Gashua Journal of Irrigation and Desertification Studies
Available online at www.gjidsfugashua.org.ng
Volume 2, Issue 1

POTENTIAL OF LEAF POWDERS OF NEEM (*AZADIRACHTAINDICA* A. JUSS) AND MORINGA (*MORINGAOLEIFERA* LAM) IN REDUCING INFESTATION OF *CALLOSOBRUCHUS MACULATUS* (COLEOPTERA: BRUCHIDAE) ON STORED COWPEA

*** Robert, O.U. II. and Blessing, A. A.**
Department of Crop Protection, Entomology Unit, Faculty of Agriculture, University of Ilorin, PMB 1515, Ilorin, Kwara State
*E-mail of corresponding author: ruddinll@yahoo.com 08033608948

Abstract

A laboratory investigation was carried out in the Department of Crop Protection, Faculty of Agriculture, University of Ilorin to determine the effectiveness of *Moringa oleifera* and *Azadirachta indica* leaf powder in reducing oviposition, adult emergence and eliciting mortality on adult cowpea beetle *Callosobruchus maculatus* in stored cowpea. The experiment was laid out in a Completely Randomized Design with three replicates**.** There were three treatments namely; *A. indica* leaf powder, *M. oleifera* leaf powder and the control applied at the following rates 0.5g, 1.0g, 1.5g and 2.0g/20g of cowpea seeds. Twenty (20) grams of treated cowpea seeds were infested with 5 newly emerged adults of *C. maculatus*. Adult mortality, oviposition rate, larvae emergence, pupae emergence and F_1 progeny emergence were used to assess the effectiveness of the treatments. There was a 73.3% and 78.7% reduction in oviposition when cowpea seeds were treated with 2.0g of moringa and 1.0g of neem respectively. Treatments resulted in a 70% and 74% reduction in larvae emergence when seeds (20g) were treated with 2.0 g moringa and 1.5 g neem leaf powders respectively. Pupae emergence was reduced by 70% and 67% when 20g seeds were treated with 2.0g each of moringa and neem leaf powders respectively. F_1 progeny emergence was reduced by 76% on 37^{th} day by 2.0 g/20 g seeds of moringa and neem leaf powder. The higher dose of 2.0g/20g seeds was more effective in both botanicals in managing *C. maculatus* and can be used as an alternative in storage of cowpea seeds.

Keywords: Cowpea, mortality rates, oviposition

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an important pulse crop native to central Africa (Coulibaly, *et al*., 2002) The drier savannah and sahelian region of West and central Africa produce 70% of the world's cowpea (Langyintuo *et al*., 2003). Global production of cowpea is 5.5 million tons on 12.6 million hectares, with Nigeria producing 2.1 million tons on 3.7 million hectares annually (FAO, 2014). Cowpea is the most economically important indigenous African legume crop (Langyintuo *et al*., 2003). As a legume it can fix up to 88 kg of nitrogen per hectare (Fatokun, 2002) thereby helping to restore soil fertility. Trades in dry cowpea grain and cowpea hay are important to the

economy of West Africa in particular with substantial quantities of cowpea grain being traded at the local and regional levels (Singh, 2002).

The production of cowpea is hampered by several biotic and abiotic factors. Abiotic factors include poor soil fertility, drought, heat, acidity and stress due to intercropping with cereals (Singh and Ajeigbe, 2002), while the biotic factors are bacteria, fungi, virus, nematode, mites and insect pests (Singh, 2005). Cowpea is attacked by insects from the seedling stage up to the fruiting stage with the stored grains sustaining considerable damage. This damage to the seeds consists of complete or partial destruction of all but the outer shell (Giga and Smith, 1983). The infested seeds are covered with eggs and with holes made by larva of *Callosobruchus maculatus* which reduce the market value, seed weight, palatability and viability of attacked seeds (Singh and Rachie, 1985). *Callosobruchus* spp. can cause damage of legume seeds up to 100 % during storage (Gbaye *et al*., 2011). *Callosobruchus maculatus*, the cowpea weevil is the most important pest of cowpea (*Vigna unguiculata* L.) during storage (Edde and Amatobi, 2003). Attack of cowpea seeds in storage by *C. maculatus* can cause grain yield loss of up to 100%, particularly in those parts of Nigeria where adequate storage facilities and effective techniques of protection are limiting (Lienard and Seek, 1994).

Over time, the management of *C. maculatus* has depended on the use of synthetic pesticides which are not available, affordable and cause serious environmental issues. This coupled with the associated hazards to man and his livestock has necessitated the search for local and sustainable alternatives for use in the control of insect pests of cowpea in storage (Dick and Mbah, 1992). Presently some of these alternative's seems to come from natural plant products which are known to be of low mammalian toxicity and highly biodegradable (Kumar, 1991). Plant species that are used as botanicals usually possess one or more useful properties such as repellence, anti-feedants, fast knock down, broad spectrum of activity and ability to reduce insect resistance (Stoll, 1988).

The damage caused by *C. maculatus* is very high in cowpea production and steps must be taken to reduce the activities of this pest. In Nigeria, farmers use synthetic insecticides which are unaffordable coupled with the contamination of ground water, persistence of the synthetic pesticide, poor knowledge of application by illiterate farmers which leads to human and animal health hazards. This therefore necessitates the need for increased research on alternative pesticides which can be produced locally, afforded by majority of the farmers and environmentally friendly. This study aims to evaluate the potential of neem (*Azadirachtaindica* A. Juss) and moringa (*Moringaoleifera* Lam) based insecticide dust in the control of *C. maculatus* in stored cowpea in Ilorin. Dust are made up of a finely ground mixture of active ingredient combined with clay, talc, or other powdered materials. They are intended for dry use. The percentage of active ingredient in a dust is generally quite low and sizes of individual dust particles varies. They are used as seed treatments and insects ingest poisonous dusts during grooming or absorb the dust through their outer body covering. Most

are ready to use, with no mixing but easily drift off-target during application.

MATERIALS AND METHODS

Seed of cowpea variety TVX 3236 sourced from the International Institute of Tropical Agriculture (IITA) Ibadan was used for the study. TVX 3236 is oval in shape, cream in colour with a rough texture. It matures in 75-80 days and is thrip resistant. It is good for full season cropping especially in savanna regions and other semi-dry areas (Singh *et al*., 2011).

A stock culture of *C. maculatus* was obtained from the Entomology Department of the Nigerian Stored Products Research Institute (NSPRI), Ilorin, Kwara State, Nigeria located on latitude 8.4966 and longitude 4.5421. From this initial culture, new cultures of the insect were reared in the laboratory at ambient temperature (25-30^{0}C, 70-75% relative humidity) on a local susceptible variety of cowpea using five Kilner jars (454 ml). Covers of the jars were removed, replaced with muslin cloth held in place with a rubber band to allow exchange of gases. The culture was to provide a ready supply of *C. maculatus* for the experiment.

Plant based insecticides used were neem, *Azadirachta indica* (A. Jus.) and moringa, *Moringa oleifera* (Lam). Older leaves at the base of *A. indica* tree were obtained from Tanke (latitude 8.4813 and longitude 4.6115) in Ilorin, Kwara State, Nigeria, while fresh leaves of *M. oleifera* were sourced from a moringa farm in the same area during the wet season of 2015. Leaves of both *M. oleifera* and *A. indica* were air dried for seven days; the resultant dried leaves were pulverized using a mortar and pestle in the laboratory to obtain the powder. Pulverized tissues were sieved using a 3.35 mm sieve to obtain a fine powder. The powders were then separately stored in a polythene bag and kept on the laboratory bench until ready for use.

Seeds were stored in the refrigerator at 40^{0}C for 4 days to prevent the development of *C. maculatus*. Twenty (20) gram of cowpea variety TVX 3236 were weighed and placed in plastic containers' (5 x 3 cm dimension). Neem and moringa leaf powder were applied at the rate of 0.5, 1.0, 1.5 and 2.0 g/20 g cowpea seed in an experiment set up as completely randomized design, with the treatments replicated three times. A control treatment without plant leaf addition was also set along with the treatments. The plant powder was then properly shaken with the cowpea seeds in order to obtain uniform coating of the seeds with the powder. Thereafter five (5) newly emerged unsexed adult *C. maculatus* selected from the culture were then introduced into each of the replicates. The containers were then covered with muslin cloth to allow for exchange of gases and prevent the escape of *C. maculatus*. The insects were allowed to mate and oviposit for 7 days before removal.

Five seeds randomly selected from each container were opened with the aid of a small knife to observe and count the number of larvae and pupae on the 12th and 17th day after treatment respectively. Data was collected on the following parameters:-adult mortality (for 10 days after treatment (DAT)), oviposition rate (7th DAT), larvae emergence (12th DAT), pupae emergence (17th DAT) and F_1 progeny emergence (30-51st DAT). The data collected were subjected to analysis of variance (ANOVA) and mean separation was carried out using Duncan multiple

range test (DMRT) at 5% level of probability. SPSS (version 21) software was used for the analysis.

RESULTS AND DISCUSSION

Powders of *A. indica* and *M. oleifera* were effective in the control of *C. maculatus*. *Moringa oleifera* at 1.5 g/20 g seeds had, 2.0 mortality amongst the moringa treatments at 1 DAT and this was significantly different ($P<0.05$), while neem leaf powder at 2.0 g/20 g seeds was the most effective amongst the neem powder treatments in increasing mortality of *C. maculatus* (Table 1).

Table 2 shows that the two plant powders deterred oviposition by *C. maculatus* when compared to the number of eggs laid in the control treatment. Moringa leaf powder applied at the rate of 2.0 g/20 g of cowpea seed was found to be significantly different ($p<0.05$) from the other treatments and control, while treatment rates of 0.5 and 1.5 were not significantly different ($p>0.05$) from 2.0 g/20 g and 1.0 g/20 g but these were significantly different ($p<0.05$) from control treatment. Treatment with neem leaf powder causes fewer numbers of eggs to be laid by *C. maculatus* on the cowpea seed. All the neem leaf powder treatments were significantly different ($p<0.05$) from the number of eggs laid in the control treatment (Table 2).

Moringa leaf powder at 1.5 g/20 g seeds had the lowest larvae emergence (1.0) and this was significantly different ($P < 0.05$) from the control (3.3) (Table 3). While neem leaf powder at 1.5 g/20 g seeds was more effective (amongst the neem treatments) in reducing larval emergence (0.6) and this was significantly different from the control (2.3) (Table 3). The trend was similar for pupae emergence when cowpea seeds were treated with moringa. In the case of neem, 1.0 g/20 g seeds (1.0) was the most effective (amongst the neem treatments) in reducing pupae emergence and this was significantly different ($P < 0.05$) from the control (3.0) (Table 4).

The F_1 progeny emergence of *C maculatus* was highly reduced when moringa was applied at 2.0 g/20 g seeds of cowpea (Table 5) and this was significantly different from the control. While neem leaf powder applied at 0.5 g/20 g seed was the most effective in reducing the F_1 progeny emergence of *C. maculatus* and it was significantly different from the control (Table 5).

DISCUSSION

Azadirachta indica and *M. oleifera* leaf powders at 1.5 g/20 g and 2.0 g/20 g of cowpea seeds were effective in managing the population of *C. maculatus*. The increase in mortality could be as a result of toxicity of the powders or abrasive action of the powders against the cuticle of the insects. Furthermore, Kemabonta and Falodu (2013) and Denloye (2010) reported that the insect spiracles become blocked by the powders and their mortality occurs due to asphyxiation. This corroborates findings of Lale (2004) while working on aqueous extracts of A. indica, who reported that the resultant high mortality of adult C. maculatus on cowpea treated with A. indica could be attributed to the presence of azadirachtins, which is toxic to stored products insect pests.

There was a reduction in the oviposition rate of *C. maculatus* in this study, which might be due to unavailability of adults for mating resulting from increased mortality observed in this study. The neem and moringa treatments might also have acted

as deterrents to oviposition. Rajapakse (2006) reported that the mechanical effects of large quantities of plant powders, the active ingredients of the powders and physiological processes of the beetle could have effect on oviposition. Shumutterer (1990) reported that neem contain triterpenoids, azadirachtin and salannin which acts in insects as anti-feedants, growth and development regulators and even as toxicant. While the leaves of moringa provide a rich source of carotenoids, vitamins, minerals, amino acids, alkaloids, and flavonoids and a rare combination of phenolic compounds including zeatin, quercetin, kaempferol and apigenin (Ali *et al*., 2015).

The treatments also reduced larval, pupal and F_1 progeny emergence of *C. maculatus*. This observed reduction in the larval emergence, pupal emergence and F_1 progeny emergence might be due to the effects of the treatments on the developmental stages of *C. maculatus*. Adebowale and Adedire (2006) stated that coating of plant products on the seeds prevent eggs to attach firmly to the seed coat and hence inhibit larval penetration into the seeds which could account for the reduced larval emergence observed. Furthermore, Abdullahi *et al.* (2011) reported that coating can also prevent entry of oxygen to the developing stages and death occurs by asphyxiation.

Jacobson (1988) reported that azadirachtin causes growth inhibition, malformation, mortality and reduced fecundity in phytophagous insects and inhibition to reproduction in adult insects. These explains' the reasons why there were reductions in fecundity, larval, pupal and F_1 progeny emergence of *C. maculatus.* Furthermore, RMRDC (2004) reported that neem powder has more than 12-15 complex constituents that have repellent, antifeedant, insect growth regulatory and pesticidal properties. Prakash and Rao (1996) further affirmed that neem powder with azadirachtin as the main constituent is used as insect repellent, feeding inhibitors, growth retardants and sterilants, having both systemic and contact action on eggs and insects thereby destroying them.

CONCLUSION

The efficacy of *A. indica* and *M. oleifera* in managing *C. maculatus* showed that *they have* potential's for use as bio-pesticide in the control of *C. maculatus*.

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Table 1**:** Effect of moringa and neem leaf powder on mortality of *C. maculatus*

Treatment	DAT*									
(g/20g)	1	2	3	4	5	6	7	8	9	10
Moringa										
0.5	1.0^{ab}	1.0^{a}	1.0^{a}	0.3^{a}	0.0^{a}	0.0^{a}	0.3^{a}	0.3^{ab}	0.6^{ab}	1.0^{ab}
1.0	1.3^{b}	0.6^{a}	1.0^{a}	0.6^{a}	0.3^{a}	0.0^{a}	0.3^{a}	0.3^{ab}	0.0^{a}	0.3^{ab}
1.5	2.0^{b}	1.0^{a}	1.0^{a}	0.3^{a}	0.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.6^{ab}	0.3^{ab}
2.0	1.0^{ab}	1.3^{a}	0.3^{a}	1.0^{a}	0.3^{a}	0.3^{a}	0.0^{a}	0.3^{ab}	0.0^{a}	0.0^{a}
Control	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.3^{b}	1.0^{b}	1.3^{b}	1.3^{b}
SED	0.5	1.0	1.1	0.6	0.2	0.7	0.2	0.2	0.4	0.4
Neem										
0.5	1.0^{ab}	0.6^{a}	1.0^{ab}	1.0^{a}	0.3^{a}	0.0^{a}	0.3^{ab}	0.0^{a}	0.3^{a}	0.3^{a}
1.0	0.6^{ab}	1.3^{a}	0.6^{ab}	1.3^{a}	0.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{a}	0.0^{a}
1.5	1.3^{ab}	0.3^{a}	1.6^{b}	1.0^{a}	0.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{a}
2.0	1.6^{b}	0.6^{a}	0.6^{ab}	1.3^{a}	0.0^{a}	0.3^{a}	0.3^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
Control	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{b}	0.9^{b}	0.6^{a}	1.3^{b}
SED	0.6	0.7	0.4	0.7	0.2	0.7	0.1	0.1	0.2	0.2

Values in the same column not followed by the same letter are significantly different at P< 0.05

*DAT= Days after Treatment

Table 2**:** Effect of moringa and neem leaf powder on oviposition at 7 DAT

Treatment (g/20g)	Moringa 7 DAT*	Neem
0.5	2.3[ab]	2.0[a]
1.0	3.0[b]	1.0[a]
1.5	2.3[ab]	1.0[a]
2.0	1.6[a]	1.3[a]
Control	6.0[c]	4.7[b]
SED	0.1	0.1

Values in a column not followed by the same letter are significantly different at P< 0.05
*DAT= Days after Treatment

Table 3: Effect of moringa and neem leaf powder on larvae emergence at 12 DAT

Treatment (g/20g)	Moringa 12 DAT*	Neem
0.5	1.3[a]	1.0[a]
1.0	2.0[b]	1.0[a]
1.5	1.0[a]	0.6[a]
2.0	1.0[a]	1.0[a]
Control	3.3[c]	2.3[b]
SED	0.1	0.1

Values in a column not followed by the same letter are significantly different at P< 0.05
*DAT= Days after Treatment

Table 4: Effect of moringa and neem leaf powder on pupae at 17 DAT

Treatment (g/20g)	Moringa	Neem
	17 DAT*	
0.5	1.7[a]	1.6[a]
1.0	1.7[a]	1.0[a]
1.5	1.7[a]	1.3[a]
2.0	1.0[a]	1.0[a]
Control	3.3[b]	3.0[b]
SED	0.5	0.1

Values in a column not followed by the same letter are significantly different at P< 0.05
*DAT= Days after Treatment

Table 5: Effect of moringa and neem leaf powder on F_1 progeny emergence of *C. maculatus*

	Moringa				Neem			
Treatment	DAT*							
(g/20g)	30	37	44	51	30	37	44	51
0.5	6.3[a]	5.3[a]	5.6[b]	1.6[b]	2.6[a]	3.3[a]	3.0[a]	0.0[a]
1.0	4.3[a]	6.3[a]	3.3[a]	0.3[a]	4.6[a]	3.6[a]	3.6[a]	0.0[a]
1.5	4.0[a]	5.3[a]	2.6[a]	0.0[a]	3.0[a]	4.3[a]	3.6[a]	0.3[ab]
2.0	4.0[a]	3.6[a]	2.0[a]	0.0[a]	3.3[a]	3.0[a]	3.3[a]	0.0[a]
Control	15.0[b]	15.0[b]	10.0[b]	3.0[b]	10.3[b]	12.3[b]	12.0[b]	1.3[b]
SED	6.3	3.5	2.4	0.5	1.1	3.1	2.6	0.3

Values in the same column not followed by the same letter are significantly different at P< 0.05
*DAT= Days after Treatment