

Full Length Research Paper

Sole and combined effect of three botanicals against cowpea seed bruchid, *Callosobruchus maculatus* Fabricius

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The bioactivity of three botanical powders in sole and combination against *Callosobruchus maculatus* (Fab.) was investigated at $29\pm 3^{\circ}\text{C}$ and relative humidity (R.H.) OF $65\pm 5\%$ in the laboratory. The appropriate mixing ratio of *Cymbopogon citratus* (C), *Alstonia boonei* (A) and *Hyptis suaveolens* involved seven combinations viz., C:A, C:H, A:H, C:A:H, H₂:C:A, A:C₂:H, H:C:A₂ in simple ratios 1:1, 1:1, 1:1, 1:1, 2:1:1, 1:2:1 and 1:1:2. The sole and combinations of botanicals were separately prepared and applied at the concentrations of 1.25% per 20 g seeds of two susceptible cowpea lines viz., Oloyin and IT845-2246 in the Kilner jars. Newly emerged ten females and five males *C. maculatus* were introduced separately into each of the Kilner jars, and replicated four times in a completely randomized design. Data were collected on adult mortality, number of eggs laid, offspring emergence, percentage seed damage, weight loss and seed viability. Results indicated that powder of *H. suaveolens* evoked significant mortality (100%) after 7 days of treatment. However, lower means were recorded in oviposition and adult bruchid emergence in cowpea seed treated with powders of *H. suaveolens* and *A. boonei*. Likewise, powder of *C. citratus* recorded the least seed damage and this implied that the three tested botanicals were observed to be effective bio-insecticide. The combination H:C:A₂ produced most desirable results causing higher adult mortality (96.33%), low offspring emergence, lower seed damage (0%), higher seed viability (88.00%), and least seed weight loss (0%) and therefore the most bio-active mixing ratio against *C. maculatus*. There was however interaction and synergism effect among the different combinations.

Key words: Bioactivity, mixing ratio, bio-insecticide, weight loss, viability.

INTRODUCTION

Cowpea, *Vigna unguiculata* is important particularly in West Africa with over 9.3 million hectares area and 2.9 million tonnes annual production (Fatokun et al., 2002).

Cowpea is grown both as a leaf and pod vegetable in the humid tropics (Steele and Mehorva, 1980). Cowpea seed is important to the income of poor farmers as well as to

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the nutritional status and diets of people in the tropics (Langyintuo et al., 2003), since animal protein sources are rarely affordable in adequate quantities by majority of the populace in developing countries. Cowpea is a highly nutritive leguminous crop which contains 22% protein, 1.5% fat and 60% carbohydrate (Dolvo et al., 1976), and a valuable source of calcium, iron, thiamine and riboflavin (Ofuya, 2001). Cowpea is a veritable source of dietary protein for the teeming human population and livestock (Murdock et al., 1997), and can serve as a useful complement in diets comprising mainly of roots, tubers or cereals. Similarly, it can be boiled and consumed directly, made into flour, puddings or weaning foods for young children and thus ameliorate malnourishment and wastage (Phillips and Dedeh, 2003). Also, it can be ploughed into soil as green manure or grown as cover crop to improve soil fertility.

Cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) is responsible for over 90% of the damage done to cowpea seed (Caswell, 1982); and if left uncontrolled for over six months in storage, 100% loss may be recorded (Singh, 1977; Seck, 1993). Thus, the damage caused during storage, shipping and transportation, is a very serious problem all over the globe (Upadhyay and Ahmad, 2011). The insect pests not only damage the grain but also depreciate the weight and quality of stored grains (Rayhan, 2014). Beetle damage also causes significant reduction in seed viability because damaged seeds are riddled with holes by adult insects. The fatty acid content of seeds infested by *C. maculatus* increases, thus caused a slight denaturation of proteins and loss of the important vitamin; thiamine (Southgate, 1978). Heat, moisture and waste products produced by the bruchid result in further deterioration and the growth of molds, which renders cowpea grains unfit for consumption (Shazia et al., 2006). The quality of grains and seeds during storage depends on various factors such as crop or variety, initial seed quality, storage conditions, seed moisture content, insect pests, bacteria and fungi (Amruta et al., 2015).

Nowadays, pest control technology is mostly dependent on synthetic insecticides (Azad et al., 2013). However, the quick and effective control of pests with insecticides convinces the farmers easily compared to the non-chemical methods of pest management. Having a knockdown effect on targets, more often insecticides form the only solution of sudden outbreak of pests. Raupp et al. (2014) reported the residual effect of insecticides on insect pests and natural enemies, while inherent high mammalian toxicity and ecological safety are of great concern to both environmentalists and researchers worldwide (Zacharia, 2011). However, the development of resistance and resurgence has limited the application of single insecticides resorting to tank mixtures. Plant products, such as aqueous or organic solvent extracts are being used in many countries as protectants of stored products (Fernando and

Karunaratne, 2012; Rajashekare et al., 2010 and 2012). Several workers have researched the use of single application of botanicals. It would however be germane to examine and determine the combinations of three botanicals in different mixing ratio for the farmer's use. This however engendered interaction and synergism effect among the different combinations which boosted more protectant ability of the botanicals. The combinations of more than one botanical would sustain optimal agricultural production through the management and control of insect pests of crops and food products.

MATERIALS AND METHODS

Plant materials

The three plants species viz., *C. citratus* (Dc ex Nees) Stapf, *Alstonia boonei* DeWild and *Hyptis suaveolens* Poit were sourced from Abeokuta, South West, Nigeria, and were identified at the Department of Forestry and Wildlife, College of Environmental Resources Management, Federal University of Agriculture, Abeokuta, Nigeria. The plant leaves were washed in clean water and were later air-dried in room temperature (25°C) and ground into fine powder using an electric grinder. The powder was further sieved in 100 µm aperture sieve. Ife Brown and IT845-2246 cowpea varieties were obtained from the Institute of Agricultural Research and Training, Ibadan and International Institute for Tropical Agriculture, Ibadan, Nigeria, respectively. The cowpea seeds were disinfested using cold shock treatment at 0 to 4°C for seven days.

Rearing of experimental insects

The initial 200 unsexed adult *C. maculatus* were obtained from the culture maintained on Ife Brown cowpea variety in the Department of Crop Protection, Federal University of Agriculture, Abeokuta, Nigeria. Fifty adults were introduced into a 500-ml Kilner jar containing 200 g of clean disinfested Ife Brown cowpea seeds, and 4 jars were prepared in this manner. The Kilner jars were covered with muslin cloth held in place by a screw cap in order to allow aeration and to prevent the insects from escaping. The set-up was kept under ambient temperature (27±3°C) and relative humidity (70-85%). The insects were allowed to mate for seven days and lay eggs in each of the jars after which they were removed to avoid multiple oviposition. The devoured seeds were replaced continuously with the same quantity of freshly disinfested seeds. Only the new adult bruchids emerging from the culture were used for the experiment.

Toxicity bioassay

The powders of each of the botanicals, *C. citratus* (C), *A. boonei* (A) and *H. suaveolens* (H) were admixed with 20 g of disinfested cowpea seeds of each variety in a Kilner jar. Similarly, seven combinations viz., C:A, C:H, A:H, C:A:H, H₂:C:A, A:C₂:H, H:C:A₂ in ratios 1:1, 1:1, 1:1, 1:1, 2:1:1, 1:2:1 and 1:1:2 were applied. The plant powders and their combinations were separately prepared and applied at lowest concentrations of 1.25%. Newly emerged ten females and five males of *C. maculatus* were introduced into each of the Kilner jars. Each treatment was replicated four times, and the control jar contained cowpea seeds admixed with plant powder prepared from *Azadiracta indica*. All Kilner jars containing the seeds and combined plant powders were arranged on work tables in the

Table 1. Effect of botanicals on the development and control of *Callosobruchus maculatus*.

Cowpea lines	Botanicals	Mortality at 7days post treatment	Number of eggs laid	Filial generations		
				F1	F3	F2
Oloyin	<i>C. citratus</i>	77.48 ^{abc}	13.50 ^{cdef}	26.25 ^{abcd}	17.75 ^{bc}	0.00 ^b
	<i>A.boonei</i>	75.83 ^{abc}	33.25 ^{bcde}	22.00 ^{abcd}	15.25 ^{bc}	0.00 ^b
	<i>H. suaveolens</i>	100.00 ^a	29.00 ^{bcde}	7.75 ^d	17.25 ^{bc}	0.00 ^b
	Control	0.00 ^e	68.08 ^{ab}	58.00 ^{abcd}	97.67 ^a	17.83 ^a
IT845-2246	<i>C. citratus</i>	95.00 ^{ab}	25.00 ^{bcde}	31.75 ^{abcd}	30.00 ^{bc}	0.00 ^b
	<i>A.boonei</i>	75.83 ^{abc}	18.00 ^{cdef}	25.75 ^{abcd}	27.75 ^{bc}	0.00 ^b
	<i>H. suaveolens</i>	78.30 ^{abc}	16.75 ^{cdef}	25.50 ^{abcd}	22.50 ^{bc}	0.00 ^b
	Control	0.00 ^e	51.00 ^{abcd}	75.33 ^{abc}	88.67 ^b	9.25 ^b

Means separated using Student Neumankeuls test ($P < 0.05$). Means followed by the same letter are not significantly different from one another across the columns.

laboratory in a completely randomized design. Also, the Kilner jars containing the treated cowpea seeds were covered with a muslin cloth and tied with a rubber band. This aerated the contents and prevented other insects from entering the containers. Records of mortality were taken at 1, 3, 5 and 7 days after treatment. Thus, bruchids that showed no visible movement after 20 s of observation were turned with forceps before considering as dead. After 7th day assessment, all adult bruchids were removed from the Kilner jars and cumulative data on percentage adult bruchid mortality were corrected using Abbott (1925) formula as:

$$P_t = P_o - \frac{P_c \times 100}{100 - P_c}$$

Where, P_t , corrected % mortality; P_o , observed % mortality; P_c , control % mortality. That is, when all the bruchids had died after 14 to 21 days, the number of egg laid was counted and recorded. The F_1 progeny population was assessed on a daily basis and removed after the Kilner jars were left until 4 weeks post treatment. At the end of the twelve weeks period, the contents of each container were sieved to remove the dust, frass and any insect present in the cowpea seeds. The number of undamaged seeds was counted to assess damage to the cowpea seeds by the bruchids. The cowpea seeds were re-weighed and the percentage loss in weight was computed, thus:

$$\%W_t \text{ loss} = \frac{(W_i \times W_f)}{W_i}$$

Where, W_i is the initial weight and W_f is the final weight. The quality of the cowpea seeds was also tested through viability test. Thus, the viability of the treated seeds was tested in Petri dishes (9 cm diameter) lined with moist filter paper. Twenty cowpea seeds were randomly selected from every treatment, watered for 48 h in the Petri-dishes until the end of experiment that is 96 h. The percentage of the germinated seeds per treatment gave an indication of the relative viability of the seeds.

Statistical analysis

All data collected were subjected to analysis of variance using SAS (2002). Significant means were separated using Student's Newman-Keuls tests $\alpha = 0.05$.

RESULTS

Irrespective of lines and botanicals, significantly higher bruchid mortality was recorded on treated cowpea seeds compared to the control. Hundred percent mortality, was recorded with *H. suaveolens* compared with *C. citratus* and *A. boonei* (Table 1). *Hyptis suaveolens* caused significant reduction in adult bruchid emergence (in the first and second filial generations) while all three botanicals tested caused outright inhibition and reduction in adult bruchid emergence in the third filial generation (Table 1). However, highest adult bruchid emergence was recorded on the untreated cowpea seeds (control).

Table 2 shows that the lowest seed damage was recorded on cowpea seeds treated with *C. citratus* compared to other botanicals. However, the highest seed damage was recorded on the control. Also, regardless of lines cowpea seeds treated with *C. citratus* powders gave significantly lower seed weight loss compared to other botanicals (Table 2). Nonetheless, the weight loss was lower on seeds treated with *C. citratus*, *A. boonei* and *H. suaveolens* compared to the untreated. Also, mortality of bruchids after three months of storage was lower on cowpea seeds treated with the botanicals compared to control. Likewise, significantly higher seed viability was recorded on cowpea seeds treated with the three botanicals compared to untreated cowpea seeds (control) (Table 2).

Irrespective of lines, bruchid mortality varied among the different combinations. The different combinations of the botanicals gave significantly higher adult mortality compared to the control. The combinations of three botanicals, *A. boonei* (A), *C. citratus* (C) and *H. suaveolens* (H), A:C₂:H (1:2:1) recorded 100% mortality followed by H:C:A₂ (1:1:2), C:A (1:1), C:H (1:1) and H₂:C:A (2:1:1); these were significantly different from A:H (1:1) and C:A:H (1:1:1) (Tables 3 and 4).

Combinations H₂:C:A (2:1:1) and C:H (1:1) recorded significantly higher number of eggs laid relative to other

Table 2. Effect of botanicals on the development and control of *Callosobruchus maculatus*.

Cowpea lines	Botanical	Mortality after 3 months storage	Seed damage	Seed weight loss	Seed viability
Oloyin	<i>C.citratus</i>	31.25 ^b	13.13 ^{cd}	3.60 ^{cd}	69.00 ^a
	<i>A.boonei</i>	29.50 ^b	41.25 ^{abcd}	4.60 ^{cd}	69.75 ^a
	<i>H. suaveolens</i>	31.25 ^b	16.66 ^{abcd}	4.60 ^{cd}	68.25 ^a
	Control	76.25 ^{ab}	78.27 ^{abcd}	42.70 ^{ab}	16.67 ^{hi}
IT845-2246	<i>C. citratus</i>	25.00 ^b	26.67 ^{abcd}	4.00 ^d	50.00 ^{def}
	<i>A.boonei</i>	19.75 ^b	40.63 ^{abcd}	4.10 ^{cd}	55.00 ^{cde}
	<i>H. suaveolens</i>	16.50 ^b	51.04 ^{abcd}	4.10 ^{cd}	60.25 ^{abc}
	Control	87.66 ^{ab}	89.09 ^{abc}	47.07 ^{ab}	16.58 ^{hi}

Means separated using Student Neumankeuls test ($P < 0.05$). Means followed by the same letter are not significantly different from one another across the columns.

Table 3. Assessment of combination ratios of two botanicals using teneral adult bruchid (*Callosobruchus maculatus*).

Parameter	Lines	C:A	C:H	A:H	Control
Mortality (7D)	Oloyin	93.32 ^a	92.00 ^a	73.30 ^{ab}	68.00 ^c
	IT845-2246	68.00 ^{ab}	69.98 ^{ab}	68.66 ^{ab}	60.00 ^c
Eggs laid	Oloyin	20.40 ^{ab}	4.60 ^d	30.00 ^a	24.71 ^e
	IT845-2246	14.80 ^{ab}	5.00 ^d	18.20 ^{ab}	19.57 ^e
F1 generation	Oloyin	0.00 ^d	0.00 ^d	1.20 ^d	42.85 ^e
	IT845-2246	0.00 ^d	0.00 ^d	2.20 ^d	31.00 ^f
F2 generation	Oloyin	0.00 ^d	0.00 ^d	4.00 ^d	48.85 ^c
	IT845-2246	0.00 ^d	4.00 ^d	2.00 ^d	58.29 ^e
F3 generation	Oloyin	0.40 ^c	0.00 ^c	2.00 ^c	30.14 ^d
	IT845-2246	0.20 ^c	0.40 ^c	1.40 ^c	28.71 ^d
Mortality (3MS)	Oloyin	58.00 ^a	18.00 ^{bc}	77.80 ^a	146.43 ^d
	IT845-2246	16.00 ^{bc}	20.00 ^{cd}	15.00 ^{cd}	142.86 ^d
Seed damage	Oloyin	4.00 ^{cd}	8.90 ^{cd}	8.10 ^{cd}	97.60 ^a
	IT845-2246	4.00 ^{cd}	8.90 ^{cd}	8.10 ^{cd}	95.00 ^a
Seed weight loss	Oloyin	12.00 ^{cde}	2.00 ^e	17.80 ^{cd}	60.86 ^f
	IT845-2246	1.80 ^e	11.80 ^{cde}	19.80 ^{cd}	62.29 ^f
Seed viability	Oloyin	72.00 ^{abcd}	88.00 ^a	72.00 ^{abcd}	30.00 ^e
	IT845-2246	84.00 ^{ab}	68.00 ^{abcd}	72.00 ^{abcd}	30.00 ^e

Means separated using Student Neumankeuls test ($P < 0.05$). Means followed by the same letter are not significantly different from one another across the columns. C = *C. citratus*; A = *A. boonei*; H = *H. suaveolens*; Mortality (3MS), mortality after 3 months of storage; mortality (7 D), mortality at 7 days post treatment.

combinations. The control however recorded the highest. Cowpea seeds treated with combinations C:H (1:1) and

C:A:H (1:1:1) recorded the lowest oviposition which was significantly different from other combinations except

Table 4. Assessment of combination ratios of three botanicals using general adult bruchid (*Callosobruchus maculatus*).

Parameter	Lines	C:A:H	A:C ₂ :H	H ₂ :C:A	H:C:A ₂	Control
Mortality (7D)	Oloyin	72.66 ^{ab}	100.00 ^a	90.00 ^a	96.00 ^a	68.00 ^c
	IT845-2246	95.00 ^a	95.00 ^a	96.00 ^a	96.66 ^a	60.00 ^c
Eggs laid	Oloyin	6.60 ^{cd}	12.00 ^{bcd}	4.00 ^d	13.00 ^{bcd}	24.71 ^e
	IT845-2246	5.00 ^d	16.80 ^{ab}	14.40 ^{ab}	9.60 ^{cd}	19.57 ^e
F1 generation	Oloyin	0.20 ^d	14.40 ^b	0.00 ^d	0.00 ^d	42.85 ^e
	IT845-2246	0.20 ^d	29.20 ^a	9.80 ^c	0.00 ^d	31.00 ^f
F2 generation	Oloyin	0.00 ^d	24.57 ^{bcd}	5.80 ^{cd}	0.00 ^d	48.85 ^c
	IT845-2246	0.00 ^d	38.80 ^a	34.40 ^{ab}	0.00 ^d	58.29 ^e
F3 generation	Oloyin	7.60 ^a	1.40 ^c	3.80 ^{ab}	0.00 ^c	30.14 ^d
	IT845-2246	1.00 ^c	0.60 ^c	1.80 ^c	0.00 ^c	28.71 ^d
Mortality (3MS)	Oloyin	58.00 ^a	52.40 ^a	62.40 ^a	0.00 ^c	146.43 ^d
	IT845-2246	30.00 ^a	62.00 ^a	35.00 ^a	0.00 ^c	142.86 ^d
Seed damage	Oloyin	15.00 ^{cd}	29.00 ^c	15.60 ^{cd}	0.00 ^d	97.60 ^a
	IT845-2246	9.50 ^{cd}	56.00 ^b	21.00 ^{cd}	0.00 ^d	95.00 ^a
Seed weight loss	Oloyin	7.60 ^{de}	29.80 ^{bcd}	11.20 ^{cde}	0.00 ^e	60.86 ^f
	IT845-2246	6.20 ^{de}	38.60 ^a	38.80 ^a	0.00 ^e	62.29 ^f
Seed viability	Oloyin	84.00 ^{ab}	54.00 ^{bcd}	76.00 ^{abc}	90.00 ^a	30.00 ^e
	IT845-2246	84.00 ^{ab}	40.00 ^{def}	44.00 ^{de}	88.00 ^a	30.00 ^e

Means separated using Student Neumankeuls test ($P < 0.05$). Means followed by the same letter are not significantly different from one another across the columns. C = *C. citratus*; A = *A. boonei*; H = *H. suaveolens*; Mortality (3MS), mortality after 3 months of storage; mortality (7 D), mortality at 7 days post treatment.

H:C:A₂ (1:1:2). The control recorded the highest number of eggs laid and was significantly different from all other treated cowpea lines.

No seed damage was recorded with combination H:C:A₂ (1:1:2). Combinations C:A (1:1), C:H (1:1) and C:A:H (1:1:1) also recorded significant reduction in seed damage compared to other lines (Tables 3 and 4). There was no seed weight loss with combinations H:C:A₂ (1:1:2). Weight loss recorded with combinations C:H (1:1) and C:A:H (1:1:1) was significantly lower than the control (Tables 3 and 4). For F₁ generation, no adult emergence of bruchids was recorded with combinations H:C:A₂ (1:1:2), C:A (1:1) and C:A (1:1) and C:H (1:1). With combinations H₂:C:A (2:1:1) and C:A:H (1:1:1), lowest values of F₁ generation emergence was recorded, which however was significantly lower than A:C₂:H (1:2:1) and control. Similarly, with combinations H:C:A₂ (1:1:2), C:A:H (1:1:1) and C:A (1:1), no bruchid emergence was recorded from both cowpea lines. Combinations C:H (1:1) and A:H (1:1) also recorded significant reduction in adult emergence compared to other lines.

No adult emergence was recorded with H:C:A₂ (1:1:2). Combinations C:A (1:1), C:H (1:1), A:H (1:1) and A:C₂:H

(1:2:1) recorded significantly lower values of F₃ generation emergence compared to C:A:H (1:1:1) and H₂:C:A (2:1:1) (Tables 3 and 4).

Combinations H:C:A₂ (1:1:2), C:H (1:1), C:A:H (1:1:1) and C:A (1:1) recorded significantly higher seed viability relative to other combinations. There were however interaction effect among the different combinations. Combination H:C:A₂ (1:1:2) recorded no bruchid mortality after three months of storage relative to other combination, while the highest percentage was recorded by the control. Combination C:H (1:1) also recorded significantly lower bruchid mortality compared to other combinations. Other combinations recorded significantly higher bruchid mortality relative to control (Tables 3 and 4).

DISCUSSION

Farmers are encouraged to resort to botanicals that have the phyto-tonic effect that would increase seed quality parameters. According to Sandeep et al. (2013), higher germination, vigour index and less infestation were

recorded during storage when *Zea may* seeds were treated with *Acorus calamus* rhizome. The results obtained from this trial showed that *H. suaveolens*, *C. citratus* and *A. boonei* caused bruchid mortality. Botanicals such as *Azadirachta indica*, *Acorus calamus*, *Lantana camara*, *Melia azadarach*, *Piper nigrum*, and *Adhatoda zeylanica* are biodegradable, non-residual, equally effective and easily available. Generally, all the botanicals tested caused significantly higher bruchid mortality compared with the untreated (control). Plant materials with medicinal and pharmacological properties have been found effective in botanical control of *C. maculatus* (Sofowora, 1982). In a similar experiment, Olaniran et al. (2013) reported the use of plant extracts of *Tephrosia vogelli* and *Azadirachta indica* in the control of foliage pests of *Hibiscus sabdariffa* L. The *C. citratus*, *H. suaveolens* and *A. boonei* caused increased in mortality, reduced progeny emergence, seed damage and weight loss. In a similar vein (Manohar et al., 2017; Azeez and Pitan 2014) reported that botanicals prove to be a better option to control field and storage pests without affecting the quality of grains or seeds and without destroying the ecosystem or environment. This is also similar to the findings of Shazia et al. (2006) who reported that black pepper powder gave significantly better results than the control in suppressing bruchid survival, higher numbers of undamaged seeds and fewer holes per cowpea seed. Rajashekare et al. (2012) however confirmed the use of botanicals as grain protectants. Previous works have demonstrated the potency of some botanicals to preserve seed quality (Khatum et al., 2011; Rana et al., 2014); reduced seed damage (Rana et al., 2014) and weight loss (Rayhan et al., 2014). Extracts of *A. boonei* possess anti-microbial activity (Omoregbe and Osaghae, 1997). Plant products, such as aqueous or organic solvent extracts are being used in many countries as protectants of stored products (Fernando and Karunarathe, 2012). Thus, some of the metabolites of plants are toxic such as pyrethrum, nicotine, rotenone etc and some are repellents, and antifeedants like azadirachtin, rape seed extract and others, like *Acorus calamus* act as sterilants (Ignatowicz and Wesolowska, 2015). *C. citratus* is effective against the yam beetle (Tobih, 2011), while the stem of *C. citratus* had been found to also cause mortality in bruchids (Dike and Mbah, 1992). Powder of *H. suaveolens* was effective in protecting cowpea seeds against insects (Adedire and Lajide, 1999). Similarly, Barbara et al. (2010) reported that topical applications of *H. suaveolens* and *H. spicigera* on insects showed that both essential oils had an effective insecticidal activity. There was neither seed damage nor weight loss in seeds treated with *A. boonei*, *H. suaveolens* and *C. citratus*. Botanicals affect only target pests, are effective in very small quantities, degrade rapidly and provide pesticide free food and a safe environment for living beings (Joseph et al., 2012; Rajashekare et al., 2010). Tobih (2011) had previously rated *C. citratus* as superior

repellent or antifeedant botanicals to the yam beetle. Oviposition deterrence was observed on seeds treated with *C. citratus*, *A. boonei* and *H. suaveolens* where significantly fewer eggs were laid on the treated cowpea seeds. Rajapakse and van Emden (1997) reported that all four oils tested (corn, ground nut, sunflower and sesame) significantly reduced the oviposition of all the three bruchid species studied (*Callosobruchus maculatus*, *C. chinensis* and *C. rhodesianus*). Boeke et al. (2004) reported that the adult beetles died soon after they came into contact with the powder of *Tephrosia vogelli* and lay few eggs, only very few developed into adults. Musa et al. (2009) reported that seed-extract of *H. suaveolens* was significantly more effective in enhancing adult mortality, reducing egg laying and suppressing larval and adult emergence. All the three botanicals recorded significantly higher seed viability compared to control because the botanicals prevented seed damage and subsequently retained the viability of the cowpea seeds. On the other hand, damage occurred on untreated seeds resulting in destruction of the embryos and subsequent reduction in the viability of the seeds. This implied that the three botanicals are potent against *C. maculatus*. This is however underscored by the findings of Misra (2014) who reported the role of botanicals, biopesticides and bioagents in integrated pest management.

The results of the study revealed that the combinations of the botanicals gave significantly higher adult mortality compared to the control. This observation is sustainable because more complex preparations such as combination of substances present in insecticide are likely to become effective to overcome development of resistance by insect pests (Regnault-Roger and Hamraini, 1993). The combinations of three botanicals A:C₂:H (1:2:1) recorded 100% mortality at 7 days. Amruta et al. (2015) recorded effective storage insect control and higher seed quality when treated with botanicals and emamectin benzoate. This is also in agreement with the findings of Emeasor et al. (2007), who reported similar work that mixture of seed powder of *Piper guineense* and *Thevetia peruviana* at different percentage caused the highest mortality of *C. maculatus* at 7 days after infestation. The percentage mortality recorded at combination A:C₂:H (1:2:1) was not significantly different from the following combinations H:C:A₂ (1:1:2), C:A (1:1), C:H (1:1) and H₂:C:A (2:1:1). Combination H₂:C:A (2:1:1) and C:H (1:1) recorded significantly lower number of eggs laid relative to other combinations. Combinations C:H (1:1) and C:A:H (1:1:1) and H:C:A₂ (1:1:2) reduced oviposition when compared with the control. Also, H:C:A₂ (1:1:2) recorded no bruchid emergence that is F₁, F₂, and F₃ generations throughout the duration of trial. This is in agreement with the work of Dawodu and Ofuya (2000), who reported that oviposition and adult emergence of *C. maculatus* were lower in seeds treated with mixed formulation of *P. guineense* and *Dennelta tripelata*

powders compared to either applied singly. Emeasor et al. (2007) reported in another study that the mixture of *P. guineense* and *Thevetia peruviana* at different percentages caused the highest mortality, least egg counts and significantly suppressed adult emergence. Also, Rayhan et al. (2014) reported that the bio-efficacy of neem, mahogoni and their mixture were able to prevent seed damage and seed weight loss by rice weevil in storage. Although there may not be differences in the bruchid mortality recorded in the combination compared with single application, the combination is desirable due to reduction in chances of resistance development.

Neither seed damage nor weight loss, was recorded with combination H:C:A₂ (1:1:2). With combination C:A (1:1), C:H (1:1) and C:A:H (1:1:1) there was significant reduction in seed damage and weight loss compared to other lines and viability was therefore preserved. These findings would be readily accepted by the local farmers because peasant farmers in sub-saharan Africa use indigenous plants either singly or in mixtures to protect cowpeas against pest damage during storage (Ibrahim, 2012; Ignatowicz and Wesolowska, 2015; Issa et al., 2011; Khatum et al., 2011). Shazia et al. (2006) found that a combination of leaf of *A. indica* and *T. vogelli* are effective in the control of cowpea seed bruchid, *C. maculatus*. Also, Ogunwolu and Idowu (1994) reported that insecticidal activity of *Zanthoxylum zanthoxyloides* root bark powder and *A. indica* seed powder was not mitigated by mixing the two against *C. maculatus*. The mixture may give best control of a complex of pests with varying levels of susceptibility to the different components of the mixtures. Insects that are resistant to one or more insecticides may be susceptible to a combination of toxicants or synergism may be exhibited by the components (Wolfenbarger and Cantu, 1975). Mixtures of insecticides could also be used because of cost efficiency (Aill et al., 1977).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Abbott SW (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18:265-267.
- Adedire CO, Lajide L (1999). Efficacy of powders of some tropical plants in the control of the pulsebeetle, *Callosobruchus maculatus* (F.) (Coleoptera : Bruchidae). *Appl. Trop. Agric.* 6:11-15.
- All JN, Ali M, Hornyak EP, Weaver JB (1977). Joint action of two pyrethroids with methylparathion, Methomyl and chlorpyrifos on *Heliothis zea* and *Heliothis virescens* in the laboratory and in Cotton and sweet corn. *J. Econ. Entomol.* 70(6):813-817.
- Amruta N, Sarika G, Umesh Maruthi JB, Basavaraju GV (2015). Effect of botanicals and insecticides seed treatment and containers on seed longevity of black gram under natural ageing conditions. *J. App. Nat. Sci.* 7(1):328-334.
- Azad AK, Sardar A, Yesmin N, Rahman M, Islam S (2013). Eco-friendly pest control in cucumber (*Cucumis sativa* L.) field with botanical pesticides. *Nat. Resour.* 4(5):6.
- Azeez OM, Pitan OOR (2014). Influence of cowpea variety on the potency and deterrent indices of six plant powders against the seed bruchid, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). *Archives Phytopathol. Plant Prot.* pp. 441-451.
- Boeke SJ, Baumgart IR, van Loon JJA, Van Huis, Dicke M, Kossou DK (2004). Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against *Callosobruchus maculatus*. *J. Stored Prod. Res.* 40:423-438.
- Caswell GH (1982). Damage stored cowpea in the Northern part of Nigeria, Samaru. *J. Agric. Res.* 1:111-191.
- Langyintuo AS, DeBoer JL, Faye M, Lambert D, Ibro G, Moussa B, Kergna A, Kushwaha S, Musa S, Ntougam G (2003). Cowpea supply and demand in West and Central Africa. *Field Crop Res.* 82:215-231.
- Dike MC, Mbah Ol. 1992. Evaluation of lemon grass, *Cymbopogon citratus* Staphs. Products in the control of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on stored cowpea. *Niger. J. Plant Protect.* 14:88-91.
- Dolvo FE, Williams CE, Zoaka L (1976). Cowpea. In: Home preparation and use in West Africa. *Int. Dev. Res. Centr, Ottawa Canada.* pp 91-96.
- Emeasor KC, Emosairue SO, Ogbuji RO (2007). Preliminary laboratory evaluation of the efficacy of mixed seed powders of *Piper guineense* (Schum and Thonn) and *Thevetia peruviana* (Persoon) Schum against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Niger. J. Entomol.* 24:114-118.
- Fatokun CA, Tarawali SA, Singh BB, Kormalwa PM, Tamo M (2002). Challenges and opportunities for Increasing cowpea production. *Proc. Wrlld Cowp. Confr. held at I.I.T.A., Ibadan, Nigeria.* 4th-8th Sept., 2002.
- Fernando HSD, Karunaratne MMSC (2012). Ethnobotanicals for storage insect pest management: Effect of powdered leaves of *Olax zeylanica* in suppressing infestations of rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *J. Trop. For. Environ.* 2(1):20-25.
- Ibrahim MY (2012). Efficacy of some plant oils against stored-product pest cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae) on chickpea seeds. *Per. Gu. Crop Prot.* 1(1): 4-11.
- Ignatowicz S, Wesolowska B (2015). Potential of common herbs as grain protectants: repellent effect of herb extracts on the granary weevil, *Sitophilus granarius* L. *Proceedings of 6th International Work. Confer. Stored-products Prot.* 2:790-794.
- Issa US, Afun JVK, Mochiah MB, Owusu-Akyaw M, Braima H (2011). Effect of some local botanical materials for the suppression of weevil populations. *Int. J. Plant Anim.. Environ. Sci.* 1(3):270-275.
- Joseph B, Sowmya, Sujatha, S (2012). Insight of botanical based biopesticides against economically important pest. *Int. J. Pharm. Life Sci.* 3(11):2138-2148.
- Khatum A, Kabir G, Bhuiyan MAH, Khanam D (2011). Effect of preserved seeds using different botanicals on seed quality of lentil. *Ban. J. Agric. Res.* 36(3):381-387.
- Manohar L, Budhi R, Prabhat T (2017). Botanicals to Cope Stored Grain Insect Pests: A Review. *Int. J. Curr. Microbiol. App. Sci.* 6(6):1583-1594.
- Misra HP (2014). Role of botanicals, biopesticides and bioagents in integrated pest management. *Odisha Rev.* pp. 62-67.
- Murdock LL, Shade RE, Kitch LW, Nittoukam G, Lowenberg-De Boer JE, Huesing JE, Moar W, Chambliss OL, Endondo C, Wolfson JL (1997). Post-harvest storage of cowpea in Sub-saharan Africa. In: Singh BB, DR Mohan Raj KE, Dashiell, LEN, Jackai (eds). *Advances in cowpea research*, IITA/JIRCAS Publication, IITA, Ibadan. pp. 302-312.
- Musa AK, Dike MC, Onu I (2009). Evaluation of Nitta (*Hyptis suaveolens* Poit.) seed and leaf extracts and seed powder for the control of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) in stored groundnut. *Am-Eur. J. Agron.* 2(3):176-179.
- Ofuya TI (2001). Biology, ecology and control of insect pests of stored cereals and pulses in Nigeria. In: pests of stored cereals and pulses in Nigeria; Ofuya TI, Lale NES, (eds). *Dave Collins Publications, Nigeria.* pp. 24-44.
- Ogunwolu EO, Idowu OT (1994). Potential of powdered *Zanthoxylum zanthoxyloides* (Rutaceae) root bark and *Azadirachta indica* (Meliaceae)

- seed for the control of cowpea seed bruchid, *Callosobruchus maculatus* (F.). Niger. J. Afr. Zool. 108:521-528.
- Omoregbe RE, Osaghe F. 1997. Antibacterial activity of extracts of *Momordica charantia* and *Alstoma boonei* on some bacteria. Niger. J. Biotechnol. 1:30-33.
- Phillips RD, Dedeh SS (2003). Developing nutritional and economic value added food products from cowpea. <http://www.isp.msu.edu/crsp/Final> Accessed 07/02/2005.
- Rajapakse R, van Emden HF (1997). Potential of four vegetable oils and ten botanical powders for reducing infestation of cowpeas by *Callosobruchus maculatus*, *Callosobruchus chinensis* and *Callosobruchus rhodesianus*. J. Stored Prod. Res. 33:59-68.
- Rajashekar Y, Bakthavatsalam N, Shivanandappa T (2012). Botanicals as grain protectants. Psyche: A J. Entomol, 13p.
- Rajashekar Y, Gunasekaran N, Shivanandappa T (2010). Insecticidal activity of the root extract of *Decalepis hamiltonii* against stored product insect pests and its application in grain protection. J. Food Sci. Technol. 47(3):310-314.
- Rana K, Sharma KC, Kanwar HS (2014). Efficacy of aqueous plant extracts on the seed quality of pea (*Pisum sativum* L.) during storage. Am. Int. J. Res. For. App. Nat. Sci. 6(1):7-11.
- Raupp MJ, Holmes JJ, Clifford SP, Shrewsbury P, Davidson JA (2014). Effects of cover sprays and residual pesticides on scale insects and natural enemies in urban forests. J. Arbori. 27(4):213-214.
- Rayhan MZ, Das S, Sarkar R, Adhikary SK, Tania SN, Islam MM, Rabbani MG (2014). Bioefficacy of neem, mahogoni and their mixture to protect seed damage and seed weight loss by rice weevil in storage. J. Biodivers. Environ. Sci. 5(1):582-589.
- Regnault-Roger C, Hamraoui A, Holeman M, Theron E, Pinel R (1993). Insecticidal effect of essential oil from Mediterranean aromatic plants upon *Acanthoscelides obtectus* Say. Coleopteran, bruchid Kidney bean (*Phaseolus vulgaris* L.) J. Chem. Ecol. 19:133-144.
- Sandeep D, Chandrashekar GS, Ranganathswamy M, Mallesh SB, Kumar HBH, Patibanda AK (2013). Effect of botanicals on storability of sweet corn (*Zea mays* L. Saccharum) seeds. Int. J. Plant Prot. 6(1):11-14.
- Seck D (1993). Resistance to *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) in some cowpea varieties from Senegal. J. Stored Prod. Res. 29:49-52.
- Shazia OWM, Minza M, Rhodes M, Robert NM, Bukheti K, Maulid M, Herman FL, Christine GI, Dastun GM, Loth SM (2006). Control of cowpea weevil (*Callosobruchus maculatus* F.) in stored cowpea (*Vigna unguiculata* L.) grains using botanicals. Asian J. Plant Sci. 5(1): 91-97.
- Singh SR (1977). Cowpea cultivars resistant to insect pests in world germplasm collection. Trop. Grain Legum. Bull. 9:1-7.
- Sofowora EA (1982). Medicinal plant and traditional medicine in Africa. John Wiley and Sons, Chichester pp. 50-55.
- Southgate BJ (1978). The importance of the Bruchidae as pests of grain legumes: their distribution and control. In: SR Singh HF van Emden and TA Taylor (eds). Pests of grain legumes: Ecology and control. Academic Press, London pp. 219-229.
- Steele WM, Mehorva KL (1980). Structure, evolution and adaptation to farming systems and environment In *Vigna* spp. In: Summer field RJ and AH Bunting (eds.). Advance in Legume Science. Royal botanical Gardens. KLEW England 667p.
- Tobih FO (2011). Evaluation of some plant materials as organic mulch for the control of yam tuber beetles (*Heteroligus* spp) in Delta State, Nigeria. Agric. J. 6(4):1-9.
- Upadhyay RK, Ahmad S (2011). Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World. J. Agric. Sci. 7(5):527-549.
- Wolfenbarger DA, Cantu E (1975). Enhanced toxicity of carbaryl when combined with synergists against larvae of the bollworm, *Heliothis zea* and the tobacco budworm, *Heliothis virescens*. Flor. Entomol. 58:103-104.
- Zacharia JT (2011). Ecological Effects of Pesticides. In: Pesticides in the Modern World - Risks and Benefits, Stoytcheva M (Ed.). InTech Publisher 560p.