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RELATIVE COMPONENTS OF NEEM BERRY AND BIOCIDAL EFFECTS OF KERNEL EXTRACTS AGAINST THE ADULT OF AGONOSCELIS PUBESCENS (THUNB.)

ABSTRACT

Neem (Azadirachta indica) seed extracts proved potent insecticidal effects against various pests in Sudan. In the course of studying neem products as rich sources of natural insecticides, this research was undertaken to quantify the relative components of neem berries that might help in estimating the portions needed in preparation of natural products, and to study the insecticidal effects of different kernel extracts against the adult sorghum bug (Agonoscelis pubescens). Three extracts (water, hexane and methanol) were bioassayed at different doses in comparison with a standard insecticide (Malathion 57% EC). The results revealed that seeds kernels, the richest neem part in active compounds, represent 20.1% and 43.4% of corticated seeds and fruit berries, respectively. Accordingly, a sack (100kg) of neem berries can provide an amount of kernels almost equivalent to one fifth of its weight. The kernels yielded 46.3% oil (hexane extracted) as the highest extract obtained. Such oil also revealed higher insecticidal effects against the adult sorghum bug than water and methanol extracts. In all extracts, the mortality rates generally increased in response to concentration and exposure time. Neem oil at 8% concentration exerted significantly the highest mortality effects as compared with the other treatments. It showed significantly comparable effect with malathion after three days post application, and scored 100% mortality after a week. Hence, neem oil is advocated for further studies to be assessed under field situations as a component of sorghum bug management program.

KEYWORDS Neem fruit components; kernels; insecticidal effect; Agonoscelis pubescens; Sudan.

INTRODUCTION

Neem tree (Azadirachta indica A. Juss), of the family Meliaceae, is thought to have been originated in Asia and Burma, and some authors believed that it is native to the whole Indian subcontinent[1]. In these areas, neem parts are used traditionally for various purposes[2]. Recently, the neem tree is distributed in several countries in tropical and sub-tropical regions of the world, particularly in Asia and Africa. The tree was imported to the Sudan about a century ago, and now is grown in almost all parts of the country as a shade or a fence tree in streets, parks and gardens. Neem trees normally begin bearing after 3-5 years and become fully productive in ten years, and may live for more than two centuries. A neem tree can produce up to 50kg of fruits annually[1]. The tree proved its richness as potential source of unique natural products for pests' management, medicine and other industrial uses at global level[3]. Locally, extracts of neem leaves and seeds gave promising results in combating various agricultural pests and diseases in the country[4,5]. Therefore, the tree is now under focusing of different research disciplines.

Sorghum bug [Agonoscelis pubescens (Thunberg)], of the order Heteroptera and family Pentatomidae, is a serious pest of sorghum and some other crops in a number of African countries south of the Sahara, including the Sudan. The pest inhabits mainly the central rain lands belt in the country where the main sorghum producing areas are found. The insect feeds on sorghum crop at the milky stage and resulted in atrophied grains. It causes 20-30% damage, but complete losses may occur in heavy infestation[6,7,8,9,10]. The control of this pest is one of tedious and costly operations that performed annually by the Plant Protection Directorate. This depends chiefly on chemical insecticides which directed mainly against the resting bugs on forest trees and sometimes on sorghum crops in the field during active period of the pest[10,11]. So, due to habitats targeted by these chemicals, great harms are manifested on biodiversity and the environment. Therefore, the situation has prompted the need for cheap, effective and environmentally safe biodegradable alternatives. Although, studies on natural biocides regarding the control of A. pubescens are almost lacking, it is proved that neem extracts are very potent in combating wide range of insects including hemipteran pests like Urentius lace bugs on eggplant[12]. Hence, it is suggested that neem would be equally effective against the sorghum bug as a member of same previous order (Hemiptera). The current preliminary laboratory research was aimed to quantify the relative components of neem berries, and to study the insecticidal effects of different kernel extracts against the adult sorghum bug (Agonoscelis pubescens), as a major pest of sorghum and other crops in Sudan.

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MATERIALS AND METHODS

Rearing of sorghum bugs

Adults of sorghum bug (A. pubescens) were collected from Sennar area during July 2009 and brought to the laboratory. Sesame (Sesamum indicum) plant was already cultivated in the field (0.42ha) at different times to secure the availability of fresh seed pods during rearing and bioassay experiment. The collected insects were reared in 60 glass jars (9.5cm diameter) fed with sesame pods. In every jar five pairs of female and male adults were kept together and left to reproduce. The eggs laid were collected in new glass jars and followed until they hatched. The emerged nymphs were fed on sesame, and followed until the adult stage. Then newly emerged adults were moved to clean glass jars, for mating and egg laying. The eggs from the first generation in laboratory were put in glass jars and allowed to hatch and develop. The obtained adults (second generation) were kept to be used as test insects in the bioassay experiment for evaluating neem extracts.

Collection of neem seeds

Neem (Azadirachta indica) berries (fruits) were collected from Shambat area, Khartoum North, during July 2009 and kept in jute sacks, after being washed in hot water to remove any contamination.

The relative components of neem berries

The neem berry consists of two parts, an outer fruit part and an inner seed. The fruit pulp is coated with a soft skin and the seed kernel with a fibrous shell (cortex). Since neem active ingredients are concentrated in seed kernels, the relative components of neem berries were quantified in order to indicate the percents of portions utilized in preparation of natural product. Accordingly, a sample of one kilogram neem berries were weighted and imbibed with water for 12h, hence the different components (fruit skin, fruit pulp and corticated seeds) were separated. The same process was repeated using two additional samples to make three replications. After drying, the seeds were gently crushed to separate the seeds kernels from cortexes. All the previous parts were weighed so that their relative quantitative percents were calculated in relation to the berries weight. The relative percents of kernel to the seed (corticated) and to the whole berry weights were also indicated.

Preparation of neem extracts

The neem fruits required for the study were soaked in water for 12 hours to facilitate removal of fruit pulps. The clean seeds were left to dry under room temperature for many days, then decorticated with a mortar and a pestle. The kernels were ground into fine powder in an electric blender (Moulinex®, model 276) and stored in tightly covered glass jars until being extracted.

For preparing water extract, 100g of neem seed powder was mixed in one liter of water in a conical flask. The mixture was thoroughly shaken manually and left to stand overnight before filtration. The obtained extract was kept as stock solution (10%w/v) in the refrigerator at 5°C till used in the bioassay experiment within 48h. The extract percent was computed in relation to the seeds weight used. However, organic extracts (hexane and methanol) of neem kernels were prepared through a soxhlet apparatus[13,14,15]. After the extraction, both solvents were removed using a rotary evaporator. The weight of extracted oil was taken to calculate its percent in neem seeds. The obtained oil and methanol extracts were kept separately in dark glass bottles, wrapped tightly with aluminum foil, and stored in a refrigerator until used.

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Bioassay experiment

The bioassay experiment was conducted at the Environment and Natural Resources Research Institute (ENNRI), Khartoum, during August-October 2009. Four concentrations (1%, 2%, 4% and 8%) were prepared serially from the water, hexane and methanol extracts. However, to make the oil suitable for application, 0.5% liquid soap was added to act as an emulsifier. The different neem treatments were evaluated against the adults of A. pubescens, in comparison with a standard insecticide Malathion 57% EC (malathion). Each ten adult insects were introduced together with fresh sesame pods into the glass jars with three replications. Both the sesame pods and insects were sprayed with 2 ml of the different concentrations of water and organic extracts[16]. The water treated control and the standard insecticide were included for comparison. The experiment was assigned in a Completely Randomized Design (CRD). The average temperatures and relative humidity percents were recorded in the laboratory during the study period. The number of dead insects and other observations were recorded at the 2nd, 3rd, 5th and 7th days from treatments. The data were analyzed (ANOVA), and means were separated according to Duncan's Multiple Range test.

RESULTS AND DISCUSSION

Components of neem berries

The flowering period of neem tree occurs between December – February and February – April in southern and northern parts of the country, respectively. In the northern and central States, including the Khartoum, mature fruits are available between June and August every year. The results of calculating the relative quantitative components of neem berries are shown in Table (1). It is clear that the fruit part (skin + pulp) constitutes the highest portion (53.7%) of neem berry, compared to the seed (cortex + kernel) part (46.3%). Since the neem fruit is rich in carbohydrates it provides suitable food source for birds and other animals like monkeys and sometimes eaten by some people in rural areas. Such fruit part may add to the soil fertility when mixed with neem cake after removal of oil. The seed kernel, the richest neem part in active compounds, represented 20.1% and 43.4% of the fruit berry and corticated seed, respectively. Accordingly, a sack (100kg) of neem fruits can provide an amount of kernels almost equivalent to one fifth (20kg) of its weight. The seed cortex was reported to be useful in facilitating grinding and water extraction of kernels[17]. However, neem oil represented the highest material extracted (46.3%) from the seed kernels. Thus, about 8.6 tons of neem berries (≈ 2.2 tons of kernels) is required in order to obtain a ton of neem oil. The results agreed with Satti and Elamin (2012) who recorded nearly similar percent of neem oil[18].

Neem insecticidal effects against the sorghum bugs

The average room temperatures and relative humidity percents during the experimental period (Aug.-Oct. 2009) were 34±2°C and 44% R.H., respectively. Table (2) summarizes the mortality effects of neem (A. indica) seeds kernels extracts against the adults of Agonoscelis pubescens at different intervals from treatments. Significant differences ($P = 0.05$) were found between treatments. In the second day (48h) of treatments, neem seeds hexane extract at 8% concentration caused the best significant mortality result (60.0±5.8%) of all extracts, but came next to malathion in activity (100% mortality). The other extracts treatments were not significantly different from the control check. However, the progressively increased effects of neem hexane extract (8%) attained the same level of significance with that of the malathion insecticide at the 3rd day, and reached 100% mortality at the 7th day post treatments. On the other hand, water and methanol extracts more or less manifested similar effects. Generally, all botanical preparations exhibited increased mortalities with increasing concentrations and exposure time. The overall results indicated that malathion has stronger knockdown effect than neem extracts within 48h of treatments, whereas neem treatments mostly induced progressively increased delayed effects on the pest.

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Currently there is a resurgence of interest in plant derived compounds for developing commercially eco-friendly insecticides. Tropical plants are more promising for the development of new insecticides[19]. Among such plants, neem derivatives receive the greatest interest nowadays and showed promising results in controlling various insect pests of different orders including some hemipteran species[12,20,21,22]. Therefore, the present study has ascertained the activity of some neem extracts against the adults of sorghum bug (A. pubescens) as a step forward to be included in the control program of such devastating economic pest. As explained in results, the mortality effects of neem treatments were significantly varied among the different extracts, and were very much dose-related and also time dependant. According to the literature, it is known that the insecticidal activities of botanical extracts are significantly based on the plant species and botanical part used, the extracting solvents, the doses and exposure time and the species and stage of the tested insect[16,23].

The results showed that the seeds hexane extract at 8% concentration was the best neem treatment which induced somehow a delayed effect as compared with Malathion 57% EC, though, comparable effects with the insecticide was attained by the neem after three days post application. Such delayed action of neem extracts could be attributed to the fact that the active ingredients (e.g., azadirachtin) in neem are not generally known to act through contact effect and may take some days to kill insect via stomach action[24,25]. Schmutterer (1990) mentioned that after application of neem products, most insect pests continue to feed on the treated plants for some time, but as a rule, the amount of food ingested is considerably reduced, owing to the influence of a secondary antifeedant effect[25]. In other way, since sucking insects feed mainly from the sieve elements of their host plants, and that the active concentration of antifeedant are required to be present in the phloem sap, hence, the complete intake of an active principle in the plant tissues requires 3-5 days after treatment[26].

Redknap (1981) showed that neem seed water extract was effective as Malathion 57% EC against Epilachna chrysomelina on cucurbit, while Satti et al. (2003) showed better effect by neem in controlling the whitefly (Bemisia tabaci)[27,28]. Moreover, Lowery et al. (1993) found that neem seeds extract and oil were effective as the botanical insecticide pyrethrum for the control of aphids on pepper and strawberry[29]. The results of screening ten indigenous plants in Sudan against the Trogoderma granarium revealed that neem seeds hexane extract was the most potent botanical used[30,31]. Schmutterer (1984) reported that water and oil extracts of A. indica are suitable for use as pesticides because of their effectiveness against various pests[32]. The current results confirmed what has been reported by Abu Bakar (2000), who showed potent activity of neem kernel extracts against this pest[33].

The insecticidal properties of neem have been attributed to a group of chemically related compounds known as terpenoids and some nonterpenoidal bioactive compounds[34]. These compounds are found in all parts of the tree, but with higher concentrations in seeds. Such vast arrays of bioactive compounds in neem affect the insects in different ways including antifeedant, repellent and growth regulatory effects. These diversified compounds even complicate the development of resistance in insects[35,36]. Hence, the differences in the toxicity levels of different neem extracts could be attributed to the presence of variable active principles that are extracted by solvents of different polarities[37,38]. The highest effect of hexane extract as compared with the other extracts of seed kernels was attributed to the occurrence of potent groups of apolar secondary metabolites such as terpenoids and steroids that are well extracted with solvents like hexane and petroleum ether[23,39]. Neem oil is reported to contain several triterpenoidal compounds, e.g., nimbin, nimbidin, azadirachtin and many others[14,40,41]. Schwinger et al. (1984) attributed the antifeedant effect in neem seeds to compounds such as salanin, salanol, salanol acetate, 3-deacetyl salannin, 4-epoxy azadirachtin, gedunin, nimbinen and deacetyl nimbinen[42]. Accordingly, more fractionation of neem oil coupled with bioassays may pin point the most effective candidates in this rich source of compounds for insecticidal purposes.

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CONCLUSION

The relative components of neem berries were indicated, and the seed oil (hexane extracted) was the highest extract obtained. Such oil also proved the highest insecticidal effects against the adult sorghum bug (Agonoscelis pubescens) as compared with water and methanol extracts. It showed significantly comparable effect with a standard insecticide (Malathion 57% EC) after three days, and attained 100% mortality one week post treatments. Therefore, the neem oil is recommended to be evaluated under field conditions after being prepared in simple form as promising alternative to hazardous and costly synthetic chemicals for the management of sorghum bug (A. pubescens) in Sudan.

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Fruit berries		Seeds		Extracted materials		
Component	(%)	Component	(%)	Sample	Extract	(%)
Skin	21.2	Cortex	56.6	Seeds (corticated)	Oil	20.1
Pulp	32.5	Kernel	43.4	Kernels	Oil	46.3
Cortex	26.2	-	-	Kernels	Water	42.5
Kernel	20.1	-	-	-	-	-
Total	100	Total	100	-	-	-

Table (1). Relative quantitative components of fruit berries and seeds of the neem tree.

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Treatments	Mortality mean (±S.E) percents at different intervals			
	2 days	3 days	5 days	7 days
NSK-water extract, 1%	3.3±3.33d	10.0±0.0c	20.0±0.0gh	30.0±5.8f
NSK-water extract, 2%	3.3±3.3d	16.7±3.3c	33.3±3.3fg	36.7±6.7ef
NSK-water extract, 4%	03.3±3.3d	20.0±5.8bc	43.3±6.7 defg	56.7±8.8cde
NSK-water extract, 8%	13.3±3.3cd	33.3±3.3b	60.0±0.0cde	66.7±3.3bc
NSK-hexane extract, 1%	03.3±3.3d	20.0±0.0bc	40.0±5.8efg	40.0±5.8def
NSK-hexane extract, 2%	20. 0±0.0c	40.0±0.0b	53.3±3.3cdef	60.0±5.8bcd
NSK-hexane extract, 4%	23.3±6.7c	36.7±14.5b	66.7±12.0cd	80.0±15.3b
NSK-hexane extract, 8%	60.0±5.8b	80.0±5.8a	96.7±3.3ab	100.0±0.0a
NSK-methanol extract,1%	10.0±10.0cd	23.3±18.6bc	36.7±14.5efg	43.3±12.0cdef
NSK-methanol extract,2%	03.3±3.3d	26.7±16.7b	46.7±21.9cdef	53.3±18.6cdef
NSK-methanol extract,4%	06.7±6.7d	33.3±8.8b	60.0±5.8cde	66.7±8.8bc
NSK-methanol extract,8%	10.0±5.8cd	30.0±5.8b	70.0±5.8bc	83.3±3.3ab
Malathion 57%EC	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a
Control	00.0±0.0d	00.0±0.0c	00.0±0.0h	00.0±0.0g
C.V.%	44.7	44.3	28.4	25.7

NSK= Neem seeds kernels.

Means followed by the same letter(s) in each column are not significantly different at (P = 0.05) according to Duncan's Multiple Range Test.

Table (2). Mortality levels of Agonoscelis pubescens adults treated with different neem (Azadirachta indica) seeds extracts, during August- October 2009.

Abdalla Abdelrahim Satti* and Hashim Ahmed El-Massad

Environment and Natural Resources Research Institute (ENRRI), National Centre for Research, Khartoum, Sudan,

Email: satisattisat@yahoo.com