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Effect of Methanol Extracts of Three Plants Seeds on the Third Larval Instar of Khapra beetle (Trogoderma granarium Evert) (Coleoptera: Dermstidae)

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Abstract

The present study was carried out under laboratory conditions of the Environment and National Resource Research Institute, Khartoum, Sudan, during the period April-May 2014. The objective of the study was to evaluate the effect of methanol extract of Neem (Azadeirachta indica), Fennel (Foeniculum vulgare) and Coriander (Coriandrum sativum) on the 3rd larvae of Khapra beetle (Trogoderma granarium). Three concentrations (5%, 2.5%, and 1.25%) were used for each extract. Five grams of sorghum seeds were treated separately with each extract and fed to 10 larvae in a Petri dish. The parameters measured after 29 days treatment included: mortality of the larvae, number of adult emerged and weight loss of grains. The results showed significant mortality action on the larvae. All treatments gave significant differences as compared to the control. The best result was obtained for Neem 5% extract which showed 43.8% mortality, then followed by Fennel (41.2%) and Coriander (32.2%). The control showed 7.5% mortality. The lowest mean of adult emerged after treatment of larvae was obtained by Neem 5% (31.02%), then Fennel 5% (41.9%), Coriander 5% (54.4%) and finally 81.7% for the control. There were no significant differences between the three treatments. However, there were significant differences between the treatments and control. There were no significant differences between treatments for mean weight of sorghum loss after treatment. The three treatments showed significant differences as compared to the control. The results obtained for seeds weight loss were: 2.8 %, 2%, 1.8% and 18% gm for Neem, Fennel, Coriander and control, respectively. It was concluded that application of methanol extracts of Neem, Fennel and Coriander was effective in Khapra beetle control. Concentration 5% for all extractions gave the best results. It is recommended to use 5% methanol extracts Neem and Fennel for Khapra beetle control.

Introduction

Sudan is agricultural country where various crop is raised by irrigation and rain fed schemes. The main crops include sorghum (Sorghum bicolor), wheat (Triticum vulgare) and pea millet (Pennisetum typhoides). Insects are the main pests which caused the greatest damage to stored grains all over the world. Loses however continued to be high throughout tropics. In Sudan stored grain insect were recorded to distribute all over the country wherever cereals are produced and stored by climate and accessibility of the produce to inset [1].

The estimated losses of food grains caused by insect have been to range between 70 to 90% in some countries lacking modern storage technologies [2].

These practices adopted at different periods included; mechanical or physical approaches, biological, chemical, ecological, cultural, legislations resistant varieties and the use of natural products. However, the most portent period in the history of the pest control was the era of synthetic pesticides which triggered by the discovery of insecticidal property of the DDT in the 1930. Recently such natural extract have received great worldwide research activities as one of the pest control alternatives to synthetic chemical. Among different tested plant neem tree Azadeirachta indica and A.juss which are widely grown in Sudan as shade avenue tree has been identified as promising source of environmentally soft and natural pesticides [3].

The aim of this study was to investigate the effect of different plants (water and ethanol) extracts: Fennel (Foeniculum vulgare

Mill), Coriander (Coriandrum sativum L) and Neem seeds (Azadeirachta indica A.juss) against the third larval instar of Khapra beetle Trogoderma granarium (Evert).

The parameters measured include:
- Mortality effect.
- Percentage of seeds damaged.

Materials and Methods

- Organic solvent: Methanol.
- **The glass wares include:** Petri- dishes measuring cylinders, pipettes, beakers, conical flasks, glass rods, funnels, jars (2liters) dark bottles (250ml) and vials.
- **Tools:** Camel hair brushes, notebook, magnifying lence, spoons, wire sieve, marker
- **Equipments:** Sensitive balance, electric blender (Moline), soxhelt refrigerator, insect repellent apparatus.
- **Insect Culture:** Larvae of the khapra beetle (Trogoderma granarium) reared on sorghum grains were provided in glass jars.
- **Plant Material:** Fennel (Foeniculum vulgare Mill), Coriander (Coriandrum sativum L) and Neem seeds (Azadeirachta indica) were used.

Rearing of the khapra beetle (T. granarium)

A culture of the Khapra beetle contained all developmental stages of the pest was obtained from Nile Valley University Faculty of Agriculture and reserved this culture at the Laboratory of Botanical Pesticides Department of Alternatives to Pesticides and Biological Control, Environment and Natural Resources Research Institute (ENRRI). Some infested grains, taken from the previous stock culture, were added to each jar (2jar) and covered with muslin cloths, fitted tightly with rubber bands. These cultures were left few months under laboratory conditions to give time for insect reproduction, hence the required numbers of larvae were secured for the different experiments. The cultures were sieved at different intervals to get rid of cast skins, excreta and grains powder accumulated in the jars through larval feedings.

- **Collection and Preparation of Plant Materials:** All plant seeds in this study were collected from various locations in Khartoum State, during different times in 2014.
- **Fennel (Foeniculum Vulgare):** Fennel seeds were collected in 2014 from Department of Medicinal and Aromatic Control, Environment and Natural resources Research Institute (ERRI). Vegetative parts were harvested in 2013 and then powdered by an electric blender.
- **Coriander (Coriandrum sativum):** Coriander seeds were collected in 2014 from Central Market of Khartoum State. Then seeds powder was prepared.
- **Neem (A. indica):** Neem seeds were collected in 2014 from the same source as above and dried under shade in the laboratory. Powder of seeds kernels were obtained as shown in the preparation of powders.

Preparation of Botanical Extracts

Organic extracts

The solvent methanol was used successively for extraction of the different plant's samples in a soxhelt apparatus, with six units and 500 ml flasks size. The sequence of extraction started with the removal of oil from a sample by using methanol to obtain the intermediate and polar compound, respectively. Therefore, this sequence was applied to extract the oil content of fennel seeds, coriander seeds, and neem seeds. A weight of 20 grams powder from each plant sample was loaded in the soxhelt thimble and extracted to 8 hours to free it from oil. The process was repeated 3 to 5 times with new samples to get required quantity, based on the degree of oil present in each plant and the amount needed to run the experiment. Upon completion of each round, the extract was left in the open air for some hours to evaporate the solvent. Similarly, the filtrate was also air dried and re-extracted by the methanol to obtain the components. However, any plant sample was re-weighed at the end of each extraction step so as to determine the percentage of the extracted material yielded. Moreover, the extract itself was also weighed for more confirmation.

Application of Treatment

Preparation of Treatments and Test Insects

Preparations of treatments were started as a pre-step for biological assays in each experiment. The intended organic extracts for an experiment was firstly diluted with water to prepare the highest extract concentration (5%). However, in methanol extract, 0.1% liquid soap was added as an emulsifier. Consequently, the other concentrations (2.5% and 1.25%) were prepared by serial dilutions with water. On the other hand, three rates of water extracted materials ware prepared on same day following the extract preparation. Accordingly, three concentrations of organic and water extracts of each plant samples were evaluated successively as natural insecticides in separate bioassay experiments.

The number of larvae allocated for each experiment was segregated from the culture with the help of small camel hairs brush, white A4 sheet and Petri dish. This was done each time taking some amount of infested grains from the jar and checked served carefully on the sheet paper, then returned back again into the jar to expose different stages of larvae clinging to the paper. Using the brush, only the therid instars larvae ware dropped from the sheet down into the dish. The process may be repeated many times to complete the required number of larvae (400 larvae in methanol extracts and 360 water extracts) in some experiments.

Evaluation of Insecticidal Effects

The experiment was conducted to test and compare the insecticidal actions (mortality effects) of the previously prepared treatments from each of the three plants under study. The experiment ware applied between April 2014 and May 2014, at the laboratory of Botanical Pesticides, ENRRI, Khartoum, using the 3rd instars larvae of T. granarium, as test insects for bioassays. The experiment was conducted, which studied the effects of Fennel and Coriander extracts compared with neem seeds extract.

However, besides the occurrence of untreated control in experiment an additional 0.1% soap treatment control and water control for the experiment it means that first control has two control.

All bioassay experiments evaluating the insecticidal effects of the different plants were executed separately in Petri dishes. According to the number of treatments, each 5 grams of clean sound sorghum (S. bicolor) ware treated with one of the different extract's concentrations, replicated four times, left for five minutes to dry and then placed into the Petri-dishes. Then 10 3rd

instars larvae of T. granarium were introduced in each Petri dish, including the control, and covered.

However, investigations of the Petri dishes under an experiment were carried out periodically on 2nd 7th, 14th .21st, and 28st days following treatments. Hence, the number of dead insects and other observations were recorded.

Weight Loss of Sorghum Seeds

Weight loss of sorghum seeds damaged by the 3rd instar larvae of T. granarium was calculated from all treatments. the same procedure adopted in regarding method of seeds weight, treatment and placement of insects in Petri-dishes was also applied in this experiment and calculating the percentages of damaged seeds based on the initial weight (5g).

Statistical Analysis

The collected data were subject to the analysis of variance (ANOVA) using software program. Whenever significant differences were detected, the least significant difference (LSD) was implemented for mean separation.

Results

Methanol Extracts

Effects of Fennel, Coriander and Neem Seeds Extracts on the 3rd Instars Larvae of Trogoderma granarium: Analysis of the variance showed that there were significant differences detected among all methanol extract. Neem 5% reflected the highest mortality mean 43.81of larvae then followed by 39.24 and 30.73for 2.5%, and 1.25% respectively. Fennel mortality means recorded 41.81 for 5%, 2.5% showed 33.86 and 1.25% recorded 33.41. While control (methanol and water) recorded 7.35&3.47. While coriander recorded 32.14, 26.6, and 22.35 for 5%, 2.5%, and 1.25% respectively. (Tableland Fig 1).

Effects of Fennel, Coriander and Neem Seeds Extracts on the Trogoderma Granarium Emerged Fed on Sorghum Seeds Treated with Methanol Extracts: The means of adult emerged induced by neem seeds extracts recorded 20.05, 21.37, and 31.02for 1.5%, 2.5%, 5% respectively. While fennel recorded 29.36, 40.67, and 41.89 for 1.5%, 2.5%, 5% respectively. However, coriander recorded 42.05, 43.41, and 55.44for 1.5%, 2.5%, 5% respectively. The control (methanol and water) recorded 81.69, 83.36 (Table 2 and Fig 2). There were significant differences between all treatments.

Weight Loss of Sorghum Seeds: The means of weight loss of sorghum seeds induced by neem seeds recorded 0.11, 0.12, and 0.14 gm for1.25%, 2.5%, 5% respectively. Fennel showed 0.6, 0.8 and 0.1 for 1.25%, 2.5%, and s5% respectively. However, coriander recorded 0.5, 0.9 and 0.9 for1.25%, 2.5%, 5% respectively. The control (methanol and water) recorded 0.86 and 0.11. No significant differences between all treatments. There were significant differences between the control and the other treatments. (Table 3 and Fig 3).

Table 1. Mortality mean of Trogoderma Granarium 3rd instar larvae fed on sorghum seeds treated with methanol extracts, during April 2014.

Treatment	Mortality mean of larvae					Mean
	2days	1weeks	2weeks	3 weeks	4 weeks	
F 1.25%	5.96 a	29.88 a	36.22 b	47.88 b	49.33 bc	33.41
F 2.5%	12.15 a	24.16 a	34.55 a	48.1 a	48.1 bc	33.85
F 5%	14.28	28.2225a	42.115b	60.645b	60.645b	41.18
Co 1.25%	1.81b	14.28 ab	28.22 b	32.89 c	34.55 c	22.35
Co 2.5%	5.96 AB	16.3ABC	25.22 bc	39.11 bc	46.44 b	26.6
Co 5%	12.15 a	22.5 a	31.39 b	43.55 b	51.11 b	32.14
N 1.25%	1.80 b	16.31 a	31.54 b	45 b	58.98 b	30.73
N 2.5%	10.12 a	24.16 a	39.1 ab	52.33 ab	70.45 ab	39.23
39.23	10.125a	24.53a	43.495a	62.145a	78.755a	43.81
Meth cont	1.81	5.96 c	9.66 c	9.66 d	9.66 d	7.35
Water cont	1.81 b	1.81 c	1.81 d	5.96 d	5.96 d	3.47
Mean	7.09	18.92	29.39	40.66	46.72	46.72
LSD 5%	11.51	9.93	11.98	15.19	15.60	15.60

F = Fennel seeds, Co =coriander seeds, N= Neem seeds, Meth cont + -Methanol control, Water cont = water control.

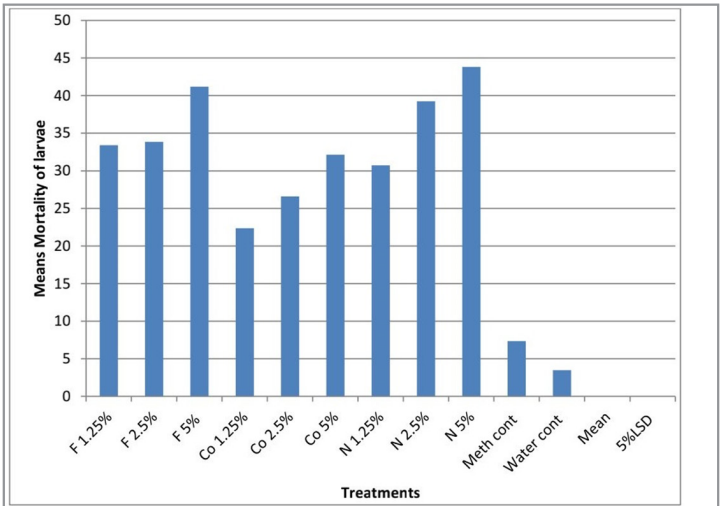

Fig 1: Comparison between different concentrations of plant extract and control on the mortality of *Trogoderma granarium* 3rd instars larvae.

Table 2. Means of Trogoderma granarium adult emerged after treatment of larvae fed on sorghum seeds treated with methanol extracts (29 days).

Treatment	Mean of a number of adult emerged
F 1.25%	29.36 d
F 2.5%	40.67 c
F 5%	41.89 c
Co 1.25%	Co 1.25%
Co 2.5%	43.41 c
Co 5%	55.44 bc
N 1.25%	20.05 d
N 2.5%	21.37 d
N 5%	31.02 cd
Meth cont	81.69 b
Water cont	83.36 a
Mean	44.57
LSD	19.24

F = Fennel seeds, Cocoriander seeds, N= Neem seeds,
Meth cont=Methanol control,. Water cont = water control.

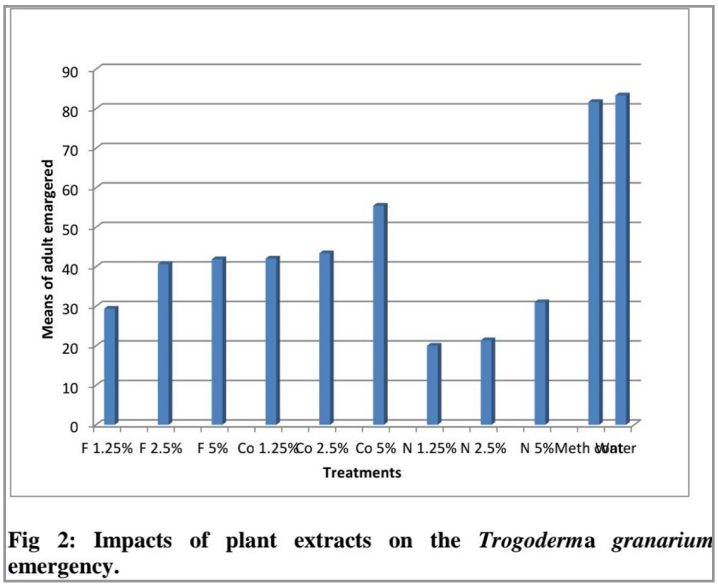

Fig 2: Impacts of plant extracts on the *Trogoderma granarium* emergency.

Table 3. Mean weight of sorghum seeds damaged by the 3rd instar larvae of Trogoderma granarium, after 29 days following treatments with different plants extracts during April 2014.

Treatment	Mean weight of sorghum damaged
F 1.25%	0.06 d
F 2.5%	0.08 cd
F 5%	0.1 c
Co 1.25%	0.05 d
Co 2.5%	0.09 c
Co 5%	0.09 c
N 1.25%	0.11 bc
N 2.5%	0.12 b
N 5%	0.14 b
Meth cont	0.86 a
Water cont	0.11 b
Mean	0.16
5% LSD	0.04

F = Fennel seeds, Cocoriander seeds, N= Neem seeds,
Meth cont=Methanol control, . Water cont = water control.

Discussion

The search for safe, naturally occurring pesticides for storage pest has been intensified. Therefore, natural products that can reduce insect pest population in a manner that is less hazardous. The most promising are those derived from plants. A number of investigations have identified and screened varieties of promising chemical compounds from leaves and seeds of many plants families as insect feeding deterrents and growth inhibitors (Siddig, 1991). In this study, methanol and water extracts of fennel, coriander and neem seeds have been stated as botanical insecticides of chemical insecticides applied on sorghum crop to investigate their role in reduction of the damage caused by Trogoderma granarium Everts. Significant differences among some treatment were detected after 14 days and after 21 days before the third readings. Neem, coriander and fennel extracts have highly significant effects on rate of mortality of the Trogoderma granarium. Hence, as step forwarded in screening indigenous plant based on previous findings, the biological properties of some extracts of neem, fennel and coriander were evaluated in a series of laboratory experiments. Shown in the result different plant samples have yielded variable level of extracted materials which performed differently regarding their toxicity, antifeedant and repellent actions against the larvae.

Considering all plant samples, the highest means of extracted material was attained by neem through methanol extraction. Followed by fennel and coriander. Such high oil constituents in the three plants were considered as added values to their highest biological activities which reflected in strong toxicant, antifeedant and repellent actions against the pest. This is particularly important when this plant, components are needed to be utilized commercially for production of natural insecticides. Although, studies on neem extract recorded the highest mortality among all of its equaling applied extracts, because of its toxic effect to larva. This report is similar to Yousif and Satti (2008). As it seems all of the extracts affected the adult Trogoderma granarium but the significant effect was caused by both the neem and fennel extract. Both of the extracts affected the physiological process of the adult which resulted in poor birth. These extracts also leading a reduction of weight of the sorghum seeds. These results agreed with Nasr and Bakeet who assured neem oil has a very high repellency effect to Trogoderma granarium [4, 5]. According to Balandrin et al the repellent action of neem extracts could result from the present of evaluated containing compound [6]. On the other hand, voluminous literature is available on neem research, showing variable level of content in seeds which found to contain numerous active ingredients (eg. Azadirachtin, salannin, nimbinen, salanol, and estaric, oleic andlinoleic acids) with pesticide properties (Bashir, 1994) [7]. The means in this study (43.81) agreed with Maydell who mentioned that the neem kernels contain about 45_60% oil [8]. As with respect to the other solvent's extracts, the methanol recorded significantly higher mortality compared with water extracts in all plant samples of neem, fennel and coriander. This result is agreed with many works in some experiments on test larvae of T. granarium. For instance, Mohamed (2004) found that under laboratory condition, Neem extracts with a concentration 5% reduced T. granarium larvae according Venkatan et al (1987). Furthermore, Sheded et al showed that Azadirachta of neem has a repellent effect on store pest [9]. However, the findings of this research disagreed with Abd Elwahid (2011) who reported the coriander was the most effective than fennel among the applied extracts it significantly reduced the level of the white fly this effect could be due to the antioxidant effect of its constituents (phenolic acid) [10]. The same effect of all of the extracts happened upon the 3rd instar of larvae, indicating that the toxic substance in the neem is very powerful and the insect couldn't make some sort of resistance [11].

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