Efficacy of *Moringa oleifera* leaf powder against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) on stored cowpea (*Vigna unguiculata* L. Walp)

Ojo J. A.[1*], Olunloyo A. A.[2] and Akanni E. O.[2]

[1]Department of Crop Production, Kwara State University, Malete, Kwara State, Nigeria
[2]Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria.
*jamesadebayoojo@gmail.com

Abstract: Cowpea is a very important grain legume that serves as a source of dietary protein for human consumption, however the grain is widely infested by a number storage insect pests among which *Callosobruchus maculatus* is a primary field to store pest causing considerably great losses to farmers. Laboratory study was conducted to assess the efficacy of *Moringa oleifera* leaf powder, used as protectant against *C. maculatus* F. infesting stored cowpea. The bioassays were conducted at six levels of concentrations (0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 g/20g seed) of test plant leaf powder and the untreated control (0g/20 g seed). All the different levels of concentrations considerably reduced oviposition and emergence of F1 progeny compared to the control. The mortality of the cowpea beetles was assessed at two days after infestation (2 DAI), 4 DAI, 6 DAI and 7 DAI and the results indicated that the mortality increased with increasing amount of *M. oleifera* leaf powder, with concentration of 2.0g /20g seed has significantly higher ($p > 0.05$) mortality compared to the control. Also, different level concentrations significantly protected the seeds in relation to seed damage and seed weight loss caused by *C. maculatus* compared to the control.

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Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of the most important grain legume in the farming systems of Nigeria and West Africa at large (Singh *et al*., 2002). It accounts for about 60% of human protein intake in Nigeria (Oparaeke, *et al*., 1998). The potential yields of improved varieties of cowpea can be as high as 3 tonnes per hectare, resource poor farmers who are the predominant growers of this important crop seldom harvest up to 500 kg per hectare of grain even when they grow such varieties. Farmers obtain low yields averaging 200 – 300 kg/ha in Nigeria (Alghali, 1992). Biotic factors are responsible for these losses in the field, most importantly insect pests infestations at all stages of the plant growth in the field and also in the store (Oparaeke, *et al*., 2004). *Callosobruchus maculatus* is a major primary pest of stored seeds of cowpea in Nigeria (Ofuya, 2001). Infestation usually starts from the field and continues in the store where the seeds are severely damaged, leading to reduced weight, low viability and poor marketability (Ofuya, 1986), these losses constitute a major threat to food security and availability. The common control method of storage pests is with synthetic insecticides such as pirimiphos methyl, fenithrithion, methyl bromide and phosphine fumigant (Cardona and Karel, 1990; Monford, 2006). Although these chemicals appear to be effective, their use is being discouraged due to associated human health and environmental problems such as pest resistance to insecticide, environmental pollution, high cost of purchase, non-availability as well as hazards to farmers (Talukder and Howse, 1995). These drawbacks have necessitated the need for sustainable alternatives that are easily biodegradable, environmentally friendly and safe to both producers and consumers (Ewete *et al*., 1996; Akob and Ewete, 2007). The challenge of finding a good alternative to replace these conventional insecticides has led to bio-prospecting for plants with natural insecticidal potency. Some plants which their parts like leaves, stems, roots have been used include *Azadirachta indica*, *Piper guineense*, *Allium cepa*, *Anethum graveolens*, *Senna species*, *Annona senegalensis* (Ewete *et al*., 1996, Ofuya, 2001) among others. The pulverized leaves of moringa have been found to be effective against storage pest like *Tribolium castaneum* (Herbst.) (Anita *et al*., 2012). This study seeks to assess the efficacy of powdered leaves of *Moringa oleifera* against cowpea beetle, *Callosobruchus maculatus* (F).

Materials And Method

The experiment was conducted in the Entomology Research Laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan at an ambient temperature of 27

± 2^0C, 70 ± 5% relative humidity and 12:12 hour photoperiod.

Source and Type of test cowpea seeds

Ife brown variety of cowpea was collected from the Seed Storage Section, Institute of Agriculture Research and Training (IAR&T), Moor Plantation, Ibadan.

Insect Culture

The cowpea seed beetle, *C. maculatus* used to establish the insect culture was obtained from infested cowpea seeds purchased locally from Bodija market in Ibadan, Nigeria, and the culture of *C. maculatus* was raised in the laboratory. Clean cowpea seeds (200g) were placed in 2 Litre (L)-capacity kilner jar to which 10 pairs of *C. maculatus* were introduced. The insects were allowed to oviposit for 5 days and then sieved out. The infested seeds were placed in the laboratory at ambient temperature and observed daily for emergence of adults. This culture was maintained and used as source of *C. maculatus*.

Preparation of powders

The *Moringa oleifera* leaves used for this study were collected and washed thoroughly, air-dried under shade for 21 days to ensure that volatile active principles are retained in the dried samples. Each was pulverized separately into fine powder in an electric grinding machine and then sieved through a 0.5 mm mesh. The pulverized sample was packaged in cellophane bags, labeled and kept in refrigerator at 5 – 8^0C for use in subsequent bioassays.

Bioassay with powdered moringa leaves

The milled sample of the moringa leaves kept in the refrigerator was used for the bioassay. It was used as direct admixtures to the cowpea at rates calculated on a weight of powdered materials per weight of grains (w/w) basis, to investigate the effectiveness of pulverized leaves of moringa on the mortality of adults, oviposition, emergence of F1 progeny, and level of damage incurred by *C. maculatus* on stored cowpea seeds. It was evaluated using the test plant at rates/concentration of 0 (control), 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0g/20g of cowpea. Twenty grammes of cowpea seeds was placed in four separate kilner jars and treated with the seven different rates of powdered plant materials including the control. Each kilner jar containing cowpea grains and moringa leaves powdered materials was shaken for even coverage of grains. Five pairs of 1-2 day-old adult cowpea beetles were introduced into each kilner jar and covered with a lid having mesh. The experiment was laid out in four replications and with the treatments arranged in a completely randomized design (CRD). Adult mortality counts were taken at 2, 4, 6 and 7 days after infestation (DAI). Insects were considered dead when they failed to respond to touch when the exposed abdomen was probed with camel hair brush. Dead adults were removed at each assessment, counted and recorded. Assessment on fertility of female or number of eggs laid was evaluated by taking the counts of the eggs laid on the grains for each replicate after 7 days of mating and oviposition. The grains were carefully placed back in the experimental jars.

After 7 days all adult weevils were removed and the kilner jars with grains left undisturbed, monitor until the emergence of F1 progeny. Data on F1 adult emergence was assessed from the commencement of adult emergence, emerged adults were removed, counted and recorded. The total number of emerged adults was calculated as a total number of adult that emerged, their means were calculated and compared among the treatments including the control. The data on emerged F1 progeny was analyzed using analysis of variance (ANOVA) and treatment means that show significant difference ($P \leq 0.05$) was separated using Least Significant Difference (LSD).

Assessment of damage to cowpea seeds

After all the adults had emerged, the numbers of damaged and undamaged seeds were counted, weighed and recorded. This was carried out by removing the grains in each treatment and separating them into damaged (grains with holes) and undamaged (grains without holes). Percentage damage was determined by a count of the damaged and undamaged seeds after visual examination and expressed as a proportion of the total number of seeds in each treatment.

$$\text{Damage \%} = \frac{\text{Number of seeds with holes}}{\text{Total number of seeds}} \times \frac{100}{1}$$

Seed weight loss was determined by taking the difference between initial and final weight of cowpea before and after the treatments. The data collected were corrected for moisture using an uninfested control set alongside the treatments (Ofuya, 1986; Ojo and Omoloye, 2012). The corrected values were expressed as proportion of the initial weight in percentage as expressed below:

$$\text{\%Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times \frac{100}{1}$$

Results

Effect of the moringa leaves powders on the mortality of adult *Callosobruchus maculatus*

The different concentrations of the test plants exhibited varying degrees of insecticidal activities killing adult *C. maculatus* more than the control ($p < 0.05$). There were significant differences in the mortalities of *C. maculatus* with treated experiment compared to the untreated control. Table 1 shows the percentage mortality counts of adult *C. maculatus* exposed to the different concentrations of the test

plants 2, 4, 6 and 7 DAI. At 2 DAI the highest dosage 2.0/20g seeds was the most effective as it caused 36.33% mortality which was significantly higher ($p < 0.05$) than what was obtained in other levels of concentrations. The same thing was observed at 4, 6 and 7 DAI, as the highest dosage caused 53.95%, 89.17% and 92.0% mortality respectively and these was significantly higher ($p < 0.05$) than the control 7.11%, 14.74% and 14.74% respectively. The higher the dosage of the moringa leaf powder applied, the higher the mortality regardless of the day after infestation. At 6 DAI, there was no significant different in the mortality rate between the concentration level at 0.2g, 0.4g, 0.6g and 0.8g/20g seeds, which were significantly higher than the untreated control. Also mean oviposition on seeds treated with 2.0g test plant (5.62 eggs) was significantly different ($p < 0.05$) from oviposition on the untreated control (14.95 eggs) (Table 2). There was no significant difference ($p > 0.05$) in the mean oviposition on seeds treated with 0.2g, 0.4g, 0.6g, 0.8g and 1.0g test plants.

Table 1:Mean percentage mortality of adult *Callosobruchus maculatus* at 2, 4, 6 and 7 days after infestation (DAI)

Doses of the powder (g)	2 DAI	4 DAI	6 DAI	7 DAI
0	0.00 ± 0.00	7.11 ± 0.00	14.74 ± 1.12	14.74 ± 0.08
0.2	31.24 ± 2.59	42.23 ± 3.92	59.92 ± 3.54	60.11 ± 2.42
0.4	31.22 ± 2.34	42.24 ± 2.75	60.11 ± 4.21	62.21 ± 3.47
0.6	32.14 ± 1.35	43.15 ± 3.95	62.03 ± 3.11	64.14 ± 5.62
0.8	32.22 ± 3.17	44.23 ± 4.96	62.95 ± 2.97	64.72 ± 6.34
1	33.34 ± 2.16	49.67 ± 3.17	66.12 ± 4.01	71.13 ± 2.81
2	36.33 ± 2.03	53.95 ± 2.58	89.17 ± 3.01	92.00 ± 0.02
$LSD_{(0.05)}$	6.4	4.9	2.4	3.9
CV (%)	7.89	8.59	6.95	7.91

Table 2: Mean oviposition count by adult female C. maculatus after application of powdered moringa leaves

Doses of the powder (g)	Number of eggs laid
0 (control)	14.95 ± 1.55
0.2	10.12 ± 0.82
0.4	8.41 ± 0.28
0.6	7.55 ± 0.29
0.8	6.00 ± 0.36
1	6.12 ± 0.35
2	5.62 ± 1.52
$LSD_{(0.05)}$	0.5
CV (%)	4.27

Table 3: Effect of powders of *M. oleifera* on the number of F1 progeny of *C. maculatus*

Doses of powder (g)	Mean number
0 (control)	59.2 ± 0.17
0.2	38.7 ± 0.11
0.4	39.1 ± 0.09
0.6	35.7 ± 0.14
0.8	33.1 ± 0.21
1	29.5 ± 0.26
2	20.3 ± 0.23
$LSD_{(0.05)}$	0.3
CV (%)	11.1

Table 4: Mean percentage seed damage after application of pulverised moringa leaves

Doses of powder (g)	Damage (%) ± SE
0 (control)	65.2 ± 0.11
0.2	50.2 ± 0.17
0.4	46.9 ± 0.32
0.6	42.5± 0.25
0.8	35.3 ± 0.41
1	30.3 ± 0.29
2	22.2 ± 0.23
$LSD_{(0.05)}$	0.4
CV (%)	12.2

Table 5: Mean percentage seed weight loss after application of pulverised moringa leaves

Doses of powder (g)	Weight loss (%) ± SE
0 (control)	4.9 ± 0.11
0.2	2.1 ± 0.27
0.4	2.0 ± 0.04
0.6	1.22± 0.17
0.8	1.11 ± 0.67
1	0.85 ± 0.27
2	0.35 ± 0.13
$LSD_{(0.05)}$	0.4
CV (%)	2.57

The effects of application of varying level of concentrations of the test plant on emergence of F1 adult of *C. maculatus* on cowpea seeds also showed

significantly ($p < 0.05$) varied effects (Table 3). The mean F1 progeny emerged on seeds treated with the highest dosage, 2.0g was significantly lower (20.3) than the control (59.2). There was significant difference ($p < 0.05$) between the treatment dosage at 2.0g (20.3) and 1.0g (29.5). The higher the dosage the lower the number of F1 progeny emerged. The mean percentages of damaged seeds differed significantly among the treatments (Table 4). The moringa leaves powders significantly reduced seed damage and weight loss caused by infestation of *C. maculatus*. The highest dosage had the lowest mean percentage seed damage (22.2%) compared to the control (65.2%) and there was significant difference ($p < 0.05$) between them. The higher the dosage, the lower the damage caused (Table 4). The concentration of the powders has a significant effect on reduction of weight loss of cowpea seeds. The highest dosage, 2.0g had the lowest weight loss (0.35%) followed by the 1.0g dosage which had the weight loss of 0.85% (Table 5). There was significant difference ($p < 0.05$) between weight loss obtained in the treated and untreated seeds.

Discussion

This study shows that the powder of *Moringa oleifera* leaf has insecticidal activity against *C. maculatus* and can be used for the control of this cowpea beetle. Moringa leaves powder, therefore conformed to the properties required in chemicals for controlling insect feeding on plants which include: toxicity to adults, reduction of oviposition, ovicidal activity and toxicity to immature stages prior to or immediately following penetration of plant tissues (Ogunwolu and Odunlami, 1996). The findings obtained in this study agreed with the earlier reports that powdered plant parts could adequately protect stored grains against storage insects (Paul *et al*., 2009). This study has shown that the leaf of *M. oleifera* is effective as oviposition deterrence. This is in conformity with earlier report by Meredia *et al*., 1992, who used neem kernel powder as oviposition deterrence and Anita *et al*., 2012 who confirmed that leaves of *Annona squamosa*, *Moringa oleifera* and *Eucalyptus globules* were effective as oviposition deterrence. In all the treatments, the moringa leaf powder at 2.0g/20g seed dosage or concentration had the highest potency of insecticidal properties, its effect was clearly observed on the mortality rate of the insect, ovipostion deterrence, seed weight loss and damage and even its suppressive effects on the emergence of F1 progeny. From the mortality studies, the insect mortality increased with increasing application rate or dosage. The percentage mortality obtained at 2.0/20g seeds application dosage was 89.17% at 6 DAI and 92% at 7 DAI, agreed with earlier report of Anita *et al*., (2012) that there was about 100% mortality at application rate of 2.0g/10g wheat at 9 DAI in grains treated with pulverized leaves of moringa.

The deterrence of oviposition and the reduction of adult emergence of *C. maculatus* by the moringa leaf powder were also concentration-dependent. Our finding that the powdered leaves of moringa plant caused significant reduction in the emergence of F1 progeny is an indication that the mode of action of the powder may not only be physical. This reduction in adult emergence could either be due to egg mortality, or larval mortality or even reduction in the hatching of the eggs. It has been reported that the larvae which hatch from the eggs of *Callosobruchus sp*. must penetrate the seeds to survive (FAO, 1999). The larvae would be unable to do so unless the eggs are firmly attached to the seed surface. It could also be as a result of feeding deterrence resulting in the death of the insects or ovicidal action leading to reduced progeny production as has been the case with other plant materials investigated (Tapondjou *et al*., 2002; Abdelgaleil and Nakatani, 2003) as it was also observed in this study. The reduction in seed weight loss and damage could be as a result of the reductive effect recorded in adult emergence. As the progenies emerged, more dead adult insects were observed than live ones which could be due to the bitter taste of moringa leaves and the biochemical constituents present which invariably deterred feeding.

This study shows that the leaf of *Moringa oleifera* is effective to protect cowpea seeds and other grains in the store, and the farmers can easily prepare it and apply it to protect their grains in the store. However, more study is required to ascertain the effectiveness of other moringa plant parts like roots, stem and stem barks, compare their effectiveness as a sole treatment and in combinations.

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References

1. Abdegaleil, S. A. M and M. Nakatani. Antifeeding activity of limonoids from *Khaya senegalensis* (Meliaceae). *Journal of Applied Entomology* 2003: 127,236-239
2. Akob, C. A. and F. K. Ewete. The efficacy of ashes of four locally used plant materials against *Sitophilus zeamais* (Coleoptera: Curculionidae) in Cameroon. *International Journal of Tropical Insect Science* 2007: 27(1): 21 – 26.
3. Alghali, A. M. Insecticide application schedules to reduce grain yield losses caused by insect

pests of cowpea in Nigeria. *Insect Scence Applied* 1992: 13: 725 – 730

4. Anita, S., Sujatha, P. and Prabhudas, P. Efficacy of pulverized leaves of *Annona squamosa* (L.), *Moringa oleifera* (Lam.) and *Eucalyptus globules* (Labill.) against the stored grain pest, *Tribolium castaneum* (Herbst.). *Recent Research in Science and Technology* 2012: 4(2):19-23
5. Ewete, F. K., J. I. Arnasan, J. Larson and B. J. R. Philogene. Biological activities of extracts from traditionally used Nigerian plants against European corn borer, *Ostrinia nubilalis* (H.). *Entomol. Exp. Appl.* 1996: 80: 531 – 537.
6. FAO. Botanical oils as grain protectants. In: The use of species and medicinal as bioactive protectants for grains. FAO Agricultural Services Bulletin, 1999: No.137. FAO, Vialledelle Terme di Caracalla, 00100 Rome, Italy.
7. Monford, K. G., Adabie – Gomez, D. A., Agyir – Yawson, A., Owusu – Biney, A. and Osae, M. Evaluation of four local plant species for insecticidal activity against *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Ghana Journal of Agricultural Science* 2006: 39: 147-154.
8. Ofuya, T. I. Use of wood ash, dry chilli pepper fruits and onion scale leaves for reducing *Callosobruchus maculatus* (F.) damage in cowpea seeds during storage. *Journal of Agricultural Science* 1986: 107: 467 – 468.
9. Ogunwolu, E. O. and Odunlami, A. T. Suppression of seed bruchid (*Callosobruchus maculatus*) development and damage on cowpea (*Vigna unguiculata* (L.) Walp) with *Zanthoxylum zanthoxyloides* (Lam.) Waterm. (Rutaceae) root bark powder when compared to neem seed powder and pirimiphos methyl. *Crop Protection* 1996: 15: 603-607.
10. Ojo, J. A. and Omoloye, A. A. Rearing the maize weevil, *Sitophilus zeamais*, on an artificial maize-cassava diet. *Journal of Insect Science* 2012: 12:69. Available online: insectscience.org/12.69
11. Oparaeke, A. M., Dike, M. C. and Amatobi, C. I. Field Evaluation of extracts of five Nigerian spices for control of post-flowering insect pests of cowpea, *Vigna unguiculata* (L.) Walp. *Plant Protection Science* 2004: 41(1): 14 – 20.
12. Oparaeke, A. M., Dike, M. C. and Onu, I. Evaluation of seed and leaf powders of neem (*Azadirachta indica* A. Juss) and Pirimiphos methyl for control of *Callosobruchus maculatus* (F.) in stored cowpea. *Entomological Society of Nigeria Occasional Publication* 1998: 31: 237 – 242.
13. Paul, U. V., Lossini, J. S., Edwards, P. J. and Hilbeck, A. Effectiveness of products from four locally grown plants for the management of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. *Journal of Stored Products Research* 2009: 45(2): 97-107
14. Singh, B. B., J. D. Ehlers, B. Sharma and F. R. Freire Filho. Recent progress in cowpea breeding. In: Fatokun, C. A., Tarawali, S. A., singh, B. B., Kormawa, P. M. and Tamo, M. (Eds.) Challenges and opportunity for enhancing sustainable cowpea production. *Proceedings of the World Cowpea Conference III held at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, IITA, Ibadan, Nigeria.* 2002.
15. Tanpondjou, L. A., C. Adler, H. Bounda and D. A. Fonlein. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectant against six stored product beetles. *Journal of Stored Products Research* 2002: 38, 395 – 402.

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