ENTOMOTOXIC EFFECT OF SOME BOTANICALS ON THE OVIPOSITION PREFERENCE OF *CALLOSOBRUCHUS MACULATUS* (FABRICIUS) (COLEOPTERA: CHRYSOMELIDAE) ON FIVE SELECTED LEGUMES.

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Abstract

Powders from ten plant materials purchased from Oja-Oba market Akure, Ondo State were evaluated for their entomocidal effects on the beetle. The evaluation of the insecticidal effects of the powders was based on oviposition and percentage weight loss of seeds caused by C. maculatus. The results from the evaluation of powders from the ten plant materials (Piper guineense, Eugenia aromatica, Nicotiana tabacum, Oryza sativa bran, Zanthoxylum zanthoxyloides, Zingiber officinale, Allium sativum, Mormordica charantia, Ocimum gratissimum, Xylopia aethiopica) tested at 2% on five legume seed types revealed that Zanthoxylum zanthoxyloides was superior to Piper guineense (77%) and Eugenia aromatica (73%) in inhibiting oviposition by C. maculatus. Zanthoxylum zanthoxyloides inhibited oviposition by (87%) which was next to the synthetic insecticide cypermethrin with (88%) inhibition. The efficacy ranking when comparing the dosage of 2% at 0.2g/10g cowpea and non-cowpea legumes was in the order of Cypermethrin (88.16%), Zanthoxylum zanthoxyloides (87.02%), Piper guineense (77.36%), Oryza sativa [Rice bran-(76.55%)], Eugenia aromatica (73%), Nicotiana tabacum (46.51%), Allium sativum (29.81), Mormordica charantia (9.29%), Xylopia aethiopica (9.07%), Zingiber officinale (6.88%) and Ocimum gratissimum (1.11%). Zanthoxylum zanthoxyloides treated seeds recorded least percentage weight loss compared with cypermethrin, indicating that it can compete favourably with the synthetic insecticides. Results from this study show that Mucuna pruriens (99%) and Sphenostylis stenocarpa (88%) of the non-cowpea legumes were tolerant to infestation by C. maculatus. Therefore, incorporation of the use of varietal resistance and powder of Z. zanthoxyloides for the management and control of C. maculatus proved a promising biopesticide for the protection of legumes as alternatives to synthetic pesticides.

Keywords: Entomotoxic, Influence, Botanicals, Oviposition, Legumes, Protection

IJAFS 2021 (3).11:1610-1628
Accepted for Publication
May, 2021

Introduction

Grain legumes are rich in protein, oil, and micronutrients such as iron and zinc – all of which tend to be deficient in the diets of the poor. However, they are heavily infested by many insect pests both in the field and during postharvest storage (Adebayo *et al*., 2013). The most important species of stored product pests of food legumes include: *Callosobruchus chinensis*, C*allosobruchus maculatus, Callosobruchus analis*, *Acanthoscelides obtectus* and *Bruchus incarnatus* (Kashiwaba *et al*., 2003). In addition, *B. rufimanus*, *B. dentipes*, *B. quinqueguttatus*, B. *emarginatus*, *B. ervi*, *B. lentis* and *B. pisorum* may also cause significant losses in some legumes (Desroches *et al*., 1995). Bruchid beetles (Coleoptera: Chrysomelidae) such as

Callosobruchus maculatus (F.) and *Callosobruchus chinensis* (L.) are peculiar to stored grain legume such as cowpea (Kashiwaba, *et al.*, 2003). *Callosobruchus maculatus* (F.) is a cosmopolitan polyphagous pest in the tropics and subtropical areas. Knowledge of host, pest and environment interaction is important to know its host range, so that storage planning can be made to avoid infestation among susceptible legume seeds species when stored in one place which will prevent a heavy build of *C. maculatus* population. Infestations on stored grains may reach 50% within 3-4 months of storage (Oparaeke and Dike, 2005).

The destructive activities of insects and other storage pests have been subdued by chemical control methods comprising fumigation of stored commodity with carbon disulphide, phosphine or dusting with malathion, carbaryl, pirimiphos methyl or permethrin. Over the years in Nigeria and in other parts of the world, management of *C. maculatus* on the field and in storage has been dominated by chemical control using fumigants and synthetic insecticides (Park *et al.*, 2003; Akinkurolere *et al.*, 2006). These chemicals have been reported to be effective against stored products pests (Oni and Ileke, 2008; Ileke and Oni, 2011). In the developed countries, conventional fumigation technology is currently being scrutinised for many reasons, such as ozone depletion potential of methyl bromide and carcinogenic concerns with phosphine. Also as a result of serious problems of genetic resistance by insect species, pest resurgence, residual toxicity, photo-toxicity, vertebrate toxicity, widespread environmental hazards and increasing costs of application of the presently used synthetic pesticides have directed the need for effective, biodegradable pesticides (Talukder and Howse 2000; Elhag 2000). In this regard several botanicals that were effective and environment friendly have been evaluated for the management of storage insect pests (Oni, 2009; Idoko, 2011; Adebayo and Eyo, 2014).

Worldwide concerns on the unjust use of chemical fumigants by farmers further lead to the development of alternative strategies, among which is the re-examination of using plant derivatives against agriculturally important insect pests. Plant-derived materials are more readily biodegradable. Most of these plant products are cheap, readily available, edible and ecologically safer means of controlling insect pest infestations of stored cereal and grains especially in the tropics (Ileke and Oni, 2011; Adebayo and Ibikunle, 2014). In the last two decades, considerable efforts have been directed at screening plants in order to develop new botanical insecticides. It was reported that when mixed with stored-grains, leaf, bark, seed powder, or oil extracts of plants reduce oviposition rate and suppress adult emergence of bruchids, and also reduced seed damage rate (Tapondjou *et al*., 2002).

This study was conducted in addition to efforts of crop researchers to minimize the indiscriminate use of synthetic chemicals and their effects. Oviposition decisions are crucial in the life cycle of bruchid beetles because they set the conditions in which an offspring must develop from egg to adult stage. Hence, the need to investigate on how oviposition can be reduced in *C. maculatus* through use of insecticidal active botanicals.

Materials and Methods

Experimental Site

The study was carried out in the Pest Management Laboratory of the Department of Crop, Soil and Pest Management, the Federal University of Technology, Akure, Ondo State Nigeria (Latitude 7° 16' N and Longitude 15° 12' E) under ambient conditions of 28 ± 2°C temperature and 70 ± 5% relative humidity.

Collection and preparation of legume seed types

Seeds of five non-cowpea legume listed in Tables 1 and 2 were collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The morphological characteristics of the five legume seeds used in this study is presented in table 1. The legume seeds were first cleaned and disinfested by keeping them in a freezer at -5^{0}C for 7 days to kill all hidden infestations.

Table 1: Morphological characteristics of the six Non- cowpea legumes.

Legumes	Common name	Texture	Colour
***Cajanus cajan* (L.) Mill-**	Pigeon pea	Rough	Brown
***Glycine max* (L.) Merrill**	Soya bean	Smooth	Milky
***Vigna subterranean* L. Verdcourt**	Bambara Groundnut	Smooth	Brown
***Sphenostylis stenocarpa* Hochst. Ex A. R.**	African yam Bean	Smooth	Glossy milk
***Mucuna pruriens* (L.)**	Devil Beans	Smooth	Black

***Preparation of botanical powders used as treatment*:**

Ten well researched insecticidal plants, *Piper guineense, Eugenia aromatica, Nicotiana tabacum, Oryza sativa bran, Zanthoxylum zanthoxyloides, Zingiber officinale, Allium sativum, Mormordica charantia, Ocimum gratissimum, Xylopia aethiopica* were purchased from various locations at Oja-Oba Akure and brought to the laboratory. The different parts of the plants underlisted in Table 2 were sun dried, separately milled into fine powders. The powders were sieved through a 4.5mm^2 mesh and stored an air-tight nylon. They were all preserved under ambient conditions prior to use. Cypermethrin dust was also obtained and used as standard.

Table 2: *Botanical Powders with the different part used including Cypermethrin used as standard*.

Scientific Name	Common name	Part Used
Eugenia aromatica **(Baill.)**	Clove plant	Pod
***Zanthoxylum zanthoxyloides* (Lam.)**	Tooth ache plant	Root bark
***Piper guineense* (Schum and Thonn)**	West African black pepper-Seed	Seed
***Oryza sativa* L.**	Rice	Bran
***Allium sativum* L.**	Garlic	Bulbs
Momordica charantia **Linn.**	Bitter melon	Leaf
***Ocimum gratissimum* (OG)**	Clove Basil	Leaf
***Xylopia aethoipica* (Dunal) A.**	Negro pepper	Pod
***Nicotiana tabacum* L.**	Tobacco	Leaf
***Zingiber officinale* Rosc.**	Ginger	Rihzomes
Cypermethrin		

Insect culture

The initial culture of cowpea storage beetle, *Callosobruchus maculatus* used was obtained from cowpea grains already infested with bruchids from Oja-Oba market in Akure, Ondo State, Nigeria and was sub-cultured on a well-known susceptible cowpea variety Ife Brown which was sourced from the International Institute of Tropical Agriculture (IITA). Ife Brown cowpea seeds were first disinfested by deep-freezing for two weeks and acclimatized in the open laboratory conditions for 24hours before subsequent use. Bruchid cultures were established according to Beck and Blumer (2011). Cleaned cowpea seed 400g were set aside in a plastic container and infested with twenty adult bruchids (10 males and 10 females) for oviposition. The condition was maintained at temperature of 28 ± 2°C and 70 ± 5% R.H. Adult *Callosobruchus maculatus* were removed 5 days after introduction an plastic container with its content was then left undisturbed for twenty one days for adult emergence. Day old teneral adults that emerged from the container were used to infest cleaned disinfested legume seeds. Insect culture was maintained for subsequent assay through regular re-culturing.

Entomotoxic test

For toxicity tests, sterilized petridishes (9-cm diamter) containing 40g of disinfected cowpeas were treated with 2g of plant powders separately. Each of the petridishes from both groups was then infested with 10 newly emerged adult bruchids of both sexes. Each treatment and control were replicated three times. Temperature and relative humidity ranges between 25-30°C and 70-75% respectively. Ten days after the death of the female, observations were made on number of eggs laid, hatched eggs, unhatched eggs were counted and the weight loss after the emergence of adult.

Calculations and data analysis

Data collected on number of eggs laid, number of hatched eggs and unhatched eggs were subjected to analysis of variance using SPSS version 16.0. Where necessary, data were transformed before analysis. Percentage data were arc-sine transformed and data based on count were square root transformed. Abbot formula was also employed to evaluate the efficacy of the botanicals (Abbot, 1925).

Abbort Formula: = (1-Ta/Ca)*100.

Where Ta=Number of eggs in treated petri dish

Ca=Number of eggs in the control petri dish.

Treatment means of the varietal parameters were separated by Tukey at 5% level of significant.

Ovipositional Response of C. maculatus reared on Six Non-cowpea Legumes.

The mean number of eggs laid, hatched, unhatched eggs and percentage pest tolerance of *C. maculatus* screened among the six (6) legume seed types were summarized in Table 5. Mean number of eggs was significantly higher in *Mucuna pruriens* (85.33) than the other legume seed types followed by *Sphenostylis stenocarpa* (72.00) while *Cajanus cajan* (46.67) gave the least. Mean hatched eggs was significantly higher in *Glycine max* (59.67) in than the other legumes, followed by *Vigna subterranean* (44.00), meanwhile *Cajanus cajan* (18.67) had the least. Mean number of unhatched eggs were significantly higher (P>0.05) in *Mucuna pruriens* (48.00), *Sphenostylis stenocarpa* (45.67) *Cajanus cajan* (28.00) and *Vigna subterranean* (27.00) than *Glycine max* (13.53) which recorded the least. A significantly higher (P<0.05) percentage pest tolerance was recorded in *Mucuna pruriens* (99.17), followed by *Sphenostylis stenocarpa* (83.33) and *Cajanus cajan* (67.50) as pest tolerance decreases in Ife Brown (20.83). It was observed that mean number of eggs was significantly higher (P>0.05) in *Mucuna pruriens*, however it recorded the highest percentage pest tolerance as most of the eggs laid were unhatched.

Table 5: Ovipositional Response of *C. maculatus* reared on Non-cowpea Legumes.

Non-cowpea Legumes	Number of eggs laid	Hatched eggs	Unhatched eggs	Percentage pest tolerance
Sphenostylis stenocarpa	72.00ab	26.33ab	45.67a	83.33ab
Glycine max	63.00ab	59.67a	13.53b	50.83cd
Cajanus cajan	46.67b	18.67b	28.00a	67.50bc
Mucuna pruriens	85.33a	37.33ab	48.00a	99.17a
Vigna subterranean	71.00ab	44.00ab	27.00a	30.83de
Ife Brown (control)	53.67ab	42.33ab	11.33bc	20.83e

Means in each column bearing the same letter are not significantly different at the 5% level of probability by Tukey test.

Mean number of eggs laid by C. maculatus on Non-cowpea legume seeds treated with botanical powders and cypermethrin.

Table 10 showed mean number of eggs laid on *Cajanus cajan* ranging from 157.33 to 4.33 across all the treatment and 177.67 with the control. Mean number of eggs was significantly lower on seeds treated with *Piper guineense* (4.33), *Eugenia aromatica* (7.67), Cypermethrin (12.67) and *Zanthoxylum zanthoxyloides* (17.00). This indicates their effectiveness in reducing oviposition by *C. maculatus* on pigeon pea. Maenwhile mean number of eggs were significantly higher ($P<0.05$) in seeds treated with *Ocimum gratissimum* (157.33), *Xylopia aethiopica* (141.00), *Zingiber officinale* (139.00) and *Allium sativum* (124.00). These treatments were proved not to be effective in reducing oviposition by *C. maculatus. Glycine max* had mean number of eggs laid ranged from 151.00 to 8.67 across all the treatment and 96.33 with the control. Mean number of eggs significantly reduced on seeds treated with *Piper guineense* (8.67), *Eugenia aromatica* (11.00) and Cypermethrin (15.67) as a result they were proved effective in reducing oviposition by *C. maculatus.* On the other hand, mean number of eggs were significantly higher ($P<0.05$) in seeds treated with *Ocimum gratissimum* (151.00), *Momordica charantia* (145.00), *Allium sativum* (144.00), *Zingiber officinale* (141.33) and *Xylopia aethiopica* (130.67). These indicated their ineffectiveness in reducing oviposition by *C. maculatus.*

It was also observed that *Vigna subterranea* had mean number of eggs laid to range from 114.67 to 9.00 across all the treatment and 201.33 with the control. Mean number of eggs was significantly lower on seeds treated with Cypermethrin (9.00). This was followed by *Piper guineense* (24.00), *Zanthoxylum zanthoxyloides* (24.00) and *Eugenia aromatica* (28.67). Meanwhile mean number of eggs was significantly higher ($p<0.05$) on seeds treated with *Xylopia aethiopica* (114.67), *Ocimum gratissimum* (106.33), and *Momordica charantia* (96.33). *Sphenostylis stenocarpa* had mean number of eggs ranging from 175.33 to 14.67 across all the treatment and 160.33 with the control. Mean number of eggs was significantly lower treated on seeds treated with Cypermethrin (14.67) than the other treatments. This was followed by *Oryza sativa* (17.33) and *Zanthoxylum zanthoxyloides* (31.33).

On the other hand, seeds treated with *Zingiber officinale* (175.33) increased mean number of eggs significantly. This was followed by *Xylopia aethiopica* (152.67), *Momordica charantia* (135.33) and *Allium sativum* (105.67). They proved ineffective at preventing egg laying in *C. maculatus*. *Mucuna pruriens* had mean number of eggs laid ranging from 142.33 to 3.67 within all the treatments and 62.67 with the control. Mean number of eggs was significantly lower ($P<0.05$) on seeds treated with *Zanthoxylum zanthoxyloides* (3.67). This was followed by Cypermethrin (16.33) and *Oryza sativa* (17.00). Meanwhile mean number of eggs were significantly higher ($P<0.05$) in seeds treated with *Zingiber officinale* (142.33), *Ocimum gratissimum* (106.33) and *Momordica charantia* (129.67). This shows that they were not effective in reducing oviposition by *C. maculatus*.

Table 10: Mean number of eggs laid by *C. maculatus* on Non-cowpea legume seeds treated with botanical powders and cypermethrin.

Plant species	*Cajanus cajan*	*Glycine max*	*Vigna subterranea*	*Sphenostylis stenocarpa*	*Mucuna pueriens*
E. aromatic	7.67d	11.00c	28.67cde	84.67abcd	44.33abc
P. guineense	4.33d	8.67c	24.00cde	82.00abcd	26.00abc
X. aethiopica	141.00ab	130.67 a	114.67ab	152.67ab	90.00abc
Z. officinale	139.00ab	141.33 a	83.33abcd	175.33 a	142.33 a
M. charantia	93.00abc	145.00 a	96.33abc	135.33abc	129.67 a
O. gratissimum	157.33ab	151.00 a	106.33abc	98.00abcd	139.33 a
O. sativa	26.33cd	15.67ab	53.00bcde	17.33cd	17.00bc
Z. zanthoxyloides	17.00d	23.00bc	24.00de	31.33bcd	3.67c
A. sativum	124.00ab	144.00 a	64.00bcde	105.67abcd	57.33abc
N. tabacum	55.00cd	45.67abc	53.33bcde	71.33abcd	104.33ab
Cypermethrin	12.67d	15.67c	9.00e	14.67d	16.33bc
Control	177.67a	96.33ab	201.33 a	160.33 a	62.67abc

Means in each column bearing the same letter are not significantly different at the 5% level of probability by Tukey test

Mean hatched eggs from eggs oviposited by C. maculatus on Non-cowpea legume seeds treated with different botanical powders and cypermethrin.

Table 11 shows mean hatched eggs on *Cajanus cajan* ranging from 140.67 to 2.33 and 153.67 with the control. Mean number of hatched eggs was significantly lower on seeds treated with *Piper guineense* (2.33), *Eugenia aromatica* (5.00), Cypermethrin (11.67) and *Zanthoxylum zanthoxyloides* (14.33). On the other hand, mean hatched eggs were significantly higher (P<0.05) in seeds treated *Ocimum gratissimum* (140.67), *Zingiber 0fficinale* (124.33) and *Xylopia aethiopica* (115.67). They proved ineffective in reducing hatchability in *C. maculatus*. Also, *Glycine max* had mean hatched eggs ranging from 130.67 to 5.00 within all the treatments and 66.33 with the control. Mean Hatched eggs was significantly lower (P<0.05) on seeds treated with *Piper guineense* (5.00) and *Eugenia aromatica* (8.33) among others treatment. This was followed by *Oryza sativa* (11.67), Cypermethrin (13.33) and *Zanthoxylum zanthoxyloides* (14.33). Meanwhile seeds treated with *Allium sativum* (130.67) were significantly higher in hatched eggs followed by *Momordica charantia* (98.67) and *Zingiber officinale* (89.67).

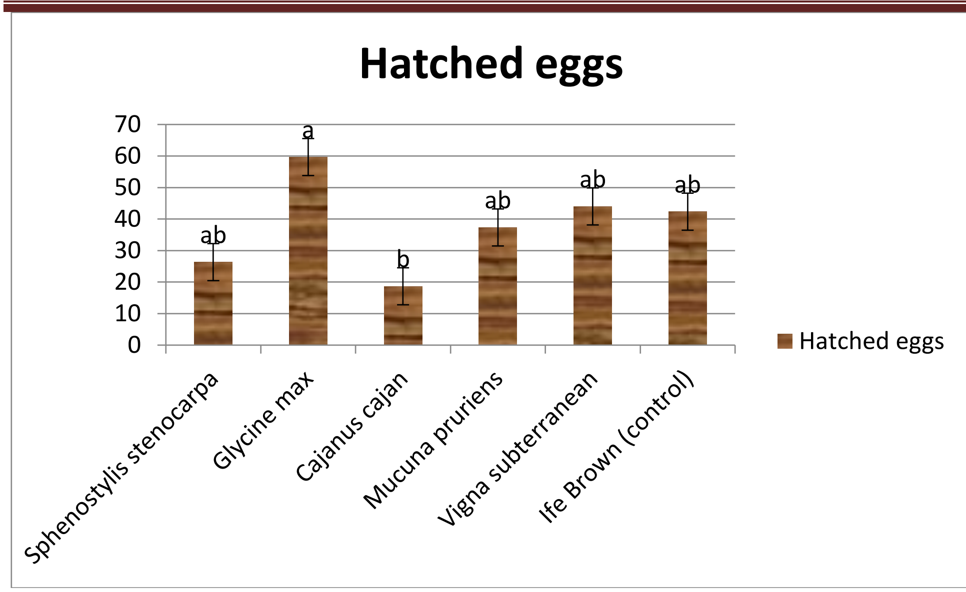
Vigna subterranean had mean hatched eggs ranging from 96.67 to 6.00 in all the treatments and 171.33 with the control. Mean hatched eggs was significantly lower (P<0.05) on seeds treated with Cypermethrin (6.00) as expected, followed by *Zanthoxylum zanthoxyloides* (17.00). On the other mean hatched eggs were significantly higher (P<0.05) in seeds treated with *Xylopia aethiopica* (96.67), *Momordica charantia* (89.33) and *Zingiber officinale* (70.67). They proved ineffective in reducing hatchability of eggs laid by *C. maculatus*. *Sphenostylis stenocarpa* had mean hatched egss ranging from 119.67 to 11.67 in all the treatment and 119.00 with the control. Mean hatched eggs was significantly lower (P<0.05) on seeds treated with Cypermethrin (11.67) and *Oryza sativa* (12.67). Meanwhile seeds treated with *Xylopia aethiopica* (119.67) had significantly higher (P<0.05) mean hatched eggs than the other treatment. This was followed by *Zingiber officinale* (108.33), *Momordica charantia* (104.00), *Allium sativum* (80.67) and *Ocimum gratissimum* (78.33). They proved ineffective in reducing hatched eggs in *C. maculatus*.

Mucuna pruriens had mean hatched eggs ranging from 109.67 to 2.33 in all the treatment used and 39.67 with the control. Mean hatched eggs was significantly lower on seeds treated with *Eugenia aromatica* (2.33) and *Zanthoxylum zanthoxyloides* (2.67). On the other hand, seeds treated with *Zingiber officinale* (109.67) and *Momordica charantia* (95.67) had significantly higher (P<0.05) mean hatched eggs indicating their ineffectiveness in reducing hatched eggs in *C. maculatus.*

Table 11: Mean hatched eggs from eggs oviposited by *c. maculatus* on Non-cowpea legume seeds treated with different botanical powders and cypermethrin

S/N	Plant species	*Cajanus cajan*	*Glycine max*	*Vigna subterranea*	*Sphenostylis stenocarpa*	*Mucuna pueriens*
1	*E. aromatic*	5.00d	8.33d	23.33bcd	57.67ab	2.33c
2	*P. guineense*	2.33d	5.00d	22.67bcd	27.00ab	5.00bc
3	*X. aethiopica*	115.67 a	98.67ab	96.67ab	119.67 a	52.00abc
4	*Z. officinale*	124.33 a	89.67ab	70.67abc	108.33ab	109.67 a
5	*M. charantia*	84.33abc	103.33ab	89.33ab	104.00ab	95.67 a
6	*O. gratissimum*	140.67 a	82.67ab	93.67ab	78.33ab	94.67ab
7	*O. sativa*	24.00cd	11.67cd	49.33bcd	12.67b	13.67abc
8	*Z. zanthoxyloides*	14.33d	14.33cd	17.00cd	18.33ab	2.67c
9	*A. sativum*	103.00ab	130.67 a	55.33bcd	80.67ab	54.00abc
10	*N. tabacum*	35.00bcd	38.33bcd	45.67bcd	12.67ab	70.00abc
11	Cypermethrin	11.67d	13.33cd	6.00d	11.67b	11.33abc
12	Control	153.67 a	66.33abc	171.33 a	119.00 a	39.67abc

Means in each column bearing the same letter are not significantly different at the 5% level of probability by Tukey test.



Mean unhatched eggs from eggs oviposited by C. maculatus on Non-cowpea legume seeds treated with different botanical powders and cypermethrin.

Mean unhatched eggs among all the treatment used on the different legume seed types were summarized in Table 12. It was observed that *Cajanus cajan* had unhatched eggs ranging from 25.33 to 1.00 across all the treatment with the control recording 24.00. Unhatched eggs were significantly lower (P<0.05) on seeds treated with Cypermethrin (1.00) and *Piper guineense* (2.00) similar result was observed with seeds treated with *Oryza sativa* (2.33), *Eugenia aromatica* (2.67) and *Zanthoxylum zanthoxyloides* (2.67). Meanwhile unhatched egg was significantly higher on seeds treated with *Xylopia aethiopica* (25.33), *Allium sativum* (21.00) and *Nicotiana tabacum* (20.00) than the other treatment.

Glycine max had mean unhatched eggs ranging from 61.67 to 2.33 across all the treatment with the control reading 30.00. Mean unhatched eggs was significantly lower (P<0.05) on seeds treated with Cypermethrin (2.33), *Eugenia aromatica* (2.67), *Piper guineense* (3.67), *Oryza sativa* (4.00) and *Nicotiana tabacum* (7.33). On the other hand, mean unhatched eggs significantly higher in seeds treated with *Ocimum gratissimum* (61.67). This was followed by *Zingiber officinale* (51.67) and *Momordica charantia* (41.67).
Also, *Vigna subterranean* had unhatched eggs ranging from 18.00 to 1.33 across all the treatment with the control recording 30.00. Seeds treated with *Piper guineense* (1.33), Cypermethrin (3.00) *Oryza sativa* (3.67) and *Eugenia aromatica* (5.33) had significantly lower number of unhatched eggs. Meanwhile seeds treated with *Xylopia aethiopica* (18.00), *Zingiber officinale* (12.67) and *Ocimum gratissimum* (12.67) had significantly higher (P<0.05) unhatched eggs.

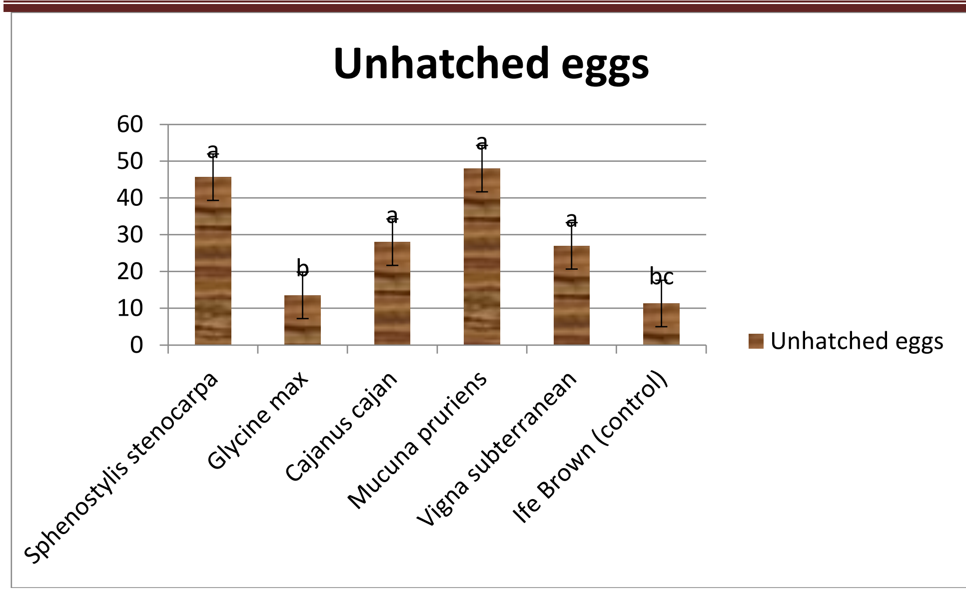
Sphenostylis stenocarpa had unhatched eggs ranging from 67.00 to 3.00 across all the treatment and 41.33 with the control. Mean unhatched eggs was significantly lower on seeds treated with seeds treated with Cypermethrin (3.00) and *Oryza sativa* (4.67). This was followed by *Zanthoxylum zanthoxyloides* (13.00). Mean unhatched eggs increased significantly on seeds treated with *Zingiber officinale* (67.00), *Nicotiana tabacum* (58.67), *Eugenia aromatica* (57.67) and *Piper guineense* (55.00) had than the other treatments.
It was discovered that *Mucuna pruriens* had mean unhatched eggs ranging from 44.67 to 1.00 across all the treatment and 23.00 with the control. Mean unhatched eggs was significantly lower on seeds treated with

seeds treated with *Zanthoxylum zanthoxyloides* (1.00). This was followed by *Oryza sativa* (3.33) and Cypermethrin (5.00). Meanwhile seeds treated with *Ocimum gratissimum* (44.67) had significantly higher (P<0.05) number of unhatched eggs followed by *Eugenia aromatica* (42.00), *Xylopia aethiopica* (38.00), *Momordica charantia* (34.33) and *Zingiber Officinale* (32.67).

Table 12: Mean unhatched eggs from eggs oviposited by *C. maculatus* on legume seeds treated with different botanical powders and cypermethrin.

S/N	Plant species	*Cajanus cajan*	*Glycine max*	*Vigna subterranean*	*Sphenostylis stenocarpa*	*Mucuna pueriens*
1	*E. aromatic*	2.67cd	2.67c	5.33b	57.67abc	42.00ab
2	*P. guineense*	2.00d	3.67c	1.33b	55.00abc	21.00abcd
3	*X. aethiopica*	25.33ab	32.00abc	18.00ab	33.00abcd	38.00ab
4	*Z. officinale*	14.67abcd	51.67ab	12.67ab	67.00ab	32.67abc
5	*M. charantia*	8.67bcd	41.67ab	7.00b	31.33abcd	34.00abc
6	*O. gratissimum*	16.67abcd	61.67a	12.67ab	19.67bcd	44.67a
7	*O. sativa*	2.33cd	4.00c	3.67b	4.67d	3.33cd
8	*Z. zanthoxyloides*	2.67cd	8.67c	7.00b	13.00cd	1.00d
9	*A. sativum*	21.00abc	13.33bc	8.67ab	25.00bcd	3.33bcd
10	*N. tabacum*	20.00abc	7.33c	7.67ab	58.67ab	34.33abc
11	Cypermethrin	1.00d	2.33c	3.00b	3.00d	5.00bcd
12	Control	24.00ab	30.00abc	30.00a	41.33abc	23.00abcd

Means in each column bearing the same letter are not significantly different at the 5% level of probability by Tukey test.



Mean percentage weight loss observed on Non-cowpea legume seeds treated with different botanical powders.

The percentage weight losses of the different legume seed type after application of the insecticides were documented in Table 13. It was observed that *Cajanus cajan* had percentage weight loss ranging from 6.43 to 0.4 across all the treatment and 5.07 with the control. There was no significant difference observed in percentage weight loss among all the treatment. However, *Allium sativum* (6.43) and *Zingiber officinale* (5.3) had increased percentage seed weight loss while seeds treated with *Piper guineense* (0.40) and *Zanthoxylum zanthoxyloides* (0.83) gave the least percentage seed weight loss. *Glycine max* had percentage weight loss ranging from 2.17 to 0.00 across all the treatment. No percentage weight loss was observed on seeds treated with *Eugenia aromatica* (0.00) *Piper guineense* (0.00) and *Xylopia aethiopica* (0.00). There was no significant difference among all the treatment used except the control which was significantly higher in percentage weight loss.

Also, *Vigna subterranean* had percentage weight loss ranging from 20.53 to 0.00 among all the treatment and 14.00 with the control. No weight loss was recorded in seeds treated with *Eugenia aromatica* (0.00) while seeds treated with Cypermethrin (3.17) had least percentage weight loss. Meanwhile percentage weight loss was significantly higher ($P<0.05$) on seeds treated with *Xylopia aethiopica* (21.50) and *Ocimum gratissimum* (20.53). *Sphenostylis stenocarpa* had percentage weight loss ranging from 3.37 to 1.63 across all the treatment applied and 2.83 with the control. There were no significant differences in percentage weight loss among all the treatment applied. *Mucuna pruriens* had zero percentage weight loss among all the treatments applied. This indicate that *Mucuna pruriens* is highly resistant to *C. maculatus* because despite the cowpea beetle laid so much eggs on the seed the insect could not bore a single hole on the seed.

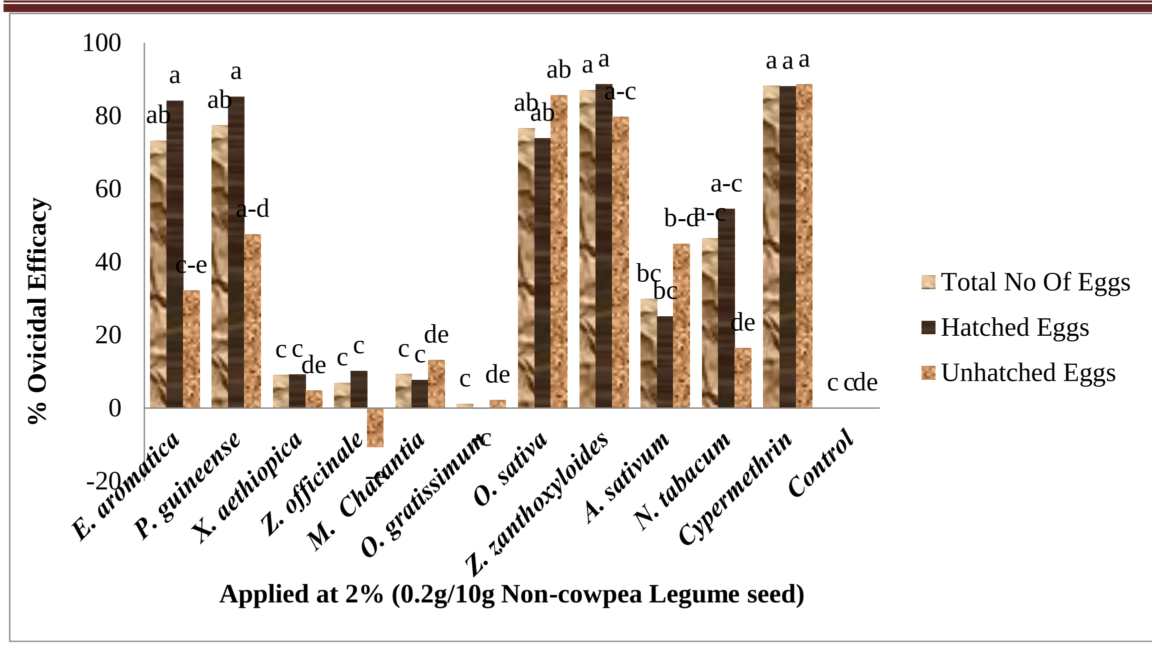
Table 13: Effect of cypermethrin dust and different plant powders on the percentage weight loss of different legume seed type.

S/N	Plant species	*Cajanus cajan*	*Glycine max*	*Vigna subterranean*	*Sphenostylis stenocarpa*	*Mucuna pueriens*
1	*E. aromatic*	1.3a	0.00b	0.00b	1.67a	0.00
2	*P. guineense*	0.4a	0.00b	13.77ab	1.87a	0.00
3	*X. aethiopica*	3.4a	0.00b	21.50a	2.23a	0.00
4	*Z. officinale*	5.3a	0.17b	6.53ab	2.60a	0.00
5	*M. charantia*	2.43a	1.53b	14.37ab	1.87a	0.00
6	*O. gratissimum*	4.6a	1.20b	20.53a	3.37a	0.00
7	*O. sativa*	1.03a	0.00b	6.67ab	2.57a	0.00
8	*Z. zanthoxyloides*	0.83a	1.23b	6.77ab	1.67a	0.00
9	*A. sativum*	6.43a	2.17b	5.30ab	3.30a	0.00
10	*N. tabacum*	4.27a	0.43b	10.03ab	1.63a	0.00
11	Cypermethrin	1.27a	0.50b	3.17ab	3.13a	0.00
12	Control	5.07a	12.33a	14.00ab	2.83a	0.00

Means in each column bearing the same letter are not significantly different at the 5% level of probability by Tukey test

Ovicidal effects of the botanical powders on eggs laid by C. maculatus on non-cowpea legume seeds.

Ovicidial efficacy of all the botanicals across the legume seed types were compared and summarized in figure 2. Ovicidal efficacy of the botanicals studied ranged from 88.16% to 1.11% with control at 0.00%. It was observed that ovicidal efficacy was significantly higher ($P<0.05$) in seeds treated with Cypermethrin (88.16%), *Zanthoxylum zanthoxyloides* (87.02%), *Piper guineense* (77.36%) and *Eugenia aromatica* (73.21%). Ovicidal efficacy among the legume types followed the same trend as that of the cowpea varieties studied amist the the aforementioned treatments. Meanwhile seeds treated with *Momordica charantia* (9.29%), *Xylopia aethiopica* (9.07%), *Zingiber officinale* (6.88%) and *Ocimum gratissimum* (1.11%) showed reduced ability in deterring oviposition in *C. maculatus.*



Means in each column bearing the same letter are not significantly different at the 5% level of probability by Tukey test.

Figure 1: **Ovicidal effects of the botanical powders on eggs laid by *C. maculatus* on non-cowpea legume seeds.**

Discussion

The present study showed that the ovipositional preference of female *Callosobruchus maculatus* varied among different types of legumes. The result of the present study has shown that some of the legume types evaluated show various degrees of resistance and susceptibility to adult *C. maculatus* attack. This supports the findings of Oke and Olajire (2012), who reported that the cowpea varieties studied, exhibited some levels of resistance and susceptibility to *Callosobruchus maculatus*. The use of plants resistant to insect attack represents a promising alternative to reduce damage, considering that plants produce chemical defenses, such as protease inhibitors, that can inhibit insect enzymes (Kuroda *et al*., 1996). Insects of the family Bruchidea have as their main digestive enzymes serine and cysteine protease. The grains of cowpea plants grown with different nitrogen sources were assimilated differently by the insects. Various type of plant proteins has been identified as being toxic to different groups of insects, for example plant lectins. These insects discriminate grains containing these proteins. Factors such as hardness of grains, seeds size, lack of nutritional factors and presence of toxic substances may decrease the damage of *C. maculatus* to cowpea seeds. The grains containing sugars, organic acids, amino acids, proteins and peptides, flavinoids, anthocyanins, alkaloids, terpenes and steroids, with a great amount of nitrogen available, it will be possible to produce more of these molecules that can participate in the defense mechanisms of the plant. Varieties of *V. unguiculata* resistant to bruchids are rich in phenolics, alkaloids and terpenes. Result obtained from this study shows, there were significant difference in the levels of resistance and susceptibility among the legume seed types to *Callosobruchus maculatus*. *Mucuna pruriens* was discovered to be the most resistant to *C. maculatus* followed by *Sphenostylis stenocarpa*, *Cajanus cajan* and *Glycine max*.

The least resistant or susceptible legume was discovered to be Ife Brown. With scanning electron microscopy, it was revealed that; solid and compressed subepidermal sclereids were found in resistant accessions of cowpea while longitudinal ridges of subepidermal sclereids were found in susceptible materials (Ramirez, Ma. C. F; Rasco, E. T. Jr, 2015). Since seed properties including seed testa colour, mass, size and moisture content generally do not influence the susceptibility of cowpea seeds and other cereals grains to *C. maculatus* in storage (Maina and Lale, 2005; Maina and Dlamini, 2009), the above observed differences were very likely due to variations in the composition or levels of chemical substances that either deter or stimulate bruchid oviposition and/or feeding in these seeds. Alternatively, seed properties such as seed coat texture (smooth or rough) and hardness might have contributed to the differences observed. For instance, studies by Messina and Renwick (1985) that evaluated the resistance of selected cowpea lines to infestation by *C. maculatus,* found that rough seed coat was less preferred for oviposition by the bruchid beetle.

Ovicidal efficacy of the Botanicals across all the legume seeds

Earlier literature indicated the importance of plant extract in protecting seeds by way of direct mixing of the dried leaves, plant powders, solvent extracts, vegetable essential oils on seeds during post-harvest storage (Ngamo *et al.*, 2007). The justification for the comparison of *Eugenia aromatica, Piper guineense, Zanthoxylum zanthoxyliodes* and *Oryza sativa* in the control of *C. maculatus* had been predicated on claims by many authors of their effectiveness in grain protection against pest depredation in storage. It is unequivocal that the powders made from these plants manifested insecticidal action against *C. maculatus* in this study. From this study it was proved that powders made from *Eugenia aromatica, Piper guineense, Zanthoxylum zanthoxyliodes* and *Oryza sativa* (rice husk ash) at 0.2g/10g of legume seed types were found to have insecticidal effect on *Callosobruchus maculatus* in stored legume seeds. From the result obtained it was obvious that *Zanthoxylum zanthoxyliodes* performed as the best botanicals to use in preserving legumes from weight loss and consequently damage caused by *C. maculatus.* These observations confirm that of Mbata (1993) who reported that weight loss is generally highly correlated with susceptibility index.

It was also observed that *Zanthoxylum zanthoxyliodes* could compete favourably with cypermethrin in its ovicidal efficacy against *C. maculatus.* The toxic effect of *Zanthoxylum zanthoxyloides* could be linked to the presence of secondary phenolic compound known as zanthoxylol and this had been reported to have mortality and ovicidal effect on stored product insect pests (Udo, 2011; Akinneye and Ogungbite, 2013). It was also discovered that *Zanthoxylum* spp. contain cuminic aldehyde. Hydroxy-alpha sanshool is a bioactive component of plants from the *Zanthoxylum* genus, including the Sichuan pepper. The relative amounts of these chemicals, their interactions and volatility of products may determine the observed bioactivities of the plants against *C. maculatus.* Some common chemicals such as limonene, eugenol and cineole found in *Eugenia* and *piper* may explain their similar action against the insects.

Some the botanical powders *Ocimum gratissimum*, *Xylopia eithopica*, *Zingiber officinale*, *Momordica charantia*, *Allium sativum* and *Nicotiana tabacum* were significantly ineffective in protecting legumes which may be due to hormoligosis. Lale (1991) reported, sub-lethal doses of botanical insecticides stimulate female oviposition and also behavioral *hormoligosis* in oviposition preference was reported by Faten A. El-Daly, (2008).

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