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Research Article

Bioactivity of medicinal plant extracts as toxicants and enzyme inhibitors against insect pests of stored commodities

Kazam Ali[1*], Muhammad Sagheer[2], Mansoor ul Hasan[2], Abdul Rashid[3] and Muhammad Shahid[4]

1. Centre for Agriculture and Bioscience International, Central and West Asia, Rawalpindi, Pakistan.
2. Department of Entomology, University of Agriculture, Faisalabad, Pakistan.
3. Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.
4. Department of Bio-Chemistry, University of Agriculture, Faisalabad, Pakistan.

Abstract: The present research was performed to evaluate the bioactivity of *Citrullus colocynthis* (L.) and *Melia azedarach* L. extracts against three major stored grain insect pests including *Tribolium castaneum* (Herbst), *Trogoderma granarium* Everts, and *Sitophilus granaries* (L.). Toxicity and enzyme inhibition activity of acetylcholinesterase (AChE), α-carboxylesterase (α-CE), β-carboxylesterase (β-CE), acid phosphatases (ACP) and alkaline phosphatases (ALP) in three insect species induced by both plant extracts were evaluated at four different dose rates viz., 5, 10, 15 and 20%. Results showed maximum mortality (34.29%) in *S. granarius* with *M. azedarach* at maximum interaction of time and dilution level. In *T. castaneum* and *T. granarium* maximum recorded values for mortality were 30.87% and 18.95%, respectively, with extract of *M. azedarach*. Plant extract of *C. colocynthis* reported a maximum mortality of 21.92%, 19.18% and 16.89% in *T. castaneum*, *S. granarius* and *T. granarium*, respectively. Findings proved that both plant extracts had decent lethal impacts on tested insect species. Exposure of studied insects to plants extracts also resulted in significant inhibition of AChE, α-CE, β-CE, ACP and ALP. All tested enzymes in three insects were maximally inhibited by plant extract of *M. azedarach* except α-CE which was slightly more inhibited in *S. granarius* and ACP which was highly inhibited in *T. granarium* and *S. granarius*, by plant extract of *C. colocynthis*. Outcomes exhibit that plant based extract of *M. azedarach* is more pronounced in stored grain insect pests and propose the capability of using these plant extracts for safety of stored commodities as a safe substitute for insecticides.

Keywords: stored product pests, enzyme inhibition, lethal effects, toxicity

Introduction

Stored grains and their products are at great risk to the infestation by insect pests (Ukeh *et al*., 2012). Stored food commodities are infested by hundreds of hexapods and other arthropods among which about 600 species belongs to order Coleoptera (Rajendran and Sriranjini, 2008). In Pakistan, Red rust flour beetle *Tribolium castaneum*, Khapra beetle *Trogoderma granarium*, Angumois grain moth *Sitotroga cerealella*, Lesser grain borer *Rhyzopertha dominica*, Rice weevil *Sitophilus oryzae* and Grain weevil *Sitophilus granarius* are reported to be the most injurious insect pests of stored commodities (Iqbal *et al*., 1992). *T. castaneum* is a secondary pest of stored

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commodities, both larvae and adults feed on grain dust and broken grain and spend entire life cycle outside the grain kernels (Karunakaran *et al.*, 2004). In severe infestation, the flour turns grayish and has a pungent smell (benzoquinone), disagreeable odour making it unfit for human consumption. This insect causes substantial loss in storage because of its high reproductive potential (Prakash *et al*., 1987). Primary stored grain pest *T. granarium* has been nominated as one of the 100 worst invasive species worldwide. It is a serious pest of stored products under hot, dry conditions (Lowe *et al*., 2000). Generally, young larvae of *T. granarium* feed on damaged seed, while older larvae feed on whole grains. Larvae attack the embryo point or a weak place in the pericarp of grain or seed. The khapra beetle can cause significant weight loss (weight loss between 5-30%, extreme cases of 70%) when left undisturbed in stored grain. Granary weevil (*S. granarius*) is another important stored grain pest which causes significant losses during storage (White and Leesch, 1996). The weevils bore into the kernels, insert their eggs within the endosperm of the grain and develop inside whole grain kernels as small, white, wrinkled, grub-like larvae (CABI, 2007), meanwhile their damage remains un-noticed until the weevils emerge from the seed with an exit hole (Niewiada *et al*., 2005).

Recently, a great attention has been paid to the use of *Citrullus colocynthis* and *Melia azedarach* extract as natural insecticids. The biological activity of these plants has been investigated against many insect pests (Soam *et al*., 2013). These plants are known to have a range of compounds, which show insecticidal, antibacterial, larvicidal, deterrent, antifeedant, growth regulating, antifertility, metamorphosis and reproduction disturbance effects (Pravin *et al*., 2013; Soam *et al*., 2013). Aqueous and methanolic extracts of plant, *C. colocynthis* demonstrated high antimicrobial activity against some bacteria and fungi. *C. colocynthis* can be used medically as an abortifacient, cathartic, purgative and vermifuge, as well as for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, tumour, and as an insect repellant (Soam *et al.*, 2013). El-Naggar *et al*. (1989) noted the impact of colocynithin and hydrated colocynithin isolated from *C. citrullus* against on seven insect species and reported effectual outcomes. Chemicals isolated from *Melia azedarach* L. species Meliaceae family have gained a particular attention from applied entomologists because of their excellent properties as insect control agents (Luo *et al*., 1995). *M. azedarach* is native to Iran, India, and China (Hong and Ellis, 1998). The plant has become the object of studies to evaluate properties from different plant structures, in particular insecticidal, antiviral, antioxidant, bactericide, and antiparasitic activities (Ahmed *et al*., 2008). The insecticidal activity of *M. azedarach* is found in leaves, fruits, and seeds, and is due to a group of biologically active triterpenoids they have antifeeding effects (Isman, 2006). Generally, extracts from green fruits and leaves have been those most efficacious because of their antifeedant effect, mainly on beetles and lepidopterans (Carpinella *et al*., 2003, 2005; Nathan and Kim, 2005; Defago *et al*., 2006).

Enzymes are main group of proteins and biomolecules which enhance metabolic activities in the organisms. Esterases and phosphatases are important components for the proper functioning of several important physiological processes in insects (Lassiter *et al*., 1995; Shanmugavelu *et al*., 2000). A little change in the levels of enzymes affects the metabolic process in organism (Roy, 2002). In insects, resistance against insecticides arises by the change of metabolic enzymes or increased detoxification (Parakrama Karunaratne, 1998). Due to the change in the target site in the insect, insecticides cannot bind to that site and insect behaves normally (Damayanthi and Karunaratne, 2005). By checking the change in metabolism of insects, the activity of the enzymes released in their plasma due to cell disturbance can be calculated (Coppo *et al*., 2002). So inhibition of enzymes is a reliable method to assess the pressure on the insects by pollutants. Esterases and phosphatases are considered as reliable bio-

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markers for assessing the toxic effects of numerous insecticidal compounds on the physiology of targeted insects (Srinivas *et al*., 2004). This study was designed to investigate the toxic and enzyme inhibitory effects of *C. colocynthis* and *M. azedarach* extracts against *T. castaneum*, *T. granarium* and *S. granarius*.

Materials and Methods

Collection and rearing of insects

The mixed populations of stored grain insect pests were collected from grain market and flour grinding mills, and were brought into the Grain Research Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad, Pakistan. In the laboratory, the target insects, red flour beetle *T. castaneum*, Khapra beetle *T. granarium* and Granary weevil *S. granarius* were separated and reared for homogeneous population.

Populations of the three coleopteran species were cultured in sterilized plastic jars (1.0 kg capacity) and 50 adults of each species were released separately into their favorite diet, these adults were allowed for copulation and were removed after 5 days. Wheat flour was used for rearing of *T. castaneum* (Sagheer *et al*., 2014), while wheat grains were used as culture medium for *T. granarium* and *S. granarius*. After removal of adults, sieved flour and grains containing eggs laid by these insects were again put into the jars and placed in incubators for getting same age population. Rearing jars of *T. castaneum* and *T. granarium* were placed in incubators (SANYO) at 30 ± 2 ºC and 65 ± 5% R.H., while *S. granarius* was cultured at 27 ± 2 ºC and 70 ± 5% R. H.

Collection of plant materials

Plant materials, such as fruits of *C. colocynthis* (Tuma) were collected from district Layyah and leaves of *M. azedarach* (Darek) were collected from the fields of University of Agriculture, Faisalabad (UAF).

Preparation of plant extracts

The fruits of *C. colocynthis* and leaves of *M. azedarach* were washed with sterilized water before placing in shade for drying. Once the plant materials were dried they were ground in electrical grinder and were brought into fine powder. The plant extracts were obtained by adding 50.0g plant powder in 100 ml acetone and solution was set on a Rotary Shaker (IRMECO, OS-10) (Sagheer *et al*., 2014) at 220 rpm for a period of 24 hours. After 24 hours rotation the solution obtained was filtered and placed on rotary evaporator to remove the extra solvent (acetone). Thus, the plant extracts obtained after evaporation were considered as stock solution and stored at 4.0 °C. Four dilution levels (5.0, 10.0, 15.0 and 20.0%) of each plant extract were prepared from the stock solutions, using acetone as solvent.

Toxic effect of plant extracts

Different dilutions (5.0, 10.0, 15.0, 20.0%) of acetone extract of *C. colocynthis* and *M. azedarach* were applied on favorite diet of each tested insect species i.e., wheat flour for *T. castaneum*, and wheat grains for *T. granarium* and *S. granarius*. After evaporation of acetone the dried weighted wheat flour (40.0 g) and wheat grains (40.0 g) were put into treatment jars separately. Fifty adults of *T. castaneum* and *S. granarius,* fifty second instar larvae of *T. granarium* were released separately into experimental jars containing treated wheat and flour. Each treatment was replicated three times and experimental jars were placed in SANYO incubator under optimum conditions as discussed above. Observations for lethal impact of *C. colocynthis* and *M. azedarach* were made after 2, 4, 6, 8 and 10 days of experiment. The alive larvae of *T. granarium* and alive adults of *T. castaneum* and *S. granarius* were stored in phosphate buffer solution to evaluate the enzymes activity.

Mortality was calculated using Abbott's (1925) formula;

$$\text{Corrected Mortality (\%)} = \frac{(\text{Mo} - \text{Mc})}{100 - \text{Mc}} \times 100$$

Where,

Mo = Mortality observed in treatments

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Mc = Mortality observed in control

Inhibition of esterases and phosphatases in survivors of toxicity assay

Preparation of whole body homogenate

The survived specimen (adults of *T. castaneum* and *S. granarius*, larvae of *T. granarium*) in toxicity experiment which were stored in buffer solution were rinsed with clean water, and the adhering water was entirely removed from insect body by blotting with tissue paper. In ice-cold sodium phosphate buffer (20 mM, pH 7.0), the survivors (larvae and adults) of test insects were homogenized separately using a teflon hand homogenizer for eventual estimation of esterases and phosphatases inhibition. For biochemical analyses, clear supernatants were used obtained by centrifuging the whole body homogenates at 8000 rpm for 20 minutes. Before using all the solutions for homogenization, glassware were stored at 4.0 ºC and homogenates were kept on ice until used for different assays.

Determination of acetylcholinesterase (AChE)

Acetylcholinesterase (AChE) activity in the whole body homogenates of *T. castaneum*, *T. granarium* and *S. granarius* were measured spectrophotometrically according to Ellman *et al*. (1961) with slight modification of using acetylthiocholine iodide instead of acetylthiocholine chloride as substrate. 50 µl of acetylthiocholine iodide (2.6 × 10-3 M) as a substrate and 1 ml of sodium phosphate buffer (20 mM, pH 7.0) was added to 100 µl of enzyme solution taken from whole body homogenates and incubated at 25 ºC for 5 minutes. Then 400 µl of freshly prepared 0.3% Fast blue B salt in 3.3% SDS (sodium dodecyl sulphonate) was added to stop reaction. Sample was run through spectrophotometer and optical density was recorded at 405 nm.

Determination of α and β-carboxylesterase (α-CE & β-CE)

The activity of α-and β-carboxylesterase in the whole body homogenates of test insects was measured by the method of van Asperen (1962). The α-carboxylesterase activity was recorded by using α-naphthylacetate as substrate. For this purpose 50 µl of α-naphthylacetate (250 µM) and 1 ml of sodium phosphate buffer (20 mM, pH-7.0) was added in 50 µl whole body homogenates. This solution was incubated at 30 ºC for 20 minutes. 400 µl of freshly prepared 0.3% Fast blue B salt in 3.3% SDS, was added to stop the reaction. Sample was run on spectrophotometer and optical density was noted at 430 nm.

Same procedure was followed for β-carboxylesterase activity, except that β-naphthylacetate was used as substrate and optical density was measured at 590nm.

Determination of acid and alkaline phosphatases (ACP & ALP)

The level of acid phosphatases (ACP) and alkaline phosphatases (ALP) were calculated by following the Asakura (1978) method. P-nitrophenyl phosphate was used as substrate for the estimation of phosphatases. For acid phosphatase (ACP), 100 µl of 20mM p-nitrophenyl phosphate (substrate) and 450µl sodium acetate buffer (50 mM, pH 4.6) were added in 50 µl enzyme solution. The solution was incubated at 37 ºC for 15 mins. Then, 100 µl of 0.5N NaOH was added to stop reaction. Optical density of sample was recorded at 405 nm.

Same procedure was followed for alkaline phosphatases (ALP) except that 450 µl Tris HCl (50 mM, pH-8) was used in place of sodium acetate buffer.

Enzyme inhibitions (%) of test enzymes were computed using the formula given by Wang *et al*., (2014).

$$\text{Enzyme Inhibition (\%)} = \frac{(OD_b - OD_o)}{OD_b} \times 100$$

OD_b = Optical density of blank (control treatment)

OD_o = Optical density of treatments

Statistical analyses

Separate two-way factorial ANOVA were performed for both *C. colocynthis* and *M. azedarach* plant extracts against each tested insect species. The differences and alterations

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in the levels of different enzymes in *Tribolium casteneum*, *Trogoderma granarium* and *Sitophilus granarius*, were computed by using mean difference Tukey-HSD test (Statistica-8.1). The means sharing similar letters within column are statistically same in Table 1. The level of acceptance for significant difference was $p \leq 0.05$ in all cases.

Table 1 Comparison of mortality in three stored grains insect species by acetone extracts of *Citrullus colocynthis* and *Melia azedarach.*

Time (Days)	Conc. (%)	Mean Mortality ± S.E (%)					
		Citrullus colocynthis			*Melia azedarach*		
		T. castaneum	*T. granarium*	*S. granarius*	*T. castaneum*	*T. granarium*	*S. granarius*
2	5	3.33 ± 0.67 j	1.34 ± 0.34 f	3.38 ± 0.68 i	3.33 ± 0.89 j	0.67 ± 0.33 j	4.05 ± 1.18 l
	10	6.00 ± 0.67 hij	2.68 ± 1.34 ef	4.73 ± 1.17 hi	5.33 ± 1.34 ij	2.01 ± 1.34 ij	6.08 ± 0.68 l
	15	8.00 ± 1.15 f-i	4.02 ± 0.67 def	6.08 ± 0.69 ghi	8.67 ± 0.67 f-j	2.68 ± 0.67 hij	10.14 ± 1.49 k
	20	8.00 ± 0.35 f-i	7.38 ± 2.01 b-f	7.44 ± 1.86 f-i	12.00 ± 2.01 d-g	4.69 ± 2.01 f-j	11.49 ± 1.34 k
4	5	5.41 ± 0.68 ij	2.68 ± 0.67 ef	6.12 ± 0.68 ghi	6.08 ± 0.67 hij	3.35 ± 0.67 g-j	7.24 ± 1.17 kl
	10	8.11 ± 0.68 f-i	5.37 ± 1.16 def	6.80 ± 1.18 f-i	6.76 ± 1.16 g-j	5.37 ± 1.16 e-j	12.93 ± 1.67 jk
	15	8.79 ± 0.59 f-i	6.04 ± 0.67 c-f	9.52 ± 2.07 d-h	12.84 ± 0.67 def	6.71 ± 0.67 e-j	15.65 ± 1.34 ij
	20	10.14 ± 0.68 efg	9.39 ± 2.32 a-e	10.20 ± 1.17 d-g	15.54 ± 2.32 d	9.39 ± 2.32 c-g	19.05 ± 1.98 hi
6	5	6.80 ± 0.34 g-j	5.37 ± 1.16 def	8.16 ± 1.78 e-i	6.80 ± 1.16 g-j	4.69 ± 1.16 f-j	15.65 ± 2.34 ij
	10	9.52 ± 1.34 e-h	6.71 ± 0.67 b-f	8.84 ± 1.51 d-h	11.56 ± 0.67 d-h	7.38 ± 0.67 e-i	21.09 ± 2.11 gh
	15	12.93 ± 0.68 de	9.39 ± 2.32 a-e	11.56 ± 1.18 c-f	16.33 ± 2.32 d	10.74 ± 2.32 b-f	24.49 ± 1.67 fg
	20	15.65 ± 1.35 cd	10.74 ± 1.78 a-d	12.93 ± 0.68 b-e	23.81 ± 1.78 bc	14.09 ± 1.78 bcd	28.57 ± 1.39 de
8	5	10.27 ± 1.37 efg	5.41 ± 0.68 def	9.59 ± 1.06 d-h	9.59 ± 0.68 e-i	5.41 ± 0.68 e-j	19.86 ± 1.34 h
	10	11.64 ± 0.36 ef	8.11 ± 0.68 b-f	13.70 ± 1.79 bcd	14.38 ± 0.68 de	8.79 ± 0.68 c-h	26.02 ± 2.17 ef
	15	17.81 ± 1.19 bc	11.49 ± 1.79 a-d	15.75 ± 1.88 abc	21.92 ± 1.79 c	11.49 ± 1.79 b-e	31.05 ± 3.11 bcd
	20	19.18 ± 1.68 abc	14.19 ± 2.44 ab	17.12 ± 1.45 ab	27.76 ± 2.44 ab	16.89 ± 2.44 ab	33.56 ± 1.68 abc
10	5	13.01 ± 1.34 de	7.44 ± 0.68 b-f	13.01 ± 0.67 b-e	11.64 ± 0.68 d-g	8.11 ± 0.68 d-i	24.65 ± 1.58 f
	10	15.75 ± 1.59 cd	8.79 ± 1.17 b-f	16.44 ± 2.05 abc	15.07 ± 1.17 de	10.81 ± 1.17 b-f	29.13 ± 2.87 cd
	15	20.55 ± 0.68 ab	13.52 ± 0.68 abc	17.81 ± 1.45 ab	24.65 ± 0.68 bc	14.87 ± 0.68 abc	33.93 ± 1.62 ab
	20	21.92 ± 1.03 a	16.89 ± 2.34 a	19.18 ± 1.86 a	30.87 ± 2.34 a	18.95 ± 2.34 a	34.29 ± 2.67 a

Separate two ways factorial ANOVA's were performed for test insects against each acetone based plant extract. The means were computed using Tukey-HSD test, similar letters within treatments (column) are not statistically different ($p < 0.05$).

Results

Toxicity experiments were performed to evaluate the lethal effects of plant extracts of *C. colocynthis* and *M. azedarach* against *T. castaneum*, *T. granarium* and *S. granarius*. Inhibition of various enzymes, acetylcholinesterase (AChE), α-carboxylesterase (α-CE), β-carboxylesterase (β-CE), acid phosphatases (ACP) and alkaline phosphatases (ALP) were studied in the survivors of the toxicity experiment. The means comparison for mortality at different interactions of time interval (2, 4, 6, 8 and 10 days) and concentrations (5, 10, 15 and 20%) induced by acetone extract of *C. colocynthis* and *M. azedarach* in *T. castaneum*, *T. granarium* and *S. granarius* are given in Table 1. Results indicated that *M. azedarach* forced a maximum 34.29% mortality in *S. granarius*, while maximum mortality evidenced in other two insect species *T. castaneum* and *T. granarium* were 30.87% and 18.95% respectively, which were also noted with *M. azedarach* at 20% dilution level after 10 days interval. The result reveals that plant extracts of *C. colocynthis* showed slightly less toxicity than *M. azedarach* except the three different tested organisms and recorded a

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maximum mortality rate of 21.92% in *T. castaneum*, 16.89% in *T. granarium* and 19.18% in *S. granarius* at 20% concentration after 10 days of application. The impact of time and concentration proves directly proportional to mortality of tested insects. Initially *C. colocynthis* was established as stronger insecticide than *M. azedarach* against *T. granarium* as it reported 7.38% mortlity which was only 4.69% with *M. azedarach* at 20% concentration after 2 days exposure, but as time went on *M. azedarach* became more lethal and showed higher values for mortality in the three insect species (Table 1).

Results evidenced that *M. azedarach* plant extract showed high inhibition of AChE (acetylcholinesterase) activity for all three test insects. Maximum AChE inhibition 37.14% was assessed in *S. granarius* at 20% concentration, while at the same dilution level 33.33% and 31.48% were the maximum inhibition values for *T. castaneum* and *T. granarium* by *M. azedarach*. Plant extract of *C. colocynthis* showed maximum inhibition (27.79%) of AChE activity in *T. castaneum* at 15% concentration, while at 20% concentration maximum inhibition (22.23% and 25.58%) of AChE acitivity were noted against *T. granarium* and *S. granarius*, respectively. Lower concentration (5%) showed minimum inhibition effect for AChE activity as it noted 25%, 16.07%, 14.67% inhibition by *C. colocynthis* plant extract; while 24.16%, 25.77%, 25.92% inhibition of AChE acitivity was forced by *M. azedarach* in *T. castaneum*, *T. granarium* and *S. granarius*, respectively (Fig. 1).

The inhibition level of α-CE (α-carboxylesterase) activity significantly increased at various concentrations, and it reached the highest level at 20% concentration in three insects when exposed to the both plant extracts ($p < 0.05$). This enzyme inhibition level steadily decreased in *T. castaneum* from 10% concentration (17.06% inhibition) to 15% concentration (13.98% inhibition) with *C. colocynthis* extract while from 5% concentration (13.94% inhibition) to 10% concentration (10.33% inhibition) with *M. azedarach* plant extract. Maximum α-CE inhibition level in *T. castaneum*, *T. granarium* and *S. granarius* noted at highest concentration (20%) were 19.67%, 17.63% and 22.34% induced by *C. colocynthis*. *M. azedarach* force a maximum 24.98% and 22.36% inhibition of α-CE activity in *T. castaneum* and *T. granarium* at 20% concentration respectively, while in *S. granarius* highest (16.79%) inhibition of α-CE activity was noted at 15% concentration (Fig. 2).

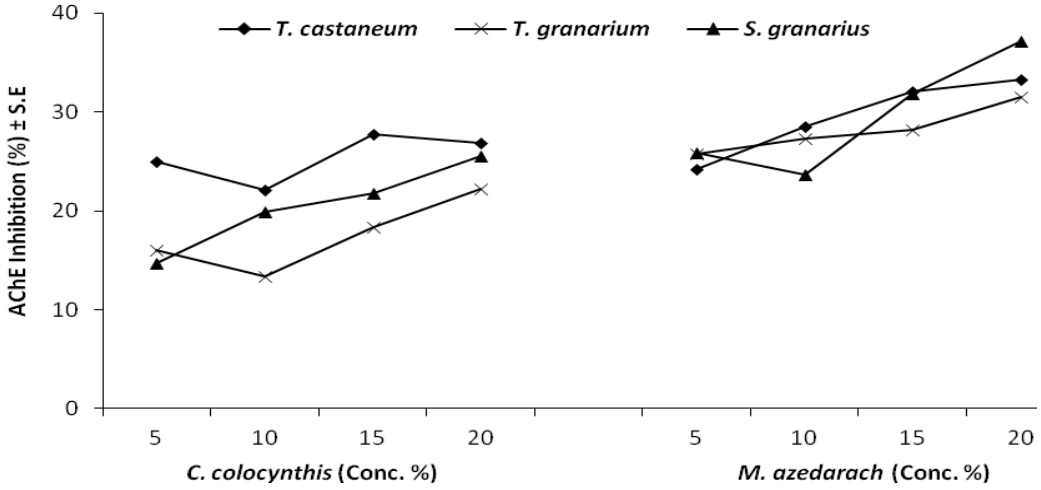

Figure 1 Impact of *Citrullus colocynthis* and *Melia azedarach* extracts at different concentrations on acetylcholinesterase (AChE) activity in three stored grains insect species.

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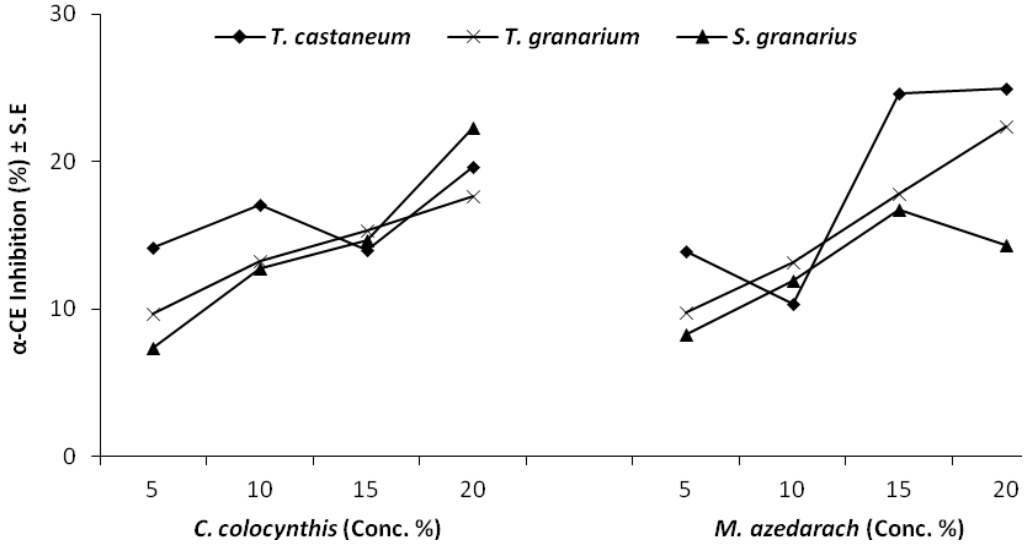

Figure 2 Impact of *Citrullus colocynthis* and *Melia azedarach* extracts at different concentrations on α-carboxylesterase (α-CE) activity in three stored grains insect species.

The exposure of the target insects to both plant extracts had great impact on the inhibition level of β-CE (β-carboxylesterase) activity. From lower (5%) to higher (20%) concentration the inhibition level of β-CE activity steadily increased from 9.14% to 17.72% in *T. castaneum*, 12.72% to 21.71% in *T. granarium* and 14.67% to 25.58% in *S. granarius* when exposed to plant extract of *C. colocynthis* ($p < 0.05$). *M. azedarach* extract showed significant effect on percent inhibition of β-CE (β-carboxylesterase) activity in *T. castaneum*, *T. granarium* and *S. granarius* ($p < 0.01$). At 20% concentration, 41.67%, 38.21% and 30.84% inhibition of β-CE activity was observed in *T. castaneum*, *T. granarium* and *S. granarius*, respectively. The effect of various concentrations of *M. azedarach* on inhibition of β-CE activity against *T. granarium* and *S. granarius* was in the following order: 20% > 15% > 10% > 5%. Minimum percent inhibition of β-CE activities noted in *T. castaneum*, *T. granarium* and *S. granarius*, were 20.83%, 30.49% and 18.47%, respectively, at 5% concentration (Fig. 3).

Inhibition of acid phosphatase (ACP) activity slightly increased from 5 to 20% dilution level of *C. colocynthis* and *M. azedarach* in three coleopteran species (Fig. 4). Maximum ACP inhibition (38.57%) was assessed in *T. castaneum* with *M. azedarach* at 20% concentration, while these values were15.38% and 13.41% for *T. granarium* and *S. granarius* at maximum dilution level of *M. azedarach* respectively. Exposure of the test insects to *C. colocynthis* treated diet significantly increased the inhibition of ACP activity ($p < 0.05$) with increase in dilution level and resulted in maximum of 29.92%, 18.65% and 15.21% ACP inhibition against *T. castaneum*, *T. granarium* and *S. granarius*, respectively at 20% concentration (Fig. 4).

Results illustrated that inhibition of alkaline phosphatase activity gradually increased from lower (5%) to higher (20%) concentration in three test insects exposed to the plant extracts ($p < 0.05$). At 20% dilution, this enzyme level was significantly inhibited ($p < 0.01$). Maximum ACP inhibition (29.44%) was noted in *T. granarium* with *M. azedarach* at 20% concentration, while extract of *C. colocynthis* forced 13.73% inhibition in *T. granarium*

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which was the lowest inhibition value in three stored grain species at the highest dose rate (20%). Exposure to *C. colocynthis* extract (20%) resulted in 17.34% and 21.70% inhibition of ACP activity in *T. castaneum* and *S. granarius*. Whereas *M. azedarach* extract affected slightly higher than *C. colocynthis* and evidenced 18.41% and 23.09% inhibition of ACP enzyme activity in *T. castaneum* and *S. granarius* respectively (Fig. 5).

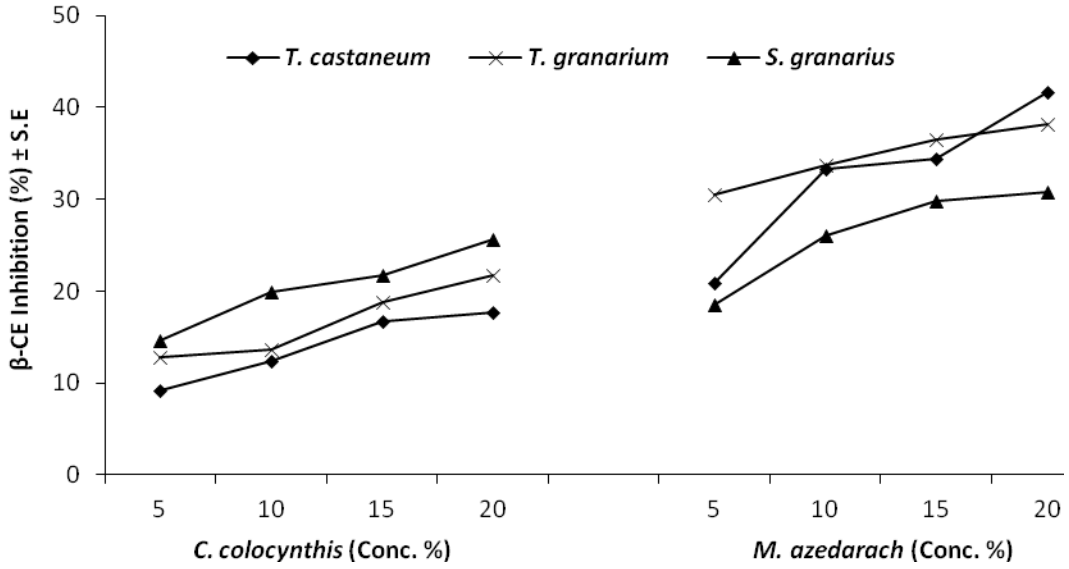

Figure 3 Impact of *Citrullus colocynthis* and *Melia azedarach* extracts at different concentrations on β-carboxylesterase (β-CE) activity in three stored grains insect species.

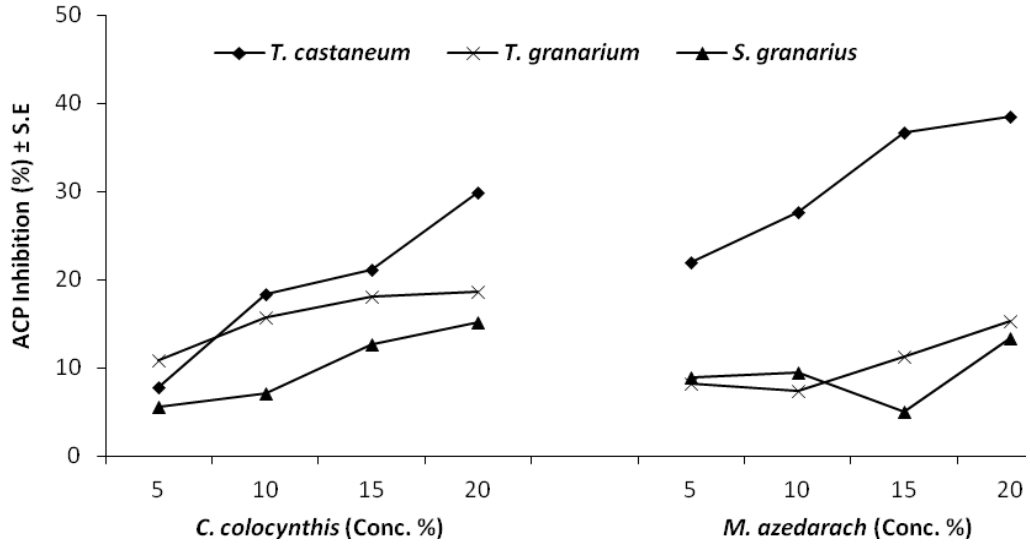

Figure 4 Impact of *Citrullus colocynthis* and *Melia azedarach* extracts at different concentrations on acid phosphatases (ACP) activity in three stored grains insect species.

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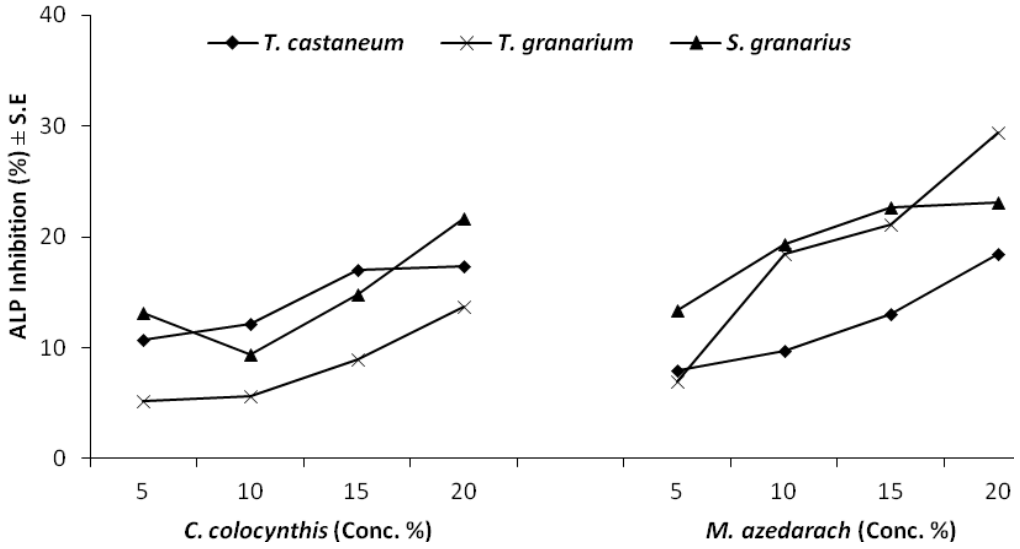

Figure 5 Impact of *Citrullus colocynthis* and *Melia azedarach* extracts at different concentrations on alkaline phosphatases (ALP) activity in three stored grains insect species.

Discussions

The findings for insect mortality evidenced that lethal impact of *C. colocynthis* and *M. azedarach* was directly related to exposure periods and concentrations. Benzi *et al*. (2009) reported similar results showing the toxic efficacy of extracts from some medicinal plants against mites and insects. Similarly, mortality of adults of *T. castaneum* increased with increase in concentration at maximum exposure period (Bibi *et al*., 2008). The insecticidal activity of *M. azedarach* is found in leaves, fruits, seeds, and is due to a group of biologically active triterpenoids that have anti-feeding effects (Valladares *et al*., 1997; Isman, 2006). *C. colocynthis* has a large range of insecticidal, antibacterial, larvicidal, deterrent, antifeedant, growth regulating and antifertility compounds (Pravin *et al*., 2013; Soam *et al*., 2013). Ali *et al*. (2017) verified same results under similar laboratory conditions that exposure of *T. castaneum*, *T. granarium* and *S. granarius* to plant extracts of *Azadirachta indica* and *Datura inoxia*, cause significant mortality in the test populations after 10 days intervals at 20.0% concentrations. Our results testify, plant extract of *M. azedarach* at tested concentrations proved more valuable as they forced higher mortality. These outcomes are supported by Anwar *et al*. (2005) who checked the neem (*A. indica*) oil in a warehouse, against four important stored grain insect pests *Rhyzopertha dominica*, *S. granarius*, *T. castaneum* and *T. granarium* at various dilution levels (5%, 10%, 15% and 20%) in natural conditions at three time periods (30, 60, and 90 days). They observed increased mortality with the increase in dose rate of the spray material.

Both toxicant, *C. colocynthis* and *M. azedarach*, showed decent anti-enzymatic activities in tested insect species, *T. castaneum*, *T. granarium* and *S. granarius*. Different insecticides targeted the AChE (Abdelgaleil *et al*., 2009; Kang *et al*., 2013), carboxylesterases also verified as principal enzyme in many tissues of a number of insects (Park and Kamble, 1999). Based on our findings, AChE activity was inhibited by both plants (*M. azedarach* and *C. colocynthis*) extracts but at minimum concentration (5%) enzyme inhibition was also low and AChE inhibition

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increases at lateral dilution level (20%). Enzyme inhibition results are supported by Wang and his co-workers (2014), as they noted the impact of *Citrus limonum*, *Litseacubeba*, *Cinnamomum cassia* and *Allium sativum* against *Alphitobius diaperinus* (darkling beetle) pest of stored poultry feed. They found that essential oils of tested plants significantly inhibited the levels of AChE activity and *A. sativum* resulted in highest inhibition (> 80%) of AChE activity. Our results are in agreement with their findings as they verified that inhibition of AChE activity increased with exposure time. Kim *et al*. (2013) reported some compounds from plant oils of apiaceae family, as inhibitor of AChE activity against *S. oryzae*, some other scientists also noted same results in number of insect pests (Breuer *et al*., 2003; Nathan *et al*., 2008). Our findings also demonstrated that *C. colocynthis* and *M. azedarach* extracts caused significant inhibition of α-carboxylesterase (α-CE) and β-carboxylesterase (β-CE) activities in three insect species. Outcomes indicated maximum inhibition of α-CE and β-CE activity in *T. castaneum*, *T. granarium* and *S. granarius* at 20% concentration. Dose dependent reactions of α-CE and β-CE activities were also noted in the larvae of *Choristoneura rosaceana* exposed to *M. azedarach* oil (Smirle *et al*., 1996). Mujeeb and Shakoori (2012) evidenced that Fury (synthetic pyrethroid) inhibits the carboxylesterase (CE) activity in all life stages of red flour beetle, *T. castaneum*. Koodalingam *et al*. (2011) proved that when the larvae of *Aedes aegypti* were released to extract of soapnut, *Sapindus emarginatus*, it significantly reduced the activities of AChE and β-CE, while no changes were observed in the level of α-CE activity.

Most of the physiological processes are phosphatases dependent as they play a vital role in the completion of their normal functions (Majerus *et al*., 1999). Our results showed that higher inhibition (38.57%) of acid phophatases (ACP) activity was noted in *S. granarius* exposed to 20% solution of *M. azedarach*, whereas 18.65% and 15.21% maximum inhibition of ACP was checked in *T. granarium* and *T. castaneum* feed on *C. colocynthis* treated diet. In case of alkaline phosphatases (ALP) maximum inhibited values 29.44%, 23.09% and 18.41% were recorded for *T. granarium*, *S. granarius* and *T. castaneum* respectively, by plant extract of *M. azedarach*. Phosphatases are deemed as reliable tools to assess the toxic impacts of various chemicals on physiological status of insects (Srinivas *et al*., 2004). These results are justified by Nathan *et al*. (2005) as they tested and concluded that the exposure of larvae of *Cnaphalocrocis medinalis* and *Spodoptera litura* to azadirachtin, resulted in significant inhibition of acid and alkaline phosphatases. Similarly acetone and ethanol based plant extracts of *A. indica* and *Datura inoxia* (Ali *et al*., 2017), *Artemisia annua* (Shekari *et al*., 2008), *Teucrium royleanum* (Ahmad *et al*., 2007a), *Andrachne cordifolia* (Ahmad *et al*., 2007b), *Cassia obtusifolia* (Kim *et al*., 2007), *Gloriosa superba* (Khan *et al*., 2007), *Paeonia emodi* (Khan *et al*., 2005) and *Corydalis incise* (Kim, 2002) were shown to have significant impact on the inhibition of AChE, ALP, urease, lipoxygenase, and amino transferase of various hexapods.

Conclusions

All the reported outcomes taken together indicate that both plant extracts *M. azedarach* and *C. colocynthis* release their anti-insecticidal activity by various modes of action as evidenced from mortality and numerous adverse changes examined in various important enzymes, including AChE, α-CE, β-CE, ACP and ALP of *T. castaneum*, *T. granarium* and *S. granarius*. Results revealed distinct differences in alterations of biochemical characteristics in the three stored grain insect species exposed to the botanical biocide tested. It also verified from the findings of the experiment that *T. castaneum* and *S. granarius* were more susceptible, while *T. granarium* was slightly tolerant to both toxicants. Conclusions suggest the use of these botanicals as a substitute for chemical insecticides for storage of wheat

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grains, flour and their byproducts.

Conflict of interests

The Authors have no conflict of interest.

Author's contribution

Kazam Ali: Collected experimental material, performed the experiments, analyzed the data and wrote the article

Muhammad Sagheer, Mansoor ul Hasan and Abdul Rashid: Developed protocols for plants extracts and their lethal impacts and also supervised the studies.

Muhammad Shahid: Developed protocols and supervised the enzyme studies of insects.

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فعالیت زیستی عصاره‌های گیاهان دارویی به‌عنوان سموم بازدارنده آنزیمی در برابر حشرات آفات انباری

کاظم علی[1*]، محمد ساغیر[2]، منصور الحسن[2]، عبدالرشید[3] و محمد شهید[4]

۱- مرکز بین‌المللی کشاورزی و علوم‌زیستی، آسیای مرکزی و غربی، راولپندی، پاکستان.
۲- گروه حشره‌شناسی، دانشگاه کشاورزی، فیصل‌آباد، پاکستان.
۳- گروه بیماری‌شناسی گیاهی، دانشگاه کشاورزی، فیصل‌آباد، پاکستان.
۴- گروه بیوشیمی، دانشگاه کشاورزی، فیصل‌آباد، پاکستان.
پست الکترونیکی نویسنده مسئول مکاتبه: k.ali@cabi.org
دریافت: ۱۳ خرداد ۱۳۹۹؛ پذیرش: ۱۹ مهر ۱۳۹۹

چکیده: پژوهش حاضر برای ارزیابی فعالیت زیستی عصاره گیاه هندوانه ابوجهل *Citrullus colocynthis* و زیتون‌تلخ *Melia azedarach* علیه سه حشره آفت انباری مهم شامل شپشه آرد *Tribolium castaneum*، لمبه گندم *Trogoderma granarium* و شپشه برنج *Sitophilus granarius* انجام شد. سمّیت و فعالیت مهار آنزیم استیل کولین استراز (AChE)، آلفاکربوکسی استراز (α-CE)، بتاکربوکسیل استراز (β-CE)، اسید فسفاتازها (ACP) و آلکالین فسفاتازها (ALP) در سه گونه حشره نسبت به دو عصاره گیاه در چهار غلظت ۵، ۱۰، ۱۵ و ۲۰ درصد ارزیابی شد. حداکثر مرگ‌ومیر در شپشه برنج (۳۴/۲۹ درصد) توسط زیتون تلخ مشاهده شد. در شپشه آرد و لمبه گندم حداکثر مقادیر ثبت شده برای مرگ‌ومیر به‌ترتیب برابر با ۳۰/۸۷ و ۱۸/۹۵ درصد توسط عصاره زیتون تلخ بود. عصاره گیاه هندوانه ابوجهل بیش‌ترین میزان مرگ‌ومیر را به‌ترتیب در شپشه آرد، شپشه برنج و لمبه گندم به‌ترتیب برابر با ۲۱/۹۲، ۱۹/۱۸ و ۱۶/۸۹ درصد بود. یافته‌ها ثابت کرد که هر دو عصاره گیاه اثرات کشنده مناسبی روی گونه‌های حشرات آزمایش شده داشتند. قرار گرفتن حشرات در معرض عصاره‌های گیاهان نیز منجر به مهار معنی‌دار آنزیم‌های AChE، α-CE، β-CE، ACP و ALP شد. همه آنزیم‌های آزمایش شده در سه حشره توسط عصاره گیاه زیتون‌تلخ حداکثر مهارکنندگی را داشتند، به‌جز عصاره گیاه هندوانه ابوجهل که α-CE در شپشه برنج و ACP در لمبه گندم و شپشه برنج را بیشتر مهار کردند. نتایج نشان می‌دهد که عصاره گیاه زیتون‌تلخ روی آفات انباری تأثیر بیش‌تری داشت. بنابراین، عصاره‌های گیاهی مطالعه شده به‌عنوان یک جایگزین مطمئن به‌جای سموم شیمیایی برای حفاظت محصولات انباری در برابر حشرات آفت پیشنهاد می‌شوند.

واژگان کلیدی: آفات انباری، مهار آنزیم، اثرات کشنده، سمّیت

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