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# Effectiveness of botanical powders against Callosobruchus maculatus (Coleoptera: Bruchidae) in some stored leguminous grains under laboratory conditions

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The study investigated the comparative efficacy of some botanicals (*Aframomum melegueta* (K. M. Schumann) seed, *Capsicum nigrum* (L.) seed, *Allium sativum* (Woodwill) bulb, *Zingiber officinale* (Roscoe) rhizome, *Azadiracta indica* (A. Juss) leaves and *Ocimum gratissimum* (L.) leaves) and pirimiphos methyl powder in the suppression of *Callosobruchus maculatus* (Fabricius) damage in some stored legume (*Vigna unguiculata* (L.), *Vigna subterranea* (L.) and *Cajanus cajan* (L.) seeds. The botanicals were applied at the rate of 0 and 1 g. The pirimiphos methyl powder was applied at 0.1 g. The experiment was laid out in split plot design of eight treatments replicated ten times. Five pairs of one-day old adult *C. maculatus* were introduced into each jar. Mortality of adult *C. maculatus* was recorded daily. *O. gratissimum* was more effective in causing *C. maculatus* mortality. The LD<sub>50</sub> revealed *O. gratissimum* powder to be the most effective biopesticide. All treatments recorded higher significant (p<0.05) mortality than the experimental control. The proximate analysis of the legumes revealed that alkaloids, steroids, glycosides and terpenoids were present in the botanicals. Out of the six botanicals investigated, *O. gratissimum* powder was the most effective biopesticide and thus recommended.

**Key words:** Botanical powders, phytochemicals, *Callosobruchus maculatus,* stored leguminous grains, mortality, proximate composition.

# INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* is a cosmopolitan polyphagous pest in most tropics and subtropics (Booth et al., 1990; Bagheri, 1996). This weevil is reported to be the most damaging pest of legume seeds and its larva infest grains such as cowpea, chickpea, Bambara groundnut, green gram, lentil, broad bean and green pea. Insect infestation is a major contributor to quality deterioration of durables legumes, pulse, roots and tubers stored in warm and humid

climates. Considerable physical and nutritional losses sustained in Nigeria are due to infestation of stored food products by bruchid weevils and other insects. Apart from the detrimental economic impact, these losses pose a major threat to food security. Currently, insect control in stored products relies primarily upon the use of gaseous synthetic fumigants and residual insecticides, both of which may pose serious hazards to humans and the environment. Residues of methyl bromide, one of the two synthetic fumigants still used in the disinfestations of stored foods, have been found to exhibit carcinogenic effects in rats (Dansi et al., 1984).

The growth of agriculture based economies of the world depends on the sustained supply of quality seed. Pulses

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have a prominent place in daily diet as a rich source of vegetable protein, minerals and vitamin B. They are of special significance to the people in developing countries who may not always afford animal protein in adequate quantities. Among the pulses, cowpea, Bambara groundnut and pigeon pea are the most common and important legume crops in the world (Mahdi and Rahman, 2008). Pulse seeds suffer a great damage during storage due to insect attack (Sherma, 1989). The extent of damage of *C. maculatus* to pulse seeds is very high both qualitatively and quantitatively (Atwal, 1976). С maculatus alone accounts for over 90% of the damage done to stored cowpea seeds by insects (Caswell, 1981). According to van Huis (1991), the control of storage insect pests using fumigants and /or residual insecticides should be discouraged, and this necessitated the search for alternative sources for the containment of storage insect pests (Dike and Mshelia, 1997; Yusuf et al., 1998). Therefore, an option that can produce satisfactory result in an acceptable and feasible manner to the farmers is necessary to achieve the desired goal. The use of plants and minerals as traditional protectants of stored products is an old practice used all over the world. Some of this knowledge has been neglected over past decades. However, there is an increasing interest and necessity to re-visit such knowledge (Stoll, 2000). In Nigerian traditional grain storage, Aframomum melegueta seed, Capsicum nigrum seed, Allium sativum bulb, Zingiber officinale rhizome, Azadiracta indica leaves and Ocimum gratissimum leaves are often employed singly or in combination as seed / grain protectants with different but encouraging results, this informed the choice of the plant materials.

This research was therefore designed to study the efficiency of six botanicals and a synthetic pesticide on their ability to control *C. maculatus* in stored leguminous grains and to proximately determine nutrient lose in grains arising from weevil infestation.

## MATERIALS AND METHODS

#### Legume grains

1 kg each of dry cowpea seed (*Vigna unguiculata*), Bambara groundnut *Vigna subterranea* and pigeon pea (*Cajanus cajan*) were collected from Crop Science Department Farm Shop and identified (Keay et al., 1964). The grains were fumigated for 24 h with phostoxin tablets (D & D Holdings, USA) in order to kill the resident insect pest. The seeds were then exposed to air in a try covered with muslin cloth for 48 h to get rid of the gas and then sieved with a 2 mm sieve to remove dead insects, exuvia and frass. The processed grains were then packaged in polythene bags and kept pending use.

#### Callosobruchus maculatus

The methods of Janzen (1977) and Ousman et al. (2007) were adopted in the mass production of *C. maculatus*. The mass production of *C. maculates* took place in the Applied Entomology

Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria with relative humidity and temperature of 66.6% and  $28 \pm 5^{\circ}$ C, respectively. Hundred adults of mixed sex (male and female) of *C. maculatus* were obtained from a laboratory stock culture and reared on 100 g each of *V. unguiculata*, *V. subterranea* and *C. cajan* in glass jars covered with muslin cloth. The food media were the whole leguminous grains selected. After one week, when oviposition had been noticed, the parent stocks of *C. maculatus* were removed by sieving the grain with a 2.00 mm sieve. The grains with the oviposited ova were left under laboratory conditions still emergence of F<sub>1</sub> progeny. The F<sub>1</sub> progenies from the cultures were used for the experiment.

#### **Plant materials**

The plant materials evaluated for biopesticidal activity against C. maculatus, the parts used and other pertinent information are provided in Table 1. The plant materials used for this study were collected from International Centre for Ethno-Medicine and Drug Development (InterCEDD), Nsukka, Nigeria and identified to species level (Keay et al., 1964). The voucher specimens numbers; AM2011, CN2011, AS2011, ZO2011, OG2011 and Al2011 were kept in the Herbarium, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria for reference purposes. The plant materials were shade-dried for three weeks until a constant weight was maintained. They were fined ground and sieve into powder using 0.20 mesh sieves (Lale, 2000). They were preserved in plastic air-tight bottles under refrigeration  $(4 \pm 2^{\circ}C)$  until needed. The pirimiphos methyl powder (synthetic compound) used in the experiment was purchased from Zhejiang Linghua, China.

#### Phytochemistry of plant materials

The 5 g of each powdered plant material was weighed using an electronic balance and mixed in 25 ml of distilled water, boiled at 60°C for 30 min on water bath and then filtered through Whatman No. 1 filter paper. The filtrates were centrifuged at 2500 rpm for 15 min, the supernatants were discarded and the residues stored in sterile bottles at 5°C (Harbone, 1973) pending qualitative phytochemical analysis to ascertain levels of alkaloids, glycosides, saponins, tannins, sugar, steroids, terpenoids, acidic compounds, flavonoids and resins in the botanicals (AOAC, 2000).

### **Experimental design**

Split plot design of eight treatments replicated ten times was used. Each of the six botanicals was used on the three different legume grains selected. A total of 240 jars (with diameter 0.09 m, volume 3.69 m<sup>3</sup>) were used for the experiment. Five pairs (five males and five females) of zero day old C. maculatus were introduced into each of the jars already with 20 g of grains (either V. subterranean / C. cajan / V. unguiculata) and 1 g of botanical (either C. nigrum / A. melegueta / O. gratissimum / A. indica / A. sativum / Z. officinale). The pirimiphos methyl powder (PMP) was applied at the rate of 0.1 g per 20 g of each legume grain (standard control). Another control group was set up with the grains and C. maculatus but no botanical (experimental control). Each jar was covered with a muslin cloth to allow air movement and prevent insects from leaving the jar. The set up was allowed for four days with daily monitoring. Dead insects in each jar was collected and counted and the percentage insect mortality was calculated thus: number of dead insect per jar x 1 divided by 100. The reason for the short period of experiment was because it has been established that adult C. maculates live and

Scientific name of plants	Common name	Local name (Igbo)	Family	Parts used
Aframomum melegueta	Grain of paradise	Ose oji	Zingibaraceae	Seed
Capsicum nigrum	Chilli pepper	Ose okpo	Solanaceae	Seed
Allium sativum	Garlic	Yabasi igbo	Liliaceae	Bulb
Zingiber officinale	Ginger	Osisi-ukpo	Zingibaraceae	Rhizome
Ocimum gratissimum	Scent leaf	Nchianwu	Lamiaceae	Leaves
Azadiracta indica	Neem	Dogo yaro	Meliaceae	Leaves

Table 1. List of botanicals tested for their effectiveness against Callosobruchus maculatus in some stored leguminous grains.

Table 2. Phytochemical composition of varied botanicals studied.

Parameter	Azadiracta indica	Aframomum melegueta	Capsicum nigrum	Allium sativum	Zingiber officinale	Ocimum gratissimum
Alkaloids	+	+++	++++	++++	++++	+
Glycosides	+++	+++	+++	+++	++++	++++
Saponins	++	-	+	+	-	+++
Tannins	++	++++	-	-	-	++
Reducing sugar	+	++	+++	+	+	++
Steroids	+	++++	+	+++	++	+
Terpenoids	+	++++	+	+++	++	+
Acidic compounds	-	-	-	+	-	-
Flavonoids	+	++++	+	-	++	+++
Resins	+	+	++++	-	+	+

-, Not present; +, present in very small concentration; ++, present in moderately high concentration; +++, present in very high concentration; ++++, abundantly present.

die within eight days under normal conditions.

least significant differences at p < 0.05.

### LD<sub>50</sub> of plant materials

The 50% lethal dose of the six botanicals was done using the methods described by Don-Pedro (1989); Ousman et al. (2007). The concentration of various botanicals that killed 50% of the 30 *C. maculatus* exposed to it every 24 h for a period of 72 h was recorded.

#### **Proximate composition**

Proximate analysis of the legumes used for the study before and after insect infestation was done (AOAC, 2000) to determine the percentage nutrient (moisture, ash, fat, crude fibre, protein and carbohydrate) benefit or loses due to *C. maculatus* infestation. Infested legumes were cleaned and sieved to remove *C. maculatus* and its body parts. Later, 10 g of each infested but cleaned legumes were drawn in triplicate for determination of their proximate compositions.

#### Data analysis

Descriptive statistics was employed to ascertain mean percentage mortality for the eights treatments. One way analysis of variance (ANOVA) was carried out to compare differences in treatment means. Significant treatment means were separated using Fisher

## RESULTS

The phytochemical studies of the six powdered plant materials used in this study indicated that alkaloids were abundantly present in C. nigrum, A. sativum and Z. officinale; it was also found to be present in high concentrations in A. melegueta and present in very small concentrations in *A. indica* and *O. gratissimum*. Similarly, glycosides were found to be abundantly present in Z. officinale and O. gratissimum, present in high concentrations in A. indica, A. melegueta, C. nigrum and A. sativum. Moreso, saponins were present in high concentration in O. gratissimum, moderately present in A. indica, present in very small concentration in C. nigrum and A. sativum and absent in A. melegueta and Z. officinale (Table 2). Tannins were found to be abundantly present in A. melegueta, moderately present in A. indica and O. gratissimum and absent in C. nigrum, A. sativum and Z. officinale. Furthermore, reducing sugar was present in high concentration in C. nigrum, moderately present in A. melegueta and O. gratissimum, present in very small concentration in A. indica, A. sativum and Z. officinale. Steroids and terpenoids were abundantly present in A. melegueta, highly present in A. sativum,

Deterical	Grain	Lethal dose (LD <sub>50</sub> )		
Botanical		24 h	48 h	72 h
	Vigna subterranea	232.90	191.06	52.24
Capsicum nigrum	Cajanus cajan	195.50	175.08	31.62
	Vigna unguiculata	300.50	224.22	91.24
	Vigna subterranea	574.49	251.57	85.00
Aframomum melegueta	Cajanus cajan	774.93	625.77	87.76
	Vigna unguiculata	881.36	783.52	97.27
	Vigna subterranea	224.65	194.85	39.62
Ocimum gratissimum	Cajanus cajan	345.88	193.70	54.67
	Vigna unguiculata	588.12	422.12	77.06
	Vigna subterranea	397.16	218.48	32.50
Azadiracta indica	Cajanus cajan	570.38	120.64	32.73
	Vigna unguiculata	992.23	246.35	53.09
	Vigna subterranea	113.71	100.13	29.11
Zingiber officinale	Cajanus cajan	186.53	159.25	39.97
	Vigna unguiculata	240.93	196.31	52.52
	Vigna subterranea	317.91	139.92	30.37
Allium sativum	Cajanus cajan	431.33	107.20	47.10
	Vigna unguiculata	843.41	150.99	49.93

**Table 3.** Lethal dose (LD<sub>50</sub>) of various botanicals against *Callosobruchus maculatus* in some stored leguminous grains.

moderately present in *Z. officinale* and present in small concentration in *A. indica, C. nigrum* and *O. gratissimum.* Acidic compounds were not present in the biopesticides studied, except in *A. sativum* where it was present in very small concentration. Flavonoids were abundantly present in *A. melegueta*, present in high concentration in *O. gratissimum*, moderately present in *Z. officinale*, present in very small concentration in *A. indica* and *C. nigrum* and was absent in *A. sativum*. Lastly, resins were abundantly present in *C. nigrum*, present in very small concentration in *A. indica, A. melegueta, Z. officinale* and *O. gratissimum* and was absent in *A. sativum* (Table 2).

The LD<sub>50</sub> showed that the percent mortality was concentration, substrate and time dependent, with higher concentration producing 50% mortality at shorter time (Table 3). For instance, a high dose of C. nigrum powder caused 50% mortality of C. maculatus inhabiting V. subterranea within 24 h, while a much lower dosage of C. nigrum produced similar mortality within 72 h in C. cajan. Furthermore, lower dosage of C. nigrum powder took longer time to caused 50% mortality in V. subterranea, while high dosage took shorter time to produce the similar percentage mortality. In V. unguiculata infested by C. maculatus, C. nigrum powder took longer time to caused 50% mortality while high dosage took shorter time to produce the similar percentage mortality (Table 3). Similarly, A. indica powder caused time, dosage and substrate dependent mortalities of C. maculatus inhabiting V. subterranea, C. cajan and V. unguiculata with higher dosage killing quicker than lower dosages. The most susceptible *C. maculatus* to *A. indica* powder was those infesting *V. subterranea* followed by those in *C. cajan* and *V. unguiculata*, respectively. The mortality of *C. maculatus* infesting different grains exposed to *Z. officinale* powder was concentration, substrate and time dependent, with higher concentration producing 50% mortality at shorter time and lower concentrations producing similar mortalities at longer time. *C. maculatus* inhabiting *V. subterranea* followed by those in *C. cajan* and *V. unguiculata* were more susceptible to *Z. officinale* powder (Table 3). Amongst the grains sampled, greater quantities of biopesticides was required to cause 50% mortality of *C. maculatus* inhabiting *V. unguiculata* more than other grains.

There was increased mortality of C. maculatus inhabiting the grains corresponding to increases biopesticide applied over the four days exposure period (Table 4). Mortality of C. maculatus was dependent on the bio-efficacies of the botanicals used, where botanicals with higher aromatic compounds produced more pest mortalities. In V. subterranea grain infested with C. maculatus, the efficacy of the biopesticide powders tested were in the order, O. gratissimum > A. melegueta > A. indica > A. sativum > Z. officinale and lastly C. nigrum. Furthermore, in C. cajan grain infested with C. maculatus, the efficacy of the biopesticide powders tested were in the order, Z. officinale > O. gratissimum > C. nigrum > A. indica > A. sativum > and lastly A. melegueta. In V. unguiculata grain infested with C. maculatus, the efficacy of the biopesticide powders

Grain	Botanical and standard insecticide	Concentration (g)	Mortality (%)
Vigna subterranea	Capsicum nigrum	1	22.73 ± 1.41
	Aframomum melegueta	1	27.26 ± 2.86
	Ocimum gratissimum	1	29.01 ± 2.90
	Azadiracta indica	1	26.36 ± 2.44
	Zingiber officinale	1	24.53 ± 2.05
	Allium sativum	1	$25.00 \pm 2.47$
	Pirimiphos methyl	0.1	49.02 ± 2.86
	Control	-	14.55 ± 1.57
	Capsicum nigrum	1	22.73 ± 1.41
	Aframomum melegueta	1	17.27 ± 1.21
	Ocimum gratissimum	1	23.00 ± 1.35
Onin marine	Azadiracta indica	1	20.01 ± 1.60
Cajanus cajan	Zingiber officinale	1	20.91 ± 0.91
	Allium sativum	1	22.73 ± 1.41
	Azadiracta indica1Zingiber officinale1Allium sativum1Pirimiphos methyl0.1Control-	0.1	38.61 ± 1.32
	Control	-	11.24 ± 2.08
	Capsicum nigrum	1	$23.73 \pm 3.04$
	Aframomum melegueta	1	21.82 ± 1.22
	Ocimum gratissimum	1	37.29 ± 3.29
Vieno unquiouloto	Azadiracta indica	1	24.55 ± 1.57
Vigna unguiculata	Zingiber officinale	1	26.00 ±2.04
	Allium sativum	1	26.36 ± 3.10
	Pirimiphos methyl	0.1	50.92 ± 3.68
	Control	-	9.09 ± 2.51
F-LSD <sub>(0.05)</sub>			6.69
P-value			0.01

Table 4. Mortality of *Callosobruchus maculatus* in three leguminous grains over a four days study in some stored leguminous grains.

**Table 5.** Combined effect botanicals on mortality of *Callosobruchus* 

 maculatus in leguminous grains over a four days period.

Botanical and standard insecticide	Mortality
Control	$34.88 \pm 6.16^{a}$
Aframomum melegueta	$66.35 \pm 5.29^{b}$
Capsicum nigrum	$69.19 \pm 5.86^{b}$
Zingiber officinale	$71.44 \pm 5.00^{\circ}$
Azadiracta indica	70.92 ± 5.61 <sup>°</sup>
Allium sativum	$74.09 \pm 6.98^{\circ}$
Ocimum gratissimum	$89.30 \pm 7.54^{d}$
Pirimiphos methyl	138.55 ± 7.86 <sup>e</sup>

Different letters on the same column = significantly different means (p < 0.05).

tested were in the order, *O. gratissimum* > *A. sativum* > *Z. officinale* > *A. indica* > *C. nigrum* and lastly *A. melegueta.* The percentage mortality of the tested biopesticides was higher than the mortality for the experimental control, but lower than the standard control

(Table 4). The combine effect of botanicals on mortality of *C. maculatus* estimated from pooled data indicated that *O. gratissimum* followed by *A. sativum* > *A. indica* > *Z. officinale* > *C. nigrum* and lastly *A. melegueta* (Table 5). The proximate composition of the leguminous grains before and after *C. maculatus* infestation in the presence of *O. gratissimum* (most effective biopesticide) indicated slight increases in moisture, ash, fat, protein contents of all the grains A drop in the carbohydrate content was reported for all the investigate legumes (Table 6).

The susceptibility of leguminous grains to *C. maculatus* infestation over a four days period indicated that *V. subterranean* was the most preferred food source of *C. maculatus* followed by *C. cajan* and *V. unguiculata* (Figure 1).

## DISCUSSION

The present study showed that *O. gratissimum* had the highest insecticidal properties against *C. maculatus*. This result suggest that *O. gratissimum* could be successfully used for the control of *C. maculatus* and may even

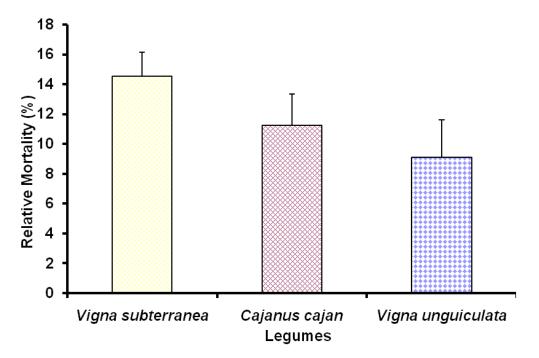


Figure 1. Susceptibility of leguminous grains to *Callosobruchus maculatus* infestation over a four days period under laboratory conditions.

Nutriont	% Proximate composition				
Nutrient	Vigna unguiculata Vigna subterrar		ea Cajanus cajan		
Before infestation					
Moisture	6.95 ± 0.25	$1.20 \pm 0.02$	8.10 ± 0.72		
Ash	$4.30 \pm 0.28$	$3.65 \pm 0.47$	$3.35 \pm 0.68$		
Fat	5.50 ± 0.31	$6.50 \pm 0.28$	$2.50 \pm 0.04$		
Crude fibre	$2.15 \pm 0.06$	1.83 ± 0.11	1.68 ± 0.14		
Protein	24.44 ± 0.15	22.60 ± 0.21	21.08 ± 0.32		
Carbohydrate	$56.66 \pm 0.48$	$64.22 \pm 0.62$	$63.29 \pm 0.59$		
After infestation					
Moisture	7.20 ± 0.15	$1.36 \pm 0.08$	$9.24 \pm 0.63$		
Ash	4.41 ± 0.01	3.95 ± 0.16	3.63 ± 0.19		
Fat	$6.22 \pm 0.07$	7.18 ± 0.12	$3.36 \pm 0.08$		
Crude fibre	$2.32 \pm 0.05$	$2.58 \pm 0.09$	2.45 ± 0.89		
Protein	25.51 ± 0.01	25.55 ± 0.03	21.10 ± 0.05		
Carbohydrate	54.42 ± 0.17	$60.87 \pm 0.38$	61.30 ± 0.29		

 Table 6. Proximate composition of leguminous grains before and after Callosobruchus maculatus infestation.

replace the synthetic insecticide. The present work agreed with the findings of Ravinder (2011) where he compared insecticidal actions of plant products with PMP and found that citrus leaf powder (CLP) was as effective as PMP in exhibiting insecticidal actions. He submitted that the synthetic insecticide PMP was superior to other treatments (except CLP) in reducing the number of eggs and in mortality than the plant products. The difference in performance of synthetic insecticide and biopesticides in this study was felt most in *C. cajan* grains with *A. melegueta* as biopesticide.

Lale (2000) have also reported the superiority of synthetic insecticide (pirimiphos methyl) in reducing oviposition and causing mortality of *C. maculatus* in an earlier study. The effectiveness of *O. gratissimum* in causing mortality could be attributed to the presence of

essential oils in its leaves and stems (Afolabi et al., 2007). Eugenol, thymol, citral, geraniol and linalool have been extracted from Ocimum oil (Sulistiarini, 1999). The antinociceptive property of the essential oil of the plant has been reported (Rabelo et al., 2003). The phytochemical analysis of the six biopesticides used revealed that they contained terpineol, alkaloids, glycosides, steroids, flavonoid as active ingredients. The active ingredients may be responsible for the noticed biopesticidal effectiveness. This finding is in line with that of Dushland (1939); Iwuala et al. (1981) that aromatic compound such as terpineol, glycosides, saponin and flavonoid has ovicidal, toxic and deterrent effects on stored product coleopterans. Onu and Aliyi (1995) worked with African nutmeg, clove, garlic, Oparaeke (1997) worked with chilli pepper, black pepper and they both obtained positive results of their insecticidal effectiveness. Mortality rate of C. maculatus was highest in V. unquiculata than for other legumes. This can be attributed to the fact that V. unquiculata was a preferred host for C. maculatus (Creadland et al., 1986; van Hius and de Rooy, 1998). Less adult pest mortalities were observed in V. subterranea and C. cajan than in V. unguiculata. Proximate studies before pest infestation revealed that legumes contain varied concentrations of moisture, protein, carbohydrates, ash and dietary fibre and make important contributions to human diet in many countries (Bressani, 1993).

Increase in moisture, ash, fat, fibre and protein in post infestation proximate composition of the legumes was observed. The increase in moisture contents over time may be due to absorption of atmospheric moisture (Ahmedani et al., 2011). Inverse correlations between ash, fat, fibre and protein in post infestation proximate composition of grains have been reported (Hameed et al., 1984; Jood and Kapoor, 1993; Jood et al., 1993; 1995; 1996 a; b; Ahmedani et al., 2009). The present investigation enunciated a negative correlation of *C. maculatus* infestation and the carbohydrate contents of *V. subterranean, C. cajan* and *V. unguiculata.* The result was in line with Ahmedani et al. (2009) that *Trogoderma granarium* significantly reduced starch digestibility in nine wheat varieties.

# Conclusion

Results of the present investigations have revealed changes in mortality and nutritional composition of different species of legumes when subjected to artificial infestation of *C. maculatus.* Apparently, varying quantity of exuviates; flour dust, live and dead adults and cast skins were found on the different legume samples indicating different levels of susceptibility or resistance. The effect of *O. gratissimum* in protecting the grains against *C. maculatus* attack was comparable to using the standard pesticide. There was a positive correlation between infestation levels and protein, fat, ash and fibre

contents of legumes studied. However, relationship between carbohydrate contents and the infestation level was negative. In general, *C. maculatus* tend to develop more slowly on resistant legume varieties. Nutritional composition of such legume was least affected by the attack of *C. maculatus* except for carbohydrate. Some authorities have argued that the high crude protein content may be made up of non-beneficial proteins.

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# REFERENCES

- Afolabi CA, Ibukun EO, Afor E, Obuotor EM, Farombi EO (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Sci. Res. Essay 2:163-166.
- Ahmedani MS, Haque MI, Afzal SN, Aslam M1, Naz S (2009). Varietal changes in nutritional composition of wheat kernel (*Triticum aestivum* L.) caused by Khapra beetle infestation. Pak. J. Bot. 41:1511-1519.
- Ahmedani MS, Haque MI, Afzal SN, Hussain T, Naz S (2011). Quantitative losses and physical damage caused to wheat kernel (*Triticum aestivum* I.) by Khapra beetle infestation. Pak. J. Bot. 43:659-668.
- AOAC (2000). Official Methods of Analysis. 17<sup>th</sup> Edition, Washington, D.C., USA.
- Atwal AS (1976). Insect pests of stored grain and other stored products. In: Agricultural pests of India and South East Asia. New Delhi, India: Kalyani Publishers, pp. 389-415.
- Bagheri ZE (1996). Pests of stored products and their control methods (Injurious Coleopterans of Food and Industrial Products), Volume 1. Tehran, Iraq: Sepehre Publishing, pp. 1-309.
- Booth RG, Cox ML, Madge B (1990). IIE Guides to Insects of Importance to Man, 3 Coleoptera. London: The Natural History Museum pp. 130-132.
- Bressani R (1993). Grain quality of common beans. Food Rev. Int. 9:217-297.
- Caswell GH (1981). The menace of *Callosobruchus maculatus* to stored cowpea seeds. Samaru J. Agric. Res. 1:1-11.
- Creadland PF, Dick KM, Wright AW (1986). Relationships between larval density, adult size and egg production in the cowpea seed beetle *Callosobruchus maculatus*. Ecol. Entomol. 11:41-50.
- Dansi L, Van Velson FL, Vander Heuden CA (1984). Methyl bromide: carcinogenic effects in the rat fore stomach. Toxicol. Appl. Pharmacol. 72:262-271.
- Dike MC, Mshelia GB (1997). Alternative sources for the containment of storage insect pests. Samaru J. Agric. Res. 14:11-18.
- Don-Pedro KN (1989). Mechanisms of action of some vegetable oils against *Sitophilus zeamais* (motsch) (Coleoptera: Curculionidae) on wheat. J. Stored Prod. Res. 25:217-223.

- Dushland RC (1939). The ovicidal, toxic and deterrent nature of botanicals on stored product coleoptera. J. Econ. Entomol. 32:430-431.
- Hameed A, Qayyum HA, Ali A (1984). Biochemical factors affecting susceptibility of flour wheat varieties to *Trogoderma granarium* Everts. Pak. Entomol. 6:57-64.
- Harbone JB (1973). Phytochemicals Methods. London: Chapman and Hall.
- Iwuala MOE, Osisiogu IUW, Agbakwuru EOP (1981). The essential oils and aromatic compounds of plant products against stored product coleoptera. J. Econ. Entomol. 74:249-252.
- Janzen DH (1977). Southern cowpea weevil larvae (Bruchidae) Callosobruchus maculatus die on non-host seeds. Ecology 85:921-927.
- Jood S, Kapoor AC (1993). Protein and uric acid contents of cereal grains as affected by insect infestation. Food Chem. 46:143-146.
- Jood S, Kapoor AC, Singh R (1993). Biological evaluation of protein quality of sorghum as affected by insect infestation. J. Plant Food Hum. Nutr. 43:105-114.
- Jood S, Kapoor AC, Singh R (1995). Amino acid composition and chemical evaluation of protein quality of cereals as affected by insect infestation. J. Plant Food Hum. Nutr. 48:159-167.
- Jood S, Kapoor AC, Singh R (1996a). Effect of insect infestation and storage on lipids of cereal grains. J. Agric. Food Chem. 44:1502-1506.
- Jood S, Kapoor AC, Singh R (1996b). Chemical composition of cereal grains as affected by storage and insect infestation. Trop. Agric. 73:161-164.
- Keay RWJ, Onochie CFA, Stanfield DP (1964). Nigerian Trees. Volume 1, Ibadan, Nigeria: Department of Forest Research.
- Lale NES (2000). Seed Powder Formulations, Stored Product Entomology and Acarology in Tropical Africa. Mole Publications, 204 pp.
- Mahdi SHA, Rahman MK (2008). Insecticidal effect of some spices on *Callosobruchus maculatus* (Fabricius) in black gram seeds. Univ. J. Zool. Rajshahi Univ. 27: 47-50.
- Onu I, Aliyu M (1995). Evaluation of powdered fruits of four peppers (*Capsicum sp*) for the control of *C. maculatus* on stored cowpea seeds. Int. J. Pest Man. 41:143-145.

- Oparaeke AM (1997). Evaluation of comparative efficacy of some plant powders for the control of *Callosobruchus maculatus* F. (Coleoptera; Bruchidae) on stored cowpea. Unpublished M.Sc. Thesis, Ahmadu Bello University, Zaria, Nigeria 105 pp.
- Ousman A, Ngassoum MB, Essia-Ngang JJ, Ngamo LST, Ndjouenke R (2007). Insecticidal activity of spicy plant oils against *Sitophilus zeamais* in stored maize in Cameroon. Agric. J. 2:192-196.
- Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA, Criddle DN (2003). Antinociceptive properties of the essential oil of *Ocimum* gratissimum L. (Labiatae) in ice Bra. J. Med. Biol. Res., 36:521-524.
- Ravinder S (2011). Bioecological studies and control of pulse beetle *C. chinensis* (Coleoptera: Bruchidae) on cowpea seeds. Adv. Appl. Sci. Res. 2:295-302.
- Sherma SS (1989). Review of literature of the losses caused by *Callosobruchus* species (Bruchidae: Coleoptera) during storage of pulses. Bull. Grain Technol. 22:62-68.
- Stoll G (2000). Natural Crop Protections in the Tropics Letting Information Come to Life. Margraf Vercey, 376 pp.
- Van Hius A (1991). Menace of fumigants and residual insecticide. Insect Sci. Appl. 12:87-102.
- Van Hius A, de Rooy M (1998). The effect of leguminous plant species on *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its parasitoid *Uscana lariophaga* (Hymenoptera: Trichogrammatidae). Bull. Entomol. Res. 88:93-99.
- Yusuf SR, Ahmed BI, Chandhary JP, Yusuf AU (1998). Laboratory evaluation of some plant products for the control of maize weevil (*Sitophilus zeamais* motsch.) in stored maize. Entomol. Soc. Nig. Occ. Pub. 31:203-213.