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INSECTICIDAL EFFECTS OF NEEM (AZADIRACHTA INDICA A. JUSS) OILS OBTAINED FROM NEEM BERRIES STORED AT DIFFERENT PERIODS

ABSTRACT

Among the studied botanicals worldwide neem tree is proved to be the richest in active compounds and one of potent sources of natural biocides. Crude extracts of neem seeds showed significant results as insecticides in Sudan where optimum doses are recommended for several vegetable pests. Since neem products of different ages are used for this purpose, laboratory experiments were conducted to compare the insecticidal effects of crude oils extracted from seeds of neem berries stored at different durations under normal room conditions. The 3rd instar larvae of Trogoderma granarium were used as test insects. Comparing all treatments, the highest insect mortalities were attained by the highest concentration (5%) used. The general performance of the different products revealed insignificant slight decreases in oil activities as the storage period of neem fruits increased from one to seven years, afterward a sudden significant drop in potency was occurred for the eight-year old product. Moreover, the same trends were attained regarding sorghum grains damaged by the larvae, as the lowest losses were shown by the highest oil concentration of the products stored between one to seven years. Accordingly, it was advised that oils of neem products stored between one to seven years can be equally used as effective natural insecticides, but newest products of one to four years old are preferable. However, the additive materials incorporated in neem oil, which seemed to improve its potency, may need additional investigations.

KEYWORDS Neem oil; storage period; insecticidal effect; Trogoderma granarium; Sudan.

INTRODUCTION

In order to counter the potential hazards associated with extensive usages of synthetic chemicals, botanical extracts have been suggested by many workers as suitable ecologically safe alternatives for combating agricultural pests and diseases in different parts of the world[1,2,3,4]. Studies on botanical pesticides in Sudan dated back to 1980s when neem (Azadirachta indica A. Juss) seeds water extract was attempted against some agricultural pests at Hudeiba Research Station in the River Nile Province[5]. But, organized research on natural pesticides was commenced in 1990s at the Environment and Natural Resources Research Institute in collaboration with some universities. Since then several indigenous plants were screened, and promising results were obtained from different species[6,7,8,9].

However, among the studied plants neem tree was proved to be the richest in active compounds and the most potent as a source of effective natural pesticides[7,10,11,12]. Enormous active ingredients are found in all botanical parts of the neem tree, but concentrated largely in the seed kernels. Among these ingredients, a group of limonoids (triterpenoids) including azadirachtin nimbin, nimbidin, salanin, salannol, quercetin, gedunin and many others are known to possess insecticidal properties, with the former one thought to be the principal active compound. Azadirachtin acts against insects in two ways; as a potent antifeedant and as an insect growth regulator through interference with the synthesis and release of the insect moulting hormone (ecdysteroids). Neem products also showed very good repellent effects on different insects; and the earlier observation of Schmutterer on neem repellent to the desert locust in Sudan was the first trigger of worldwide research on neem insecticides[1,13]. Hence, neem extracts of various polarities were investigated and proved effective in controlling wide range of insects among Lepidoptera, Homoptera, Hemiptera, Diptera and Coleoptera. Moreover, various neem extracts were also showed comparable or sometimes better effects than different synthetic insecticides[6,14,15,16,17]. Due to the fact that neem ingredients principally act through a stomach action rather than contact effects, they proved to be safe for the general predators in the field[13,15,16,17,18,19,20].

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In Sudan, neem water and organic extracts were tested under laboratory and field conditions for controlling various agricultural pests, and optimum doses of crude preparations were indicated and recommended for some vegetable crops[5,17,20]. Therefore, mature neem seeds are collected during a short period between June and July every year and stored to be used whenever needed in the same season or in subsequent years. However, it is questionable to what extent such seeds can remain effective under storage conditions. The lonely first study conducted on this aspect showed that water extracts of neem seeds stored for up to four years were effective as new seeds in controlling the larvae of Trogoderma granarium[21]. Notably, neem oil represents the highest portion (>45%) of neem seeds that can be extracted and used in crude form, besides water extract, as effective natural insecticides[22]. Hence, the objective of this research was to compare the insecticidal effects of neem oils obtained from seeds of neem berries stored at different years under normal room conditions, using the khapra beetle (T. granarium) as a test insect.

MATERIALS AND METHODS

Two laboratory experiments were conducted at the Environment and Natural Resources Research Institute (ENRRI), National Centre for Research (NCR), Khartoum-Sudan, to compare the insecticidal effects of neem oils prepared from seeds of neem fruits stored at different durations under normal room conditions. The first experiment was conducted during October-November 2006 comparing the products of three years (2003, 2004 and 2005), whereas the second experiment was performed during October-November 2012 using the products of eight years (2005-2012).

Preparations of neem treatments

The neem fruits indicated for the two experiments were already collected from Shambat area, Khartoum North, during the fruiting period (June-July) in each year and stored under normal room conditions in the laboratory. Neem seeds were cleaned from their fruiting parts after being soaked in water for 6hrs, then dried under shade and ground into fine powders using an electric blender (Moulinex®, Type MS-223). In both experiments, all samples of neem powders were extracted with hexane solvent in a soxhlet apparatus. The obtained neem oils for the different studied products were emulsified with a cattle's bile and a liquid soap in the first and second experiments, respectively. Moreover, 0.5% gum Arabic was added in the second experiment so as to improve the adhesive and emulsification properties of oils. Three concentrations (5%, 2.5% and 1.25%v/v) were used in the first experiment, but in the last experiment only two concentrations (5%, 2.5%) were applied.

Bioassay tests

The 3rd instar larvae of the khapra beetle (Trogoderma granarium) were used as test insects to evaluate the insecticidal effects of the different neem oil treatments in each experiment. Such larvae were segregated from a culture already preserved at the Botanical Pesticides Unit in ENRRI. Petri dishes were used to accommodate each experiment where ten grams of sound and clean sorghum grains were placed after being treated with the respective extract concentration. Ten larvae were introduced in each Petri dish with the seeds and covered. Three replications were prepared for the treatments assigned in a Completely Randomized Design (CRD). Records of larval mortalities and other observational were taken at regular intervals from treatments. The sorghum grains were weighed again after half a month for the first experiment, and after one month for the second, so as to compare the loss in seeds weights among different treatments. The ANOVA analysis was performed for each experiment and treatments were compared using Duncan's Multiple Range test.

RESULTS AND DISCUSSION

The results of the first experiment are shown in Table 1. It compares the effects of oils extracted from neem seeds stored at one (product of 2003), two (2004) and three (2005) years, on mortality of the tested insects and the consequent sorghum damage. No

significant effects were found at the fourth day of treatments. At the 7th day, all products exerted significant mortality effects, as compared with the untreated control. At this stage, some variations were detected between the different extracts, with slight decrease in mortality levels as the storage period increased. The highest mortalities were generally attained by the highest concentrations. Thereafter, no significant differences were found between all extracts at 14 days interval. However, due to such short period of the experiment (2 weeks) in addition to the high mortalities of insects, the very poor damage reflected on sorghum grains couldn't be assessed (Table 1). But, the important fact is that no significant differences were recognized between the three years seeds extracts.

Since no significant variations were obtained between the extracted oils of three years (Table 1), products of wide range (1-8 years) were tested in the second experiment (Table 2). The treatments were also evaluated at 48h (2days) to check for the knockdown effects, and followed for a further extended period (21 days) to observe the delayed actions in the latter experiment. However, it is obvious that superior results were recorded by oils treatments in this experiment since the first count (48h), compared to the previous experiment. This might be related to the kind of additive materials (gum Arabic and soap) incorporated in the second experiment. The real effects of these materials, in relation to quantities added, on oil activity should be evaluated in future studies.

As explained in Table (2), the first count showed significant larval mortalities by most neem treatments compared with the untreated control. The highest concentrations (5%) of seven years (2012-2006) products showed significantly comparable results, with higher effects than the lowest concentrations. However, the oldest product (2005) was not different from the control. During the subsequent counts the same trends were maintained, but gradual increases in mortality percents were appeared with time for all products, proving the delayed actions of neem[13,20]. Similarly, the highest concentrations of oils for seven products (2012-2006) revealed the best significant effects during all counts. Although, slight decreases in mortality percents were detected between these treatments with an increase in storage period from one year to seven years, but they were significantly alike as mentioned above. Nevertheless, the newest products of the last four years (2012-2009) were the best treatments, as they showed 100% larval mortalities after one week of application. Again, the oil treatments of the oldest eight years' neem seeds (2005) showed the lowest effects during the 7, 14 and 21 days intervals without significant differences from the control, except in some late counts.

The results of sorghum grains damaged by T. granarium larvae during the second experiment are presented in Table (3). All neem extracts reflected significant reduction in grains damage with variable levels (1-5% damage) as compared with the control check (8% damage). In correspondence with the above bioassay mortality results, the amounts of grains consumed were significantly lower in the highest oil concentrations of seven years (2012-2006) products (1-3% damage) than in the rest of neem treatments (4-5% damage). The two oil concentrations of the oldest seeds (2005) came next in order without significant differences from the lowest concentrations of the other extracts.

Oil represents the highest portion that can be extracted from neem seeds. Maydell (1986) reported that neem kernels contain about 45 – 60% oil, hence more than 46% was obtained in Sudan through hexane extraction[22,23]. This is an added value when commercialization of such neem product is considered. Since pure neem oil free of water can be extracted with organic solvents, it was suggested that more concentration of active ingredients and more insecticidal activities can be achieved from neem oil than from seed water extract. As indicated in several literatures, neem oil proved superior mortality effects over water and other organic solvents extracted polar materials, which attributed to potent apolar secondary compounds largely of triterpenoidal derivation in the oil[22,24]. It was demonstrated that neem oil is rich in active ingredients mainly azadirachtin and many other terpenoids such as salannin, nimbin, meliantrol, isonimolicinolide, azadirone, azadiradione, meldenin, nimbonin, nimbidinin, vepinin and mahmoodin[1,10]. These active substances showed considerable potentialities against various pests of stored and field crops, primarily through repellent, antifeedant, growth regulatory and toxic effects[1,25,26,27,28,29,30].

Another credit which can be added to the potentiality of neem oil according to the current results is the high tolerance to long storage durations of neem fruits, as there were no significant variations found among oils extracted from berries stored between one to seven years. In other way, very minor insignificant deterioration of insecticidal activities of oil was occurred over seven years of fruit storage, but significant drop in activities abruptly appeared after eight years. Accordingly, neem fruits stored for up to seven years can be used fairly to obtain oil for insecticidal purpose, though fruits of one to four years old are preferable. These results also manifested the superiority of polar compounds in neem oil over apolar extracts when the age of neem fruits is considered. It was found that the activities of water and ethanol extracts of seeds were significantly deteriorated in neem fruits stored for more than four and three years, respectively[21,31]. Therefore, the present results advocated the potentiality of neem oil and invite more research for proper exploitation in pests' control.

CONCLUSIONS

The results indicated that seeds oils obtained from neem berries stored for one to seven years under normal room conditions gave significantly comparable insecticidal effects against the 3rd instar larvae of Trogoderma granarium, though the newest products of one to four years old were relatively the best. However, significant reductions in activities were reported in oil treatments prepared from the oldest fruits of eight years old. Therefore, fruits stored for up to seven years were advocated for preparing crude oil extracts, but additional studies are needed regarding the additive materials for quality improvement.

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Treatments	Mortality mean (±S.E.) percents at different intervals			Grains consumed	
	4 days	7 days	14 days	X±S.E.	%
NSO-2003, at 5%	14.3±1.2 a	52.9±2.3 cd	88.0±4.0 a	0.1±0.0 ab	1.0%
NSO-2003, at 2.5%	10.0±0.6 a	47.1±1.7 de	70.0±4.0 a	0.1±0.1 ab	1.0%
NSO-2003,at 1.25%	14.3±0.6 a	38.6±1.7 e	70.0±3.5 a	0.1±0.1 ab	1.0%
NSO-2004, at 5%	32.9±1.2 a	61.4±2.9 bc	88.0±3.5 a	0.1±0.0 ab	1.0%
NSO-2004, at 2.5%	28.6±0.6 a	61.4±1.7 bc	70.0±4.0 a	0.1±0.1 ab	1.0%
NSO-2004,at 1.25%	04.3±0.3 a	52.9±1.7 cd	70.0±4.6 a	0.1±0.1 ab	1.0%
NSO-2005, at 5%	14.3±0.6 a	71.4±2.9 a	88.0±3.5 a	0.1±0.0 ab	1.0%
NSO-2005, at 2.5%	04.3±0.0 a	67.1±2.3 ab	70.0±4.0 a	0.1±0.1 ab	1.0%
NSO-2005,at 1.25%	04.3±0.0 a	67.1±1.7 ab	70.0±4.0 a	0.1±0.1 ab	1.0%
Control (untreated)	00.0±0.0 a	00.0±0.0 f	00.0±0.0 b	0.2 ± 0.1 a	2.0%
CV%	100.0	25.0	9.5	44.4	

NSO = Neem seeds oil extract.

Table 1. Effects of neem oil extracted from seed products of three years (2003-2005) on mortalities and feedings of the 3rd instar larvae of Trogoderma granarium, at different intervals from treatments, during October-November 2006.

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Treatments	Mortality mean (±S.E.) percents at different intervals			
	2 days	7 days	14 days	21 days
NSO-2005, at 5%	00.0 ± 0.0 g	06.7 ± 0.3 f	26.7 ± 0.3 fg	46.7 ± 0.3 d
NSO-2005, at 2.5%	03.3 ± 0.3 fg	03.3 ± 0.3 f	13.3 ± 0.3 gh	23.3 ± 0.3 e
NSO-2006, at 5%	36.7 ± 0.3 cde	80.0 ± 0.6 ab	96.7 ± 0.3 a	96.7 ± 0.3 a
NSO-2006, at 2.5%	23.3 ± 0.3 def	40.0 ± 0.0 de	60.0 ± 0.6cde	70.0 ± 0.6 bc
NSO-2007, at 5%	40.0 ± 0.6 cd	76.7 ± 0.9 ab	90.0 ± 1.0 ab	96.7 ± 0.3 a
NSO-2007, at 2.5%	26.7 ± 0.3 de	63.3 ± 0.9 bc	73.3 ± 0.7abc	76.7 ± 0.3 b
NSO-2008, at 5%	46.7 ± 0.3 c	86.7 ± 0.9 ab	93.3 ± 0.3 ab	96.7 ± 0.3 a
NSO-2008, at 2.5%	30.0 ± 0.6 cde	53.3 ± 0.9 cd	63.3 ± 0.7 cd	73.3 ± 0.3 bc
NSO-2009, at 5%	73.3 ± 0.3 b	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0a
NSO-2009, at 2.5%	26.7 ± 1.2 de	43.3 ± 1.9 cde	63.3 ± 1.3 cd	66.7 ± 1.2 bc
NSO-2010, at 5%	73.3 ± 0.3 b	90.0 ± 0.6 a	93.3 ± 0.3 ab	100.0 ± 0.0 a
NSO-2010, at 2.5%	10.0 ± 0.5 fg	33.3 ± 0.3 de	43.3 ± 0.9 ef	60.0 ± 0.6bcd
NSO-2011, at 5%	80.0 ± 0.6 ab	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
NSO-2011, at 2.5%	20.0 ± 0.6 ef	36.7 ± 0.3 de	43.3 ± 0.3 ef	56.7 ± 0.9 cd
NSO-2012, at 5%	93.3 ± 0.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
NSO-2012, at 2.5%	23.3 ± 0.3 def	30.0 ± 0.6 e	53.3 ± 0.7 de	60.0 ± 1.0bcd
Control (untreated)	00.0 ± 0.0 g	00.0 ± 0.0 f	00.0 ± 0.0 h	03.3 ± 0.3 f
CV%	23.9	21.4	15.6	12.7

NSO = Neem seeds oil extract.

Table 2. Effects of neem oil extracted from seed products of eight years (2005-2012) on mortalities of the 3rd instar larvae of Trogoderma granarium, at different intervals from treatments, during October-November 2012.

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Treatments	Amount (g) of grains consumed	
	X±S.E.	%
NSO-2005, at 5%	0.5 ± 0.0 b	5.0
NSO-2005, at 2.5%	0.4 ± 0.2 bcd	4.0
NSO-2006, at 5%	0.2 ± 0.0 de	2.0
NSO-2006, at 2.5%	0.4 ± 0.0 bcd	4.0
NSO-2007, at 5%	0.3 ± 0.1 cde	3.0
NSO-2007, at 2.5%	0.4 ± 0.0 bcd	4.0
NSO-2008, at 5%	0.2 ± 0.0 de	2.0
NSO-2008, at 2.5%	0.4 ± 0.0 bcd	4.0
NSO-2009, at 5%	0.1 ± 0.0 e	1.0
NSO-2009, at 2.5%	0.5 ± 0.1 b	5.0
NSO-2010, at 5%	0.3 ± 0.0 cde	3.0
NSO-2010, at 2.5%	0.5 ± 0.0 b	5.0
NSO-2011, at 5%	0.1 ± 0.1 e	1.0
NSO-2011, at 2.5%	0.5 ± 0.1 b	5.0
NSO-2012, at 5%	0.3 ± 0.0 cde	3.0
NSO-2012, at 2.5%	0.5 ± 0.1 b	5.0
Control (untreated)	0.8 ± 0.0 a	8.0
CV%	26.3	

NSO = Neem se

Table 3. Sorghum grains damaged by the 3rd instar larvae of Trogoderma granarium, after one month post treatments with seeds oils of eight neem products (2005-2012), during October-November 2012

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