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Fumigant bioactivity of extracts of *Citrulus colocynthes, Moringa oleifera* and *Azadirachta indica* against *Tribolium castaneum* and *Alphitobius diaperinus* under laboratory conditions

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ABSTRACT

Tribolium castaneum (Herbst) and *Alphitobius diaperinus* (Panzer) are pests of stored cereals, their products and poultry feed. They cause qualitative and quantitative losses to agricultural produce and products during storage. The present research activity was carried out to evaluate the bioactivity of extracts of *Azadirachta indica* (L.), *Citrullus colocynthes* (L.) and *Moringa oleifera* (Lam.) against *T. castaneum* (Herbst) and *A. diaperinus* (Panzer). Three concentrations (10, 20 and 30%) of leave extract of *A. indica*; fruit extract of *C. colocynthes* and leaf extract of *M. oleifera* prepared in acetone solvent were evaluated. There were three replications of each treatment. Two bioassays were conducted to evaluate the toxicological and repellent effect of these extracts. The collected data were analyzed statistically for analysis of variance. Tuckey HSD test was used to compare the means of significant treatments. Results revealed that plant extracts were more effective against *A. diaperinus* (Panzer) relative to *T. castaneum.* Mortality and repellency increased with the increase in relative dose and time. In case of *A. diaperinus* the highest mortality and (64%) was caused by the extract of *M. oleifera* (Lam.) leaves was with 30% concentration. In case of *T. castaneum,* the highest mortality of *T. castaneum* (35.40%) with 30% concentrations of *C. colocynthes* whereas minimum mortality of *T. castaneum* (16.01%) was caused by *A. indica* with 10% concentration. *A. diaperinus* was more susceptible as compared to *T. castaneum* and 64% mortality of *A. diaperinus* was caused by 30% extract of *M. oleifera.* This insect was more killed by the *M. oleifera* as compared by other two plant extracts. Results showed that with the increase of dose rate and exposure time, repellence caused by the plant extracts increased. In case of repellency of test insects caused by plant extracts, results showed that *T. castaneum* was more repelled by these extracts as compared to *A. diaperinus.* Neem was more repellent plant extracts showing 88.88% repellency of *T. castaneum* and 71.10% repellency to *A. diaperinus* after 168 h exposure time with 30% dose of the extract. Overall results revealed that these plant extracts can be used in IPM of stored grains for sustainable management of stored product insect pests

Key words: *Alphitobius diaperinus, Azadirachta indica, Citrulus colocynthes,* Mean mortality, *Moringa oleifera,* Percent repellency, Proximate composition, Refraction analysis, *Tribolium castaneum*

Insect pests cause serious food grain losses in storage, particularly at farm level in tropical and subtropical countries. Foodgrain losses in India during storage at farm level approximate 10% of the production (Lal, 1988). In sub-Saharan Africa, foodgrain losses during storage at farm level can reach as high as 25-40% (Dichter, 1976). Such high level of foodgrain losses generally result from adequate postharvest management practices and poorly design

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storage structures. In Pakistan, wheat is a major cereal crop used as staple food. It suffers from heavy losses during storage due to the attack of insect pests, which results not only in the deterioration of the quality but quantity of attacked commodity is also destroyed (Nadeem et al., 2012). Insect infestation can result in significant weight loss, considerable reduction in volume and reasonable decrease in germination percentage of grains (Phillips and Throne, 2010).

The lesser mealworm or darkling beetle, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is reported to be the most abundant beetle inhabiting poultry, feed, litter and manure (Pfeiffer and Axtel 1980, Stafford et al.,1987). *A. diaperinus* can cause structural damage when last instar larvae tunnel into insulation and structural materials (Vaughan et al., 1984) and are capable of harbouring several types of poultry pathogens (Elowni and Elbihari, 1979).

Red flour beetle (*Tribolium castaneum*) is an important pest of stored grain commodities throughout the world (Sokoloff, 1977). This species has had a long association with human stored food and is found associated with diverse range of commodities such as grain, flour, peas, beans, cocoa, nuts, dried fruits, and spices, but milled grain products such as flour is the most preferred food (Good, 1936). Both the larvae and adults cause damage. In severe infestation, the flour turns grayish and mouldy, and has a pungent, disagreeable odour making it unfit for human consumption (Atwal and Dhaliwal, 2005). Direct feeding on grains of host promotes mould growth and excretion of hydroxyquinone compounds that causes contamination and damage to grains (Campbell and Runnion, 2003).

During the last few decades, fumigants mainly phosphinne and methyl bromide have played significant role in the control of stored product insect pests. But now phase out of methyl bromide has started and its worldwide abandonment as a fumigant of public sector storages is programmed for the year 2015 (Bell, 2000). Conversely, there are several limitations for the use of phosphine like corrosiveness, resistance, need of specialized equipment etc. (Bell, 2000; Champ and Dyte, 1976). Currently one of the main methods for controlling insect pests of stored products is the mixing of chemicals protectants with grains (Oberlander et al., 1997; Kostyukovsky and Trostanetsky, 2004). However, owing to loss of fumigants, resistance to organo phosphate insecticides, and serious ecological and health concerns, it is obvious that there is urgent need for alternative control measures, which are safe to humans and environment friendly (Kostyukovsky and Trostanetsky, 2004; Oberlander et al., 1997).

Present research activities were planned to evaluate the insecticidal and repellent impact of plant extracts against two tenebrionid beetles (*Tribolium castaneum* and *Alphitobeus diaperinus*).

MATERIALS AND METHODS

Rearing of homogenous insect culture

Population of red flour beetle was collected from flour mills and storages of grain market from the district of Faisalabad, whereas beetles of *Alphitobius diaperinus* (Panzer) were gathered from poultry sheds, feed mills and grain markets. Collected insects were reared to make homogenous population in laboratory at optimum conditions of temperature (30 ± 2ºC) and relative humidity (65 ± 5%) in Stored Grain Pest Management Laboratory of Department of Entomology, University of Agriculture Faisalabad. After 5 days adults were sieved out from diet of insects (flour for *T. castaneum* while poultry feed for *A. diaperinus).*

Preparation of plant extract

Fruits of *Citrullus colocynthes* (L.), leaves of *Azadirachta indica* and leaves, twigs and pods of *Moringa oleifera* were collected. After collection, these were shade dried at room conditions. The plant materials were ground into powder using a grinding mill. The powder was added in a conical flask along with acetone (1:3 W:V ratio). The conical flasks were placed on rotary shaker and shook at 220 rpm for 48 h, after that the solution was filtered out. The filtrate was having acetone and plant extract was placed in rotary evaporator to get pure extract by evaporating the acetone. The plant extract was considered as stock solution and three concentrations/dilutions (10, 20 and 30%) were prepared using acetone as solvent. These concentrations of plant extracts were used to check their toxic and repellent effect against two stored product insect pests.

Bioassay 1: Toxicological impact of plant extracts on adults of Alphitobius diaperinus *and* Tribolium castaneum

Three concentrations (10, 20, 30 %) of extracts of *Azadirachta indica, Moringa oleifera* and *C. colocynthes* were evaluated against adults of *A. diaperinus* and *T. castaneum.* Each extract was applied on a filter paper. The treated filter paper was allowed to dry at room temperature. These filter papers were placed in plastic jars having thirty adults of test insects feeding on the grains. The lids of the jars were closed and were made air tight using cellophane tape. Data of percent mortality of test insect were observed after three, five and seven days. Corrected mortality from data were calculated using Abbot's Formula (Abbot, 1925).

Bioassay 2: Repellent effect of plant extracts against Alphitobeus diaperinus *and* Tribolium castaneum

Percent repellency of different concentrations of plant extracts was evaluated using Area preference method. Whatman No 1 filter paper was used for this purpose. Two halves were made of each filter paper. The respective dose of each extract was applied on one half of each paper, while other filter paper was treated with acetone alone. Both the treated and untreated halves were allowed to air dried. These were united together with adhesive tape on their lower side. The filter paper was placed in petri dishes. Beetles (30) of test insect were placed atop, in the center of the filter paper. Petri dishes were covered and wrapped with adhesive tape to avoid the escape of beetles. Data were observed after three, five and seven days. Percent repellency was calculated using following formula

Percent Repellency = (Nc - Nt/Nc + Nt) ×100

Nc , insects on untreated half; Nt , insects on treated half.

Analysis of data

Analysis of all the collected data regarding percent mortality and repellency was analyzed using statistical software. Tuckey HSD test was used for the comparison of means of significant treatments.

RESULTS AND DISCUSSION

The main aim of the present study was to check the repellency and mortality of *Tribolium castaneum* and *Alphitobius diaperinus* against botanical extract of *Azadirachta indica*, *Citrullus colocynthis*, and *Moringa oleifera*

Overall results showed that concentrations and exposure time had significant effect on the mortality and repellency caused by plant extracts. Results regarding toxic effect of plant extract revealed that mortality of both insect species increased with the increase and exposure time. The highest mortality (Table 1) of *T. castaneum* (35.40%) with 30% concentrations of *C. colocynthus,* whereas minimum mortality of *T. castaneum* (16.01%) was caused by *A. indica* with 10% concentration. *A. diaperinus* was more susceptible as compared to *T. castaneum*. Extract (30%) of *M. oleifera* caused 64% mortality of *A. diaperinus.* This insect was more killed by the *M. oleifera* as compared by other two plant extracts.

The results shows the effect of exposure interval (Table 2) on the toxic effect of plant extracts showed that the mortality of both insect species increased with the increase of exposure time. Exposure period of 168 h was considered responsible for 32% mortality of *T. castaneum* and 87% mortality of *A. diaperinus* using the extract of *M. oleifera.* In case of *C. colocynthus* 34% mortality of *T. castaneum* and 62% mortality of *A. diaperinus* was reported after highest exposure duration (168 h).

Results regarding the effect of concentration and exposure times on the repellent effect of plant extracts are given in Tables 3, 4 respectively. Results showed that with the increase of dose rate and exposure time, repellence caused by the plant extracts increased. Regarding the effect of concentrations, *M. oleifera* extract was more effective as compared to other two plant extracts. Repellent effect of plant extracts was higher against red flour beetle as compared to darkling beetle. The highest repellence up to 80% was observed in experiment of red flour beetle whereas maximum repellence due to plant extracts in *A. diaperinus* was 61% with highest dose of *A. indica.*

Many scientists have worked on the insecticidal

Table 1 Toxic effect of different concentrations of three plant extracts against *Tribolium castaneum* and *Alphitobius diaperinus*

Plant extract	Conc.	Mortality (%)			
		Tribolium castaneum		*Alphitobius diaperinus*	
Azadirachta indica	10%	d.f.=2	16.01 ± 1.84 b	d.f.=2	36.62 ± 1.14 b
	20%	F-val=5.45	24.28 ± 3.20 ab	F-val=5.91	41.57 ± 6.86 ab
	30%		26.57 ± 3.76 a		50.77 ± 8.77 a
Citrulus colocynthus	10%	d.f.=2	18.55 ± 2.04 c	d.f.=2	22.44 ± 5.81 b
	20%	F-val= 37.22	27.19 ± 2.77 b	F-val=9.42	38.98 ± 6.37 ab
	30%		35.40 ± 2.81 a		41.68 ± 9.41 a
Moringa oleifera (leave extract)	10%	d.f.=2	16.97 ± 2.57 b	d.f.=2	51.09 ± 9.64 a
	20%	F-val=18.37	21.90 ± 3.14 b	F-val=5.42	58.01 ± 9.30 ab
	30%		32.18 ± 3.10 a		64.19 ± 1.91 b

Table 2 Effect of exposure time on the toxic effectiveness of plant extracts against *Tribolium castaneum* and *Alphitobius diaperinus*

Plant extract	Exp. time (h)	Mortality (%)			
		Tribolium castaneum		*Alphitobius diaperinus*	
Azadirachta indica	72	d.f.=2	15.41 ± 2.33 b	d.f.=2	13.31 ± 2.41 c
	120	F-val=10.22	21.00 ± 2.52 b	F-val=126.20	36.88 ± 4.01 b
	168		30.45± 3.05 a		78.77 ± 3.72 a
Citrulus colocynthus	72	d.f.=2	19.18 ± 5.18 c	d.f.=2	13.94 ± 3.07 c
	120	F-val= 31.02	27.41 ± 3.84 b	F-val= 55.28	26.58 ± 4.20 b
	168		34.55± 3.91 a		62.57 ± 5.78 a
Moringa oleifera (leave extract)	72	d.f.=2	15.33 ± 2.07 c	d.f.=2	28.18 ± 6.32 c
	120	F-val=22.31	23.31 ± 2.91 b	F-val=20.98	57.21 ± 7.54 b
	168		32.42 ± 3.43 a		87.91 ± 3.51 a

Table 3 Repellent effect of different concentrations of three plant extracts against *Tribolium castaneum* and *Alphitobius diaperinus*

Plant extract	Conc.	Repellence (%)			
		Tribolium castaneum		*Alphitobius diaperinus*	
Azadirachta indica	10%	d.f.=2	40.73 ± 7.65 c	d.f.=2	49.16 ± 8.72 b
	20%	F-val=42.13	62.21 ± 6.47 b	F-val=6.76	52.66 ± 5.39 b
	30%		80.73 ± 4.22 a		77.03 ± 4.02 a
Citrulus colocynthus	10%	d.f.=2	58.51 ± 5.64 b	d.f.=2	32.64 ± 6.85 b
	20%	F-val= 9.80	65.92 ± 4.50 b	F-val=3.26	47.49 ± 6.59 ab
	30%		82.21 ± 2.72 a		54.78 ± 9.09 a
Moringa oleifera	10%	d.f.=2	63.70± 4.17 b	d.f.=2	19.99 ± 6.29 b
	20%	F-val=6.05	77.03 ± 2.96 a	F-val=7.57	38.66 ± 5.77 ab
	30%		79.99 ± 4.00 a		59.25 ± 7.57 a

Table 4 Effect of exposure times on the repellent effectiveness of plant extracts against *Tribolium castaneum* and *Alphitobius diaperinus*

Plant extract	Exp. time (h)	Repellence (%)			
		Tribolium castaneum		*Alphitobius diaperinus*	
Azadirachta indica	72	d.f.=2	44.44 ± 7.53 c	d.f.=2	51.10 ± 7.93 a
	120	F-val= 35.21	58.51 ± 7.83 b	F-val=3.24	57.03 ± 7.21 a
	168		80.73 ± 4.07 a		61.10 ± 5.21 a
Citrulus colocynthus	72	d.f.=2	59.99 ± 6.75 b	d.f.=2	35.53 ± 7.78 a
	120	F-val= 5.27	68.88 ± 4.58 ab	F-val=1.06	47.38 ± 7.25 a
	168		77.77 ± 3.14 a		50.36 ± 8.96 a
Moringa oleifera	72	d.f.=2	65.99 ± 4.90 b	d.f.=2	31.48 ± 9.01 a
	120	F-val=3.80	75.55 ± 4.15 ab	F-val=1.00	42.21 ± 7.40 a
	168		79.25 ± 2.82 a		45.92 ± 8.46 a

properties of plant extracts (Gakuru and Foua-Bi, 1996; Kimura et al., 1981; Meehan, 1980) and their essential oils (Haubruge et al., 1989; Singh et al., 1978). Although the chemical composition of the essential oils of the plants was reported (Arora and Kohli, 1993; Lamaty et al., 1992), their isolation and biological evaluation tests are necessary to determine the efficacy of their active principles. Studies revealed that insecticidal properties of the essential oils of *Ageratum conyzoides* are mainly associated with their precocene content (Menut et al., 1993; Vera, 1993). 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 all the concentration induced more than 82.99% mortality after the interval of 30 days, and mortality increased with an increase in relative concentration of the spray material. Odeyemi and Ashamo (2005) evaluated same conclusions that exposure of larvae and adults of *T. granarium* to neem extract, mortality rate of both stages of the insect was increased with the increase in dose rate, ultimately the infestation of this insect reduced and overall quantitative loss was also decreased in stored groundnuts. Similar results were reported by Sagheer et al. (2013) who checked the impact of four medicinal plants and concluded that with increase in dose rate and exposure time mortality of red flour beetles (*Tribolium castaneum*) also increased. They also noted that *Nicotiana tabacum* plant extract proved most effective at higher concentration (10.0%) than *Pegnum hermala, Saussurea costus* and *Salsola baryosma* extracts.

Finally it is concluded that these botanical extracts could find a place in IPM strategies, especially where the emphasis is on organic approaches for control of stored product insect pests.

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