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Bio-Insecticidal Potency of Five Plant Extracts against Cowpea Weevil, *Callosobruchus maculatus* (F.), on Stored Cowpea, *Vigna unguiculata* (L)

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Abstract

It is estimated that the global post-harvest grain losses caused by insect damage and other bio-agents ranged between 10 to 40%. Small-scale farmers may lose up to 80% of their stock due to insect pest infestation after several months of storage. Control measures are necessary; they are proactive, cost effective and safe. The present study was conducted to evaluate the insecticidal effect of ethanol extracts from five locally available aromatic plants, namely *Allium sativum* L. (Garlic), *Cordia millenii* Baker (Manjack), *Monodora myristica* (Gaertn.) (Nutmeg), *Xylopia aethiopica* (Dunal) (Negropepper) and *Zingiber officinale* Roscoe (Ginger) against *Callosobruchus maculatus* (Cowpea weevil) infesting cowpea seeds (*Vigna unguiculata* L.). Bioassay was done by a direct contact application of the extracts using three concentrations (50, 75 and 100mg/ml) of each extract in 10g of previously disinfested cowpea seeds containing 10 adults of *C. maculatus* of 1-2 days old. The results revealed that all the plant species had lethal effects against the insect as compared with the untreated check. Considering the LC_{50} of the extracts 96 hours post-treatment as a main index *C. millenii* appeared superior (LC_{50} = 36.3mg), followed by *Z. officinale* (LC_{50} = 37.5mg), *X. aethiopica* (LC_{50} = 43.8mg), *M. myristica* (LC_{50} = 47.5mg) and *A. sativum* (LC_{50} = 55.0mg). All the tested plant species exhibited a toxic action against the cowpea weevils. These results have implications for cost effectiveness and safety that even the local farmers can use to protect stored cowpea seeds against the weevil.

Key words: Bio-insecticide, Cowpea weevil, Potency, Plant extract,Stored cowpea

1. Introduction

Cereals and grain legumes are the most commonly stored durable food commodities in the tropics (Odeyemi and Daramola, 2000). Cowpea grain (*Vigna unguiculata* L.) is a pulse crop produced and consumed largely by subsistence farmers in the semi-arid and sub-humid regions of Africa (DeBoer, 2003). It is an important cash and food crop for many poor farmers and also noted for its high nutritional value. It forms a major part of the diets of the people in West and East Africa, Latin America and the Carribean basin (DeBoer, 2003). It is a source of dietary protein in some parts of the world especially where there is a low availability and consumption of animal protein (Ofuya, 1991).

Insect pests are a major constraint on crop production, especially in developing countries. The cowpea weevil, *Callosobruchus maculatus*, F., (Coleoptera: Bruchidae) is a serious pest of stored grains in sub-sahara Africa (Al-Moajel and Al-Fuhaid, 2003). Postharvest losses of cowpea 3-4 months in storage caused by *C. maculatus* infestation have been reported as high as 50% in Northern Nigeria (Caswell, 1981) and 60% in Northern Ghana (Tanzubil, 1991). The loss of cowpea is a serious problem in Africa where as much as 20-50% of the grain is damaged by *C. maculatus* (FAO, 1985). The damage of this magnitude is incredibly high and demonstrates the destructive nature of the pest which can threaten food security at both household and national levels. This is a major agricultural problem for farmers in developing countries.

Weevil infestation causes weight loss, quality deterioration resulting in overall unacceptability in markets and impaired germinability of grains (Keita *et al.*, 2001). Infested grains are rendered unfit for consumption and sale. Consequently, farmers are compelled to sell their products early after harvest when prices are still low partly because of anticipated losses of the grain in storage.

Over the years, the destructive activities and menace of storage pests have been effectively suppressed with synthetic organochlorine and organophosphate compounds like carbon disulphide, phosphine, malathion, carbaryl, pirimiphos methyl and permethrin (Adedire *et al.*, 2011). The application of these chemicals as pest control agents is, however, fraught with problems, such as high persistence of the compounds, resurgence and genetic resistance of pests, negative effects on non-target

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organisms, poor knowledge of application, direct toxicity to the users, non-availability of the chemicals and increasing costs of application (Berger, 1994; Sharma *et al*., 2006).

These deficiencies of synthetic insecticides have caused a shift of opinion away from their usage towards plants products in the control of pests with varying levels of effectiveness. The use of plants to protect agricultural products against insect pests is an age-long practice in many parts of the world (Dales, 1996). Botanicals are non-persistent and are known to have broad spectrum insecticidal properties. Plants with insecticidal properties offer a cheaper sustainable fumigation and thermal distribution methods. Besides, they are ecologically safer to non-target species (Ito and Ighere, 2017; Ellis and Baxandele, 1997), easily available and can be produced within farmer's vicinity, thereby providing a more sustainable approach to pest control (Berger, 1994). These qualities make plants ideal candidates for incorporation into an integrated pest management strategy.

It is common practice in traditional African communities to use locally available plants for medicinal purposes and in agriculture (Obeng-Ofori *et al*., 2006). Natural plants products possess insecticidal properties against a wide range of insect pests. For instance cowpea seeds treated with cashew nut liquid (Echendu, 1991), fruit powder from *Piper guineense* (Ivbijaro and Agbaje, 1986), certain spices (Igbai and Poswal, 1995), essential oil from sandbox (*Hura crepitans* L.) (Ajayi and Adedire, 2003) and leaves of Eucalyptus (Berger, 1994) were better protected than untreated seeds. Therefore, more investigations are necessary to explore the natural protectants available within the locality for a more sustainable approach in controlling storage pests.

The present study was undertaken to (1) evaluate the toxicity of five aromatic plant species against the cowpea weevil, *C. maculatus*, on stored cowpea, (2) ascertain whether the plants extracts could be used as protectants of cowpea against *C. maculatus*, (3) identify effective botanicals available within the farmers environment that can be recommended as alternative low cost technique for minimizing postharvest losses of cowpea from cowpea weevils, and (4) increase the data bank of natural products used in the control of stored insect pests.

2. Materials and Methods

2.1. Plants Materials and Extracts Preparation

Five plant species (bulb of garlic - *Allium sativum*; seeds of manjack - *Cordia millenii*; seeds of Africa nutmeg – *Monodora myristica*; fruit of negro pepper –*Xylopia aethiopica* and rhizome of ginger - *Zingiber officinale* identified by botanical taxonomist were selected for this insecticidal study. All the plants materials were obtained locally from markets in Abraka, Delta State, Nigeria. The materials were cut into pieces including the Africa nutmeg seeds which were hulled before slicing. The materials were sun-dried for seven days (Sowunmi, 1983) and later dried to constant weight in an oven maintained at 60^oC for three hours. After drying, each plant material was milled using an electric blender (Model BLG-400) and the powder sieved repeatedly through a $1mm^2$ perforation mesh to obtain the finest powder which was kept separately in a glass container with screw cap and stored at room temperature prior to use.

2.2. Extract Preparation

Fifty gram (50 g) powder of each plant species was soaked in 1000 ml ethanol solvent and macerated for 72 h with regular shaking and stirring with glass rod thrice daily. The mixture of solvent and powder was filtered through cheesecloth and Whatman No. 1 filter paper. The filtrate was extracted using Soxhlet apparatus for 5-6 h and concentrated under pressure to dryness in a rotary evaporator at $25\text{-}30^0C$. The weights of the extracts were determined. *A. sativum* yielded 12.5 g, *C. millenii* 20.5 g, *M. myristica* 10.6 g, *X. aethiopica* 14.6 g and *Z. officinale* 15.8 g. The extracts were stored in a refrigerator maintained at 5-10 ^0C until ready for use. A technique described by (Ogunsina *et al*., 2011) was used to determine the percentage extract yield of each plant species. This involved dividing the extract mass obtained by sample mass used multiplied by 100. From each stock extract 1.5 g (=1500 mg) was weighed and dissolved in 30 ml ethanol solvent to give 50.0 mg/mL concentration. Two thousand two-hundred and fifty milligrams (2250 mg = 2.25 g) were dissolved in 30 ml ethanol to produce 75.0 mg/mL concentration. Similarly, three thousand milligrans (3000 mg = 3.0 g) were dissolved in 30 ml solvent to yield 100.0 mg/mL concentration. The extract concentrations (50.0, 75.0 and 100.0 mg/mL) of each plant species were used for the study.

2.3. Insect Culture

The insect pest used in this study was cowpea weevil, *C. maculatus* (F) (Coleoptera: Bruchidae). The weevils were reared on cowpea seeds (*V. unguiculata*) (L.) Walp in the laboratory to adapt them to the laboratory conditions using the method described by Sowunmi (1983). Cowpea seeds already infested with *C. maculatus* were collected locally in Abraka, Delta State, from traders of the food commodity. The adult weevils were isolated from the infested seeds. The cowpea seeds used as substrate to culture the weevils were thoroughly cleaned and exposed in an oven to ensure the absence of insects, mites and disease-causing microorganisms. Batches of one thousand (1000) treated seeds were placed in five different plastic containers previously washed, sterilized and dried. One hundred (100) weevils isolated from the infested cowpea seeds were introduced into each plastic container and covered with polythene net fastened tightly with rubber band. The cowpea seed-weevil mixtures were kept in the laboratory for 4 days to allow mating and oviposition to occur after which the parent weevils were removed. The rearing was given sufficient time (25-30 days) until new adult insects emerged. The first filial generations (F_1) adult weevils used for the experiment were 1-2 days old after emergence.

2.4. Toxicity Bioassay of Extracts

The plant extracts were assayed for insecticidal potency using the method described by Dharmasena *et al*. (2001). Cowpea seeds previously disinfested were divided into three lots of 10g each and replicated thrice. Each set of seeds was placed in a test tube (14.7x 2.4 cm) and treated with the plant extracts of different concentrations (50.0, 75.0 and 100.0 mg/mL). The tubes were manually rocked for two minutes to ensure that the seeds were coated with the extracts after which they were removed from the tubes and placed on filter papers for 24 h to allow the solvent to evaporate. Then each lot of seeds was placed in separate fresh test tubes and ten (10) adult weevils were introduced into the tubes and closed with plastic stoppers bearing gauze windows for ventilation. A control experiment, also replicated thrice, was constituted with identical amount of cowpea seeds and number of weevils but without the plant extracts. Mortality count of the insect pest was taken every 24 h for the exposure period of 96 h. The insect which did not respond when touched with a brush were considered killed and removed.

2.5. Statistical Analysis

Mortality data recorded every 24 h for 96 h exposure period were corrected for natural mortality of the insect pests in the control treatment using the formula proposed by Abbott (1925). The data were subjected to Analysis of Variance (ANOVA) and where significant differences existed treatment means were compared at 0.05 significant level using the New Duncan's Multiple Range Test (DMRT) (Zar, 1984). LC_{50} for each plant species at 96 h observation period was computed using regression analysis model (Finney, 1971).

3. Results

3.1. Mortality of C. maculatus

The result indicated that *C. millenii* gave the highest extract yield of 41.0%, followed by *Z. officinale* (31.6%), *X. aethiopica* (29.2%), and *A. sativum* (25.0%). The least extract yield of 21.2% was obtained from *M. myristica*. Table 1 shows the data of percentage mortality of the cowpea weevil, *C. maculatus*, observed on different days of treatment with ethanol plants extracts over 96 h.

Each value is a mean of triplicate data ± Standard Error with 10 weevils per replicate. Percentage values are in parenthesis. Column means followed by the same superscript letter are not significantly different (p<0.05) from each other using new Duncan Multiple range Test (DMRT).

Table 1. Cumulative mortality effect of five ethanol plant extracts on adult *C. maculatus*

Plant extract	Conc. (mg/ml)	Mean (%) mortality ± S.E at 24 to 96h Post-treatment*				
		24 h	48 h	72 h	96 h	Mean/24 h
A. sativum	50.0	2.33 ± 0.88 (23.3)a	3.66 ± 0.33 (36.6)a	4.66 ± 0.67 (46.6) a	4.66 ± 0.67 (46.6)a	3.83 ± 0.55 (38.3)a
	75.0	2.66 ± 0.33 (26.6) a	3.66 ± 0.33 (36.6) a	5.0 ± 0.0 (50.0)b	5.66 ± 1.20 (56.6)b	4.25 ± 0.67 (42.5)b
	100.0	3.03 ± 0.06 (30.3)b	4.33 ± 0.33 (43.3)b	5.66 ± 1.20 (56.6)c	6.66 ± 1.20 (66.6)c	4.92 ± 0.79 (49.2)c
C. millenii	50.0	3.0 ± 1.0 (30.0) a	4.83 ± 0.60 (48.3)a	5.66 ± 1.20 (56.6)a	6.36 ± 0.86 (63.6)a	4.96 ± 0.58 (49.6)a
	75.0	3.66 ± 0.33 (36.6)a	4.99 ± 0.01 (49.9)a	5.66 ± 1.20 (56.6 a	6.66 ± 0.67 (66.6)a	5.24 ± 0.63 (52.4)b
	100.0	4.66 ± 0.67 (46.6)c	6.33 ± 0.33 (63.3)b	6.66 ± 0.67 (66.6)b	8.36 ± 0.36 (83.6)b	6.5 ± 0.76 (65.0)c
M. myristica	50.0	1.33 ± 0.33 (13.3)a	2.66 ± 0.88 (26.6)a	4.03 ± 0.57 (40.3)a	5.33 ± 1.33 (53.3)a	3.41 ± 0.86 (34.1)a
	75.0	2.33 ± 0.33 (23.3)b	3.36 ± 0.32 (33.6)b	4.36 ± 1.86 (43.6)a	5.66 ± 1.20 (56.6)a	3.93 ± 0.71 (39.3)b
	100.0	2.66 ± 0.33 (26.6)b	4.03 ± 0.57 (40.3)c	4.66 ± 0.67 (46.6)b	5.66 ± 1.20 (56.6)a	4.25 ± 0.67 (42.5)c
X. aethiopica	50.0	2.33 ± 0.88 (23.3)a	4.66 ± 0.67 (46.6)a	5.33 ± 1.33 (53.3)a	6.36 ±0.86 (63.6)a	4.67 ± 0.85 (46.7)a
	75.0	3.33 ± 0.67 (33.3)b	5.33 ± 1.33 (53.3)b	6.0 ± 1.53 (60.0)b	7.33 ± 1.45 (73.3)b	5.50 ± 0.83 (55.0)b
	100.0	3.33 ± 0.67 (33.3)b	5.83 ± 1.15 (58.3)c	6.33 ± 0.33 (63.3)b	7.33 ± 1.45 (73.3)b	5.71 ± 0.85 (57.1)b
Z. officinale	50.0	2.33 ± 0.88 (23.3)a	3.33 ± 0.67 (33.3)a	4.66 ± 0.67 (46.6)a	5.66 ± 1.20 (56.6)a	4.0 ± 0.73 (40.0)a
	75.0	3.13 ± 1.15 (31.3)b	4.96 ± 0.58 (49.6)b	5.33 ± 1.33 (53.3)b	5.66 ± 1.20 (56.6)a	4.77 ± 0.57 (47.7)b
	100.0	3.33 ± 0.67 (33.3)b	5.86 ± 0.94 (58.6)c	6.33 ± 0.33 (63.3)c	7.66 ± 1.33 (76.6)b	6.0 ± 1.53 (60.0)c

The insecticidal efficacies of the plant species on the adult *C. maculatus* over 96 h are presented in Figure 1.

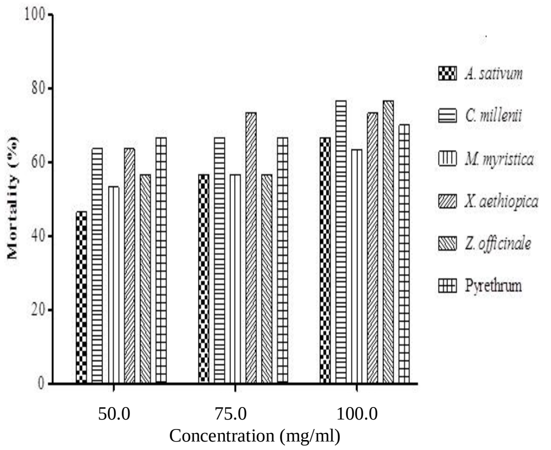

Figure 1. Cumulative mean mortality of *C. maculatus* exposed to various concentrations of plant extracts over 96 hours

The result showed that *C. millenii* and *X. aethiopica* extracts were most effective at 50 mg/mL concentration against the pest with respective mortality value of 63.3% at 96 h post-treatment. *Z. officinale* and *M. myristica* gave 56.6 and 53.3% mortality, respectively. The least percentage mortality of 46.6% over the 96 h exposure period was recorded in *A. sativum* extract. The highest mortality of 73.3% was observed in 75.0 mg/mL of *X. aethiopica* ethanol extract in 96 h exposure, followed by *C. millenii* (66.6%), while 56.6, 56.6 and 56.6% pest kill was exhibited by *A. sativum, M. myristica* and *Z. officinale* extracts, respectively, over 96 h. The extract of *C. millenii* at 100 mg/mL concentration gave the highest mortality of 83.6% compared to *Z. officinale* that caused 76.6% mortality of the pest after 96 h treatment. This was followed by *X. aethiopica* (73.3%), *A. sativum* (66.6%) and *M. myristica* (56.3%).

The overall mean percentage mortality data at all concentrations indicated that *C. millenii* extract gave better control of the cowpea weevil than the other extracts. The daily mean mortality of the pest at 100 mg/mL of *C. millenii* was 65.0%, followed by *Z. officinale* (60.0%), *X. aethiopica* (57.1%) and *A. sativum* (49.2%). *M. myristica* extract gave the least mortality of 42.5%. However, all the five treatment proved significantly better than the untreated check. Considering the mean daily percentage mortality of *C. maculatus* as main index the insecticidal efficacy of the five plants species is indicated as follows: *C. millenii* > *Z. officinale* > *X. aethiopica* > *A. sativum* > *M. myristica* (Table 2).

The efficacy and economic value of the five tested plants species are corroborated by the percentage yield of the extract (Table 1). The higher the yield the more efficacious was the plant extract.

Probit analysis of extract concentrations lethal to 50% (LC_{50}) of the adult cowpea weevil over 96h exposure is presented in Table 2.

Table 2. LC_{50} (mg/mL) of extracts on adult *C. maculatus*

Plants extract	LC_{50} at different time intervals			
	24h	48h	72	96h
A. sativum	-	-	75.0	55.0
C. millenii	-	75.0	42.5	36.3
M. myristica	-	-	-	47.5
X. aethiopica	-	50.0	46.3	43.8
Z. officinale	-	77.5	62.5	37.5

LC_{50} analysis of the extracts considered in 96 h post-treatment indicated that *C. millenii* was the most effective control agent of the target pest (LC_{50} = 36.3 mg/mL) followed by *Z. officinale, X. aethiopica, M. myristica* and *A. sativum* with respective LC_{50} of 37.5, 43.8, 47.5 and 55.0 mg/mL. The result revealed that the length of exposure time was a determining factor of the LC_{50}. The longer the period of treatment, the less was the LC_{50} of the extracts.

4. Discussion

The solubility of active ingredients in plants varies during extraction as observed in the extract mass and percentage yield of the tested plant species. Differences in toxicity may be related to the proportion of the active chemicals in the extracts due to differential solubility in the ethanol solvent. This probably was the reason for the high mortality of *C. maculatus* in *C. millenii* (manjack), *Z. officinale* (ginger) and *X. aethiopica* (negropepper) extract at all concentrations used since they gave higher percentage yield arising from their solubility.

The current study revealed that all the tested plants extracts were toxic and could be used as protectant against *C. maculatus*. However, the high toxicity of *C. millenii* (LC_{50} = 36.3mg/mL) was close to *Z. officinale* (LC_{50} = 37.5 mg/mL) against the target pest showed that it would be economical to carryout mass production of these plant species for use as protectant of grains since they were able to achieve 50% *C. maculatus* mortality with the least extract concentration within 96h exposure.

The results of this study are in conformity to some degree with the report of some workers, like Opareke and Dike (2005), Adedire *et al*. (2011), Mukanga *et al*. (2010), Ileke and Oni (2011), who observed that certain botanicals are effectively toxic against storage insect pests including *C. maculatus*. The resultant mortality rates of *C. maculatus* in this investigation could be attributed to the toxic effects of the chemicals in the tested plant species. Although all the plants showed promise as insecticides their toxicity against *C. maculatus* varied probably because of the different phytochemical contents.

C. millenii exhibited the strongest insecticidal effect due to oleanoic and triterpene derivatives (Chen *et al*., 1983), betulic and terpenoid quinones (Moir and Thomsom, 1973) as the main constituents. The high toxicity of *C. millenii* could be attributed to the terpenoids, which act as insecticides, repellants and antifeedants against insects (Detheir *et al*., 1996). According to Grøntved and Pittler (2000) *Z. officinale* has alpha-zingiberine as the major phytochemical which is believed to be toxic. The toxicity of ginger in the present study is somewhat corroborated by the report of Bandara *et al*.

(2006) that the ginger species, *Z. purpureum*, was ovicidal against *C. maculatus*. Ginger has been reported toxic to storage insect pests (Igbai and Poswal, 1995; Owolabi *et al*., 2009). Ginger has become a promising future alternative to expensive and toxic therapeutic agent because of the chemopreventive potentials of zingiberine (Duke and Ayensu, 1985). The principal chemicals in *X. aethiopica* are mono- and sesquiterpene hydrocarbons (Karioti *et al*., 2004) which may have caused the pest mortality. Terpenoids are the main chemical components in *A. sativum* (garlic) (Duke and Ayensu, 1985). Terpenoids act as fumigant causing insect death owing to anorexia arising from drastic reduction in insect respiratory activities as reported by Don-Pedro (1996e). Allitin, another chemical in garlic, inhibits cholinesterase activity in insects (David and Ananthakrishna, 2004). Garlic toxicity may have been caused by one or a combination of these chemicals. The mortality of *C. maculatus* by garlic and ginger in this study agrees with earlier reports that plants in the genera *Allium* and *Zingiber* to which garlic and ginger belong are insecticidal against various insect pests (Owusu *et al*., 2008). African nutmeg (*M. myristica*) has myristicine (Dales, 1996), p-cymene, alpha-phellandrene and other terpene hydrocarbons (Owolabi *et al*., 2009) as chemical constituents. Nutmeg toxicity may be due to the terpenes and their derivatives which are known to influence insect respiratory metabolism by disturbing mono-oxygenase enzymes activity (Bernard *et al*., 1989) and affect nervous system by inhibiting acetylcholinesterase enzyme activity (Keane and Ryan, 1999). Terpene 1,8-cineole is toxic against rice weevil (Byung-Ho *et al*., 2001) and cowpea weevil (Aggrawal *et al*., 2001). These chemicals in nutmeg notwithstanding the reportedly low toxicity of the extract may be as a result of low solubility of the active ingredients in the ethanol solvent.

5. Conclusion

All the tested plant species exhibited toxic action against the cowpea weevils. Therefore, they could be used by local farmers to protect cowpea seeds in storage against the weevil based on the available data. It is recommended that similar investigation on different parts of the tested plants species be carried out to further assess their efficacy against the weevil and other storage pests. Besides, the use of other extraction solvents for the tested plants is recommended in order to evaluate further their insecticidal potency. It is, therefore, expedient to control pest populations to low or zero levels in storage since higher bruchid populations result to higher level of stored grain damage.

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