

COMPARATIVE EFFICACY OF *Azadirachta indica* AND *Moringa oleifera* IN THE CONTROL OF *Callosobruchus maculatus* (F.) AGAINST STORED COWPEA (*Vigna unguiculata*)

Uddin II*, R. O. and Ibironke, T. O.

ABSTRACT

Department of Crop Protection, Faculty of Agriculture, University of Ilorin. Email:-ruddinll@yahoo.com; 234-803-3608948

Aqueous extracts of Neem (*Azadirachta indica*) and Moringa (*Moringa oleifera*) leaves and their mixtures in a ratio of Moringa/Neem (1:1); (1:3) and Neem/Moringa (1:3) were evaluated in the laboratory of the Department of Crop Protection, University of Ilorin for their efficacy in the control of the cowpea storage pest *Callosobruchus maculatus*. Cowpea seeds purchased from a local market were used for the study. The aqueous leaf extracts were applied to 18.6 g (100 seeds) of cowpea at the following rates, 0.5 ml; 1.0 ml; 1.5 ml and 2.0 ml. The experiment was conducted using a randomized complete block design. There were five treatments and each treatment was replicated 3 times. The effect of the aqueous extracts were assessed using adult mortality after 24 hours, oviposition at the 2nd and 4th day after treatment (DAT), number of larvae at the 20th and 22nd DAT, number of pupae at the 27th and 29th DAT and the F₁ progeny emergence at the 36th, 43rd, and 50th DAT. The data obtained were analyzed using the analysis of variance and the means separated using the Least Significant Difference. The results showed that the aqueous extracts were able to suppress oviposition, larval and pupal emergence and inhibit adult emergence of *C. maculatus*. The aqueous extracts at 2.0 ml had the best effect as it reduced oviposition, larval and pupal emergence and the F₁ progeny that emerged when compared to the control. All the treatments were effective in the control of stored cowpea seeds at 2.0 ml. The use of these treatments could be encouraged in the storage of cowpea seeds by resource poor farmers.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is one of the several species of the widely cultivated genus of the most important food legume crop in the semi-arid tropics covering Asia, Africa, Southern Europe, Central and South America (Scott, 2008). It is shade tolerant and hence makes it compatible for intercrop, thus making it an important component of traditional intercropping systems especially in complex and elegant subsistence farming system of the dry savanna in sub-Saharan Africa (Scott, 2008). Cowpea can be used at all stages of growth as a vegetable crop. It is very useful for both human and livestock consumption (Davies *et al.*, 1991). More than 5.4 million tons of dried cowpeas are produced worldwide, with Africa producing nearly 5.2 million tons. Nigeria, the largest producer and consumer, accounts for 61% of production in Africa and 58% worldwide. Cowpea is susceptible to attack by pests and diseases at all stages of its growth. One of the major constraints to cowpea production is insect pest infestation. These pests attack the cowpea plant during every stage of its life cycle.

Damage is done by several types of insect both on the field and in storage. Infestation by these pests begins on the field and rapidly builds up during storage causing substantial damage and post-harvest losses (Singh *et al.*, 1990). The cowpea seed beetle *Callosobruchus maculatus* F. is a multivoltine, principal storage pest of cowpea in the tropics and it is responsible for about 30-60% of the storage losses incurred on cowpeas stored for 3-6 months in Nigeria (Adedire and Ajayi, 2003). Caswell (1973) estimated that in Nigeria alone, the dry weight loss due to *C. maculatus* is approximately 70% of the annual production, while Huignard *et al.* (1985) stated that *C. maculatus* was responsible for 90% of the pod infestation in Nigeria. This high loss has contributed immensely to cowpea's secondary position in the farming systems of several countries in the tropics (Mortimore *et al.*, 1997).

Preservation of stored products from insect damage is primarily dependent upon continued application of liquid and gaseous insecticides (White and Leesch, 1995). Although it is efficient, the repeated use of insecticides for several decades has disrupted the biological control system by natural enemies and led to outbreaks of insect pests, undesirable effects on non-target organisms, environmental and health concerns (White and Leesch, 1995). Due to the dangers inherent in the use of chemicals such as environmental pollution, toxicity to mammals, hazard to the user and consumer (Alabi *et al.*, 2003), alternative control measures are being sought. The traditional cowpea farmers who are resource poor and can neither afford efficient and effective storage systems or the synthetic chemical insecticides used for the protection of the cowpeas from bruchid infestation and damage (Adedire and Ajayi, 2003), have resorted to using local traditional methods of grain storage which involves the use of plant parts or products in the storage of their grains. There is a tremendous wealth of traditional local knowledge on the use of plant materials in storage protection (Govindan *et al.*, 2010). Some of this knowledge has been neglected over past decades. However, there is an increasing interest and necessity to reactivate the knowledge (Stoll, 2000).

Karungi *et al.* (2000) reported the use of plant material extracts as an option to minimize the use of chemical insecticides in controlling field and storage pests of cowpea. Other studies have also shown that some botanical

products (bio-pesticides) such as tobacco, tagetes and tophrosia have good potential for controlling storage pests of cowpea. However extracts of some plants are toxic to some species of insect pests of crop and others are less toxic (Ivbijaro, 1983). These extracts with lethal activity on insects may be applied sole or in mixture with less toxic plant extracts to ascertain their complimentary or synergistic attributes in the management of crop pests. Therefore, it has become necessary to search for an option that can produce satisfactory result in a way that such option is not only acceptable to the farmers, but must also be feasible from a socio-economic stand point (Yusuf *et al.*, 2011). The aim of this study is to determine the efficacy of aqueous leaf extracts of neem and moringa in the protection of stored cowpea against *C. maculatus* in order to proffer a cheap and readily available alternative method for cowpea preservation during storage.

MATERIALS AND METHODS

Preparation of seeds and insect culture

The cowpea seeds used for the experiment were sourced from Ipata market, a local market in Ilorin. The seeds are white in colour with a smooth coat. Prior to the commencement of the experiment, all the seeds were placed in a freezer at 10°C for 96 hours in order to kill any insects present in the seeds. The seeds were later brought to room temperature and sorted to remove any dead insects, exuviae, frass or broken seeds. The resultant clean whole seeds were used for the experiment. The initial insect culture of *C. maculatus* was obtained from the Nigerian Stored Product Research Institute (NSPRI) Ilorin. More cultures were established from the initial stock culture in the laboratory by placing ten pairs of *C. maculatus* in 1lb Kilner jars (4 jars were used) containing Ife-brown cowpea seeds. Bandera and Saxena's (1995) technique for handling and sexing of bruchids was used for the experiment. Each kilner jar was covered with a piece of muslin cloth held in place with the aid of a rubber band. This was done to provide ventilation for the insect pest and to prevent their escape. The culture was maintained in the laboratory of the Department of Crop Protection at ambient temperature and relative humidity for the duration of the experiment. The freshly emerged F₁ progenies of *C. maculatus* from the cultures were used for the experiment.

Preparation of the plant materials

The fresh leaves of Moringa were obtained from a Moringa plantation at Tanke area, Ilorin, Kwara State while fresh leaves of Neem were sourced from the Faculty of Agriculture Pavillion, University of Ilorin. Five hundred grams (500g) of fresh leaves of Moringa and Neem were separately blended in the laboratory using a kitchen blender. The blended leaves were separately placed in a 3 litres capacity plastic container each, into which 1 litre of water was added. It was properly stirred and allowed to stand for 24 hours in the laboratory. The mixtures were sieved using a muslin cloth and the resultant extracts each of Neem and Moringa were then poured into separate plastic containers (3 litres capacity) and properly labeled. Different ratios of the extracts were also prepared i.e. Moringa/Neem in a ratio 1:1; Moringa/Neem in a ratio 1:3 and Neem/Moringa in a ratio 1:3. The treatments were applied at 0.5, 1.0, 1.5 and 2.0ml/100 seeds (18.6 g). Each treatment was replicated three (3) times.

Test method

The extracts were applied to the seeds in a plastic container (5cm x 3cm) and allowed to coat the surface of the seeds. The seeds were then air dried in the laboratory for 20 minutes. Four (4) freshly emerged adults of *C. maculatus* in a ratio 2:2 (2 males:2 females) were introduced into each replicate which were covered using baft cloth held in place with the aid of a rubber band to prevent the escape of the adult insects and to allow for proper ventilation. The replicates were properly labelled and left at room temperature and humidity. The experiment was laid out in a completely randomized design for 8 weeks and the following parameters were collected: counting of adult mortality of *C. maculatus* after 24 hours; oviposition of the cowpea beetle at the 2nd and 4th day after treatment (DAT); number of larvae at the 20th and 22nd day after treatment; number of emerged pupae 27th and 29th day after treatment and the number of F₁ progeny at the 36th, 43rd and 50th day after treatment.

Data analysis

The data collected had zero values. A value of 0.5 was added to all entries and transformed using the square root transformation. The transformed data were analyzed using the analysis of variance (ANOVA). Significantly different means at P=0.05 were separated using the Least Significant Difference (LSD) using the IBM SPSS statistic version 21.

RESULTS

All the treatments resulted in an increase in mortality of *C. maculatus*. Moringa/Neem mixture in a ratio 1:3 had the highest mortality (1.95) at 2.0 ml when compared to the other treatments a day after treatment (1 DAT). It was significantly different from the control but not significantly different from the other treatments (Table 1).

Table 1: Effect of aqueous extracts on mortality of adult *C. maculatus*

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	0.70a	1.34a	1.22a	1.16a	0.70a
0.5	1.34b	1.34a	1.34ab	1.46ab	1.34a
1.0	1.55bc	1.34a	1.34ab	1.46ab	1.46ab
1.5	1.67ab	1.55a	1.55ab	1.55ab	1.67cd
2.0	1.87c	1.67a	1.67b	1.95b	1.87d
LSD	0.34	0.18	0.52	0.19	0.41

Figures followed by the same letters in a column are not significantly different at $P < 0.05$

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

All the treatments at 2.0ml/100seeds had no eggs laid on the seeds at 2 DAT. (Table 2). This was significantly different from the control which had the highest number of eggs at 2 DAT. The trend was similar at 4 DAT (Table 3).

Table 2: Effect of aqueous extracts on oviposition of *C. maculatus* 2 Days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	1.56c	1.50c	1.34b	1.87b	1.77c
0.5	1.34bc	1.22bc	1.22b	1.34ab	1.22b
1.0	1.05ab	1.05ab	1.17ab	1.04ab	1.04ab
1.5	0.87ab	0.87ab	0.87ab	0.87a	1.04ab
2.0	0.70a	0.70a	0.70a	0.70a	0.70a
LSD	0.25	0.19	0.12	0.15	0.16

Figures followed by the same letters in a column are not significantly different at $P < 0.05$

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

Table 3: Effect of aqueous extracts on Oviposition of *C. maculatus* 4 Days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	1.95b	2.03d	2.03d	2.03c	1.87c
0.5	1.55b	1.67c	1.77cd	1.46b	1.67c
1.0	1.04a	1.22b	1.58bc	0.87a	1.22b
1.5	0.87a	0.87a	1.34b	0.70a	1.04b
2.0	0.70a	0.70a	0.87a	0.70a	0.70a
LSD	0.15	0.21	0.18	0.23	0.15

Figures followed by the same letters in a column are not significantly different at $P < 0.05$

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

At 20 DAT, all the treatments at 1.5 and 2.0 ml were able to suppress larval emergence and this was significantly different from the control which had the highest larval emergence (Table 4). The trend was similar at 22 DAT (Table 5).

Table 4: Effect of aqueous extracts on larval emergence 20 Days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	1.46b	1.34ab	1.58b	1.58b	1.46b
0.5	1.04a	1.04ab	0.87a	0.87a	1.22b
1.0	0.87a	0.87a	0.87a	0.87a	0.87a
1.5	0.70a	0.70a	0.70a	0.70a	0.70a
2.0	0.70a	0.70a	0.87a	0.70a	0.70a
LSD	0.17	0.17	0.15	0.16	0.13

Figures followed by the same letters in a column are not significantly different at $P < 0.05$

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

Table 5: Effect of aqueous extracts on larval emergence 22 days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	1.50c	1.34b	1.58b	1.50c	1.46b
0.5	1.22b	1.22b	1.04a	1.22b	1.04a
1.0	0.87ab	0.87a	0.87a	0.87a	0.87a
1.5	0.87ab	0.70a	0.70a	0.70a	0.70a
2.0	0.70a	0.70a	0.70a	0.70a	0.70a
LSD	0.16	0.13	0.16	0.10	0.17

Figures followed by the same letters in a column are not significantly different at $P < 0.05$

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

There was a total suppression of pupa emergence at 27 DAT at 1.5 ml and 2.0 ml rate of application for all the treatments. This was significantly different from the control at $P=0.05$ (Table 6). The trend was the same at 29 DAT (Table 7).

Table 6: Effect of aqueous extracts on pupal emergence 27 days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	1.22b	1.34b	1.34b	1.34b	1.58c
0.5	1.22b	1.22b	1.22b	1.22b	1.22b
1.0	0.87a	1.04b	0.87a	0.87a	1.04b
1.5	0.70a	0.70a	0.70a	0.70a	0.70a
2.0	0.70a	0.70a	0.70a	0.70a	0.70a
LSD	0.10	0.13	0.13	0.13	0.10

Figures followed by the same letters in a column are not significantly different at $P < 0.05$

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

Table 7: Effect of aqueous extracts on pupal emergence 29 Days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	1.22b	1.22b	1.22b	1.22b	1.34b
0.5	1.22b	1.22b	1.04ab	1.22b	1.22b
1.0	0.87a	0.87a	0.87ab	0.87a	0.87a
1.5	0.70a	0.70a	0.70a	0.70a	0.70a
2.0	0.70a	0.70a	0.70a	0.70a	0.70a
LSD	0.10	0.10	0.16	0.10	1.30

Figures followed by the same letters in a column are not significantly different at $P < 0.05$

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

All the treatments at 2.0ml completely suppressed F_1 progeny emergence of *C. maculatus* at 36 DAT (Table 8), 43 DAT (Table 9) and 50 DAT (Table 10) and it was significantly different from the control at $P = 0.05$.

Table 8: Effect of aqueous extracts on F_1 progeny emergence 36 Days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	5.07a	4.97c	4.66d	4.87d	5.23d
0.5	2.38c	2.59b	2.47c	2.46c	2.33c
1.0	1.55b	1.93b	1.55b	1.46b	1.67b
1.5	1.04a	1.09a	0.70a	0.70a	0.70a
2.0	0.70a	0.70a	0.70a	0.70a	0.70a
LSD	0.21	0.30	0.15	0.14	0.10

Figures followed by the same letters in a column are not significantly different at $P < 0.05$

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

Table 9: Effect of aqueous extracts on F₁ progeny emergence 43 Days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	6.74d	6.95d	6.60d	6.63d	6.67d
0.5	3.97c	3.97c	3.92c	3.84c	3.70c
1.0	2.59b	1.88b	2.84b	2.32b	2.49b
1.5	1.04a	0.87a	0.70a	0.70a	0.70a
2.0	0.70a	0.70a	0.70a	0.70a	0.70a
LSD	0.24	0.25	0.17	0.13	0.14

Figures followed by the same letters in a column are not significantly different at P< 0.05

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

Table 10: Effect of aqueous extracts on F₁ progeny emergence 50 Days after treatment

Concentration (ml)	Moringa	Neem	M/N	1:1M/N	1:3N/M 1:3
Control	4.55d	4.73c	4.80d	4.52d	4.73d
0.5	2.29c	2.53b	2.52c	2.39c	2.47c
1.0	1.95b	1.87b	1.85b	1.85b	1.77b
1.5	0.70a	0.70a	0.70a	0.70a	0.70a
2.0	0.70a	0.70a	0.70a	0.70a	0.70a
LSD	0.21	0.30	0.15	0.14	0.10

Figures followed by the same letters in a column are not significantly different at P< 0.05

M/N 1:1= Moringa/Neem mixture in a ratio 1:1; M/N 1:3= Moringa/Neem mixture in a ratio 1:3;

N/M 1:3= Neem/Moringa mixture in a ratio 1:3

DISCUSSION

The aqueous extracts were able to protect the cowpea seeds from attack and damage by the chrysomelid beetle, *C. maculatus*, with the treated seeds having the least number of emerged adults of *C. maculatus*. The aqueous extract treated seeds had fewer eggs laid when compared with the control. All the treatments were effective in the control of *C. maculatus* with 2.0 ml per 100 seeds being the most effective rate of application followed by 1.5 ml per 100 seeds. This is in agreement with the work of Makanjuola (1989) who stated that the production of eggs, hatching and adult emergence of *C. maculatus* were significantly reduced when raised on cowpea seeds treated with extracts of neem leaf. Similarly, Lale and Abdulrahman (1998) also reported a significant reduction in egg laying of *C. maculatus* on cowpea mixed with neem seeds. The efficacy of the treatments in the protection of stored cowpea is due to its ovicidal effect which led to a near total suppression of both larval and pupal emergence. Sadeghi (2006) demonstrated that an application of plant lectins on the surface of chicken peas dramatically reduced oviposition by *C. maculatus*. Lectin has not been reported on *Moringa oleifera* leaves (Makkar and Becker, 1996). That azadirachtin inhibits the release of prothoracicotropic hormones and allatropins, thereby affecting metamorphosis in insects (Schmutterer and Rembold, 1995) is well documented. It also alters the insects' behavior because of its antifeedant and repellent action, and it modifies insect development by inhibiting the release of prothoracicotropic hormones and allatropins (Williams and Mansingh, 1996). The study shows that pure neem and moringa extracts and their mixtures were also able to protect the cowpea seeds effectively from *C. maculatus* infestation. The moringa and neem extracts and the moringa/neem mixture in a ratio 1:3 applied at 2.0 ml/18.6g seeds were the most effective. This result is consistent with those reported by Aliyu and Ahmed (2006) who used groundnut extracts at the rate of 2-8 ml/kg of cowpea to treat cowpea seeds for storage. The efficacy of vegetable extracts against *C. maculatus* has been attributed to ovicidal effects of the extracts, reduced oviposition and reduced adult longevity. Lale (2002) stated that some essential extracts are highly lipophilic and therefore have the ability to penetrate the cuticle of the insect and that fatty vegetable extracts kill insects by flooding their spiracles thus causing asphyxiation. Fatty vegetable extracts are mainly active against eggs and so a uniform spread of the extract is necessary to maximize effectiveness.

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