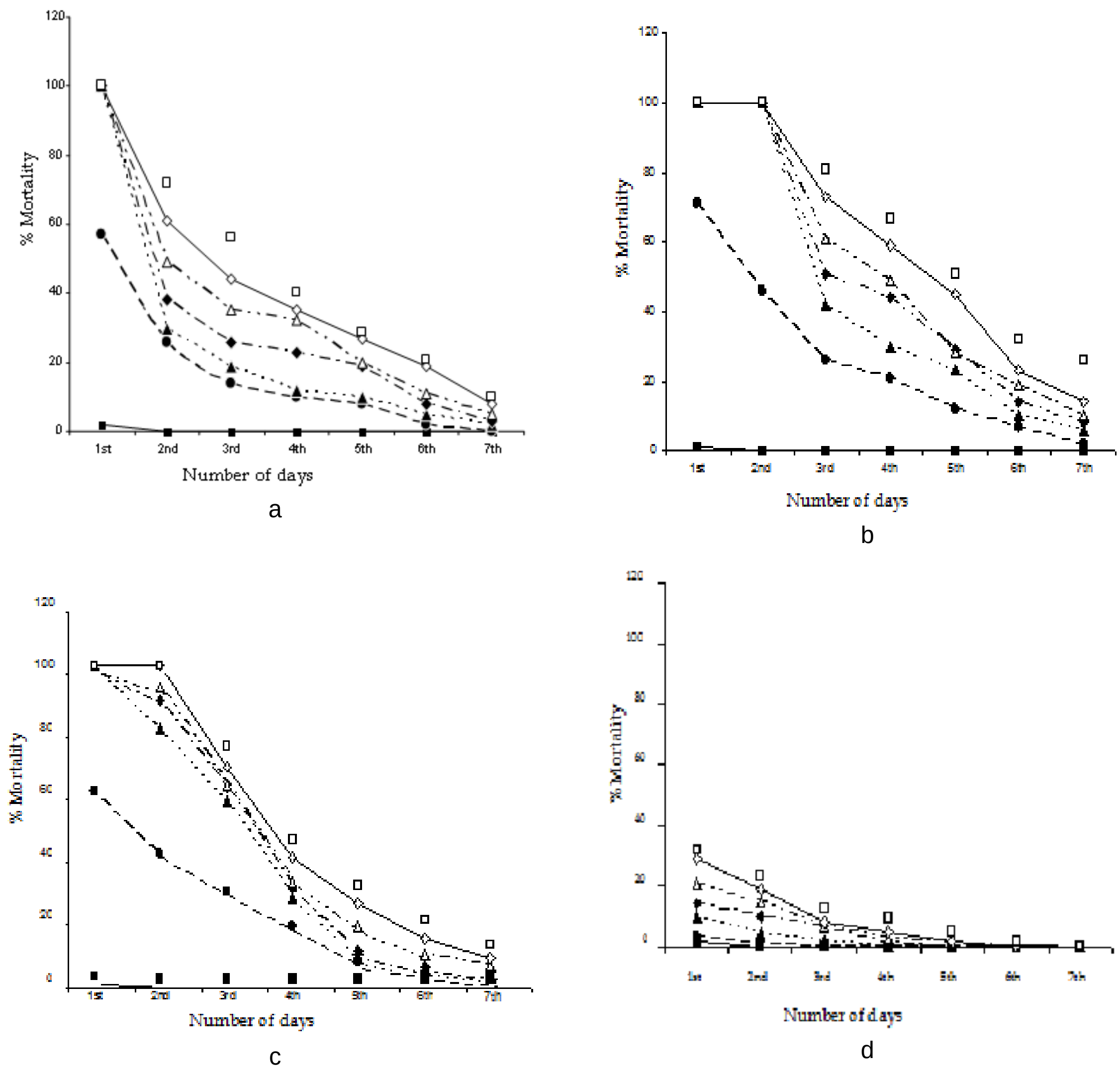


Figure 4. Residual effects of the hill toon, *Cedrela serrata* Royle extract against 5th instar of the red flour beetle, *Tribolium castaneum* (Herbst.): 4 different fractions of extract, a: methanolic extract (ME); b: n-butanol fraction (NBF); c: ethyl acetate fraction (EAF); d: aqueous fraction (AQF); when applied 5 different doses, ▲: 20.4; ♦: 25.5; ∆: 30.6; ◊: 35.7 and □: 40.8 in µl/cm^2; with ■: C_1 [untreated filter paper (control)]; ●: C_2: [treated with solvent (environmental factors control)]; during day 1 to 7; mortality was observed after each 24 h; %: percentage of 3 replications of 7 d consecutive experiments for each fraction and dose

dose 204.1 µl/cm^2 after 24 h of n-butanol fraction (NBF), LD_{50} was found 383.98 µl/cm^2 of aqueous fraction (AQF), during 1st day, the highest mortality was 100% at dose 40.8 µl/cm^2 and during 7th day, the highest mortality was 11% at maximum dose 204.1 µl/cm^2 of ethyl acetate fraction (EAF) of *C. serrata* methanolic leaves extract against *T. castaneum*, however, effects on fertility will be observed in future.

Despite the favorable characteristics of the use of botanical extracts few products have been commercially available lately (Forim et al., 2012). Azmi et al. (1998) showed contact toxicities of neem, *Azadirachta indica* L. extract RB-a and coopex using impregnated paper, the activity was found to be 34% with a 1257.1 µg/cm^2 dose of RB-a while 24, 28, 32 and 50% mortalities were observed by applying 1.1, 2. 5, 3.7 and 6.1 µg/cm^2 coopex (Bioallethrin: Permethrin) against rice weevil, *Sitophilus oryzae* (L), respectively. In the present research, it is reported that toxicity of methanolic extract of C. serrata using contact method, was found to be 10, 18, 35, 51 and 59% mortalities with 20.4, 51.0, 102.0, 153.1 and 204.1 µg/cm2 doses were observed against *T. castaneum*, respectively. The difference may be due to difference in method used or may be due to different plant and insect species used.Jbilou et al. (2006) reported significant insecticidal activity on *T. castaneum* larvae by crude methanol extract of harmal flower, Peganum harmala L; birthworts, Aristolochia baetica L, pipevines, Aristolochia iva L. and wild radish, Raphanus raphanistrum L having mortality count 58, 34, 31 and

26%, respectively, as extracts were mixed with the diet at concentration of 10%. In the present research, methanolic extract of C. serrata showed 59.3% mortality against 5th instar of T. castaneum by filter feeding mechanism. These variations may be due to different plant species used in two experiments or due to difference in procedure used.

Khanam et al. (2006) investigated the efficacy of sugarcane bagasse-based lignin against 4 stored grain insect pests, viz., T. castaneum, S. oryzae and cowpea weevil, Callosobruchus maculatus (F.) The susceptibility of the insect species was in the following order: T. castaneum > T. confusum > S. oryzae > C. maculatus at 24 h interval. The present studies revealed that methanolic extract and fractions of C. serrata showed toxicity against T. castaneum in the following order: NBF > ME > EAF > AQF. The difference in both studies is that they used sugarcane bagasse-based lignin against 4 insect pests, while in the present research work four extracts of C. serrata were used against one species, i.e., T. castaneum.Ogunleye and Adefemi (2007) tested dust and methanol extracts of bitter kola, Garcinia kolae Heckel on C. maculatus and maize weevil, Sitophilus zeamais (Motschulsky). They found the dust had no significant effect on the two insects while the methanol extracts had rapid lethal effects on both C. maculatus and S. zeamais. The mortality of C. maculatus by the lowest concentration of methanol extracts ranged from 95~100% whereas in S. zeamais, the mortality ranged from 87.5~100 and 70~100% in concentrations of 3 ml methanol was added to 1.0 g extract and 5 ml methanol was added to 1.0 g extract, respectively, from 24-48 h. The present results showed that the methanolic extract as well as all the fractions of C. serrata had rapid lethal effects on T. castaneum. The mortality of *T. castaneum* was ranged by the lower to higher concentration of methanolic extracts ranged from 10~59.3% whereas in other fractions, the mortality ranged from 73~99 (n-butanol), 35.3~79 (ethyl acetate) and 5.3~17% (aqueous fraction). The difference might be due to different plant and insect species used or due to difference in concentration of extract.

The results of residual effects showed that methanolic extract and ethyl acetate fraction of C. serrata have significant results during 1st d and showed 100% mortality while NBF showed very significant results as it showed 100% mortality during first two days and after that % mortality decreases during next 7 days as readings were taken after every 24 h. The least toxic fraction was aqueous as it did not show any significant % mortality even during 1st day. It means that n-butanol fraction is the most toxic one while aqueous fraction was least toxic. Daglish et al. (2003) assessed 5 organophosphorus compounds, 5 synthetic pyrethroids and 3 insect growth regulators (IGR) against *T. castaneum* in peanuts, *Arachis hypogaea* L. stored. Activity against adults and progeny was assessed separately. Of the insecticides tested, chlorpyrifos-methyl, methacrifos and deltamethrin applied completely prevented the development of progeny. In other studies, sublethal doses of chlorfluazuron were found to effect the size of ovaries, testes, viability, reproductivity etc against common cutworm, *Spodoptera litura* F. (Perveen, 2000a, 2000b,2006, 2008, 2009a, 2009b, 2009c, 2009d, 2010, 2011 a, b, c, d, e; Perveen and Miyata, 2000; Perveen et al., 2010a; Perveen et al., 2011). In the present research work, out of 4 fractions of *C. serrata* NBF gave high effects against 5th instars of *T. castaneum*. The 3 results are similar to some extent as each effects different aspects of insect pests, but differences lie as different extracts were applied, two synthetic while other plant origin.

Mondal and Khalequzzaman (2006) tested contact and fumigant toxicity of the three essential oils, viz., green cardamom, *Elettaria cardamomum* (L), toothache grass, *Ctenium aromaticum* (Walter) and cloves, *Syzygium aromaticum* (L) against *T. castaneum* larvae and adults. The results revealed that cardamom oil was generally a more effective contact poison and fumigant against the adults *of T. castaneum.* In the present study, different fractions of *C. serrata* were tested against *T. castaneum* and n-butanol fraction was found to be the most toxic than others. A direct comparison of the potency of contact toxicities of the essential oils could not be made because different experimental methods were employed.

Epidi and Odili (2009) tested the efficacy of powders of plant parts from white cedar, *Thuja occidentalis* L., ginger, *Zingiber officinale* Roscoe; large Christmas bell, *Blandfordia grandiflora*, Brown and angel's trumpet, *Brugmansia arborea* (L) using completely Randomized Design (CRD) against *T. castaneum* in groundnut andfound that *V. grandifolia* and *D. arborea* both can serve as protectants against *T. castaneum.* In present studies, 4 fractions of *C. serrata* were tested against *T. castaneum* and NBF was found to be most toxic than others. A direct comparison of the potency of contact toxicities of the essential oils could not be made (Leyva et al., 2012) because different experimental methods were employed. The plant origin substances are economic, less hazardous and specific; therefore, their use should be increased for controlling of insect pests.

CONCLUSION

The fractions of *C. serrata* leaves extracts showed significant toxicity and residual effects against *T. castaneum*. The most toxic fraction was found to be NBF showed the highest mortality 99% at the highest dose 204.1 μl/cm^2. The LD_{50} of fractions of *C. serrata* leaves extracts are in ascending order: AQF: 384.0 μl/cm^2 > ME: 143.0 μl/cm^2 > NBF: 69.8 μl/cm^2 > AQF: 17.9 μl/cm^2. The residual effects of the same fractions are in ascending order: at 1st d: ME ~ NBF ~ EAF > AQF; at 7th d: NBF > EAF > ME > AQF.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Salmam Akbar Malik, Head of Department, Quaid-i-Azam University, Islamabad and Dr Mukhtar Hasan and Sumaira Zaib, Departments of Biochemistry, Hazara University, Garden Campus, Mansehra-21300, Pakistan for providing chemicals and laboratory facilities throughout the present research work. They extend thank to Miss Gulnaz Bibi for her help throughout the work for providing all possible information and cooperation during the work. The experiments comply with the current laws of the country in which they were performed.

REFERENCES

Akhtar N, Jilani G, Mahmood R, Ashfaque M, Iqbal J (2004) Effects of plants derivatives on settling response and fecundity of peach fruit fly *Bactrocera zonata* (Saunders). Sarhad J. Agric. 20:269-274.

Arulprakash R, Veeravel R, Senthilkumar P (2005) Studies on the management of red flour beetle, *Tribolium castaneum* (Herbst.) in groundnut by using plant parts of milkweed plant *Calotropis gigantea* R. Madras Agric. J. 92(7-9):504-507.

Azmi MA, Naqvi SNH (1998) Comparative toxicological studies of RB a (neem extract) and coopex (permethrin + bioallethrin) against *Sitophilus oryzae* with reference to their effects on oxygen consumption and Got, Gpt activity. Turk. J. Zool. 22:307-310.

Banerji B. Nigam SK (1984) Wood constituents of saponins, Meliaceae: A review.Fitoterapia 55: 3-36.

Baris O, Gulluce M, Sahin F, Ozer H, Kilic H, Ozkan H, Sokmen M, Ozbek T (2006) Biological activities of the essential oil and methanol extract of *Achillea biebersteinii* Afan. (Asteraceae). Turk. J. Biol. 30:65-73.

Blaney WM, Simmonds MSJ, Ley SV, Jones PS (1988) Insect antifeedants: a behavioural and electrophysiological investigation of natural and synthetically derived clerodane diterpenoids. Entomologia Experimentalis et Applicata 46: 267–274.

Chiam WY, Huang Y, Chen SX, Ho HS (1999). Toxic and antifeedant effects of allyl disulfide in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus zeamais* (coleoptera: curculionidae). J. Econ. Entomol. 92:239-245.

Daglish GJ, Wallbank BE, Nayak MK (2003) Synergized bifenthrin plus chlorpyriphos-methyl for control of beetles and psocids (Psocoptra: Liposcelididae) in sorghum in Australia. J. Econ. Entomol.2:525-532

Duke SO (1990) Natural pesticides from plants. In: Advances in New Crops. (Janick J. and Simon J. E. Eds.) Timber Press, Oregon, Portland 511-517.

Duke SO, Dayan FR, Romaine JG, Rimando AM (2000) Natural products as sources of herbicides: status and future trends. Pak. J. Weed Sci. Rese. 40:99-111.

Epidi TT, Odili EO (2009) Biocidal activity of selected plant powders against *Tribolium castaneum* Herbst. in stored groundnut (*Arachis hypogaea* L.). Afri. J. Environ. Sci. Technol. 3(1):1-5.

Forim MR, Fátima DM, Fernandes G, Silva D, Fernandes JB (2012) Secondary metabolism as a measurement of efficacy of botanical Extracts: The use of *Azadirachta indica* (Neem) as a model. In: Insecticides-Advances in Integrated Pest Management. (Perveen, F. Ed.), InTech Book Publisher, Rijeka, Croatia 367-390.

Ge XS, Weston PA (1995). Ovipositional and feeding deterrent from Chinese prickly ash against angoumois grain moth (Lepidoptera: Gelechiidae). J. Econ. Entomol. 88: 1771–1775.

Hashim MS, Devi KS (2003). Insecticidal action of the polyphenolic rich fractions from the stem bark of *Streblus asper* on *Dysdercus cingulalus*. Fitoterapia 74(7-8): 670-676.

Herbst (1797) online: http://www.hear.org/starr/images/species/?q= cedrela+serrata&o=plants&s=-date (Retrieved: 11/7/2010).

Huang Y, Chen SX, Ho SH (2000). Bioactivities of methyl and allyl disulfide diallyl tisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). J. Econ. Entomol. 93:537-543.

Ishaaya I, Degheele D (1998). Insecticides with Novel Modes of Action: Mechanisms and Application. Narosa Publishing House, New Delhi, India 289

Jilani G, Saxena RC (1990). Repellent and feeding deterrent effects of turmeric oil, sweet flag oil, neem oil and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). J. Econ. Entomol. 83: 629-634.

Jilani G, Ullah N, Ghiasuddin A, Khan MI (1993). Repellency of some plant extracts against *Tribolium castaneum* (Herbst) (Coloeptera: Tenebrionidae)-V. Pak. J. Entomol. Karachi 15:103-105.

Khanam LAM, Talukder D, Hye MA (2006). Toxic and repellent action of sugarcane bagasse-based lignin against some stored grain insect pests. University Journal of Zoolog, Rajshahi University, Rajshahi, India Rajshahi 25:27-30.

Khattak SUM, Shafique M (1986) Varietal susceptibility studies of ten heat cultivars flour to red flour beetle *Tribolium castaneum*. Pak. J. Zool. 18(3):257-261.

Leyva M, Tiomno O, Tacoronte JE, Marquetti MDC, Montada D (2012). Essential plant oils and insecticidal activity in *Culex quinquefasciatus*. In: Insecticides-Pests Engineering. (Perveen, F. Ed.), InTech Book Publisher, Rijeka, Croatia 221-238.

Lohar MK, Hussainy SW, Juno GM, Lanjar AG, Shah AA (1997). Estimation of quantitative losses of wheat, rice and maize caused by *Tribolium castaneum* (Herbst.) under laboratory condtions. Pak. J. Entomol. Karachi 19:32-35.

Mancebo F, Hilje L, Mora GA, Salazar R (2000). Antifeedant activity of *Quassia amara* (Simaroubaceae) extracts on *Hypsipyla grandella* (Lepidoptera: Pyralidae) larvae. Crop Protection 19:301–305.

Meena R, Suhag P, Prates HT (2006). Evaluation of ethanolic extract of *Baccharis genistelloides* against stored grain pests. J. Stored Produ. Res. 34(4):243-249.

Mondal M, Khalequzzaman M (2006). Toxicity of essential oils against Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Biosci. 14: 43-48.

Mordue AJ, Simmonds MSJ, Ley SV, Blaney WMW, Mordue MAJ, Nasiruddin N (1998). Actions of azadirachtin, a plant allelochemical, against insects. Pesticide Science 54:277–284.

Naqvi SNH, Perveen F (1991) Toxicity and residual effect of *Nerium indicum* crude extract as compared with coopex against adults of *Tribolium castaneum* (Coleoptera : Tenibrionidae). Pak. J. Entomol. Karachi 6(1-2):35-44.

Naqvi SNH, Perveen F (1993). Toxicity of some plant extracts in comparison to coopex (Bioallethrin : Permethrin) against stored grain pest (*Callosobrucus analis*) (Coleoptera : Bruchidae). Pak. J. Entomol. Karachi 8(1):5–15.

Ndungu M, Lawndale W, Hassanali A, Moreka L, Chhabra SC (1995) *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellents. Entomologia Experimentalis et Applicata 76: 217-222.

Ogunleye RF, Adefemi SO (2007). Evaluation of the dust and methanol extracts of *Garcinia kolae* for the control of *Callosobruchus maculatus* (F.) and *Sitophilus zeamais* (Mots). J. Zhejiang Univ. Sci. Biol. 8(12):912–916.

Papachristos DP, Stamopoulos DC (2002). Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscescelids obtectus* (Say) (Coleoptera: Bruchidae). J. Stored Products Res. 38: 117-128.

Papachristos DP, Stamopoulos DC (2003). Selection of *Acanthoscescelids obtectus* (Say) for resistance to lavender essential oil vapour. J. Stored Prod. Res. 39:433-441.

Perveen F (2000a). Effects of sublethal dose of chlorfluazuron on testicular development and spermatogenesis in the common cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Physiological Entomology 25:315–323.

Perveen F (2000b). Sublethal effects of chlorfluazuron on reproductivity and viability of *Spodoptera litura* (F.) (Lep., Noctuidae). J. Appl. Entomol. 124:223–231.

Perveen F (2006). Reduction in egg hatch after sublethal dose of chlorfluazuron to larvae of the common cutworm, *Spodoptera litura*. Physiological Entomology 31:39–45.

Perveen F (2008) Effects of sublethal doses of chlorfluazuron on the number of inseminated sperm in *Spodoptera litura* (Lepidoptera: Nocutidae). Entomological Science 11 (1): 111–121.

Perveen F (2009a). Biochemical analyses of action of chlorfluazuron in *Spodoptera litura*. VDM Verlag House Ltd., Germany 1–144.

Perveen F (2009b). Chlorfluazuron and embryonic development of *Spodoptera litura*. VDM Verlag House Ltd., Germany 1–115.

Perveen F (2009c). Effects of chlorfluazuron on the reproduction of *Spodoptera litura.* VDM Verlag House Ltd., Germany 1–184.

Perveen F (2009d). Effects of residual chlorfluazuron on haemolymph-borne oviposition-stimulating factors in *Spodoptera litura* (Lepidoptera: Noctuidae). Entomologia Experimentalis et Applicata 132: 241–249.

Perveen F (2010) Effects of sublethal doses of chlorfluazuron on embryogenesis in *Spodoptera litura.* Journal of Agricultural Science and Technology USA 5(2:33): 127–138.

Perveen F (2011a). Effects of chlorfluazuron sublethal doses on spermatogenesis of adult common cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). J. Agric. Sci. Technol. USA 5:933-941.

Perveen F (2011b). Effects of sublethal doses of chlorfluazuron on the ovarian biochemical constituents of *Spodoptera litura*. Afr. J. Biotechnol. 1 0(62): 13598-13604.

Perveen F (2011c). Effects of sublethal doses of chlorfluazuron on the testicular biochemical constituents of *Spodoptera litura* (Lepidoptera: Noctuidae). Afr. J. Biotechnol. 10(44): 8956-8964.

Perveen F (2011d). Effects of sublethal doses of chlorfluazuron on ovarioles in the common cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). J. Life Sci., USA 5: 609-613.

Perveen F (2011e). Effects of sublethal doses of chlorfluazuron on the biochemical constituents of the eggs of *Spodoptera litura* (Lepidoptera: Noctuidae). The Canadian Entomologist 143(2): 178-184.

Perveen F, Hussain Z (2012). Use of statistical techniques in analysis of biological data. Basic Res. J. Agric. Sci. Rev. 1(1):01-10

Perveen F, Khan A (2012). Effect of the yellow-berried nightshade against the red flour beetle. Lambert Academic Publisher (LAP), Germany 1–58

Perveen F, Miyata T (2000). Effects of sublethal dose of chlorfluazuron on ovarian development and oogenesis in the common cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Annals of the Entomological Society of America 93(5):1131–1137

Perveen F, Shah M (2012). Reduction in fertility by *Nerium indicum* leaves extract in adults of red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) compared with Coopex (Bioallethrin: Permethrin). J. Agric. Sci. Technol. USA 155-160.

Perveen F, Shah M (2013). Nest architectural patterns by three wasp species (*Vespa velutina*, *Polistes flavus* and *Sceliphron formosum*) with reference to their behavior. Int. J. Insect Sci. 51-58.

Perveen F, Ahmed H, Abbasi FM, Yasmin N, Gul A (2010a) Characterization of embryonic stages through variations in the egg's contents in *Spodoptera litura.* J. Agric. Sci. Technol. USA 4 (5:30): 24–36.

Perveen F, Mehmood SA, Yasmin S (2011) Effects of sublethal doses of chlorfluazuron on pupal-testis of the common cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). J. Agric. Sci.Technol. USA A 1:890-896.

Perveen F, Naqvi SNH, Yasmin N, Mehmood T (2010b) Toxicity and residual effect of yellow-berried nightshade, *Solanum surrattense* leaves extract against red flour beetle, *Tribolium castaneum*. Pak. J. Entomol., Karachi 25(2): 2–29.

Perveen F, Zaib S, Irshad S, Hassan M (2012c). Antioxidant and DNA protection activities of the hill toon, *Cedrela serrata* (Royle) leaves extract and its fractions. J. Nat. Products 5:207- 213.

Perveen F, Khan A, Zaib S (2013). Repellency of the hill toon, Cedrela serrata against the red flour beetle, Tribolium castaneum. Int. J. Entomol. Res. 01(01): 01-10.

Perveen F, Zaib S (2013). Phytochemical and spectrophotometric analyses of the hill toon, *Cedrela serrata* Royle methanolic leaves extract and its fractions**.** Inte. J. Agric. Innovat. Res. 1(5):140-145.

Raja N, Albert S, Ignacimuthu S, Dorn S (2001). Effects of plant volatile oils in
stored cow pea *Vigna unguiculata* L. (Walpers) against *Callosobruchus maculatus* (F.) (Coleopteran: Bruchidae) infestation. J. Stored Products Res. 37:127-132.

Riebeiro BM, Guedes RNC, Oliveira EE, Santos JP (2003). Insecticide resistance and synergism in Brasilian populations of *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Stored Products Res. 39: 21-31.

Shaaya E, Kostyukovsky M (2006) Essential oils: Potency against stored product insects and mode of action. Stewart Postharvest Review 2(4): 34-40. Online: http://www.stewartpostharv est.com/vol2_2006/August_2006/Shaaya. Pdf. (Retrieved: 2/8/2006).

Siddiqui AR, Jilani G, Rehman JU, Kanvil S (2006). Effect of turmeric extracts on settling response and fecundity of peach fruit fly (Diptera: Tephritidae). Pak. J. Zool. 38:131-135.

Villalobos PJM (1996). Evaluation of the insecticidal activity of *Chrysanthemum coronarium* L. plant extracts. Boletin de Sanidad Vegetal Plagas 22:411-420.

Wakil W, Hassan M, Javed A, Anwar S (2003) Comparison of nutritional losses of insects infested wheat in laboratory and public storages. Pak. J. Arid Agric. 6:1-6.