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Efficacy of Hargel (*Solanostemma argel* (Del) hayne) Shoots Extract Against the Broad Bean Beetle (*Bruchidius incarnatus*)

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Abstract

Laboratory studies were conducted to evaluate the efficacy of aqueous and organic extracts of the Hargel shoots against the adult stage of the broad bean beetle *Bruchidius incarnatus* in the Sudan. Hargel shoots were extracted sequentially by organic solvents of increasing polarity (Petroleum ether, Ethyl acetate and Ethanol) as well as directly by with distilled water or ethanol. Extracts were tested at concentrations ranging between 1% to 10%. The evaluated efficacy parameters included; mortality, repellency, antifeedant and effects on weight loss in stored faba bean seeds (*Vicia faba* L.). The tests were conducted in Petri dishes (9 cm i.d) and plastic cups (capacity 200 ml) and the obtained data were subject to the analysis of variance (ANOVA) and further by probit analysis.The results of the mortality data indicated that the sequential extraction with ethanol was the most potent against the test insect as shown by its low LD_{50} of 0.33%. Various types of Hargel shoot extracts induced significant dose dependent repellency against the broad bean beetle. The highest 24 hours repellency was caused by the aqueous extract as indicated by its low ED_{50} value of 10.6 %.The different Hargel shoot extracts also induced significant antifeedant action against the test insect. The lowest Feeding ratio (Fr) of 0.017 was recorded in aqueous extract treated faba bean seeds while the lowest percentage weight loss (1.7%) was noticed on stored faba bean treated with petroleum ether extract.

Keywords

Tribolium castaneum, Hargel Shoots, Mortality, Repellency and Antifeedant Effects

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1. Introduction

Faba bean (*Vicia faba* L.) is one of the most important winter crops for human consumption in the Middle East and has been consider as meat and skim-milk substitute. The nutritive value of faba bean is high, and is considered in some areas to be superior to field peas or other legumes. Large-seeded cultivars are used as vegetable. Roasted seeds are eaten like peanuts in India (Duke, 1981). The straw of harvested faba bean is used for brick making and as a fuel in some parts of Sudan and Ethiopia (Bond *et al*., 1985).

Faba bean seeds are subject to attack by many coleopteran insects of the family Bruchidae during storage (El Kifl and Metwally, 1971 and Elsonoussy, 2009). Cardona *et al.* (1985) stated that *Bruchidius incarnatus* is an important pest of faba bean in Egypt and Sudan. Chemical control of these pests can be achieved by DDT and BHC (Gamaxane) dust or by spraying with dimenthoate (Rogar), carbaryl (Sevin) or by fumigation with phostoxin (Bushara and Abu-Elgasim, 1982).

Sudan with its variables geographical regions is rich in endogenous plants which may represent a promising reservoir of naturally occurring toxicants that can used as

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effective components of integrated pest management (IPM) programmes. In the Sudan examples of the promising botanical pesticides includes, neem tree *Azadirachta indica* (A), sadom apple (Usher) *Calotropis procera* (J), fenugreek (Hilba) *Trigonella foenum* (G), garlic *Allium sativum* (L), sesame *Sesamum indicum* (L), Hargel plant and sweet basil (Rehan) *Ocimum basilicum* (L) (Fageer, 1999 and Sidahmed (B) *et al*, 2009).

The promising results obtained with hargel, initiated our interest to further develop the method of extraction and bioassay. The expected law mammalians toxicity of the plant, being a common human beverage and the safety limitation of stored products to conventional insecticides further strengthen our goals.

The main objectives of the present study are to investigate the potential of Hargel (*Solanostemma argel* (Del) hayne) shoot extracts in the control of the broad bean beetle *B. incarnatus* (Boh). The efficacy will be evaluated through the following criteria;

- The toxicity of organic and aqueous extracts to target pest
- Its repellancy action.
- Its antifeedant effect.
- Its effect on weight loss of stored faba bean.

2. Materials and Methods

2.1. Preparation of Hargel Shoots Powder

The vegetative parts of Hargel were collected from the northern state at Al Robatab area (Alshereig) in June 2008. Collected samples were cleaned and washed under water tap, then spread to dry under room temperature. The dried parts were first crushed by hand and then powdered by an electric blender type Braun: Mx 32. The powder was stored in a tightly covered glass jars wrapped with Aluminium foil until needed for preparation of extracts.

2.2. Organic Extracts Consecutive Extraction

Sub samples (30g) of Hargel shoot powder were consecutively extracted with three organic solvents of increasing polarities (petroleum ether, ethyl acetate and ethanol). The samples were extracted with Soxhlet apparatus for eight hours. Defatted powder was dried under room condition before extraction with next solvents. The solvent was stripped off by rotary evaporator and the extracts were stored in a refrigerator at 4°C until needed for bioassay. The same method was followed for each solvent. Four concentrations (v/v) 10%, 5%, 2.5% and 1% were prepared by serial dilution and used for bioassay.

2.3. Direct Extraction with Ethanol

Sub samples (30 g) of Hargel powder were extracted with ethanol. The samples were extracted with soxhlet apparatus for eight hours. The solvent was stripped off by rotary evaporator and the extracts were stored in a refrigerator at 4°C until needed for bioassay. Four concentrations (v/v) 10%, 5%, 2.5% and 1% were prepared by serial dilution and used for bioassay.

2.4. Preparation of Hargel Aqueous Shoot Extracts

Aqueous solutions of Hargel shoot powder were prepared by mixing 20 grams powder with 180 ml distilled water in a conical flask (500 ml) following the method of Ascher (1981). The mixture was left to stand for 24 hours at room temperature and shaken thoroughly (by hand) for 5 minutes every 8 hours for 24 hours. The mixture was then strained through a light cloth and then filtered through a Whatman filter paper No 1 (24 cm). The stock solution (10% w/v) was kept in the refrigerator at 4°C for further work. Four concentrations (w/v) 5%, 2.5% and 1% were prepared by serial dilution by adding distilled water.

2.5. Rearing of *Bruchidius Incarnatus* (Boh.)

The stock culture was obtained from the Dept. of Crop Protection, Faculty of agriculture U of K, and sieved with mesh No. 10 to remove adult insects. Adults were reared in rearing glass jars, capacity 3Kg, half filled with sound grain of Faba bean local variety, covered with muslin cloth, fixed with rubber bands and kept in the laboratory at room temperature. Four week later the culture was sieved, the old adults were removed and the newly emerged adult (0-2 days old) were collected and used for bioassay.

2.6. Bioassay

2.6.1. Toxicity of Hargel Shoot Extracts to Test Insects

The method described by (Udo and Epidi, 2009) was followed; Petri dishes 9 cm in diameter were used to confine insects during the experiment. Filter paper 9 cm diameter was treated with 2ml of Hargel shoot extract. The filter papers were allowed to dry for 30 min at room temperature. Twenty adults of the test insects were introduced into each Petri dish using a camel hair brush. Hargel shoot extracts were tested at 4 concentrations (10%, 5%, 2.5% and 1%). Solvents (petroleum ether, ethyl acetate and ethanol) and distilled water and untreated controls were included. The number of dead insects in each Petri-dish was counted every day for three days. The experimental units were arranged in a completely randomized design with four replicates.

2.6.2. Repellency Test

The method described by Koko *et al.* (2009) was followed; Petri dishes 9cm in diameter were used to confine insects during the experiment. Filter paper with a 9 cm diameter was cut to two half and 1 ml of each concentration was applied separately on one half of the filter paper as uniformly as possible with pipette. The second half (control) was treated with 1 ml of solvent or distilled water. Both filter paper halves were allowed to dry for 30 min at room temperature. A full disc was carefully remade by attaching the two halves with tape. Care was taken so that the attachment did not prevent free movement of insects from the one half to another, but the distance between the two halves remained sufficient to prevent seepage of test extracts from one half to another. Test insects 20 adult were released in the centre of each filter-paper and Petri dish were covered immediately. Hargel shoot extract was tested at 4 concentrations (10%, 5%, 2.5% and 1%).Solvent (petroleum ether, ethyl acetate and ethanol) and untreated control was included. Each treated was replicated Four times and units were arranged in CRD. Insects in each half were counted every day for three days. The percentage repellency of each extract was then calculated using the formula:

$$PR\ (\%) = [(Nc - Nt)/(Nc + Nt)] \times 100$$

Where:

Nc ≡ the number of insects present in the control half,

Nt ≡ the number of insects present in the treated half.

2.6.3. Antifeedant Test

Antifeedant test was done following the method of Owusu *et al.* (2007). Fifty grams of faba bean grains was placed in 200-ml plastic cups and mixed with 2 ml of various concentrations from each extract and left for 1hr to dry at room temperature. The control was treated with solvent and water alone. Twenty adults of *Bruchidius incarnatus* (10 male and 10 female; 1-2 days old) were introduced. The cups were covered with muslin cloth, held in place with rubber bands and then placed in the laboratory at room temperature. Experimental units were arranged in CRD with Four replicates. After 30 and 60 days, the remaining grains were reweighed and feeding ration (*Fr*) was calculated as follows:

$$Fr = 1 - FW/50$$

Where: *FW* ≡ the final grain weight

2.6.4. Effect of Hargel Shoots Extract on Weight Loss

For grain damage assessment, samples of 50g of faba bean grains were taken from each cup and mixed with 2 ml of 10, 5, 2.5 and 1% of each extracts and left for 1hr to dry at room temperature. The control was treated with solvent or distilled water. Twenty adults of *Bruchidius incarnatus* (10 male and 10 female; 1-2 days old) were introduced. The cups were covered with muslin cloth, held in place with rubber bands and then placed in the laboratory at room temperature. The number of damaged grains (grains with characteristic holes) and intact grains were counted and weighed. Experimental units were arranged in CRD with Four replicates. Pest damage was computed using the method of Owusu *et al.* (2007) as follows:

$$\%\ \text{Weight loss} = [UaN - (U+D)]/UaN \times 100$$

Where:-

U ≡ weight of undamaged fraction in sample

N ≡ total number of grains in the sample

Ua ≡ average weight of one undamaged grain

D ≡ weight of damaged fraction in the sample

2.6.5. Statistical Analysis

Collected data were expressed as percentage and subjected to the analysis of variance following the procedure described by Gomez and Gomez (1984) using SAS software for windows version 9 (2004). Lethal doses (LD) and effective doses (ED) were calculated following probit analysis method according to Finney (1971) using Minitab software version 13.3 (2000).

3. Results

Various extracts from Hargel shoot system were tested as potential source of control agent for broad bean beetle (*Bruchidius incarnatus)*. Parameters tested include mortality, repellency, antifeedant actions and weight loss. Extracts were obtained by consecutive extraction with solvents of different polarities (petroleum ether, ethyl acetate and ethanol) as well as directly with ethanol and distilled water. Results can be summarized as follows:

3.1. Effect on Mortality

All extracts caused significant mortality compared to the control and effects were dose and time dependent (fig.1 and 2). The highest effects were noticed after three days of exposure. Generally aqueous extracts was the most potent followed by ethanol, direct extraction with ethanol, petroleum ether and ethyl acetate extracts after second days while after the third day ethanol extract showed the most potent effects followed by direct extraction with ethanol, aqueous extract, ethyl acetate and petroleum ether extract.

The probit analysis of the 48 hours indicated an LD_{50} values (%) of 16 for Petroleum ether extract, 60 for ethyl acetate extract, 7 for ethanol extract, 8 for direct extraction with

ethanol and 2 for aqueous extract. Since the slopes of LD_{50} probit lines are identical, the efficacy can be ranked based on the relative potency measured at the LD_{50} as aqueous extract> ethanol extract > direct extraction with ethanol > petroleum ether extract > ethyl acetate extracts. Fudicial limits are generally narrow at the LD_{50}. Slopes are relatively flats and LD_{90}/ LD_{50} ratio is relatively high indicating a relatively hetrogenous population with respect to sensensitivity to test extracts. Chi-square values were small indicating good execution of the experiments (figure 3).

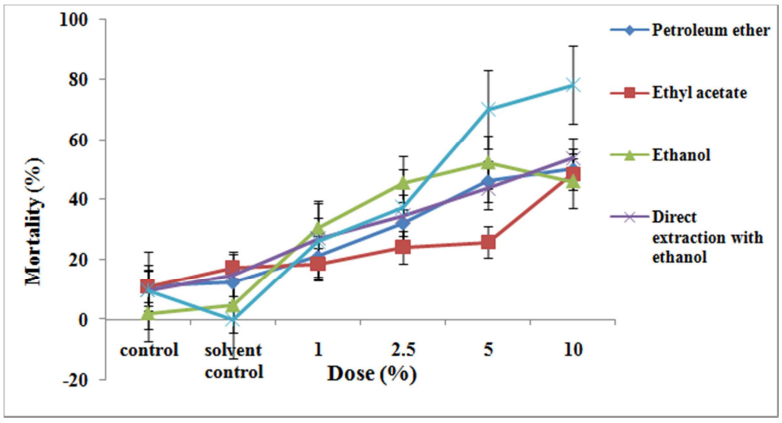


Fig. 1. Percentage mortality of *Bruchidius incarnatus* following two days exposure to various type s of Hargel shoot extracts.

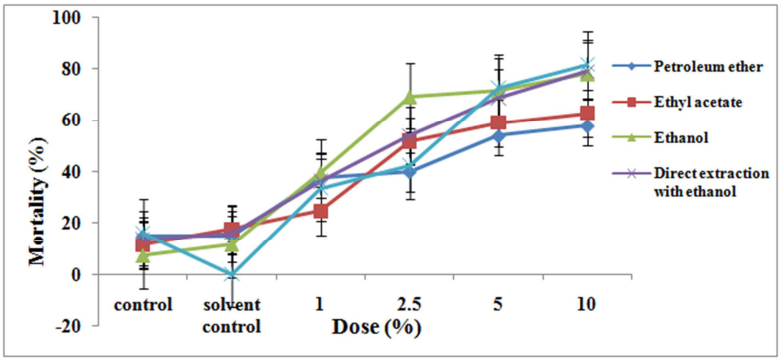


Fig. 2. Percentage mortality of *Bruchidius incarnatus* following three days exposure to various types of Hargel shoot extracts.

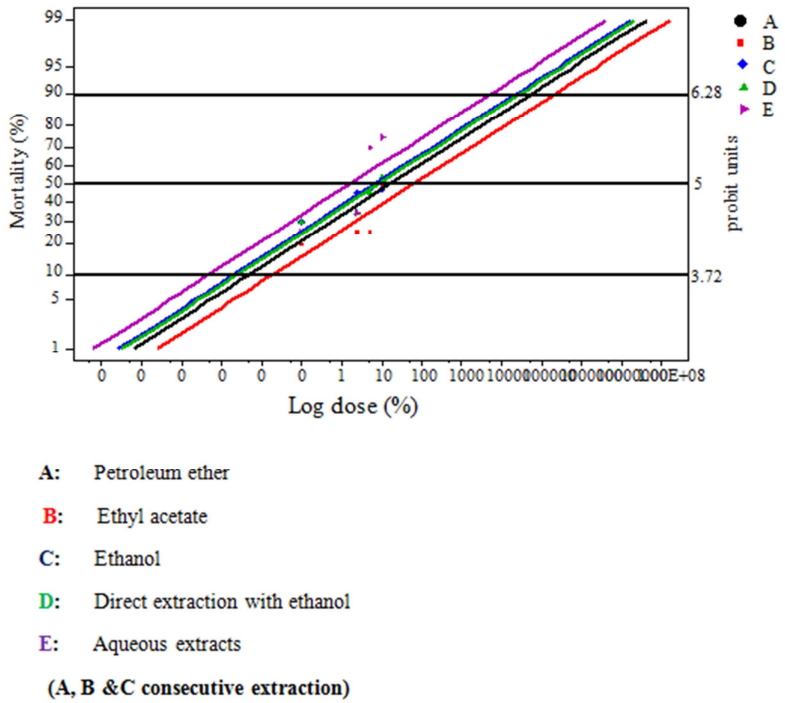


Fig. 3. Mortality response probit lines of *B.incarnatus* (adult) exposed to Hargel shoot extracts for 48 hours.

The probit analysis for the 72 hours (figure 4) indicated an LD_{50} value of 2.62 % for Petroleum ether extract, 1.95 % for ethyl acetate extract, 0.33 % for ethanol extract, 0.54 % for direct extraction with ethanol and 0.87 % for aqueous extract. Since the slopes of LD_{50} probit lines are identical, the efficacy can be ranked based on the relative potency measured at the LD_{50} as ethanol extract > direct extraction with ethanol > aqueous extract > ethyl acetate extract > petroleum ether extracts. Fudicial limits are generally narrow at the LD_{50}. Slopes are relatively flats and LD_{90}/ LD_{50} ratio is relatively high indicating a relatively hetrogenous population with respect to sensitivity to test extracts. Chi-square values were small indicating good execution of the experiments.

3.2. Repellent Actions

All concentrations of various extracts caused significant repellency to test insect and effects were dose and time dependent (fig. 5 and 6). The highest effects were noticed after the first and second day of exposure. Generally aqueous extract was the most potent followed by ethanol extract, direct extraction with ethanol, ethyl acetate extract and petroleum ether extracts after first day while after the 2nd day aqueous extract showed the most potent effect followed by direct extraction with ethanol, petroleum ether extract, ethyl acetate and ethanol extracts.

The probit analysis of the first 24 hours indicated an ED_{50} value of 54 % for petroleum ether extract, 35 % for ethyl acetate extract, 18 % for ethanol extract, 32 % for direct extraction with ethanol and 11% for aqueous extract. Since the slopes of ED_{50} probit lines are identical, the efficacy can be ranked based on the relative potency measured at the ED_{50} as aqueous extract > ethanol extract > direct extraction with ethanol > ethyl acetate > petroleum ether extracts. Fudicial limits are generally narrow at the ED_{50}. Slopes are relatively flats and ED_{90}/ ED_{50} ratio is relatively high indicating a relatively hetrogenous population with respect to sensitivity to test extracts. Chi-square value was small indicating good execution of the experiments (figure 7).

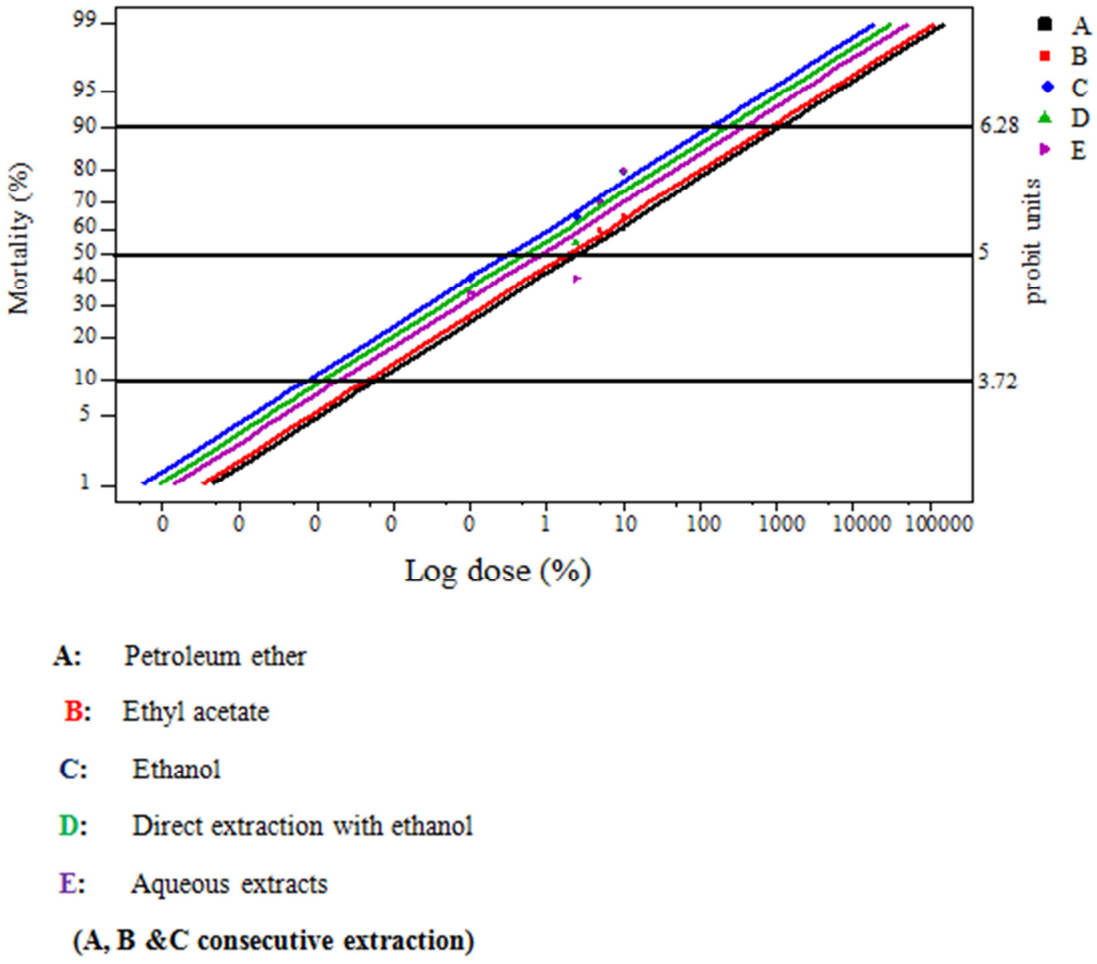


Fig. 4. Mortality response probit lines of *B.incarnatus* (adult) exposed to Hargel shoot extracts for 72 hours.

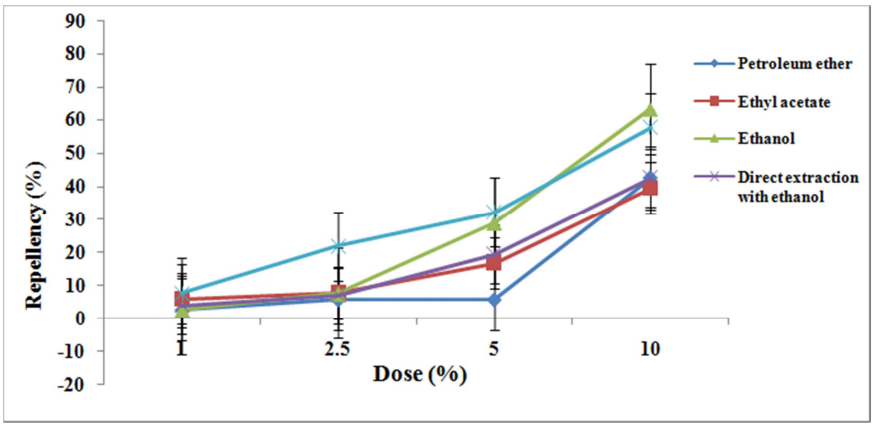


Fig. 5. Repellency percentage of *Bruchidius incarnatus* following one day exposure to various types of Hargel shoot extracts.

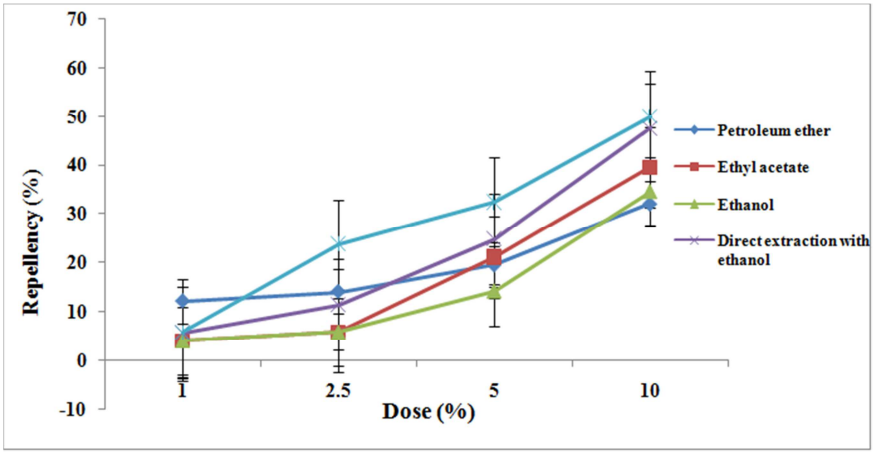
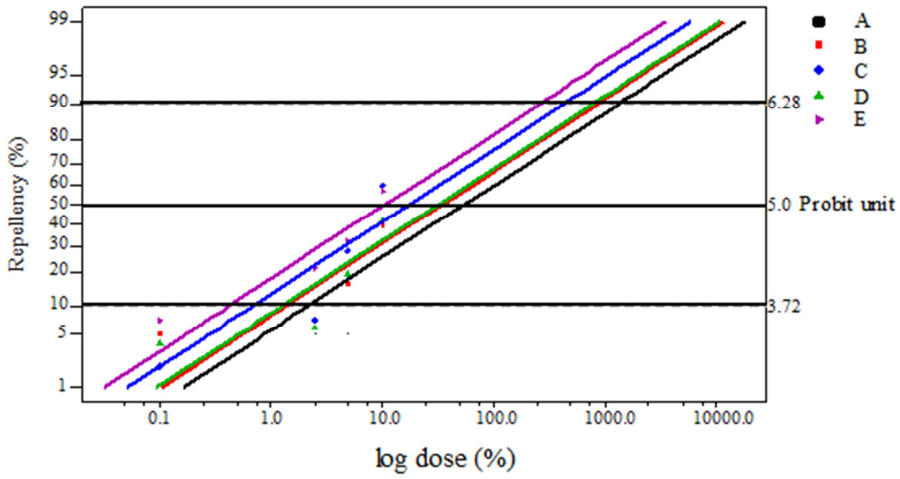


Fig. 6. Repellency percentage of *Bruchidius incarnatus* following two days exposure to various types of Hargel shoot extracts.



A: Petroleum ether

B: Ethyl acetate

C: Ethanol

D: Direct extraction with ethanol

E: Aqueous extracts

(A, B &C consecutive extraction)

Fig. 7. Repellency response probit lines of B. incarnatus (adult) exposed to Hargel shoot extracts for 24 hours.

The probit analysis for the 48 hours (figure 8) indicated an ED_{50} value of 54 % for petroleum ether extract, 79 % for ethyl acetate extract, 125 % for ethanol extract, 43 % for direct extraction with ethanol and 21 % for aqueous extract. Since the slopes of ED_{50} probit lines are identical, the efficacy can be ranked based on the relative potency measured at the ED_{50} as aqueous extract > direct extraction with ethanol > petroleum ether extract > ethyl acetate extract > ethanol extract. Fudicial limits are generally narrow at the ED_{50}. Slopes are relatively flats and ED_{90}/ ED_{50} ratio is relatively high indicating a relatively hetrogenous population with respect to sensitivity to test extracts. Chi-square value was small indicating good execution of the experiments (figure 8).

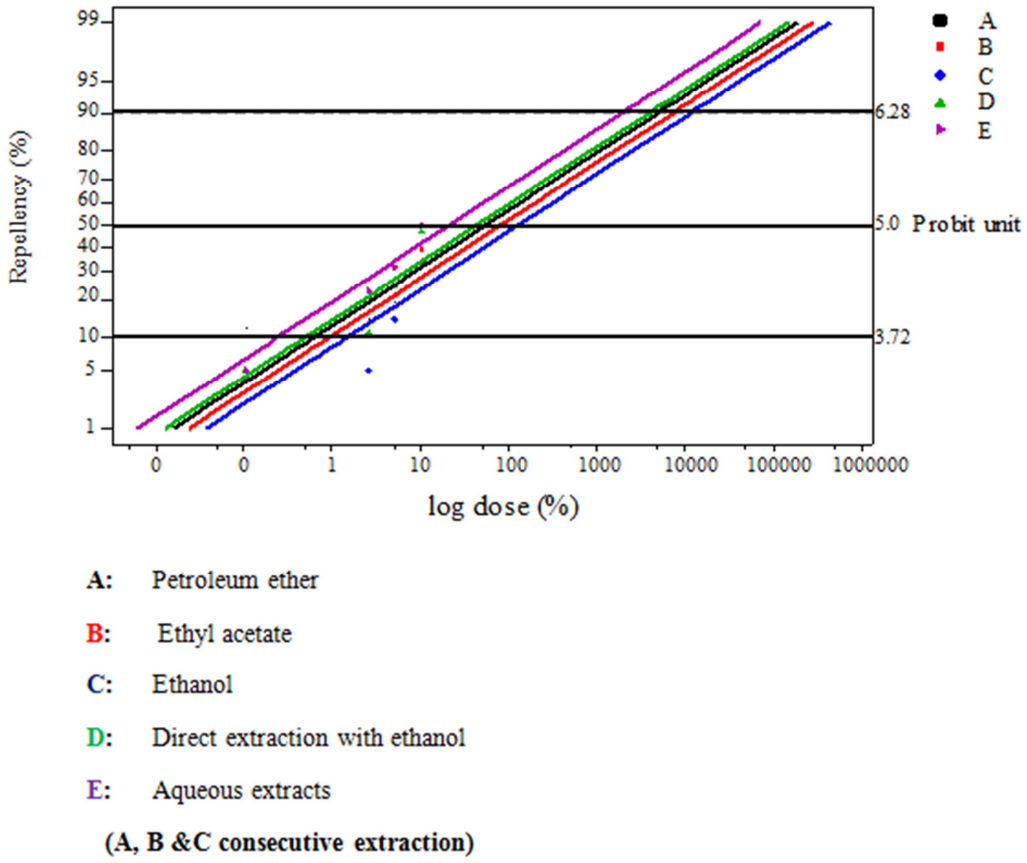


Fig. 8. Repellency response probit lines of *B. incarnatus* (adult) exposed to Hargel shoot extracts for 48 hours.

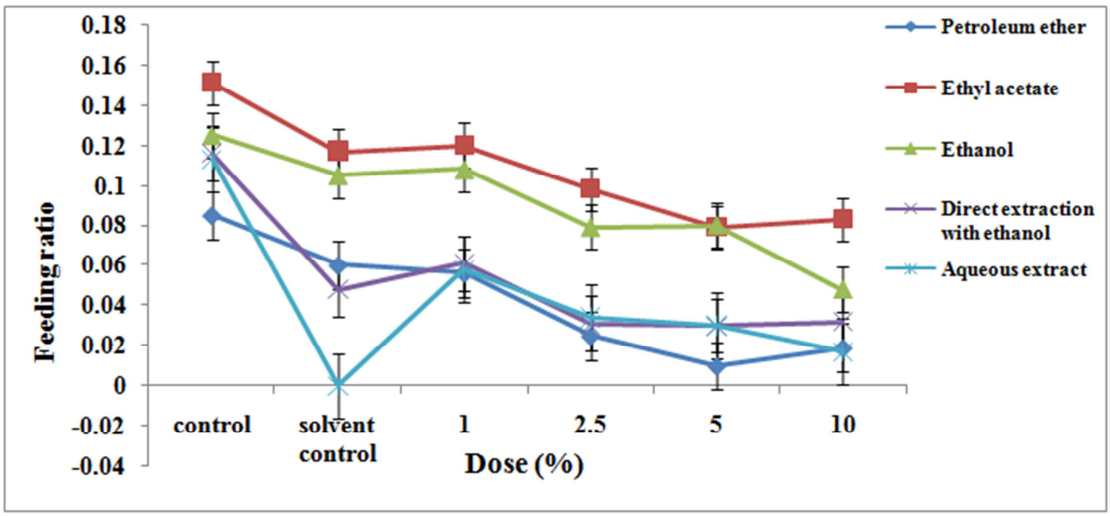


Fig. 9. Feeding ratio of *Bruchidius incarnatus* following 60 days exposure to various types of Hargel shoots extracts.

3.3. Antifeedant Effects

All extracts caused significant antifeedant effects compared to the control and effects were dose and time dependent (fig.9). Generally aqueous extract showed the most potent effects followed by petroleum ether extract, direct extraction with ethanol, ethanol extract and ethyl acetate extracts. The feeding ration (Fr) decreased with the increase of the concentration and increased when the exposure period increases (fig.9).

3.4. Weight Loss Effects

All extracts caused significant effects on weight loss compared to the control and effects were dose and time dependent (fig.10). Generally aqueous extract showed the most potent effects followed by petroleum ether extract, direct extraction with ethanol, ethanol extract and ethyl acetate extract. The weight loss effect decreased with the increase of the concentration and increased when the exposure period increases.

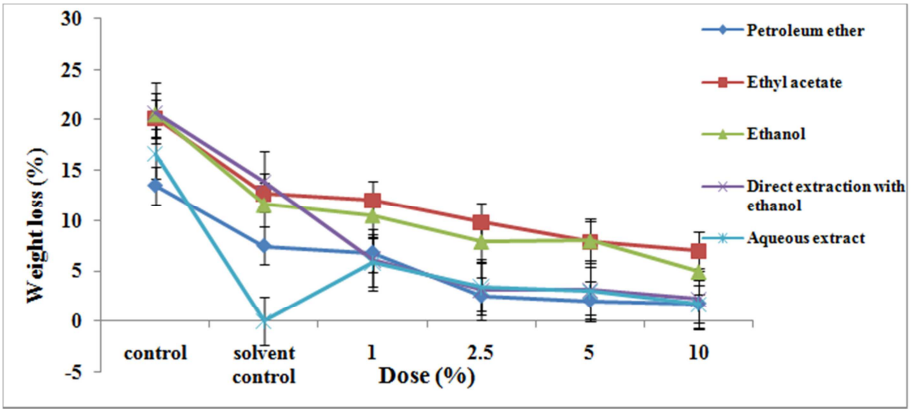


Fig. 10. Weight losses in stored faba bean seeds caused by *B. incarnatus* following sixty days exposure to various types of Hargel shoot.

4. Discussion

Grain legumes are usually stored for certain period under different conditions, which render them susceptible and vulnerable to damage by a variety of insect pests. The most troublesome of these insects in the Sudan are *Callosobruchus maculatus* and *Bruchidius incarnatus*. The later is considered the most important insect pest in the family Bruchidae that attacks stored Faba bean (Shazali, 1985). Unless measures are taken to control this insect excessive damage and losses could occur. Existing infestations are usually controlled by fumigation, whereas protection against re-infestation is achieved through the use of contact insecticides. Chemical control of stored product insect pests although is the most efficient and effective means of protection of stored produce (Bakheit, 2004 and Elsonoussy, 2009), yet it has many drawbacks which necessities the search for safe naturally occurring alternatives. Many natural products were tested in the Sudan with promising results such as; Neem *Azadirachta indica* (Siddig, 1991; Schumutterer and Ascher 1987), Usher *Calotropis procera* (Fagger, 1999), Rehan *Ocimum basilicum* (Stoll, 2001), Garlic *Allium sativum* (Abdalla, 2003; Khiralla, 2007; Elsonoussy, 2009 and Ahmed, 2010) and Hargel *Solenostemma argel* shoot, (Sir ElKhatim, 2005 and Sidahamed(B) *et al.* 2009).

In the present work various aspects of the efficacy of five extracts of Hargel shoots were tested against the broad bean beetle *Bruchidius incarnatus*. The investigations included evaluation of the following parameters; mortality, repellency, antifeedant and weight loss effects. The results showed that the mortality of adult stage of the test insect increased with the increase in the concentration of Hargel shoot extracts. Most of the tested concentrations were significantly better than control. The probit analysis indicated that aqueous extract of Hargel shoot was the most effective after 2nd day of exposure as indicated by its low LD_{50} value (less than 2%) with narrow fudicial limits. While after the 3rd day, ethanol extract was most effective as indicated by its low LD_{50} value (0.33%) with narrow fudicial limits. Generally the mortality rate (as measure by the LD_{50}) of other extracts after the 3rd day was less than 3%. These results are in accordance with the previous study carried by Bakheit (2004) who reported that powder and aqueous extract of Hargel shoot induced mortality against the adult stage of the *B. incarnatus* when used at the concentrations 10%. The current results were also in line with findings obtained by Sir Elkhatim (2005), Al-Doghairi *et al* (2004) and Sidahmed (A) *et al* (2009) who reported that hargel shoot products possesses insecticidal activities to *T. castaneum*; *Culex pipiens* and *Microtermes thoracalis* correspondingly.

The test population of adults was relatively hetergenous as evident by the low slope and high LD_{90}/LD_{50} ratio. This is also clear when comparing the LD_{90} to the LD_{10}. This

heterogeneity might be of significance for future work where highly susceptible individuals (LD_{10} range between 0.000 % to 0.02 % for 2nd day and 0.001% to 0.01% for third day) may be found in other sources of stock culture specially if collected from other regions of the Sudan.

The results showed that all extracts of Hargel shoot induced significant dose dependent repellency to the test insects with the highest effects clearly noticed after the 1st day of exposure. The aqueous extract was the most effective as indicated by its low ED_{50} value (11%) with narrow fudicial limits. However after 48 hours of exposure the aqueous extract maintained its superior efficacy but at a relatively higher dose (ED_{50} less than 21%) with narrow fudicial limits. The ED_{90} / ED_{50} ratio is relatively narrow, especially in the first day, compared to the corresponding mortality ratio which indicated a better homogeneity response to repellency compared to mortality effects. These findings were in line with results obtained by Sir Elkhatim (2005) who reported that hargel shoot powder and aqueous extract has shown repellant activities against *T. castaneum*. Further farmers in some parts of Northern Sudan (Shaygia area) used to soak hargel shoots in the main irrigation canals to repel insect of vegetables particularly bollworms (Sir Elkatim, 2005).

The results showed that the different types of Hargel shoot extracts caused significant antifeedant effects to the adults of *C. maculatus* compared to the control. Effects were dose and time dependent with the feeding ration (Fr) decreased with the increase of the concentration and increases when the exposure periods increase. Petroleum ether extract is the most potent. The current study also demonstrated that hargel shoot extracts significantly reduced the weight loss induced by the test insect on stored broad bean seeds. This study was the first in the Sudan. The only available work found in the literature was on the 4th larval instar of cotton leaf worm *Spodoptera littoralis* (Boisd.) where the petroleum ether extract exhibited significant antifeedant activity (Abdel-Rahman and Al-Mozini ,2007).

5. Conclusion and Recommendations

- The present study revealed that Hargel shoot extracts caused different significant mortality and repellency to the broad bean beetle.
- The various types of Hargel extracts induced close and significant antifeedant and weight loss effects to the test insect.
- The aqueous extract of Hargel shoots was the effective against the *B. incarnatus*.
- Effects were dose and time dependent.
- Hargel is cheap and available for farmers in rural areas of Northern Sudan and therefore deserve further work in assessment as pest control agent for small farmer in these areas.
- Improving extractions methods, clean-up, separation and identification of the active materials in Hargel shoot is equally important and should be considered in future lines of research.

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