# BIOLOGICAL RESPONSE OF COWPEA BRUCHID, CALLOSOBRUCHUS MACULATUS (FAB.) (COLEOPTERA: BRUCHIDAE) TO COUMARIN EXTRACTS

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**Abstract**

Cowpea beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae), is the most important storage pest of cowpea. The effect of ethanol and chloroform extracts of murraya, kumquat and celery plants on the various biological aspects of Cowpea beetles was studied. Kumquat, ethanol extract was most efficient extract which affected significantly the fecundity of the females followed by murraya. Celery may have an attractant effect on adults; however, it induced the higher mortality. Longest longevity was recorded in the treatment with 1% murraya chloroform extract. On the other hand, the shortest longevity was obtained with the higher concentration of kumquat and celery alcohol and chloroform extracts. Kumquat was the potent one to deter the insect from laying eggs as confirmed by the higher calculated oviposition Deterrence index. The minimum values were gained from chloroform and ethanol extracts at concentration of 4.0% of celery. On the other hand, highest percentage of egg hatchability was recorded for chloroform and ethanol extracts of murraya at 1% concentration, respectively. The data revealed insignificant extension of the duration of immature individuals on using different extracts in comparison with the control. Also, no significant difference existed between different extracts. Maximum percentage of adult emergence (survival) was obtained on using 1% chloroform extract of Kumquat. The least survival was obtained on using 4% ethanol extract of murraya and celery.

**Keywords:**Callosobruchus maculatus, Coumarins, fecundity, oviposition deternce.

## Introduction

Cowpea, (*Vignaunguiculata*)., is the most important legumes in the tropics andsubtropics regions for human as well as for animal food. Its value lies in its high protein content (23-29%, with potential for perhaps 35%); and its ability to fix atmospheric nitrogen, which allows it to grow on, and improve poor soils, **Steele, 1972** and **Duke, 1990.**

Cowpea plant, (*Vignaunguiculata*) greatly suffered from the attack by several insect pests, especially of family Bruchidae, *Callosobruchus maculatus* (Fab.) which induce higher damage to the yield of one of great protein source.

Cowpea seed beetle, *C. maculatus* (Fab.), is a major insect pest of stored legumes, in Africa and Asia, **Mohamed, *et al.* 2009**. *C. maculatus* consumed 50-90% of cowpea in storage annually, **IITA, 1989**. Insecticides are available to control losses in cowpea but chemical control is impractical at the subsistence level. Insecticides are known to possess side effects like resistance, residual effect, environmental pollution, high mammalian toxicity, ecological imbalance and harm to pesticides appliers. Consequently, there is a need for safe and convenient methods of pest control particularly in small-scale farming level. Control

methods should also be inexpensive. Protection of stored products by means of plant materials is one of the oldest traditional methods in Africa, and plant derivatives were used for insect pest control before the synthetic insecticides. There are many plants in the tropics with potential for insect such as murraya, kumquat and celery belonging to *Rutaceae* and *Apiaceae* known to contain coumarins were chosen to elucidate their anti-feedant and protectant against cowpea beetles (*Callosobruchus maculatus*) that destroy cowpea seeds. To achieve this goal, biological tests were conducted on the ethanol and chloroform extracts of these plants on the *Callosobruchus maculatus* during its immature and adult stage.

**Materials and methods**

The stock culture of *C. maculatus* Fab. (Coleoptera, Bruchidae) was collected from infested cowpea (*Vignaunguiculata*) seeds. Newly emerged adults were kept in glass jars of 1-liter capacity and each containing about 100 grams of cowpeas seeds covered with muslin. The stock culture and bioassay tests were carried out under laboratory conditions (30±1$^{o}$C and 70±5 % R.H.).

To study the effect of different extracts on the various biological aspects of *C. maculatus*, two pairs of the newly emerged beetles were taken from the stock culture and kept in cups containing 10 grams cowpea seeds treated with different plant extracts and covered with muslin under laboratory conditions, (30±1$^{o}$C and 70±5 % R.H.). The beetles were left for mating and laying eggs. The fecundity and longevity of the adults were observed.

To evaluate the efficiency of different extracts on oviposition preference by *C. maculatus* females, two pairs of the newly emerged beetles were introduced in middle of an arena (a petri-dish 20 cm in diameter 3cm in barrier. The barrier was cut in the middle to allow the beetles to move freely in the arena. On half had 10 gm of untreated seeds and the other contained the same amount of treated seeds with the different concentrations (1%, 2% and 4%) of the tested extracts.

The seeds were fixed in the arena using melted paraffin wax. The beetles were left to lay eggs for 24hours, and then the eggs laid were counted on the surface of the both seeds. Each experiment was repeated three times. An analysis of oviposition deterrence was conducted by counting the number of eggs laid on untreated and treated seeds after 24 hours. The oviposition deterrence of the tested extracts on *C. maculatus* adults, the oviposition was calculated using the following equation (**Lundgren, 1975**) ODI = B-A / A+B X 100.

Where: ODI = oviposition deterrence index

A = the number of eggs laid on treated *V. unguiculata* seeds.

B = the number of eggs laid on untreated *V. unguiculata* seeds.

According to the above mentioned equation, one hundred percent means complete deterrence whereas zero percent means an equal number of egg deposited on treated and untreated seeds.

To study the effect of extracts on egg stage, the seeds with eggs on them were collected from stock culture, in new plastic cups and kept at laboratory conditions previously described. The incubation periods as well as the percentage of hatching were calculated by counting the empty egg shells on the surface of the seeds.

The development of the larval and pupal stages was followed up and the duration of larval and pupal stages was recorded from the time of egg hatching until emergence of adults. Also, the percentages of adult's survival were recorded.

Data were statistically analyzed by ANOVA using the Instat V2.03 computer programme test and mean values were separated by the least significant differences (LSD) procedure (**Snedecor and Cochran, 1980**) at probability = 5%.

**Results and discussion**

**Effect of the tested plant extracts (ethanol and chloroform) on the fecundity of C. maculates:**

The fecundity of the adult female as affected by the two extracts were studied by calculating the eggs deposited in the different concentrations of both ethanol and chloroform extracts.

The results obtained are shown in table (1). It is obviously clear that the fecundity of *C. maculatus* females was highly affected by ethanol extracts of all the tested plants at all concentrations used. Compared with the chloroform extracts. Generally speaking the higher the concentration used the higher effect on fecundity obtained.

Adult females offered seeds treated with ethanol extract of murraya at concentration of 4% of the extract laid the least number of eggs (3.16) versus (8.66) with kumquat at the same concentration. Oppositely the adult laid higher eggs with higher concentration of celery ethanol extract which was nearly like that laid by control insects (53.1) at 1% concentration.

In conclusion, kumquat, ethanol extract was most efficient extract which affected significantly the fecundity of the females followed by murraya. Celery may have an attractant effect on adults; however, it induced the higher mortality.

Females given seeds treated with chloroform extract were influenced by different plants at various concentrations.

Murraya has the effective role followed by celery and finally kumquat. With the extracts of the three plants the higher concentrations caused the higher effects.

These results are in good agreement with those obtained by **Abbass, (1993)** who indicatedthatseeds treatedwith different extracts had rather various effects on the fecundity of *B. incarnatus* females during itswhole life. He, also, added that water extract of the different leguminous tested seeds was superior to all other extracts tested as it reduced the fecundity of the females drastically at all the concentration tested. **El-Sayed and Abdel-Razik, (1987)** stated that cowpea seeds (*Vignaunguiculata*) treated with oil extracted from the outer peel of grape fruit, naval or sweet orange and offered to *C. maculatus* adults. These treatment decreased the number of egg laid or inhibited deposition of viable eggs according to the concentration of the oil used. **Chander and Ahmed, (1986)** assayed the role of oils and extracts from five medicinal plants against *C. chinensis* L. infestationcowpea seeds. They found that fecundityof insect female was significantly reduced when oils and extracts from *Acorus calamus*, *Curcuma amada* , *Carum copticum* and *Bassia longifolia* were applied at dose of 0.25 and 0.50 ml/kg seeds. **Pandy *et al.*, (1986)** checked several plant extracts against *C. chinensis* and found that petroleum ether extracts of neem leaves when mixed with seeds of green gramat 0.5, 1.0 and 1.5 parts / 100 parts seeds inhibited oviposition of *C. chinensis* female.

**El-Ghar and El-Sheikh, (1987)** observed that petroleum etherextracts of four plant species collected from Egypt reduced the fecundity of *C. chinensis* adults. **Verma and pandey, (1988)** indicated that oviposition of*C. maculatus*was completely inhibited when seeds treated with coconut and mustard oils. **Lale and Abdulrahman, (1999)** studied the effect of neem seed oil and neem powder on the oviposition of *C. maculatus* on cowpea treated with different dosages of neem oils. They found that egg-laying was significantly reduced s the dosage of neem seed oil increased. However, but the different between 75.1 mg and 150mg oil / 10 gm cowpea was not significant. **Ileke, *et al.* 2013** found that methanol, ethanol, acetone, petroleum ether and n-hexane extracts of *A. boonei* stem bark were effective in controlling *C. maculatus* and could serve as an alternative to synthetic insecticides for the protection of stored cowpeas against bruchids.

**1- Effect on longevity of adults:**

The data obtained on the effect of different extracts on the longevity of the adult bruchids are presented in table (1). The data revealed that the longevity increased with chloroform extracts of the three plants than the ethanol one. In the two different extracts the longevity decreased with increasing the concentration of the two extracts.

Table (1) Effect of tested plant extracts on the fecundity and longevity of *C. maculatus*.

| Plant extract | Concentration %, w/v | Total number of deposited eggs/ female (Fecundity) | | Longevity of adults (days) Mean± SE | |
|---|---|---|---|---|---|
| | | Ethanol extract | Chloroform extract | Ethanol extract | Chloroform extract |
| Murraya | 1 | 36.83±3.12 | 56.00±4.14 | 4.33±0.33 | 5.66±0.33 |
| | 2 | 33.50±2.28 | 43.16±4.37 | 3.37±0.33 | 4.66±0.33 |
| | 4 | 3.16±0.16 | 18.0±1.81 | 3.33±0.33 | 4.0±0.0 |
| Kumquat | 1 | 30.50±2.08 | 77.33±3.36 | 4.0±0.57 | 5.33±0.33 |
| | 2 | 21.00±2.29 | 45.33±3.48 | 4.0±0.0 | 5.0±0.0 |
| | 4 | 8.66±0.36 | 45.00±5.77 | 3.0±0.0 | 4.66±0.33 |
| Celery | 1 | 53.10±2.7 | 63.66±1.26 | 3.66±3.33 | 4.66±0.33 |
| | 2 | 27.66±2.24 | 44.66±5.24 | 3.33±3.33 | 3.33±0.88 |
| | 4 | 26.33±2.02 | 31.83±4.53 | 3.0±0.0 | 3.0±0.57 |
| Control | | 58.50±2.14 | 81.16±3.50 | 6.66±0.33 | 6.0±0.0 |
| F. value | | 16.283*** | 36.67*** | 4.107*** | 4.73*** |
| L.S.D (5%) | | 4.0 | 5.1 | 1.2 | 1.1 |
| L.S.D (1%) | | 5.5 | 6.9 | 1.7 | 1.6 |

Longest longevity was recorded in the treatment with 1% murraya chloroform extract. On the other hand, the shortest longevity were obtained with the higher concentration of kumquat and celery alcohol and chloroform extracts.

The increase or decrease in longevity represents a disturbance in the pest cycle which in turn may influence the cycles of the insect, and they may be attributed to the presence of substance had a growth inhibitor or growth stimulants in different plant extracts.

**Effect on oviposition preference:**

To evaluate the effect of different plant extracts on oviposition preference, two pairs of newly emerged beetles were introduced in the middle of an arena to choose between untreated cowpea seeds and treated ones with the required concentration of the tested extracts, as described before. The beetles were left to lay eggs for 24 hours, and then the eggs laid were counted on the free surface of the seeds.

Results obtained in table (2) indicate that the female bruchid can discriminate between the untreated and treated cowpea seeds present in the arena. From the first sight, it was observed that kumquat extracts were unsuitable for oviposition as the majority of eggs were laid on the untreated seeds which few numbers were laid on the untreated ones. The ODI linearly with the concentrations used. Extracts, chloroform and ethanol induced high deterency effect on oviposition. The higher ODI in the two extracts was obtained as the concentrations of extract increased. In the second rank, murraya extracts came producing high deterrence activity, with the same trend as in kumquat, increasing with concentration. It is interesting to note that kumquat contained fewer amounts of coumarins than murraya, but the latter had intensive odour due to its volatile oil than murraya, and hence higher deterrence effect on ovipositon was observed.

Celery extracts show other picture, both its two extracts induced negative ODI values which mean that the numbers of eggs laid on untreated seeds less than treated ones, table (2, 3).

The results presented in previous tables revealed that kumquat was the potent one to deter the insect from laying eggs as confirmed by the higher calculated ODI.

These results agreed with those obtained by **Wassermann, (1981),Fitzner *et al.*, (1985)** and **Abass, (1993)** who found that the extend of oviposition of *C. maculatus*wasknown to be influenced by the surface area, and the kind of treatments on seeds.

**Bhaduri *et al.* (1985)** indicated that some plant extracts were significantly efficient in reducing the population of *C. maculatus*. Whereas the extract of *Tiridaxc procumbens* in petroleum ether was most effective treatment. **Pandey *et al.*, (1986)** and **El-Ghar and El-Sheikh, (1987)** indicated that some plant extracts had ovipositional deterrence against *C. chinensis.*

**Ashamo, *et al.*(2013)** Evaluation of protectant ability of *Newbouldia laevis* (Seem.) extracts, wood ash, leaf, stem and root bark against infestation by *Callosobruchus maculatus* in cowpea, *Vigna unguiculata* L. (Walp.) in the laboratory at different concentrations of 0, 1, 2, 3, 4 and 5%. All the extracts significantly ($p < 0.05$) reduced oviposition and adult emergence of *C. maculatus* when compared with the controls although the reduction was higher at 5% concentration than others. Adult beetle emergence was completely prevented at higher concentrations (4 and 5%) except in wood ash.

Table (2) Effect of chloroform extracts of tested plants on the oviposition preference of *C. maculatus*.

| Plant extract | Concentration %, w/v | Number of eggs laid / female /day. Mean±S.E | | |
|---|---|---|---|---|
| | | Control | Treated seeds | ODI* |
| **Murraya** | 1 | 10.67±1.40 | 5.665±1.85 | 30.62 |
| | 2 | 16.50±1.15 | 7.50±1.157 | 37.51 |
| | 4 | 17.67±2.00 | 6.50±2.11 | 46.20 |
| **Kumquat** | 1 | 8.50±1.15 | 2.67±1.2 | 52.27 |
| | 2 | 9.17±0.88 | 2.83±0.88 | 52.77 |
| | 4 | 6.17±1.20 | 1.67±0.88 | 57.47 |
| **Celery** | 1 | 11.33±1.6 | 16.33±1.45 | -18.0766 |
| | 2 | 5.33±1.15 | 11.50±1.16 | -36.660 |
| | 4 | 5.33±1.67 | 11.67±0.88 | -37.275 |

*ODI refers to oviposition Deternce index.

Table (3) Effect of ethanol extracts of tested plants on the oviposition preference of *C. maculatus*.

| Plant extract | Concentration %, w/v | Number of eggs laid / female /day. Mean±S.E | | |
|---|---|---|---|---|
| | | Control | Treated seeds | ODI* |
| **Murraya** | 1 | 13.33±2.19 | 9.83±1.763 | 15.112 |
| | 2 | 10.33±1.85 | 6.0±1.54 | 26.515 |
| | 4 | 16.5±2.31 | 6.50±2.11 | 38.481 |
| **Kumquat** | 1 | 12.83±4.67 | 9.0±2.1 | 17.545 |
| | 2 | 11.16±1.45 | 5.83±1.763 | 31.93 |
| | 4 | 14.83±1.76 | 3.65±2.02 | 60.51 |
| **Celery** | 1 | 14.165±0.53 | 14.5±0.3 | -1.168 |
| | 2 | 15.5±1.73 | 20.5±1.543 | -13.888 |
| | 4 | 15.5±1.73 | 21.0±1.52 | -15.068 |

*ODI refers to oviposition Deternce index.

## Effect on egg stage

Data obtained in table (4) showed that the both extracts of the three plants had inconsiderable effect on the incubation period of *Callosobruchus maculatus* (Fab.) eggs. A slight increase in the incubation period of eggs to 7.66 and 7.5 days while was 6.66 days in control of the extracts of the different plants over control, these increases was no significant. As regards to the percentage of egg hatchability, the minimum values were gained from chloroform (7.31%) and ethanol (8.81%) extracts at concentration of 4.0% of celery.

On the other hand, highest percentage of egg hatchability was record (84.59% and 69.96%) for chloroform and ethanol extracts of murraya at 1% concentration, respectively.

Table (4) Effect of tested plant extracts on egg stage of *C. maculatus*.

| Plant extract | Concentration %, w/v | Incubation period (Days)Mean± SE | | %Hatchability | |
|---|---|---|---|---|---|
| | | Ethanol extract | Chloroform extract | Ethanol extract | Chloroform extract |
| **Murraya** | 1 | 7.33±0.59 | 7.00±0.57 | 69.96 | 84.59 |
| | 2 | 7.33±0.33 | 7.08±0.38 | 66.16 | 83.33 |
| | 4 | 7.66±3.51 | 7.08±0.38 | 15.79 | 68.51 |
| **Kumquat** | 1 | 7.06±0.33 | 7.00±0.55 | 69.39 | 82.76 |
| | 2 | 7.33±0.33 | 7.33±0.88 | 68.25 | 72.79 |
| | 4 | 7.33±0.33 | 7.33±0.88 | 64.17 | 71.85 |
| **Celery** | 1 | 6.66±0.33 | 6.66±0.33 | 58.31 | 48.0 |
| | 2 | 7.50±0.28 | 7.33±0.88 | 42.78 | 38.73 |
| | 4 | 7.66±0.33 | 7.33±0.33 | 8.81 | 7.31 |
| **Control** | | 6.66±0.33 | 6.66±0.33 | 93.73 | 90.34 |
| **F. value** | | 2.993 (ns) | 2.173 (ns) | - | - |

**Effect on the immature stages**

The developments of larval and pupal sages were followed up to study the effect of different extracts on these stages.

The duration of larval and pupal stages were recorded from the time the larval were hatched until emergence of the adults. Also, the percentage of adult emergence (survival) was recorded.

The results obtained in table (5) show that the time required to reach the adult stage of *C. maculatus* was generally increased as a result of treatment cowpea seeds with different extracts.

In this respect, the effect was more pronounced with ethanol extracts of celery where the larval and pupal duration prolonged to 15.66 days at 2% and 4% concentrations. Chloroform extract of murraya were more potent than ethanol extract. An ethanol and Chloroform extract of Kumquat was found to be the least effective as it show no effect or reduce the duration.

Statistical analysis of the data revealed insignificant extension of the duration of immature individuals on using different extracts in comparison with the control. Also, no significant difference existed between different extracts.

As regard to the percentage of adult emergence, the results obtained in table (5) show that treatment of cowpea seeds with all extracts reduced the survival of the larvae and pupal of *C. maculatus* in comparison with control.

Maximum percentage of adult emergence (survival) was 71.77 obtained on using 1% chloroform extract of Kumquat. The least survival (15.79% and 23.82%) were obtained on using 4% ethanol extract of murraya and celery, respectively (table5).

The percent survival decreased as the percentage of extract increased with the three plants.

The resulting progeny of *C. maculatus* suffered greatly from treatment of cowpea seeds with different extracts. The hatchability of the egg was differently affected through the three plants but reduced as the percentage of extracts increased.

The results of the percent work are in agreement with **Sharma, (1985)** who pointed that extracts of flowers of oak (*Calotropis procera*) when mixed with wheat flour and provided to different instar larvae of the stored products of pest *Rhyzopertha dominica* increased larval mortality and decreased adult emergence as the concentration of extracts increased from 0.1 to 1000 ppm. The younger larvae were susceptible than the other one.

**Abbass, (1993)** pointed on that powders of *Abrus precatorius*, *Lupinus termis* and *Trigonella foenum* had different effects on the pupal duration of *Bruchidius incarnatus*. Both powders of *A. precatorius* and *L. termis* decreased the pupal duration to 3.6 and 3.8 days, respectively using concentration of 5%, meanwhile *T. foenum* at the same concentration prolonged duration of the resulting pupae to 6.6 days.

Table (5) Effect of tested plant extracts on the duration period of immature stages (larval and pupal stages) of *C. maculatus*.

| Plant extract | Concentration %, w/v | Larval and pupal duration ( in days) Mean± SE | | Adult's emergence (%) (Survival) | |
|---|---|---|---|---|---|
| | | Ethanol extract | Chloroform extract | Ethanol extract | Chloroform extract |
| Murraya | 1 | 14.66±4.34 | 14.66±0.33 | 60.64 | 62.55 |
| | 2 | 14.66±0.66 | 15.33±0.66 | 44.77 | 58.51 |
| | 4 | 15.00±0.57 | 15.66±0.66 | 15.79 | 44.44 |
| Kumquat | 1 | 12.33±0.33 | 12.33±0.33 | 69.84 | 71.77 |
| | 2 | 13.33±0.33 | 13.66±0.66 | 53.55 | 58.82 |
| | 4 | 13.33±0.33 | 13.66±0.33 | 43.41 | 52.59 |
| Celery | 1 | 15.33±0.88 | 13.33±0.33 | 62.65 | 71.12 |
| | 2 | 15.66±0.33 | 13.33±0.33 | 61.44 | 67.12 |
| | 4 | 15.66±1.20 | 13.33±0.33 | 23.82 | 52.88 |
| Control | | 13.33±0.33 | 13.63±0.33 | 91.73 | 92.34 |
| F. value | | 2.054 (ns) | 1.95 (ns) | - | - |

## Conclusion

According to the obtained results, it could be stated that the tested compounds played an important role in controlling the bruchid *C. maculatus*. These compounds may be used as components in (IPM) programmes for controlling this insect pest and to avoid pollution of environment and hazards to man or animals.

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## References:

Abass, M.H. (1993). Biological studies on *Bruchidus incarnatus*Schm. And the control using plant extracts Ms. Thesis, Faculty of Science, Cairo University.

Ashamo,M. O.; O. O. Odeyemi and O. C. Ogungbite(2013). Protection of cowpea, *Vigna unguiculata* L. (Walp.) with *Newbouldia laevis* (Seem.) extracts against infestation by *Callosobruchus maculatus* (Fabricius) Archives of Phytopathology and Plant Protection, 46 (11): 1295-1306.

Bhaduri, N.;Ram, S. and Patil, B.D. (1985). Evalution of some plant extracts as protectants against the pulse beetle, *Callosobruchus maculatus* (Fabricius) infesting cowpea seeds. J. Ent. Res., 9(2): 183-187.

Chander, H. and Ahmed, S.M. (1986). Efficiency of oils from medical the pulse beetle *Callosobruchus chinensis*. Entomol, 11(1): 21-25.

Duke, J. A. (1990): Introduction to food legumes. *In* Singh, S R (ed.), Insect Pests of Tropical FoodLegumes. Chinchester. John Wiley & Sons, pp 1-42.

El-Ghar, G.S.A. and El-Sheikh, A.E. (1987). Effectiveness of some plant extracts as surface protectants of cowpea seeds against the pulse beetle *Callosobruchuschinensis*. Phytoparasitica, 15(2): 109-113.

El-Sayed, F. M.A. and Abdel-Razik, M. (1987). Effect of three plant oils as protectants of three legumes against infestation by cowpea weevil, Agric. Res. Rev., 65(1):53-59.

Fitzner, M.S.; Hagtrum, d.w.; Knauft, D.A.; Bhur, K.L. and Mchlaughlin, J.R. (1985). Genotypic diversity in the suitabilityof cowpea (*Rosales: Leguminosae*) pods and seeds for cowpea weevil (Coleoptera: Bruchidae) oviposition and development. J. Econ. Entomol. 78: 806-810.

IITA (International Institute of Tropical Agriculture) (1989): Annual Report 1988/89. Ibadan, Nigeria.

Ileke,K.D.; O.O. Odeyemi and M.O. Ashamo (2013). Response of cowpea bruchid, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) to cheese wood, *Alstonia boonei* De Wild stem bark oil extracted with different solvents Archives of Phytopathology and Plant Protection 46(11): 1359-1370.

Lale, N. E. S. and Abdulrahman, H.T. (1999). Evolution of neem (*Azadriachta indica A. Juss*) seed oil obtained by different methods and neem powder for the management of *Callosobruchus maculatus* (F.) (Coleoptera, Bruchidae) in stored cowpea. J. stored products research, 35: 135-143.

Lundgren, L. (1975). Natural plant chemicals activity as oviposition deterrents on cabbage butterflies (*Pieris brassicae* L.) *P. rapae* L. and *Pieris napi* L.). Zool. Sci.4: 253-258.

Mohamed, H.A.; O.A. El-Sebai and S.F. Hafez (2009). Effect of lufenuron and methylamine avermectin on growth , development and reproductive performance of *Callosobruchus maculatus* (F.) (Coleoptera, Bruchidae). Bull. ent. Soc. Egypt, Econ. Ser., 35:75-90.

Pandey, N.D.; Mathur, K.K.; Pandey, S. and Tripathi, R. A. (1986). Effect of some plant extracts against pulse beetle, *Callosobruchuschinensis* Linensis. Indian .J.Ent., 48 (1): 85-90.

Sharma, Y. (1985). A new indigenous plant antifeedant against *Rhyzopertha dominica* Fab.Bull. Grain Technol., 21(3):223-225.

Snedecor, G.W.; Cochran, W.G. (1980). Statistical method. 7th ed. Ames (IA): lowa State University Press; USA. p. 507.

Steele, W. M. (1972): Cowpea in Africa. Ph.D.thesis,University of Reading, United Kingdom.

Verma, B. K. and Pandey, G.P. (1988). Treatment of stored green grain seed with edible oils for protection from *Callosobruchus maculatus*,(Fab.) Indain Agric. Sci., 48(2): 72-75.

Wassermann, S. (1981). Host–induced oviposition preferences and oviposition markers in the cowpea weevil *Callosobruchus maculatus*.Ann. Ent. Soc. An. 74:242-245.