PRELIMINARY SCREENING OF SELECTED TROPICAL BOTANICALS AS COWPEA PROTECTANTS AGAINST COWPEA SEED BRUCHID, *CALLOSOBRUCHUS MACULATUS* FABRICIUS (COLEOPTREA: CHRYSOMELIDAE: BRUCHINEAE)

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ABSTRACT: A preliminary screening was conducted to investigate the potentials of thirteen botanical powders as cowpea seed protectant against cowpea seed bruchid, *Callosobruchus maculatus,* Fabricius using seed damage parameters. The botanicals included *Azadirachta indica, Ekebergia senegalensis, Urginea altissima, Ancistrophyllum secundiflorum, Pseudocedrela kotschyi, Lannea welwitschii, Xylopia parviflora, Usteria guineensis* and *Antiaris toxicaria*. Others were *Indigofera arrecta, Hoslundia opposita, Cleome ciliata* and *Lagerra aurita*. All the botanicals, except *L. aurita,* showed potentials for cowpea protectant ability against the seed bruchid. Using Bruchid Perforation Index (BPI) values, the most effective powders were *A. indica* (2.95), *A. toxicoria* (2.07) and *H. opposita* (2.64) which BPI values were significantly ($p<0.05$) lower than that of *L. aurita* (26.46). Percentage Seed damage (PSD) varied with the studied botanicals (2.10-41.32%) and significantly ($p<0.05$) lower in the botanical-treated seeds compared to the untreated control (98.28%). The effective botanicals are, therefore, recommended for tropical resource-poor subsistent farmers for use in their small scale cowpea postharvest storage and for further studies to elucidate other effective formulations and their active ingredients.

KEY WORDS: Botanical powders, Bruchid Perforation Index, *Callosobruchus,* grain protectant, seed damage

Cowpea, *Vigna unguiculata* (L.) Walpers, is an important food legume and an essential component of cropping systems in many developing countries. Rich in protein and carbohydrate, it is the preferred pulse in large parts of Africa, where the seeds are processed into various products for human consumption or to appease to gods among the traditional worshippers. Seeds are medicinally used as a poultice to treat skin infections and boils. Despite its various uses, the post-harvest infestation by bruchids, especially the genus *Callosobruchus* (Coleoptera: Chrysomelidae: Bruchinae) poses a serious threat to its all-year round availability (Tuda et al., 2005).

The use of plant products to protect stored products from insect pest infestation is an age-long practice in developing world and is recently receiving a renewed attention as an important component of integrated pest control scheme. The reasons for this renewed interest include their abundance and cost effectiveness. Also, the use of botanicals reduces the ecological problems and health hazards of over-dependence on synthetic pesticides. Thirdly, some botanical formulations like powder and ash could be prepared by local resource-poor farmers, because they require no skilled technicality. Although, a large array of plant species has been documented for their insecticidal properties against bruchids (Dales, 1996; Ileke & Bulus, 2012; Ashamo et al., 2013; Musa et al., 2015; Babarinde et al., 2016a,b; Chauhan et al., 2016; Vijayaraghavan & Zadda, 2016; Kosini & Nukenine, 2017; *Usman* et al., 2017), screening more botanicals for potential efficacies cannot be inappropriate in bio-rational innovations for bruchids control. This is because bioactivity of botanicals could be species-specific which necessitates the attempt to establish the spectrum of bioactivity of any chosen botanical species. Plants selected for the study were those known to possess medicinal, pesticidal or nutritional values.

In this study, powder formulation was used being a preliminary study which was designed to provide baseline information for further studies on the insecticidal properties of the selected botanical species. Interestingly, the selected species are naturally available in many tropical countries. Therefore, the aim of the study was to evaluate thirteen selected tropical botanicals for their protectant ability of cowpea seed against the seed bruchid, *Callosobruchus maculatus* using seed damage parameters due to the bruchid's infestation.

MATERIALS AND METHODS

Insect culture

C. maculatus was reared on clean seeds of "Ife Brown", a bruchid-susceptible cowpea cultivar, under ambient environmental temperature of 30±2°C and 70±5% using standard method earlier described by Babarinde and Ewete (2008).

Botanical procurement and preparation

Thirteen botanicals were collected from different towns in south western Nigeria, where they are found in abundance (Table 1). Identification of the botanicals was done with the help of local ethno-botanists and matching of the vernacular names with the scientific names contained in Gbile (2006). The root and stem bark of the woody species used for the study were exposed to sun drying for 2 days and subsequently air-dried, while the leaves were air-dried under shade until crisp to prevent destroying the thermo-labile compounds in them. Thereafter, the dried plant parts were pulverized with the aid of a hammer mill and sieved with the aid of 50 µm sieve. The plant powder were then stored in labelled plastic airtight jars until use.

Botanical screening for insecticidal potentials

The plant powders were screened according to Fatope et al. (1995) with some modifications. Cowpea seeds (30 g each) were put in a 1 L Kilner jar covered with muslin cloth into which 3 g plant powder corresponding to 10% (w/w) was added to the cowpea seeds. A Kilner jar containing 30 g cowpea seeds without botanical treatment served as control. Three pairs (sex ratio 1:1) 1- to 3-day old *C. maculatus* were introduced into each covered jar. Six replicates of the setup was maintained for seven days in order to infest the stock after which the insects were removed from the stock. At 3 months after infestation, data were collected on the number of damaged (NDS) and number of undamaged seeds (NUdS), weight of damaged and undamaged seeds from both treated and untreated grains.

Percentage seed damage (PSD) was calculated as

$$PSD = \frac{NDS \times 100}{NDS+NUdS}$$

Bruchid perforation index (BPI) was calculated to determine the seed damage level according to Fatope et al. (1995), using the formula:

$$BPI = \frac{(\%TP) \times 100}{(\%TP+\%CP)}$$, where

%TP = % treated cowpea seeds perforated
%CP = % control cowpea seeds perforated
BPI > 50 = negative protectant of plant material tested (i.e. enhancement of infestation of the bruchid) BPI < 50 = positive protectant (i.e. prevention of infestation of the bruchid).

Experimental design and data analysis

The experiments were laid out in completely randomized design. Data were subjected to analysis of variance and significant treatment means were separated using Tukey's HSD at 5% probability level, with the aid of SPSS Software (SPSS, 2006).

RESULTS AND DISCUSSION

The highest BPI was observed in cowpea treated with *Lagerra aurita* (26.46), which was not significantly different from the BPI observed in cowpea treated with *U*riginea *altissima*, *Lannea welwitschii, Xylopia parviflora, Usteria guineensis, Indigofera arrecta* and *Cleome ciliata*. The BPI obtained from cowpea treated with *Azadirachta indica* (2.95), Antiaris *toxicoria* (2.07) and *Hoslundia. opposita* (2.64), *Ancistophyllum secundiflorum* (6.88), *Ekebergia senegalensis* (5.57), *H. opposita* (2.64) were not significantly different from one another but were significantly lower than the BPI obtained from cowpea treated with *L. aurita* (26.46) (Table II). Based on the BPI, *A. indica, A. toxicoria* and *H. opposita* were ranked to possess very strong grain protectant effect; while *E. senegalensis, U. altissima, A. secundiflorum, P. kotschyi U. guineensis* and *I. arrecta* were ranked to possess strong grain protectant effect. Three of the studied botanicals, *L. welwitschii, X. parviflora* and *C. ciliata* were ranked to possess fairly strong grain protectant effect; while only one (*Laggera aurita)* was ranked to be weak in its grain protectant potential. Seed damage varied significantly with the botanicals used (2.10 - 41.20%), but generally lower in the botanical-treated seeds compared to the untreated control (98.28%). The most effective powders were *A. toxicaria* (2.10%)*, H. opposita* (2.68%) *A. indica* (3.05%), *E. senegalensis* (5.94%) and *A. secundiflorum* (7.59%). The least effective botanical was *L. aurita* (with 41.32% PSD) (Fig. I).

According to Fatope et al. (1995), of the various screening procedures available, the cowpea bruchid bioassay is the most convenient for general use in the laboratory. A BPI value of 50 shows that equal amounts of botanical-treated and untreated cowpea seeds were perforated. This bioassay procedure thus allows plant materials with strong, weak or negative grain protectant effects to be identified, irrespective of their mode of action. In this study, all the tested botanicals showed varying levels of protection potentials of cowpea seeds against *C. maculatus*. BPI value of ≤ 15 is good and considered to be a strong effect. The low BPI values obtained from the seed treated with *A. toxicaria, A. indica* and *H. opposita,* made them good candidates for further study towards establishment of their bioactivity against *C. maculatus*. Earlier studies on the insecticidal potentials of *A. indica* against *C. maculatus* were on its seeds (Lale & Mustapha, 2000; Tofel et al., 2016), known to possess azadirachtin. This work examines the insecticidal potentials of the leaves. The study of Cepeda Palacios et al. (2014) reported the bioactivity of the neem leaves against insect. Based on their results, we included the leaf to investigate its bioactivity against the cowpea seed bruchid. Of the ten species assayed at 10-30% w/w by Fatope et al. (1995), *Hyptis suaveolens* (Labiatae) and *Spenoclea zeylanica* (Sphenocleceae) were the only ones with a BPI value of < 15 when the botanical powders were assayed at 10% w/w. The result from this study where the BPI of the majority of the tested materials was < 15 suggests that the majority of the plants have cowpea protectant potentials against the bruchid. The result of this study agrees with previous authors on the efficacy of botanical powders in controlling *C. maculatus* (Ileke & Bulus, 2012; Ojo & Ogunleye, 2013; Tefsu & Amana, 2013).

Plant species with lower PSD had lower BPI. *Xylopia parviflora* had a BPI of 15.76, despite the fact that some members of its family (Annonaceae) had been reported to be insecticidal against stored product pests (Babarinde et al., 2008; Babarinde & Adeyemo, 2010; Akinyemi et al., 2016; Babarinde et al., 2017). Similar report exists for another member of Annonaceae family (*Annona senegalensis)* included in Fatope et al. (1995), that was not effective in the protection of cowpea seeds against *C. maculatus*. The insecticidal properties of Meliaceae against *C. maculatus* has been reported by Babarinde and Ewete (2008). However, this is the first report of *A. toxicaria* (Family Moraecea), for its pesticidal potentials against stored product insect. The fact that the powders showed protectant ability justifies their recommendation for local farmers who may not have the technicality of essential oil extraction or production of inorganic extracts.

CONCLUSION

Majority of the screened species showed insecticidal potentials against bruchids. Since the formulation investigated in this study was powder, it is necessary to investigate other formulations like organic and inorganic extracts and essential oil. Also, their modes of action and bioactive ingredients should be well studied as prerequisites to the understanding of their mechanism of actions and the production of synthetic products from the species.

LITERATURE CITED

Akinyemi, A. O., Babarinde, S. A., Oyerinde, R. M. & Aderonbi, A. A. 2016. Bioactivity of acetone and chloroform extracts of *Xylopia aethiopica* (Dunal) A. Rich seed against *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae). Journal of Biologically Active Products from Nature, 6 (5-6): 412-423.

Ashamo, M. O., Odeyemi, O. O. & Ogungbite, O. C. 2013. Protection of cowpea, *Vigna unguiculata* L. (Walp.) with *Newbouldia laevis* (Seem.) extracts against infestation by *Callosobruchus maculatus* (Fabricius). Archives of Phytopathology and Plant Protection, 46 (11): 1295-1306.

Babarinde, S. A., Adebayo, M. A. & Oduyemi, K. 2008. Integrating varietal resistance with *Xylopia aethiopica* (Dunal) A. Richard seed extract for the management of *Sitophilus zeamais* Motschulsky in stored maize. African Journal of Biotechnology, 7 (8): 1187-1191.

Babarinde, S. A., Adesina, G. O., Oladoye, S. A. & Adebayo, M. A. 2016a. Chemical composition and bioactivity of *Lippia adoensis* Hochst ex. Walp (Verneneaceae) leaf essential oil against *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae). Journal of Northeast Agricultural University, 23 (4): 8-14.

Babarinde, S. A. & Adeyemo, Y. A. 2010. Toxic and repellent properties *of Xylopia aethiopica* (Dunal) A. Richard on *Tribolium castaneum* Herbst infesting stored millets, *Pennisetum glaucum* (L.) R. Br. Archives of Phytopathology and Plant Protection, 43 (8): 810-816.

Babarinde, S. A., Akinyemi, A. O., Odewole, A. F., Yisa, S., Oke, A. A. & Adeyemo, A. O. 2016b. Phytochemical composition and insecticidal properties of mechanically extracted castor seed oil against cowpea seed bruchid (*Callosobruchus maculatus* Fabricius) infesting bambara groundnut. Elixir Agriculture, 92: 39086-39092.

Babarinde, S. A., Esan, E. O., Olatunde, O. Z., Ajayi, D. S. & Olaniyi, J. P. 2017. Combination of *Piper guineense* essential oil with cowpea varietal resistance in control of cowpea seed beetle, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae). Journal of Northeast Agricultural University, 24 (3): 10-18.

Babarinde, S. A. & Ewete, F. K. 2008. Comparative bioactivity of three *Khaya* species (Meliaceae) on *Callosobruchus maculatus* Fabricus (Coleopteran: Bruchidae). Journal of Entomological Research Society, 10 (1): 27-35.

Chauhan, A., Ranjan, A. & Jindal, T. 2016. Insecticidal Activity of Methanolic Extract of *Calotropis Procera* against *Callosobruchus Maculatus* using Moong Seeds (*Vigna Radiata*). Journal of Biomedical and Pharmaceutical Research, 5 (6): 75-82.

Cepeda-Palacios, R., Servín, R., Ramírez-Orduñ, J. M., Ascencio, F., Dorchies, P. & Angulo-Valadez, C. E. 2014. In vitro and in vivo effects of neem tree (*Azadirachta indica* A. Juss) products on larvae of the sheep nose bot fly (Oestrus ovis L. Diptera: Oestridae). Veterinary Parasitology, 200: 225-228.

Dales, M. J. 1996. A review of plant materials used for controlling insect pests of stored products. Natural Resources Institue (NRI) Bulletin, 65, Chatham, United Kingdom.

Fatope, M. O., Nuhu, A. M., Mann, A. & Takada, Y. 1995. Cowpea weevil bioassay: a simple prescreen for plants with grain protectant effects. International Journal of Pest Management, 41 (2): 84-86.

Gbile, Z. O. 2006. Vernacular names of Nigerian plants: Yoruba. (Revised by Soladoye, M.O.). Forestry Resaerch Institute of Nigeria, Ibadan, Nigeria. 101 pp.

Ileke, K. D. & Bulus, D. S. 2012. Evaluation of contact toxicity and fumigant effect of some medicinal plant and pirimiphous methyl powders against cowpea bruchid, *Callosobruchus maculatus* (Fab.) (Coleoptera, Chrysomelidae) in stored cowpea seeds. Journal of Agricultural Science, 4 (4): 279-284.

Kosini, D. & Nukenine, E. N. 2017. Bioactivity of novel botanical insecticide from *Gnidia kaussiana* (Thymeleaceae) against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in stored *Vigna subterranea* (Fabaceae) grains. Journal of Insect Science, 17 (1): 1-7.

Lale, N. E. S. & Mustapha, A. 2000. Potential of combining neem (*Azadirachta indica* A. Juss) seed oil with varietal resistance for the management of the cowpea bruchid, *Callosobruchus maculatus* (F.). Journal of Stored Products Research, 36: 215-222.

Musa, A. K., Odunayo, A. & Adeyeye, O. E. 2015. Effects of initial infestation levels of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) on cowpea and use of *Nicotiana tabacum* L. aqueous extract as grain protectant. Journal of Northeast Agricultural University, 22 (4): 1-6.

Ojo, D. O. & Ogunleye, R. F. 2013. Comparative effectiveness of the powders of some underutilized botanicals for the control of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Journal of Plant Diseases and Protection, 120 (5/6): 227-132.

SPSS. 2006. Statistical Package for Social Sciences. Version 15.0 for Windows, SPSS Inc. 2335, Walker Drive, Chicago, Illinois 60606.

Tefsu, F. & Amana, G. 2013. Evaluation of *Parthenium hysterophorus* L. powder against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) on chickpea under laboratory conditions. African Journal of Agricultural Research, 8 (44): 5405-5410.

Tofel, K. H., Nukenine, E. N., Stahler, M. & Adler, C. 2016. Degradation of azadirachtin A on treated maize and cowpea and the persistence of *Azadirachta indica* seed oil on *Callosobruchus maculatus* and *Sitophilus zeamais*. Journal of stored Product Research, 69: 207-212.

Tuda, M., Chon, L. Y., Niyomdham, C., Buranapanichpan, S. & Tateishi, Y. 2005. Ecological factors associated with pest status in *Callosobruchus* (Coleoptera: Bruchidae): high host specificity of non-pests to Cajaninae (Fabaceae). Journal of stored Product Research, 41: 31-45.

Usman, L. A., Bello, S. O., Babarinde, S. A., Olanrewaju, I. R. & Ogundele, V. A. 2017. Effect of collection time on phytochemical profile and insecticidal activity of essential oil from leaf of *Ocimum gratissimum* grown in Nigeria. Biokemistri, 29 (2): 76-86.

Vijayaraghavan, C. & Zadda, K. 2016. Evaluation of botanical powders and oils on pulse beetle, *Callosobruchus maculatus* in black gram. Annals of Plant Protection Sciences, 24 (2): 307-311.

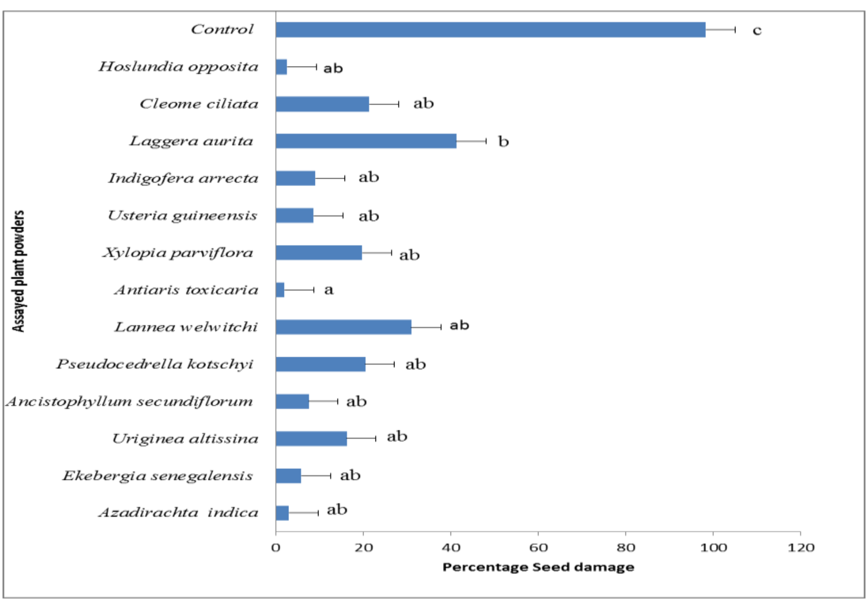

Figure I. Percentage seed damage by *Callosobruchus maculatus* of cowpea seeds treated with selected plant powder {Number of replicates = 6; ANOVA Result: $F = 9.929$; d f =13, 70; $p<0.0001$}.

Table 1. List of the thirteen plant species screened for insecticidal properties against *Callosobruchus maculatus*.

Plant species	Common name	Family	Part used	Bioactivity information	Point of collection
Azadirachta indica	Neem	Meliaceae	Leaf	Medicinal, insecticidal	Ogbomoso
Ekebergia senegalensis	Stavewood	Meliaceae	Leaf	Antibacterial	Ibadan
Urginea altissima	Tall squill	Liliaceae	Leaf	Medicinal	Ibadan
Ancistrophyllum secundiflorum	Large Benin rattan	Arecaceae	stem bark	Chewing stick	Ogbomoso
Pseudocedrela kotschyi	Cedar mahogany	Meliaceae	root bark	Antibacterial, chewing stick,	Ogbomoso
Lannea welwitschii	Kumbi	Anacardiaceae	Leaf	Antibacterial, medicinal, furniture	Ibadan
Antiaris toxicaria	False iroko	Moraceae	Stem bark	Insecticidal, medicinal	Ibadan
Xylopia parviflora	Bushveld bitterwood	Annonaceae	root bark	Medicinal, Chewing stick	Alapa-Ilorin
Usteria guineensis	-	Loganiaceae	Aerial	Medicinal	Akure
Indigofera arrecta	Indigo	Papilonaceae	Leaf	Dye production	Ogbomoso
Lagerra aurita	Laggera	Asteraceae	leaf	Antibacterial, insecticidal	Ogbomoso
Cleome ciliata	Wild mustard	Capparaceae	seed	Green manure, vegetable	Ogbomoso
Hoslundia opposita	Hoslundia	Lamiaceae	Leaf	Medicinal, insecticidal	Ilorin

Table 2. Cowpea grain protectant potentials of the selected botanicals against *Callosobruchus maculatus* using Bruchid Perforation Index.

Plant powder	Bruchid Perforation Index	Grain Protectant Potentials*
Azadirachta indica	2.95±.1.05a	Very strong
Ekebergia senegalensis	5.57±1.48ab	Strong
Uriginea altissina	13.25± 3.78ab	Strong
Ancistophyllum secundiflorum	6.88 ± 2.45ab	Strong
Pseudocedrella kotschyi	12.51 ± 7.71ab	Strong
Lannea welwitchi	16.48 ± 10.04ab	Fairly strong
Antiaris toxicaria	2.07 ±0.76a	Very strong
Xylopia parviflora	15.76 ±3.97ab	Fairly strong
Usteria guineensis	7.76 ± 2.79ab	Strong
Indigofera arrecta	7.66 ± 3.67ab	Strong
Laggera aurita	26.46 ± 6.75b	Weak
Cleome ciliata	17.30 ± 3.16ab	Fairly strong
Hoslundia opposita	2.64 ± 0.37a	Very strong
ANOVA Results	F=2.586; df=12, 65; p=0.007	

Means followed by same alphabet along a column are not significantly different from one another using Tukey's HSD test (p<0.05).

*BPI of < 15 depicts a strong grain protectant effect {Adapted with modification from Fatope et al. (1995)}