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Efficacy of Hargel (*Solanostemma argel* (Del) hayne) shoots extract for the control of the cowpea beetle (*Callosobruchus maculatus*) (Coleoptera: Bruchidae)

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Abstract: **Studies were conducted to evaluate the efficacy of aqueous and organic extracts of the Hargel shoots against the adult stage of the cowpea beetle *Callosobruchus maculatus* in 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% and 10%. The evaluated efficacy parameters included; mortality, repellency, antifeedant and effects on weight loss in stored cowpea (*Vigna unguiculata*). 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 direct extraction with ethanol was the most potent against the test insect as shown by its low LD_{50} of 0.39%. Various types of Hargel shoot extracts induced significant dose-dependent repellency against the C. maculatus. The highest 24 hours repellency was caused by the aqueous extract as indicated by its low ED_{50} value of 8%. The different Hargel shoot extracts also induced significant antifeedant action against the test insect. The lowest Feeding ratio (Fr) of 0.007, as well as the lowest percentage weight loss (0.7%), was recorded in ethyl acetate extract treated cowpea seeds.**

Keywords: **Cowpea beetle, Hargel, mortality, repellency, antifeedant.**

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is one of the five most important legumes in the tropics which provides protein for most people and nitrogen in the soils [1]. The cowpea seed beetle, *Callosobruchus maculatus* (Fab.) is the most important storage pest of cowpea throughout the tropics [2]. The quantity of cowpea lost annually through *C. maculatus* is substantial, although no accurate figures are available. For instance, Caswell [3] indicated 50% loss at Ibadan and

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minimally above 30% at Zaria, also he added later (1980) that *C. maculatus* damage to cowpea reached 10% by January and July. Throughout tropical Africa, *C. maculates* consumed 50-90% of cowpea in storage annually [4]. Frequently, farm storage for six months was accompanied by 70% seed infestation and about 30% weight loss and virtually seeds become unfit for consumption [5]. In contrast to field crops, stored products cannot compensate losses, further, the treatment of storage pests with chemical pesticides deserved special consideration and precautionary measures should be taken to protect consumer safety. Store products represent a ready form of human and/or animal food, therefore, only safe chemicals are allowed for use in such product. There are many organic compounds of plant origin that has been identified to affect pest population in different ways. They are cheap, of low mammalian toxicity, and are environmentally friendly. Generally, a number of plants product, e.g. oils, powders, ashes, and others, are commonly used by traditional farmers in villages to protect cowpeas from damage in storage [6-9]. They include; Neem, Usher and Hargel shoot extracts which were reported effective against many insect pests [10-14]. These promising reports, initiated our interest to further develop the method of extraction and bioassay.

The main objective of the present study is to investigate the potential of Hargel shoot extracts in the control of the cowpea beetle *Callosobruchus maculatus*. The efficacy will be evaluated through the following criteria;

- The toxicity of organic and aqueous extracts to the target pest
- It's repellent action.
- It's antifeedant effect.
- It's effect on weight loss of stored cowpea.

2. MATERIALS AND METHODS

2.1 Collection and preparation of Hargel shoot 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, spread out 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 tightly covered glass jars wrapped with Aluminum foil until needed for preparation of extracts.

2.2 Organic extracts:

2.2.1 Consecutive extraction:

Sub samples (30g) of Hargel shoot powder were consecutively extracted with three types of 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.

2.2.2 Direct extraction with ethanol:

Subsamples (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.

2.2.3 Preparation of Hargel aqueous shoot extracts:

Aqueous solutions of Hargel shoot powder were prepared by mixing 20 grams of powder with 180 ml distilled water in a conical flask (500 ml) following the method of Ascher [15]. 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 with distilled water.

2.3 Insects Rearing:

The primary stock of *Callosobruchus maculatus* was obtained from the Department of Crop Protection, Faculty of Agriculture, University of Khartoum. The collected insects were placed in three glass jars (capacity 3 kg), half filled with the media (cowpea seeds) to allow flight space for adults. Jars were covered with muslin cloth fixed with rubber bands [16]. Four weeks later the culture was sieved. Sieving was done 24 hrs prior to the test. The old adults were removed and newly emerged adult (0-24 hrs old) were collected for bioassay.

2.4 Bioassay:

2.4.1Toxicity of Hargel shoot extracts to test insects:

The method described by Udo and Epidi [17] was followed; Petri dishes 9 cm in diameter were used to confine insects during the experiment. Filter paper 9 cm diameter was treated with 2 ml 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 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.

2.4.2 Repellancy test:

The method described by Koko *et al.* [18] was followed; Petri dishes 9 cm 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 center 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:

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

Where:

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

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

2.4.3 Antifeedant test:

Antifeedant test was done following the method of Owusu *et al.* [19]. Fifty grams of cowpea 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 adult *C. maculatus* (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

2.4.4 Effect on weight loss:

For grain damage assessment, samples of 50g grains of cowpea were taken into cups and mixed with 2 ml of 10, 5, 2.5 and 1% organic and aqueous extracts and left for one hr to dry at room temperature. The control was treated with solvent and water alone. Twenty adult *C. maculatus* (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

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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.* [19] as follows:

% Weight loss = [UaN- (U+D)]/UaN x 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

2.5 Statistical analysis:

Collected data were expressed as percentage and subjected to the analysis of variance following the procedure described by Gomez and Gomez [20] using SAS software for windows version 9 (2004). Lethal doses (LD) and effective doses (ED) were calculated following probit analysis method according to Finney [21] using Minitab software version 13.3 (2000).

3. RESULTS

Various extracts from Hargel shoot system were tested as potential source of control agent for *C. maculatus.* 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 adult 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 3 days of exposure. Generally direct extraction with ethanol is the most potent followed by aqueous extracts, ethanol, ethyl acetate and petroleum ether extracts.

The probit analysis for 72 hours of exposure indicated an LD_{50} value of 74 % for Petroleum ether extract, 3.87 % for ethyl acetate extract, 1.37 % for ethanol extract, 0.39 % for direct extraction with ethanol and 0.56 % 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 direct extraction with ethanol > aqueous extract > ethanol extract > ethyl acetate extract > petroleum ether extract. 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 heterogeneous population with respect to sensitivity to test extracts. Chi-square values were small indicating good execution of the experiments (Fig. 3).

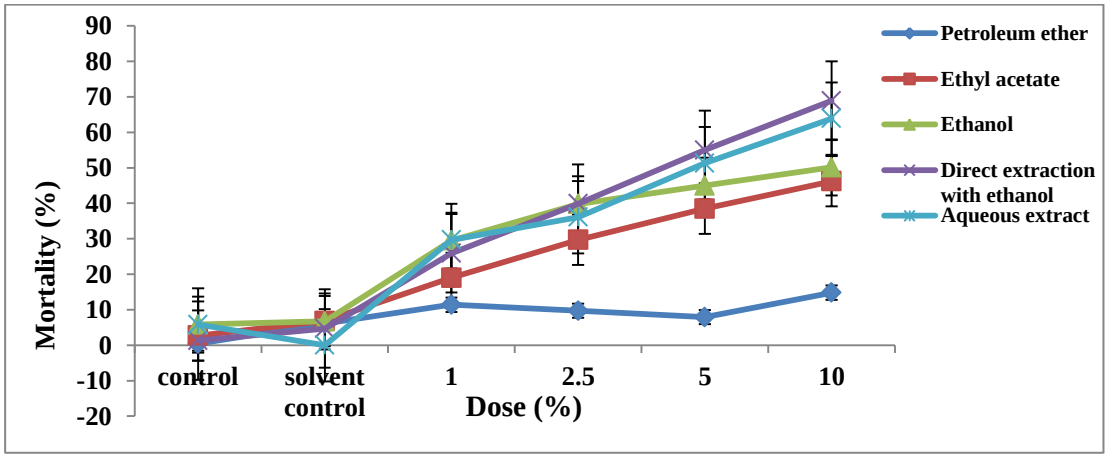

Figure 1: Percentage mortality of Callosobruchus maculatus following two days exposure to various types of Hargel shoot extracts.

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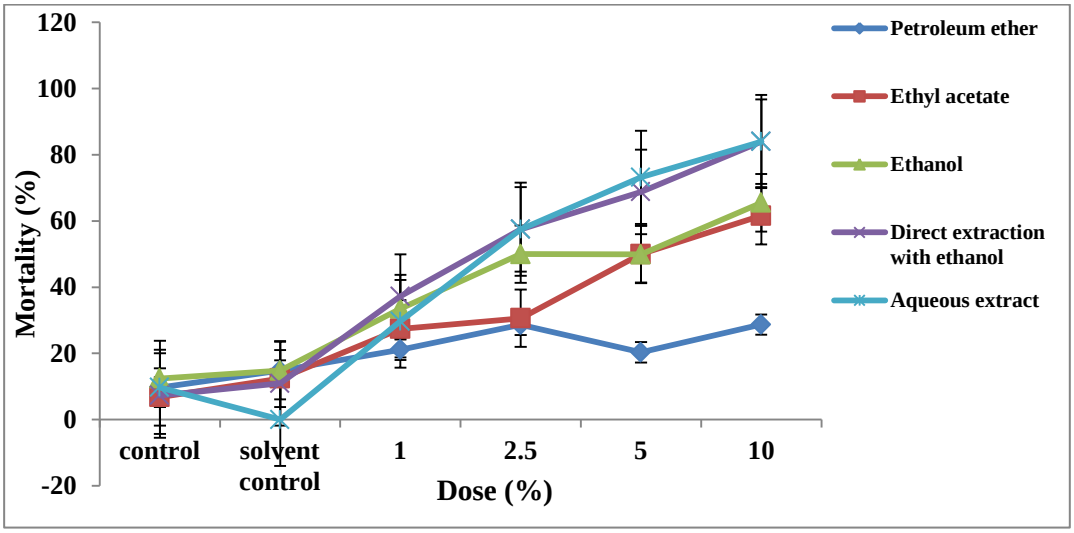

Figure 2: Percentage mortality of C. maculatus following three days exposure to various types of Hargel shoot extracts.

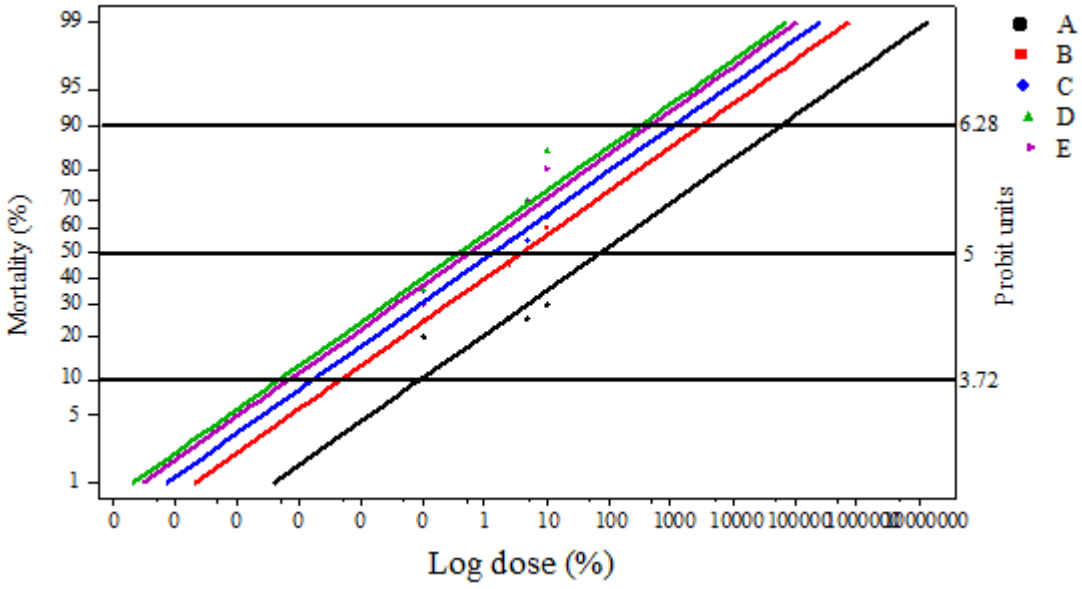

Figure 3: Mortality response probit lines of C. maculatus (adult) exposed to Hargel shoot extracts for 72 hours.

A: Petroleum ether

B: Ethyl acetate

C: Ethanol

D: Direct extraction with ethanol

E: Aqueous extracts

(A, B &C consecutive extraction)

3.2 Repellent actions:

All concentrations of various extracts caused significant repellency and effects were dose and time dependent (Fig. 4 and 5). The highest effects were noticed at first and second day after exposure. Generally aqueous extract is the most potent followed by direct extraction with ethanol, petroleum ether, ethyl acetate and ethanol extract after first day while after the 2nd day ethyl acetate extract is the most potent followed by petroleum ether, ethanol extract, aqueous extract and direct extraction with ethanol.

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The probit analysis of the first 24 hours indicated an ED_{50} value of 24 % for petroleum ether extract, 29 % for ethyl acetate extract, 36 % for ethanol extract, 14 % for direct extraction with ethanol and 8% for aqueous extract. Since the slopes of the 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 > ethyl acetate > ethanol 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 heterogeneous population with respect to sensitivity to test extracts. Chi-square value was small indicating good of the execution experiments (Fig. 6).

The probit analysis for 48 hrs (Fig. 7) indicated an ED_{50} value of 15 % for petroleum ether extract, 12 % for ethyl acetate extract, 15.12 % for ethanol extract, 43 % for direct extraction with ethanol and 16 % 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 etheyl acetate extract > petroleum ether extract > ethanol extract > aqueous extract > direct extraction with ethanol. 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 heterogeneous population with respect to sensitivity to test extracts. Chi-square value was small indicating good execution of the experiments.

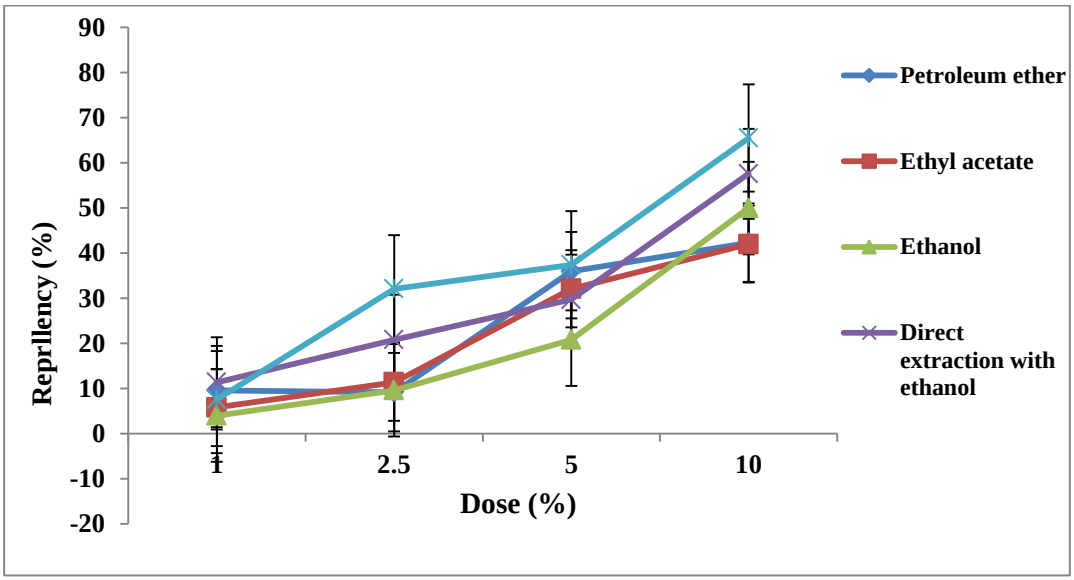

Figure 4: Repellency percentage of C. maculatus following one day exposure to various types of Hargel shoot extracts

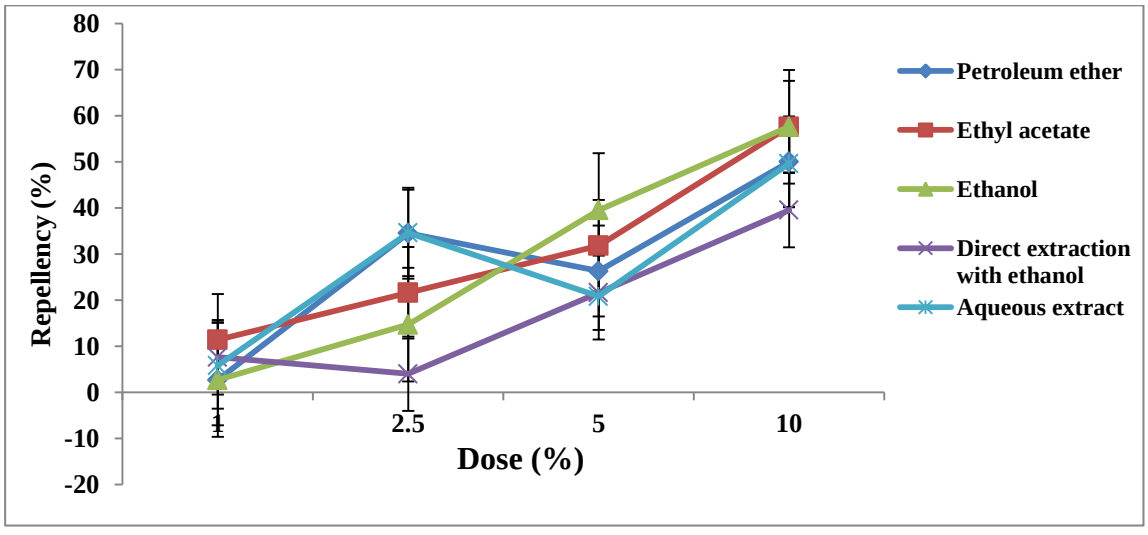

Figure 5: Repellency percentage of C. maculatus following two days exposure to various types of Hargel shoot extracts.

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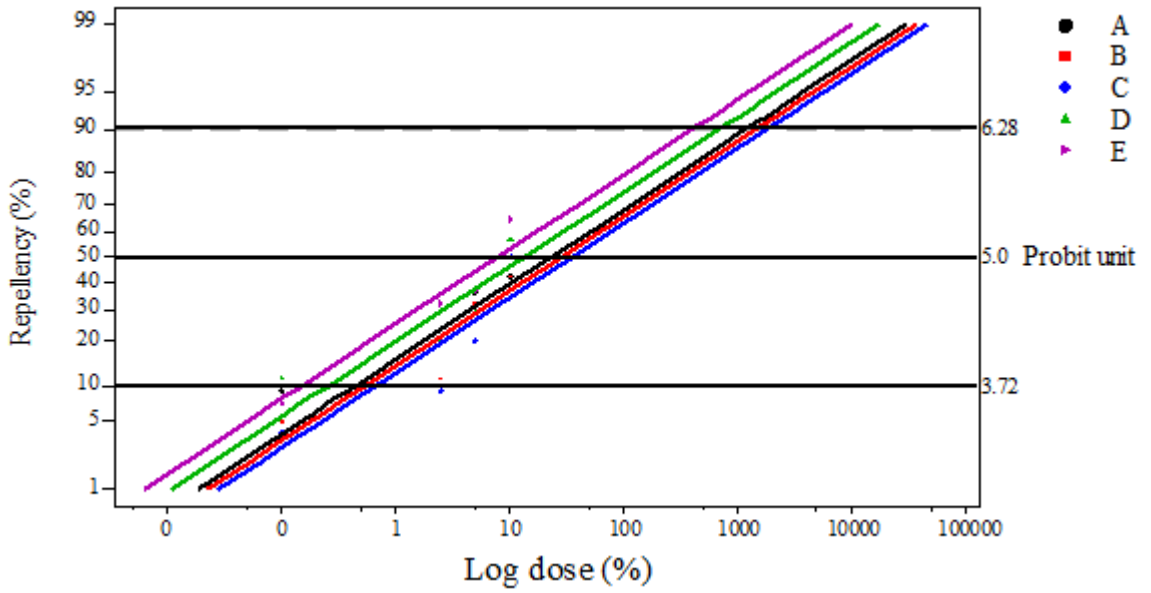

Figure 6: Repellency response probit lines of C. maculatus (adult) exposed to Hargel shoot extracts for 24 hours.

A: Petroleum ether
B: Ethel acetate
C: Ethanol
D: Direct extraction with ethanol
E: Aqueous extracts
(A, B &C consecutive extraction)

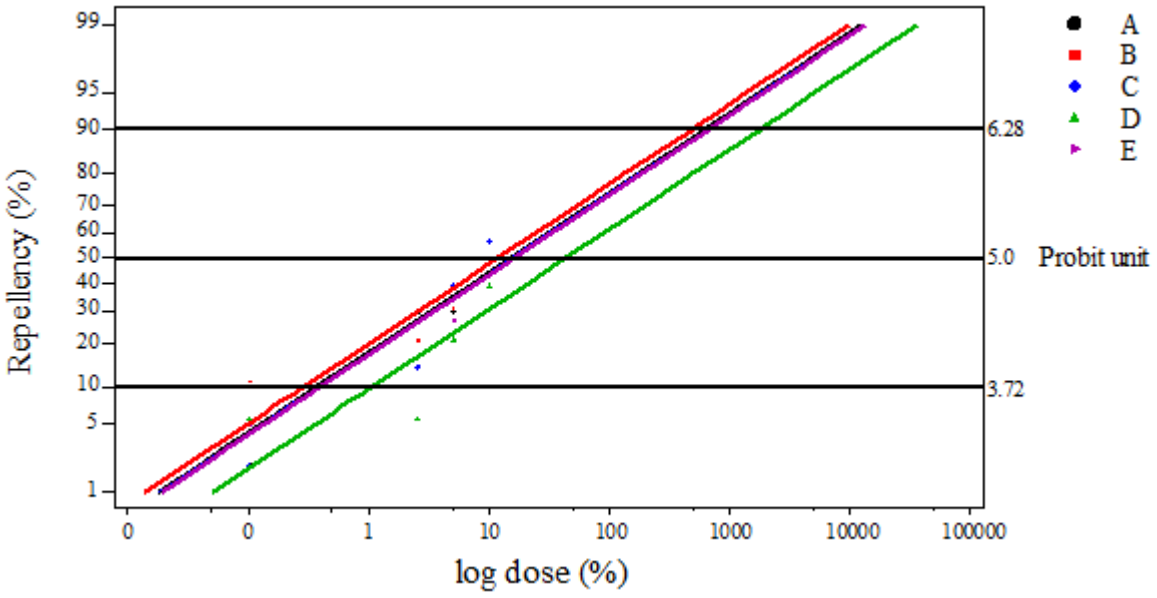

Figure 7: Repellency response probit lines of C. maculatus (adult) exposed to Hargel shoot extracts for 48 hrs.

A: Petroleum ether
B: Ethel acetate
C: Ethanol
D: Direct extraction with ethanol
E: Aqueous extracts
(A, B &C consecutive extraction)

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3.3 Antifeedant effects:

All extracts caused significant antifeedant effects compared to the control and effects were dose and time dependent (Fig.8). Generally direct extraction with ethanol showed the highest potency followed by ethyl acetate extract, aqueous extract, ethanol extract and petroleum ether extracts. The feeding ration (Fr) decreased with the increase of the concentration and increased when the exposure period increases (Fig.8).

3.4 Weight loss effects:

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

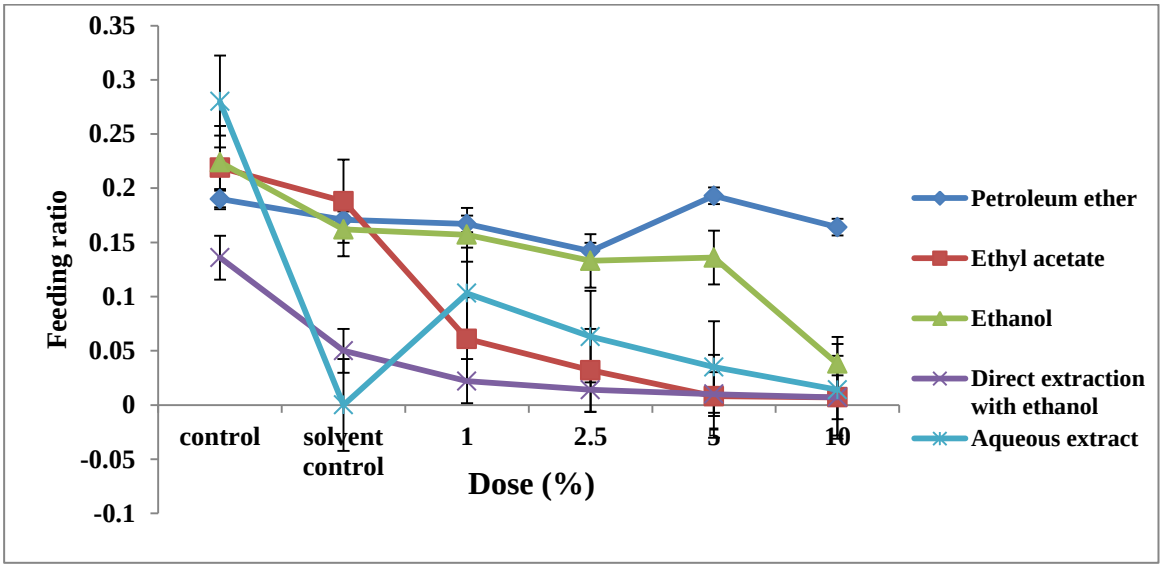

Figure 8: Feeding ratio of C. maculatus following 60 days exposure to various types of Hargel shoots extracts.

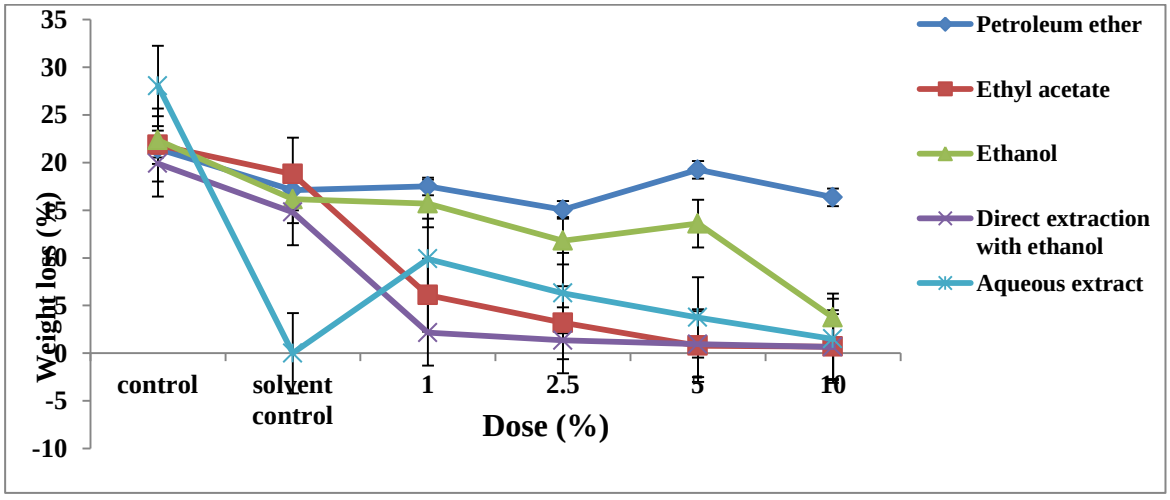

Figure 9: Weight losses in stored cowpea seeds caused by C. maculatus following sixty days exposure to various types of Hargel shoot.

4. DISCUSSION

The use of synthetic pesticides have caused serious problems to the environment, such as; contamination of the biosphere, toxicity to man, animals, beneficial insects and other non-target organisms. These problems had drawn the attention of public and policy makers to the need to adopt new pest management strategies, based on safe naturally occurring products [22]. The most promising natural products tested in the Sudan included; Neem *Azadirachta indica* [23], Usher *Calotropis procera* [24], Rehan *Ocimum basilicum* [25] and Hargel *Solenostemma argel* shoot, garlic oil/and or aqueous [14, 28].

In the present work various aspects of the efficacy of five extracts of Hargel shoots were tested against the adult stage of *C. maculatus*. The investigations included evaluation of the following parameters of efficacy such as 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 the control. The probit analysis indicated that direct extraction of Hargel shoot with ethanol gave the superior effects as indicated by its low LD_{50} value with narrow fudicial limits. The highest mortality rate for four types of extracts was achieved after the third day of exposure with an LD_{50} less than 4%. The current result was the first study in the Sudan which evaluates the efficacy of hargel shoot extracts on the adult of *C. maculatus.* Previous studies in the Sudan and the region reported on the effects of hargel shoot powder, aqueous or ethonolic extracts on different types of insects; *B. incarnatus*, *T. castaneum*, *Culex pipiens* [14, 29, 29-30] who reported on *Microtermes thoracalis.* Generally the current result agrees with their findings although test insects and type of products tested is different.

The test population of adults was relatively heterogeneous as evident by the low slope and high LD_{90}/LD_{50} ratio. This is clear also 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 from 0.0005 % to 0.09 %) may be found in other sources of stock culture specially if collected from other regions of the Sudan.

The results of the repellent effects showed that all extracts of Hargel shoot induced significant dose dependent repellency to the test insects with the highest effects clearly noticed after the 2nd day of exposure (ED_{50} less than 16% for four types of extracts). The ethyl acetate extract was the most effective as indicated by its low ED_{50} value (12%) with narrow fudicial limits. However after 24 hours of exposure the aqueous extract was the best with an ED_{50} less than 8% with narrow fudicial limits. These findings were in line with results obtained by Sir Elkhatim and Abdelbagi [26] who reported that hargel shoot powder and aqueous extract has shown repellant activities against *T. castaneum*. The ED_{90} / ED_{50} ratio is relatively narrow, especially in the 2nd day, compared to the corresponding mortality ratio which indicated a better homogeneity response to repellency compared to mortality effects. 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 the bollworms [26].

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 period increases. Direct extraction with ethanol 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 cowpea 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 [31].

5. CONCLUSION

The results of the current study indicated that the all Hargel shoot extracts caused significant mortality, Repellency and antifeedant action to all test insect. The hargel shoot in all different extracts forms decrease of weight loss of *Vigna unguiculata* seeds infested by *C. maculatus*. Based on the current results 84% mortality and 65.5% repellency action can be achieved at higher concentration (10%) of all hargel shoot extracts at the adult stage of *C. maculatus*. Effects were related to dose and exposure period.

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