Original Research Article

Insecticidal Activity of Five Plant Species on Cowpea Weevil [*Callosobruchus maculatus* (Fabricius)]

Abstract

A study was conducted to evaluate the insecticidal activity of five locally available plants, namely: *Azadirachta indica* (Neem), *Cymbopogon citratus* (Lemon grass), *Lantana camara (*Lantana), *Occimum gratissimum* (Scent leaf) and *Tagetes erecta* (African marigold) on cowpea weevil, *Callosobruchus maculatus* (Fab.) using the repellency, adult mortality, anti-oviposition and growth inhibition tests. Results revealed that all test materials exhibited repellency against cowpea weevils. Powdered leaves of neem and scent leaf were noted to be slightly more repellent than the others. Cowpea grains treated with powdered leaves of *T. erecta* exhibited the lowest mortality of the cowpea weevils. Again, *A. indica* and *O. gratissimum* treatment were most effective in anti-oviposition and growth inhibitory action, as well as adult mortality of the weevils. Some of the examined treated cowpea grains (except those treated with *A. indica* and *O. gratissimum*), had larval tunnels. The total development period of the cowpea weevils that emerged from such treated and the untreated seeds was the same (37days). Botanicals are known to have varying degrees of insect inhibition, and in this case *O. gratissim*um and *A. indica* were the most effective.

Keywords: Insecticidal, cowpea weevil, repellency, anti-oviposition, growth inhibition, adult mortality

Introduction:

Cowpea [*Vigna unguiculata* (L.)] of the Family *Mimosaceae* and Order Leguminales is a legume grain that is related to groundnut, pigeon pea, soybean and bambara nut. Generally, legumes are a major source of diet in the nutrition of many Nigerians, because their edible seeds serve as alternatives to protein, as well as a source of calcium, iron, thiamine and riboflavin (Messina, 1999). However, a major problem with cowpea production is infestation by weevils.

The bean weevil [*Callosobruchus maculatus* (Fab.)] is an economically important pest of stored grains of cowpea and pigeon pea, among others (Ofuya and Bamigbola, 1991), resulting to loss in weight, seed viability and nutritive quality of affected seeds. A loss in weight of 30-40% has been reported (Law-Ogbomo, 2007). *C. maculatus* of the family Bruchidae, is a small compact brownish beetle of about 2.5-3.5 mm long. The male beetles are smaller in size than the female (Arotolu et al., 2018).

The cowpea weevil is a major pest of *Vigna unguiculata* (L.), which attacks cowpea seeds during storage and transport (Ofuya and Bamigbola, 1991). Weevil attack may begin either in the field or storage and may completely destroy the seed. Further infestation in storage is as result of the transfer of infested seeds into the store or from flying weevils making contact with storage facilities, probably attracted by the odour of the stored seeds (Law-Ogbomo, 2007)

In storage, pests are difficult to control, however this is often done by burying the entire sac of cowpea in a deep hole or by using insecticides. Presently, effective pest control of stored products are no longer feasible due to high cost of pesticides, environmental pollution, evolution of resistant forms of the pest under control, and contamination of food materials.

Many conventional insecticides have been effective against storage insects, either as dusts or fumigants. Insecticidal dust of malathion, chlorpyrifos-methyl, fenitrothion, carbamate and deltamethrin can protect shelled cowpeas stored in bags or in airtight containers. The use of inert materials like wood ash, silicates and sand, wood ash, diatomaceous earth (diatomite), etc for the control of bruchids, especially *C. maculatus* has been reported by Kalpna (2022). Grains are mixed with sieved ash and placed in a mud granary of clay bar tapped-down to compress the mixture, often covered with an additional layer of ash. A minimum ratio of 3 part or wood ash to 4 part of cowpea significantly reduced the population of *C. maculatus*.

The use of botanical pesticides to protect plants and plant products from pests has gained prominence due to obvious advantages: Pesticidal plants are generally much safer than conventional/synthetic pesticides (Ahmad et al, 2019). Plant-based pesticides are renewable in nature and cheaper, they are easily biodegradable or ecologically friendly and non-toxic to other useful organisms. Therefore, they can be transferred into practical applications in natural crop protection, especially among small-holders (Ahmad et al., 2019; Malik et al., 2012; Mahdi and Rahman, 2009).

A number of indigenous plants in Nigeria have been reported to have pesticidal properties. Ilesanmi and Gungula(2010) used neem kernel powder to protect cowpea seeds from *C. maculatus* damage. Rosulu et al (2022) observed that dry chili (pepper) resulted to moderate adult mortality in *C. maculatus*. Tiroesele et al (2015) reported that dry chili fruit (*Capsicum annnum*) and onion scale leaves (*Allium cepa*) afforded some degree of protection against *C.*

maculatus in stored cowpea seeds. Law-Ogbomo (2007) reported that powdered *Nicotiana tabaccum*, *Erythrohleum suaveolens*, *Ocimum gratissimum* and *Lantana camara* significantly reduced oviposition and egg hatching in *S. zeamais*, *Sitophilus oryzae* and *C. maculatus*. Powdered root bark of *Zanthoxylum zanthoxyloides* in water solution was toxic to adult *C. maculatus* by protecting cowpea seeds from predation by the pest (Okwute, 2012). Short exposure of adult bruchids to *Zanthoxylum zanthoxyloides* powder reduced their reproductive fitness (Kalpna, 2022). The rate of application of these insecticidal plant powders ranges generally from less than 1g/kg to 20g/kg of seed (Lale and Vidal 2003). The use of plant powders is impracticable in large scale storage because of the high quantities required. Many of the plants used have known medicinal and pharmacological properties and have been subjected to empirical verification for their effectiveness against *C. maculatus* (Mahdi and Rahman, 2009). The objective of this study is to ascertain the insecticidal properties of *Azadirachta indica* (Neem), *Cymbopogon citratus* (Lemon grass), *Lantana camara* (Lantana), *Ocimum gratissimum* (Scent leaf) and *Tagetes erecta* (African marigold) as grain protectants against cowpea weevil (*C. maculatus),* using repellency, adult mortality, anti-oviposition and growth inhibition tests.

Materials and Method

This experiment was conducted in the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria, located on latitude 4^{o}79 N and longitude 6^{o} 98 N. Infected cowpea seeds were obtained from the 'Mile Three' market in Port Harcourt, and about Five Hundred (500) of the infected seeds were put into a transparent plastic container, covered tightly and maintained at a temperature of 30-35^{o}C and 50% relative humidity in the laboratory for fast breeding of insects. This was left undisturbed for 35 days, during which period the weevils had grown into full adults.

Fresh and mature leaves of the respective plant materials: *Azadirachta indica*, *Cymbopogon citratus*, *Lantana camara*, *Ocimum gratissimum* and *Tagetes erecta* were sourced and authenticated from the Departmental garden and the Taxonomy Unit of the Department, respectively. The leaves were air dried until crispy, then they were blended and sieved through a 0.5mm mesh. The powders were stored separately in sealed containers until use.

Repellency Test

The method used was that employed by Garcia (1990) with some modifications. Transparent plastic tubing, 17cm long, were used as test cylinders. Each tubing was plugged at one end (B) with cotton wool containing 2 grams of treatment powder made from the leaves of each test plant, while the other end (A) was plugged with clean cotton ball without the treatment powder. A mixture of ten male and ten female weevils were introduced at the middle of each tubing through a hole at the middle. The hole was covered to keep the weevils inside. Each of the five plant treatments and control were replicated three times, thus making a total of 18 tubing. They were left undisturbed, and the number of weevils that moved towards the untreated half of the tubing were counted every hour for the first five hours, then at 24 hours, 48hrs and 96 hrs. Repellency rating was calculated following the formula:

$$\text{Repellency rating} = \frac{n(1) + n(3) + n(5) + n(7)}{N}$$

Where n = number of insects staying at 0, 1-2, 3-4 and 5-6 cm from the center of the tubing towards the untreated cotton plug, respectively.

1, 3, 5 and 7 = rating scale on the reaction of the insects on different test materials.

N = Total number of insects introduced per tubing.

Anti-oviposition and growth inhibition test

Two hundred grams of cowpea were placed in 20cm high transparent plastic containers. Ten grams of powdered leaves of each treatment material was thoroughly mixed with the grains in each container. Each treatment was done in three replicates. A mixture of twenty male and twenty female cowpea weevils were introduced in each container. The female adults were allowed to oviposit on the seeds for 48hrs, after which they were discarded. Five days after, thirty cowpea seeds were taken from each container for close examination using a dissecting microscope. Since the eggs were not very visible, rough jelly-like spots on the grains were used to indicate egg deposition. A tunnel was formed inside the grain when the deposited egg hatched and the larva starts feeding. This was the basis for anti-oviposition effect of the test materials. The total development period of the weevils was gathered by counting the number of days from when the eggs were laid (a day after removal of parent weevils from the jar) to adult emergence.

$$\text{Percent insect survival} = \frac{\text{number of insects that emerged into adult stage}}{\text{total no. of seeds with eggs on them (represented by seeds with tunnels)}} \times \frac{100}{1}$$

Adult Mortality Test

One hundred grams of cowpea seeds were admixed with 5g of powdered leaves of each plant treatment into glass jars. The admixtures were shaken manually for 5 mins, and then left undisturbed for an hour. Thereafter, a mixture of twenty male and female weevils were introduced per jar. The untreated cowpea seeds served as control. Each treatment and control were made in three replicates. Adult mortality was monitored at 4, 7, 14 and 24 days after exposure to the treatment.

$$\text{Percent adult mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects introduced}} \times \frac{100}{1}$$

Results

Repellency Test

With the aid of the scale (Table 1) as a guide, the degree of repellency of each plant test material was ascertained.

Table 1. Scale for the determination of the degree of repellency of the test materials

Rating	Distance (cm) from the center of the tubing towards the untreated cotton plug.	Description
1	0	Ineffective
3	1-2	Slightly repellant (SR)
5	3-4	Moderately repellant (MR)
	5-6	Highly repellant (HR)

Kumar, et al., 2004

The repellency effect of the plant powder treatment on the weevils is shown in Table 2, and the mean values reveal that *O. gratissimum* had the highest repellency rating of 0.91, followed by *A. indica* (0.88). The least value among the treatments was that of *Tagetes erecta* (0.73), while the Control was lower than them all (0.58). All the values fell below 1.0, but were all higher than that of the control, so they can be said to be slightly repellant. It was also observed from Table 2 that repellency values dropped after 24 hrs for *C. citratus*, *L. camara* and *T. erecta*, and after 4 days (96 hrs) values dropped for all of them, including the Control.

Table 2. Distance (cm) over time (hrs) of the weevils from the center of the tubing towards the untreated cotton plug

	Exposure duration (hours) to treatment effect								Mean distance (cm)towards untreated
Treatments	1	2	3	4	5	24	48	96	
A. indica	0.99	0.97	0.97	0.97	0.90	0.92	0.95	0.34	0.88±0.0113
C. citratus	1.00	1.00	1.00	1.00	1.00	0.83	0.63	0.40	0.86±0.0076
L. camara	1.00	1.00	1.00	0.83	1.00	0.63	1.00	0.4	0.86±0.0076
O. gratissimu	1.00	1.00	1.03	1.00	0.97	0.97	0.73	0.60	0.91±0.0075
T. erecta	0.9	1.00	1.00	1.00	1.00	0.30	0.30	0.30	0.73±0.0151
Control	0.37	0.83	0.77	0.73	0.83	0.67	0.33	0.1	0.58±0.0037

Anti-oviposition and growth inhibition test

It was observed that some test plants did not indicate anti-oviposition action as shown in Table 3. Despite the deposition of eggs that were observed on the seeds, only a few treated with *C. citratus, L. camara* and *T. erecta* indicated larval tunnels. Nevertheless, observation of larva tunnels was used to determine the deposition of eggs.

Zero percent insect survival was recorded in some of the treatments viz: *A. indica* and *O. gratissimum. T. erecta* had the highest percentage (10) of weevils from tunnels, after Control; while both *C. citratus* and *L. camara* had 5%. The recorded total development period (TDP) for these three treatments and the Control was 37 days.

Table 3. Treatment effect on weevil reproduction

Treatments	No. of infected seeds with eggs (%)	No. of seeds with tunnels (%)	Total Development period (days) (%)	No. of weevils from tunnels (%)
A. indica	6	0	0	0
C. citratus	6	2	37	5
L. camara	8	1	37	5
O.gratissimum	4	0	0	0
T. erecta	11	4	37	10
Control	36	13	37	22

Adult Mortality Test

The mortality rate of the treatment effect on the adult weevils recorded on the fourth day was the highest in all the treatments, except for Control that had its highest on the 24th day (Table 4). By

Day 14, the jars treated with the test plants: *A. indica, C. citratus* and *O. gratissimum* already had all the adult weevils dead. At 24 days after insect introduction (DAII), the cumulative percentage mortality of adult weevils over time show that the cowpea treated with *A. indica*, *O. gratissimum* and *L.camara* had higher mortality (100%, 99.98% and 99.96% respectively) than the rest. *C. citratus* and *T. erecta* had lower mortality rates (88.37% and 89% respectively), while the Control gave the lowest mortality rate of 56.66% (Table 5).

Table 4. Treatment effect on percentage mortality of adult weevils over time

Treatments	Day 4 (%)	Day 7 (%)	Day 14 (%)	Day 24 (%)
A. indica (Neem leaf)	75	3.33	21.67	0
C. citratus (Lemon grass)	65	11.7	11.67	0
L. camara (Lantana)	73.3	15	3.33	8.33
O. gratissimum (Scent leaf)	78.3	8.34	13.34	0
T. erecta (African marigold)	30.7	20	20	18.3
Control	3.33	18.33	8.33	26.67

Table 5. Treatment effect on Cumulative percentage mortality of adult weevils over time

Treatments	Day 4	Day 7	Day 14	Day 24
A. indica (Neem leaf)	75	78.33	100	100
C. citratus (Lemon grass)	65	76.7	88.37	88.37
L. camara (Lantana)	73.3	88.3	91.63	99.96
O. gratissimum (Scent leaf)	78.3	86.64	99.98	99.98
T. erecta (African marigold)	30.7	50	70.7	89
Control	3.33	21.66	29.99	56.66

Discussion

The repellency test shows that all the plant test materials had values that fell slightly below 1.0, implying that they were all slightly repellent. *O. gratissimum* however had the highest value of 0.91. Mc Gowan (1999) mentioned that *O. gratissimum* and its volatile oil were particularly effective in repelling flies, and were found effective as repellant against cowpea weevils. The repellency of the treatments was however observed to decrease as exposure duration lengthened (Table 2). This could be as a result of either the volatilization of chemical compounds present in the powdered leaves, or the weevils adjusting to the odour of the plants. Many plant extracts are known to possess biological compounds such as flavonoids, alkaloids, phenols and similar constituents. The repellency of all the test plants dropped by the end of 24 hrs, except that of *A. indica* that lasted till the end of 48 hrs, and *O. gratissimum* that dropped in repellency during the 48 hr period. Repellency of an average of 8hrs was observed against Anopheles mosquitoes by Asadollahi et al (2019) using similar plants.

A. indica and *O. gratissimum* were the most effective test plants for anti-ovipository/growth inhibition, and these two test plants again were the most effective on percentage mortality of adult weevils by Day 4; and by Day 24, as all the adult weevils had died in these two treatments as well as in that of *C. citratus*. In all the three tests carried out with the test plants, *O. gratissimum* competed favorably with *A. indica*, and was always a little bit ahead in performance.

A. indica is however the most promising source of biopesticide. Its Azadirachtin content being the most potent of its insecticidal compounds, was found effective against more than 200 pests of agriculture, horticulture, vegetable crops and household pests (Isman, 2006; Morgan, 2009), including *C. maculatus*. However, leaves of *A. indica* contain lesser amount of the major active ingredient, azadirachtin than *A. indica* kernels (Busungu and Mushobozy., 1991). Kernel of *A. indica* is the richest source of meliacins and contains 0.2 to 0.3% azadirachtin and 30 to 40% oil (Isman, 2006; Saxena et al., 1989). Varma and Dubey (1999) found that the essential oils of *O. gratissimum* showed its insecticidal activity against *Sitophilus oryzae*, *Stegobium panicum* and *T. castaneum*. Also, the essential oil of *A. indica* showed its in vitro fumigant activity in the management of storage fungi and insects of some cereals without exhibiting mammalian toxicity in albino rats. *C. citratus* essential oil was found effective in deterring a wide variety of insects (Mc Gowan, 1999), including cowpea weevils, *C. maculatus* (Boeke et al., 2004).

Marigold (*T. erecta*), *L. camara*, garlic, chili, lemon grass (*C. citratus*) and *O. gratissimum*, among others, repel insects, thus farmers use them as companion crops to food crops because in some cases, their smell acts as repellant.

CONCLUSION

In conclusion, the powdered leaves of all the test plants exhibited repellency, anti-oviposition/growth inhibition and adult mortality against cowpea weevils, however those of *O. gratissimum* and *A. indica* were more effective. Further studies comparing the other plant parts will be even more effective.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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