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INSECTICIDAL ACTIVITIES OF TWO MELIACEOUS PLANTS AGAINST Trogoderma granarium Everts (Coleoptera: Dermestidae)

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ABSTRACT

The khapra beetle (*Trogoderma granarium*) is one of the major economic store pests in Sudan. Application of chemical insecticides, either in form of fumigation or spraying, is the sole measure of control adopted. Since these chemicals were linked with several drawbacks, attempts are in progress to find environmentally sound and economically feasible alternatives. Therefore, laboratory experiments were carried out to evaluate the insecticidal activities of several extracts prepared from two meliaceous plants, viz., *Khaya senegalensis* (mahogany) and *Azadirachta indica* (neem), against the 3rd instar larvae of *T. granarium*. The results showed variable insecticidal activities by the tested extracts. Mahogany leaves exerted better actions than those of the neem leaves. Nevertheless, all leaves extracts manifested relatively low and delayed mortalities as compared with neem seeds. Accordingly, the neem seeds hexane extract (oil) at 5%v/v, was the best treatment induced significant knock down effect on the larvae 48hrs post treatments. It showed a progressive increase in effectiveness with time to attain 92.5% mortality on the third week of exposure. This extract also repelled the pest (85.4%) and saved sorghum seeds (45.5%) significantly as compared with the untreated control. Moreover, in spite of what has been mentioned about the low mortality effect of mahogany leaves water extract, it recorded the superior saving of sorghum grains (54.5%) suggesting the presence of potent repellent and/or antifeedant active constituents in this extract. Therefore, additional studies are required to ascertain the actual bioactivities in mahogany leaves, and to proceed forwards in formulating botanical insecticides from the neem oil.

KEYWORDS: Mahogany; neem; *Trogoderma granarium*; water extract; organic extracts.

INTRODUCTION

Pests control in most tropical countries, as in many other regions of the world, depends largely on the usage of conventional pesticides. But, due to several drawbacks linked with these chemicals, including mainly the development of resistant pest strains, toxicity to man and animals, environmental pollution and the increasing cost of pesticides, hence the search for new alternatives was thought to be important. Botanical pesticides were considered among important promising tools attempted and showed compatibility with other tactics for storage pests' management (Ahmedani et al., 2007). Although natural products were recognized since ancient times, their usages have been suspended after the appearance of synthetic pesticides. Examples of plants exhibiting pesticidal properties are numerous starting with Chrysanthemum, Nicotiana and Derris species in earlier times and ending with neem extracts in recent decades (Whitehead and Bowers, 1983; Schmutterer, 1995).

In the Sudan, various studies were performed to evaluate the insecticidal activities of different indigenous plants for controlling agricultural pests of field crops and store products (Siddig, 1991; Satti *et al.*, 2003; Sir El Khatim, 2005; Yousif and Satti, 2008). Among these plants, extract of neem (*Azadirachta indica* A. Juss.) tree (Meliaceae) has received the greatest attention during the last decades as potent source for biologically active ingredients. The neem tree is widely distributed in the country, and its total numbers were now approaching ten million trees. However, another meliaceous species of economic importance is the mahogany tree, Khaya senegalensis (Des.) A. Juss., which is abundant along rivers and seasonal streams especially in western and southern parts of the country (El Amin, 1990 and Bein et al., 1996). This species is also known to contain active ingredients with some biological activities (Bamaiyi et al., 2006 and Ibrahim et al., 2006), but it has got little research as compared to the neem tree. Although, both of the foregoing trees are utilized for many socio-economic purposes, particularly as timbers, but no real exploitation is recognized regarding their rich phytochemical constituents. Therefore, in the course of studies aiming to find potent plants for preparing natural pesticides, the current research was intended to evaluate and compare the insecticidal activities of different extracts prepared from neem and mahogany trees. However, the $3^{\bar{r}d}$ instar larvae of the Khapra beetle (Trogoderma granarium Everts) were used in the bioassays, as test insects.

MATERIALS AND METHODS

Plant materials used

All plants parts used in this study were collected from various locations in Khartoum State, during different times between 2009 and 2011. They included leaves of mahogany tree (*K. senegalensis*) and leaves and seeds of the neem tree (*A. indica*). The neem fruits were already stored in the laboratory since 2009. They were collected

from the ground after being dropped under neem trees during the fruiting period (June-August). However, the leaves of both tree species were picked fresh just one week before the commencement of the bioassay experiments, and dried under shade. All samples were ground into fine powders using an electric blender (Moulinex) and kept in dark bottles until being extracted.

Preparation of botanical treatments and test insects

Regarding organic solvent extractions, 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 neem seeds, respectively. A weight of 20 grams seeds powder was loaded on the soxhlet thimble and extracted to 9 hours to free it from oil. The process was repeated 3 times with new samples to get the required quantity. Upon completion of each round, the filtrate was air dried and reextracted successively by the ethyl acetate and the methanol to obtain the other components of the sample, in the same way. However, the leaves samples of the two plants were extracted only with ethyl acetate and methanol in the same sequence. All extracts were kept separately in dark bottles and placed in 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 seed weight. Moreover, the extract obtained was also weighed for more confirmation. Water extraction was done for all the above mentioned plant materials of the neem and mahogany trees. Plant powders were prepared one day before the start of each experiment. Accordingly, the amount of powder needed was weighed in a conical flask where 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 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%). However, in case of oil extract, 0.1% liquid soap was added as an emulsifier. Consequently, the other concentrations (2.5 and 1.25%) were prepared by serial dilutions with water. On the other hand, 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. Based on these experiments, the concentration which reflected the best mortality performance was selected for a repellent test. The number of larvae allocated for each experiment was segregated from the culture with the help of a small camel hairs brush, white A4 paper sheet and a Petri dish. This was done each time by taking some amount of infested grains from the jar and spread carefully on the sheet paper, then returned back again into the jar to expose different stages of larvae clinging to the paper. Using the brush, only the 3rd instar larvae were dropped from the sheet down into the dish. The process may be repeated many times to complete the required number of larvae in some experiments.

Evaluation of mortality and repellent effects

Three experiments were conducted successively to compare the insecticidal actions (mortality effects) of the previously prepared treatments against the 3rd instar larvae of T. granarium, as test insects. The first experiment was conducted in January 2010 (winter season) to test the water and organic leaves extracts of mahogany and neem, and then supported by another experiment during summer period in May 2010 using merely leaves water extracts. The third experiment in February 2011 was dealt with neem seeds organic and water extracts. However, the means room temperatures (maximum and minimum) and relative humidity were recorded for the study period. All the above experiments were executed separately in Petri dishes. According to the number of treatments, each 10 grams 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 3^{rd} instar larvae of T. granarium were introduced in each Petri dish, including the untreated control, and covered. All experiments were assigned in Completely Randomized design. However, inspections of the Petri dishes were carried out periodically on the 2nd, 7th, 14th and 21st day following treatments. Hence, the number of dead insects and other observations were recorded. This allowed to evaluate the knock down and delayed effects of different treatments. The collected records were tabulated and statistically analyzed. Whenever significant differences were achieved, means separation was performed using Duncan's Multiple Range Test. The best botanical treatment (i.e., 5% neem seed kernel hexane extract) that achieved from mortality experiments was selected for repellent test against the same pest. A locally made repellent apparatus was prepared and utilized in this study as described by Berndt (1963), but slightly modified in the control hole of platform to accommodate a Petri dish 5cm in diameter. The experiment was carried out in April 2011. Sorghum grains treated with 5% neem seed kernel hexane extract, in addition to the untreated control were placed randomly in the peripheral holes of the repellent apparatus. A number of 200 3rd instar larvae 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. In the second day (24 hours) the number of insects detected in each peripheral hole was recorded. Such experiment was repeated successively in subsequent two days, following the same procedures. Hence, three counts were taken and 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 seeds according to larval damage during the insecticidal bioassay experiment was calculated for four selected treatments. These included; mahogany leaves water extracts (5% w/v), neem leaves methanol extract (5% w/v), neem seeds hexane extract (5% w/v) and the control (untreated sorghum seeds). Evaluation of damaged seeds 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 seeds) were weighed again for calculating the percentages of damaged seeds based on the initial weights (10g/dish).

RESULTS AND DISCUSSION

Extraction yields of plant samples

The result of extraction yields (quantity and percent) of the studied plant samples were explained in Table (1).

Regarding mahogany leaves, methanol (20.5%) and water (17.4%) extracts gave the best significant yields. However, neem seeds produced higher yields than the leaves extracts of the two plants (mahogany and neem). Neem seeds hexane extract gave the highest significant yield (46.7%), followed by neem seeds methanol extract (28.9%) and neem leaves methanol extract (16.9%). It is clear that in both plants ethyl acetate extracts gave the lowest yields. These results suggested that the content of polar materials in most tested plant parts were higher than the intermediate polarity compounds. Also, the higher oil content in neem seeds was considered as an indicator for rich apolar constituents with diversified biological activities in this extract. However, literature showed variable levels of oil content in seeds which reported to contain numerous active ingredients (e.g. azadirachtin, salannin, nimbinen, salannol, salannolacetate, and stearic, oleic and linoleic acids) with pesticidal properties (Schwinger, 1984 and Bashir, 1994). The oil level achieved in this study (46.7%) agreed with Maydell (1986) who mentioned that the neem seed kernels contain about 45 - 60% oil.

TABLE 1. Yields of extracted materials from different parts of mahogany (*Khaya senegalensis*) and neem (*Azadirachta indica*) trees, using water and organic solvents.

Plant samples	Extracted materials per 20 g	
	Mean±S.E.	(%)
I. Mahogany:		
M-L. eth.ac. extract	1.7±0.0b	08.7
M-L. meth. extract	4.1±0.1a	20.5
M-L. water extract	3.5±0.1a	17.4
C.V. %	5.6	
IV. Neem:		
N-S. hex. extract	9.3±0.0a	46.7
N-S. eth.ac. extract	2.0±0.0d	09.9
N-S. meth. extract	5.8±0.0b	28.9
N-L. eth.ac. extract	1.4±0.3e	07.0
N-L. meth. extract	3.4±0.2c	16.9
C.V. %	6.1	

M-L.= mahogany leaves; eth.ac.= ethyl acetate; ext.= extract; meth.= methanol; N-S.= neem seeds; hex.= hexane; N-L.= neem leaves.

Insecticidal effects of botanical treatments

Table (2) showed the mortality effects of mahogany and neem leaves extracts on the 3^{rd} instar larvae of *Trogoderma granarium*, as a result of winter experiment (average $28.6\pm1.4^{\circ}$ C and $29.6\pm4.1\%$ R.H.). No significant differences were detected between all plants extracts up to seven days post treatments. However, mahogany water extract at 5% concentration reflected the highest significant mortality means at 14 days interval (15.0% mortality) and onwards, followed by 5% neem methanolic extract. The rest of treatments exerted no significant differences from the control. Similarly, Table (3) showed the mortality results of leaves water extracts of the two plants which performed in summer time ($36.7\pm0.2^{\circ}$ C and 31.2 \pm 4.6%R.H.). The highest concentration (5%) of both plants gave the best significant results since the second day of treatment, with mahogany extract being superior at all intervals. Notably, summer experiment manifested higher mortality rates than the winter experiment. Such seasonal difference in results could be connected to variation in temperature degrees between the two seasons; a factor seems to affect the activity of the pest. It was observed that, during hot times the larvae of *T. granarium* were activity feeding and crawling, while in cold they tend to be more quiescent. Whatsoever, the superiority of mahogany leaves water extract could be attributed either to high concentration or more potent active ingredients in this extract compared with the other plant.

TABLE 2. Mortality levels of *Trogoderma granarium* 3rd instar larvae as affected by mahogany (*Khaya senegalensis*) and neem (*Azadirachta indica*) leaves organic and water extracts, during winter season (January 2010).

Treatments	Mortality percent means (±S.E.) at different intervals			
	2 days	7 days	14 days	21 days
M-L. eth.ac.ext.1.25%	0.0±0.0a	00.0±0.0a	00.0±0.0c	02.5±0.2bc
M-L. eth.ac.ext. 2.5%	0.0±0.0a	02.5±0.2a	02.5±0.2c	05.0±0.3abc
M-L. eth.ac.ext. 5%	0.0±0.0a	00.0±0.0a	00.0±0.0c	07.5±0.5abc
M-L. meth.ext. 1.25%	0.0±0.0a	00.0±0.0a	00.0±0.0c	02.5±0.2bc
M-L. meth.ext. 2.5%	0.0±0.0a	00.0±0.0a	00.0±0.0c	00.0±0.0c
M-L. meth.ext. 5%	0.0±0.0a	02.5±0.3a	02.5±0.2c	05.0±0.3abc
M-L. water ext. 1.25%	0.0±0.0a	02.5±0.3a	02.5±0.2c	05.0±0.3abc
M-L. water ext. 2.5%	0.0±0.0a	05.0±0.3a	07.5±0.2abc	07.5±0.2abc
M-L. water ext. 5%	2.5±0.2a	10.0±0.4a	15.0±0.5a	15.0±0.5a
N-L. eth.ac.ext. 1.25%	0.0±0.0a	02.5±0.3a	05.0±0.5bc	05.0±0.5abc
N-L. eth.ac.ext. 2.5%	0.0±0.0a	07.5±0.3a	07.5±0.2abc	07.5±0.2abc
N-L. eth.ac.ext. 5%	0.0±0.0a	05.0±0.3a	07.5±0.2abc	10.0±0.4abc
N-L. meth.ext. 1.25%	0.0±0.0a	05.0±0.3a	05.0±0.3bc	05.0±0.3abc
N-L. meth.ext. 2.5%	2.5±0.2a	05.0±0.3a	07.5±0.2abc	07.5±0.5abc
N-L. meth.ext. 5%	0.0±0.0a	07.5±0.2a	12.5±0.2ab	12.5±0.2ab
N-L. water ext. 1.25%	0.0±0.0a	05.0±0.3a	05.0±0.3bc	05.0±0.3abc
N-L. water ext. 2.5%	0.0±0.0a	00.0±0.0a	00.0±0.0c	00.0±0.0c
N-L. water ext. 5%	0.0±0.0a	02.5±0.2a	05.0±0.3bc	05.0±0.3abc
Control	0.0±0.0a	00.0±0.0a	00.0±0.0c	00.0±0.0c
C.V. %	577.4	138.9	121.7	115.0

M-L.= Mahogany leaves; eth.ac.= ethyl acetate; ext.= extract; meth.= methanol; N-L.= neem leaves.

TABLE 3. Mortality levels of *Trogoderma granarium* 3rd instar larvae as affected by mahogany (*Khaya senegalensis*) and neem (*Azadirachta indica*) leaves water extracts, during summer period (May 2010).

Treatments	Mortality percent means (±S.E.) at different intervals			
	2 days	7 days	14 days	21 days
M-L. water ext. 1.25%	0.0±0.0b	00.0±0.0c	10.0±0.4b	10.0±0.4bc
M-L. water ext. 2.5%	0.0±0.0b	00.0±0.0c	10.0±0.4b	17.5±0.6ab
M-L. water ext. 5% N-L. water ext. 1.25%	7.5±0.2a 0.0±0.0b	17.5±0.2a 00.0±0.0c	22.5±0.2a 00.0±0.0c	27.5±0.5a 00.0±0.0c
N-L. water ext. 2.5% N-L. water ext. 5% Control	0.0±0.0b 5.0±0.3a 0.0±0.0b	00.0±0.0c 05.0±0.3b 00.0±0.0c	02.5±0.2bc 05.0±0.3bc 00.0±0.0c	02.5±0.2bc 12.5±0.5b 00.0±0.0c
C.V. %	157.3	92.5	78.4	69.3

M-L.= Mahogany leaves; ext.= extract; N-L.= neem leaves.

TABLE 4. Mortality levels of *Trogoderma granarium* 3rd instar larvae as affected by neem seeds organic and water extracts, during February 2011.

Treatments	Mortality percent means $(\pm S.E.)$ at different intervals			
	2 days	7 days	14 days	21 days
N-S. hex.ext. 1.25%	00.0±0.0b	00.0±0.0b	02.5±0.2d	05.0±0.3f
N-S. hex.ext. 2.5%	02.5±0.2b	05.0±0.3b	12.5±0.5cd	22.5±0.5e
N-S. hex.ext. 5%	45.0±0.9a	80.0±1.2a	87.5±1.0a	92.5±0.5a
N-S. eth.ac.ext. 1.25%	00.0±0.0b	00.0±0.0b	12.5±0.2cd	27.5±0.5e
N-S. eth.ac.ext. 2.5%	00.0±0.0b	00.0±0.0b	10.0±0.4cd	32.5±0.5cde
N-S. eth.ac.ext. 5%	00.0±0.0b	07.5±0.5b	35.0±0.5b	47.5±0.5b
N-S. meth.ext. 1.25%	00.0±0.0b	00.0±0.0b	07.5±0.2d	20.0±0.4e
N-S. meth.ext. 2.5%	00.0±0.0b	02.5±0.2b	12.5±0.5cd	25.0±0.3e
N-S. meth.ext. 5%	00.0±0.0b	07.5±0.2b	30.0±0.4b	42.5±0.2bcd
N-S. water ext. 1.25%	00.0±0.0b	00.0±0.0b	10.0±0.4cd	30.0±0.4de
N-S. water ext. 2.5%	00.0±0.00b	00.0±0.0b	22.5±0.2bc	45.0±0.3bc
N-S. water ext. 5%	00.0±0.0b	05.0±0.2b	30.0±0.4b	52.5±0.8b
Control	00.0±0.0b	00.0±0.0b	00.0±0.0e	00.0±0.0f
C.V.%	123.7	77.4	40.6	24.2

hex.= hexane; ext.= extract; N-S.= neem seeds; eth.ac.= ethyl acetate; meth.= methanol.

According to Ibrahim *et al.* (2006), mahogany leaves contain several active compounds such as saponin, phenols and tannins. On the other hand, Biu *et al.* (2009)

reported that neem leaves contain moderate concentration of tannins. The effects of organic and water extracts of neem seeds were explained in Table (4). Contrary to what have been shown by leaves extracts, relatively higher knock down effects were exerted by seeds, particularly the hexane extract, 48hrs of treatments. As a result, the 5% neem seeds hexane extract showed significant mortalities at all investigation intervals, as compared with the other treatments and the untreated control. It is obvious that this treatment showed gradual increase in mortality with time from the second day (45.0% mortality) up to the third week (92.5%). In the same context, Table (5) proved the repellency effect of neem seeds hexane extract (5%), as the potent treatment against the pest. It reflected significant effect with 85.4% repellency on the larvae. However, the current results demonstrated that the latter extract constitutes very good repellent as well as toxicant active ingredients against the tested insect. This result agreed with Nasr (1993) and Bakheet (2008) who assured that neem oil has a very high repellency effects to *T. granarium*. According to Balandrin *et al.* (1988) the repellent action of neem oil could result from the presence of volatile sulphur containing compounds.

TABLE 5. Repellency percent of neem seed hexane extract (5%) against the 3rd instar larvae of *Trogoderma granarium*, during April 2011.

Treatments	Mean (±S.E.) insects/ treatment.	Repellency percent (%)
N-S. hexane extract 5%	5.0±0.6b	85.4
Control	63.3±5.7a	
C.V. %	29.1	

N-S.= Neem seeds.

Moreover, the results of sorghum seeds lost after 45days as a result of larval feeding on certain treatments were shown in Table (6). The mahogany leaves water extract (5%) followed by neem seeds hexane extract (5%), were the best treatments reduced sorghum grains damage significantly compared with the untreated control. This may be resulted from the multi-activities (viz, insecticidal, repellency and antifeedant effects) induced by these extracts (Ahmed, 1993; Jood et al., 1993; Sharma, 1999 and Mohamed, 2003). However, the antifeedant actions of mahogany and neem extracts were reputed to the presence of certain active compounds in the two plants (Schwinger, 1984 and Ibrahim et al., 2006). However, the superior saving of sorghum grains showed by the mahogany leaves water extract (54.5%) need to be investigated in further studies. Although, inferior mortality result was shown by this extract, it may constitute powerful repellent and/or antifeedant effects, compared with those of the neem extracts. As shown previously, the different plant samples have yielded variable levels of extracted materials which performed differently in the bioassay tests. The highest mortality and repellent effects exerted by neem hexane extract could be attributed to the variable active ingredients reported in the ample oil content of the seeds,

including mainly several triterpenoid compounds as mentioned before (Butterworth and Morgan, 1968; Gill and Lewis, 1971 and Schwinger, 1984). Such high oil constituent in neem seeds was considered as an added value to its highest biological activities against the pest. This is particularly important whenever this plant component is needed for commercial production of natural insecticides.

CONCLUSION AND RECOMMENDATION

The study revealed that extracts of mahogany, *Khaya* senegalensis, leaves were more effective than those of the neem leaves in controlling the larvae of *Trogoderma* granarium. Nevertheless, neem seed oil (5%v/v) was proved to be the most potent extract of the studied materials which reflected the best significant mortality action against the pest. This extract also significantly repelled the pest and saved sorghum seeds as compared with the untreated control. But, due to superiority of mahogany leaves water extract in saving sorghum grains, this extract required additional studies to clarify such finding. Mean while, the neem seeds hexane extract (5%) was advocated to be formulated as natural biocide.

TABLE 6. Sorghum grains (seeds) damaged by the 3rd instar larvae of *Trogoderma granarium*, after 45 daysfollowing treatments with different plants extracts.

Treatments	Seeds weigh	Seeds weight loss	
	Mean (±S.E.)	(%)	(%)
N-S. hexane extract 5%	0.6±0.0b	06.0	45.5
N-L. methanol extract 5%	1.0±0.0a	10.0	09.1
M-L. water extract 5%	0.5±0.0c	04.8	54.5
Control	1.1±0.1a	11.0	-
C V %	10.5		

N-S.= Neem seeds; N-L.= neem leaves; M-L.= mahogany leaves.

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