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Full Length Research Paper

Insecticidal potentialities of *Balanites aegyptiaca* extracts against the khapra beetle (*Trogoderma granarium*)

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Desert date (*Balanites aegyptiaca*) is a widely distributed natural tree in Sudan and many other tropical countries especially in Africa and Asia. The tree is rich in useful products with multi-uses in rural lives and industry. Hence, the current laboratory study was aimed to evaluate the insecticidal potentiality of *B. aegyptiaca* against the third instar larvae of *Trogoderma granarium*. Water and organic solvents extracts of leaves, branches and seeds were tested at different concentrations. The extracts yields, mortality and repellent effects and the consequent damage on sorghum grains were the main parameters studied. The highest extractive yield (56.7%) was obtained by the seeds hexane extract (oil). This extract also scored better mortality effects than the other treatments. The highest concentrations (2.5% and 5%) of oil showed comparably the best significant effects since the second day of treatments, and revealed progressive activities with exposure time. The latter concentration (5%) manifested 72.7% repellency for the insect larvae, and saved sorghum grains damage by 30.0% within 45 days post treatments. Nevertheless, the highest savings of grains (50.0%) that attained by the water seeds extract in spite of its inferior mortality results may needs to be clarified in further research. The findings obtained proved the insecticidal potentialities of *B. aegyptiaca* and encouraging a new forward plan.

Keywords: Desert date, extracts, bioassay, repellent, *Trogoderma granarium*.

INTRODUCTION

The uses of synthetic insecticides for controlling insect pests have been associated with several problems which became more apparent in recent years. This necessitated the search for eco-friendly alternatives for pest management. Among the proposed alternatives are the uses of biocides specially those of plant origin. However, research on botanical pesticides in Sudan was firstly performed sporadically during the last two decades of the 20^{th} century (Siragelnour, 1986; Siddig, 1987). Later on, this kind of research has been adopted by different institutions. The results obtained so far reflected the presence of several active plants that need meticulous investigations, and proved the potentiality of the country in this field (Satti, *et al*., 2010). Nevertheless, it is worthy to state that most of the previous works were

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concentrated mainly on neem tree (*Azadirachta indica*) as one of the most active plants studied (Yousif and Satti, 2008). Hence, screening of other indigenous plants is urgently looked-for.

Desert date (*Balanites aegyptiaca* (L.) Del.), of the family Balanitaceae, is one of the prevailing natural trees in Sudan and many tropical countries in Africa and Asia (Vogt, 1995; El Ghazali *et al*., 2004). It is an evergreen tree adapted to various climatic conditions especially in arid regions with extremely high temperatures and scarce water; thus it was advised to be promoted for combating desertification (Gour and Kant, 2012). So, the tree is widely distributed in the Sudan under rainfall of less than 1000mm, with higher occurrence in zones of 200 – 800mm (Badi *et al*., 1989). The flowering time generally occurs during November – April, while the fruiting takes places during December – July (El Amin, 1990; El Ghazali *et al*., 1994; Bein *et al*., 1996). The fruit yield was estimated to be about 100 to 150 kg/ tree/ annum. This tree is rich in medicinal ingredients such as diosgenin, and contains other useful products with multi-uses in rural lives and industry. Among such useful products, high level of oil (30-60%) can be extracted from seeds with valuable application as cooking oil as well as biofuel (Hall and Walker, 1991; Khidir, 1997; Chapagain *et al*., 2009). Secondary metabolites like rotenone, coumarin, bergopin, steroids yamogenin and the flavonoids isorhamntetin-3-rutinoside and 3-rhamnoglactoside were detected in different parts of the tree (El Ghazali, *et al*., 1997). Also, *B. aegyptiaca* is named as an African-Asian saponin-producing plant due to its high constituent of saponin compounds. These multiple chemicals have proved different biological activities including molluscicidal, mosquitocidal and insect antifeedant properties, besides other industrial uses (Hall and Walker, 1991; Osman *et al*., 2003; Wiesman and Chapagain, 2003; Chapagain and Wiesman, 2005).

Therefore, preliminary laboratory studies were carried out to evaluate the insecticidal potentialities of *B. aegyptiaca* against the third instar larvae of *Trogoderma granarium* Everts (Coleoptera: Dermestidae), as one of the major economic store pests in Sudan. Different extracts of the plant were assessed for their mortality and repellent effects on the pest, and the consequent damage on sorghum grains.

MATERIALS AND METHODS

Collection and preparation of plant materials

Mature desert date (*B. aegyptiaca*) fruits (Laloub) were collected from naturally grown trees at Ommarrahi area, North of Omdurman city, during December 2009 and January 2011. The seeds kernels were released from such fruits using a mortar and a pistil. On the other hand, leaves and branches were picked from a desert date tree at Khartoum city in 2009, and dried under shade. One day before the extraction process, all samples were pulverized into fine powders using an electric blender (Moulinex). The resultant powders were kept in dark bottles (size 250 ml) until being extracted.

Preparation of plant extracts

Three solvents (viz., hexane, ethyl acetate and methanol) were used successively in a soxhlet apparatus to extract the apolar (oil), intermediate and polar compounds in desert date seeds, respectively. Upon completion of each extraction round, the filtrate was air dried and re-extracted successively by the next solvent, in the same way. However, the leaves and branches samples were extracted only with ethyl acetate and methanol in the same sequence. All extracts were kept separately in dark bottles and placed in a refrigerator until used. However, any plant sample was re-weighed at the end of each extraction step so as to determine the percentages of the extracted materials in relation to samples weights. Moreover, the extract obtained was also weighed for more confirmation.

Water extraction was done for all the three parts (seeds, leaves and branches) of the desert date. Powders of the previous parts were prepared one day before the start of each experiment. Accordingly, the amount of powder needed from each sample was weighed in a conical flask where a half volume of water was added, mixed thoroughly with a glass rod and allowed to stand overnight. In the next day the mixture was agitated manually for few seconds before filtration, using fine mesh. The volume of the extract was completed with water to attain the highest concentration (5%w/v) indicated for the study. Consequently, the other concentrations (2.5 and 1.25% w/v) were prepared through serial dilutions.

Preparation of treatments and test insects

Preparation of treatments was performed as a pre-step for biological assays in each experiment. Therefore, the intended organic extracts were firstly diluted with water to prepare the highest extract concentration (5%v/v). However, in case of oil extract, 0.1% liquid soap was added as an emulsifier. The other concentrations (2.5 and 1.25%) were prepared by serial dilutions with water. On the other hand, the three rates of water extracted materials were prepared on the same day following the extract preparation as shown previously. The different extracts concentrations were thus ready to be evaluated in bioassay experiments. So, the concentration which reflected the best mortality performance in the first experiment was selected for the second experiment to test its repellent effect.

The number of larvae (3rd instar) allocated for each experiment was segregated from a culture of khapra beetle found already preserved at the Laboratory of Botanical Pesticides, Environment and Natural Resources Research Institute (ENRRI), Khartoum.

Evaluation of mortality and repellent effects

A laboratory experiment was conducted in March 2010 to compare the insecticidal actions (mortality effects) of the previously prepared treatments against the 3rd instar larvae of *T. granarium*, as test insects. However, the means room temperatures (maximum and minimum) and relative humidity were recorded for the study period.

The experiment was executed in Petri dishes. According to the number of treatments, each 10g of clean sound sorghum (*S. bicolor*) grains were treated with one of the different extracts concentrations, replicated four times, left for five minutes to dry, and then placed into the Petri dishes. Ten 3rd instar larvae of *T. granarium* were introduced in each Petri dish, including two controls (untreated + liquid soap 0.1%), and covered. The experiment was assigned in a Completely Randomized design. However, inspections of the Petri dishes were carried out periodically on the 2nd, 7th, 14th and 21st day following treatments, so as to record the number of dead insects and other observations. This allowed to evaluate the knock down and delayed effects of different treatments. Data analysis was carried out and means separation was performed using Duncan's Multiple Range Test.

The best desert date treatment (i.e., 5% seeds hexane extract) that achieved from the mortality experiment was selected for a repellent test against the same pest in a second experiment (April 2011). A locally made repellent apparatus (Satti and Elamin, 2012) that prepared according to Berndt (1963) was used. Sorghum grains treated with 5% desert date seeds hexane extract, in addition to the untreated control were placed randomly in the peripheral holes of the repellent apparatus. A number of 200 larvae (3rd instar) of *T. granarium* were introduced in a Petri dish located in the central hole of the platform, then closed by a glass cover provided with a glass rod fitted into the top opening cover of the equipment. After a moment, the glass rod was pulled up to permit releasing of the insects. After 24h, the number of insects detected in each peripheral hole was recorded. This trial was repeated three times in subsequent days, as replicates. The data was analyzed to calculate the attractancy or repellency effect of the treatment according to Leonard and Ehrman (1976) formula, as shown below:

$$A = \frac{NO-Nb}{Nt}$$

Where; A = attractancy (+) or repellency (-); No = number of insects in the test hole; Nb = number of insects in the control hole; Nt = the total number of insects in both holes.

The output of this equation ranges from +1 (100% attractant) to -1(100% repellent) when compared to the control.

Weight loss of sorghum grains

Weight loss of sorghum grains according to larval damage during the insecticidal bioassay experiment was calculated for two selected extracts (leaves water extract 5% and seeds hexane extract 5%), which reflected the highest mortalities, besides the control (untreated sorghum grains). Evaluation of damaged grains was carried out 45 days following the treatments. Firstly, the insect larvae were removed from Petri-dishes, then the infested grains were subjected to sieving to get rid of cast skin, grain dust, insect excretion and other debris occurred due to insect feeding and development. Lastly, Petri-dishes contents (sorghum grains) were weighed again for calculating the percentages of damaged grains based on the initial weights (10g/dish).

RESULTS AND DISCUSSION

Extraction yields of plant samples

The results of extraction yields of the studied samples were explained in Table (1). It is clear that, hexane extract (oil) gave the highest significant yield (56.7%) of all extracts. Methanol extracts revealed significantly higher yields than those of ethyl acetate, and in all cases seeds extracts were richer than the leaves. The results of seeds extracts suggested that the content of apolar materials was quantitatively higher than the polar compounds, while the intermediate compounds were the lowest. The oil level detected in this study agreed with Hall and Walker (1991) who reported that desert date seeds contain about 30 – 60% oil. Although, studies on this tree is very scanty in Sudan, the current level of oil obtained was nearly equal to the maximum limit of the range (40 – 55%) recorded by Khidir (1997). However, as reported by Elfeel (2010), the oil content in kernels of desert date generally differs according to the location. But, such quantitative levels may not always correspond to levels of bioactivities (Elamin and Satti, 2012).

Insecticidal effects of botanical treatments

The mean (maximum and minimum) temperature and relative humidity recorded for the laboratory during the

Table 1. Yields of materials extracted from different parts of desert date (*Balanites aegyptiaca*), using water and organic solvents.

Plant samples	Extracted materials per 20 g	
	Mean±S.E.	(%)
DD-S. hexane extract	11.3±0.1a	56.7
DD-S. ethyl acetate extract	03.2±0.1e	16.0
DD-S. methanol extract	06.6±0.1b	32.8
DD-L. ethyl acetate extract	02.3±0.0f	11.6
DD-L. methanol extract	05.2±0.1c	25.8
DD-B. ethyl acetate extract	04.5±0.1d	22.4
C.V. %	1.8	

DD-S.= desert date seeds; DD-L.= desert date leaves; DD-B. = desert date branches;

Table 2. Mortality of *Trogoderma granarium* 3rd instar larvae fed on sorghum grains treated with different extracts of desert date (*Balanites aegyptiaca*), during March 2010.

Treatments	Mortality percent means (±S.E.) at different intervals			
	2 days	7 days	14 days	21 days
DD-L. eth.ac.ext.1.25%	0.0±0.0b	10.0±0.0def	15.0±0.3defg	17.5±0.2cdef
DD-L. eth.ac.ext. 2.5%	0.0±0.0b	07.5±0.2def	15.0±0.3defg	25.0±0.6bcde
DD-L. eth.ac.ext. 5%	0.0±0.0b	07.5±0.2def	25.0±0.3cde	25.0±0.3bcde
DD-L. methanol ext. 1.25%	0.0±0.0b	05.0±0.3def	10.0±0.0fgh	10.0±0.0efg
DD-L. methanol ext. 2.5%	0.0±0.0b	10.0±0.0def	10.0±0.0fgh	15.0±0.3 def
DD-L. methanol ext. 5%	0.0±0.0b	10.0±0.0def	25.0±0.3cde	27.5±0.2bcd
DD-L. water ext. 1.25%	0.0±0.0b	00.0±0.0f	02.5±0.2gh	02.5±0.2fg
DD-L. water ext. 2.5%	2.5±0.2ab	05.0±0.3def	10.0±0.0fgh	10.0±0.0 efg
DD-L. water ext. 5%	2.5±0.2ab	10.0±0.0def	22.5±0.2cdef	32.5±0.2bc
DD-B. eth.ac.ext. 1.25%	0.0±0.0b	10.0±0.0def	20.0±0.0def	20.0±0.0cde
DD-B. eth.ac.ext. 2.5%	5.0±0.3ab	15.0±0.3bcd	27.5±0.2bcd	27.5±0.2 bcd
DD-B. eth.ac.ext. 5%	5.0±0.3ab	15.0±0.3bcd	32.5±0.5abc	32.5±0.5bc
DD-B. methanol ext. 1.25%	0.0±0.0b	05.0±0.3def	15.0±0.5defg	15.0±0.5def
DD-B. methanol etx. 2.5%	0.0±0.0b	10.0±0.0def	17.5±0.5def	22.5±0.6cde
DD-B. methanol ext. 5%	2.5±0.2ab	10.0±0.4def	20.0±0.4def	27.5±0.5bcd
DD-B. water ext. 1.25%	0.0±0.0b	07.5±0.2def	15.0±0.3defg	17.5±0.2cdef
DD-B. water ext. 2.5%	0.0±0.0b	05.0±0.3def	10.0±0.0fgh	12.5±0.2defg
DD-B. water ext. 5%	2.5±0.2ab	07.5±0.5def	20.0±0.0def	20.0±0.0 cde
DD-S. hexane ext. 1.25%	5.0±0.3ab	12.5±0.2cde	15.0±0.5defg	15.0±0.5 def
DD-S. hexane ext. 2.5%	7.5±0.2a	20.0±0.0abc	42.5±0.2a	47.5±0.5a
DD-S. hexane ext. 5%	7.5±0.2 a	25.0±0.3a	42.5±0.2 a	50.0±0.4a
DD-S. eth.ac.ext.1.25%	0.0±0.0b	07.5±0.2def	10.0±0.0fgh	12.5±0.2defg
DD-S. eth.ac.ext. 2.5%	2.5±0.2ab	10.0±0.4def	15.0±0.5defg	15.0±0.5def
DD-S. eth.ac.ext. 5%	2.5±0.2ab	07.5±0.5def	12.5±0.7efg	15.0±1.0 def
DD-S. methanol ext. 1.25%	0.0±0.0b	12.5±0.5cde	22.5±0.5cdef	25.0±0.5bcde
DD-S. methanol ext. 2.5%	0.0±0.0b	10.0±0.4def	20.0±0.4def	22.5±0.5 cde
DD-S. methanol ext. 5%	5.0±0.3ab	12.5±0.5cde	22.5±0.8cdef	25.0±1.0bcde
DD-S. water ext. 1.25%	2.5±0.2ab	05.0±0.3def	10.0±0.4fgh	10.0±0.4efg
DD-S. water ext. 2.5%	2.5±0.2ab	05.0±0.3def	15.0±0.3defg	22.5±0.2 cde
DD-S. water ext. 5%	2.5±0.2ab	02.5±0.2ef	17.5±0.6def	17.5±0.6cdef
Liquid soap 0.1%	0.0±0.0b	00.0±0.0f	00.0±0.0h	00.0±0.0g
Control (untreated)	0.0±0.0b	00.0±0.0f	00.0±0.0h	00.0±0.0g
C.V. %	200.3	63.4	41.2	42.5

DD-L.= desert date leaves; eth.ac.= ethyl acetate; ext.= extract; DD- B. = desert date branches; DD-S.= desert date seeds.

Table 3. Repellency percent of desert date (*Balanites aegyptiaca*) seeds hexane extract (5%) against the 3rd instar larvae of *Trogoderma granarium*, during April 2011.

Treatments	Mean (±S.E.) insects/ treatment.	Repellency percent (%)
DD-S. hexane extract 5%	10.0±0.0b	72.7
Control (untreated)	63.3±5.7a	
C.V. %	29.1	

DD-S.= desert date seeds.

Table 4. Sorghum grains damaged by the 3rd instar larvae of *Trogoderma granarium*, after 45 days of treatments with some desert date extracts, during Jan. – Feb. 2011.

Treatments	Seeds weight loss		Seeds weight saved
	Mean (±S.E.)	(%)	(%)
DD-L. water extract 5%	0.5±0.0c	05.0	50.0
DD-S. hexane extract 5%	0.7±0.0b	07.0	30.0
Control	1.0±0.1a	10.0	-
C.V. %	10.5		

DD-L.= desert date leaves; DD-S.= desert date seeds.

study period were, (29.0±1.4C° and 28.3±1.4C°) and (31.0±3.9% and 28.3±4.3%), respectively. The mortality effects of treatments on the 3rd instar larvae of *T. granarium* are shown in Table (2). The tested extracts manifested more or less variable results throughout the course of the experiment when compared with controls. Regarding leaves extracts, no significant differences were obtained between all concentrations and the controls until the 7th day of treatments. At the 14th day interval the three concentrations of ethyl acetate and the highest concentration (5%) of both methanol and water extracts showed significant results. The latter (water) extract gave the best result (32.5±0.2% mortality) after 21 days of treatments. Similarly, branches extracts also started to show significant larval mortalities after two weeks (14 days) of treatments. Ethyl acetate extract reflected the best treatment up to the 3rd week, where the 5% concentration exerted the highest mortality mean (32.5±0.5%).

However, the desert date seeds seemed to be the most potent investigated components of the plant. Besides, methanol, ethyl acetate and water extracts, hexane extract represents an additional important fraction obtained from seeds due to the high oil content in the seeds kernels. Such oil showed significant mortality effect since the first count (48h). It also significantly surpassed all other treatments during the subsequent investigation intervals. The larval mortality rates due to the oil treatments increase progressively with exposure time and concentration. Therefore, the experiment revealed the highest mortality mean by the 5% seeds hexane extract during all counts, but without significant differences from the medium concentration (2.5%) of seeds hexane extract. Therefore, such high oil constituent in desert date seeds was considered as an added value to its highest biological activities against the pest. This is important for commercial production of natural insecticides. However, the results also proved that the added liquid soap (0.1%) had no apparent effect on the larvae.

Table (3) shows the repellency effect of seeds hexane extract (5%) of desert date on the 3rd instar larvae of *T. granarium*, as the best performed treatment in the previous bioassay experiment. Highly significant repellency percent (72.7%) was obtained by this treatment compared to the control. Moreover, this extract also reduced sorghum grains damage significantly after 45 days, compared with the untreated control (Table 4). Although, the leaves hexane extract had induced significantly lower mortality effect than the seeds hexane extract, it showed significantly better gains savings (50%) than the latter seeds extract (30%). Since very meager research data is available on these aspects, no clear reasons were known. However, it can be stated that water extract may be richer in active principles with antifeedant and/or repellent properties rather than stomach toxicity action against the pest. These aspects need to be clarified in future investigations.

Generally, *B. aegyptiaca* was reported to contain various classes of secondary metabolites with different biocidal effects (Hall and Walker, 1991; El Ghazali, *et al*., 1997; Wiesman and Chapagain, 2003), but the characterization of active compounds in each class and their biological efficacies should be well ascertained. In the short run, these studies are recommended to be done at least for the two previously mentioned extracts (viz., water and hexane extracts of seeds) so as to evaluate their possible exploitation in pest control. On the other hand, the detected superior insecticidal activities of the desert date oil extract may suggests the presence of potent active compounds in this extract with stomach toxicant effects to the pest which also need to be ascertained. Anyhow, the results partially confirmed what has been recorded by Mohamed (2003) who verified the insecticidal effect of desert date seed hexane extract against *T. granarium*. The literature also reflected various examples of botanicals with superior effects by materials extracted through non polar solvents like hexane and petroleum ether compared to intermediate and high polarity extracts (El-Massad *et al*., 2012; Satti and Elamin, 2012).

CONCLUSION

The study revealed significant variations in insecticidal effects of different desert date extracts on *T. granarium* larvae. Seeds hexane extract (oil) achieved significantly the highest extractive yield and the best larval mortality compared with the other intermediate and high polarity extracts. The oil extract also exerted significant repellent effect on the pest larvae and significant reduction in sorghum damage compared with control. Nevertheless, seeds water extract has attained the highest savings of sorghum grains in spite of its inferior mortality results compared to seeds hexane extract. Therefore, chemical characterizations of active compounds in both the foregoing extracts coupled with careful bioassays are wanted for better exploitation of such eco-friendly available natural products.

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