Evaluation of Moringa *(Moringa oleifera* Lamp) powders and seed aqueous extract for the control of Khapra Beetle (*Trogoderma granarium* Everts)

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Abstract:

The study investigated the comparative efficacy of Moringa (*Moringa oleifera* Lamp*)* powders from different plant parts (leaf, flower, seed and branch) on the suppression of khapra beetle (*Trogoderma granarium* (Everts.) (the 3rd larval instar) damage in sorghum grains. Three rates 1%, 2.5%, and 5% w/w of the plant powders were used under laboratory conditions. The experiment was laid out in Completely Randomized Design (CRD) of four treatments. Each treatment was replicated four times .Ten larvae of khapra beetle were introduced into each petridish . Mortality of khapra beetle larvae was recorded after 1, 3, 7 and 30 days. Result indicated that Moringa seeds powder was more effective in causing khapra beetle larvae mortality. The sorghum grains treated with Moringa seed powder gave a high range of 7.5- 2.5% larvae mortality compared to the low range of 2.5- 0.0% recorded in sorghum grains treated with Moringa leaves powder at 1 day. A progressive increase in mortality ranging from 15.0-42.5% in sorghum grains treated with Moringa seed powder and 12.5- 22.5% sorghum grains treated with Moringa leaves powder were achieved at 5% rate after 3 and 30 days respectively. The LC50 and LC90 revealed that Moringa seed powder to be the most effective one. Also result revealed that Moringa seed powder is more effective than seed aqueous extract in the control of khapra beetle larvae. Out of the four powder parts of the Moringa investigated , seed powder was the most effective biopesticide and thus will be recommended.

INTRODUCTION:

Many beetles have been implicated as pests of field crops and stored products. Notable among them are Khapra beetle, *Trogoderma granarium* Everts; pod borer, bean beetle, *Callosobruchus maculatus* (F.); maize weevil, *Sitophilus zeamais* Motschulsky; larger grain borer, *Prostephanus truncates* Horn; lesser grain borer, *Rhizopertha dominica* F. etc. whose larvae/adults are capable of causing damage on seeds either in the field or in storage. Among the stored product insect pests, Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), has been considered a member of the 100 worst invasive species in the world (Musa, 2013). It is one of the most notorious primary insect pests for stored grains (Banks, 1977 and Hill, 1983). The khapra beetle larvae are one of the most serious stored seed pests. When attack of this pest is very serious in grain, it makes grains unable to germination or unmarketable because larvae consume specific nutrients during feeding on grain (Jood and Kapoor, 1993). Khapra beetle native to the Indian sub-continent and now a serious pest of stored grain in most parts of the

world (Konemann , 1993). The main infestation of the khapra beetle is the loss of stored grain. Losses occur due to *T. granarium* infestation range from 0.2 to 2.9% over a period of 1 to 10.5 months (Irshad and Baloch, 1985). The larva bore into the grain, where they feed, leaving the grains hollow. The insect also contaminate the produce with their moulds and frass. Oil extracted from infested groundnut is also contaminated (Asawalam and Onu, 2014). In addition to direct losses caused by the insect, secondary feeding and infestation is often followed by colonization by secondary insect pest especially *Ephestia cautella* Walker, fungi (*Aspergillus flavus*) and consequently leading to deterioration in grain characteristics (Nadi *et al*, 2001). Insect infestation causes dry mass loss and increases the level of free fatty acids in the kernels which results to a reduction in quality (Lale, 2002). The adult, a poor flyer, is important for its reproductive ability. The adult measures 3 – 4 mm long. It is oval in shape and dark brown with lighter bands on the elytra. Adults and larvae have numerous fine hairs on the body surface. The larval instars have varying colours from whitish yellow to dark brown at the last instar. The male is distinguished by the elongate apical segment of the clubbate antennae (Beal, 1956). The life cycle of dermestid beetles shows a typical holometabolous development; the lavae being the destructive stage of the insect pest. The adults possess wings but are not capable fliers (Ofuy and Lale, 2001) and do not feed. The control and management of stored grain insect pests by the use of insecticides and fumigation technique has caused a number of difficulties in managing these insect pests. Among various problems faced by using chemical and synthetic insecticide, most serious are resistance in insect pest species, pest resurgence, widespread environmental hazards, residual toxicity and increase cost of application (Zettler and Cuperus, 1990: Talukder and Howse, 2006), hence the search for new alternatives was thought to be important. Botanical pesticides were considered among important promising tools attempted and showed compatibility with other tactics for storage pests' management (Ahmedani *et al*., 2007). alternative chemicals (biocide) for pest control are being sourced from plants (Berger, 1994). In the Sudan, various studies were performed to evaluate the insecticidal activities of different indigenous plants for controlling agricultural pests of field crops and store products (Siddig, 1991; Satti *et al*., 2003; Sir El Khatim, 2005; Yousif and Satti, 2008). *Moringa oleifera* leaves possess some antibacterial and antifungal qualities. Incorporation of the green leaves into the soil had been successfully used in preventing damping off disease caused by *Pythium debaryanum* in young crop seedlings (Fuglie, 2005). Study in Nigeria reveaed that The *Moringa oleifera* leaf extract effect on the morphological and physiological growth characteristics of cassava (*Manihot utilissima* Pohl.), the extract gave the highest percentage stem height difference at the eighth week after treatment (Ndubuaku *et al*, 2015). Therefore, in the course of studies aiming to find potent plants for preparing natural pesticides, the current research was intended to evaluate and compare the insecticidal activities of different powders (leaf, flower, seed and stem) prepared from Moringa trees against the 3rd larval instars of the Khapra beetle (*Trogoderma granarium* Everts).

MATERIALS AND METHODS:

The experiment was carried out during 2016 at the College of Agricultural Studies, Department of Pant Protection, Sudan University of Science and Technology Shambat, Khartoum-Sudan.

Collection of the insects: The insect species used in the present study was the Khapra beetle, *Trogoderma granarium* (Everts). The adults collected from both, College of Agricultural Studies, Sudan University of Science and Technology, Khartoum State and White Nile State were reared in the laboratory for a homogenous population.

Rearing of insects: Adults of *Trogoderma granarium* (Everts.), were cultured in a 2L glasses container covered with a muslin cloth and held by the side with an expansible rubber band to allow for aeration and avoid suffocation of the insects and equally prevent escape of the insects. The culture was raised under ambient temperature and relative humidity condition (28+3c and 70+5%) respectively. Newly emerged 3rd larval instars of *T. granarium* were used in this experiments.

Collection and* Preparation *of different plant part powders: *Moringa oleifera* Lamp was collected from the Khartoum state, Sudan and separated into different plant parts (leaf, flower, seed and branch), washed with tab water and they were left to dry under shade for 7 days, then grounded using a motor pestle and sieved to obtain a fine powder. Each power was kept separately in air-tight jar prior to use.

Preparation of seed aqueous extract:

250g of only seeds powder were mixed with 500 ml of destilled water placed on electric shaker for 8 hrs. Plant extract were filtered then the seed equaous extract was kept in air tight jars prior to use.

Bioassay:

1/ Effect of *Moringa olifera* powders from different pant parts on the mortality of khapra beetle larvae:

Four experiments were conducted to evaluate the insecticidal action (mortality effects) of the different plant parts (leaf, flower, seed and branch) of *M. oleifera* against the3red larval instars of *T. granarium* as the test insect. All above experiments were executed separately in Petri-dishes according to the number of treatments. All four plant powders were applied separately at rates 1%, 2.5%, and 5% w/w of the plant powders on the test insect. The Petri-dishes were shaking manually to enable the powder to spread evenly over the grain. Each concentration was replicated four times. Ten larvae the 3red instars of *T. granarium* were interoduced in each Petri-dish and covered. Mortality counts of the larvae beetles were recorded after 1, 3, 7 and 30 days. Insects were taken as dead of they did not move away when touched. Another control group set up with the grains and *T. granarium* as untreated control.

2/ Effect of *Moringa olifera* seed aqueous extract on khapra beetle larvae:

Different concentration (5%,2.5% and 1% w/v) were prepared from seed aqueous extract, then each filter paper (9cm-1d.) were soaked for one minute in the desired concentration and left for one hour at room temperature (35c°) to dry and then transferred each to petridish. Ten 3rd larval instars of khapra beetle and 100 seeds of sorghum were placed into each petridish. Control petridishs containing untreated filter paper were included. Each treatment was replicated four times and units were arranged in a Completely Randomized Design. Mortality counts of the larva were recorded at the 1, 3, 7 and 30 days. Insects were taken as dead if they did not move away when touched. Another untreated control group was set up. Percentage larvae mortality was determined by the method of Parugrug and Roxas (2008) using the following formula:

$$\text{Mortality \%} = \frac{\text{No of dead insects}}{\text{Total no of insects}} \times 100$$

Toxicity regression lines of different plant powders were drew manually and LC50 and LC90 values were recorded.

Data Analysis:

Data collected were subjected to Analysis of Variance (ANOVA). Significantly different means were separated using Least Significant Difference (LSD) at probability (0.05).

Results:

1/ Effect of *Moringa olifera* powders on larval mortality of *Trogoderma granarium*:

Table (1) showed the mortality effects of *M. olifera* powders from different plant parts (leaf, flower, seed and branch) on the 3rd larval instars of the khapra beetles (*Trogoderma granarium).* up to three days post treatment, no significant difference were detected between all plant powders and the control, but at seven and thirty days after exposure all rates of *M. olifera* powders had significant effect ($p \leq 0.05$) on mortality of *Trogoderma granarium* larvae. The mortality effect increased with the rates of treatments. At 5% level rate of *M. olifera,* seed powder showed the highest percentage mortality of 37% on larvae of *T. granarium* after exposure duration of 7 days, while branches, leaves and flowers powders gave 25%, 17.5% and 15.5% percentage mortality respectively. Thirty days after exposure of *T. granarium* larvae to *M. olifera*, the highest percentage mortality on *T. granarium* larvae of 42.5% were achieved also by *M. olifera* seed powder, However the lowest percent mortality of 22.5% was achieved by *M. olifera* leaves powder (Table 1).

Table (1): Percentage mortality of *Trogoderm granarium* larvae feed on stored sorghum seeds treated with different rates of *Moringa oleifera* powders (Leaves, Flowers, Seeds and branches) after 1, 3, 7 and 30 day.

Within each column data followed with the same letter were not significantly different at p≤.05 according to Least Significant Difference(LSD).

Treatment	Log-concentration	
	LC50	LC90
Seeds	3.64	7.20
leaves	11.11	70.80
flowers	44.74	115.67
branches	6.45	21.64

2/ Probit analysis

Table (2) showed the LC50 and LC90 value at 95% confidence, low value of LC50 and LC90 of 1.84% and 7.20% respectively was achieved by *M. olifera* seed powder (Fig, 2)**.**

Plant part	Concentration (W/W) (%)	Larvae mortality after indicated days			
		1	3	7	30
Leaves	**5** **2.5** **1**	2.5A 0.0A 0.0A	5.0A 5.0 A 5,0A	17.5A 17.5A 12.5AB	22.5A 17.5AB 12.5AB
Flowers	**5** **2.5** **1**	5.0A 2.5A 2.5A	7.5A 7.5A 7.0A	15.5A 15.0A 12.0AB	23.5A 15.0AB 12.5B
Seeds	**5** **2.5** **1**	7.5A 2.5A 2.5A	15.0A 5.0A 5.0A	37.5A 17.5B 10.0BC	42.5A 20.0B 12.5B
Stems	**5** **2.5** **1**	5.0A 0.0A 0.0A	7.5A 0.0A 0.0A	22.0A 15.0B 7.5BC	25.0A 15.0B 12.5BC
Control	-	0.0A	0.0A	2.5B	7.5B

Table 2: LC50 and LC90 value of powders from different parts of *Moringa olifera* against 3rd larval instars of khapra beetle after 30 days.

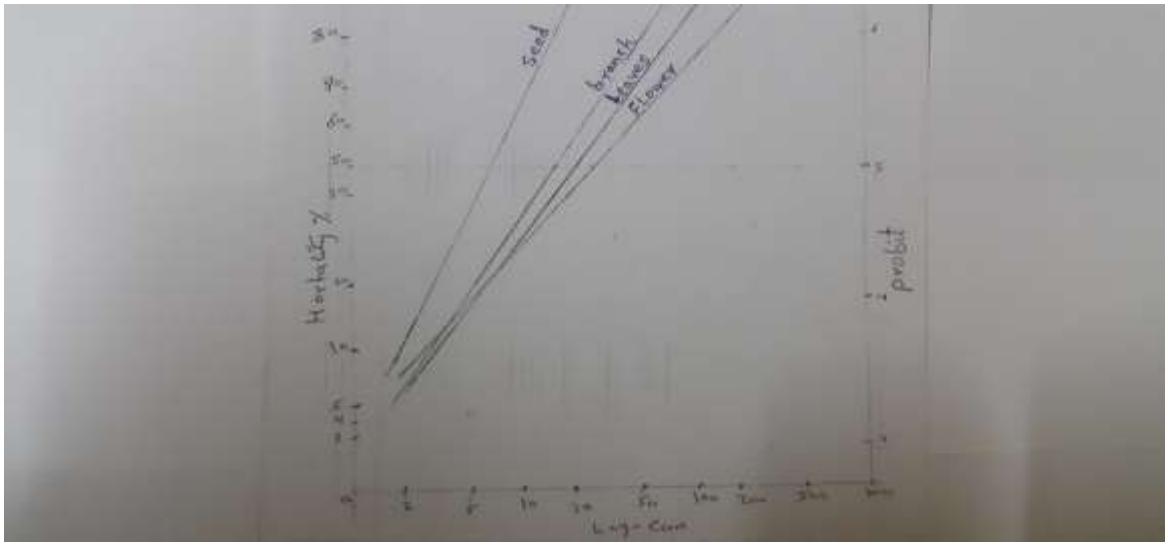

Fig 1: Toxicity regression lines of powder from different parts of *Moringa olifera* against 3rd larval instars of khapra beetle.

3/ Effect of *Moringa olifera* seed aqueous extract on *T. granarium* larvae:

Table(3), showed that *M. oliefera* seed aqueous extract at all rates, were not significantly different at ($P \leq 0.05$) after one day from application when compared with the control, but after 3, 7 and 30 days after application there were significant ($p \geq .05$) effect on the 3rd larval instars of *T. granarium.* No mortality was observed in the control until one 7 days after application and the rate of the mortality increase with time of exposure. The highest range of the percentage mortality among the *T. granarium* larvae (5.00-15.00%) achieved with 5% concentration and the lowest range of the percentage mortality (2.6-11.25%) was achieved with 1% concentration of *M. oleifera* seed aqueous extract after 3 and 30days respectively (Table3).

Table (3) Percentage mortality of *Trogoderm granarium* larvae feed on stored sorghum seeds treated with different concentrations of *Moringa oleifera* seed aqueous extract after 1, 3.7 and 30 days.

Concentration	Larvae mortality after indicated days			
	1	3	7	30
5	0.00 A	5.00 AB	10.0 A	15.00 A

2.5	0.00 A	7.50 A	10.0 A	13.13 A
1	0.25 A	2.50 B	2.50A B	11.25A
Control	0.00 A	0.00 B	0.00 B	6.88 B
LSD (0.05)	0.515	0.702	0.73	4.60
SE±	0.171	0.233	0.24	1.56

With in each column data followed with the same letter were not significantly different at $p \leq .05$ according to Least Significant Difference(LSD).

DISCUSSION:

In the current investigation , powders from the different parts of *Moringa oleifer*a plant (leaf, flower, seed and branch) were tested against the 3rd instar larvae of khapra beetle *Trogoderma granarium*. The result indicated that all parts studied have insecticidal activity against the test insect comparing with control and response varied with plant parts and exposure time. This may be due to the fact that *M. oleifera* was found to contain Phytochemicals. Phytochemicals present in *M. oleifera* include catechol tannins, Gallic tannins, steroids, triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars as reported by Kasolo *et al* (2010).

This study shows that *M. oleifer*a seeds powder gave highly significant toxic effects against *Trogoderma granarium* larvae (3rd instars), gave high range (37.5- 42.5%) of percentage mortality at 5% concentration (w/w) after week and month respectively with low LC50 0f 3.64% followed by branches , flower and leaves they gave percent of mortality range (22.0-25.0%), (15.0-23.0%) and (17,0-22.0%) respectively. The low value of LC50 value of 3. 64% acheieved by *M. oleifer*a seeds powder and this agreed with previous study by Ashfaq *et al*. (2012),they found that *M. oleifer*a seeds powder have insecticidal effects against *Culex quinquefasciatus* larvae , gave 98.89 % mortality within 24 hours exposure. Also studies with Water extract of *M. oleifera* seeds against 3rd instars larvae of *Aedes aegypti*, showed 24-hour-LC_{50} value of 1260 ug/ml (Ferreira *et. al*., 2009). While, Adenekan, *et al* (2013)**,** reported that cowpea seeds treated with *M. oleifera* flower, leaf and stem powders were effective against the bruchid beetles and showed 100 %, 85% and 70% mortality respectively at 24 hrs of insect infestation compared with 10 % mortality achieved with the control. Musa (2013), mentioned

that groundnut seeds treated with two plant powders, *M. oleifera* leaves and *Allium sativum* cloves at 6% (w/w) gave 100% mortality of *T. granarium* adult after 5 days from treatment.

This study also revealed that the highest range of mortality (5.00-15.00%) larvae mortality achieved with *M. olifera* seed aqueous extract at the highest concentration (5%) after week and month respectively, but it seems that *M. olifera* seed powder better than *M. olifera* seed aqueous extract in controlling *T.granarium* larvae on the sorghum grains .However, experiment conducted with Alao and Adebayo, (2015) who tested the insecticidal effect of Moringa leaf extract on the *Phylotreta cruciferae* (insect pest of watermelon) , they found that *M. olifera* gave the high mortality compared to control. Results of this study indicated that the powder of *M. oleifera* seed showed potentials in the control of 3rd larval instars of *T.granarium* larvae on sorghum seeds as bio-insecticide.

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