Effects of Medicinal Plants *Moringa olifera, Calotropis porcera, Citrullus colocynthis* as Toxicants against *Tribolium Castaneum.*

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Abstract: *Tribolium castaneum* is the most destructive insect pest of stored products. The heavy infestation of this insect pest causes massive damage to cereals both qualitatively and quantitatively. This experiment was conducted to evaluate mortality and repellency effect of *Moringa olifera, Calotropis porcera, Citrullus colocynthis* against *Tribolium castaneum* on filter papers. Three different concentrations of extracts of each plant viz. 2.5.%, 5%, 7.5%,10% 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 *Tribolium castaneum* (*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 10% 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 *T. castaneum*.

[Muhammad Ishtiaq Sarwar, Muhammad Nabeel saleem, Muhammad Umair Gulzar, Muhammad Muneeb Shabbir, Aneeb Ali, Mirza Aizaz Asim, Muhammad Hamza Khaliq and Tamoor Ali. **Effects of Medicinal Plants *Moringa olifera, Calotropis porcera, Citrullus colocynthis* as Toxicants against *Tribolium Castaneum.*** *Nat Sci* 2019;17(2):71-76]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 7. doi:10.7537/marsnsj170219.07.

Key words: medicinal plants, repellant, toxicant

Introduction

Safe storage of grains and food products against insect damage is a serious concern (Haq *et al*., 2005). Stored grain infestation is a very serious problem as various life stages of insects cause economic damage and deteriorates the quality of food grains and food products. (Sarwar *et al*., 2013). It has been estimated that about 9% of the world's grain production is lost to post harvest insect and mite's infestations (Tooba *et al*., 2005). Traditional grain storage facilities may not offer protection, but promotion of the use of metal silos and resistant varieties for grain storage is an alternative approach to reduce losses (Tadele *et al*., 2011). The drying of the foods helps in reducing the moisture content to about 9-12% in the drier areas, thus, minimizing the activities of storage insect pests and pathogens (Okunade *et al*., 2001).

There are a number of stored grain insect pests that infest food grains in farmer stores and public warehouses and massively surge due to un-controlled environmental conditions and poor warehousing technology used (Sarwar *et al*., 2012). Primary stored grain insect pests include Lesser Grain Borer (*Rhyzopertha dominica*) (Fabricius), Granary, Rice and Maize Weevils (*Sitophilus* spp.), Angoumois Grain Moth (*Sitotroga cerealella*) (Oliver) Secondary Pests of Stored products are Flour Beetles (*Tribolium confusum* and *Tribolium castaneum*), Saw-toothed Grain Beetle (*Oryzaephilus surinamensis*) (Linnaeus), Flat Grain Beetle (*Cryptolestes ferugineus*) Steph, Warehouse Moth (*Ephestia elutella*) (Hubner), Indian Meal Moth (*Plodia interpunctella*) (Hubner) and Khapra beetle (*Trogoderma granarium*) Everts (Sarwar *et al.,* 2015). All stages (egg, larva, pupa, and adult) of each stored grain insect pest may be present simultaneously in infested stored products (Ali *et al*., 2011).

Rust-red flour beetle (*T. castaneum*) (Herbst) is frequently found on farms and it is a reddish brown beetle about 3 mm long. The final three segments of its antennae are greatly enlarged to form a club shape. Young adults are pale brown in color becoming darker with age. Females lay up to 1000 eggs loosely scattered throughout infested grain. Cream-colored larvae with biting mouth parts and three pairs of legs hatch, and remain free from the grain, feeding on cereal dust and damaged grains. A generation takes about one month to complete under summer conditions, but longer in cold weather. The adult is winged and may fly and can live up to a year. (Sarwar *et al*., 2013)

Tribolium castaneum is a very destructive insect pest of food grains and stored grain products (Nadeem *et al.,* 2012). The Larvae of *Tribolium castaneum* destroy 12.5-14.60 % of weight of individual seeds and approximately 88 grains are attacked by larvae

(Wakil *et al.,* 2003). The entire life cycle of *Tribolium castaneum* lasts about 30-80 days while adult can live for 3 years into grain storage containers (Singh *et al.,* 2006). It is necessary to conserve the stored food grains reserves so that the supply food remain continuous and the prices of food grains and derived products remain stable. For this purpose, the occurrence of *Tribolium castaneum* is primarily controlled by fumigant insecticides. However, resistance has been developed due to their consistent and repetitive use against this pest (Kumar *et al.,* 2011). Though a huge progress in pest control has been achieved by chemical measures, its adverse effect on ecological system and human life has stressed the need to develop alternative methods for controlling the various arthropod pests (Risk *et al.,* 2001).

Under such conditions, the use of bioactive pesticides for protection of stored grains would be a safe alternative (Epidi *et al.,* 2009). The easiest way to use botanical insecticides against stored grain pest consist on drying the foliage and then mixing it with the grain. However, if fumigant effect is required, the essential oil is a better option (Juan *el al.,* 2016). The botanical insecticides have been used as powders, extracts, and essential oils for many years. These pesticides have shown contact, fumigant, antifeedant, and repellent activity against insects (Silva *et al*., 2003). Many plants like *Annona squamosa* (L.)*, Lantana camara, Clerodendrun inerme, Cassia fistula, Azadirachta indica* and *Calotropis procera* are proved to be lethal to various stored grain pests and delay the developmental stages by interfering with their apolytic and molting processes (Deka *et al*., 2005). These plants have shown insecticidal, antifeedant, repellant and growth regulating properties (Sankari *et al.,* 2007). Studies of essential oils extracted from other plants that contain terpinen-4-ol, 1, 8-cineole or cymol as the main components, have shown insecticidal activity against insect pests of stored products (Tapondjou *et al*., 2005). The main secondary metabolite identified with insecticidal properties is the (*E*)-beta-bergamotene which is a sesquiterpene identified as a volatile compound released by plants in response to insect herbivory (Zhuang *et al*., 2012). Huang *et al*. (2002) indicated that safrole has insecticidal activity against *S. zeamais* and *Tribolium castaneum* (Herbst). Similarly leaf powders of *Annona squamosa* and *Balanites aegyptica* (L.) caused high mortality in *T. castaneum* and provided protection against seed damage. So, botanicals are traditionally and widely used as stored grain protectants due to their easy accessibility and biodegradable nature (Dwivedi *et al.,* 2003). Production of repellents from plants is less expensive and easy as compared to synthetic chemicals. Many plant extracts are used in different forms such as *essential* extracts and powders and they are proved to be used as stored products repellents that are economically important (Mondal *et al.,* 2014).

There is a need to produce such environment friendly alternatives which have the potential to replace highly toxic chemicals. The present study is being performed to evaluate the biological activity of plant extracts against the *Tribolium castaneum.* The reason for selection of plants is that they are easily available in Punjab and these plants are also cheaper source of bio-chemicals against *Tribolium castaneum.* So this project will be carried out to demonstrate the effect of botanicals with combination and alone against *Tribolium castaneum*. The outcomes of this study will be helpful for planning the grain protection in the storage.

Objectives

These experiments were conducted keeping in view the following objective.

- ❑ To determine the mortality effect of plant extracts against *Tribolium Castaneum.*

Materials And Methods

The research project was carried out in the Stored Grain Laboratory at Department of Entomology, UAF. The material comprised of *Moringa olifera, Calotropis porcera, Citrullus colocynthis* leaves & fruits and insect (*Tribolium castaneum)*. The insects were collected from different godowns. 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.

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.

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

Bioassay

Mortality Effect of Plant Extracts against *Tribolium Castaneum*

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 *Tribolium Castaneum* 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.

Repellent effect of plants extracts on *Tribolium castaneum*

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.

Statistical Analysis

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

Mortality noted in *Tribolium Castaneum* was computed using Abbot's formula (1925);

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

Mo = Mortality observed in treatments

Mc = Mortality observed in control

Repellency was computed using following formula;

$$\text{Repellency (\%)} = \left(\frac{C - T}{C + T}\right) \times 100$$

(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 T.Castaneum*

Time (Days)	Concentration %			
	2.5	5	7.5	10
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 T.Castaneum*

Time (Days)	Concentration %			
	2.5	5	7.5	10
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 T.Castaneum*

Time (Days)	Concentration %			
	2.5	**5**	**7.5**	**10**
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

2. Repellency of *T.Castaneum* 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 %			
	2.5	**5**	**7.5**	**10**
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 %			
	2.5	**5**	**7.5**	**10**
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 %			
	2.5	**5**	**7.5**	**10**
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

Mortaility & Repellency

In Table no. 1 *Moringa olifera solution used against Tribolium castaneum* at different concentrations viz 2.5%, 5%, 7.5% and 10% minimum mortaility (9.56) was observed after 2 days of interval at 2.5% concentration and maximuom mortaility (37.07) was observed, after 10 days at 10% 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 *Tribolium castaneum* at different concentrations viz 2.5%, 5%, 7.5% and 10% minimum mortaility (12.23) was observed after 2 days of interval at 2.5% concentration and maximuom mortaility (53.33) was observed after 10 days at 10% 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 2.5%, 5%, 7.5% and 10% 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 2.5%, 5%, 7.5% and 10% concentrations. The maximum mortality percentage after 10 days was at 10% 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 *Tribolium castaneum* was observed at 2.5%, 5%, 7.5% and 10% respectively. These digits shows more significancy than 2 days results as among these, 10% 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 10% concentration.

Discussion

From the all conducted experiment, different botanicals (*Moringa olifera, Calotropis porcera* and *Citrullus colocynthis*) were used to check their insecticidal properties against *Tribolium castaneum* (Coleoptera; Tenebrionidae). 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 2.5%, 5%, 7.5% and 10% were prepared from extracted plant solutions in Acetone. Filter papers were treated at each concentration of each botanical and adults of *Tribolium castaneum* were released in petridishes 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 *Tribolium castaneum.* 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 *T. castaneum.*

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 *T. castaneum* 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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12/14/2018