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Efficacy of Argel (*Solenostemma argel* (Del) hayne) Shoots' Extract in the Control of the Red Flour Beetle (*Tribolium castaneum* Herbst)

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Abstract: Laboratory studies were conducted to evaluate the efficacy of aqueous and organic extracts of the argel (*S. argel*) shoots against the 4th larval instar and adult stages of the red flour beetle (*Tribolium castaneum*) in the Sudan. Argel shoots were extracted sequentially by organic solvents of increasing polarity (petroleum ether, ethyl acetate and ethanol) as well as directly by distilled water or ethanol. Extracts were tested at concentrations ranging between 1% and 10%. The evaluated efficacy parameters were mortality, repellency and antifeedant effects. The tests were conducted in Petri dishes (9 cm i.d) and plastic cups (capacity 200 ml) and the obtained data were subjected to the analysis of variance and to the probit analysis. The results of the mortality data indicated that petroleum ether extracts was the most potent against the 4th larval instar of *T.castaneum* as shown by its low LD_{50} of 8.2%, while ethyl acetate extract was the most potent against the adult of this pest with an LD_{50} value of 16%. The results showed that various types of argel shoot extracts induced significant dose dependent repellency against the *T. castaneum*. The highest 24 hours repellency was caused by the petroleum ether extract on the adult stage of *T. castaneum* as indicated by its low ED_{50} value of 1.58%.The different argel shoot extracts induced significant antifeedant action against the test insect. The lowest feeding ratio (0.02) was recorded in ethyl acetate extract- treated crushed wheat grains.

Key words: *Tribolium castaneum*; argel shoots; mortality; repellency and antifeedant effects

INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst), is a major pest in anthropogenic structures used for the processing and storage of grain-based products (*e.g*., flour mills, warehouses, retail stores). This species had a long association with human stored food and has been found in association with a wide range of commodities including grain, flour, peas, beans, coco, nuts, dried fruits and spices, but milled grain products such as flour appear to be their preferred food (Good 1936). The ability of this species to find and colonize patches of food and to persist on small amounts of food that accumulated in mass, contributes to its pest status (Campbell and Hagstrum 2002). *T. castaneum* is considered a major pest of stored grains (Howe 1965).

Annual post-harvest losses, resulting from insect damages, microbial deterioration and other factors, are estimated to be 10%-25% of worldwide production (Matthews 1993). Control of these insects relies heavily on the use of synthetic insecticides and fumigants, the widespread use of which has led to some serious problems including development of insect resistance to insecticides (Rachid *et al*. 2006), toxic residue on stored grain, and toxicity to consumers and increasing costs of application. Therefore, there is an urgent need to develop safe alternatives of low cost, convenient to use and environmentally friendly. Considerable efforts have been focused on plant derived materials as alternative to conventional insecticides for insect control. Many plants and their secondary metabolites are known to have various activities against different species of insect (Grainge and Ahmed 1988).

The use of plants and minerals as traditional protectants of stored products is an old practice used all over the world (Golob and Webley 1980). These traditions have been largely neglected by farmers, after the Second World War, with the advent of synthetic or petroleum based insecticides. However, the potential hazards for mammals from synthetic insecticides, the ecological consequences and the increase of insect resistance to pesticides have led to search for new classes of insecticides with lower mammalian toxicity and a lower persistence in the environment (Mahadi and Rahman 2008). One of the promising sources of botanical insecticides in Sudan is argel (Sidahamed *et al*. 2009b). The expected low mammalian

toxicity of this plant, being a common human beverage, further strengthens our goals.

This work is intended to evaluate the efficacy of organic and aqueous extracts of argel [*S. argel* (Del) Hayne] shoots in the control of *Tribolium castaneum*.

MATERIALS AND METHODS

Collection and preparation of argel shoot powder

The vegetative parts of argel were collected from the Northern State at 'Al Robatab' area (Alshereig) in June 2008. The collected samples were cleaned and washed under tap water, 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 Aluminum foil until needed for preparation of extracts.

Organic extracts

Consecutive extraction: Sub samples (30g) of argel shoot powder were consecutively extracted with three organic solvents (petroleum ether, ethyl acetate and ethanol) of increasing polarities. The samples were extracted with Sox let 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.

Direct extraction with ethanol: The samples (30 g) 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.

Preparation of argel aqueous shoot extracts: Aqueous solutions of argel shoot powder were prepared by mixing 20 grames 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)10%, 5%, 2.5% and 1% were prepared by serial dilution by adding distilled water.

***Tribolium castaneum* culture:** The primary stock of *Tribolium castaneum* was obtained from the Department of Crop Protection, Faculty of Agriculture, University of Khartoum. The stock was reared in a plastic container (35x30x98cm) containing wheat flour mixed with yeast (12:1 w/w). The culture was kept at room temperature. About 500 adults were collected from the stock cultures and reared in small containers (glass jars). Each container was covered with muslin cloth fixed with rubber band. Paper balls were placed on the culture surface to enhance the mating rates and left for about 3 weeks under room temperature for oviposition. When cultures started to emerge, they were separated from the old adults, and the newly emerged ones were collected over one-week period. Therefore, adults collected would be 0-1 week old. The collected adults were then transferred to fresh flour for another week. Hence, tested adults would be 1-2 weeks old; also the 4^{th} larval instars were collected from the culture for the efficacy tests.

Bioassay

Toxicity of argel shoot extracts to test insects: The method described by Udo and Epidi (2009) was followed; Petri dishes of 9 cm diameter were used to confine insects during the experiment. Filter paper of 9 cm diameter was treated with 2 ml of argel shoot extract. The filter papers were allowed to dry for 30 min at room temperature. Twenty adults or 4^{th} larval instar of the test insects were introduced into each Petri dish using a camel hair brush. Argel 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 seven days for adult and for three days for the 4^{th} larval instars of *T. castaneum*. The experimental units were arranged in a completely randomized design (CRD) with four replicates.

Repellency test: The method described by Ko ko *et al.* (2009) was followed; Petri dishes of 9 cm diameter were used to confine insects during the experiment. Filter paper of a 9 cm diameter was cut to two halves 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 at room temperature. A full disc was carefully remade by attaching the two halves with tape. Care was taken so that the attachment does not prevent free movement of insects from one half to another, but the distance between the two halves remained sufficient to prevent seepage of test extract from one half to another. Twenty adult test insects were released in the center of each filter paper and covered immediately by Petri dish. Argel shoot extract was tested at 4 concentrations (10 %, 5%, 2.5% and 1 %). Solvent (petroleum ether, ethyl acetate and ethanol) and untreated control were included. Each treatment was replicated four times and units were arranged in a Completely Randomized Design. Insects in each half were counted every day for three days. The percentage repellency of each extract was then calculated using the following 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

Antifeedant test

Antifeedant test was done following the method of Owusu *et al.* (2007). Fifty grames of crushed wheat grains were 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 *T. castaneum* 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 experimental units were arranged in a CRD with four replicates. After 30 and 60 days, the remaining grains were reweighed and feeding ratio (*Fr*) was calculated as follows:

$$\mathbf{Fr = 1 - FW/50}$$

where: **FW** ≡ the final grain weight

RESULTS

Effect on mortality of the 4th larval instar of *T. castaneum*

All extracts caused significant mortality compared to the control (Fig.1), and the effects were dose and time dependent. The highest effects were noticed after 3 days of exposure. Generally petroleum ether extract was the most potent, followed by direct extraction with ethanol, consecutive extraction with ethanol, ethyl acetate and aqueous extracts.

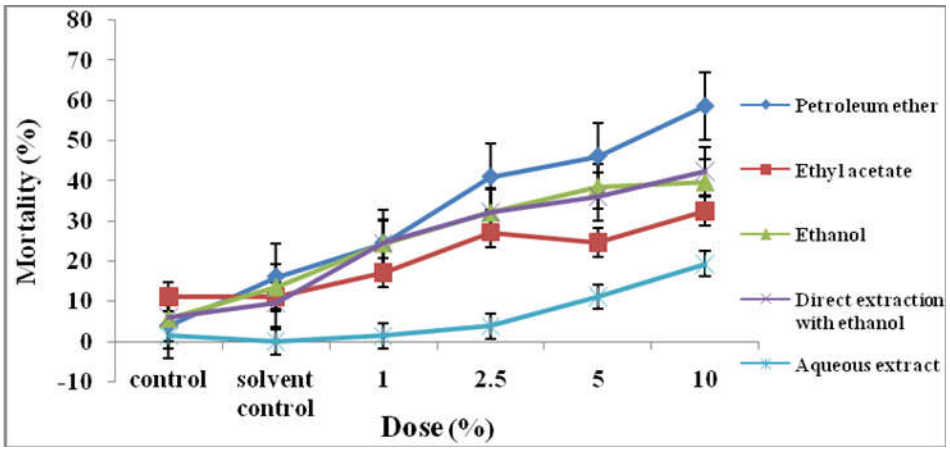

Fig. 1. Percentage mortality of 4th larval instars of *T. castaneum* after three days of exposure to various types of argel shoot extracts

The probit analysis indicated an LD_{50} value of 8.2% for petroleum ether extract, 182% for ethyl acetate extract, 47% for ethanol extract, 37 % for direct extraction with ethanol and 687111% 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 petroleum ether > direct extraction with ethanol > consecutive extraction with ethanol > Ethel acetate > Aqueous extracts. Fudicial limits are generally narrow at the LD_{50}. Slopes are relatively flat 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. 2).

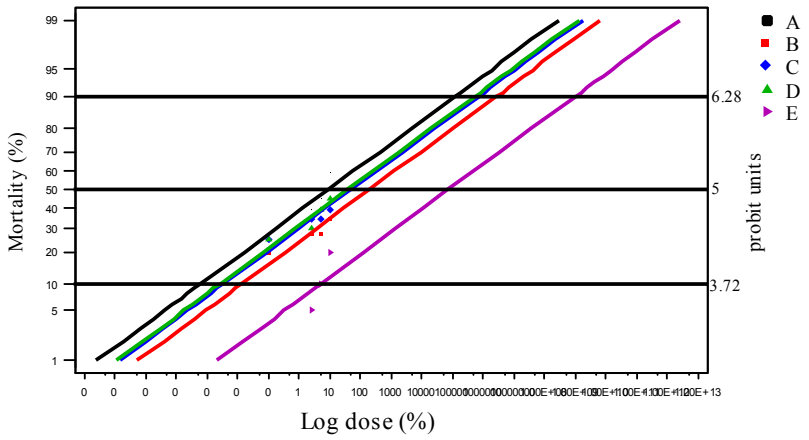

Fig. 2. Mortality response probit lines of *T. castaneum* (larvae) exposed to argel shoot extracts for 72 hours

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

Effect on mortality of the adult stage of *T. castaneum*

All extracts caused significant mortality compared to the control, andthe effects were dose and time dependent (Fig.3). The highest effects were noticed at seven days after exposure. Generally, ethyl acetate extract was the most potent followed by aqueous extract, petroleum ether, direct extraction with ethanol and ethanol extracts.

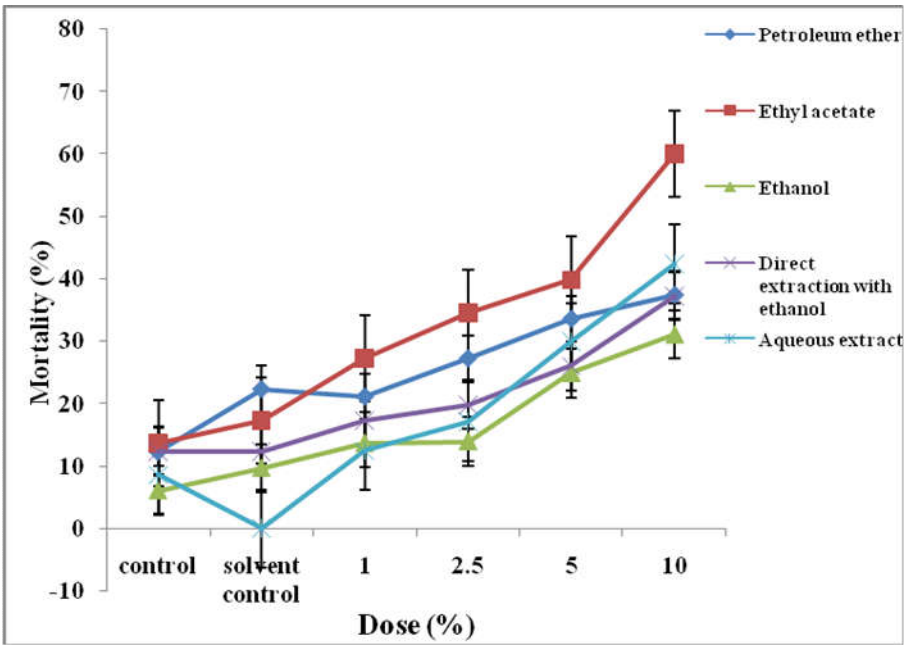

Fig. 3. Percentage mortality of *T. castaneum* (adult) after seven days of exposure to various types of argel shoot extracts.

The probit analysis indicated an LD_{50} value of 115% for petroleum ether extract, 16 % for ethyl acetate extract, 650% for ethanol extract, 358% for direct extraction with ethanol and 280% for aqueous extract. Since all 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 ethyl acetate extract > petroleum ether extract > aqueous extract > direct extraction with ethanol > ethanol extract. Fudicial limits are generally narrow at the LD_{50}. Slopes are relatively flat and LD_{90}/ LD_{50} ratio was 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. 4).

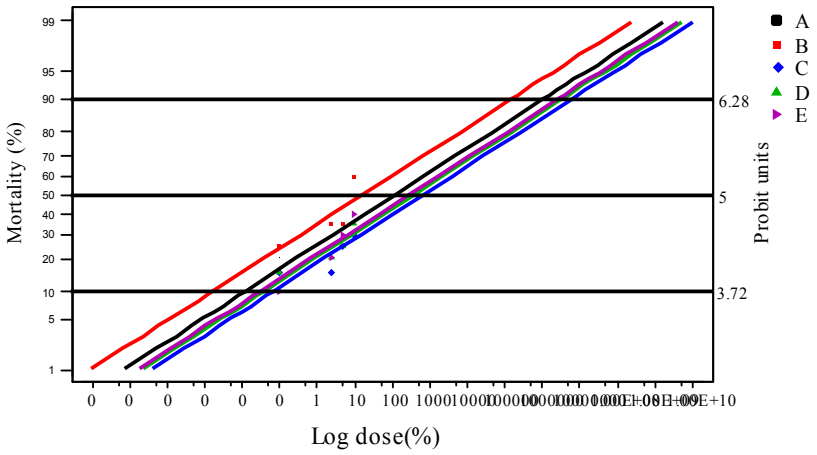

Fig. 4. Mortality response probit lines of *T. castaneum* (adult) exposed to argel shoot extracts for seven days

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

Repellent actions

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

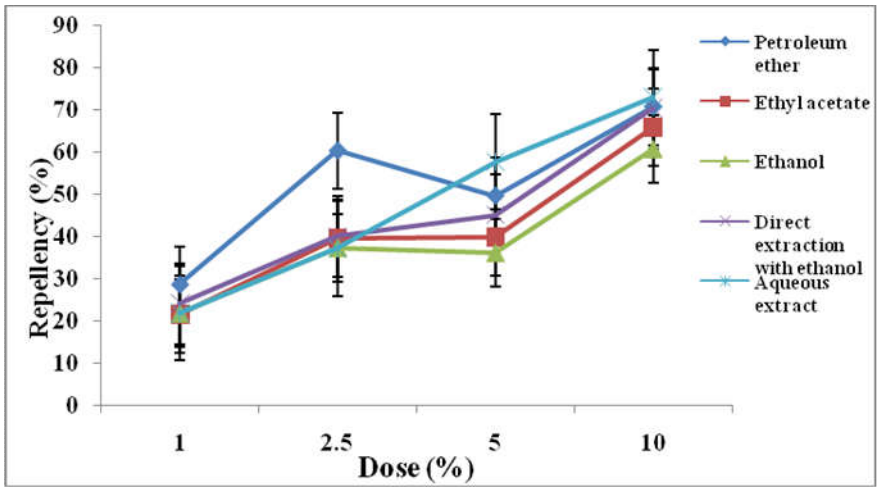

Fig. 5. Repellency percentage of *T. castaneum* (adult) after one day of exposure to various types of argel shoot extracts

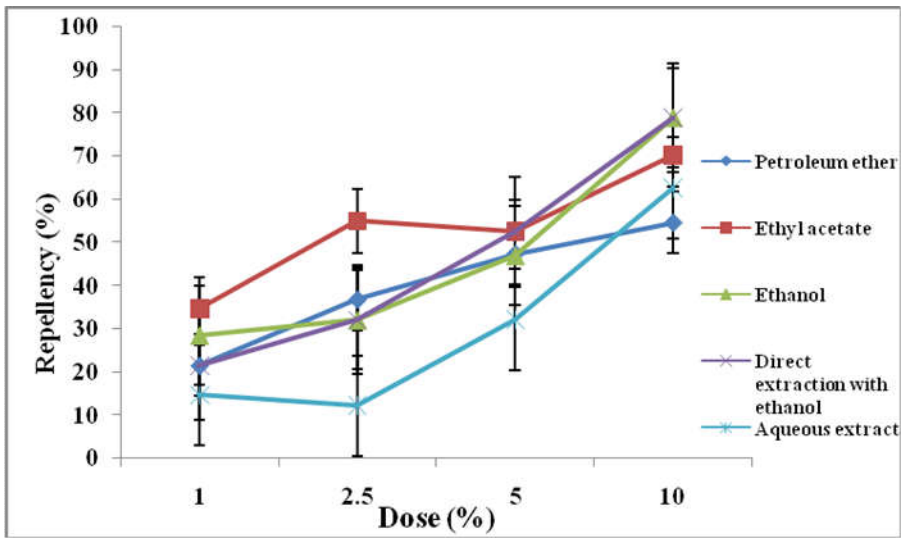

Fig. 6. Repellency percentage of *T. castaneum* (adult) after two days of exposure to various types of argel shoot extracts

The probit analysis of the first 24 hours indicated an ED_{50} value of 1.58% for petroleum ether extract, 5.78% for ethyl acetate extract, 7.82% for ethanol extract, 3.77 % for direct extraction with ethanol and 2.899% 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 petroleum ether > aqueous extract > direct extraction with ethanol > ethyl acetate > ethanol extracts. Fudicial limits are generally narrow at the ED^{50}. Slopes are relatively flat and ED90/ ED50 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 (Fig.7).

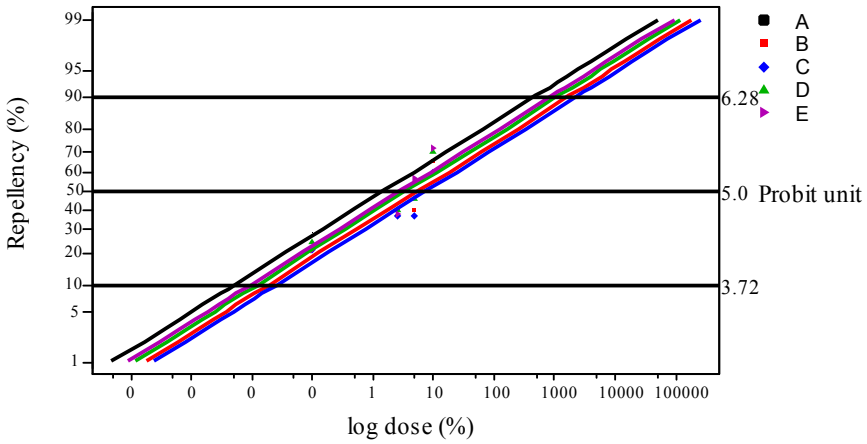

Fig. 7. Repellency response probit lines of *T. castaneum* (adult) exposed to argel shoot extracts for 24 hours

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

The probit analysis for the 48 hours (Fig. 8) indicated an ED_{50} value of 6.18% for Petroleum ether extract, 1.45% for ethyl acetate extract, 3.26% for ethanol extract, 3.13 % for direct extraction with ethanol and 19.09% 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 ethyl acetate extract > direct extraction with ethanol > ethanol extract > petroleum ether extract > aqueous extract. Fudicial limits are generally narrow at the ED_{50}. Slopes are relatively flat 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 (Fig.8).

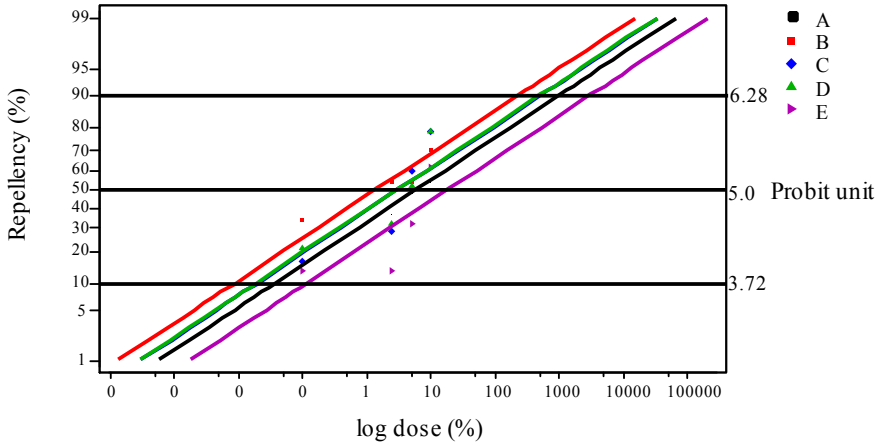

Fig. 8. Repellency response probit lines of *T. castaneum* (adult) exposed to argel shoot extracts for 48 hours

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

Antifeedant effects

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

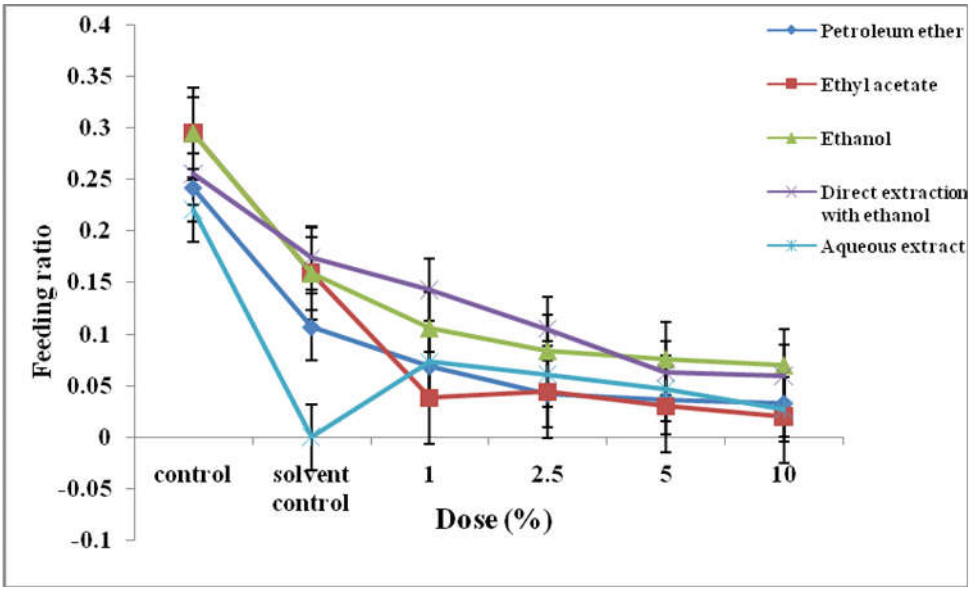

Fig. 9. Feeding ratio of *T. castaneum* (adult) after 60 days of exposure to various types of argel shoot extracts

DISCUSSION

The use of synthetic pesticides for pest control has led to serious hazards to the environment including pollution, destruction of beneficial organisms and natural enemies in addition to development of insect resistance and the high cost (Sir El Khatim 2005; Elsonoussy 2009). This necessitated the search for safe naturally occurring alternatives. Among the promising sources are those derived from plants (Siddig 1991;

Elsonoussy 2009).The most promising natural products tested in the Sudan included Neem (*Azadirachta indica)* (Schumutterer and Ascher 1987), Osher *Calotropis procera* (Fagger 1999), Rehan (*Ocimum basilicum*) (Stoll 2001) and Argel (*S. argel*) shoot, (Sir Elkhatim 2005; Sidahamed *et al.* 2009b). In the present work, various aspects of the efficacy of five extracts of argel shoot were tested against the larva and adult of the red flour beetle [*Tribolium castaneum* (Herbst)]. The present investigation targeted the evaluation of the mortality, repellency and antifeedant effects.

The results showed that the mortality of 4^{th} larval instars of test insect increased with the increase in the concentration of argel shoot extracts. Most of the test concentrations were significantly better compared to the control. The probit analysis indicated that petroleum ether extract was the most effective as indicated by its low LD_{50} value with narrow fudicial limits. These results are in accordance with a previous study carried out by Sir Elkatim (2005) who reported that argel products caused significant effects on mortality of adults and larvae of *T. castaneum*. On the other hand, all extracts of argel shoot induced relatively less effects on the mortality of the adult stage of *T. castaneum,* whereas the highest effects were noticed after 7 days of exposure compared to three days for larvae. The probit analysis indicated that ethyl acetate extract of argel shoot was the most effective against the adult stage as indicated by its low LD_{50} value with narrow fudicial limits. These findings arein line with results of Al-Doghairi *et al.* (2004); Sir Elkhatim (2005); Abdel-Rahman and Al-Mozini (2007) and Sidahmed *et al.* (2009a) who reported that argel shoot products possess insecticidal activities to *T. castaneum*; *Spodoptera littoralis*; *Culex pipiens* and *Microterm thoracalis*.

The test population of both larvae and adults were relatively heterogeneous as evident from the low slope and high LD_{90}/LD_{50} ratio. This was also clear when comparing the LD_{90} to the LD_{10}. This heterogeneity may be of significance for future work where highly susceptible individuals (LD_{10} range between 0.001% to 5% for larvae and 0.002% to 0.04% for adults) 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 argel shoot induced significant dose dependent repellency to the test insects with the highest effects noticed after the first 24 hours of exposure (ED_{50} less than 8%). The petroleum ether extract was the most effective as indicated by its low ED_{50} value (1.6%) with narrow fudicial limits. However, after 48 hours of exposure all extracts generally maintained their efficacy at an ED_{50} of less than 6% except the aqueous extract which showed an ED_{50} of 20%. Also, the 48 hours data showed that the ethyl acetate extract was the best with an LD_{50} of 1.4% with narrow fudicial limits. These findings are in line with the results obtained by Sir Elkhatim (2005) who reported that aqueous extract of argel shoot powder had shown repellant activities against *T. castaneum*. The ED_{90}/ ED_{50} ratio was 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 argel shoots in the main irrigation canals to repel insect of vegetables particularly the bollworms (Sir Elkatim 2005).

The results of this study showed that the different types of argel shoot extract caused significant antifeedant effects on the adults of *T. castaneum* compared to the control. The effects were dose and time dependent with the Fr decreasing with the increase of the concentration and exposure period. Ethyl acetate extract was the most potent.

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كفاءة مستخلصات نبات الحرجل
Solenostemma argel (Del) Hayne
فى مكافحة خنفسانة الدقيق الحمراء Tribolium castaneum (Herbst)

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المستخلص: أجريت دراسات مختبرية لتَقييم كفاءةِ المستخلصات المائيةِ والعضويةِ لنبات الحرجل ضدّ الطور اليرقي الرابع والطور الكامل لخنفساء الدقيق الحمراء (*Tribolium castaneum*) تم استخلاص المجموع الخضري لنبات الحرجل بصورة متتالية وبمذيبات عضوية تتدرج في القطبية (البترولیم إيثر وإيثايل أستيت والايثانول) ومباشرة بالماء المقطر وبمذيب الايثانول كلٌ على حده . أجريت الاختبارات بتراكيز تراوحت ما بين 1% و 10% . إشتملت معايير تقيم الكفاءة على دراسة تقييم التأثير القاتل والطارد والمانع للتغذية. أجريت الاختبارات الحيوية في اطباق بتري (قطرها 9 سم) واكواب بلاستيكية (سعة 200 مل) . وأخضعت البيانات لتحليل التباين (ANOVA) وتحليل البروبت (probit analysis) . أشارتْ بيانات التاثير القاتل بأنّ مستخلص البترولیم ايثر كان الأكثر فعالية ضدّ الطور اليرقي الرابع لخنفساء الدقيق الحمراء . ودل على ذلك اقل جرعة قاتلة نصفية LD_{50} (8.2%) ، بينما مستخلص الايثايل استيت كَانَ الأكثر فعالية ضدّ الطور البالغ لهذه الحشرةِ باقل جرعة قاتلة نصفية LD_{50} (16%) . أشارت النتائج على أن جميع مستخلصات المجموع الخضري احدثت تأثيراً طارداً يتناسب مع الجرعة ضد الحشرة موضوع الإختبار . أحدث مستخلص البترولیم إيثر أعلى نسبة طرد بعد 24 ساعة وذلك علي الطور الكامل لخنفساء الدقيق الحمراء كما دل على ذلك جرعتة الفعالة النصفية الأقل (ED_{50}) 1.58% . أحدثت مستخلصات المجموع الخضري منعاً معنوياً متناسباً مع الجرعة في تغذية الحشرة المختبرة . سُجّلتْ اقل نسبة تغذية لحبوب قمح مدروش ومعامل بمستخلص الايثايل استيت .