Effect of Medicinal Plants *Moringa olifera, Calotropis porcera, Citrullus colocynthis* as Toxicants against Khapra Beetle (Trogoderma granarium).

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Abstract: *Trogoderma granarium* is the most harmful insect pest of stored products. The heavy infestation of this insect pest causes very big injure to cereals both qualitatively and quantitatively. This experiment was conducted to evaluate mortality and repellency effect of *Moringa olifera, Calotropis porcera, Citrullus colocynthis* against *Trogoderma granarium* on filter papers. Three different concentrations of extracts of each plant viz. 5%, 7.5%, 10%, 12.5% were taken after different time periods (2,4,6,8 and 10 days) from stock solution prepared. All concentrations of each botanical showed well effectiveness as repellent against *Trogoderma granarium* (*Moringa olifera*; 37.07%, *Calotropis porcera*; 53.33%, and *Citrullus colocynthis*; 75.06 %). Among these, the better result of repellency was observed in *Citrullus colocynthis*. Mortality effect was maximum observed in *Citrullus colocynthis* at 12.5% concentration after 10 days of interval (89.11%). Other results of *Moringa olifera* and *Calotropis porcera* were also significant. These results suggest that the plant extracts evaluated in this study may be useful in repellent and toxicant formulations against *Trogoderma granarium*.

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Key words: medicinal plants, repellant, toxicant

Introduction:

Wheat is the staple food of Pakistan, harvested from 9.1 million hectares area yielding 25.5 Million metric tons annually (Anonymous, 2015). During life cycle, wheat plants may face various kinds of perturbations such as plant growth problems, water shortage, nutrient imbalances, disease attacks, yield and postharvest impediment including insect pest attacks (Hagstrum and Subramanyam, 2009; Arshad et al., 2015; Noman et al, 2015; Ali et al., 2016). Due to such type of perturbations along with post-harvest damages, losses increase at exponential rate as insect pest results in heavy quality losses to stored grains (Fornal et al., 2007). In hot and dry areas, Khapra beetle; *Trogoderma granarium* (Everts) is one of the most devastating stored grain pest as its larvae causes more damage than adults (Parashar, 2006; Burges, 2008; Mark et al., 2010). The presence of insect and its larvae in wheat flour reduce the product market value (Perez et al., 2003). The khapra beetle, *Trogoderma granarium* (Coleoptera, Dermestidae) is considered to be one of the most serious pests of stored grain products, various leguminous crops, rice, oat, barley, and rye throughout the world (Lowe et al., 2000). It is originally occurred in India, and spread to Africa, Europe, South America and East Asia (Harris, 2009). According to FAO estimate, 10 to 25% of the world harvested food is destroyed annually due to insects and rodent pests (Anonymous, 1980). Chemical insecticides such as malathian, cypermethrin, bifenthrin are used for rapid control, but are expensive, not readily available and may be poisonous to humans and environment (Tsumura et al., 1994). Moreover, malathian and cypermethrin have gone ineffective due to development of resistance in insect pests of stored grain, particularly in *Trogoderma granarium* (Saxena and Sinha, 1995). The larval period of development of Khapra beetle had been prolonged after treatment with DDT (Shantaram, 1958). Local alternatives such as the natural products are cheaper, easily available way for controlling pests, which are safe for humans and environment. Most pesticide plants also have medicinal values, and some are consumed by humans as spices (Okonkwo and Okoye, 1996). There is a growing interest in entomological research to identify and evaluate plant species with insecticide properties for control of various insect pests, including *S. zeamais* (Odeyemi et al., 2008). In stored products pest control, extracts of plant and essential oils may have various types of effects (Papachristo and stamopouls, 2002), they may have fumigant activity and used as contact insecticides or they may act as repellents (Huang *et al.,* 1997) or they may affect some biological parameters of insects such as reproduction, growth rate and life span. In developed countries, plant extracts and essential oils could be useful they can be a mean of low cost protection (Isman, 2006). Plants may provide potential alternatives to currently used insect control agents because they constitute a rich source of bio active chemicals. Plant products have played an important part in traditional methods of protection against insect infestation. Plant derived chemicals such as rotenone, pyrethrum and nicotine used economically for pest control in the west since decades. Many plant extracts are known to possess repellent activities against storage insect pests (Nazli *et al.,* 2003). Plant extracts constitute a rich source of bioactive chemicals with a potential for development as successful pest control agent (Padin *et al.,* 2002) which can affect insect in different ways: they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants, anti feedants or modify oviposition, also retard or accelerate development or interfere with the life cycle of the insects.

Objectives

These experiments were conducted keeping in view the following objective.

To determine the mortality effect of extracts against *Trogoderma granarium.*

Materials And Methods

3.1. Experimental Site

The research project was carried out in the Stored Grain Laboratory at Department of Entomology, College of Agriculture, Bahauddin Zakariya University Bahadur Sub-Campus Layyah. The material comprised of *Moringa olifera, Calotropis porcera, Citrullus colocynthis* leaves & fruits and insect (*Trogoderma granarium).*

3.2. Collection of Insects

The insects were collected from different godowns located in Bhakkar district.

3.3. Rearing of Insects

The insect culture was maintained in jars placed in the incubator at 30±2°C and 60± 5% R.H to get the homogenous population. The culture medium was wheat flour sterilized at 60 °C for 60-90 minutes.100 beetles from the heterogeneous population were liberated in 250gm of wheat flour placed in different jars. The jars were covered with muslin cloth, tied with rubber bands to avoid the escape of beetles. Beetles were allowed to remain in the culture medium for 3 days for egg laying and then removed from jars with the help of sieves and fine camel hair brush for continuation of culture. The flour containing eggs was placed again in the same jars. This newly emerged culture was considered as homogeneous for the use of experimentation.

3.4. Preparation of Plant Extracts

Collection and Preparation of Plant Extracts

Plant leaves and plant parts were collected from district Layyah (*Moringa olifera, Citrullus colocynthis, Calotropis porcera*). Leaves/fruits were cleaned by washing in water and dried them under the shade. Grinding of leaves/fruits was done in electrical grinder for getting plants powder.

3.5. Preparation of different Concentration

Acetone was used for preparing stock solutions of each plant.50 g plant powder + 100 ml acetone (stock solution). Kept on rotary Shaker at 220 rpm for 24 hours. From each solution four concentrations viz; 2.5 %, 5%, 7.5 % and 10% were prepared.

Preparation of Various Concentrations:

- 2.5% = 2.5 ml stock solution + 97.5 ml solvent
- 5% = 5 ml stock solution + 95 ml solvent
- 7.5% = 7.5 ml stock solution + 92.5 ml solvent
- 10% = 10 ml stock solution + 90 ml solvent
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3.6. BIOASSAY

Mortality Effect of Plant Extracts against Trogoderma granarium

Filter paper was used in petri dishes. Various concentrations (2.5 %, 5%, 7.5 % and 10%) of each plan extract were applied on filter paper (each treatment was replicated thrice). In control treatment Filter paper were treated with acetone (solvent). Solvent was allowed to evaporate. Thirty larvae of *Trogoderma granarium* were released into each Petri dish. Treated Filter papers were kept in incubator under optimum conditions (30±2 ºC and 65±5% R.H). Data regarding mortality was checked after 2, 4, 6, 8 and 10 days.

3.6.2. Repellent effect of plants extracts on *Trogoderma granarium*

In this experiment, filter papers and petri dishes were used. Filter paper was cut into two halves. One half was treated with plant extract cane 2.5 %, 5%, 7.5 % and 10% respectively. While other half was treated with acetone with the help of pipette. Cane viz. 2.5 %, 5%, 7.5 % and 10% of plant extracts were taken. The treated and untreated halves were attached to their opposite sites using adhesive tape and placed in Petri dishes. Twenty adults were released separately at the centre of each filter paper. The dishes then covered and placed in dark. Three replications were used for each concentration. Observations on the number of insects present on both treated and untreated halves were recorded after 24 hours in mild light.

3.6.3. Statistical Analysis

Statistical analysis of mortality effects of botanicals against *Trogoderma granarium* was carried out. The effect of treatments was computed following CRD analysis of variance using software Statistix 8.1.

Mortality noted in *Trogoderma granarium* was computed using Abbot's formula (1925);

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

Mo = Mortality observed in treatment Mc = Mortality observed in control

Repellency was computed using following formula;

Repellency (%) = $\frac{C - T}{C + T} \times 100$ (Wang et al., 2014)

C = number of insects present on the control area (repelled insects)

T = number of insects present on the treated area

Results And Discussion Mortality Experiment

1. Mortality of *T.Castaneum* induced by plant extracts of *Moringa olifera, Calotropis porcera* and *Citrullus colocynthis.*

Table 1: Mean of percent mortality induced by various interactions concentration and time of intervals of plant extract of *Moringa olifera against Trogoderma granarium*

Time (Days)	Concentration %			
	5	7.5	10	12.5
2	9.56 ± 1.11f	13.89 ± 1.11d	17.34 ± 1.92c	21.45 ± 2.22d
4	13.00 ± 1.92d	19.45 ± 2.93c	18.89 ± 2.93c	26.12 ± 2.93c
6	17.13 ± 2.97c	20.85 ± 2.24c	28.09 ± 2.97b	32.09 ± 2.97b
8	22.60 ± 2.97b	25.59 ± 2.97b	29.33 ± 2.96b	33.70 ±1.98b
10	26.85 ± 2.97a	29.21 ± 1.94a	33.70 ± 2.49a	37.07 ± 2.05a

Table 2: Mean of percent mortality induced by various interactions concentration and time of intervals of plant extract of *Calotropis porcera against Trogoderma granarium*

Time (Days)	Concentration %			
	5	7.5	10	12.5
2	12.23 ± 1.99d	16.67± 1.11e	19.00 ± 1.92e	28.03 ± 1.92d
4	21.67 ± 1.11c	22.00 ± 1.92d	24.34 ± 1.9d	29.67 ± 1.92d
6	22.74 ± 1.12c	26.48 ± 1.12c	29.60 ± 2.24c	32.59 ± 2.97c
8	28.23 ± 1.97b	31.85 ± 2.97b	35.47 ±1.94b	39.96 ± 2.97b
10	35.48 ± 1.12a	40.47 ± 2.01a	47.09 ± 2.97a	53.33 ± 2.97a

Table 3: Mean of percent mortality induced by various interactions concentration and time of intervals of plant extract of *Citrullus colocynthis against Trogoderma granarium*

Time (Days)	Concentration %			
	5	7.5	10	12.5
2	22.45 ± 1.11d	25.78 ± 1.11d	29.11 ± 2.22d	37.56 ± 2.93e
4	28.89 ± 1.11c	34.61± 1.92c	38.78 ± 2.22c	44.34 ± 1.92d
6	33.73 ± 1.94b	35.59 ± 1.12c	39.46 ± 1.12c	51.95 ± 2.94c
8	39.22 ± 2.24a	38.09 ± 1.94b	42.20 ± 2.05b	62.57 ± 1.92b
10	40.84 ± 1.96a	44.33 ± 2.92a	59.45 ± 2.97a	75.06 ± 2.01a

1. Repellency of *Trogoderma granarium* in response of *Moringa olifera, Calotropis porcera* and *Citrullus colocynthis*

Table 4: Analysis of Variance Table for Corrected by Repellency *M. olifera*

Time (Hours)	Concentration %			
	5	7.5	10	12.5
24	31.11±2.11c	43.56±2.94b	56.67±3.85b	62.78±2.94c
48	59.11±1.78a	68.89±3.01a	80.22±2.94a	83.22±2.94a
72	51.33± 1.93b	70.00±2.92a	82.22±4.01a	78.89±3.67b

Table 5: Analysis of Variance Table for Corrected Repellency by *C. procera*

Time (Hours)	**Concentration %**			
	5	**7.5**	**10**	**12.5**
24	41.78 ±2.94b	43.67 ±5.09c	49.00 ±4.67b	59.00 ±4.94b
48	42.78 ±1.11b	49.00 ±3.85b	51.33 ±3.94b	67.89 ± 2.88a
72	52.00 ±3.67a	57.00 ±4.72a	60.89 ±4.01a	70.67 ±1.62a

Table 6: Analysis of Variance Table for Corrected Repellency by *C. colocynthis*

Time (Hours)	**Concentration %**			
	5	**7.5**	**10**	**12.5**
24	49.89±2.22b	65.89±5.18b	73.33±1.77b	75.67±2.34c
48	61.11 ±1.94a	66.89±4.44b	75.56±3.01b	83.89±3.84b
72	63.11±2.22a	74.22±2.87a	82.67±2.77a	89.11±1.88a

Mortality & Repellency

In Table no. 1 *Moringa olifera solution used against Trogoderma granarium* at different concentrations viz 5%, 7.5%,10% and 12.5% minimum mortaility (9.56) was observed after 2 days of interval at 5% concentration and maximuom mortaility (37.07) was observed, after 10 days at 12.5% concentrarion of *Moringa olifera.* This results was also significant after 10 days at different concentrations but having variations among them at different days of intervals and at different concentrations.

In table no. 2 *Calotropis porcera* solution used against *Trogoderma granarium* at different concentrations viz 5%, 7.5%, 10% and 12.5% minimum mortaility (12.23) was observed after 2 days of interval at 5% concentration and maximuom mortaility (53.33) was observed after 10 days at 12.5% concentrarion of *Calotropis porcera*. This results was also significant after 10 days at different concentrations but having variations among them at different days of intervals and at different concentrations.

In Table 3**,** mortaliliy effects were observed at different concentrations of *C. colocynthis* viz 5%, 7.5%,10% and 12.5% after different intarvals. After minimum days of intervals, 22.45 %, 25.78%, 29.11% and 37.56% was observed which is significant. These percentages were recorded according to parameters 5%, 7.5%, 10% and 12.5% concentrations. The maximum mortality percentage after 10 days was at 12.5% concentration of *C. colocynthis* solution. According to the time interval of 10 days, 40.84 %, 44.33 ±2.24 %, 59.45 % and 75.06 % mortality of *Trogoderma granarium* was observed at 5%, 7.5%, 10% and 12.5% respectively. These digits shows more significancy than 2 days results as among these, 12.5% concentration of *C. colocynthis* after 10 days showed more significant results than other concentrations and other days of intervals.

In table 4 Moringa olifera, table 5 Calotropis porcera and table 6 *Citrullus colocynthis* shows maximum repellency after maximum hours and at maximum concentrations but among all these *Citrullus colocynthis shows maximum repellency (*89.11%) after 72 hours and at 12.5% concentration.

Discussion

From the all conducted experiment, different botanicals (*Moringa olifera, Calotropis porcera* and *Citrullus colocynthis*) were used to check their insecticidal properties against ***Trogoderma granarium***. These botanicals have been used previously against different stored grain insect pests for their management and showed good insecticidal properties of repellency and mortality. Concentration of each botanical viz 5%, 7.5%, 10% and 12.5% were prepared from extracted plant solutions in Acetone. Filter papers were treated at each concentration of each botanical and adults of ***Trogoderma granarium*** were released in petri dishes with filter papers. Mortality and repellency was observed by each plant concentration and significant results were obtained. The best results of mortality were observed in *Citrullus colocynthis* at each interval as compared with other while secondary results were also significant in *Moringa olifera and Calotropis porcera.* The repellency was maximum observed *Citrullus colocynthis*. According to previous studies, botanicals have been used since long as oil fumigant mostly while extracts and solutions were rarely used to control *Trogoderma granarium.* All these results showed that the use of these botanicals evaluated in this study may be useful in repellent and toxicant formulations specially *Citrullus colocynthis* against *Trogoderma granarium.*

The findings of this investigation revealed *Citrullus colocynthis*, *Moringa olifera and Calotropis* *porcera* have good insecticidal activity. They can be used in the control of *Trogoderma granarium* population with integrated pest management system which seems to be economically feasible and ecologically sound. However, more research should be directed towards isolation of bioactive compounds as well as field trials must be conducted before these extracts are used in grain storages. This task is further complicated by the possibility that some of the compounds in these mixtures may act as synergists (Fields *et al*., 2010) or as antagonists (Kordali *et al*., 2006). Ideally, insecticides should be very toxic to target insects, yet should be not toxic to non-target organisms such as plants, insects (e.g., parasites, predators, and pollinators) and other animals, such as fish and birds (Tomlin, 2003). Above all, the risk to workers and consumers must be very low. Since the use of any plant materials with insecticidal activity is likely to involve some unwanted exposure of human and domestic animals to toxic substances, toxicity studies on the effects of the plant materials and their extracts on non-target organisms need to be undertaken. Therefore, the toxicity of plant materials either directly admixed into foodstuffs or of extracts which are applied onto the produce, may be unacceptable and even long-standing use does not guarantee a level of safety which can be recommended unconditionally (Golob *et al*., 1999).

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