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# Controlling bruchid pests of stored cowpea seeds with dried leaves of *Artemisia annua* and two other common botanicals

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**Insecticidal activities of dried *Artemisia annua* L. leaves were evaluated against bruchid (*Callosobruchus maculatus* F.) pests in comparison with those of *Azadirachta indica*, *Ocimum gratissimum* and a conventional grain storage insecticide, Actellic® 2% dust. Each treatment was added to a mixture of 250 g cowpea seeds and 25 adult bruchids and laid out in a completely randomized design with four replicates. Irrespective of the concentration tested, all three plant materials significantly ( $P < 0.05$ ) increased mortality rate of adult insects earlier than the control. Higher concentrations of the botanical pesticides equally resulted in an increased reduction in the number of surviving bruchids and reduction to seed damage through a lower number of eggs laid and weevil perforation index (WPI) after 90 days. Comparatively, *A. annua* was more effective than the other plant materials at all levels evaluated though it was not as effective as Actellic 2% dust. Moreover, differences amongst the efficiency rates and interactions between *A. annua* and *A. indica* in three treatment combinations produced a significant ( $P < 0.05$ ) effect on two of the parameters evaluated. Taken together, all plant materials evaluated here were seen to have significant insecticidal properties and could therefore, be used as environmentally friendly products for controlling bruchid pests during storage of cowpeas with no adverse effects on eventual mammalian consumers as observed during an animal feeding trial in this study.**

**Key words:** Actellic 2% dust, botanical pesticides, *Callosobruchus maculatus*, *Vigna unguiculata*, weevil perforation index.

## INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most versatile food legumes in the tropical and sub-tropical regions of the world where it is cultivated. The seeds are processed and consumed as an inexpensive source of very high vegetable protein by almost half a billion people; especially in Africa, an area of the world where

the green revolution has had very little impact. On the average, cowpea seeds contain about 23 to 25% protein and are also a major source of other nutrients (Quin, 1997). The plant foliage itself can be dried and stored until needed as fodder for livestock (Rachie, 1985). As a nitrogen-fixing legume, cowpea improves soil fertility and consequently, enriches the soil in which it is grown for subsequent crops.

At the moment, Africa accounts for about 75% of the world's cowpea production, with Nigeria and Niger predominating. Unfortunately, cowpea rarely reaches its

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yield potential in the continent, primarily due to diseases and the limited use of fertilizers and irrigation inputs. Moreover, investments in breeding programmes aimed at enhancing yield in the field and especially reducing post-harvest losses due to bruchid infestation of the crop (Emosairue et al., 1994; Jackai, 1993; Singh et al., 1990) are very limited in many African countries. Consequently, about 30 to 80% of the total production of cowpea valued at over US 300 million dollars is either lost or suffers damage annually as a result of bruchid infestation (Ohiagu, 1985). This is a very serious agronomic constraint to cowpea production in sub-Saharan Africa, where chemical control of pests, though feasible, is equally very expensive and often unaffordable to many resource-poor farmers (Afun et al., 1995).

Recently, a number of plant materials have been explored as sustainable alternatives for controlling short-lived insect pests during storage of grains and found to be quite effective. Some of these botanical pesticides include powders from *Piper guineensis* (Ivbijaro and Agbaja, 1986), *Piper nigrum* (Rajapakse, 1990), *Zanthoxylum xanthoxyloides* (Ogunwolu and Odunlami, 1996), root bark of *Annona senegalensis* (Aku et al., 1998), *Capsicum frutescens* (Echezona, 2006; Ofuya, 1986), plant mixtures (Arannilewa et al., 2006) and essential oil derived from *Artemisia sieberi* (Negahban et al., 2007). Expectedly, some of the advantages of these botanical pesticides are their availability and user-friendliness as biological control agents with no adverse effects on the environment. For instance, empirical reports from the past indicated that the dried leaves of annual wormwood (*Artemisia annua*) were used to address the great flea epidemic, which ravaged parts of the United Kingdom in the 18<sup>th</sup> century while the same plant materials are usually burnt in China as a fumigant to kill mosquitoes (Foster and Chongxi, 1992) without leaving any residues toxic to the environment.

*A. annua* is a vigorous growing annual weedy herb, usually single-stemmed, reaching up to 2 to 3 m in height. The plant produces a beautiful portfolio of biologically-active secondary metabolites including flavonoids, coumarins, steroids, phenolics, purines, lipids, aliphatic compounds, monoterpenoids, triterpenoids and sesquiterpenoids. Thus far, the most important of the sesquiterpenoids seems to be artemisinin, dihydroartemisinic acid, artemisinic acid and arteannuin B which are stored in glandular trichomes found in the leaves and inflorescence of the plant (Ferreira and Janick, 1995). Artemisinin is a cadinane-type sesquiterpene lactone with an internal peroxide bridge that is well known for its antimalarial properties (Klayman, 1989). It is presently the most potent and efficacious compound in the global fight to eradicate malaria (Brisibe et al., 2008). Together with its semi-synthetically prepared derivatives such as dihydroartemisinin, artesunate and artemether, artemisinin has also displayed unique pharmacological activities against a wide range of parasitic organisms including bacteria (Bone and Morgan,

1992) such as *Enterobacter* species, *Klebsiella pneumoniae*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Escherichia coli* and *Pneumocystis carinii* (Chen et al., 1994), an opportunistic pathogen which causes pneumonia in HIV/AIDS and other immune-compromised patients. Recent studies have demonstrated that artemisinin derivatives are equally potent and efficacious against several other common infectious parasites of man including *Toxoplasma gondii* (Jones-Brando et al., 2006) which causes toxoplasmosis that is responsible for behavioral abnormalities in patients, *Trypanosoma* and *Schistosoma* species (Mishina et al., 2007; Utzinger et al., 2001) that are responsible for human trypanosomiasis or 'sleeping sickness' and schistosomiasis, respectively. Artemisinin derivatives are also effective against some other pathogens including those responsible for cryptosporidiosis, amoebiasis, giardiasis, clonorchiasis and leishmaniasis (Ma et al., 2004; Yang and Liew, 1993). Moreover, artemisinin has been recently indicated as a potential and effective compound against a number of viruses including hepatitis B, C and others (Efferth et al., 2008).

We observed from a series of preliminary studies that in addition to these very unique pharmacological properties of artemisinin and its derivatives, the dried aerial parts of *A. annua* also contain fairly high levels of bioactive constituents with fumigant activity, leading us to suspect that this crop could probably have potent insecticidal properties with low toxicity to mammals. Consequently, to encourage resource-poor farmers in developing countries to increase their productivity of grains without losses in storage due to bruchid pests, this study was therefore, designed to compare the effects of dried leaves of *A. annua* with two other common plant materials in their ability to control *Callosobruchus maculatus* in stored cowpeas.

## MATERIALS AND METHODS

Three trials were undertaken in a laboratory at the Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria, with a mean temperature and relative humidity of  $28 \pm 3^\circ\text{C}$  and  $65 \pm 5\%$ , respectively. Each trial lasted for 90 days. The susceptible Ife Brown variety of seeds was used to maintain the insect cultures in 1 L plastic containers. Various levels of shade-dried (usually for 5 days under normal environmental conditions) and pulverised (1 to 4 mm) leaves of *A. annua*, *Azadirachta indica* and *Ocimum gratissimum*, obtained courtesy of Molecular Bio/Sciences Limited, 124 MCC Road, Calabar, Nigeria and a conventional insecticide (Actellic® 2% dust containing pirimiphos methyl; produced by Chemical and Allied Products Plc, Lagos, Nigeria) were used in comparison with an untreated control. Each pesticide treatment was added to a mixture of 250 g of dry cowpea seeds and 25 adult bruchids.

### Experiment 1

Each of the three plant materials was weighed out at four rates of

2.5, 5, 10 and 20 g, which corresponded to 1, 2, 4 and added initially to 250 g of cowpea seeds, free from any weevils, in 500 ml plastic containers. Actellic 2% dust at 0.25 g was also separately added to 250 g of cowpea seeds in a plastic container. Perforated muslin cloth, held tightly in place with several rubber bands, was used to cover each container to ensure adequate ventilation. The seeds and pesticides were shaken thoroughly in the container until the pulverized leaf materials and Actellic 2% dust, respectively, were evenly distributed among the seeds. The content of the plastic containers was allowed to settle down for two hours before the introduction of the insects to each treatment. Twenty five (25) newly emerged adult stock (1 to 4 days old) of *C. maculatus* was obtained from insect-infested cowpea seeds from Watt market in Calabar, Cross River State, Nigeria and introduced into each plastic container, which were arranged in a completely randomized design on laboratory tables. The control treatment comprised of containers with no botanical pesticides or Actellic 2% dust. Each of the treatments was replicated four times and thereafter, the number of adult bruchids that survived in each container was recorded every month (30 days) after infestation. The percentage survival of the adult weevils was later calculated. Also recorded was the number of eggs laid as well as the number of exit holes per seed (weevil perforation index) taken randomly from 100 seeds per container after three months (90 days) of treatment. Visual inspection was used to identify infested seeds.

## Experiment 2

This experiment involved proportional mixtures of *A. annua* and *A. indica* as a result of certain observations that were made in experiment 1. Essentially, the whole idea behind this experiment was to establish the best combination of the two plant materials such that there would be no over-dependence on *A. annua*, a plant material which is not commonly available to most resource-poor farmers in the tropics. In this second experiment, therefore, varying levels of dried pulverised *A. annua* leaves used in experiment 1 were selected and mixed with different levels of *A. indica* in the ratios of 75:25, 50:50 and 25:75%, respectively, since the latter is readily available in most parts of the developing world. The mixtures were applied to the fumigated wholesome cowpea seeds at the rate of 8% of the seed weight (20 g). Cowpea seeds without treatment with any plant materials again served as the control. Each treatment was replicated four times just as both the procedure and data collected were the same as in experiment 1.

## Animal feeding assay and biochemical analysis

This bioassay was performed to determine the *in vivo* effect of consumption of cowpea seeds treated with the three botanical pesticides. Three groups of albino rats (6 per group) of both sexes, each weighing between 120 and 160 g were allowed to acclimatize for two weeks in ventilated cages before the start of the experiments. All the animals were fed with commercial rat chow (Pfizer Nigeria Plc, Ikeja, Nigeria) and provided with water *ad libitum* throughout the two weeks duration of acclimatization on a 12 h dark/light cycle. After this period, animals in all the groups were continuously fed diets of dried pulverised cowpea that had been treated with the highest concentration (20/250 g seed) of each of the three botanical pesticides evaluated in this investigation for a period of 28 days. Animals in a fourth group, which served as control, were fed with untreated cowpea seeds in order to compare the effects of the various treatments on the safety of subsequent human consumers.

At the end of the animal feeding experiment, rats from the various treatment groups were sacrificed by direct heart puncture and blood was collected into a clean centrifuge tube and allowed to

stand for 30 min before being centrifuged at 1,500 rpm for 10 min to obtain the serum. The levels of alkaline phosphatase (ALP) were determined according to Shahsavani et al. (2010), while those of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated as described by the Randox reagent kit manufacturer (Randox Laboratories Ltd., Ardmore, United Kingdom) using 2,4-dinitrophenylhydrazine as substrate according to a combination of the methods of Reitman and Frankel (1957) and Mohun and Cook (1957). Similarly, serum electrolytes and urea and creatinine levels were determined according to Xu et al. (2010).

## Statistics analysis

Statistical analysis was performed with Student's *t* test (Sigma Stat; Scientific, Erkrath, Germany) to analyze treatments in relation to the control. To analyze differences between different treatments, the one-way analysis of variance at 5% level of probability ( $P < 0.05$ ), which was followed by a Dunnett test (Sigma Stat; Scientific) was performed.

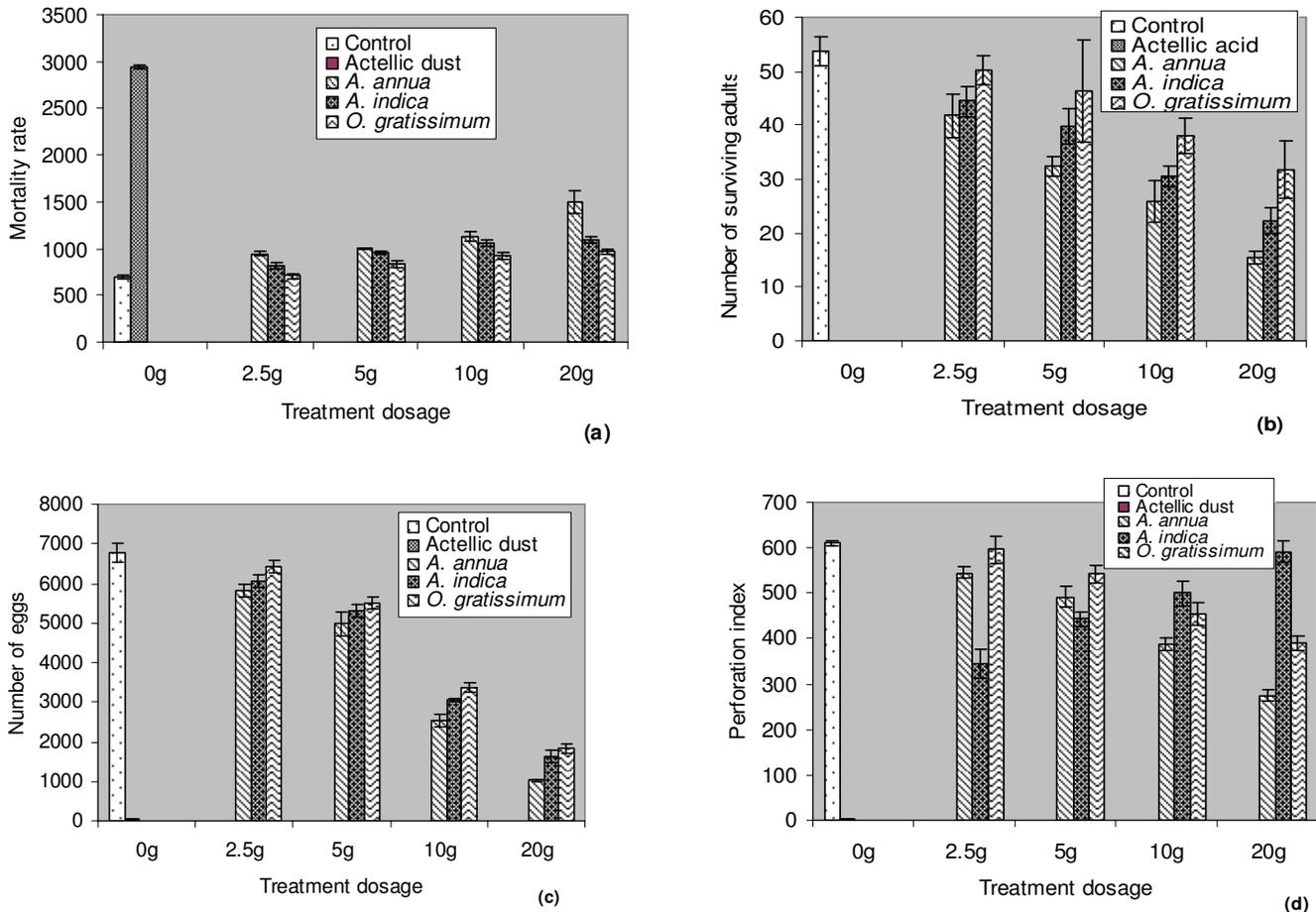
## RESULTS

### Effect of botanical pesticides on the performance of *C. maculatus*

The results of the effects of different concentrations of the plant materials on adult mortality and survival rates of *C. maculatus* as well as the number of eggs laid and weevil perforation index (WPI) on stored cowpea seeds are presented in Figure 1. Differences among the botanical pesticides were significant ( $P < 0.05$ ) for all the parameters evaluated in this study even though each of the plant materials was generally less effective than Actellic 2% dust at 0.25/250 g applied to cowpea seeds. From the results in Figure 1, all the three plant materials were effective against the insect, albeit to significantly varying degrees of efficiency. In each treatment, there was a higher weevil mortality rate following an increase in the concentration and duration of exposure to the plant material. Mortality rates of adult bruchids taken at 30 days after infestation showed that Actellic 2% dust was the most effective, resulting in over 95% mortality of the adult insects, perhaps on account of the fact that this product is a conventional synthetic insecticide specifically formulated with high insecticidal activities on stored product pests (Anon, 1993).

Of the three botanical pesticides tested, the highest adult insect mortality rate was recorded in the treatment with the highest concentration (20 g) of dried and pulverized leaves of *A. annua*; the same concentration of *A. indica* and *O. gratissimum*, respectively, had considerably lesser effect during the same duration of storage. Except for the treatment with *A. annua*, there were no statistically significant differences ( $P > 0.05$ ) in the mortality rates between the lowest dosages of *A. indica* and *O. gratissimum* used and the untreated control.

The mortality rates of the different test materials were



**Figure 1.** Effect of different concentrations of *A. annua*, *A. indica* and *O. gratissimum* on: (a) adult mortality rate; (b) number of surviving adults; (c) number of eggs laid; (d) weevil perforation index (WPI) of *C. maculatus* in stored cowpea seeds 90 days after infestation.

expressed as the survival percentage of bruchids at varying concentrations of the plant materials and periods of exposure. All the botanical pesticides significantly reduced adult survival of *C. maculatus* 90 days after infestation, relative to the control at  $P < 0.05$  (Figure 1). Differences in percentage survival amongst the botanical pesticide rates and their interactions with the different plant materials did not attain any level of statistical significance.

The effects of the different plant materials and exposure periods on the number of eggs deposited on the seeds and adult emergence (weevil perforation index) were also tested and recorded. There were no significant differences between the various treatments and the control regarding the number of eggs deposited on the seeds early in the experiment while there were fewer eggs laid but with none hatched (as there was no adult emergence holes) in the seeds protected with Actellic 2% dust at 30 days, all the botanical pesticides and their interactions did not produce any significant ( $P > 0.05$ ) effect on the number of eggs deposited on the seeds at

this period. At 60 days after infestation, the treatment with 20 g of *A. annua* leaves resulted in a lower number of eggs laid and about 12% perforation index compared with the seeds in the control without any treatment (data not shown). However, at 90 days the pulverized leaves of both *A. annua* and *A. indica* significantly reduced the number of eggs laid by the insects relative to the control ( $P < 0.05$ ) though expectedly, seeds treated with Actellic 2% dust had the least perforation index (Figure 1). The number of eggs laid on seeds treated with Actellic 2% dust was statistically lower than that on seeds treated with *A. indica* and *O. gratissimum*, respectively, but not significantly lower than the counts on seeds treated with *A. annua* after 90 days.

Interestingly, the proportional combination of either 75% *A. annua* and 25% *A. indica* or 50% *A. annua* and 50% *A. indica* equally led to an enhanced performance of the plant materials for both adult bruchid control and a lower weevil perforation index than when seeds were treated with 100% of either of the botanical insecticides used in this study (Table 1). Taken together, the

**Table 1.** Effect of different combinations of *A. annua* and *A. indica* on adult mortality rate and weevil perforation index (WPI) of *C. maculatus* in stored cowpea seeds 90 days after infestation.

Treatment	Number of dead adult weevil	Weevil perforation index
75% of <i>A. annua</i> + 25% of <i>A. indica</i>	1901.35 <sup>a</sup> ±15.92	268.02 <sup>e</sup> ±0.12
50% of <i>A. annua</i> + 50% of <i>A. indica</i>	1809.00 <sup>b</sup> ±7.70	228.98 <sup>e</sup> ±8.21
25% of <i>A. annua</i> + 75% of <i>A. indica</i>	1426.12 <sup>c</sup> ±10.12	332.52 <sup>ef</sup> ±9.18
0% of <i>A. annua</i> + 0% of <i>A. indica</i>	201.42 <sup>d</sup> ±1.01	628.02 <sup>g</sup> ±12.06

\*Means having the same superscripts within the same column do not differ significantly from one another at 5% level of probability as determined by LSD test.

**Table 2.** Mean\* and standard error of mean (S.E.M.) values of biochemical indices and other parameters obtained in rats following administration of cowpea seeds treated with 20 g of the three botanical pesticides.

Parameter	Control (0/250 g)	<i>Artemisia annua</i> (20/250 g)	<i>A. indica</i> (20/250 g)	<i>O. gratissimum</i> (20/250 g)
AST (iu/l)	129 <sup>c</sup> ±0.5	130 <sup>c</sup> ±0.2	132 <sup>c</sup> ±0.9	132 <sup>c</sup> ±0.2
ALT (iu/l)	42.4 <sup>d</sup> ±0.1	41.6 <sup>d</sup> ±0.9	42.2 <sup>d</sup> ±0.1	42.6 <sup>d</sup> ±0.9
ALP (iu/l)	114 <sup>a</sup> ±0.5	112 <sup>a</sup> ±0.3	114 <sup>a</sup> ±0.8	112 <sup>a</sup> ±0.3
Creatinine (μmol/l)	44.2±0.5	44.2±0.5	44.2±0.5	44.2±0.5
Urea (mmol/l)	9.7 <sup>a</sup> ±0.1	9.9 <sup>a</sup> ±0.6	9.7 <sup>a</sup> ±0.3	9.9 <sup>a</sup> ±0.6
Sodium (mmol/l)	125 <sup>a</sup> ±0.6	124 <sup>a</sup> ±0.1	122 <sup>a</sup> ±0.8	124 <sup>a</sup> ±0.1
Potassium (mmol/l)	10.6 <sup>a</sup> ±0.3	10.4 <sup>a</sup> ±0.2	10.4 <sup>a</sup> ±0.3	10.4 <sup>a</sup> ±0.2
Calcium (mmol/l)	24.3 <sup>a</sup> ±0.7	22.3 <sup>a</sup> ±0.5	21.8 <sup>a</sup> ±0.2	22.3 <sup>a</sup> ±0.5

\*Means having the same superscripts within the same row do not differ significantly ( $P > 0.05$ ) from one another.

effectiveness of the different single pesticides evaluated in this study was therefore ranked as: Actellic 2% dust > *Artemisia annua* > *Azadirachta indica* > *Ocimum gratissimum* > no protection.

### Mammalian feeding trial

Cowpea seeds treated with the three botanical pesticides were tested *in vivo* and fed continuously as the only diet to rats for 28 days, during which period they were examined for possible adverse symptoms. All the experimental animals appeared completely normal with no adverse symptoms in spite of having been placed only on a diet of cowpea seeds. The average body weight gain of the animals fed with cowpea seeds treated with each of the botanical pesticides was not significantly different ( $P > 0.05$ ) from that of rats fed with pesticide untreated seeds (data not shown). Moreover, all the rats in the three treatment groups and control appeared healthy throughout the duration of this experiment, indicating that treatment of the seeds with the various botanical pesticides had no toxic effects on the animals that had been fed continuously with the treated seeds for 28 days, as also confirmed by the results of the kidney and liver function tests. The serum levels of ALT, AST as well as those for the electrolytes, urea and creatinine in the animals treated with plant materials showed no significant ( $P < 0.05$ ) increases compared with those in the control

group (Table 2).

### DISCUSSION

Recently, post-harvest loss of grain due to insect pests has become a major concern all over the world such that demand for good quality products, which are free from chemical residues, is high and increasing rapidly (Kashi, 1981). In fact, such products are now known to attract premium prices at international grain auctions. It is obvious from the results of this study that pulverised dried leaf materials of the three botanicals can be used as inexpensive and very effective biological pesticides for the control of bruchid pests in stored cowpeas. They also deterred oviposition by the bruchid pest, albeit to significantly varying degrees, probably on account of the toxicity of the phytochemicals they contain on the potential egg laying adults. This result supports earlier observations by Opareke et al. (1998) and Lale (1994), who demonstrated a high degree of oviposition deterrence by neem and pepper against *Callosobruchus maculatus*. Taken together, these results are not overtly surprising since dried leaves of some aromatic Rocky Mountain plants, including *Artemisia tridentata*, have been reported to decrease insect infestation in stored grains (Weaver et al., 1995), while those of *Artemisia pallens* were used for repelling moth in the preservation of delicate and expensive fabrics (Sastry, 1946). Aside

from these, essential oil extracted from *Artemisia absinthium* has equally been reported to either kill house flies (Kaul et al., 1978) or repel flies, fleas (Erichsen-Brown, 1979) and mosquitoes (Morton, 1981). Incidentally, all the three botanical pesticides used in this study (that is, *A. annua*, *A. indica* and *O. gratissimum*) have been described as plants known to possess bioactive secondary metabolites present mainly in leaf extracts and essential oils. The essential oil in *A. annua*, for example, contains many volatile compounds and several non-volatile sesquiterpenes, some with antimalarial, antibacterial, anti-inflammatory, plant growth regulatory and antitumour properties (Bhakuni et al., 2001) with the major ones including (in relative percentage of the total)  $\beta$ -pinene (0.032%), camphene (0.049%), p-pinene (0.882%), myrcene (3.8%), 1,8-cineole (5.5%), artemisia ketone (66.7%), linalool (3.4%), camphor (0.6%), borneol (0.2%) and  $\beta$ -caryophyllene (1.2%) according to Simon et al. (1990). Many of these biologically active ingredients like triterpenoids, phenolic compounds, carotenoids, steroids, ketones, tannins, and saponins, some of which are present within the essential oil fraction of *A. annua*, are also known to be present in the leaves of *A. indica* (Schmutterer, 1990) and *O. gratissimum* (Junaid et al., 2006) though at different concentrations.

The use of essential oils derived from plant materials in the control of weevil pests of stored products is legendary and it is a practice quite as old as civilization itself. Essential oils are commonly used because they are quite efficacious against all life stages of insects. Moreover, it has been widely reported that terpenes from a variety of essential oils derived from vegetative sources have potent insect pest control properties, which affect the biology of target insects in different ways by acting either as ovicides, repellants, antifeedants, fumigants, contact toxicants or insecticides (Hough-Goldstein, 1990; Watanabe et al., 1993; Rice and Coats, 1994; Tsao et al., 1995). It is therefore, possible that the efficacy seen with the dried leaves of the three botanicals against the bruchid pests could be attributable to these and other constituents. It is equally possible that when combined, as was the case of *A. annua* and *A. indica* in this study, these constituents in both plant materials may have produced a more remarkably synergistic, toxic effect on the bruchid pests than when used alone as the insecticidal effects obtained from the mixture of the two plant materials appeared significantly higher than those of either *A. annua* or *A. indica*, respectively.

It is clear from the data generated in this study and other reports that the different plant materials evaluated here have significant insecticidal properties that are relevant from the agricultural point of view. However, what has been difficult to predict from these studies is the safety and overall acceptability of stored grains, which have been treated with the various plant materials. A feeding trial where rats were provided with only cowpea

seeds treated with the plant materials used in this study clearly demonstrated no acute toxicity or other adverse reactions to the botanicals by the study animals. This is perhaps an indication that even though they were insecticidal, the phytochemicals in the different plant materials used in this study were either non-toxic or the concentrations were far too low to elicit any meaningful adverse reactions in mammals. Of course, this in itself is not surprising as each of these three botanicals is presently consumed by humans without any reported adverse symptoms. For example, *A. annua* has been used for several millennia as an herbal tea preparation in China, where it is renowned for its treatment of haemorrhoids and fevers associated with malaria without any known adverse effects. Lately, young stems of the plant have equally become a delicacy in parts of the country where they are used as food in a salad-like manner and for the preparation of an herbal tonic. On the other hand, *A. indica* has also been used for centuries in the treatment of several digestive problems, malaria and skin diseases in India and Africa while fresh and dried leaves of *O. gratissimum* are used as condiments in the preparation of some traditional West African cuisines. Recent studies have equally demonstrated that *O. gratissimum* leaves are commonly used in Nigerian folk medicine for the treatment of upper respiratory tract infections, skin diseases, ophthalmia, headache and pneumonia and diarrhoea (Sofowora, 1993; Junaid et al., 2007), especially for people living with the human immunodeficiency virus (Elujoba, 2000).

Based on the results reported here, it could be concluded that even though the three plant materials used in this study had insecticidal properties against bruchid pests, however, there is no evidence of adverse reactions associated with any of them by mammals at the moment suggesting that the leaves, especially, of *A. annua* either alone or in combination with those of *A. indica*, can be used as cost-effective, biodegradable and user friendly tools for controlling *C. maculatus* in stored grains, particularly at the household level in technology-poor surroundings.

## REFERENCES

- Afun JVK, Jackai LEN, Hodgson CJ (1991). Calendar and monitored insecticide application for the control of cowpea pests. *Crop Prot.*, 10: 363-370.
- Aku AA, Ogunwolu EO, Attah JA (1998). *Annona senegalensis* L. (Annonaceae): performance as a botanical insecticide for controlling cowpea seed bruchid *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) in Nigeria. *J. Plant Dis. Prot.*, 105: 513-519.
- Anon (1993). Zeneca Agrochemical Product Specifications. Imperial Chemical House, Millbank, London, p. 4.
- Arannilewa ST, Ekrakene T, Akinneye JO (2006). Laboratory evaluation of four medicinal plants as protectants against the maize weevil, *Sitophilus zeamais* (Mots). *Afr. J. Biotechnol.*, 5: 2032-2036.
- Bhakuni RS, Jain DC, Sharma RP, Kumar S (2001). Secondary metabolites of *Artemisia annua* and their biological activity. *Curr. Sci.*, 80: 35-48.
- Bone K, Morgan M (1992). Clinical applications of Ayurvedic and

- Chinese Herbs: Monographs for the Western Herbal Practitioner. Warwick, Australia: Phytotherapy Press, pp. 7-12.
- Brisibe EA, Uyoh EA, Brisibe F, Magalhães PM, Ferreira JFS (2008). Building a golden triangle for the production and use of artemisinin derivatives against falciparum malaria in Africa. *Afr. J. Biotechnol.*, 7: 4884-4896.
- Chen YT, Ma L, Mei Q, Tang Y, Liao XG (1994). An experimental trial of artemether in treatment of *Pneumocystis carinii* in immunosuppressed rats. *Chin. Med. J.*, 107: 673 - 677.
- Echezona BC (2006). Selection of pepper cultivars (*Capsicum* spp.) for the control of bruchids *Callosobruchus maculatus* (F.) on stored cowpea (*Vigna unguiculata* (L.) Walp.) seeds. *Afr. J. Biotechnol.*, 5: 624-628.
- Efferth T, Romero MR, Wolf DG, Stammering T, Marin JJG, Marschall M (2008). The antiviral activities of artemisinin and artesunate. *Clin. Infect. Dis.*, 47: 804-811.
- Elujoba AA (2000). Studies on the antiarrhoeal activity of *Ocimum gratissimum* quavu. University of Ile-Ife Press, Ile-Ife, Nigeria.
- Emosairue SO, Eze OO, Okorie IK (1994). Timing of insecticide application in *Vigna unguiculata* (L.) Walp: CVT845-2246-4 and its potential as late season crop in the Calabar area. *J. Appl. Chem. Agric. Res.*, 1: 6-11.
- Erichsen-Brown C (1979). Use of plants for the past 500 years. Breezy Creek Press, Aurora, Ontario, Canada.
- Ferreira JFS, Janick J (1995). Production and detection of artemisinin from *Artemisia annua*. *Acta Hort.*, 390: 41-49.
- Foster S, Chongxi Y (1992). Herbal Emissaries: Bringing Chinese Herbs to the West. Healing Arts Press, Rochester, Vermont, USA.
- Gill LS (1992). Ethno-medicinal uses of plants in Nigeria. University of Benin Press, Benin City, Nigeria.
- Hough-Goldstein JA (1990). Antifeedant effects of common herbs on the Colorado potato beetle. *Environ. Entomol.*, 19: 234-238.
- Ivbijaro MF, Agbaje M (1986). Insecticidal activity of Piper guineense Schum and Thonn, and *Capsicum* species on cowpea bruchid, *Callosobruchus maculatus* (F.). *Insect Sci. Appl.*, 7: 521-524.
- Jackai LEN (1993). The use of neem in controlling cowpea pests. International Institute of Tropical Agriculture, Ibadan. *Res. Rep.*, 7: 5-11.
- Jones-Brando L, D'Angelo J, Posner GH, Yolken R (2006). *In vitro* inhibition of *Toxoplasma gondii* by four new derivatives of artemisinin. *Antimicrob. Agents Chemother.*, 50: 4206-4208.
- Junaid SA, Olabode AO, Onwuliri FC, Okwori AEJ, Agina SE (2006). The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *Afr. J. Biotechnol.*, 5: 2315-2321.
- Kashi KP (1981). Controlling pests in stored grain with carbon dioxide. *Span*, 24: 69-71.
- Kaul VK, Nigam SS, Banerjee AK (1978). Insecticidal activities of some essential oils. *Indian J. Pharmacol.*, 40: 22.
- Klayman DL (1989). Weeding out malaria. *Nat. History*, 10: 18-91.
- Lale NES (1994). Laboratory assessment of the effectiveness and persistence of powders of four spices on cowpea bruchid and maize weevil in air-tight storage facilities. *Samaru J. Agric. Res.*, 11: 79-84.
- Ma Y, Lu D, Lu X, Liao L, Hu X (2004). Activity of dihydroartemisinin against *Leishmania donovani* both *in vitro* and *vivo*. *Chin. Med. J.*, 117: 1271-1273.
- Mohun AF, Cook IJV (1957). Simple methods for measuring serum level of glutamic oxaloacetic and glutamic pyruvic transaminases in routine laboratories. *J. Clin. Pathol.*, 10: 394-399.
- Mishina YV, Krishna S, Haynes RK, Meade JC (2007). Artemisinins inhibit *Trypanosoma cruzi* and *Trypanosoma brucei* rhodesiense *in vitro* growth. *Antimicrob. Agents Chemother.*, 51: 1852-1854.
- Morton JF (1981). Atlas of medicinal plants of Middle America. Charles C. Thomas, Springfield, Illinois, USA.
- Negahban M, Moharrampour S, Sefidkon F (2007). Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. *J. Stored Prod. Res.*, 43: 123-128.
- Ofuya TI (1986). Use of wood ash, dry chill-pepper fruits and onion scale leaves for reducing *Callosobruchus maculatus* (F.) damage in cowpea seeds during storage. *J. Agric. Sci.*, 107: 467-468.
- Ogunwolu EO, Odunlami AT (1996). Suppression of seed bruchid (*Callosobruchus maculatus* (F.)) development and damage on cowpea (*Vigna unguiculata* (L.) Walp.) with *Zanthoxylum xanthoxyloides* (Lam.) Waterm. (Rutaceae) root bark powder when compared to neem seed powder and pirimiphos-methyl. *Crop Prot.*, 15: 603 - 607.
- Ohiagu CE (1985). Arthropod invaders as a threat to food and raw material security. Paper presented at a symposium of the 18th Annual Conference of the Entomological Society of Nigeria. Ahmadu Bello University, Zaria, Nigeria 6-9th October, 1985.
- Opareke AM, Dike MC, Onu I (1998). Evaluation of seed and leaf powder of neem (*Azadirachta indica* A. Juss) and pirimiphos-methyl for control of *Callosobruchus maculatus* (F.) in stored cowpea. *Entomol. Soc. Nigeria. Occasional Pub.*, 31: 237-242.
- Quin FM (1997). Introduction. In: Advances in cowpea research, Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Centre for Agricultural Sciences (JIRCAS), eds., Singh BB, Mohan Raj DR, Dashiell KE, Jackai LEN, IITA, Ibadan, Nigeria, pp. 4 -14.
- Rachie KO (1985). Introduction In: Cowpea research production and utilization. International Institute of Tropical Agriculture, Ibadan, Nigeria, eds., Singh SR, Rachie KO, John Wiley and Sons Ltd., pp. 21-28.
- Rajapakse RH (1990). Effect of five botanicals as protectants of greengram against the pulse beetle, *Callosobruchus maculatus*. In: Bruchids and Legumes: Economics, Ecology and Co-evolution. Eds., Fujii K, Gatehouse AMR, Johnson CD, Mitchell R, Yoshida T, Kluwer Academic publishers, Dordrecht, pp. 85-90.
- Reitman S, Frankel S (1957). Colorimetric method for the determination of serum transaminases. *Am. J. Clin. Pathol.*, 28: 56-63.
- Rice PJ, Coats JR (1994). Insecticidal properties of several monoterpenoids to the houseful, red flour beetle on Southern maize root worm. *J. Econ. Entomol.*, 87: 1172-1179.
- Sastry SG (1946). Davana oil. *Indian Soap J.*, 11: 242-244.
- Schmutterer H (1990). Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annu. Rev. Entomol.*, 35: 271-297.
- Shahsavani D, Mohri M, Gholipour Kanani H (2010). Determination of normal values of some blood serum enzymes in *Acipenser stellatus* Pallas. *Fish Physiol Biochem.*, 36: 39-43.
- Simon JE, Charles D, Cebert E, Grant L, Janick J, Whipkey A (1990). In *Artemisia annua* L.: A promising aromatic and medicinal. *Advances in New Crops*, eds. Simon JE and Janick J, Timber Press, Oregon, USA, pp. 522-526.
- Singh SR, Jackai LEN, Dos Santos JHR, Adalla CB (1990). Insect pests of cowpea. In: *Insect Pests of Food Legumes*, ed., Singh, SR. IITA/John Wiley and Sons Ltd., pp. 43-89.
- Sofowora LA (1993). Medicinal plants and traditional medicine in Africa, 3. Monograph, Spectrum Books Ltd., Ibadan, Nigeria.
- Tsao R, Lee S, Rice PJ, Jensen C, Coats JR (1995). Monoterpenoids and their synthetic derivatives as leads for new insect control agents. *Synthesis and Chemistry of Agrochemicals IV. ACS Symposium Ser.*, 584: 312-324.
- Utzinger T, Shuhua X, Keiser J, Minggan C, Jiang Z, Tanner M (2001). Current progress in the development and use of artemether for chemoprophylaxis of major human schistosome parasites. *Curr. Med. Chem.*, 8: 1841-1859.
- Watanabe K, Shono Y, Kakimizu A, Okada A, Matsuo N, Satoh A, Nishimura H (1993). New mosquito repellent from *Eucalyptus camaldulensis*. *J. Agric. Food Chem.*, 41: 2164-2166.
- Weaver DK, Philips TW, Dunkel FV, Weaver T, Grubb RT, Nance EL (1995). Dried leaves from Rocky Mountain plants decrease infestation by stored-product beetles. *J. Chem. Ecol.*, 21: 127-142.
- Xu QQ, Huang XB, Ma K, Chen L, Yu CF, Yu LP, Zhu JC, Wang XF, Na YQ (2010). Analysis of serum electrolytes in urolithiasis patients. *J. Peking Univ. Health Sci.*, 42: 451-453.
- Yang DM, Liew FY (1993). Effects of qinghaosu (artemisinin) and its derivatives on experimental cutaneous leishmaniasis. *Parasitology*, 106: 7-11.