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Contact toxicity of *Canarium schweinfurthii* Engl. tissues against *Callosobruchus maculatus* in stored bambara groundnut

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Key words: *C. schweinfurthii, C. maculatus,* mortality, toxicity, bambara groundnut.

Abstract

The production of bambara groundnut is in the hands of peasant farmers and its improvement is militated by storage pests, such as *Callosobruchus maculatus*. Laboratory experiments were conducted on powders, extracts and oils of *Canarium schweinfurthii* for their insecticidal activity against *C. maculatus* in bambara groundnut. This was done at ambient conditions (30 – 35°C and 70 – 80% r.h) between January 2011 to December 2011. Randomized completely block design in four replications was used. The results showed that contact toxicity of *C. schweinfurthii* tissues (cotyledon and mesocarp powder) caused 25 - 97% and 42.5 - 95% mortality, rerspectively, commercial prossed mesocarp oil caused 55 - 100% and laboratory processed cotyledon oil caused 62 - 100% mortality to *C. maculatus*. The highest mortality against *C. maculatus* were observed in methanol extract and petroleum ether of the mesocarp tissues which caused 80 - 100% and 90 – 100% mortality, respectively at the application rates of 1.25 and 2.5mg/ml/50g grain within 3 days post-treatment. In conclusion, *C. schweinfurthii had* insecticidal activities against *C. maculatus* using contact toxicity. The highest activities were observed in mesocarp and cotyledon tissues. These suggest that *C. schweinfurthii* can serve as alternative botanicals in protecting stored bambara groundnuts against *C. maculatus*.

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Introduction

Bambara groundnut [*Vigna subterranean* (L.) Verdcourt] is an indigenous African leguminous crop belonging to the family Fabaceae. It ranked the third most important among the grain legume crops in term of its protein content. It is processed into various types of food, industrial products and animal feed (Atiku *et al.* 2004). The production of bambara groundnut is in the hands of peasant farmers and full scale production to meet the need of the populace is militated by storage pests. Among the storage pests, *Callosobruchus maculatus* is the more destructive due to its shorter life cycle and higher fecundity (Haines,1991). The infestation of this bruchid starts in the field and the population increases rapidly in the store, causing quantitative and qualitative damage to the products as well as reducing viability, and aesthetic value of grains (Lale, 2001). Obeng-Ofori and Danquah (2004) observed that bruchid beetles are the major storage insect pests of bambara groundnuts in Africa. With respect to grain loss during storage, Golob *et al.* (1996) reported that up to 10% losses per month can be incurred. Food grain losses, such as seen in bambara groundnut, due to insect infestation during storage are serious problem in Nigeria and Africa at large.

Synthetic insecticides (such as pyrethrins, malathion, carbamate, methyl bromide, phosphine, cyanogens, ethyl formate, or sulfuryl fluoride) have been used extensively to control stored product insect pests (Rajashekar *et al.*, 2012; Ogunwolu and Odunlami, 1986). However, the residues of these products have direct or indirect toxic effects on humans, animals and on the environment (Habiba *et al.* 2010). Botanicals which are pesticides derived from plants, are also useful for the control of storage insect pest. They degrade rapidly and therefore are considered safer to the environment than the common synthetic chemicals. According to Arnason *et al.* (1989), these botanicals minimize the problems associated with the use of synthetic chemicals. Various plants tissues have been used as botanicals to protect stored grains like cowpea and bambara groundnut against the infestation of insect pests. For example, local plants such as pepper and citrus peels have been used to protect stored cowpea against *C. maculatus* (Habiba *et al.*, 2010; Bocke *et al.*, 2004). It has been shown that powders of *Piper guineense* seeds caused 100% eggs mortality (Ajayi and Wintola, 2006), *Eugenia aromatica* killed adults within 48hours (Ofuya *et al.*, 2010), and West African black pepper, Ethiopian pepper and clove were able to reduce the oviposition of *C. maculatus* (Ajayi and Wintola, 2006).

The leaves and oils of *Canarium schweinfurthii* Engl. have been reported to have antimicrobial, antifungal (Ngbede *et al.*, 2008) and insecticidal properties (Gill, 1992) . In the same vein, Philip *et al.* (2009) reported that this plant has repellent properties and toxic effect on the heart muscles in insects. In addition, Vera *et al.* (2009) documented that *C. schweinfurthii* conferred resistance against diamond back moth and flea beetles. Hence, *C. schweinfurthii* can be considered as a good botanical insecticidal agent that needs more investigation on different crops for its activities. As at the time of writing this report, there was no access to information in the literature on the use of *C. schweinfurthii* tissues against *C. maculatus* in stored legumes. Therefore, the aim of this research was to investigate whether *C. schweinfurthii* tissues (leaves, fruit mesocarp and seed cotyledon) have insecticidal properties against *C. maculatus* in stored bambara groundnuts.

Materials and methods

Study Area

The tests were conducted in the Crop Science Research Laboratory, University of Agriculture, Makurdi at ambient temperature of 30 – 35^{o}C, relative humidity (r.h) of 70 – 80%. (Longitude 8° 20' N and 9° E and Latitude 7° 20' N and 8' N of equator) between the months of January and December, 2011.

Insect Culture

The insects used to establish the laboratory colony of *C. maculatus* came from batches of infested bambara groundnut grains purchased at Wadata market, Makurdi. Immature stages of the bruchids were reared on Maifarinhanci variety of bambara

groundnut, in Petridishes with perforated cover to allow air circulation. Devoured grains were constantly replaced and accumulated grain dust sieved out

Plant materials

Powder

Mature fruits of *C. schweinfurthii* were de-pulped, the seeds cracked to obtained cotyledons. The mesocarp, seed cotyledon as well as young leaves obtained from mature trees were air- dried and ground to powder using GX 160 electric grinder and sieved using 3mm mesh. The products were kept in plastic containers in the laboratory until needed for use.

Extract

One hundred grammes (100g) each of leaf, mesocarp and seed cotyledon powders were extracted separately with 200ml of methanol or petroleum ether in 500ml conical flask, shaken mechanically for 24hr and filtered. The solvent was evaporated and the resulting extract kept in laboratory cupboard until needed for use.

Laboratory oil extraction

One hundred grammes (100g) of each of leaf, mesocarp and seed cotyledon were extracted with 200ml of methanol M_r ($67 - 56^{-1}$) in a soxhlet apparatus, the mantle was heated for 8hr after which the solvent was recovered by distillation. The crude extracts were then poured into a 100ml beaker and placed on a water bath to evaporate methanol still left. The concentrated oil extracts were stored in beaker in the fume cupboard covered with paraffin wax until needed for use.

Experimental Design

Experiments were carried out in randomized completely block design in four replications.

Test with powdered products

Fifty grammes of bambara groundnut grains was admixed with leaf, mesocarp and seed cotyledon powders at the rate of 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2g. An equal weight of bambara groundnut grains was treated with the same rate of permethrin (Rambo 0.60%) or left untreated as control. These treatments and untreated control were replicated four times. All treatments were infested with five pairs of 1 – day old bruchids and mortality was recorded over a 5-day period. Bruchids not responding to a probe were counted as dead.

Test with extracts

Fifty grammes of bambara groundnut contained in 250ml glass beaker was treated with 1.25 or 2.5mg/ml of *C. schweinfurthii* leaf, mesocarp or seed cotyledon extract. A glass rod was used to stir to ensure uniform coating of grains with extract before air-drying the seeds for 24hr. Grains were infested with five pairs of 1-day old bruchids and mortality was recorded over a 5-day period.

Test with oils

Oils extracted in the laboratory from the mesocarp and from the seed cotyledons as well as oil extracted commercially from the mesocarp of *C. schweinfurthii* oil were admixed with 50g of bambara groundnut at the rate of 0.1, 0.2, 0.3, 0.4 and 0.5ml. An equal weight of bambara groundnut was treated with the same rate of commercially- processed groundnut oil or left untreated as control. The oils were dispensed in 2ml of acetone unto the grains contained in glass beakers and the beakers shaken mechanically for 15minutes. Thereafter, grains were air dried, transferred into bags, infested with five pairs of 1- day old bruchids and mortality was recorded over a 5-day period. Bruchids not responding to a probe were counted as dead.

Data Analysis

Data were subjected to analysis of variance, using SAS (2000) statistical package; Duncan's multiple range test was used to separate significantly different means.

Results

Results on contact toxicity of *Canarium schweinfurthii* tissues powder to *C. maculatus* is presented in Table 1. No mortality occurred in control over the 5-day observation period. Across all rates

over this period of observation, permethrin caused the highest mortality ($\overline{X}$ = 96.9%) to *C. maculatus*, exceeding the mortality caused by *C. schweinfurthii* mesocarp powder and leaf powder by 23.0 and 43.1%, respectively. No plant material cause mortality as rapidly as permethrin and no rate of leaf powder caused up to 50% mortality at day 1 post- treatment. One rate of cotyledon powder (1.6g/50g grain) caused 50% mortality. Mortality increased with number of days post-treatment irrespective of application rate and by day 5, all plant powder treatments caused mortality comparable with those caused by permethrin (Table 1).

Table 1. Comparison of the contact toxicity of *Canarium schweinfurthii* tissues powder to *C. maculates*.

Rate		*% mortality at indicated hour***			*Treatment*
Treatment* (g/50g seed)		24	72	120	Mean
LP	0.05	20.00f	72.50c	97.50a	63.3
	0.1	35.00ef	75.00bc	87.50a	65.8
	0.2	35.00ef	82.50abc	92.50a	70.0
	0.4	37.50ef	80.00abc	95.00a	70.8
	0.8	30.00ef	82.50abc	95.00a	69.2
	1.6	25.00ef	75.00bc	92.50a	64.2
	3.2	40,00def	82.50abc	90.00a	70.8
MP	0.05	55.00bcdef	85.00abc	97.50a	77.5
	0.1	62.50bcde	80 .00abc	87.50a	80.0
	0.2	42.50def	90.50abc	92.50a	75.2
	0.4	60.00bcde	92.50abc	95.00a	83.3
	0.8	50.00bcdef	95.00abc	95.00a	81.7
	1.6	57.50bcdef	90.00abc	92.50a	80.8
	3.2	50.00bcdef	77.50abc	90.00a	73.3
CP	0.05	27.50ef	77.50abc	92.50a	66.7
	0.1	25.00ef ,	77.50abc	97.50a	65.8
	0,2	47.50cdef	92.50abc	92.50a	78.3
	0.4	42.50def	90.00abc	97.50a	76.7
	0.8	47.50cdef	97.50ab	100.00a	80.8
	1.6	50.00cdef	82.50abc	95.00a	74.2
	3.2	25.00ef	87.50abc	92.50a	69.2
Permethrin	0.05	77.50abcd	90.00abc	95.00a	89.2
	0.1	82.50abc	97.50ab	97.50a	93.3
	0.2	90.00ab	97.50ab	97.50a	95.8
	0.4	100.00a	100.00a	100.00a	100
	0.8	100.00a	100.00a	100.00a	100
	1.6	100.00a	100.00a	100.00a	100
	3.2	100.00a	100.00a	100.00a	100
UTC	0.0	0.00f	0.00f	0.00f	0.0

*LP= leaf powder, MP = mesocarp powder, CP= seed cotyledon powder, UTC = untreated control, ** = Means within the column followed by the same letter(s) are not significantly different at p = 0.05 according to Duncan's new multiple range test.

Table 2 showed the results on contact toxicity methanol extract of *Canarium schweinfurthii* mesocarp powder to *C. maculatus*. No mortality of *C.maculatus* in the control treatment over the 5-day period of observation. At day 1, toxicity of 1.25mg/ml of methanol extract of *C. schweinfurthii l*eaf was the lowest differing significantly from values for other treatments. The higher rate of 2.5mg/ml matched the efficacy of other extracts excluding the leaf extract. At day 3 post- treatment, all test insects died except

those on grains treated with 1.25mg/ml of leaf extract.

The results of comparative contact toxicity with petroleum ether extract of *C. schweinfurthii* tissues to *C. maculatus* is presented in Table 3. There was no mortality in control over the 5- day period of observation. Petroleum ether extract caused 50-95% mortality across all rates at day 1 post-treatment with cotyledon extract being the highest (95%) at 1.25mg/ml. At day 3, mortality of *C maculatus* had considerably increased in all treatments and by day 5, all test insects had died (100% mortality) in all treatments.

Table 2. Comparative contact toxicity of methanol extract of *C. schweinfurthii* tissues *C. maculates.*

Rate		*% mortality at indicated hour***			*Treatment*
Treatment * (mg/ml/50g seed)		24	72	120	Mean
LE_m	1.25	25.00d	77.50b	100.00a	67.5
	2.5	65.00c	100.00a	100.00a	83.3
ME_m	1.25	80.00abc	100.00a	100.00a	93.3
	2.5	95.50bc	100.00a	100.00a	97.5
CE_m	1.25	72.50bc	100.00a	100.00a	90.8
	2.5	85.00ab	100.00a	100.00a	95.0
UTC	0	0.00e	0.00e	0.00e	0

*LE_m = leaf extract in methanol, ME_m = mesocarp extract in methanol, CE_m = seed cotyledon extract in methanol, UTC = Untreated control, ** = Means within the column followed by the same letter(s) are not significantly different at p = 0.05 according to Duncan's new multiple range test.

Table 4 compares contact toxicity of the various oils to *C. maculatus*. In this test, there was no mortality observed in control over the 5-day period of observation. In each type of oil, there was an observed increase mortality of *C.maculatus* with increase days post- treatment irrespective of application rates. Mesocarp oil processed commercially and laboratory-processed mesocarp oil caused 11.5 and 18% higher mortality than the groundnut oil at 24 hours ($\overline{X}$ =56.5%); however, by days 3 and 5 post- treatment, difference among treatments excluding the control were not significant.

Table 3. Comparative contact toxicity with petroleum ether extract of *C. schweinfurthii* tissues to *C. maculatus*

Rate		*% mortality at indicated hour***			*Treatment*
Treatment * (mg/ml/50g seed)		24	72	120	Mean
LE_{pe}	1.25	50.00c	95.00a	100.00a	81.7
	2.5	52.50c	95.00a	100.00a	82.5
ME_{pe}	1.25	90.00ab	95.00a	100.00a	85.0
	2.5	75.00b	95.00a	100.00a	90.0
CE_{pe}	1.25	95.00a	100.00a	100.00a	98.3
	2.5	85.00ab	100.00a	100.00a	95.0
UTC	0	0.00d	0.00c	0.00b	0.0

*LE_{pe} = leaf extract in petroleum ether, ME_{pe} = mesocarp extract in petroleum ether, CE_{pe} = seed cotyledon extract in petroleum ether, UTC = untreated control. ** = Means within the column followed by the same letter(s) are not significantly different at p = 0.05 according to Duncan's new multiple range test.

Discussion

The *Canarium schweinfurthii* Engl. products have been reported to contain insecticidal properties (David, 1989; Gill, 1992; Abayeh *et al.* 1999; Georges *et al.* 1992). High rate of *C maculatus* mortality on exposure to *C. schweinfurthii* powder treatments may be attributed to the chemical composition of the products. David (1989) reported that tannic acid contained in *C. schweinfurthii* leaf act as toxin and feeding deterrent to insects. Philip *et al.* (2009)

reported that saponnins that is found in *C. schweinfurthii* affect the respiratory system of insects and causes emetic effect due to their detergent action on them; Ranaweera (1986) in his report showed *Canarium zalannicum* (Rtz) BL displayed significant larvicidal activity against 3rd instar larvae of *Culex quinquefasciatus*. The action of the powder treatments of *C. schweinfurthii* may also be attributed to their smell which corroborates the report of Lale (1993) that mortality of storage bruchids could be associated with the pungent ordour produced by plant powders used against them.

Table 4. Comparative contact toxicity of *C. schweifurthii* tissues oil to *C. maculates*.

Rate		% mortality at indicated hour**			Treatment
Treatment*	(ml /50g seed)	24	72	120	Mean
CPMO	0.1	55.00bcd	82.50a	95.00a	77.5
	0.2	77.50abc	95.00a	100.00a.	90.3
	0.3	70.00abcd	92.50a	95.00a	87.5
	0.4	60.00abcd	100.00a	100.00a	86.7
	0.5	77.50a	92.50a	100.00a	90.0
LPMO	0.1	52.50bcd	82.50a	92.00a	75.8
	0.2	57.50bcd	85.00a	92.00a	77.5
	0.3	37.50d	82.500a	92.50a	70.8
	0.4	60.00bcd	90.00a	90.00a	82.5
	0.5	57.50bcd	82.50a	97.50a	77.5
LPCO	' 0.1	62.50abcd	95.00a	95.00a	84.2
	0.2	77.50abc	95.00a	100.00a	90.0
	0.3	77.50abc	97.50a	100.00a	91.7
	0.4	75.00ab	97.50a	100.00a	92.5
	0.5	80.00abc'	100.00a	100.00a	92.5
G/Nut oil	0.1	57.50bcd	82.50a	90.00a	76.7
	0.2	52.50bcd	92.50a	97.50a	80.8
	0.3	40.00d	77.50a	90.00a	69.2
	0.4	47.50cd	92.50a	100.00a	80.0
	0.5	85.00ab	100.00a	100.00a	95.0
UTC	0	0.00e	0.00e	0.00e	0.0

*CPMO= commercially- processed mesocarp oil, LPMO= laboratory - processed mesocarp oil, LPCO= laboratory-processed seed cotyledon oil, UTC= untreated control. ** = Means within the column followed by the same letter(s) are not significantly different at p=0.05 according to Duncan's new multiple range test.

The mortality of *C. maculatus* caused by *C. schweinfurthii* powder treatments were comparable with those caused by permethrin at application rates < 2.0g/50g grain. Yusuf and Mohammed (2009) showed that leaf powders from bitter melon (*Monordica balsamina*) were as effective as pirimiphos methyl in suppressing *C. maculatus* population and growth in cowpea storage.

The toxicity of *C. schweinfurthii* powders used in this study suggest that they could serve as alternative to synthetic chemical insecticide to protect stored produce against *C. maculatus*. Toxicity of the application rates adopted in this study corroborates the reports by Ogunwolu and Idowu (1994) and Ivbijaro and Agbaje (1996) which showed that effective rates of plant powders against storage bruchids range from <1g/kg to 20g/kg of grains. The findings of Lale (1995) showed plant powders applied as grain protectants should normally not exceed 2% of the grain weight Lale (1995). Virtually all tissue types applied at <0.8g/50g grain showed efficacy in causing contact mortality to adult bruchids. Denloye (2010), in his report stated that powders of *Allium sativum* were highly toxic to *C. maculatus* in stored cowpea.

Extracts of *C. schweinfurthii* were highly toxic to *C. maculatus* on contact, with the petroleum ether extracts being the more effective than the methanol extracts. In this study, *C. schweinfurthii* oils showed potency comparable to groundnut oil and thus can

serve as surface protectant of grains against storage bruchids. High mortality of *C. maculatus* caused by mesocarp oil processed commercially corroborates the report by Pereira (1993) on the efficacy of traditionally- extracted vegetable oils in controlling *C. maculatus* in stored bambara groundnut and cowpea. Lale and Abdulrahaman (1990) have shown that neem seed oil obtained by the traditional kneading method significantly reduced *C. maculatus* adult emergence in stored cowpea. Neem oil, cotton oil, castor oil, and clove oil were also effective in controlling *C. maculatus* in cowpea, or bambara groundnut through inhibition of oviposition (Lale and Maina, 2003; Ajayi and Lale, 2001).

The result of this study also corroborates the report by Rajapakse and Ratnosekera (2008) that *Anona recticulata* oil inhibited oviposition and adult emergence of *C. maculatus* in cowpea storage. The efficacy of plant oils against *C. maculatus* has been reported (Ahmed and El-Salam, 2000; Bamaiyi *et al.* 2006; Yahaya *et al.* 2009). As documented by Obame *et al.* (2007), *C. schweinfurthii* act as antimicrobial and antifungal agent against insects. The findings of Koudou *et al. (*2005) and Agbo *et al.* (2007) revealed that *C. schweinfurthii* oil contains oleic acid which was toxic to several animal species. Shaaya *et al.* (1977) showed that *C. schweinfurthii* oils are potential control agents against *C. maculatus.*

The toxicity of alkaloids in *C. schweinfurthii* products was reported by Philip *et al.* (2009). In study reported by Bamaiyi *et al.* (2009), 1.0-3.0ml of *Khaya senegalenses* oil/100g seed used caused almost 100% mortality of *C. maculatus*. The same level of efficacy was achieved in this study but at relatively lower rates of application (0.1- 0.5ml/50g grain) of *C. schweinfurthii* oils. In similar report, Ajayi and Anda (2008) showed olive oil applied at 0.125g/10g seed caused 100% mortality of *C. maculatus* within 24 hours in cowpea storage.

In conclusion, *C. schweinfurthii* had insecticidal activities against *C. maculatus* using contact toxicity. The highest activities were observed in mesocarp and cotyledon tissues. These suggest that *C. schweinfurthii* can serve as alternative botanicals in protecting stored bambara groundnuts against *C. maculatus.* Nevertheless, there is need for further study to evaluate the insecticidal active ingredients of the *C. schweinfurthii* tissues.

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