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Research article

Bioactivities of *Moringa oleifera* leaf powder towards the cowpea beetle *Callosobruchus maculatus* F. under laboratory conditions

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Key words: *Moringa oleifera, Callosobruchus maculatus* adults, insecticidal efficacy, biological efficacy, oviposition deterrency.

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Abstract

Potency of *Moringa oleifera* leaf powder was tested against the cowpea beetle *Callosobruchus maculatus* (F.) in a choice and non-choice tests. The insecticidal and the biological efficacy were tested against the adult bruchid stage. Orientation of the adult beetles towards cowpea seeds treated with moringa leaf powder is tested at different concentrations. The results obtained show that in the choice test, the adult beetles after one day preferred to stay in the control. After 3 and 6 days from treatment, adults could not discriminate between the treated and control seeds for orientation. On counting the number of eggs laid on cowpea seeds, it was found that as the concentration of leaf powder on the seeds increased, the number of eggs laid increased.
In non-choice experiment, mortality of *C. maculatus* increased significantly as the concentration of moringa leaf powder decreased to 0.1 gm. The total number of eggs laid decreased significantly as the concentration of moringa leaf powder decreased. On the contrary, at the highest concentration of moringa leaf powder, the number of eggs increased to 52.25±9.12 compared with 59.0±3.78 for the control. On calculating the percentage of egg oviposition deterrency, 0.1gm resulted in high percentage deterrence 95.76 % compared with 11.44 % on using 1.0 gm. Also, the percentage of adult emergence increased as the concentration of leaf powder increased. The foregoing results laid to the conclusion that Moringa leaf powder at the lowest concentration tested could be successfully used for the control of cowpea beetle under storage conditions.

Introduction

Moringa tree (*Moringa oleifera*) (Family Moringaceae) is native to India, Pakistan, Afghanistan and Bangladesh. It is also cultivated in Egypt. Moringa leaves, seeds, flowers and roots are used in different medicinal treatments. Oil that has been extracted from moringa seeds is used in Agriculture, foods, perfume and hair care [1]. Different parts of this tree contain a profile of various important minerals and are a good source of proteins, vitamin A, B, C, D & E and minerals which include potassium, calcium, iron, selenium and magnesium and various poly phenolic compounds [2] (Wikipedia, the free encyclopedia en. Wikipedia, org [3-5]. According to Madukwe *et al.,* (2012) [6] moringa leaves are completely safe for consumption and have no negative effect or toxic elements. The leaves are also rich source of essential amino acids such as methionine, cystine, tryptophan and lysine with a high content of proteins [7]. Moringa leaves are a good source of natural antioxidants [8]. In spite of all the benefit uses of moringa leaves, its effect in pest control is meager and scanty, with the exception of the work of Butani and Verma (1981) [9] who recorded different pests attacking drumsticks tree and it is also used for pest control [10-11]. Cowpea (*Vigna unguiculata* (L.) always suffer postharvest losses both in the field and under the storage conditions after harvest [12-13]. Reduction in the yield caused by insect pests can reach as high as 95 % if not controlled and depends on location, year and cultivar [14]. Several species of bruchids have been mentioned as pests of cowpea [15]. *Callosobruchus maculatus* Fabricius is the most serious pest of stored cowpea crop "*V. unguiculata* (L.) Walp in Egypt and throughout the tropics [16, 10]. Infestation often occurs in the field when pods are near maturity. After the crop is harvested, the bruchids multiply and do considerable damage to stored cowpeas [17].
The use of synthetic chemicals in pest control is the cause of much concern owing to the undesirable side effect emanating from their uses [18]. Also, they are expensive, polluting and potentially dangerous. Their repeated uses for several decades has disrupted biological control system by natural enemies and led to outbreaks of secondary insect pests. Insecticides especially the dust and gaseous forms of methyl bromide and phostoxin are recommended for storage. Methyl bromide is prohibited and phostotoxic a fumigant that kill humans and animals [19]. Fumigation is the most effective method in insect pest elimination in stored products. Currently phosphine and methyl bromide are the two common fumigants used

for stored product protection world over. However, because of its ozone depletion potential, methyl bromide is being phased out completely [20]. Therefore, it is very important to search for safe, cheap and effective method of pest control. Green plants and their extracts, also essential oils extracted from plants are safe alternative for pest control which are mammalian non toxic and easily biodegradable in comparison to chemical pesticides. The use of moringa for controlling insect pests during storage has not been studied in Egypt according to the best of our knowledge. The objective of the present study is directed towards evaluating repellency, insecticidal and biological efficacy of moringa leaf powder for the control of the cowpea beetle in choice and non choice tests under laboratory conditions.

Experimental

Materials and methods

Insect colony

The cowpea beetle, "*C. maculatus* (F.) was reared under laboratory conditions (27±2ºC. and 65- 75 % R.H. and a photoperiod of 16: 8 L :D for several generations on cowpea seeds in glass containers of 1 liter capacity, each containing about 200 gm of cowpea seeds and covered with muslin. Cowpea seeds were heated in an oven at 65 - 75ºC for five hours to kill the microorganisms or any other form of pests before starting any experiment.

M. oleifera leaves were kindly obtained from Prof. Dr. Abou El Fetoh Prof. at Technology and Horticulture Crop Department, NRC, Cairo, Egypt. The leaves were washed thoroughly and left to dry under laboratory conditions. The leaves were crushed and grinded completely to be fine powder

Choice experiments

A glass arena (3.5cm high X 21.0 cm. in diameter) was divided into two equal parts by using hard card board sheet put inside the arena with an opening in the middle to give free movement for the adult beetles to choose the proper part of the Petri dish to rest and laid eggs according to Jermy (1966) [21] and Dimetry *et al.* (2017) [22] with some modifications. Equal quantities (10 gm) of clean cowpea seeds without any treatment as well as 10 gm cowpea seeds mixed thoroughly with moringa leaf powder were put each in each side of the arena using different concentrations of moringa leaf powder. The whole experiment lasted 6 days and the concentrations of the powder used are 0.1, 0.2, 0.3 and 0.5 gm. To assess the preference of adults, two pairs of *C. maculatus* about 1–2 days old were put in the centre of each arena and given the choice between the two different parts either treated seeds or clean seeds without any treatment. The orientation preference of the adult beetles was recorded after 1, 3 and 6 days. If the adult was not recorded on the seeds on the different recording time, it was recorded as a non choice and was not selected in determining the preference. Four replicates were done for each concentration. The data obtained determined the relative percentage repellency and it was calculated according to Nerio *et al.* [23].

% repellency according to Nerio *et al.* [23].

$$PR = (NC - NT / NC+ NT) X 100$$

Where NC= the number of adults on the control (not treated seeds after exposure interval)

Nt= the number of adults of treated seeds after exposure interval

Also, the number of dead insects was recorded in each partition of the Petri dish. After seven days, all the beetles were removed and discarded and the number of deposited eggs on treated and untreated seeds was recorded and followed up till the emergence of the adults from infested seeds.

Non- choice test

Pesticidal efficacy

To test the toxicity of the different concentrations of moringa leaf powder, newly emerged ten adult beetles were placed into a glass cup 120 ml with 10 gm cowpea seeds each mixed thoroughly with the tested concentrations (0.05, 0.1, 0.2, 0.3, 0.5 gm.) Another group of beetles were placed into a glass cup 120ml together with 10 gm of cowpea seeds free from any treatments. The mortality of adults were recorded after 72 hours and corrected according to Abbott's formula (1925) [24]. The LC_{50} was calculated through probit analysis after Finney (1971) [25].

Biological efficacy

Newly emerged *C. maculatus* beetles (male and female) were placed into a cup 120 ml. with 10 gm cowpea seeds mixed thoroughly with the different tested concentrations separately. Total number of eggs laid was counted daily till seven days. The laid eggs were separated and incubated under the previously mentioned conditions until the emergence of the adults from the infested seeds. The percentage of adult emergence was recorded at the different concentrations of moringa leaf powder used and compared with that of the control without any treatment. The percentage of deterrence of egg deposition was calculated according to the formula of Lwande *et al.* [26].

D= (1 –T/C) X 100) where T and C represent the mean number of deposited eggs per female of the treated and control individuals. Four replicates were done for each concentration.

Statistical analysis

Abbott's formula [24] was carried out to correct the percentage of mortality. Data were subjected to analysis of variance (ANOVA) using SPSS computer program;

means were compared using Duncan's Multiple Range Test. Analysis of T-test was applied through the SPSS Computer program to discriminate between treatments and/or control. The LC values were calculated according to Finney (1971) [25].

Results and discussion

Choice test

The results obtained in Table 1 show that *C. maculatus* adults can't discriminate between treated and untreated seeds after the different periods studied 3 & 6 days for the different concentrations of moringa leaf powder. However, at the concentrations of 0.1gm moringa leaf powder, the beetles preferred to settle on the control seeds where 40 beetles were directed to the control seeds one day after treatments and only 24 adults settled in the treated section. After 3 days more than 50 % of the adult beetles were directed to the treated cowpea seeds. After 6 days, the beetles could not discriminate between treated or control seeds where nearly equal number of the beetles was found in the two sections of the arena (Table 2). This may be due to the presence of different nutritive substances like amino acids, anti-oxidants and many vitamins in the dried moringa leaf powder and also may be due to the variation of seed texture owing to the presence of moringa powder covering the treated seeds. On calculating the percentage of repellence of the adult bruchid beetles towards the treated seeds, the adults show very low percentage of repellency (7%) after the sixth day from seed treatments. After the third day from seed treatment, no repellency was present and the adults could not discriminate between treated and control seeds. After the first day from treatment only 25 % of the adults were repelled from treated seeds.

Concerning the mean number of eggs laid per two females on the different seeds either treated with the different concentrations of moringa leaf powder or control seeds without treatment, maximum number of eggs were found on seeds treated with the highest concentration of leaf powder (0.5gm) 32 eggs compared with 14.75 eggs on using 0.1 gm leaf powder (Figure 1). However, comparing the number of eggs laid in the treated section of the arena and the control section seeds without treatment the results show great reduction in the number eggs in the treated section for all the concentrations used compared with control section. This is to ascertain the previous data about the orientation of the adults. From the foregoing results, it could be concluded that decreasing the concentration of leaf powder to 0.1gm proof to be the least suitable for laying eggs in comparison to the biggest concentration of moringa leaf powder (0.5 gm) were found to be the most suitable to lay eggs on. The present findings are in agreement with Madukwe *et al.,* (2012) [6] who reported that moringa leaves are completely safe for consumption and have no known negative side effects or toxic elements. This statement clarifies why *C. maculatus* adults prefer to settle on cowpea seeds treated with the highest concentration (0.5gm) of moringa leaf powder covering cowpea seeds. Again, the texture of the cowpea seeds covered with the biggest concentration of moringa leaf powder may be a preferable surface for the adults to lay their eggs on.

Using different concentrations of Moringa leaf powder mixed thoroughly with cowpea seeds, the female beetles lay eggs normally on these seeds and as the concentration of the leaf powder increased on the seeds, *C. maculatus* female laid eggs normally on these seeds. On increasing the concentration of moringa leaf powder gives significant protein against oxidative damage molecules and increasing the concentration of moringa leaf powder to 0.5gm, the female bruchid laid the biggest number of eggs (32 eggs) compared with 14.75 eggs only on using 0.1 gm leaf powder of moringa. The leaves of *M. oleifera* have been reported by Loebel (2002) [27] to demonstrate antioxidant activity due to its high amount of poly phenols. It prevents oxidative damage to major bio-molecules and gives significant protein against oxidative damage. This explains why the cowpea beetle laid maximum number of eggs on using the highest concentration of moringa leaf powder (0.5 gm /10 gm seeds). The results of the present findings are in agreement with Shazal (1990) [28] who stated that the relationship between pest number and damage is necessary as bases for pest management.

Table 1. Distribution of *Callosobruchus maculatus* adults in treated and control sections under laboratory conditions (28±0.5°C., 70 – 75% R. H. & 16: 8 L:D Photoperiod).

Concentrations of leaf powder in gm	After one day				After 3 days				After 6 days			
	Treated		Control		Treated		Control		Treated		Control	
	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live
0.1	0	6	0	10	7	2	7	0	1	0	1	0
0.2	3	5	1	7	4	3	2	3	1	3	0	2
0.3	0	3	0	13	6	3	2	5	0	3	1	4
0.5	2	5	0	9	2	3	2	7	3	1	6	0

Table 2. Total orientation of adult beetles *C. maculatus* in treated and control sections of the arena.

Adults	after one day		After 3 day		after 6 day	
	T	C	T	C	T	C
Live	19	39	11	15	7	6
Dead	5	1	19	13	5	8
Total	24	40	30	28	12	14
% repellency	25		-3.45		7.69	

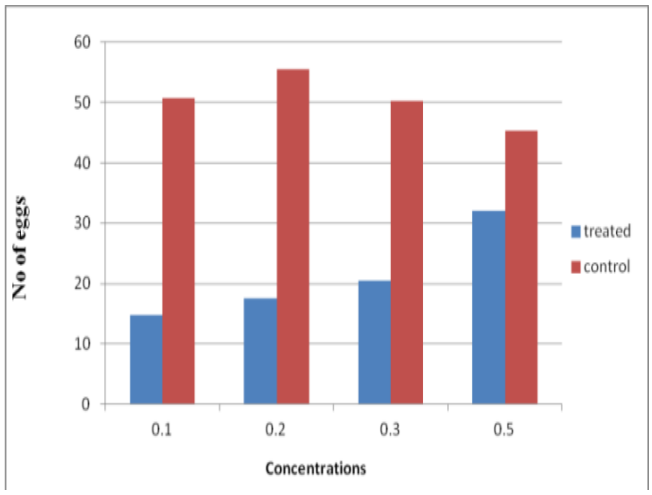

Figure 1. Mean Number of deposited eggs per two females

Non- choice test

The pesticidal activity

The results obtained in Table (3) show that the mortality of *C. maculatus* adults was maximum 93.33% after exposure period of 72 hours on using cowpea seeds treated with the lowest concentration of moringa leaf powder (0.05). This data are in consistent with Joana and Daniel (2010) [29] who concluded that pure neem oil, mixture of neem and moringa oils could be used for the preservation of cowpea. However, if only moringa oil is to be used, the concentration should not be less than 1.5 ml/ 200 gm cowpea. Increasing the concentration of leaf powder to 1% decreased the mortality of the adults to 61.59 %. The mortality of the beetles on the control seeds was 7.77 %.

All the data obtained are statistically significant. Susceptibility to insecticides may vary in different arthropod species as a result of physiological selectivity. This type of selectivity is rare and derives from excretion mechanisms, detoxification processes, penetration, rates or variations in enzymes that are inhibited by the active ingredients of an insecticide [30]. The difference in the susceptibility is likely to be based on mobility. *C. maculatus* adults were mobile and showed an active searching behavior when they were exposed to plant surfaces, thus they came into contact with the lower doses of insecticide residue than the relatively static.

Table 3. Mortality % of adult beetles *C. maculatus* on using different concentrations of *M. oleifera* leaf powder under laboratory conditions.

Concentrations of moringa leaf powder in gm.	Mortality %
0.05	93.33±4.08 a
0.1	83.85±4.36 ab
0.3	72.38±5.99 bc
0.5	65.53±7.25 c
1	61.59±7.58 c
Control	7.77±2.96 d
F	31.680**
Sig.	0.000

**Highly significant * Significant

Means in a Colum followed with the same letter(s) are insignificantly different at 5% level of probability

Based on LD_{50} values, the data obtained in Table (4) show that LD_{50} increased to 2.54 gm compared with 0.05 gm responsible for LD_{90} values So moringa leaf powder at the highest dose used (0.5gm/10 gm cowpea seeds) acted as attractant for *C. maculatus* adults to lay eggs on and could not be used as an insecticide. The foregoing results are in agreement with the data mentioned by Adenekan *et al.,* (2013) [11] who stated that *M. Oleifera* leaf and stem powder recorded 85 and 70 % insect mortality

Table 4. LD_{50} and LD_{90} of *M. oleifera* leaf powder for *C. maculatus* adults after 72 hours from treatment.

LD	Dose	Lower limit	Upper limit
	gm	gm	gm
50	2.5428	1.1733	19.2242
90	0.058	0.0103	0.115
Slope	-0.7807+/- 0.1923		
p	0.1684		

Biological activity

The results obtained in Table (5) show that the different biological aspects of *C. maculatus* adults given treated cowpea seeds with moringa leaf powder at different concentrations were greatly affected. The highest number of eggs laid was on cowpea seeds treated with 1% concentration which was insignificant in comparison with the control seeds without any treatment. This results may be due to the difference in the seed pod wall toughness

resistance to bruchid. Owusu-Akyaw [31] agreed with the present findings and stated that cowpea variety with a tough pod wall suffered the least damage. Also, Adjadi *et al.*, [32] reported that two recessive genes are required in the homozygous conditions to give resistance to bruchid. On the other hand, cowpea seeds treated with the lowest concentration of moringa leaf powder, there was a sharp reduction in egg deposition and the adults laid the least number of eggs (2.5) compared with 59 eggs for the control. This means that the number of eggs laid increased significantly as the concentration of moringa leaf powder increased. The preference for egg deposition of the cowpea beetle could be arranged in a descending order as follows: Control > 1 > 0.5 > 0.3 > 0.1 % concentration. This condition can be explained by the fact that using the lowest concentration of moringa leaf powder caused deterrence of the females to lay eggs on. These results are in accordance with that reported by Lopez *et al.* (2008) [33] who stated that moringa leaf powder exhibited volatile toxicity to *C. maculatus* beetles. On the other hand, highest concentration of moringa leaf powder attracted the female bruchid to lay eggs. The percentage of deterrence of egg deposition show that the least concentration used (0.1) gave the highest percentage deterrence for the adults to lay eggs (95.8 %) compared with the least percentage of deterrence on using the highest concentration of moringa leaf powder (1%) where the percentage repellency was 11.44 % only. The present findings were in agreement with Eleirt *et al.*, (1980) and Madukwe (2012) [34, 6] who pointed out that *M. oleifera* can be used as biopesticide and moringa leaves are completely safe for consumption and have no known negative side effect or toxic elements.

Table 5. Effect of different concentrations of *M. oleifera* leaf powder covering cowpea seeds on oviposition of adult beetles under laboratory conditions.

Concentrations of moringa leaf powder in gm.	Mean No of eggs laid/ female	D=100x(1-T/C)
0.1	2.5±0.29 c	95.76
0.3	13.67±3.18 bc	76.84
0.5	29.3±10.71 b	50.28
1	52.25±9.12 a	11.44
Control	59±3.78 a	
F	16.736**	
Sig.	0.000	

**Highly significant * Significant
Means in a Colum followed with the same letter(s) are insignificantly different at 5% level of probability

The Percentage of adult emergence (Table 6) increased as the percentage of moringa leaf powder increased. This result may be due to the fact that hatched larvae inside the treated seeds fed regularly and benefit from the nutritive substances from vitamins and amino acids present in moringa leaf powder. These results are in agreement with Loebel, 2002 [24] who stated that moringa tree has indigenous source of highly digestible protein, calcium, iron, vitamin C and carotenoids.

Table 6. Effect of different concentrations of *M. oleifera* leaf powder on the percentage of emergence of *C. maculatus* adults under laboratory conditions.

Concentrations of moringa leaf powder in gm.	% emergency
0.1	70.8±10.49 b
0.3	91.6±6.01 a
0.5	93.097±1.67 a
1	95.35±0.8 a
Control	98.41±0.45 a
F	4.417
Sig.	0.016

On using cowpea seeds treated with moringa leaf powder at 0.1gm, the percentage of adult emergence decreased and reached the lowest value compared with the control seeds. In this case, the nutritive substance present in moringa leaf powder is not sufficient for the development of the larvae inside the seed and the secondary plant substances present in the powder act as botanical insecticide and this is responsible for the less percentage of adult emergence compared with the control.

Conclusion

Using different concentrations of moringa leaf powder as a botanic insecticide for the control of *C. maculatus* adult under the laboratory conditions show that very low concentrations between 0.05 and 0.1 gm / 10 gm cowpea seeds were efficient to decrease the number of eggs laid as well as decreasing the percentage of adult emergence in the next generation. This is in accordance with Isman (2000) [35] who stated that botanicals have wide range of bioactivity and possess contact and fumigant toxicity and repellent oviposition and feeding deterrence. Accordingly, it can be used successfully in IPM program.

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