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Effects of *Moringa oleifera* root and leaf powder on reproductive capacity and damage caused on stored cowpea seed by *Callosobruchus maculatus* (F)

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A laboratory study was conducted to investigate the effect of Moringa (*Moringa oleifera*) root and leaf powder on reproduction and damage to cowpea seed by *Callosobruchus maculatus* F at Botswana College of Agriculture in Gaborone, Botswana, in March 2012. Two experiments were conducted to test the effects of moringa leaf and root powder on *C. maculatus* using susceptible cowpea variety blackeye. The root powder was applied at dosages of 10%, 15%, 20%, 25%, and 30% of 8.00g seed while leaf powder was applied at 10%, 15%, 20%, 25%, 30%, 35% and 40% of 8.00g of seed. Malathion was included in both experiments as a check, applied at dosage of 0.01g per kg of seed. The results showed that moringa root powder significantly protected cowpea seed against damage by *C. maculatus*. We observed significant reduction in number of eggs laid, percentage of eggs hatched percentage of adult emerged, number of exit holes and seed weight loss when seeds were protected by moringa root powder. Mixing seeds with leaf powder did not significantly affect the number of eggs laid, the number of hatched eggs, the number of adults emerged, the number of exit holes and percentage seed weight loss. It is therefore considered that based on the dosages used in this experiment, moringa leaf powder may not be effective in reducing seed damage by *C. maculatus.* In contrast, moringa root powder has potential to reduce damage by *C. maculatus* on stored cowpea seed.

Key words: Moringa, powder, *Callosobruchus maculatus*, (*Vigna unguiculata),* oviposition, exit holes

Introduction

Cowpea, *Vigna unguiculata* (L.) Walpers, is a staple grain legume of worldwide importance (Jackai and Adalla, 2003). Cowpea provides more than

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half of the plant protein are consumed by many poor people in the tropics and is a source of income. It is particularly important in West Africa with over 9.3 million hectares and 2.9 million tones annual production (Fatokun *et al.*, 2002). The dry savannas of west and central Africa account for about 70% of the estimated 3 million metric tons of annual global grain production (Singh, 1987). The crop is normally grown as a mixture with millet or sorghum and rarely grown as a sole crop because of production constraints, the most important of which are drought, parasitic weeds and pests (Fatokun *et al.*, 2002).

The cowpea weevil, *Callosobruchus maculatus* (F.), Coleoptera: bruchidae is considered a major insect pest for grain legumes in many tropical and subtropical regions (Singh, 1987). Annual losses associated with cowpea weevil in West Africa were estimated at more than $50 million (Shade *et al.*, 1986). In stored seeds, cowpea bruchid causes irreparable damage to the tissue, damage that can reduce nutritive value and quality of seeds for planting in many areas of developing countries. Within a few months of storage, losses of up to 100% may occur rendering the seeds unfit for human consumption due to fungal growth associated with increased temperatures in storage (Singh and Jackai, 1985). The damage is caused by larvae feeding and developing inside the seed and when adults emerge they leave circular exit holes (Davidson and Lyson, 1979).

A variety of pest management options are available to the cowpea farmers to reduce losses associated with cowpea weevil in Africa. However management of storage pests in cowpea seed heavily relies on the use of chemical insecticides (Jackai and Adalla, 2003). Over reliance on synthetic insecticides is not sustainable due to long term negative impact on the environment and the safety of food and livestock feed (Zettler and Cuperus, 1990; Silver, 1994).

The search for safer and cheaper alternatives to insecticides has continued over the years. Resource-poor farmers in Africa employ a range of traditional methods such as use of ash, sand dry pepper and botanical extracts. The naturally occurring plant products have been used to protect agricultural products against pests for many years in some parts of the world. Many authors have reported insecticidal effects of plant products against a broad range of pests. The use of plant product may offer a sustainable, environmentally friendly and safer alternative to synthetic insecticides.

Moringa (*Moringa oleifera*, Family: Moringaceae) is a multipurpose plant cultivated in tropical regions. Moringa tree has significant economic importance because there is several industrial and medicinal application and various products used as food and feed which can be derived from its leaves and fruits. Root, bark, pods and leaves of this tree are used in traditional medicine for the treatment of human diseases, whereby the leaves are enriched in vitamin A and C. Pods and young leaves of the plant are primarily used for vegetative purpose (Mughal *et al.*, 1999). Its various parts are identified for innumerable pharmacological properties viz. antimicrobial activity (Dahot, 1998), analgesic activity (Marugandan *et al.*, 2000) and antihypertensive activity (Dangi *et al.*, 2002). Moringa has an antimicrobial activity which can make it eligible to be used as an insecticide (Dahot, 1998).

The effects moringa leaf powder on *Tribolium Castaneum* (Herbst), no work have been directed towards the use of moringa leaf powder to control C. *maculatus* on stored cowpea seeds. The use of plant extract or powder can play an important role in developing an Integrated Pest Management (IPM) for control of C. *maculatus* in stored cowpea seeds. IPM is sustainable because it emphasizes the use of compatible multiple pest management tactics with limited use of synthetic pesticides.

In this study, the potential of moringa root and leaf powders for control of *C. maculatus* on cowpea were evaluated. Our prediction were that cowpea weevil reared on seed treated with moringa root and leaf powder would have lower oviposition, adult emergence and seed damage.

Materials and methods

Insect cultures and rearing

Experiment was performed in a laboratory using reared population of *C. maculatus* that was originally reared from susceptible seeds of cowpea variety blackeye collected from infested seeds provided by the Botswana College of Agriculture. The stock culture of *C. maculatus* was raised by placing about 100 unsexed adults on two liter jars three quarter full of disinfected blackeye seed, a commonly grown cowpea variety in Botswana. The jars were covered with open screw caps having muslin cloth to prevent bruchids from escaping. The beetles were allowed to mate for seven days at 25 to 28°C and 60 to 90% relative humidity and lay eggs after which they were removed.

Experimental procedure

The first generation of adults emerging from stock culture was used in this study. The 2 kg seed of blackeye were placed in a cold room (0-5°C) for 14 days to eliminate any possible contamination with insects. Before the beginning of the experiment seeds were stored in sealed plastic bags in the laboratory at room temperature for 15 days to condition them.

The evaluated materials were powder derived from Moringa leaves. A conventional insecticide Malathion (1.5% dust) was included in both experiments as a check and applied at dosage rate of 0.01g per kg of seed. The leaves and roots of moringa were ground to powder using mortar and pestle. The root powder was applied at dosage rates of 10%, 15%, 20%, 25%, and 30% of 8.00g of blackeye cowpea seed. In the other experiment using leaf powder the dosages were 10%, 15%, 20%, 25%, 30%, 35% and 40% of 8.00g of blackeye cowpea seed.

In both experiments, fifty seeds of locally grown blackeye cowpea variety which is susceptible to *C. maculatus* which were weighed and put in clear glass jars. In each glass jar a particular amount of Moringa leaf and root powder based on above dosages was added and thoroughly mixed with the seeds. All treatments in both experiments were replicated four times and arranged in completely randomized design in the laboratory. Two pairs (4 females and 4 males) of newly emerged (0-24 hrs.) adults from the stock culture were introduced into 50 seeds (8.00g) of each treatment. The insects were allowed to mate and lay eggs for 36 hours and removed thereafter to prevent further breeding. Temperature and relative humidity ranged between 25-28°C and 60-70%, respectively.

Data collection

The total number of eggs laid and number of eggs hatched were counted under dissecting microscope. After seven days unhatched eggs remained transparent and those hatched and from which larvae entered seed became opaque, thus helping in differentiating them when recording. After observation of the first beetle emergence, F_1 adults were removed daily; their number recorded and discontinued 90 days after initial oviposition (Ofuya and Credland 1995). Damage to seeds was scored by counting the number of adult exit holes per replication. Weight loss of cowpeas due to feeding by cowpea weevil was calculated as the difference between initial weight and final weight of seeds after adjusting for soil moisture changes.

Data analysis

All percentage data were transformed using the arcsine square root [arcsine √(percent x/100)] to stabilize variance. Counts of eggs and adult exit holes and seed weight loss were transformed using log (x+1) transformation. Data was analyzed using mixed model procedures (PROC MIXED in SAS (SAS Institute, 2003). Multiple comparisons were made on least square means.

All the comparisons were based on LSD and considered significant when $P \leq 0.05$.

Results

Leaf powder

The analysis of the data showed no significant effect of moringa leaf powder on reproductive parameters of the cowpea weevil and damage caused (Table 1). There was no significant difference in the number of eggs laid ($F_{8, 24}$ = 1.57; P = 0.1872), no of eggs hatched ($F_{8, 24}$ = 1.68; P = 0.1558), number of adult emerged ($F_{8, 24}$ = 1.72; P = 0.1448). Moringa leaf powder also did not show any significant effect on the number of exit holes ($F_{8, 24}$ = 1.68; P= 0.1547) and percentage seed weight loss ($F_{8, 24}$ = 1.54; P= 0.1956). The results showed that Malathion completely protected the seed from cowpea weevil infestation because no eggs were obtained (Table 1).

Table 1. Reproductive and developmental parameters of cowpea weevil at different treatments

Dosage	No eggs	% eggs hatched ±SE	% adult emerged ±SE	No holes
Control	13.75±0.41abc	42.13±25.15ab	17.13±7.10ab	7.00±0.37ab
Malathion	0.00±00c	0.00±00b	0.00±00b	0.00±00b
10%	24.00±0.38ab	71.91±23.97a	71.91±23.97a	18.00±0.35a
15%	0.75±0.15bc	8.33±.3.32b	8.33±.3.32b	0.25±0.08b
20%	21.5±0.08abc	81.97±7.37ab	75.62±4.243ab	14.75±0.14ab
25%	31.00±0.52a	46.00 ± 26.58a	43.09±24.92a	23.50±0.48a
30%	11.25±0.31abc	58.39±20.47ab	57.45± 0.03ab	6.75±0.28ab
35%	16.00±0.35abc	38.33±22.30ab	36.77 ±21.28	8.25±0.35ab
40%	19.00±0.37abc	48.72±28.15ab	43.91±25.43ab	10.50±0.39ab

Means with the same letter are not significantly different (P≤0.05 LSD).

The emerged number of adults was highest on 25% followed by 10% and the lowest was obtained from Malathion treatment. Like with other parameters (number of eggs and adult emergence) the hatched number of eggs was highest on 25% followed 10% dosage and the lowest was obtained from Malathion treatment (Table 1). The 25% dosage had the highest number of adult exit holes because it had more adults that emerged and 15% had the lowest number of exit holes. The number of holes depends on the number of adults that emerge. The highest weight loss was obtained from seed treated with 25% dosage followed by 10% and the lowest was from seed treated with Malathion (Fig. 1). Regression analysis showed no significant relationship between response variable (number of eggs, number of emerged adult, number of eggs hatched, number of exit holes and seed weight loss) (Table. 2). This confirmed that moringa leaf powder had no significant effect on reproductive capacity of *C. maculatus* and subsequent seed damage.

Table 2. The relationship between Moringa leaf powder and reproductive parameters of *Callosobruchus maculatus* and seed damage

Response	Intercept + SEM	Slope + SEM	R^2	P
Number of eggs	9.10±7.27	1.23+1.30	0.02	0.35
Number of hatched	7.63± 6.65	1.10± 1.18	0.02	0.36
Number of emerged adult	7.73±6.34	0.94±1.13	0.02	0.41
Number of exit holes	6.60±5.33	0.66±0.95	0.01	0.49
Percentage weight loss	1.34±1.02	0.14±0.18	0.02	0.42

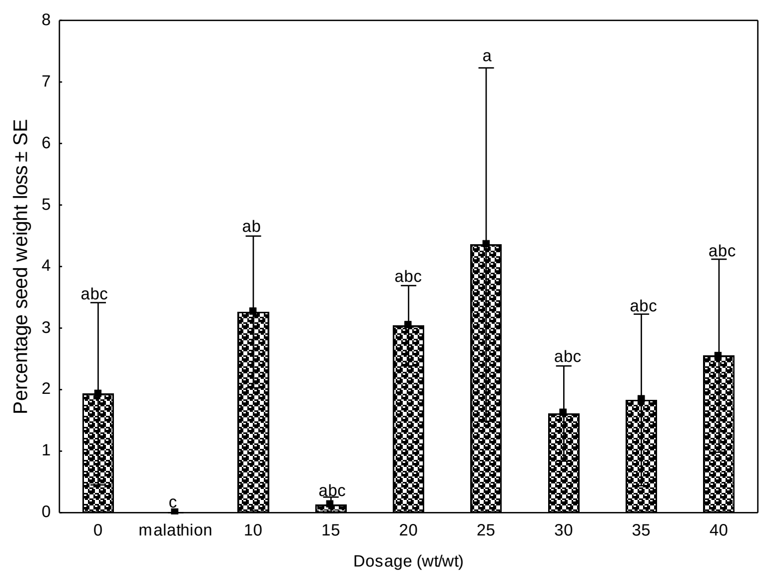

Fig. 1. Mean percentage seed weight loss of blackeye treated with deferent dosages of Moringa leaf powder. Means with the same letter are not significantly different (P≤0.05 LSD).

Root powder

Moringa root powder unlike leaf powder significantly affected the number of eggs laid ($F_{7,\ 24}$ = 4.32, P = 0.0032), number of eggs hatched ($F_{7,\ 24}$=3.55, P=0.0092), number of adult emerged ($F_{7,\ 24}$=3.72; P=0.0073), number of exit holes ($F_{7,\ 24}$ = 2.98; P = 0.021) but had no significant effect on percentage weight loss ($F_{7,\ 24}$ = 2.27; P = 0.069) (Table 1). The 25 and 30% dosage levels had significantly lower mean number of eggs laid compared to other dosages (Table 3). Mean separation showed no significant difference between 25%, 30 percent dosages and Malathion. A significant reduction in number of eggs that hatched was observed on 25 and 30 % dosage which was comparable to Malathion. Conversely the mean number of eggs laid, eggs hatched, adult emerged, number of exit holes were significantly higher on the control and the first three dosage levels (5, 10, 15%).

The number of adult exit holes was significantly lower on 20, 25, and 30% dosage levels and higher on the first three dosages (5, 10, 15%) indicating that the number of exit holes was significantly reduced by the treatment with moringa root powder (Fig 2). While mixed model analysis did not detect any significant reduction in percentage seed weight loss mean separation showed that the dosage level of 25 and 30% significantly reduced seed weight loss to a level comparable to Malathion (Table 3).

Table 3. The effect of moringa root powder on reproductive capacity of cowpea weevil and seed damage

Dosage level (wt/wt)	**No. Eggs ±SE**	**% eggs hatched ±SE**	**% adult emerged ±SE**	**% weight loss ±SE**
0 (control)	44.5±13.63a	53.96±5.30a	49.60±4.08 ab	7.96±3.22a
5	39.25±9.32ab	39.89±6.90a	29.82±6.76a	6.01±2.30ab
10	40.75±8.05ab	62.82±10.45a	61.15±11.40ab	7.00±2.24a
15	23.00±8.88abc	49.08±7.09ab	36.13±3.61abcd	5.25±2.17ab
20	21.53±3.43bcd	43.39±15.14ab	34.76±12.20abc	3.89±0.83ab
25	15.75±7.49cd	17.36±10.78bc	13.19±10.60cd	1.35±0.43b
30	7.50±2.53cd	38.46±19.14bc	28.86±17.40cd	2.91±1.80ab
Malathion	0.00±0.00d	0.00±00c	0.00±00d	0.79±.19b

Means with the same letter are not significantly different ($P \leq 0.05$ LSD).

Table 4. The relationship between Moringa root powder and reproductive parameters of *Callosobruchus maculatus* and seed damage

Response	**Intercept + SEM**	**Slope + SEM**	**R^2**	**P**
Number of eggs	50.54±6.15	-5.89+1.22	0.44	<0.0001
Number of hatched	25.32±3.17	-3.03±0.63	0.44	<0.0001
Number of emerged adult	22.27±2.92	-2.73±0.58	0.43	<0.0001
Number of exit holes	26.92±3.45	-3.19±0.68	0.42	<0.0001
Percentage weight loss	8.23±1.47	-0.85±0.29	0.22	0.007

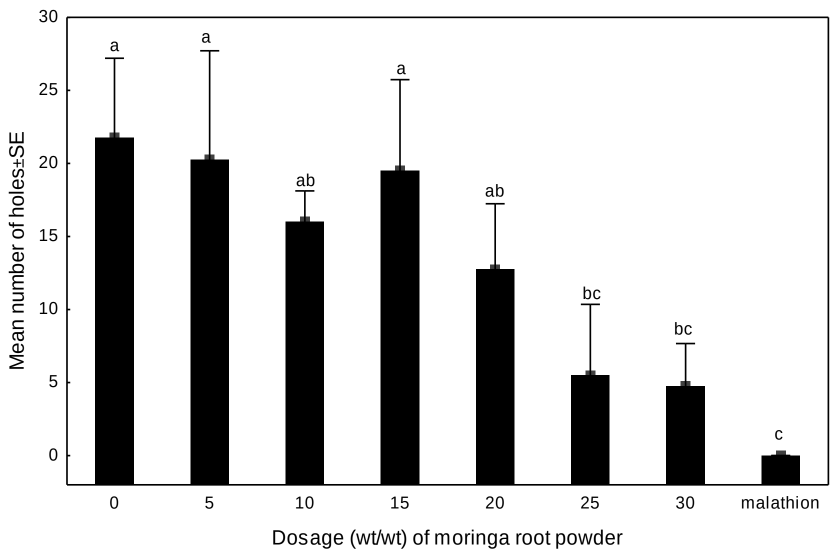

Fig. 2. Mean number of exit holes (±SE) on cowpea seeds exposed to different levels of moringa root powder.

The regression analysis showed that the response parameters (the number of eggs laid, number of eggs hatched, number of adult emerged, and the number of exit holes) decreased significantly with the increase in dosage level (Table 2). The analysis indicated that the Moringa root powder had significant effect on the reproductive capacity and damage to cowpea seed. An increase in the dosage of moringa root powder resulted in significant reduction in seed weight loss (Table 2). Moringa root powder accounted for more than 40% of variation in reproductive capacity of cowpea weevil.

Discussion

Leaf powder

As expected, Malathion provided a complete protection of cowpea against C. *maculatus*. The results showed that moringa leaf powder did not control damage caused by *C. maculatus* on cowpea. This was shown by no differences that were detected between untreated (seeds not mixed with powder) and treated seeds (seeds mixed with powder). A study done by Sadeghi *et al*. (2006) demonstrated that an application of plant lectins to the surface of chickpeas dramatically reduced oviposition by *C. maculatus*. Lectins had not been reported on *Moringa oleifera* leaves (Makkar and Becker, 1996). The lower levels of phenols on moringa leaves might explain lack of control of cowpea weevils on cowpea seeds in the current study due to reduce deterrent activities. However more detailed studies are required to confirm these observations.

The other possible reason for lack of control of cowpea weevil could be due to lower dosage of moringa leaf powder used in the current study. Previous reports using plant ashes have shown that higher doses of ashes (50-100%) mixed with cowpea seeds were most effective against cowpea weevil (Zehrer 1985; Dorlo *et al*., 1976; Wolfson *et al*., 1991) than lower doses. In an experiment using ash from leadwood tree (*Combretum imberbe* Wawra) provided better protection to cowpeas than lower doses. The effectiveness of the ash depended upon dosage of *C. imberbe.* In future studies, an increased dosage of moringa leaf powder could be tested.

Root powder

In contrast to leaf powder, moringa root powder provided significant seed protection against *C. maculatus* which was comparable to conventional insecticides Malathion. This was confirmed by significant reduction in seed damage and lower weight losses on seeds protected with higher dosages of root powder compared to untreated control. The reduction in seed damage was due to reduced oviposition and number of eggs that hatched. There was therefore reduced larval feeding on seed protected by higher dosages and consequently lower damage than seed not mixed with root powder.

The current results agrees with previously ones which showed that other plants extract and powders protected the stored seeds against storage pests. The moringa root powder therefore can serve as an alternative to insecticides which are too costly and not sustainable in the long run due to environmental contamination (Silver, 1994). The use of moringa would be cost effective and sustainable especially that the tree is easy to plant and grows very fast. Moringa tree is currently been introduced and widely adopted and can be planted in famers fields for harvesting the roots to treat cowpea seeds and leaves used for medicines and food and drinks and preparations. Treatment of cowpea seeds with moringa root powder can be incorporated into an Integrated Pest Management option for control of *C. maculatus.* Currently cowpea varieties with relative resistance to *C. maculatus* have been identified (Obopile *et al*., 2006). These varieties can be planted by famers to reduce damage by *C. maculatus* and when infestation is high they can be treated with moringa root powder thus reducing reliance on synthetic insecticide. This is plausible especially when cowpea seeds are stored for consumption because moringa is readily used as medicine and food (Mughal *et al.,* 1999). Therefore this implies that moringa root powder can be used as part of IPM program to positively manage the pests. Thus the role of moringa root powder in IPM has can be hailed as a worthy measure of pest control.

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