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Full Length Research Paper

Insecticidal efficacy of neem seed kernels ethanolic extract prepared from neem products of different years

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A laboratory experiment was conducted during October 2011 to compare the insecticidal effects of neem seed kernels ethanolic extracts prepared from neem products of five years (2007, 2008, 2009, 2010 and 2011) against the 3rd instar larvae of *Trogoderma granarium*. Three concentrations (5%, 2.5% and 1.25%) were used from each product extract. The results of larval mortalities were recorded at four weekly intervals (7, 14, 21 and 30 days) post treatments. Although, some variations were observed between treatments after one week, no significant differences were detected. After 2 weeks, the highest rates (5%) of three products (2008, 2009 and 2010) extracts showed significantly the highest mortality percents, with the former one being relatively the best. After the 3rd and 4th weeks the three previous treatments also kept comparably superior significant effects, but the product of the year 2010 was relatively the best. It is clear that the fresh seeds (product of 2011) and the oldest seeds (2007) were the least effective during all counts. In general, all extracts exerted increased mortalities in relation to concentrations and exposure time. No significant variations were detected regarding seeds damage due to the short period of the study. Hence, it can be concluded that storage of neem berries at periods ranging from one to three years under normal room temperature were optimum for obtaining the highest effect of ethanol extract from seed kernels.

Keywords: Neem, Storage durations, Seeds age, Insecticidal effect, *Trogoderma granarium.*

INTRODUCTION

The khapra beetle (*Trogoderma granarium* Everts) (Coleoptera: Demestidae) is an exotic serious pest of stored grains in Sudan. It is originated in South Asia and considered one of the World's most destructive pests of grain products, and therefore placed among the hundred worst invasive species in the world (Lowe *et al*., 2000; Ahmedani *et al*., 2007; Stibick, 2007). Infestation by this pest is difficult to control because of the insect's ability to survive without food for long periods, adaptability to dry conditions and low moisture food, and its resistance to many insecticides. However, botanical insecticides were suggested among suitable alternatives of control, and proved promising results in controlling different field and store pests (Siddig, 1991; Sir El Khatim, 2005; Yousif and Satti, 2008).

The neem tree (*Azadirachta indica* A. Juss), family Meliaceae, has certain distinct advantages over most botanical active plants based on its richness in diversified secondary metabolites of variable biocidal effects against

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different phytophagous pests. Although, it is native to south eastern Asia, *A. indica* nowadays is cultivated in most tropical and subtropical parts of the world. It is widely grown in Sudan as a shade tree. The tree acts as a major source of botanical insecticides. The environmental problems of synthetic insecticides that currently used in agriculture are obvious worldwide and particularly in Sudan (Leidholm and Amisi, 1986; Abdelbagi *et al*., 2006). It was reported that the characteristic garlic odor of neem materials presumably repelled insects in closed storage, and that the bitter components present in neem extracts deterred feeding. Neem materials were found to cause toxicant, repellent, antifeedant and growth regulatory effects on wide range of insect pests (Schmutterer, 1995).

In Sudan, neem extracts were recommended for combating different agricultural pests, including store pests (Siddig, 1987, 1991; Satti and Nasr, 2006). Since neem trees generally produce one time during the year (June-August), the collected fruits may be stored for many years to be used in pest control. The objective of this experiment was to compare the effect of various storage durations of neem berries (fruits) on insecticidal activities of seeds kernels. Ethanolic extracts of seeds stored at 4, 3, 2, 1, and 0 years were bioassayed under laboratory conditions against the khapra beetle (*T. granarium*).

MATERIALS AND METHODS

Preparation of treatments

Neem fruits produced in five successive years (2007, 2008, 2009, 2010 and 2011) were collected from Shambat area, Khartoum North, and stored under laboratory conditions to be used in a comparative bioassay test against the 3rd instar larvae of *Trogoderma granarium*. Hundred grams of seed kernel powder from each product were extracted in a soxhlet apparatus using the ethanol solvent. The extracts were air dried and stored in black bottles in a refrigerator until used. As a pre-step for the bioassay experiment, three concentrations (5%, 2.5% and 1.25%) were prepared serially from each extract. Liquid soap (0.1%) was added as an emulsifier due to the presence of traces oils on the different extracts.

Bioassay experiment

A laboratory bioassay experiment was conducted during October 2011. Sixty eight clean Petri dishes were arranged and labeled to accommodate the above prepared treatments with four replications. Such treatments included two controls (untreated + 0.1% soap solution). Five grams of sorghum (cv. fetarita) grains were allotted to each Petri dish, after being mixed with the respective extract concentration and dried.

The 3rd instar larvae of *T. granarium* were segregated from a culture of the pest found kept at the Environment and Natural Resources Research Institute, Khartoum. Ten larvae were introduced with the sorghum grains in each Petri dish, as indicated above, and covered. Periodical counts were conducted per weekly intervals to record the dead insects and any other observations. The experiment was ended after four counts, then the remained larvae were removed and the content of each Petri dish was sieved to get rid of any remnants found as a result of insect feedings and development. Hence, the grains were re-weighed so as to compare the losses occurred due to larval feeding. Mortality and damage data were analyzed statistically based on Completely Randomized Design and compared using Duncan's Multiple Range test.

RESULTS AND DISCUSSION

The results of the bioassay experiment comparing seeds ethanolic extracts prepared from neem products of five years against the larvae of *T. granarium* were presented in Table (1). In general, the results showed clear variations in mortality levels among the different treatments based on seeds age and concentrations. All extracts exerted increased mortalities in relation to concentrations and exposure time. This proved the delayed effect of neem extracts which appeared gradually with time, post treatments. Therefore, slight variations were observed between treatments after one week, but significant differences were achieved from the second week onwards. After 2 weeks, the highest doses (5%) of seed ethanolic extracts of the years 2008, 2009 and 2010 showed significantly the highest mortality percents, with the former product being relatively the best. After the 3rd and 4th weeks the three previous treatments also kept significant superior effects, but the product of 2010 was relatively the best. It is clear that the fresh seeds (product of 2011) and the oldest seeds (2007) were the least effective compared to the other products, though they showed significant differences from controls for most concentrations particularly during the last two counts. This indicated that neem seed can retain significant insecticidal activities for more than four years when stored under normal room temperature, but seeds ages ranged from one to three years were optimum. However, the results may indicate that deterioration of neem active ingredients start after three years, but it was unclear why fresh seeds were less effective.

The losses in sorghum grains resulted from the different treatments of the bioassay experiment were compared in Table (2). It is obvious that very slight damages were recorded due to the short time of the study. Therefore, no significant differences were detected

Table 1. Effects of neem kernels ethanolic extracts (NK-OH.E) prepared from neem berries stored at different years, against the 3rd instar larvae of Trogoderma granarium, during October 2011.

Treatments	Mortality mean percents at different periods			
	1 week	2 weeks	3 weeks	4 weeks
NK-OH.E, 2007 (5%)	02.3 a	31.2 bc	47.1 ab	63.0 bcd
NK-OH.E, 2007 (2.5%)	00.0 a	02.7 de	12.7 ef	26.0 ef
NK-OH.E, 2007 (1.25%)	02.3 a	10.6 cde	31.2 bcde	31.2 de
NK-OH.E, 2008 (5%)	07.2 a	57.7 a	57.7 ab	81.5 ab
NK-OH.E, 2008 (2.5%)	02.3 a	18.0 bcde	26.0 cde	44.5 de
NK-OH.E, 2008 (1.25%)	02.3 a	13.0 cde	18.3 def	55.1 bcde
NK-OH.E, 2009 (5%)	00.0 a	41.8 ab	55.1 ab	76.2 abc
NK-OH.E, 2009 (2.5%)	02.3 a	18.3 bcde	23.3 cdef	39.2 de
NK-OH.E, 2009 (1.25%)	04.9 a	26.0 bcde	47.1 ab	49.7 cde
NK-OH.E, 2010 (5%)	07.2 a	44.5 ab	65.6 a	92.1 a
NK-OH.E, 2010 (2.5%)	00.0 a	28.9 bcd	52.4 ab	60.3 bcd
NK-OH.E, 2010 (1.25%)	09.8 a	28.9 bcd	36.5 bcd	60.3 bcd
NK-OH.E, 2011 (5%)	12.1 a	18.3 bcde	39.2 bcd	55.0 bcde
NK-OH.E, 2011 (2.5%)	04.9 a	18.3 bcde	31.2 bcde	36.5 de
NK-OH.E, 2011 (1.25%)	02.3 a	18.3 bcde	26.2 bcd	36.5 de
Control with 0.1% soap	00.0 a	00.0 e	00.0 f	00.0 f
Control, untreated	00.0 a	00.0 e	00.0 f	00.0 f
C.V.%	93.6	57.0	35.4	33.3

Table 2. Effects of neem kernels ethanolic extracts (NK-OH.E) prepared from neem berries stored at different years, on sorghum seeds damaged by the 3rd instar larvae of Trogoderma granarium (October 2011).

Treatments	Damaged seeds (%)
NK-OH.E, 2007 (5%)	0.6a
NK-OH.E, 2007 (2.5%)	0.5a
NK-OH.E, 2007 (1.25%)	1.0a
NK-OH.E, 2008 (5%)	1.0a
NK-OH.E, 2008 (2.5%)	0.7a
NK-OH.E, 2008 (1.25%)	0.7a
NK-OH.E, 2009 (5%)	0.1a
NK-OH.E, 2009 (2.5%)	0.5a
NK-OH.E, 2009 (1.25%)	1.6a
NK-OH.E, 2010 (5%)	0.4a
NK-OH.E, 2010 (2.5%)	3.1a
NK-OH.E, 2010 (1.25%)	0.7a
NK-OH.E, 2011 (5%)	0.7a
NK-OH.E, 2011 (2.5%)	1.6a
NK-OH.E, 2011 (1.25%)	0.6a
Control with 0.1% soap	1.2a
Control, untreated	1.0a
C.V.%	145.8

among all treatments including the controls. This aspect may needs to be assessed at a longer period.

Although, studies on storage conditions of neem fruits are very scanty in the country, the current results are in agreement with some investigators. Neem has been used as an effective postharvest protectant for many crops. In

a study made by Ahmed (2010) to examine the effect of time and storage methods on the efficacy of neem seeds for controlling the red flour beetle *Tribolium castaneum* (Herbst) and the mosquito *Culex quinquefasciatus* (Say), he proved three facts: 1) the insecticidal effects of neem seeds against the both species, 2) indicated the occurrence of significant losses in efficacy of neem seeds with storage time from one to two years, and 3) showed that storage of neem seeds in jute sacks or exposed under room conditions can reduce the negative effect of storage on efficacy of neem seeds. The results also more or less confirmed what have been stated by Satti *et al*. (2010), who showed that neem seeds water extracts obtained from neem fruits stored at different years revealed the best results from products stored at two to four years, as compared with those of one and five years old.

Moreover, neem extracts were also reported to withstand certain levels of temperature degrees. Jenkins *et al*. (2003) reported that neem kernels extract can be stored at high temperatures for at least five months without significant reduction in overall effectiveness. These authors showed reduced infestations by *Callosobruchus maculatus* even after the neem kernels extract had been stored at high temperatures (50^{o}C) for two weeks, followed by up to five months storage at 28^{o}C. Analysis with HPLC revealed that azadirachtin A which present in unheated or fresh neem kernel extract was dissipated when stored at 50^{o}C for two weeks. They attributed the observed mortality, which was maintained even when azadirachtin A was absent in neem kernels extract, to physical effects of the neem oil. Such properties of neem place this tree as the first candidate for commercial uses in natural pesticides.

CONCLUSION

Based on the prevailing environmental conditions in Sudan, the study concluded that the optimum storage durations for neem berries to obtain the best insecticidal effects from kernel ethanolic extract are one to three years under normal room temperature. Products of less than one year or more than three years old can also be of significant results, but with relatively lower activities.

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