Efficacy of *Chrozophora plicata* and *Trianthema portuclacastrum* weed plant extracts against *Trogoderma granarium* Everts under laboratory conditions

Kishwar Sultana[1], Muhammad Kashif Zahoor[1]*, Muhammad Sagheer[2] and Farhat[3]
[1]Entomology Lab, Department of Zoology, Government College University, Faisalabad, Pakistan
[2]Grain Research, Training and Storage Management Cell, Department of Entomology,
University of Agriculture, Faisalabad, Pakistan
[3]Department of Zoology, Government College University, Faisalabad, Pakistan

Abstract: The efficacy of *Chrozophora plicata* and *Trianthema portuclacastrum* extracts was investigated against *Trogoderma granarium* at 10%, 20% and 30% concentrations and 2, 4 and 6 days of exposure periods. It was found that *T. portuclacastrum* extract caused significantly higher larval mortality (37.47%) than *C. plicata* (27.03%). Maximum number of *T. granarium* larvae (91.11% and 82.22%) was repelled when exposed to 30% concentration. A significant reduction in percentage larval emergence was also found in F1 generation. A decrease in the activity of Acetylcholine Esterase (AChE), Acid Phosphatase (ACP), Alkaline Phosphatase (AKP), α-Carboxyl and β-Carboxyl was also found. The FTIR analysis showed the presence of polyphenolic compounds in *T. portuclacastrum* extract. The overall results revealed that *T. portuclacastrum* extract was very effective against *T. granarium* than *C. plicata*.

Keywords: Weed plants, extracts, *Chrozophora plicata*, *Trianthema portuclacastrum* and *Trogoderma granarium.*

INTRODUCTION

Stored grain insect pests are the major problem which deteriorates the quantity as well as quality of stored grain (Madrid *et al*., 1990). The storage losses are extremely high in both developing and developed countries (Sagheer *et al*., 2013; Hasan *et al*., 2014; Kulkarni *et al*., 2015; Forghani *et* al., 2015; EPPO, 2015). About 10% of world stored grains are lost, i.e. 13 million tons of grain by insect attack or 100 million tons due to improper storage (Hasan *et al*., 2014).

The khapra beetle, *Trogoderma granarium* Everts is considered as a serious pest of stored grains particularly of wheat grains throughout the world (Moreira *et al*., 2007; Kulkarni *et* al., 2015; Forghani *et al*., 2015; EPPO, 2015). Due to high infestation potential of *T. granarium*, it is also a major threat in Pakistan (Ahmad *et al*., 2011; Ahmad, 2013; Sagheer *et al*., 2013, 2013a; Hasan *et al*., 2014). The development of *T. granarium* depends on physical conditions such as temperature, light, moisture. High humidity has a depressing effect on population buildup. It has one to nine or more generations per year (Ramzan and Chahal, 1986). At favorable temperatures; eggs, pupae and adults, each take about a week for development while the larval stage may survive a month to several years under diapause condition (Burges, 1962; Peacock, 1993).

Mostly, synthetic insecticides are used for the control of stored product pests (Arthur, 1996; Forghani and Marouf, 2015; EPPO, 2015). Besides their effectiveness; there are major health and environmental concerns due to insecticides (Benhalima *et al*., 2004). Hence, effective biodegradable and non toxic control measures are needed (Alves *et al*., 2014; Rafiee-Dastjerdi *et al*., 2014; Asma *et al*., 2015).

Several natural substances from plant origin are being used as an alternative to pesticides against many insects (Moreira *et al*., 2007; Satti and Elmin, 2012; Talukder, 2006; Montenegro *et al*., 2013; Alves *et al*., 2014; Rafiee-Dastjerdi *et al*., 2014; Mahmoud *et al*., 2014 & 2015; Asma *et al*., 2015). These plant-derived products are not involved in the inhibition of photosynthesis, growth or other process of plant physiology; however, their biological activity against insect pests is widely reported (Subramanyam and Roesli, 2000; Moreira *et al*., 2007; Sagheer *et al*., 2013; Hasan *et al*., 2014). Moreover, the insecticidal, repellent and anti-feedant effect of plant products have also been well documented so far (Huang *et al*., 1998; Sagheer *et al*., 2013; Hasan *et al*., 2014). Recently, the plant extracts are gaining tremendous importance to protect the stored grain commodities (Sultana *et al*., 2016; Pugazhvendan and Elumalai, 2012). Sometimes, biochemical changes have also been reported due to the use of different plant extracts. The activity of various enzymes such as Acetylcholine esterase (AChE), Acid phosphatase (ACP) and Alkaline phosphatase (ALP) are decreased by plant extracts. Lipid, glucose and protein contents are also affected due to a change in the corresponding metabolism (Younes *et al*., 2011).

**Corresponding author:* e-mail: kashif.zahoor@gcuf.edu.pk

Weeds are unwanted and undesirable plants commonly found in and around the crop lands (Samad *et al*., 2008; Hegab *et al*., 2008). One of the most common genus, *Chrozophora* (Euphorbiaceae) comprises of 11 species, which are mostly shrubs and distributed in Pakistan, India, West Africa and Mediterranean regions. *Chrozophora plicata* is a monoecious, annual or perennial herb, up to 50cm; and grows in warmer climate and temperate regions throughout tropical Africa to Northern South Africa, Egypt, Syria, Palestine, and North-Western India to the Mediterranean (Forster and Welzem, 1999; Chopra, 1988). This weed plant is medicinally used in Saudi Arabia, Pakistan and India for the purification of blood and to cure gastro, jaundice and ulcer diseases (Kadiri *et al*., 2013; Kadiri and Avanapu, 2013). The leaves of *C. plicata* contain triterpenoids and related compounds such as sterols, alcohols and hydrocarbons and phenolic compounds like flavonoids, lignans, coumarins, tannins, phenanthrenes, quinines and phenolic acids (Kadiri *et al*., 2013). *Trianthema portulacastrum* Linn. (Family: Aizoacae) is commonly known as black pigweed or It-Sit. It is an annual herb commonly found in moist soil and spreads on the ground in circle not more than 4-6 ft. in length. Its leaves are more commonly used as therapeutic agent for diverse pathological conditions, viz. Mudirre Baul (diuretic), Mudirrehaiz (emmenagogue) jali (detergent), muqawwiebaah (aphrodisiac), musakkhin (calorific), used in colitis, jaundice and ascites. Its juice is used in corneal ulcer, night blindness and dribbling of urine (Ghani, 2010; Kirtikar and Basu, 2003).

Since there are no reports on the evaluation of insecticidal activities of weed extracts against stored grain insect pests, the present study was planned to exploit the insecticidal potential of weed plants named *Trianthema portulacastrum* Linn and *Chrozophora plicata*, for the control of *T. granarium.*

MATERIALS AND METHODS

The bioassay was performed in Entomology Lab, Government College University Faisalabad to investigate the insecticidal effects of the selected weed plants against larvae of *Trogoderma granarium* E.

Mass rearing of experimental beetles

The khapra beetles were reared on healthy food commodities apparently free from insect infestation, kept in the sterilized plastic jars (1.0kg capacity), under optimum conditions of temperature and relative humidity (30±2ºC and 65±5%, respectively). Common wheat (*Triticum aestivum*, var. Nela), was utilized as culture media for *Trogoderma granarium*. Adults were sieved out and hundred beetles were released in properly labeled 500ml glass jars having 200gm of sterilized food commodities with 14% moisture contents and covered with muslin cloth to avoid interbreedingof the strains. Adults were allowed to mate and lay eggs (kept under the uniform experimental conditions inside the incubator). After 5 days, adults were sieved out in order to obtain homogenous larval population. The 3rd instar larvae of *T. granarium* were used in the experiments (Sultana *et al*., 2016; Sagheer *et al*., 2013, 2013a; Hasan *et al*., 2014; Rafiee-Dastjerdi *et al*., 2014).

Preparation of plant extracts

Chrozophora plicata and *Trianthema portulacastrum* Linn were collected from the vicinity of Faisalabad and identified by the Department of Botany, Government College University Faisalabad. The whole weed plants were cleaned by washing in water and dried under the shade to get the desired dried form. Grinder was used to crush the plant material to fine powder. The extraction was made by mixing 100g of ground sieved sample and 300 ml of petroleum ether (40-60%) in 1:3 ratio (w/v) and shaking was ensured for 24hours with the help of Rotary Shaker, adjusted at 220 revolutions per minutes (rpm). After 24hours, filtration was made with the help of Whatman filter paper. After filtration, the obtained extracts were put into clean and air tight lid bottles and were stored in refrigerator before use. Different concentrations viz.10.0%, 20.0% and 30.0% were prepared using petroleum ether as solvent from the stock solution of each plant (Sultana *et al*., 2016; Sagheer *et al*., 2013, 2013a; Hasan *et al*., 2014).

Mortality bioassay (Diet incorporation method)

The bioassay was carried out to observe the toxic effect of weed extracts on the larvae of *Trogoderma granarium*. Three different concentrations (10, 20 and 30%) of botanical extracts in petroleum ether were applied on 50gm of wheat. For control group, the food commodity was only treated with petroleum ether which was then air dried to evaporate the petroleum ether and then poured in 250ml sterilized plastic jars. Thirty larvae were released in each jar and were covered with muslin cloth with the help of rubber band. Then, these jars were placed in incubator under optimum conditions (30±2ºC and 65±5%, RH). Experiment was replicated three times and completely randomized design was followed (Sultana *et al*., 2016; Sagheer *et al*., 2013, 2013a; Hasan *et al*., 2014; Rafiee-Dastjerdi *et al*., 2014). The insects were confirmed dead when there was no response to probing with sharp pin at the abdomen. Data regarding percentage larval mortality was recorded after 2, 4 and 6 days of treatment. Mortality in control groups was also recorded to correct the mortality according to Abbot's formula (Abbot, 1925).

Repellency bioassay

The repellent effect of the weed extracts was checked against the test beetles by using a modified methodology of area preference as described by McDonald et al.,

(1970). For this purpose, eight centimeter diameter Whatman No. 1 filter paper was cut into two equal halves. Different dilutions (10%, 20% and 30%) of weed extracts were separately applied on one half of the filter paper with the help of micropipette; the other half of filter paper was treated with solvent alone. After air drying for 10 minutes, each treated half of the filter paper was attached lengthwise to untreated half with the help of adhesive tape and was adjusted in the Petri dishes. Twenty larvae of *T. granarium* were released separately at the center of both halves in each petri dish. Petri dishes were covered with lid to prevent the escape of test insects and kept under controlled experimental conditions. Each treatment was replicated thrice and the repellency data was taken after a period of 24, 48 and 72hours. A little diet was provided on both sides to decrease the mortality due to starvation (Sultana *et al*., 2016; Sagheer *et al*., 2013, 2013a; Hasan *et al*., 2014).

Percent repellency (RP) were calculated by using the following formula:

$$PR = \left[\frac{(NC - NT)}{(NC + NT)}\right] \times 100$$

Where NC= number of beetles present on control half, NT= number of beetles present on treated half.

Growth regulatory impact of plant extracts on the larvae of Trogoderma granarium

In order to investigate the growth regulatory impact on F1 generation, wheat grains were sterilized and various concentrations of plant extracts were applied on the wheat grains including a control treatment. Solvent was allowed to evaporate and 50g wheat grains were put into each treatment jar. Thirty larvae of 3rd instars were released into each treatment jar. The treatment jars were kept in incubator under optimum conditions. Data regarding growth regulation was collected after 35 days (Sultana *et al*., 2016; Sagheer *et al*., 2013).

Enzyme assay

Preparation of whole body homogenate

For enzymatic estimation, larvae and adults of *T. castaneum* remained alive in mortality and growth regulatory assays were washed thoroughly with distilled water and the adhering water was removed by using the bloating paper. The insects were separately homogenized in eppendrof tubes in 1.5ml ice-cold sodium phosphate buffer (20mM. pH7.0) with the help of Teflon hand homogenizer. Then, the homogenate was centrifuged at 8000×g and 4°C for 20minutes and supernatant was used for the estimation of Esterases or Phosphatases. Solutions and glassware used for homogenization were kept at 4ºC prior to use, and the homogenates were held on ice until used for various assays (Younes *et al*., 2011).

Quantitative determination of esterases and phosphatases

Estimation of Acetylcholinesterase activity

In the 50µl of enzyme solution, 50µl of acetylcholine chloride (2.6mM) as a substrate and 1ml of sodium phosphate buffer (20mM, pH7.0) were added. It was incubated at 25ºC for 5mins. Then 400µl of 0.3% Fast blue B salt was added to stop reaction. Blank and sample were run through spectrophotometer. Optical density (OD) was recorded at 405nm (Younes *et al*., 2011)

Estimation of Carboxylesterase activity

The activity of α-carboxylesterase and β-carboxylesterase in the larvae and adult was measured as devised by Van Asperen (1962). In 50µl enzyme solution (homogenates), 1ml of sodium phosphate buffer (20mM, pH7.0) and 50µl of each α-naphthyl acetate and β-naphthyl acetate (substrate) were added separately to determine the activities of α-carboxylesterase and β-carboxylesterase respectively. The solutions were incubated at 30ºC for 20mins. After incubation 400µl of freshly prepared 0.3% Fast blue B in 3.3% SDS was added in each reaction mixture to stop the enzymatic reaction and the color was allowed to develop for 15min at 20◦C. Blank and sample were run on spectrophotometer. Optical density (OD) was recorded at 430 and 590nm for α-carboxylesterase and β-carboxylesterase, respectively.

Estimation of acid and alkaline phosphatase activity

The levels of these two phosphatases in the beetle homogenates was measured following the procedure of Asakura (1978). The acid phosphatase activity was estimated by mixing 50µl larval or adult homogenate with 50µl sodium phosphate buffer (50mM, pH7.0) and 100µl of 20mM p-nitrophenyl phosphate (substrate). For the estimation of alkaline phosphatase activity, 50µl larval or adult homogenate were mixed with 50µl Tris HCl buffer (50mM, pH9.0) and 100µl of 20mM p-nitrophenyl phosphate (substrate). After that both solution of acid phosphatase and alkaline phosphatase were incubated at 37ºC for 15mins in water bath, the enzymatic reaction was stop by adding 0.5N NaOH solution. The absorbance (OD) of the resulting clear supernatants of sample and blank was recorded at 440nm.

The percentage inhibition of the enzyme activity by the test extracts was calculated as follows:

$$\%\ \text{Enzyme inhibition} = \frac{\text{OD of Control - OD of treated}}{\text{OD of Control}} \times 100$$

Where, OD of Control is the absorbance of untreated beetles; OD of Sample is the absorbance of treated *Trogoderma* beetle.

Fourier transform infrared spectroscopy (FTIR) analysis

The *T. portuclacastrum* extracts was examined by FTIR spectroscopy for the detection of the characteristic

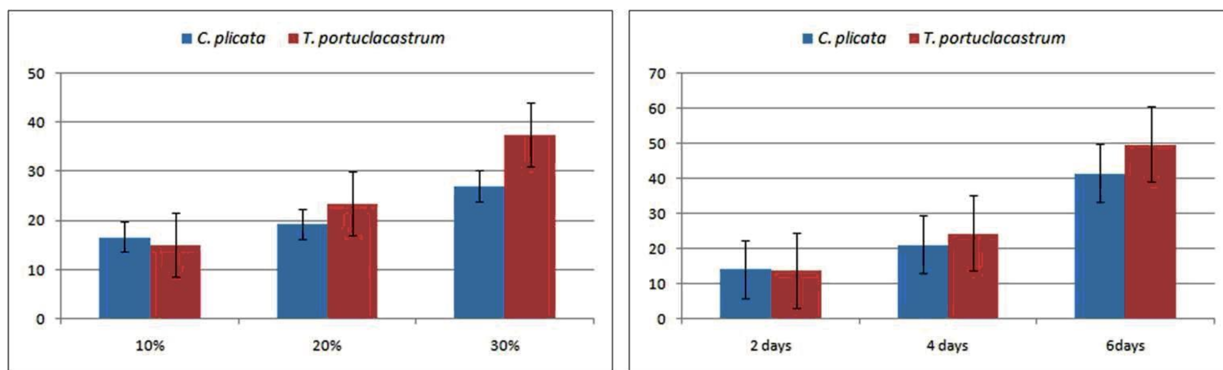

Fig. 1: Comparison of percent larval mortality in *T. granarium* exposed to different concentrations and exposure time of *Chrozophora plicata* and *Trianthema portuclacastrum*

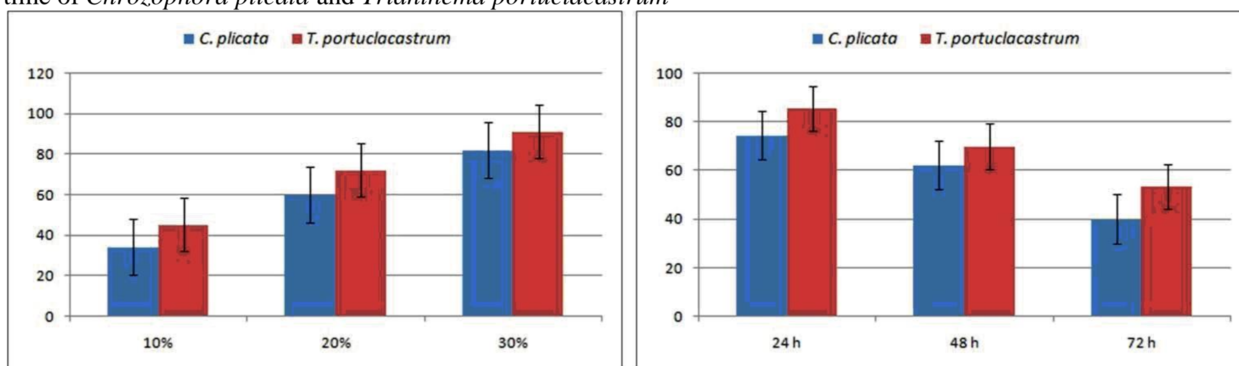

Fig. 2: Comparison of percent larval repellency in *T. granarium* exposed to different concentrations and exposure time of *Chrozophora plicata* and *Trianthema portuclacastrum*

functional groups. The extracts prepared in n-Hexane were frozen at -80°C followed by lyophilization. Infrared absorption spectrum of the lyophilized extract was recorded on a FTIR spectrophotometer (Alpha, Bruker, California, USA) in the region 4000 to 500 cm^{-1} (Bina *et al*., 2003; Kassim *et al*., 2011)

STATISTICAL ANALYSIS

Mortality data obtained at various time intervals was corrected by using Abbot's formulae. The data of corrected mortality and repellency was subjected to ANOVA using Statistics 13.0 for Windows. The means were separated using Tuckey's HSD test with $P< 0.05$ considered statistically significant (Pandir & Bas, 2016; Sultana *et al*., 2016; Sagheer *et al*., 2013).

RESULTS

Mortality of Trogoderma granarium larvae

The mortality data for the insecticidal activity of *C. plicata* and *T. portuclacastrum* against *T. granarium* larvae was observed at different concentrations and exposure period. The comparison of mean mortality data in *T. granarium* larvae induced by various concentrations and duration of exposure of *C. plicata* and *T. portuclacastrum* extracts is shown in table 1. It is shown that *C. plicata* induced maximum mean mortality (27.03%) in *T. granarium* larvae at 30% concentration which significantly differed from the minimum mortality found at 10% concentration (16.67%). In case of *T. portuclacastrum*, maximum mortality (37.47%) was found at 30% extract concentration while 10% concentration resulted in minimum mortality (15.12%).

The mortality at 20% concentration, intermediate values were obtained (19.37 & 23.54%). The results indicated that the larval mortality increased with the increase in extract concentration.

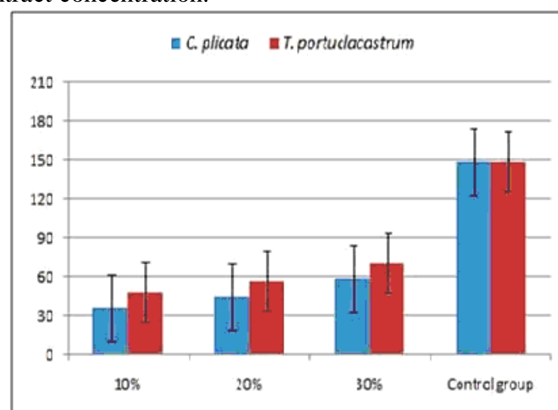

Fig. 3: Comparison of percent progeny reduction in F1generation of *T. granarium* exposed to different concentrations of *Chrozophora plicata* and *Trianthema portuclacastrum.*

Table 2 shows that exposure time had a significant effect on the mortality of *T. granarium* larvae exposed to 30% concentration of *C. plicata* and *T. portuclacastrum* extracts. The highest mortality (41.57%) was found after 6 days while the lowest mortality (3.33%) was recorded after 2 days with *C. plicata*. Similarly, highest mortality was observed with *T. portuclacastrum* extract after 6 days (49.80%), whereas lowest mortality was found after 2 days (8.15%). The results indicated that the larval mortality increased with increasing exposure time (table 2). The overall mortality effect of both tested weed plants indicated that *T. portuclacastrum* performed better than *C. plicata* due to high mortality in *T. granarium* (fig. 1).

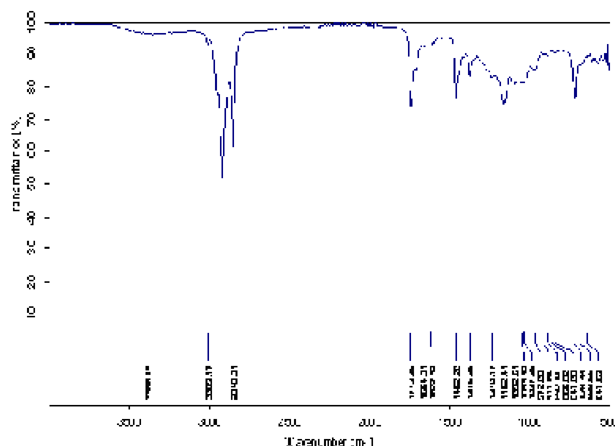

Fig. 4: FTIR spectrum of *Trianthema portulacastrum* The spectrum shows a range of 4000 to 400 cm^{-1}wave number (along X-axis) the function of % transmittance (along Y axis). Following peaks can be observed: 1622.32 cm^{-1}-C=O (carbonyl) group, 1092.61 cm^{-1}-C-O linkage in -C-OH, 2848.81 cm^{-1} and 3009.17 cm^{-1} C-H stretching.

Repellency in Trogoderma granarium

The repellency of *C. plicata* and *T. portuclacastrum* in *T. granarium* larvae at various concentrations and exposure period is shown in table 3. *Chrozophora plicata* showed higher repellency (82.22%) at 30% than 20% and 10% concentrations (60.00% and 34.44%, respectively). In case of *T. portuclacastrum* extract, maximum repellency was observed (91.11%) at 30% concentration whereas 20% and 10% concentrations gave 72.22% and 45.56% repellency, respectively. The statistical analysis showed that 30% extract resulted in higher percentage of larval repellency and the results also indicated that percent larval repellency increased with the increase in concentration (table 3)

The repellency effect of *C. plicata* and *T. portuclacastrum* in *T. granarium* larvae at various intervals at 30% concentration is shown in table 4. The higher repellency was observed with *C. plicata* after 24h exposure (74.44%) followed by 62.22% and 40.00% after 48 and 72h, respectively. *T. portuclacastrum* caused highest larval repellency (85.56%) after 24h followed by 70.00% and 53.33% after 48 and 72h, respectively. The results indicated that the repellent activity of *C.plicata* and *T. portuclacastrum* extracts decreased with the increasing exposure period (table 4). The overall comparison of repellent effect of *C. plicata* and *T. portuclacastrum* is shown in fig. 2 which indicates that *T. portuclacastrum* had more repellent effect than *C. plicata*.

Effect of extracts of Chrozophora plicata and Trianthema portuclacastrum on progeny of Trogoderma granarium

The mean larval emergence and inhibition of *T. granarium* at various concentrations of *C. plicata* and *T. portuclacastrum* were observed after 35 days of infestation period (table 5). It was found that lower number of larvae emerged in F1 generation compared to the control group. The lowest mean number of larvae (43.67) and highest larval inhibition (70.63%) in F1 generation was observed at 30% concentration of *T. portuclacastrum* followed by *C. plicata* (61.67) inhibiting the F1 progeny by 58.52%. Moreover, 77.33 and 109.67 larvae with 10% *C. plicata* and *T. portuclacastrum* were emerged and induced 47.98% and 26.24% larval inhibition, respectively. The highest number of larvae (148.67) in F1 generation was obtained when the grains were left untreated for 35 days of experimental period. Thus, it is clear that the progeny inhibition effect of *C. plicata* and *T. portuclacastrum* decreased with the increase in applied concentration.

Effect of extracts of Chrozophora plicata and Trianthema portuclacastrum on the enzymatic activity in Trogoderma granarium

The effect of *C. plicata* and *T. portuclacastrum* extracts on the enzymatic activity in *T. granarium* was observed at various concentrations and exposure period (Table 6). The maximum decrease was observed at 30% concentration of both extracts. *C. plicata* induced a decrease in acetylcholine esterase, ACP, α-Carboxyl and β-Carboxy enzymes by 27.72%, 55.02%, 44.70% and 52.35, respectively with 30% concentration. In addition, a decrease in the activity of acetylcholine esterase (29.01%), ACP (59.88%), α-Carboxyl (53.13%) and β-Carboxyl (56.28%) was found with 30% concentration of *T. portuclacastrum* extract. A maximum decrease in the activity of AkP (51.01%) was recorded at 30% concentration of *C. plicata* as shown in table 6.

The effect of *T. portuclacastrum* and *C. plicata* extracts on the enzymatic activity in *T. granarium* larvae with respect to time duration is shown in table 7. It was found that *T. portuclacastrum* induced maximum reduction in the activity of AChE (20.62%, 25.48%), ACP (43.76%, 2.02%), α-Carboxyl (40.86%, 50.35%) and β-Carboxyl (42.35%, 44.31%) except AkP (36.93%, 39.84%) which was highly affected by *C. plicata* at both recorded exposed durations (table 7). It is clear from the data that

Table 1: Mean percent larval mortality in *T. granarium* exposed to different concentrations of *C. plicata* and *T. portuclacastrum* extracts

No.	Plant name	Concentrations (%)		
		10%	20%	30%
1	*Chrozophora plicata* (F=4.33; d.f =2; P<0.05)	16.67 ± 5.22 a	19.37 ± 6.05 ab	27.03 ± 6.73 b
2	*Trianthema portuclacastrum* (F=31.58; d.f =2; P<0.05)	15.12 ± 4.76 a	23.54 ± 5.76 b	37.47 ± 9.12 c

Table 2: Mean percent larval mortality in *T. granarium* exposed to 30% concentration of *C. plicata* and *T. portuclacastrum* for different exposure intervals

No.	Plant name	Exposure intervals (Days)		
		2 days	4 days	6 days
1	*Chrozophora plicata* (F=55.77; d.f =2; P<0.05)	3.33 ± 1.47 a	18.18 ± 1.80 b	41.57 ± 4.25
2	*Trianthema portuclacastrum* (F=117.09; d.f =2; P<0.05)	8.15 ± 1.85 a	18.18 ± 3.55 b	49.80 ± 6.05

Table 3: Mean percent larval repellency in *T. granarium* exposed to different concentrations of *C. plicata* and *T. portuclacastrum* extracts

No.	Plant name	Concentrations (%)		
		10%	20%	30%
1	*Chrozophora plicata* (F=18.09; d.f =2; P<0.05)	34.44 ± 7.29 a	60.00 ± 8.98 b	82.22 ± 4.34 c
2	*Trianthema portuclacastrum* (F=23.57; d.f =2; P<0.05)	45.56 ± 8.84 a	72.22 ± 5.72 b	91.11 ± 3.89 c

Table 4: Mean percent larval repellency in *T. granarium* exposed to 30% concentration of *C. plicata* and *T. portuclacastrum* extracts for different exposure intervals

No.	Plant name	Exposure intervals (Days)		
		2 days	4 days	6 days
1	*Chrozophora plicata* (F=9.62; d.f =2; P<0.05)	74.44 ± 7.84 b	62.22 ± 8.30 b	40.00 ± 9.43 a
2	*Trianthema portuclacastrum* (F=11.69; d.f =2; P<0.05)	85.56 ± 6.26 b	70.00 ± 7.64 ab	53.33 ± 9.72 a

Table 5: Mean number of emerged larvae and their percent inhibition in F1 progeny of *T. granarium* exposed to different concentrations of *C. plicata* and *T. portuclacastrum* extracts

S. No.	Conc. (%)	F1 progeny ± S.E.			
		C. plicata		*T. portuclacastrum*	
		Mean No. of Larvae (F=38.68; d.f =3;	Percent larval inhibition (F=20.28; d.f =2;	Mean No. of Larvae (F=52.72; d.f =3;	Percent larval inhibition (F=8.92; d.f=2;
1	10%	109.67 ± 7.84 b	26.24 ± 5.27 a	77.33 ± 6.94 b	47.98 ± 4.67 a
2	20%	82.00 ± 3.46 a	44.84 ± 2.33 b	64.33 ± 5.24 ab	56.73 ± 3.52 ab
3	30%	61.67 ± 3.53 a	58.52 ± 2.37 b	43.67 ± 4.63 a	70.63 ± 3.12 b
4	Control	148.67 ± 7.80 c			

According to Tukey's HSD test, means sharing the identical letter(s) in a column are not different significantly from each other when P=0.05

the enzyme inhibitory activity of *T. portuclacastrum* and *plicata*plant extracts increased with the increase in applied concentrations. Moreover, *T. portuclacastrum* and *C. plicata* plant extracts effectively decreased the enzyme contents in test grubs as well as in newly emerged larvae. This inhibition in the enzymatic activity could possibly cause an obstruction in their chemical pathways which lead to the formation of abnormal state in *T. granarium* larvae making them unable to survive.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a powerful molecular spectroscopic tool which helps in and chemical analysis. It is frequently used for pharmaceutical analysis, which provides both quantitative and qualitative analysis of diverse inorganic and organic compounds. It provides result in the form of absorption spectrum. Generally, the FTIR analysis looks at the vibration of functional groups present in organic molecules and explores the structural alterations as the function of shifts in wave number (Bina *et al*., 2003).

FTIR spectroscopic analysis of *T. portulacastrum* extract revealed the presence of various chemical constituents (fig. 4). The intense absorption bands at 2848.81 cm^{-1} and 3009.17 cm^{-1} represents C-H stretching

Table 6: Effect of different concentrations of *T. portuclacastrum* and *C. plicata* extracts on the enzymatic activity of *T. granarium*

S. No.	Enzymes	*C. plicata*			*T. portuclacastrum*		
		10%	20%	30%	10%	20%	30%
1	A Ch E	12.66±1.40a	19.63 ± 1.69 b	27.72±0.80 c	17.31 ± 2.08 a	22.84 ± 2.40 b	29.01 ± 0.52
		(F=12.61;d.f =2; P<0.05)			(F=20.05; d.f =2; P<0.05)		
2	ACP	28.32 ± 2.83 a	42.33 ± 1.86 b	55.02±1.44 c	35.10 ± 3.23 a	48.67 ± 2.64 b	59.88 ± 1.78
		(F=23.73; d.f =2; P<0.05)			(F=45.56; d.f =2; P<0.05)		
3	AKP	27.44 ± 2.72 a	34.68 ± 0.81 b	51.01 ± 1.78 c	27.27 ± 2.65 a	37.21 ± 1.57 b	50.67 ± 2.01
		(F=8.84; d.f =2; P<0.05)			(F=29.86; d.f =2; P<0.05)		
4	*α*-Carboxyl	16.84 ± 1.89 a	32.47 ± 2.54 b	44.79 ± 2.07 c	37.33 ± 2.21 a	46.35 ± 3.00 b	53.13 ± 2.64
		(F=19.64; d.f =2; P<0.05)			(F=21.13; d.f =2; P<0.05)		
5	*β*-Carboxyl	27.45 ± 2.31 a	38.24 ± 1.00 b	52.35 ± 1.48 c	32.16 ± 1.76 a	41.57 ± 1.12 b	56.28 ± 2.26
		(F=17.71; d.f =2; P<0.05)			(F=45.57; d.f =2; P<0.05)		

A Ch E= acetylcholine esterase, ACP= Acid phosphatase, AKP= alkaline phosphatase, *α*-Carboxy= *α*-Carboxylesterase and *β*-Carboxyl = *β*-Carboxylesterase

Table 7: Effect of *T. portuclacastrum* and *C. plicata* extracts on the enzymatic activity of *T. granarium* in the parental and F1 generations

Sr. No.	Enzymes	*C. plicata*		*T. portuclacastrum*	
		6 days	35 days	6 days	35 days
1	A Ch E	18.54 ± 2.84 a	21.47 ± 1.76 b	20.62 ± 2.73 a	25.48 ± 1.05 b
		(F=6.01; d.f =1; P<0.05)		(F=10.38; d.f =1; P<0.05)	
2	ACP	39.43 ± 4.98 a	44.35 ± 3.00 b	43.76 ± 4.40 a	52.02 ± 3.26 b
		(F=1.79; d.f =1; P<0.05)		(F=15.14; d.f =1; P<0.05)	
3	AKP	38.83 ± 4.39 a	36.59 ± 3.07 a	36.93 ± 3.70 a	39.84 ± 3.80 a
		(F=0.93;d.f =1; P<0.05)		(F=1.38; d.f =1; P>0.05)	
4	*α*-Carboxyl	29.51 ± 4.50 a	33.22 ± 4.19 a	40.86 ± 2.30 a	50.35 ± 2.87 b
		(F=14.59; d.f =1; P<0.05)		(F=22.72; d.f =1; P<0.05)	
5	*β*-Carboxyl	38.56 ± 3.50 a	40.13 ± 4.14 a	42.35 ± 3.47 a	44.31 ± 4.04 a
		(F=0.72; d.f =1; P<0.05)		(F=0.89; d.f =1; P>0.05)	

A Ch E= acetylcholine esterase, ACP= Acid phosphatase, AKP= alkaline phosphatase, *α*-Carboxy = *α*-Carboxylesterase and *β*-Carboxyl = *β*-Carboxylesterase

and the absorption band at 1092.61 cm^{-1} denotes the presence of -C-O linkage in -C-OH. Moreover, the band around 1622.32 cm^{-1} show - C=O (carbonyl) group. The presence of these functional groups indicated the presence of polyphenolics (-OH) in *T. portuclacastrum* extract (Kavitha *et al*., 2014).

DISCUSSION

The extracts of *T. portuclacastrum* and *C. plicata* weed plants were used in order to evaluate the efficacy of these plants against *T. granarium*. Although, no work has been yet performed on these weed plants for their insecticidal activity against stored grain insect pests. However, the toxic potential of *T. portuclacastrum* has been reported in mosquitoes (Sing *et al*., 2011). The current results regarding high mortality in *T. granarium* due to *T. portuclacastrum is* in accordance with Sing *et al*. (2011) who suggested that crude aqueous and acetone extract of *T. portuclacastrum* leaves had excellent larvicidal activity against mosquitoes; causing 100% mortality in third instar larvae of *Anopheles culicifacies*, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* at 1.0, 0.75, 0.75 and 1.0% concentrations, respectively. Asma *et al*., (2015) tested different concentrations (2.5, 5.0 and 10.0%) of *Azadrichta indica*, *Calotropis procera, Soleno stemma argel* and *Aristolochia bracteolata* against *T. granarium* and found that each plant extract showed increased mortality with increasing the concentration. Similar trend was also illustrated by Dwivedi and Sharma (2002) who described repellency of five plant extracts against *T. granarium* and showed that the repellency increased with the increase in extract concentration which tends to decrease with the passage of exposure time. In addition, the present results are in agreement to Dwivedi and Nidhi (2004) who reported the repellent activity of six aboriginal plant species using olfactroometer.

Shanker and Uthamasamy (2010) studied the bio-efficacy of some medicinal plants; *Cassia tora, Clerodendron Inermi, Calotropis gigantea, Aloe vera, Vitex negundo* and *Andrographis paniculata* against stored product pests, *Callosobruchus chinensis*. Sagheer *et al*., (2013) invest

tigated the repellent potential of acetone extracts of *Nicotiana tobaccum, Peganum harmala, Saussurea costus* and *Salsola barysoma* using different concentrations against *T. granarium* and found that the repellent behavior of the tested plants increased by increasing the extract concentrations.

Boeke *et al*. (2004) used traditional african plant powders and found 13volatile and 2non-volatile oils and 8 slurries against *Callosobruchus maculates*in stored cowpea. The volatile oils caused a reduced number of eggs on treated beans whereas non-volatile oils had no repellent effect. The current results of progeny reduction are consistent with the findings of Talukder (1995). They reported 43 plant species which caused progeny inhibition in stored product insects. Ahmed (2011) also reported the progeny reduction effect of seed powders of four plants (hamal, black pepper, radish and celery). In accordance to the present results, the number of F1 progeny was found significantly decreased with the increase in concentration.

The petroleum ether extract of *C. plicata* and *T. portuclacastrum* plants also induced enzyme inhibitory activity against *T. granarium*. These findings are in agreement with Falak *et al*. (2004) who reported a significant reduction in the activity of acetylcho linesterase, total esterase (TE) and arylesterase (AE) in 4th instar larvae of *T. granarium* treated for 80h exposed period with Phosphine. Nathan *et al*. (2008) and Caballero *et al*. (2008) also reported the inhibition of esterase activity in insects by plant products. Our results are also in accordance with Zibaee and Bandani (2010) who reported that *Artemisia annua* extract inhibited the AChE activity in higher doses in treated Sunn pest. Similalry, Younes *et al*. (2011) reported the biochemical effects of seven culinary and medicinal plant oils; garlic (*Allium sativum* L.), onion (*Allium cepa* L.), olive (*Olea europaea* L.), rosemary (*Rosmarinus officinalis* L.), sunflower (*Helianthus annuus*L.), peppermint (*Mentha piperita* L.) and camphor (*Eucalyptus globulus*) against *Trogoderma. Granarium* 4th instar larvae. They found less glucose and lipid contents in treated larvae while observed higher protein contents. They also found that these plant oils caused less alkaline phosphatase (AKP) activity and low Acid phosphatase (ACP) content. Cholinesterase was found to be increased whereas Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activity in 4th instar larvae were found to be decreased Geethalakshmi *et al*., (2010) reported that the leaves of *T. portulacastrum* contain many active compounds including trianthenol, C-methylflavone, leptorumol, trianthemine etc. Subsequently, Kassim *et al*. (2011) used FT-IR to analyze the various components from different medicinal plants. IR spectra showed the presence of phenolic compounds which were further evaluated for their antioxidant activity. The peak values through FTIR analysis of *T. portuclacastrum* indicated the presence of phenolic compounds which are consistent to the findings of Kavitha *et al*., (2014). They obtained similar IR spectra corresponding to different functional groups with similar band stretching. Thus, on the basis of the absorption values the presence of polyphenolics (-OH) and flavonoid type compounds were confirmed in the methanolic extract.

Although, the efficacy of phytochemicals from various plants to evaluate the toxicity, repellency, progeny inhibition have been reported in *T. granarium*. However, efficacy of *C. plicata* and *T. portuclacastrum* and their effect on enzymatic activity in stored grain insects has not been reported yet. Thus, the present study is the first report to describe these parameters.

CONCLUSION

The present results indicate that *C. plicata* and *T. portuclacastrum* extracts have insecticidal, repellent and progeny reduction potential against *T. granarium*. In addition, the extracts also induced enzyme inhibitory activity against *T. granarium* at all tested concentrations. Furthermore, petroleum ether extract of *T. portuclacastrum* had higher insecticidal activity than *C. plicata*. FTIR analysis of *T. portuclacastrum* showed the presence of polyphenolic compounds. More research work is needed so that a weed plant-extract based biopesticides can be produced for their use in stored grain insect pest management programs.

ACKNOWLEDGEMENTS

The facilities and support provided by the Department of Zoology, Govt. College University Faisalabad, and Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad are highly acknowledged. The authors declare that there is no conflict of interest.

REFERENCES

Abbott WS (1925). A method of computing the effectiveness, of an insecticide. *J. Econ. Entomol.,* **18**: 265-267.

Ahmad F, Sagheer M, Hammad A, Rahman SM and Hasan MU (2013). Insecticidal activity of some plant extracts against *Trogoderma granarium* (E.). *The Agriculturists,* **11**(1): 103-111.

Ahmed JM (2011). The efficacy of four seed powders on some biological aspects and mortality of Khapra beetle. *The Iraqi J. Agri. Sci*., **42**(6): 112-123.

Alves APC, Corrêa AD, Alves DS, Saczk AA, Lino JB and Carvalho GA (2014). Toxicity of the phenolic extract from jabuticabeira (*Myrciaria cauliflora* (Mart.) O. Berg) fruit skins on *Spodoptera frugiperda*. *Chilean J. Agri Res*., **74**(2): 200-204.

Arthur FH (1996). Grain protectants: current status and prospects for future. *J. Stored Prod. Res.,* **32**: 293-302.

Asakura K (1978). Phosphatase activity in the larva of the euryhaline mosquito, *Aedes togoi* Theobald with special reference to sea-water adaptation. *J. Exp. Mar. Biol. Ecol.,* **31**: 325-337.

Asma KM, Suad MB and Abdalla AS (2015). Efficacy of some botanical extracts in the control of khapra beetle (*Trogodermagranarium*). *J. Science,* **5**(4): 213-217.

Benhalima H, Chaudhry MQ, Mills KA and Price NR (2004). Phosphine resistance in stored product insects collected from various grain storage facilities in Morocco. *J. Stored Prod. Res.,* **40**(3): 241-249.

Bina SS, Afshan F, Gulzar T and Sultana R (2003). Tetracyclic triterpenoids from the leaves of *Azadirachtaindica* and their insecticidal activities. *Chem. Pharm. Bull*., **51**(4): 415-417.

Boeke SJ, Barnaud C, van Loon JJ, Kossou DK, van Huis A and Dicke M (2004). Efficacy of plant extracts against the cowpea beetle, *Callosobruchus maculatus*. *Int. J. Pest Management*, **50**(4): 251-8.

Burges DH (1962). Diapause, pest status and control of the Khapra beetle, *Trogoderma granarium* Everts. *Ann. Appl. Biol*., **50**: 614-617.

Caballero C, Lopez-Olguin J, Ruiz M, Ortego F and Castanera P (2008). Antifeedant activity and effect of terpinoids on detoxification enzymes of the beet armyworm, *Spodoptera exigua* (Hubner). *Span. J. Agri. Res.,* **6**:177-84.

Chopra GL (1988). Angiosperm.Pradeep Publications, India, 452.

Dwivedi SC and Sharma Y (2002). Investigation on repellent responses of khapra beetle: *Trogoderma granarium* (Coleoptera: Dermestidae) to five plant species. *Indian Biologists,* **34**(2): 55-58.

Dwivedi SC and Shekhawat NB (2004). Repellent effect of some indigenous plant extracts against *Trogoderm agranarium* (Evert). *Asian J. Exp. Sci*., **18** (1& 2): 47-51.

EPPO, 2015.EPPO Global Database (available online). https://gd.eppo.int

Falak S, Ali and Shakoori AR (2004). Phosphine Induced Changes in Various Esterase levels in 4th Instar Larvae of Trogodermagranarium. *Pak. J. Zool.,* **36**(4): 257-260.

Forghani SH and Marouf A (2015). An introductory study of storage insect pests in Iran. *Biharean Biologist*., **9**(1): 59-62.

Forster PI and Welzem PCV (1999). Revision and phylo geneny of subtribes Chrozophorinae and Doryxylinae (Euphorbiaceae) in Malaysia and Thailand. *Blumea.,* **44**: 411-436.

Ghani N (2010). KhazainulAdvia. Ed. 1st, New Delhi: Idara Kitab-us-Shifa; (YNM): **231**, 371, 409, 1053, 1114.

Geethalakshmi R, Sarada DVL and Ramasamy K (2010). Trianthema decandra L: a review on its phytochemical and pharmacological profile. *Int. J. Eng. Sci. Technol.*, **2**: 976-979.

Hasan M, Sagheer M, Ali MRQ, Hanif CM and Anwar H (2014). Evaluation of some plant essential oils as repellent and toxicant against *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). *J. Glob. Inn Agri. Soc. Sci.,* **2**: 65-69.

Hegab MM, Khodary SEA, Ola H and Ghareib HR (2008). Autotoxicity of chard and its allelopathic potentiality on germination and some metabolic activities associated with growth of wheat seedlings. *Afr. J. Biotech.,* **7**(7): 884- 892.

Huang Y, Tan JMWL, Kini RM and Ho SH (1998). Toxic and antifeedant action of nutmeg oil against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. Stored Products Res*., **33**: 289-298.

Kadiri SK and Avanapu SR (2013). Evaluation of antiulcer activity of plant *Chrozophora plicata. Int. J. Pharm*., **3**(4): 774-778.

Kadiri SK, Srinivasa AR and Satyanarayana K (2013). Evaluation of in vitro antioxidant activity of plant *Chrozophora plicata. Bio. Med. Res*., **1**(1): 47-50. Kassim MJ, Hussin MH, Achmad A, Dahon, NH, Suan TK and Hamdan HS (2011). Determination of total phenol, condensed tannin and flavonoid contents and antioxidant activity of *Uncaria gambir* extracts. *Indones*. *J. Pharmacy*., **22**(1): 50-59.

Kavitha D, Parvatham R and Padma PR (2014). Assessment of Trianthema portulacastrum for its antimicrobial potential and investigation of their phytochemicals using HPTLC, GC-MS, and IR. *Int. J. Pharm. Pharm. Sci*., **6**(1): 675-686.

Kirtikar KR and Basu BD (2003). Indian medicinal plants with illustrations. Ed. 2nd. Dehradun: *Oriental Enterprises*, **5**: 1640.

Kulkarni NV, Kataria, R and Gupta S (2015). Evaluation of various oils on Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) in terms of survival of adulthood, grain damage and population build-up. *Int. J. Agri. Sci. Res*., **5**(5): 11-22.

Madrid FJ, White NDG and Loschiavo SR (1990). Insects in stored cereals and their association with farming practices in Southern Manitoba. *Canad. Entomologist*, **122**: 515-523.

Mahmoud AK, Bedawi SM and Satti AA (2015). Efficacy of some botanical extracts in the control of Khapra beetle (*Trogoderma granarium*). *J. Sci.,* **5**(4): 213-217.

Mahmoud AK, Satti AA, Bedawi SM and Mokhtar MM (2014). Combined insecticidal effects of some botanical extracts against the khapra beetle (*Trogodermagranarium* Everts). *Res. J. Engineer Applied Sci.,* **3**(6): 388-393.

McDonald L, Guy R and Speirs R (1970). Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects. *Marketing Res. Report*, 882.

Montenegro I, Pino L, Werner E, Madrid A, Espinoza L,

Moreno L and Cuellar M (2013). Comparative study on the larvicidal activity of drimane sesquiterpenes and nordrimane compounds against *Drosophila melanogaster* til-til. *Molecules*, **18**(4): 4192-4208.

Moreira MD, Picanço MC, Barbosa LCDA, Guedes RNC, Campos MRD, Silva, GA and Martins JC (2007). Plant compounds insecticide activity against Coleoptera pests of stored products. *Pesquisa Agropecuária Brasileira*, **42**(7): 909-915.

Nathan SS, Choi MY, Seo HY, Paik CH, Kalaivani K and Kim JD (2008). Effect of *azadirachtin* on acetylcholine esterase activity and histology of brown planthopper *Nilaparvata lugens* (Stal). *Ecotox. Environ. Safety,* **70**: 244-250.

Pandir D and Bas H (2016). Compositional analysis and toxicity of four plant essential oils to different stages of Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Turk. J. Ent.*, **40**(2): 185-195. Peacock ER (1993). Adults and larvae of hide, larder and carpet beetles and their relatives (Coleoptera: Dermestidae) and of derodontid beetles (Coleoptera: Derodontidae). Handbooks for the Identification of British Insects London, UK; *Natural History Museum*, **5**(3): 144.

Pugazhvendan SR, Ross PR and Elumalai K (2012). Insecticidal and repellant activities of plants oil against stored grain pest, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Asian Paci. J. Trop. Dis*., **2**: S412-S415.

Rafiee-Dastjerdi H, Khorrami F, Nouri Ganbalani G, Fathi AA and Esmaielpour B (2014). Efficacy of some medicinal plant extracts and essential oils against Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). *Arch. Phytopath. Plant Protect*., **47**(10): 1175-1178.

Ramzan M and Chahal BS (1986). Effect of interspecific competitionon the population build-up of some storage insects. *Ind. J. Ecol.,* **13**: 313-317.

Sagheer M, Hasan M, Ali Z, Yasir M, Ali Q, Ali K, Majid A and Khan FZA (2013). Evaluation of essential oils of different citrus species against *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) collected from Vehari and Faisalabad. *Pak. Entomol.,* **35**: 37-41.

Sagheer M, Hasan M, Majid A, Ali Q, Shahid MI and Ali K (2013a). Repellent effect of four medicinal plant extracts to *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *J. Glob. Innov. Agri. Soc. Sci.,* **1**: 9-11.

Samad MA, Rahman MM, Hossain AKMM, Rahman MS and Rahman SM (2008). Allelopathic effects of five selected weed species on seed germination and seedling growth of Corn. *J. Soil. Nature,* **2**(2):13-18.

Satti AA and Elamin MM (2012). Insecticidal activities of two meliaceous plants against *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Int. J. Sci. Nature*., **3**(3): 696-701.

Shanker C and Uthamasamy S (2010).Evaluation of some medicinal plants and their mixtures for their bio-efficacy against crop and stored product pests. *Arch. Phytopath. Plant Protect*., **43**(2): 140-148.

Singh SP, Raghavendra K and Thomas TG (2011). Mosquito larvicidal properties of aqueous and acetone extracts of *Trianthema portulacastrum* Linn. (Family: Aizoaceae) against vector species of mosquitoes. *The J. Comm. Dis.,* **43**(4): 237-241.

Subramanyam BH and Roesli R (2000). Inert dusts. *In*: Bh. Subramanyam and DW. Hagstrum, Editors, Alternatives to Pesticides in Stored-Product IPM, Kluwer Academic Publishers, Dordrecht, pp.321-380.

Sultana K, Zahoor, MK, Sagheer M, Nasir S, Zahoor MA Jabeen F and Bushra R (2016). Insecticidal activity of weed plants, *Euphorbia prostrata* and *Chenopodia strum murale* against stored grain insect pest *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae). *Turk. J. Ent.,* **40**(3): 291-301.

Talukder FA (1995). Isolation and characterization of the active secondary pithraj (*Aphanamixis polystachya*) compounds in controlling stored-product insect pests (PhD thesis), University of Southampton, Southampton, UK, 1995.

Talukder FA (2006). Plant products as potential stored product insect management agents: A mini review. *Emirates J. Agri. Sci.,* **18**: 17-32.

Van Asperen K (1962). A study of house fly esterase by means of a sensitive colorimetric method. *J. Insect Physiol*., **8**:401-416.

Younes MW, Othman SE, Elkersh MA, Youssef NS and Omar GA (2011). Effect of seven plant oils on some biochemical parameters in Khapra beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Egypt. J. Expt. Biol.* (Zool)., **7**(1): 53-61.

Zibaee A and Bandani AR (2010). A study on the toxicity of the medicinal plant, *Artemisia annua* L. (Astracea) extracts the Sunn pest, *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae). *J. Plant Protect. Res.,* **50**: 48-54.