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IMPACT OF EXTRACTS OF *Azadirachta indica* AND *Datura inoxia* ON THE ESTERASES AND PHOSPHATASES OF THREE STORED GRAINS INSECT PESTS OF ECONOMIC IMPORTANCE

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Research was carried out for the appraisal of toxic and anti-enzymetic action of acetone extracts of *Datura inoxia* and *Azadirachta indica* against *Tribolium castaneum*, *Trogoderma granarium* and *Sitophilus granarius* in the grain research training and storage management cell of department of entomology and in protein molecular biology laboratory (PMBL) department of biochemistry (UAF) during the year 2014. Various dilution levels viz., 5.0, 10.0, 15.0 and 20.0% of both plants extracts were assessed against test insects. Toxic effect of plant extracts in *T. castaneum*, *T. granarium* and *S. granarius* was observed after an exposure period of 2, 4, 6, 8 and 10 days while inhibition of different enzymes acetylcholinesterase (AChE), α-carboxylesterase (α-CE), β-carboxylesterase (β-CE), acid phosphatases (ACP) and alkaline phosphatases (ALP) were calculated spectrophotometrically in the survivors of toxicity assay. The results evidenced that both plant extracts had lethal effects against three stored grain insect species. Comparison of means of mortality in *T. castaneum*, *T. granarium* and *S. granarius* proved that *A. indica* extract was more effective, causing maximum 38.41, 24.35 and 28.56% mortality, respectively. *D. inoxia* resulted in 15.12, 13.52 and 14.07% maximum mortality in *T. castaneum*, *T. granarium* and *S. granarius*, respectively. The results also revealed significant inhibition of AChE, α-CE, β-CE, ACP and ALP upon exposure to various concentrations of tested plants. Plant extract of *A. indica* proved more efficient for the inhibition of all test enzymes in three stored grain insect pests. The results suggest the ability of using these plants extracts for wheat flour and grains protection as a safe alternative to insecticides.
Keywords: Acetone extract, anti-enzymatic, inhibition, lethal effects, toxicity.

INTRODUCTION

In Pakistan, *Tribolium castaneum* (Herbst.), *Trogoderma granarium* (Everts), *Sitophilus granarius* (L.), *Rhizopertha dominica* (F.), *Sitotroga cerealella* (Oliv.) and *Sitophilus oryzae* (L.) are reported as important insect pests of stored grains and cereals (Iqbal *et al*., 1992). *T. castaneum* H. (Coleoptera: Tenebrionidae), *S. granarius* L. (Coleoptera: Curculionidae) and *T. granarium* E. (Coleoptera, Dermestidae) are most common and highly destructive pest of stored cereals and their products all over the world (Lowe *et al*., 2000). The larvae and adults, of red flour beetle (*T. castaneum*), not only feed on wheat flour but they can also feed on previously damaged grains and dried fruits attacked by other insect pests (Sharaby, 1988). Granary weevil (*S. granarius*), is capable of causing significant losses (White and Leesch, 1996). Grubs of khapra beetle (*T. granarium*) usually occur in high numbers, cause significant losses and can persist for a long time period as inactive state (Dwivedi and Shekhawat, 2004).

Currently, protection of stored grain from insect damage is highly dependent on synthetic pesticides more commonly phosphine fumigation. Scientists resulted that use of these pesticides introduced the hazardous impacts on almost all life forms (Bhaduri *et al*., 1989; Brown, 1968; Georghiou and Taylor, 1977, Desneux *et al*., 2007). These synthetic formulations also induce the problems like resistant pest populations, pesticide residues and insecticides deregulations (Rajeskaran and Baker, 1994). They also had harmful effects on non-target organisms (Jembere *et al*., 1995; Okonkwo and Okoye, 1996). These problems have highlighted the need for the development of new types of selective insect-control alternatives. The plant kingdom could be a rich source of a large number of chemicals which could be developed as control agents against stored grain pests successfully (Arnason *et al*., 1989). Repellent, toxicant (Sagheer *et al*., 2014), anti-feedants (Saxena *et al*., 1984; Banchio *et al*., 2003) and anti enzymatic (wang *et al*., 2014) effects from several plant products have been reported against a number of insects that attack stored products (Hasan *et al*., 2006; Ali *et al*., 2012).

In hexapods, many essential physiological processes like digestion, reproduction, moulting, and metabolism of juvenile hormone make use of esterases as a vital component (Lassiter *et al*., 1995; Shanmugavelu *et al*., 2000). Esterases are also involved in the detoxification of synthetic insecticides by

converting them into metabolites of low toxicity (Wheelock *et al*., 2005). In this manner, esterases are the enzymes which are exploited as markers to estimate the effect of toxicants at variety of target insects (Fourcy *et al*., 2002; Wheelock *et al*., 2005; Smirle *et al*., 2010). In addition to estrases, phosphatases are the enzymes identified to perform a key role in diverse physiological mechanisms (Majerus *et al*., 1999). These enzymes are also believed to be trustworthy markers for measuring the toxic impacts of several lethal compounds on the physiology of test insects (Srinivas *et al*., 2004; Nathan *et al*., 2005a). Thereby, to assess the toxicity of synthetic and botanical insecticides, phosphatases and esterases have been recognized as sensitive and precise biomarkers (Galloway *et al*., 2002; Bonacci *et al*., 2004; Shekari *et al*., 2008).

Keeping in view the whole scenario, present research was designed with the objective to evaluate the insecticidal and anti-enzymatic potential of plant extracts of *Azadirachta indica* and *Datura inoxia* in three stored grain insects species *T. castaneum*, *T. granarium* and *S. granarius*.

MATERIALS AND METHODS

***Collection and rearing of insects*:** The heterogeneous populations of *Tribolium castaneum* H. (Red flour beetle), *Trogoderma granarium* E. (Khapra beetle) and *Sitophilus granarius* L. (Granary weevil) were collected from grain market and flour mills located in the district Faisalabad, Punjab Province of Pakistan. The population was brought back for rearing in the laboratory of Stored Grain Management Cell, Department of Entomology, University of Agriculture, Faislabad at room temperature for getting homogeneous population.

Insect culture was maintained in sterilized plastic jars (1.0 kg capacity). Wheat flour (prefer food of *T. castaneum*) was used as culture medium for *T. castaneum* (Imura, 1991), while wheat grains were used for the rearing of *T. granarium* and *S. granarius*. Jars were kept in incubators (SANYO) at 30±2°C and 65±5% R.H. for *T. castaneum* and *T. granarium*, while *S. granarius* population was maintained at 27±2°C and 70±5% R.H. Adult beetles were sieved out by sieves (60mm mesh size for *T. castaneum*, 40mm mesh size for *T. granarium* and *S. granarius*) after 5 days. Sieved flour and grains along with eggs were shifted into jars (1.0 kg capacity) and placed in incubators at optimum conditions for getting homogenous population.

***Collection and preparation of plant extracts*:** Leaves of *Azadirachta indica* were collected from the farm fields of University of Agriculture, Faisalabad, and fruits of *Datura inoxia* were collected from district Layyah of Punjab Province of Pakistan. The collected leaves of *A. indica* and fruits of *D. inoxia* were washed with sterilized water, and placed in shade until leaves and fruits of plants are dried. Dried leaves and fruits were brought in form of fine powders by grinding them into electrical grinder (Pascall, 20069). The stock solution of plant extracts was obtained by mixing 50.0g powder in 100 ml acetone for a period of 24 h at 220 rpm using Rotary Shaker (IRMECO, OS-10) (Khan *et al*., 2013). After 24 hours, the filtration was done by filter paper, and then extracts were set on rotary evaporator until all the solvent has been evaporated as discussed by Hasan *et al*. (2006) and Sagheer *et al.* (2014). Thus, chemical extracts obtained were considered as stock solution and were put into clean and air tight plastic lid bottles (250 ml capacity). For each plant, stock solution was stored at 4.0°C in refrigerator (Haier) from which various concentrations (5.0, 10.0, 15.0 and 20.0%) were prepared with acetone as solvent.

Bioassays:

***Toxic effect of plant extracts*:** Various concentrations (5.0, 10.0, 15.0, 20.0%) of plant extracts of *A. indica* and *D. inoxia* were applied uniformly on wheat flour for *T. castaneum* and on wheat grains for *T. granarium* and *S. granarius*. Acetone was allowed to evaporate and the air dried treated wheat flour (40.0g) and wheat grains (40.0g) were put into treatment jars (250 ml capacity). Fifty adults of *T. castaneum* and *S. granarius*, while larvae in case of *T. granarium* were released separately on the treated flour and grains in each jar, separately. These jars were placed in incubator under optimum conditions. Three replications of each treatment were made and data regarding mortality effects induced by plant extracts of *A. indica* and *D. inoxia* (space) was observed after 2, 4, 6, 8 and 10days. The survivors, larvae of *T. granarium* and adults of *T. castaneum* and *S. granarius* were stored in phosphate buffer solution for various enzymes assays.

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

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

Mo = Mortality observed in treatments, Mc = Mortality observed in control

Inhibition assay of esterases and phosphatases in survivors (*T. castaneum*, *T. granarium* and *S. granarius*) of toxicity assay*:*

***Preparation of whole body homogenate*:** The survivors (adults of *T. castaneum* and *S. granarius*, larvae of *T. granarium*) of toxicity bioassay stored in buffer solution were washed with distilled water, and the adhering water was completely removed from the body surface by blotting with tissue paper. The larvae and adults were separately homogenized using a teflon hand homogenizer in ice-cold sodium phosphate buffer (20 mM, pH 7.0) for eventual estimation of esterases and phosphatases inhibition. The whole body homogenates were centrifuged (8000 rpm) for 20 minutes and the clear supernatants were used for the biochemical analyses. Solutions for homogenization and glassware were kept at 4.0°C prior to use, and the homogenates were held on ice until used for various assays (Koodalingam *et al*., 2011).

***Determination of acetylcholinesterase (AChE)*:** Acetylcholinesterase (AchE) activity in the whole body homogenates of *T. castaneum*, *T. granarium* and *S. granarius* were spectrophotometrically measured according to Ellman *et al.* (1961) with slight modification using acetylthiocholine chloride as substrate. For the determination of acetycholinesterase, 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 in the 100 µl of enzyme solution taken from whole body homogenates, it was incubated at 25°C for 5 mins. Then 400 µl of freshly prepared 0.3% Fast blue B salt in 3.3% SDS (sodium dodecyl sulphonate) was added to stop reaction. After that the sample was run through spectrophotometer. 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 were measured by the method of van Asperen (1962).The α-carboxylesterase activity was recorded by using α-naphthylacetate as substrate. For this purpose, 50µl of α-naphthylacetate (250µM) and 1 ml of sodium phosphate buffer (20 mM, pH-7.0) was added in 50µl whole body homogenates. This solution was incubated at 30°C for 20 mins. 400µl 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 430nm. Same procedure was followed for β-carboxylesterase activity, except 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 50µl enzyme solution. The solution was incubated at 37°C 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 450µl Tris HCl (50mM, pH-8) was used in place of sodium acetate buffer.

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

Enzyme Inhibition

$$\text{Enzyme Inhibition (\%)} = \frac{(\text{ODb} - \text{ODo})}{\text{ODb}} \times 100$$

ODb = Optical density of blank (control treatment), ODo = Optical density of treatments

***Statistical analyses*:** In all trials, separate ANOVA was performed for each insect against plant extract of *Datura inoxia* and *Azadirachta indica*. The variations in the inhibition levels of various enzymes of *T. castaneum*, *T. granarium* and *S. granarius* and differences among various experimental units recorded from toxicity assays were tested by using mean difference Tuckey-HSD test for statistical significance by using Statistica-6.0. The acceptance level of statistical significance was $p\leq0.05$ in all cases.

RESULTS

The outcomes showed that various interactions (time and concentration) of plant extract of *D. inoxia* had significant effect on the mortality of *T. castaneum*, *T. granarium* and *S. granarius* (Table 1). The results revealed that after 10d interval, 15% concentration proved more effective in case of *T. castaneum*, while for *T. granarium* and *S. granarius*, 20% concentration was proved more lethal. It was notified that *D. inoxia* extract reported almost same toxic effect to *T. castaneum*, *T. granarium* and *S. granarius*, as it induced maximum mean mortality of 15.12% in *T. castaneum* at 15% concentration, 13.52% in *T. granarium* at 20% dilution level and 14.07% in *S. granarius* at 20% concentration, after an interval of 10 days. At 20% concentration, after 10 days the calculated mean mortality for *T. castaneum* was 8.22%, which is statistically lower than 15% concentration after same time interval (10d). Findings, proved that with increase in interaction of concentrations and time intervals the percent mean mortality of *T. granarium* and *S. granarius* also increased, while in case of *T. castaneum* mortality increases up to 15% concentration, while at 20% dilution level it start to decrease. At 5% concentration, after 2d interval, *D. inoxia* reported no mortality against *T. granarium* and *S. granarius*, while the same interaction (5% concentration after 2d) reported 2.0% mean mortality against *T. castaneum*. Table-1, also described that after 10d exposure to the treated diet of *D. inoxia* at 5, 10, 15 and 20% concentrations resulted in 8.22, 13.69, 15.12, 8.22% mean mortality in *T. castaneum*, 6.76%, 7.44%, 10.81%, 13.52% mean mortality in *T. granarium* and 6.85, 8.90, 12.01 14.07% mean mortality in *S. granarius*, respectively. Results indicating that in case of *T. castaneum* higher mean mortality were noted at 5% dilution of *D. inoxia* than 20% dilution level after 4,6,8 and 10d interval, as these intervals forced 4.06, 4.76, 6.85 and 8.22% mean mortality at 5% concentration, 2.71, 4.08, 6.16 and 8.22% mean mortality at 20% concentration, respectively.

Table 1 indicates that the population of *T. castaneum*, *T. granarium* and *S. granarius* effected greatly when exposed to the plant extract of *A. indica* at various interactions of time intervals (2, 4, 6, 8 and 10d) and concentration (5, 10, 15 and 20%). Higher mean mortality (38.41%) was observed in *T. castaneum* after 10d interval at 20% concentration. *A. indica* reported a maximum 24.35% mortality against *T. granarium* while 28.56% in *S. granarius* after 10d interval at 20% dilution level, 32.87% mean mortality observed at 15% dose rate, after 10d intervals was even higher than the *T. granarium* (24.35%) and *S. granarius* (28.56%) mean mortalities which

Table 1. Mean comparison of percent mortality in *T. castaneum*, *T.granarium* and *S. granarius*induced by various interactions of time and concentration of plant extracts of *D. inoxia* and *A. indica* using Abott's Formula (1925).

Time (Days)	Conc. (%)	Mean Mortality (%) ± S.E					
		Datura inoxia			*Azadirachta indica*		
		T. castaneum	*T. granarium*	*S. granarius*	*T. castaneum*	*T. granarium*	*S. granarius*
2	5	2.0±0.46j	0.00 k	0.00k	8.67±1.17l	1.34±0.67k	4.73±1.17k
	10	4.67±0.67hij	1.34±0.33jk	2.71±0.67ijk	15.33±0.67hijk	2.68±1.31jk	8.11±0.68jk
	15	7.33±1.15efgh	2.68±0.40hijk	4.06±0.68hi	16.0±1.33hijk	7.78±0.33gh	9.46±1.34jk
	20	2.71±0.67j	2.68±1.67hijk	4.73±1.18ghi	20.33±1.15efgh	10.06±0.67efg	12.84±1.81hi
4	5	4.06±1.17hij	0.67±0.67ijk	0.68±0.34jk	9.46±0.67l	3.35±1.67ijk	6.12±0.67jk
	10	7.43±0.83efgh	3.35±0.69ghij	4.76±1.33ghi	15.54±1.67hijk	6.04±0.57hi	12.93±1.68hi
	15	8.79±1.68def	4.69±1.44efgh	5.44±0.47ghi	18.25±3.13ghij	9.39±2.11fg	15.65±1.59efg
	20	2.71±0.67j	6.71±0.77def	6.80±1.18fgh	23.65±1.67defg	12.75±1.89de	19.73±1.67de
6	5	4.76±1.18ghij	3.35±1.33ghij	3.40±0.68ij	12.24±1.79kl	5.37±1.33hij	10.20±1.11ij
	10	10.20±1.53cde	5.37±1.47defgh	6.80±1.17fgh	19.05±1.18fghi	8.05±1.67gh	16.33±1.18efg
	15	12.93±0.67bc	6.04±0.67defg	9.52±2.32def	24.49±1.68def	11.41±0.75ef	17.69±2.59ef
	20	4.08±0.33hij	8.05±1.33bcd	10.20±0.34cde	29.25±2.33cd	18.12±1.83c	21.53±1.21cd
8	5	6.85±1.33efgh	4.06±1.43fghi	3.42±1.53ij	13.01±1.68jkl	6.08±1.59hi	13.01±2.34hi
	10	11.64±0.68bcd	7.44±0.86cde	7.53±2.24efg	20.55±2.34efgh	10.14±1.67efg	18.54±0.98de
	15	13.69±2.88ab	7.44±1.59cde	11.64±1.79bcd	28.08±3.33cd	14.87±2.58d	22.34±1.30bc
	20	6.16±1.67fghi	10.14±0.67bc	13.70±1.15ab	36.29±2.05ab	21.62±0.88b	25.81±1.96ab
10	5	8.22±1.17defg	6.76±1.67def	6.85±1.18fgh	13.69±0.67ijkl	8.11±0.58gh	15.07±2.67gh
	10	13.69±1.33ab	7.44±1.59cde	8.90±1.77def	25.34±2.55de	11.49±1.44ef	23.28±1.81bc
	15	15.12±1.59a	10.81±0.86ab	12.01±2.34bc	32.87±1.68bc	19.59±1.33bc	25.39±2.79ab
	20	8.22±0.68defg	13.52±0.68a	14.07±1.68a	38.41±2.37a	24.35±2.37a	28.56±1.63a

ANOVA's were performed separately for each insect against each plant extract and similar letters within treatment are statistically same ($p < 0.05$).

were induced by their maximum interaction (after 10d at 20% concentration). The results evidenced that *A. indica* extract proved more effective in case of *T. castaneum*, while for *T. granarium* and *S. granarius* also affected adversely. The mean mortality observed after 2d interval, at 5, 10, 15 and 20% were 8.67, 15.33, 16.0 and 20.33% in *T. castaneum*, 1.34, 2.68, 77.78 and 10.06 % for *T. granarium*, 4.73, 8.11, 9.46 and 12.84% for *S. granarius*, respectively. In *T. castaneum* at 20% concentration, 29.25% mean mortality after 6d and 36.29% mean mortality after 8d were checked, while after 6 and 8d, at 20% dilution observed mean mortality were 18.12, 21.62% for *T. granarium* and 21.53, 25.81 for *S. granarius*, respectively. The outcomes also reveal that, after 10d at 5, 10, 15 and 20% concentrations, the calculated mean mortality were in the following order 13.69<25.34<32.87<38.41% for *T. castaneum*, 8.11<11.49<19.59<24.35% for *T. granarium*, while in case of *S. granarius* mean mortality was 15.07<23.28<25.39<28.56%, respectively.

Effect of both plant extracts on the inhibition of acetylcholinesterase (AChE) activity is shown by Figure 1. Findings proved that maximum inhibition (30.01%) on AChE activity was studied in *T. castaneum* at 15.0% concentration of *D. inoxia*, while at 20.0% concentration of *D. inoxia* maximum inhibition (13.57 and 25.16%) on AChE acitivity was noticed in *T. granarium* and *S. granarius*, respectively. Minimum dilution level (5.0%) of *D. inoxia*, induced lower inhibition (6.22 and 13.12%) of AChE activity in *T. granarium* and *S. granarius*, respectively. While 22.78% inhibition of AChE activity was found in *T. castaneum* at 5.0% concentration level of *D. inoxia*.

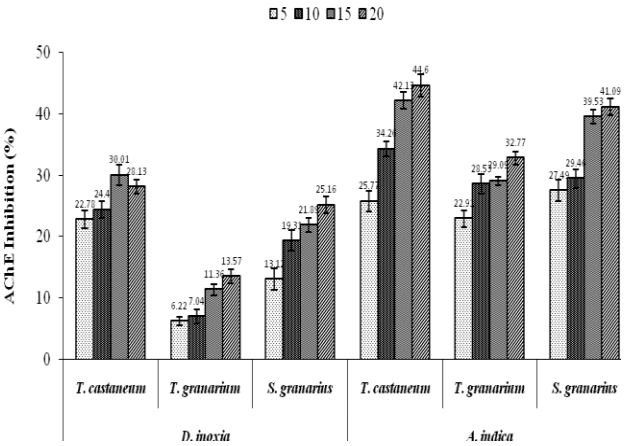

Figure 1. Effect of various concentrations of *D. inoxia* and *A. indica* extracts on the inhibition of AChE activity of *T. castaneum*, *T. granarium* and *S. granarius.*

Furthermore, in 10.0 and 20.0% concentrations of *D. inoxia*, 24.40 and 28.13% inhibition of AChE activities were checked by the plant extract of *D. inoxia* against *T. castaneum*. Outcomes signifying that the effect of *A. indica*'s concentration is highly efficient to the inhibition of AChE (acetylcholinesterase) activity in *T. castaneum*, *T. granarium* and *S. granarius*. Initial concentration (5.0%) of *A. indica* extract reported 27.49% inhibition of AChE activity in *S. granarius*, which is slighty higher than *T. castaneum* (25.77%) at same concentration (5.0%). *A. indica* evidenced 22.91% inhibition of AChE activity in *T. granarium* at 5.0% concentration. But, maximum dilution level (20.0%) induced slightly higher inhibition of AChE activity (44.60%) in *T. castaneum* than *S. granarius* (41.09%) and for *T. granarium* 32.77% inhibition of AChE activity was noticed. (space) In *T. castaneum*, 34.26 and 42.13% inhibition of AChE activity was recorded at 10.0% and 15.0% concentrations of *A. indica* (space) respectively, while 28.53% at 10.0% of *A. indica*, and 29.09% inhibition of AChE activity was observed at 15.0% concentration of *A. indica* (space) in *T. granarium*. For *S. granarius*, 29.46 and 39.53% inhibition of AChE activity were examined at 10.0 and 15.0% dilution levels of *A. indica*. In case of α-carboxylesterase (α-CE) plant extract of *D. inoxia* proved less effective for *T. castaneum* as it induced maximum 12.13% inhibition at 15.0% concentration and showed higher effect against *S. granarius* as it reported 25.92% inhibition of α-CE activity at 15.0% concentration (Fig. 2). In *T. granarium* 19.17% was the highest value for the inhibition of α-CE activity at 20.0% concentration of *D. inoxia*. In case of *T. castaneum*, higher concentration 20.0% (10.17% inhibition of α-CE) of *D. inoxia* proved less effective than 10.0 and 15.0% concentrations for inhibition of α-CE activity, as both these concentrations reported higher values (11.91 and 12.13%, respectively) for the inhibition of α-CE activity. Lower concentration (5.0%) of *D. inoxia* forced 7.38, 4.69 and 6.33% inhibition of α-CE activity in *T. castaneum*, *T. granarium* and *S. granarius*, respectively. Fig. 2 is also representing that *A. indica* extract showed better results against *T. castaneum* as it reported 37.81% inhibition of α-CE activity at 20.0% concentration, while in other two insect species (*T. granarium* and *S. granarius*) the observed values for inhibition of α-CE activity at 20.0% concentration of *A. indica* were 25.67 and 23.19%. *A. indica* proved less effective against *S. granarius* as it showed low percent inhibition of α-CE enzyme than *T. castaneum* and *T. granarium*. At 5.0% concentration, 17.04, 11.43 and 8.36% inhibition of α-CE activities were checked in *T. castaneum*, *T. granarium* and *S. granarius*, respectively. Medium concentrations (10.0, 15.0%) of *A. indica* forced 22.67 and 29.44% inhibition of α-CE activity in *T. castaneum*, while 19.57 and 21.93% inhibition of α-CE activity in *T. granarium*, and in *S. granarius* reported inhibition of α-CE activities were 16.54 and 22.81%, respectively.

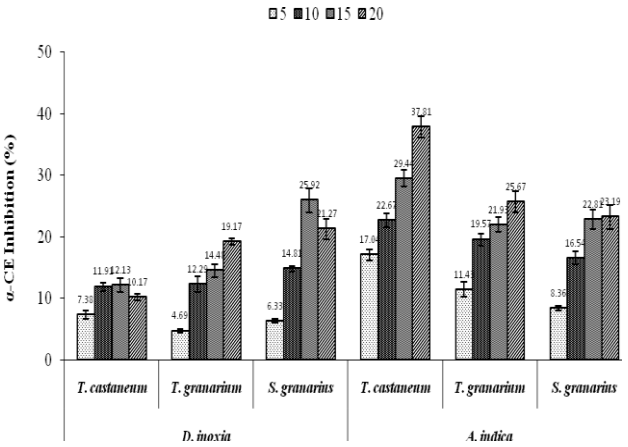

Figure 2. Effect of various concentrations of *D. inoxia* and *A. indica* extracts on the inhibition of α-CE activity of *T. castaneum*, *T. granarium* and *S. granarius.*

The results verified that 5.0, 10.0, 15.0 and 20.0% concentrations of *D. inoxia* (space) forced 14.88, 12.70, 21.13 and 27.50% inhibition of β-CE (β-carboxylesterase) activity, in (space) *T. castaneum* respectively (Fig. 3). All dilution level (5.0, 10.0, 15.0, 20.0%) of *D. inoxia* showed almost same effect against *T. granarium* on inhibition of β-CE activity as they reported 19.02, 21.06, 20.24 and 22.48% inhibition respectively. In *S. granarius*, observed values for inhibition of β-CE activity were 16.83, 24.31, 26.42 and 28.79% at 5.0, 10.0, 15.0 and 20.0% concentration of *D. inoxia*, respectively.

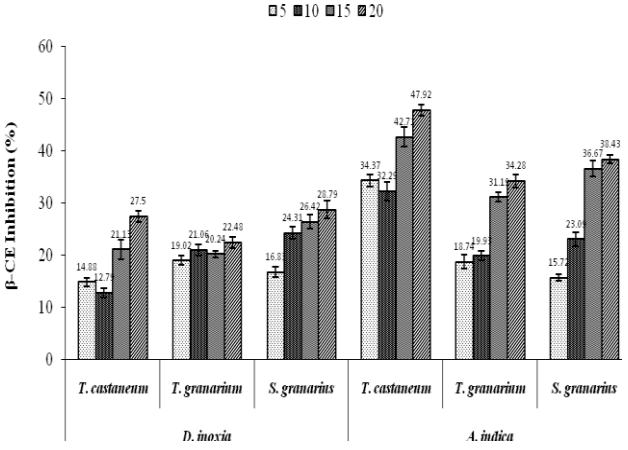

Figure 3. Effect of various concentrations of *D. inoxia* and *A. indica* extracts on the inhibition of β-CE activity of *T. castaneum*, *T. granarium* and *S. granarius.*

A. indica reported high effect on percent inhibition of β-CE (β-carboxylesterase) activity in *T. castaneum*, *T. granarium* and *S. granarius* than the plant extract of *D. inoxia*. Fig. 3, showing comparison of means that higher concentration induced high values of percent inhibition of β-CE activity. At 20.0% concentration of *A. indica*, 47.92, 24.28 and 38.43% inhibition of β-CE activity was observed in *T. castaneum*, *T. granarium* and *S. granarius*, respectively. For *T. castaneum* 42.71% inhibition of β-CE activity was noticed at 15.0% concentration which was higher than the percent inhibition of β-CE activity reported at 20.0% concentration of *A. indica* in *T. granarium* and *S. granarius*. *A. indica* reported 32.29% inhibition of β-CE activity at 10.0% which was slightly lower than the 34.37% inhibition of β-CE activity reported at 5.0% concentration in *T. castaneum*. The effect of various concentrations of *A. indica* 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 for *T. granarium* and *S. granarius*, were 18.74 and 15.72%, at 5.0% dilution level of *A. indica* respectively.
Inhibition of acid phosphatases in *T. castaneum*, *T. granarium* and *S. granarius* resulted by various dilution levels of both plant extracts (*D. inoxia* and *A. indica*) is represented by Fig. 4.

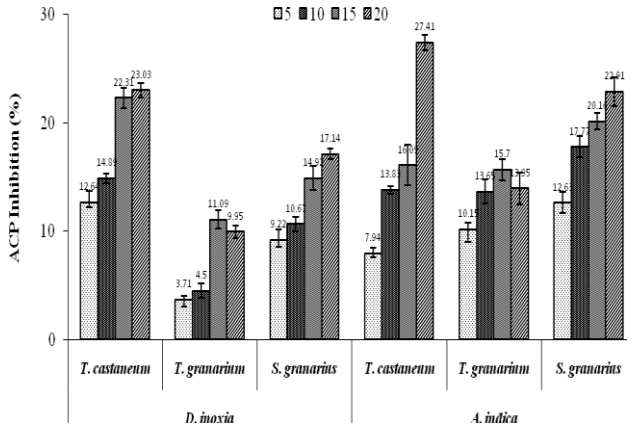

Figure 4. Effect of various concentrations of *D. inoxia* and *A. indica* (space) extracts on the inhibition of ACP activity of *T. castaneum*, *T. granarium* and *S. granarius.*

Plant extract of *D. inoxia* proved less effective for *T. granarium* as it reported a maximum inhibition (11.09%) on ACP activity at 15.0% concentration, while at 20% concentration 23.03 and 17.14% inhibition of ACP activity were noticed against *T. castaneum* and *S. granarius* respectively. At 20.0% concentration rate of *D. inoxia*, 9.95% inhibition of ACP activity was observed in *T. granarium.* Lower concentration (5.0%) *D. inoxia* confirmed 12.64% (*T. castaneum*), 3.71% (*T. granarium*) and 9.22% (*S. granarius*) inhibition of ACP activity. Again *T. castaneum* was most affected insect species by the plant extract of *A. indica* for percent inhibition of ACP (acid phosphatase) activity, as it reported maximum 27.41% inhibition of ACP activity at 20.0% concentration. In *T. granarium* and *S. granarius* 13.95% and 22.91% inhibition of ACP activity was noted at 20.0% concentration *A. indica*, respectively. Initially *T. castaneum* was less affected for inhibition of ACP activity by plant extract of *A. indica* at 5.0% concentration. Lower concentration (5.0%) *A. indica* imposed 7.94%, 10.15%, and 12.63% inhibition of ACP activity in *T. castaneum*, *T. granarium* and *S. granarius*, respectively.
Only 2.67%, 4.67% and 8.33% inhibition of ALP (Alkaline Phosphatase) activity was observed at 5.0% concentration of *D. inoxia* in *T. castaneum*, *T. granarium* and *S. granarius*, respectively (Fig. 5).

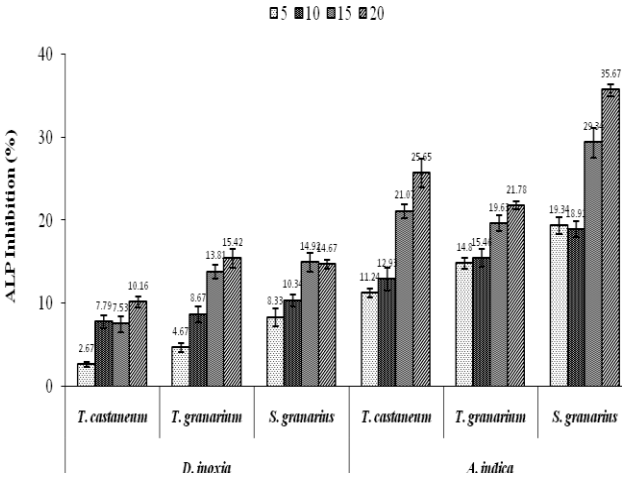

Figure 5. Effect of various concentrations of *D. inoxia* and *A. indica* extracts on the inhibition of ALP activity of *T. castaneum*, *T. granarium* and *S. granarius.*

Higher concentration (20.0%) of *D. inoxia* reported 10.16% inhibition of ALP activity in *T. castaneum*, 15.42% inhibition of ALP activity in *T. granarium*, and 14.67% inhibition of ALP activity in *S. granarius*. 10.0 and 15.0% dose rate of *D. inoxia* reported almost same (7.79 and 7.53%) inhibition of ALP activity in *T. castaneum*, while these dilution levels (10.0 and 15.0%) forced 8.67 and 13.81% inhibition of ALP activity in *T. granarium* and 10.34 and 14.92% inhibition of ALP activity in *S. granarius*, respectively. Plant extract of *A. indica* showed maximum inhibition of ALP (Alkaline Phosphatase) activity (35.67%) in *S. granarius* at 20.0% concentration (Fig. 5). The reported values for the inhibition of ALP activity in *T. castaneum* and *T. granarium* at 20.0% dilution of *A. indica* were 25.65 and 21.78%, respectively. Lesser concentration (5.0%) of *A. indica* stated 11.24, 14.80

and 19.34% inhibition of ALP activity in *T. castaneum*, *T. granarium* and *S. granarius*, respectively. Other two dilution levels (10.0 and 15.0%) of *A. indica* reported 12.93 and 15.46% inhibition of ALP activity in *T. castaneum*, 15.46% and 19.63% inhibition of ALP activity in *T. granarium*, while 18.91 and 29.34% inhibition of ALP activity was checked in *S. granarius*, respectively (Fig. 5).

DISCUSSION

Present studies reported that toxicity of plant extracts of *Azadirachta indica* and *Datura inoxia* were relatively increased with increasing exposure times and amount of dilution levels. Our outcomes revealed that plant extract of *A. indica* induced maximum mortality 38.41% in *T. castaneum* at maximum interaction of time (10d) and concentration (20.0%), while at same combination of time and dilution level of *A. indica*, 24.35% and 28.56% mortality were the maximum noted values for *T. granarium* and *S. granarius*, respectively. *D. inoxia* showed almost same results against three insect species and reported maximum 15.12%, 13.52% and 14.07% mortality in *T. castaneum*, *T. granarium* and *S. granarius* respectively, after 10d interval. Similar findings were also achieved in other studies showing the toxic efficacy of extracts from some aromatic plants against insects and mites (Benzi *et al*., 2009). Bibi *et al*. (2008) recorded similar results and concluded that mortality of adult of *T. castaneum* increased with increase in concentration at maximum exposure period. *Datura inoxia* plays insecticidal activities against some pests in different parts of the world (Khalequzzman and Islam, 1992; Lohra *et al*., 2002). Extracts and powders achieved from neem plant (*Azadirachta indica* A. Juss) seeds have been accounted to supply reasonable management of stored grains (Lale and Mustapha, 2000; Maina and Lale, 2004). The *Azadirachta indica* and *Nicotiana tabacum* has long been known for its pesticidal properties (Debashri and Tamal, 2012). In my findings, plant extract of *A. indica* proved more effective as they induced higher mortality. Our results are supported by the Anwar *et al.* (2005), as they evaluated the neem (*Azadirachta indica*) oil against four insect pests of stored grains *Rhyzopertha dominica*, *Sitophilus granarius*, *Tribolium castaneum* and *Trogoderma granarium* at different dose rates (5%, 10%, 15% and 20%) under natural conditions at various time points (30, 60, and 90 days) in a warehouse. They recorded that mortality increased with the increase in concentration of the spray material. Odeyemi and Ashamo (2005) evaluated same conclusions that exposure of larvae and adults of *T. granarium* to neem extract decreases infestation and injury to groundnut seeds by increasing the percent mortality of both stages (larvae and adults) at higher dilution levels. Mamun *et al*. (2009) evaluated the toxicity of six botanicals, Bazna (*Zanthoxylum rhetsa*), Ghora-neem (*Melia sempervirens*), Hijal (*Barringtonia acutangula*), Karanja (*Pongami apinnata*), Mahogoni (*Swietenia mahagoni*) and Neem (*Azadirachta indica*) using three solvents acetone, methanol and water, against *Tribolium castanium* (Herbst). They reported, seed extract of all plants had direct toxic effect in *T. castanium*. Neem seed extract showed the highest toxic effect (mortality, 52.50%).

Plant extracts of *D. inoxia* and *A. indica* verified decent anti-enzymetic activities in three insect species (*T. castaneum*, *T. granarium* and *S. granarius*) of stored grains and their products. Present studies proved that increased exposure and higher concentrations induced maximum inhibition of tested enzymes (AChE, α-CE, β-CE, ACP and ALP) in *T. castaneum*, *T. granarium* and *S. granarius*, which resulted in higher mortality of insects. As a sequel to our results, it was noted that plant extracts forced significant inhibition of α-carboxylesterase (α-CE) and β-carboxylesterase (β-CE) in *T. castaneum*, *T. granarium* and *S. granarius*. Reported findings indicated, a maximum 37.81% inhibition of α-CE activity in *T. castaneum*, 25.67% inhibition of α-CE activity in *T. granarium* by plant extract of *A. indica* at 20.0% concentration, while 15.0% dilution level of plant extract of *D. inoxia* induced 25.92% inhibition of α-CE activity in *S. granarius*. Phosphatases are identified to play a key role in most physiological processes (Majerus *et al*., 1999). These enzymes also considered as reliable marker enzymes to assess the deleterious effects of various toxicants on physiological status of insect pests (Srinivas *et al*., 2004; Nathan *et al*., 2005a). At 20.0% concentration, our findings tested that maximum inhibition 27.41%, 15.70% and 22.91% of ACP activity reported in *T. castaneum*, *T. granarium* and *S. granarius* (space) exposed to *A.indica.*

Acetylcholinesterase (AChE) is a main target for different insecticides, and its inhibition by chemicals obtained from plant essential oils reported in previous research (Abdelgaleil *et al*., 2009; Kang *et al*., 2013), while Carboxylesterases evidenced as predominant enzyme in many tissues of a number of insects (Park and Kamble, 1999). Yeom *et al*. (2013) reported isoeugenol found in Myrtaceae plant essential oils and they evidenced that acetylcholinesterase inhibition was exhibited in male of cockroaches, with LC_{50} of 0.22 mg/mL. Kim *et al*. (2013) also noticed some compounds from essential oils of Apiaceae family, as inhibitor of acetylcholinesterase activity against *Sitophilus oryzae*. Same findings by other biopesticides were also reported in numerous insect pests (Wanchun *et al*., 1999; Breuer *et al*., 2003; Nathan *et al*., 2008).Based on our results all the plants inhibit the AChE activity but initially at lower dilution levels inhibition was also lower and inhibition of AChE increases with increase in dilution rate. These studies are supported by Wang *et al*. (2014), they checked four essential oils extracted from *Citrus limonum*, *Litsea cubeba*, *Cinnamomum cassia* and *Allium sativum* against *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) pest of stored poultry feed. They reported that all essential oils reported significant inhibition

of AChE activity, but essential oil of *A. sativum* forced maximum inhibition (>80%) of AChE activity, they also verified that with passage of time inhibition of AChE activity also increased. (space) Concentrations dependent responses of α-CE and β-CE activities were checked in the larvae of *Choristoneura rosaceana* exposed to *Melia azedarach* oil (Smirle *et al*., 1996). Our studies resulted in 47.92% inhibition of β-CE activity in *T. castaneum*, 34.28% inhibition of β-CE activity in *T. granarium* and 38.43% inhibition of β-CE activity in *S. granarius* by plant extract of *A. indica*. Koodalingam *et al*. (2011) stated that exposure of the larvae of *Aedes aegypti* to the plant extract of soapnut, *Sapindus emarginatus*, significantly reduce the activities of AChE and β-CE, while α-CE activities remained unchanged. Mujeeb and Shakoori (2012) reported that synthetic pyrethroid Fury inhibit the carboxylesterase (CE) activity in all four stages of three strains of red flour beetle, *T. castaneum*. Nathan *et al*. (2005a,b) verified that the introduction of the larvae of two major insect pests of economic importance, *Cnaphalocrocis medinalis* and *Spodoptera litura*, to azadirachtin has been established to significantly decrease the activity of acid and alkaline phosphatases. Similarly aqueous and solvent extracts of *Gloriosa superba* (Khan *et al*., 2007), *Paronia emodi* (Khan *et al*., 2005), *Corydalis incise* (Kim, 2002), *Cassia obtusifolia* (Kim *et al*., 2007), *Artemisia annua* (Shekari *et al*., 2008), *Teucrium royleanum* (Ahmad *et al*., 2007a), *Andrache cardifolia* (Ahmad *et al*., 2007b), *Angelica archangelica* and *Geranium sylvatica* caused significant inhibition in the level of acetylcholinesterase (AChE), lipoxygenase, urease, alkaline phosphatase (ALP) and amino transferase of insects (Sigurdsson and Gudbjarnason, 2007).

From these results it is concluded that the plant extracts of *D. inoxia* and *A. indica* has natural potential of insecticidal and anti-enzymatic activities against *T. castaneum*, *T. granarium* and *S. granarius*. Insecticidal activity was confirmed in both tested plant species, although the results showed variation in their effectiveness in test populations. The outcomes propose the ability of using these plants extracts as an alternative to insecticides for safe storage of wheat flour and grains. Moreover, these botanical extracts could find a place in IPM strategies, especially where the emphasis is on environmental, food safety and on replacing the more dangerous toxic insecticides.

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