

Comparative study on the effects of storage period of neem (*Azadirachta indica* A. Juss) seed oil in controlling *Callosobruchus maculatus* (F.) infestation of stored cowpea grains

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Research Paper

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ABSTRACT

The efficacy of the storage period of neem (*Azadirachta indica* A. Juss.) seed oil (NSO) for the management of *Callosobruchus maculatus* (F.) infestation of stored cowpea grains was investigated. Fifteen gram cowpea grains of Banjara, a local cowpea cultivar, were treated with NSO stored for 0/freshly prepared, 2, 8, 10 and 15 years, each applied at four dosages (0.00/untreated control, 0.02, 0.04 and 0.08 ml) and in four replicates laid out in a completely randomized block design (CRBD) in the laboratory. One-way analysis of variance (ANOVA) was used to analyze data collected, and significant means at $P < 0.05$ were separated using the least significant difference (LSD). At 0.02 and 0.04 ml dosages, the mean number of *C. maculatus* eggs laid, severity of grain damage and developmental period of *C. maculatus* were significantly lower on cowpea grains

treated with NSO stored for 2 and 8/10 years, but not amongst NSO storage periods when treated at 0.08 ml. For cowpea grains treated with 0.04 and 0.08 ml dosages, the mean number of emerged *C. maculatus* adults, percentage grain damage and susceptibility index were not significantly different amongst all NSO storage periods. Also, for each of these parameters, no interaction existed between the storage period of NSO and dosages applied. These results indicate that when applied at moderate to high dosages, NSO, freshly prepared or stored for between 2 and 15 years can effectively manage *C. maculatus* infestation of cowpea grains.

Key Words: Cowpea grain, *Callosobruchus maculatus*, neem seed oil, storage period, dosages and Banjara.

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INTRODUCTION

The role of synthetic pesticides in increasing agricultural productivity especially in developing countries cannot be overemphasized. Although, these pesticides have the advantage of giving reliable control of crop pests whenever and wherever required (Asogwa, 2006; Ntow *et al.*, 2006; Ngowi *et al.*, 2007), their usage have been gravely associated with ecological damage and health hazards. For instance, to handlers/farmers and consumers during formulation or field application and consumption of treated commodities (Akunyili and Ivbijaro, 2006; Bashir, 2007; Asogwa and Dongo, 2009; Salami *et al.*, 2010). The use of botanicals with insecticidal properties that are biodegradable, less toxic to the environment/non-target organisms and handlers,

less persistent and are readily available/economical to obtain and prepare have therefore continued to gain prominence (Rajashekar, 2006; Obeng-Ofori, 2010; Oruonye and Okrikata, 2010; Mochiah *et al.*, 2011; Dimetry, 2012; Rajashekar *et al.*, 2012). Of the over 2000 plant species belonging to 60 families possessing insecticidal properties (Dev and Koul, 1997; Dimetry, 2012), the neem tree, *Azadirachta indica* A. Juss., stands out. In addition to having antiviral, antibacterial, antifungal properties, neem products have insecticidal properties that are effective against pests of field crops (Chakraborti and Sarkar, 2011; El Atta *et al.*, 2011; Degri and Sodangi, 2013; Shannag *et al.*, 2014) and stored grains (Oparaeke *et al.*, 1998; Lale and Mustapha, 2000; Maina and Lale,

2004; Ileke and Bulus, 2012; Maina *et al.*, 2012a; Yahaya, 2013). Although all parts of the *A. indica* tree possess insecticidal activity, the seed kernel is reported to be most effective, and has several pesticidal active ingredients which together are called triterpene or limnoids. Major limnoids include Azadirachtin, Salannin, Meliatriol and Nimbin (Debashri and Tamal, 2012). Azadirachtin alone is biologically active and possess repellent, antifeedant, insect growth regulator, oviposition deterrent and toxic/insecticidal effects against 400 to 500 insect species in at least 10 to 13 orders (Dimetry, 2012; Debashri and Tamal, 2012).

Botanicals that can be stored for long periods of time without losing potency will be of great advantage to users. In that, such pesticides may not necessitate too frequent re-applications of prepared/formulated botanicals to avoid wastage, nor require large storage space for keeping sufficient quantities of plant materials for processing/preparation whenever needed for application. In addition to the cumbersome handling/preparation of the botanicals, adequate storage space can be a big challenge, particularly to subsistence farmer. Yet, information on the efficacy of neem products stored for long periods of time on economically important insect pests such as *Aphis* sp., *Callosobruchus maculatus*, *Coniesta ignefusalis*, *Helicoverpa armigera*, *Heteroligus meles*, *Maruca vitrata*, *Mylabris* sp., *Riptortus dentipes*, *Sesamia calamistis*, *Sitophilus zeamais* etc remain scarce. This study, therefore, determined the efficacy of neem seed oil (NSO) stored for different periods (0, 2, 8, 10 and 15 years) against *C. maculatus* infestation on one moderately infested local cowpea cultivar, Banjara.

MATERIALS AND METHODS

Experimental site and insect rearing

The experiment was carried out in the Entomology Laboratory, Department of Crop Protection, University of Maiduguri under ambient temperature of 30-35°C and 65-70% relative humidity. Stock culture was raised using adult *C. maculatus* obtained from infested cowpea in the laboratory on 100 g cowpea grains of Borno brown placed in a 1 L kilner jar. The jar was then capped with a piece of mushin cloth, 10 mesh/cm, to allow ventilation and at the same time preclude the entry or exit of insects. Prior to setting up the culture, cowpea grains used were obtained in Maiduguri-Monday-Market and sterilized by refrigeration at about 0°C for 14 days.

Materials sourcing and preparation

Grains of Banjara, a local cowpea cultivar was obtained from IITA/PROSAB, Maiduguri, Nigeria. To avoid hidden

infestation grains obtained were immediately sterilized by refrigeration as stated above. Ripe and fallen fruits of neem trees were collected at the Sanda-Kyarimi Park or Zoo in Maiduguri during November and December, 2010. The fruits were air-dried and decorticated to obtain the kernels.

Neem seed oil extraction was carried out using the Soxhlet extraction process in the Chemistry Laboratory, Department of Chemistry, University of Maiduguri. Forty gram of the pulverized neem seed was weighed using a mehlher balance and wrapped in a double layer of Whatman No.1 filter paper. The paper was stapled adequately at the seams to prevent any leakage. Each pack was then extracted in 200 ml of analytical grade of acetone for 1½ hours at 56°C. The freshly extracted oil was pressed out manually and then poured into a 250 ml volumetric flask and stored at room temperature. This procedure was repeated until adequate quantity of oil was obtained. NSO of different ages/stored for 2, 8, 10 and 15 years were obtained from the laboratory.

Experimental set up and data analysis

Using the complete randomized block design (CRBD), four replications each of 15 g of cowpea grains in a 100 ml glass jar were treated with three dosages (0.02, 0.04 and 0.08 ml) of freshly prepared NSO or the untreated control and also NSO of four different ages, following storage for 2, 8, 10 and 15 years. Each dose was applied separately in 0.02 ml of analytical grade of acetone. Treated grains were stirred with a glass rod until the acetone evaporated completely. All controls (untreated) were treated with 0.02 ml pure acetone only and then stirred. Each experimental jar was then infested with three pairs of newly emerged (0-1 day old) adults of *C. maculatus*. Oviposition was allowed for five days, after which all introduced adult insects were removed and the number of eggs laid on the grains counted using a tally counter. The contents of each experimental jar were returned and kept for the observation of progeny emergence. Upon emergence, the number of all adult beetles per replicate was counted on daily basis and throughout the first filial generation (F₁). After which, the total number of emerged adults was recorded. The number of damaged grains and emergence holes were counted and used to calculate the following:

Percentage grain damage = $\frac{\text{Number of damaged grains}}{\text{Total number of grains}} \times 100$

Severity of grain damage = $\frac{\text{Number of emergence holes}}{\text{Number of damaged seeds}}$

Developmental period of *C. maculatus* was estimated as the time in days from the mid-point of oviposition to the emergence of 50% of the F₁ progeny.

Table 1. Mean number of eggs laid by *C. maculatus* on Banjara cowpea grains treated with NSO of different storage periods and dosages.

NSO storage period (years)	NSO dosage (ml/15 g grain)				Mean
	0.00	0.02	0.04	0.08	
0	73.67	32.33	32.67	15.00	38.42
2	53.33	49.00	46.33	11.67	40.08
8	42.00	36.67	29.33	15.67	30.92
10	44.00	26.67	25.67	4.67	25.25
15	67.67	60.00	24.33	7.67	39.92
Mean	70.17	51.17	39.58	13.67	

SED = 10.025, LSD (0.05) = 20.261 (Age of NSO); SED = 8.966, LSD (0.05) = 18.122 (Dosage of NSO); SED = 20.050, LSD (0.05) = 40.521 (Interaction).

Table 2. Mean number of adult *C. maculatus* that emerged from Banjara cowpea grains treated with NSO of different storage periods and dosages.

NSO storage period (years)	NSO dosage (ml/15g grain)				Mean
	0.00	0.02	0.04	0.08	
0	16.00	1.33	0.67	0.00	4.50
2	8.00	0.00	0.00	0.00	2.00
8	12.33	0.00	0.00	0.00	3.08
10	14.00	0.67	0.33	0.00	3.75
15	14.00	6.33	0.33	0.00	5.17
Mean	16.08	2.08	0.33	0.00	

SED = 1.140, LSD (0.05) = 2.304 (Age of NSO); SED = 1.020, LSD (0.05) = 2.061 (Dosage of NSO); SED = 2.280, LSD (0.05) = 4.609 (Interaction).

Data obtained were subjected to one-way analysis of variance (ANOVA). Significantly different means at $P < 0.05$ were separated using the least significant difference (LSD) (Gomez and Gomez, 1984).

RESULTS

The mean number of eggs laid by *C. maculatus* was highest (73) on control/untreated cowpea grains of freshly extracted NSO and lowest (5) on grains treated at 0.08 ml with 10 years old NSO (Table 1). The number of bruchid eggs laid was significantly lower on cowpea grains treated with the highest test concentration of 0.08 ml for all ages (0, 2, 8, 10 and 15 years) of NSO. At lower concentrations (0.00, 0.02 and 0.04 ml), however, the number of eggs laid by *C. maculatus* were significantly lower on cowpea grains treated with NSO stored for 8 and 10 years.

The mean number of emerged *C. maculatus* adults ranged from 16 on control cowpea grains under freshly extracted NSO to zero on grains treated with NSO stored for 0, 2, 8, 10 and 15 years (Table 2). The mean number of emerged *C. maculatus* adults was significantly lower on cowpea grains treated with 0.02, 0.04 and 0.08 ml of NSO stored for 0, 2, 8, 10 and 15 years than the untreated ones.

The mean percentage grain damage caused by *C.*

maculatus and the severity of damage were highest (23) on grains untreated with freshly extracted NSO to zero on grains treated with NSO stored for 0, 2, 8, 10 and/or 15 years (Tables 3 and 4). The mean percentage grain damage caused and the severity of grain damage were significantly lower on cowpea grains treated with 0.02, 0.04 and 0.08 ml of NSO stored for 0, 2, 8, 10 and 15 years than the untreated ones.

The mean susceptibility index of Banjara cowpea grains and developmental period of *C. maculatus* respectively ranged from zero and zero on untreated grains to 8 and 23 on grains treated with NSO stored for 0, 2, 8, 10 and 15 years (Tables 5 and 6). The mean susceptibility index of cowpea grains and developmental period of *C. maculatus* were significantly lower on cowpea grains treated with 0.02, 0.04 and/or 0.08 ml of NSO stored for 0, 2, 8, 10 and 15 years than the untreated ones.

The effect of each dosage amongst NSO storage periods was also compared. At 0.02 and 0.04 ml dosage, the mean number of *C. maculatus* eggs laid, severity of grain damage and developmental period of *C. maculatus* were significantly lower on cowpea grains treated with NSO stored for 2 and 8/10 years, but not significantly different amongst NSO storage periods when treated at 0.08 ml (Tables 1, 4 and 6). For cowpea grains treated with 0.04 and 0.08 ml dosage, the mean number of emerged *C. maculatus* adults, percentage grain damage

Table 3. Mean percentage damage caused by *C. maculatus* to Banjara cowpea grains treated with NSO of different storage periods and dosages.

NSO storage period (years)	NSO dosage (ml/15 g grain)				Mean
	0.00	0.02	0.04	0.08	
0	23.20	2.07	1.07	0.00	6.59
2	12.50	0.00	0.00	0.00	3.13
8	18.07	1.00	0.00	0.00	4.77
10	16.00	1.00	0.50	0.00	4.38
15	18.40	8.97	0.53	0.00	6.98
Mean	22.04	3.26	0.53	0.00	

SED = 1.721, LSD (0.05) = 3.447 (Age of NSO); SED = 1.539, LSD (0.05) = 3.110 (Dosage of NSO); SED = 3.441, LSD (0.05) = 6.955 (Interaction).

Table 4. Mean severity of seed damage caused by *C. maculatus* to Banjara cowpea grains treated with NSO of different storage periods and dosages.

NSO storage period (years)	NSO dosage (ml/15 g grain)				Mean
	0.00	0.02	0.04	0.08	
0	23.20	2.07	0.07	0.00	6.34
2	12.50	0.00	0.00	0.00	3.13
8	13.07	1.00	0.00	0.00	3.52
10	16.00	1.00	0.50	0.00	4.38
15	18.40	8.97	0.53	0.00	6.98
Mean	20.79	3.26	0.28	0.00	

SED = 0.133, LSD (0.05) = 0.269 (Age of NSO); SED = 0.199, LSD (0.05) = 0.241 (Dosage of NSO); SED = 0.267, LSD (0.05) = 0.539 (Interaction).

Table 5. Mean susceptibility index of Banjara cowpea grains to *C. maculatus* infestation when treated with NSO of different storage periods and dosages.

NSO storage period (years)	NSO dosage (ml/15 g grain)				Mean
	0.00	0.02	0.04	0.08	
0	8.13	0.83	0.00	0.00	2.24
2	4.23	0.00	0.00	0.00	1.06
8	4.67	0.33	0.00	0.00	1.25
10	5.83	0.00	0.00	0.00	1.46
15	3.40	0.00	0.00	1.06	1.12
Mean	6.57	0.29	0.00	0.27	

SED = 0.437, LSD (0.05) = 0.883 (Age of NSO); SED = 0.391, LSD (0.05) = 0.990 (Dosage of NSO); SED = 0.874, LSD (0.05) = 1.766 (Interaction).

and susceptibility index were not significantly different amongst all tested NSO storage periods (Tables 2, 3 and 5). At 0.08 ml dosage, in particular, the mean number of *C. maculatus* eggs laid, number of emerged *C. maculatus* adults, percentage grain damage, severity of grain damage, susceptibility index and developmental period of *C. maculatus* were not significantly different amongst all tested NSO storage periods (Tables 1, 2, 3, 4, 5 and 6).

For treated grains, no interaction was observed between the storage period of NSO and dosage applied for all the parameters tested (Tables 1, 2, 3, 4, 5 and 6).

DISCUSSION

Indeed, the mean number of eggs laid, severity of grain damage or developmental period of *C. maculatus* were lower on cowpea grains of Banjara treated with NSO than the untreated ones, and at the same time, these means were not significantly different amongst all NSO storage periods (0-15 years) when applied at 0.04 and/or 0.08 ml/15 g grains. Interestingly also, the mean number of *C. maculatus* adults emerged, percent grain damage and susceptibility index were lower on cowpea grains treated

Table 6. Mean developmental period of *C. maculatus* on Banjara cowpea grains treated with NSO of different storage periods and dosages.

NSO storage period (years)	NSO dosage (ml/15 g grain)				Mean
	0.00	0.02	0.04	0.08	
0	21.67	21.67	10.33	0.00	13.42
2	21.33	0.00	0.00	0.00	5.33
8	20.67	8.33	0.00	0.00	7.25
10	22.67	16.33	6.33	0.00	11.33
15	21.33	15.30	34.00	0.00	17.66
Mean	26.92	15.41	12.67	0.00	

SED = 2.935, LSD (0.05) = 5.932 (Age of NSO); SED = 2.625, LSD (0.05) = 5.306 (Dosage of NSO); SED = 5.870, LSD (0.05) = 11.863 (Interaction).

with NSO than those untreated, and at the same time, the means were not significantly different amongst all NSO storage periods (0-15 years) at all applied dosages including 0.02, 0.04 and 0.08 ml/15 g grains. Altogether, these results suggest that NSO, either freshly prepared or stored for between 2 and 15 years can effectively manage *C. maculatus* infestation of cowpea grains, especially when treated with moderate and high concentrations of the product. These imply that the active pesticidal ingredient(s), triterpene/limnoids, in NSO remains potent even when prepared and stored for up to 15 years.

Earlier studies by Maina et al. (2011), Main and Lale (2005) and Maina et al. (2012b) respectively indicated that NSO could remain potent particularly against *C. maculatus* infestation on different cowpea cultivars following storage for 1-3 months, 7 years and 14 years. Lower percentage grain damage and severity of grain damage, as well as higher susceptibility index from cowpea grains treated with NSO and at moderate to high dosages were attributed to the oviposition deterrence/reduction, insect growth regulation, antifeedant and/or toxicity effects of NSO to the bruchids. The application of *A. indica* oil at low concentration of 0.1% (wt/wt) to wheat grain has for instance been reported to reduce egg laying by *Sitotroga cerealella* Oliver as effectively as a 5% malathion dust treatment (Verma et al., 1985).

Also, the use of *A. indica* oil at 8 ml/kg seed of cowpea and bambara groundnut reduced oviposition by *C. maculatus* and killed the larvae, whilst the activity persisted for more than 90 days on cowpea and 180 days on bambara groundnut (Pereira, 1983). Neem seed kernel oil applied straight or in mixture with cotton, groundnut, castor and desert dates, was found to even at very low rates of 0.5 ml/50 g cowpea grains reduce the number of eggs laid by each female *C. maculatus*, whilst no single egg was laid by the bruchids maintained on grains treated with the highest (4.0 ml) dosage of each oil combination (Yahaya, 2013). El Atta et al. (2011) likewise found that high dosages, 5 and 10% v/v, of neem seed kernel oil significantly reduced the ovipositioning of the tree locust, *Anacridium melanorhodon melanorhodon*

Walker, on gum Arabic, as well as reduced their feeding and molting, whilst the mortality of all developmental stages (larval instars 4, 5 and 6) of the pest increased. Low dosages of neem seed kernel oil, 0.5 and 1.0% v/v, however had no significant effect on the test parameters. Results of both El Atta et al. (2011) and Yahaya (2013) somewhat align with that from this study where higher concentration of the NSO gave better protection of crops against *C. maculatus*.

Besides having the ability to penetrate the chorion of bruchid eggs via the microphyle and cause the death of developing embryos through asphyxiation (Don-Pedro, 1989; Credland, 1992), the NSO could have been responsible for the death of some first instar larvae upon contact with the botanical after eclosion. Furthermore, NSO might have caused a reduction in bruchid feeding and/or disturbed the neuro-endocrine or other physiological system of the insects (Koul, 1996). Feeding of the larvae of Lepidoptera and Coleopteran pests, for example, have been shown to suffer impaired development under the influence of neem preparations (Saxena, 1993). Concentrations ranging from 0.001 to 0.4% of various neem seed kernel extracts have generally been found to deter feeding in some insect pests such as *Aphis* spp. and *Callosobruchus* spp. (Arora and Dhaliwal, 1994; Dimetry and Abd-El Salam, 2005, Dimetry et al. 2007; Sammour et al., 2011). Yadav (1973) also found that *A. indica* kernel powder protected legumes against *Callosobruchus chinensis* (Linn.) and *C. maculatus* infestation, and stopped the development of their progeny even 12 months after *C. chinensis* was released on treated grains. The powder of *A. indica* together with *Alstonia boonei* have further been shown to provide higher protection by exerting high mortality rates at 2.5, 5.0, 12.5 and 25.5% (w/w) concentration within 24 to 96 hours compared to the effects of *Garcinia kola* and *Moringa oleifera* powders (Ileke and Oni, 2011). The quality of NSO to keep for long periods of time without losing its biological efficacy, make the botanical reliable and convenient to use. In that, once its storage does not exceed 15 years, it can effectively protect stored commodities and would also not warrant frequent extraction of the oil for application as at when needed. Its

handling therefore would not require large space for storing huge quantities of neem seeds, which can be a very big challenge to resource-poor farmers. Altogether, these results should increase the popularity, desirability and adoption of NSO for use by farmers and grain merchant to economically protect stored grains.

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