Bioscience Research Communications
Vol. 14, No. 2, April 30, 2002
Printed in Nigeria

0795-8072/2002 $6.00 + 0.00

BRC 2001058/14213

Control of Seed Bruchid [*Callosobruchus maculates* F.] Infestation in Cowpea [*Vigna unguiculata* (L.) Walp.] with *Clausena anisata Hook (Rutaceae).*

E. O. Ogunwolu*; A. A. Aku, J. Attah and J. O. Igoli[+]

Department of Crop Production, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria.
[+]Department of Chemistry, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria.

(Received April 12, 2001)

ABSTRACT: *Clausena anisata* Hook has shown repellency and antifeedant effects on some insect pests. In this paper, we report toxicity of *C. anisata* leaf powder and its extract (applied at 2.5% concentration) to cowpea seed bruchid (CSB) [*Callosbruchus maculatus* F.], deterrence of oviposition and suppression of development to adult stage. Bioactivity persisted for over 4½ months (140 days). The leaf powder had acute toxicity inferior to permethrin (Cooper 0.5% dust) in short duration test but its long term control was equivalent with the pyrethroid insecticide. The leaf powder was, however, more potent ($P<0.05$) than either the stem or root bark powder in reducing bruchid population and seed damage. The minimum effective rate of application of *C. anisata* leaf powder or its extract was 0.62% concentration.

Keywords: *Callosobruchus maculatus*; Cowpea; *Vigna* u*nguiculata; Clausena anisata*.

Introduction

Profiles of chemical constituents of *Clausena anisata* Hook show the occurrence of coumarins (1), carbazole alkaloids (2,3) and limonoids (4). They provide basis for the use of *C. anisata* products in traditional health care in Africa and Asia (5). Equally important is the pesticidal property of these chemicals. They have potential useful application in the management of crop pests and disease vectors as exemplified by: (i) bioactivity of clausenol isolated from *C. anisata* stem bark on fungi and gram positive as well as gram negative bacteria (2); (ii) feeding deterrency to *Spodoptera litura* of coumarins from the root bark (1); (iii) molluscicidal activity of root extract on *Bulinus globosus* (6); (iv) repellency of the leaves to mosquito (7); and (v) the control of *Zonocerus variegatus* (8).

---

*To whom correspondence should be addressed.

Clausena anisata occurs in forest and savanna eco-zones of West Africa (9). It showed grain protection properties in our preliminary screening of plants for bioactivity against storage insect pests. We tested it further because documentation of insecticidal efficacy of *C. anisata* against storage insect pests is scanty and because plant-derived insecticides still provide, almost exclusively, reprieve from pest damage to stored food grains in many developing countries. The cowpea seed bruchid (CSB), *Callosobruchus maculatus* F., was chosen as test insect for its major pest status in stored cowpea (10). In conducive environments, CSB population grows rapidly thereby accentuating storage losses and food insecurity, as well as markedly influencing marketability and retail price of cowpea (11, 12).

Materials and Methods

Test materials

Young stands of *C. anisata* uprooted from Ikwue Forest Reserve, Igbor, Benue State, were washed and their leaves, stem bark and root barks detached. These were sun-dried and milled to powder of ca. 350 µm particle size in a Culatt TZ electric grinder. To obtain extracts, 50g samples of each powdered plant product was homogenized in 500ml of methanol:water (4:1) solvent for 5 min and filtered. The suspension was evaporated to one 10^{th} its initial volume, acidified with 2 M H_2SO_4 and extracted with chloroform. Each extract residue, 0.28, 0.22 and 0.24g for the root bark, leaf and stem bark respectively, was dissolved in appropriate quantity of acetone to make a 10% stock solution.

The test insects were 1-day old 1^{st} or 2^{nd} generation progeny of a laboratory culture of CSB raised on cowpea var. IAR 48 in a Gallenkamp incubator set to 30 ± 2°C. The same environment was used for the bioassay. Undamaged cowpea seeds which had been disinfested with phosphine gas (from Phostoxin pellets) and aired were used in all tests.

Bioactivity testing

Short duration bioactivity tests were conducted in Petri dishes (9.0 cm wide) on 20 g of cowpea seeds admixed with 0.5 g of powders of *C. anisata* leaf, stem bark or root bark or admixed with 1 ml of their 2.5% extracts (obtained by serial dilution of the 10% stock in acetone). In tests with powders, permethrin (Coopex 0.5% dust) insecticide, applied at the rate of 0.5 g/20g of seeds, and untreated checks were included. In tests with extracts acetone served as control. The solvent was evaporated in air before infesting each of four replicates of treated seeds with six male and four female CSB. We quantified mortality and oviposition/female at 7 days after infestation (and then discarded the bruchids), F_1 progeny/female 14 days after date of first emergence, and percentage seed perforation. In another test, batches of cowpea seeds carrying 100 one-day to three-day old bruchid eggs were treated as described above and F_1 progeny/female and seed damage were recorded.

Persistence of toxic action of powdered products of *C. anisata* to CSB was compared to permethrin by infesting (n = 10 pairs of CSB) 100 g of seeds treated with 2.5 g of a test product or treated with 2.5 g of permethrin contained in 250 ml conical flask. Muslin cloth was fastened to the mouth of the flask to confine the bruchids. CSB population was monitored at 25-day intervals starting at 40 days after infestation in each of three treatment replicates. After 140 days (ca. 41/2 months) of storage, seed weight loss was computed after sieving out bruchids and dust and reweighing the seeds.

In all preceding tests, the leaf powder showed high insecticidal activity. Therefore, a wider rate of application was bioassayed to determine the minimum effective rate; the same was done with the leaf extract.

In analyzing data, Abbott's formula (13) was used to correct for mortality in control. Oviposition count and the number of F_1 progeny/female were transformed to $\sqrt{x} + 0.5$ while percentages of mortality, emergence, seed perforation and seed weight loss were arcsine-transformed. Analyses of variance for randomized complete block experiments were carried out and significantly different treatment means were separated by Duncan's multiple range test. Means of untransformed data were reported in the text.

Results

Five percent of CSB adults died in untreated control treatment within 7 days. All powdered products except *C. anisata* stem bark powder significantly increased mortality; permethrin was the most toxic although it was not significantly better than the leaf powder (Table 1). Oviposition/female was significantly reduced in all treatments but the stem bark powder was not as effective as permethrin.

Each female in the control reproduced itself 45 times whereas the females exposed to *C. anisata* products or permethrin were significantly less productive (89.5 – 100.0% reduction relative to control). When *C. anisata* products were applied to seeds pre-infested with 100 CSB eggs, they similarly suppressed CSB development to adult stage (29.2-54.4% reduction in emergence relative to control; Table 2) but not to the same extent as when bruchid infestation followed seed treatment. The leaf powder was inferior to permethrin in acute toxicity ($P>0.05$) but the stem and root bark treatments were significantly ($P<0.05$) less effective in reducing bruchid emergence and damage (Tables 1 and 2).

The leaf extract showed slight contact action on CSB, causing more than twice the mortality in control, but it strongly deterred oviposition (84.5% reduction relative to control vs. 41.4% for stem bark and 73.4% for root bark). All extracts suppressed development to adult stage regardless of whether seeds were treated before (70.7 – 99.1% reduction of F_1 progeny/female) or after (72.4% reduction of adult emergence) bruchid infestation. In either case, the leaf extract was the most effective. The general trend in reduction of seed perforation due to seed treatment with extracts was leaf > root bark> stem bark (Tables 1 and 2) with more significant treatment differences in the seeds treated before infestation.

At day 140 of storage, CSB population in the control had increased 177-fold over the parental population (n=20), i.e. a daily increase of 25.1 bruchids. The population growth rates in the stem bark and root bark treatments generally approached the rate in control (Fig. 1) but differed significantly. With the leaf powder treatment, population growth was not noticed until day 90, when it had just 4 adults above the parental population, and it progressed slowly thereafter (maximum of 28 adults at day 140). Permethrin killed all individuals of the parental population precluding the establishment of an infestation.

Table 1: Effects of treatment of cowpea seeds with permethrin or *Clausena anisata* on *Callosobruchus maculatus* reproduction and damage.

Treatment	% Mortality++	Mean No. eggs/female	Mean No. progeny/female	% Seeds perforated
		Powder+*		
Control	5.0c	47.2a	45.0a	96.7a
Permethrin	100.0a	0.0c	0.0b	0.0d
Leaf	76.8ab	12.4bc	0.3b	2.0d
Root bark	63.2b	13.2bc	4.0b	8.9c
Stem bark	29.5c	21.6b	4.7b	27.4b
		Extract+*		
Control	15.0b	42.5a	34.1a	92.7a
Leaf	38.8a	6.6c	0.3d	1.4d
Root bark	11.8b	11.3c	3.3c	22.8c
Stem bark	15.3b	24.9b	10.0b	49.2b

+ Rate of application was 2.5% concentration

++ Percentages corrected by Abbot's formula (13) for mortality in control

*Means within columns with different letter(s) significantly differ at P = 0.05

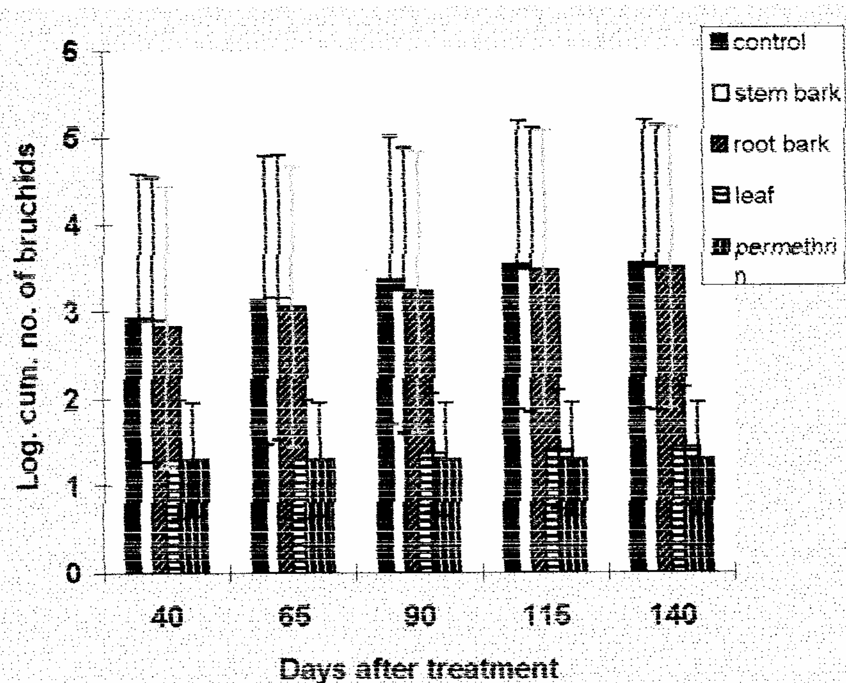

Fig. 1. Population trend of Callosobruchus *maculatus on cowpea seeds treated with* Clausena anisata powdered products or permehrin and stored for 140 days.

At termination of storage, CSB had consumed seed materials weighing 97.3 – 98.1 g, out of the initial 100 g, in the control, stem bark and root bark treatments. In contrast, the seed material consumed in leaf powder treatment was limited to 3.1 g; there was no weight loss in permethrin.

Graduated increases in the rate of application caused CSB adult mortality to increase and oviposition and number of progeny/female as well as seed perforation to decrease (Table 3). The only exception noted to this trend was bruchid mortality in the 2.5% leaf powder. The minimum effective rate was 0.625% concentration; this rate caused significant reductions in F_1 progeny/female and seed damage despite failing to reduce oviposition below control value (Table 3).

Table 2: Effects of treatment of cowpea seeds with permethrin or *Clausena anisata* on *Callosobruchus maculatus* emergence and damage.

Treatment	% Emergence[++]	% Perforated seeds[++]
	Powder[+]	
Control	95.9a	93.6a
Permethrin	42.3d	29.6a
Leaf	43.7cd	31.0c
Root bark	67.9b	51.4b
Stem bark	58.5c	31.0c
	Extract[+]	
Control	92.1a	91.1a
Leaf	25.4c	24.3c
Root bark	52.1b	46.4b
Stem bark	49.7b	46.8b

[+] Application rate was 2.5%
[++] Means within columns with different letter(s) significantly differ at P = 0.05

Discussion

Secondary chemicals from *C. anisata* and other rutaceous plants have, apart from direct toxicity and growth inhibition properties, shown repellency and feeding deterrency to some insect pests (1, 8, 12, 14-17). In this study, permethrin was toxic to CSB by contact and possibly fumigant action (10). The leaf powder of *C. anisata* and its extract to some extent also were toxic to CSB but the contact action showed variation (Tables 1-3). Variation in chemical composition of plants have been related to intra- and interplant variation, time and place of collection, as well as to method of storage and extraction (5, 18). Plant sampling/collection and extraction methods for *C. anisata* products would have to be better standardized to reduce variability of their toxicity.

The leaf powder and its extract might not have caused as high mortality by contact action to adults as permethrin, however, their deterrence of oviposition are notable just as their suppression of development. These are indicators of the products' chronic toxic effects upon adults, and growth inhibiting and insecticidal action (acute contact and/or oral) on CSB larvae as they hatched and ate their way into cowpea seed. Data on population growth seemingly point to the importance of toxic action of *C. anisata* leaf products more so that we did not observe significantly longer duration of development in these treatments as one would expect to happen were they to act mainly as insect growth regulators and antifeedants.

Table 3: Effects of rate of application of *Clausena anisata* leaf powder or extract on reproduction and damage by *Callosobruchus maculatus*

Rate (%)	% Mortality	Mean No. eggs/female	Mean No. progeny/female	% Perforated seeds
		Powder+		
Control	5.0c	50.0a	42.2a	99.6a
0.625	5.2c	43.8a	15.0b	44.4b
1.25	15.5bc	25.7b	1.8c	4.0c
2.50	12.4bc	12.0c	0.4c	1.2c
5.0	27.8b	10.9c	0.3c	0.8c
10.0	53.6a	5.7c	0.1c	0.4c
		Extract+		
Control	5.0c	50.0a	44.2a	99.6a
0.625	15.5b	30.0b	11.6b	33.2b
1.25	23.7b	12.4c	0.7c	1.6c
2.50	34.0ab	6.6c	0.3c	1.0c
5.0	36.1ab	4.0c	0.2c	0.6c
10.0	51.5a	2.6c	0.1c	0.4c

+Percentages corrected by Abbott's formula (13) for mortality in control
+ Means within columns followed by different letter(s) differ significantly at P = 0.05

The root and stem bark powders and their extracts were less insecticidally active. While all parts tested elaborate coumarins and carbazole alkaloids, the leaf may be richer in volatile/essential oils, e.g. estragol, trans-anethol, sabiene and foeniculin which studies have shown have ovicidal, toxic and deterrent effects on stored products Coleoptera and other insects (20-21). The extracts tended to be more effective in reducing oviposition and progeny production (Tables 1 and 2). The toxic principle(s) may have been concentrated in the extract making higher dose available for acute and chronic toxic action.

Refined bioassays by topical, residual contact or oral application to reveal dose-response relationships are needed to fill gaps in our knowledge of the major and adjunct mechanisms of insecticidal actions (acute contact toxicity, post-ingestion toxicity and antifeedant action) of *C. anisata* leaf powder and its extract.

Isman (22) in discussing prospects for botanical insecticides has stressed the importance of "availability of the natural resource in substantial quantitites". Prospecting for insecticidal chemicals from *C. anisata* leaves looks more promising compared with the stem or root bark because the leaves are more readily renewable. Equipotency of *C. anisata* leaf powder with permethrin and the persistence of its bioactivity against CSB led to a suggestion of its use as a substitute protectant especially in areas where the plant can be procured cheaply. However, as little is known about the hazard of *C. anisata* residue on food, the caution by Isman (23) not to assume safety of botanical insecticides to vertebrates is worth reiterating.

In conclusion, the leaf powder of *C. anisata* had insecticidal effects upon the CSB exemplified by contact action upon adults, strong deterrence of oviposition and suppression of development to adult. Persistence of efficacy is adequate to maintain good quality of seeds stored between one planting season and another.

ACKNOWLEDGEMENT: The authors thank the Research Board of the University of Agriculture, Makurdi (UAM), for a grant to EOO that defrayed a part of the research costs, as well as Mr. P. Ekwuno of the Department of Forestry, Wildlife and Range Management, for identification and collection of plant materials. The junior author (AAA) is grateful to Plateau State Government, for granting him leave to study at UAM; Professor A.O. Nwankiti, for co-supervising his thesis research; and Mrs. A.A. Ogunwolu for support services.

References

1. Mester, I; Szendrei, K.; Johannes, R. (1977). Studies on location of natural chemicals. Part 59. Constituents of *Clausena anisata* (Willd.) Oliv. (Rutaceae) I. Coumarins from root bark. Planta Medica 32: 81 – 85.
2. Chakraborty, A.; Chowdhury, B.K.; Bhattacharyya, P. (1995). Clausenol and clausenine-two carbazole alkaloids from *Clausena anisata*. Phytochemistry 40: 295 – 298.
3. Okorie, D.A. (1975). A new carbazole alkaloid and coumarins from roots of *Clausena anisata*. Phytochemistry 14: 270 – 272.
4. Ayedoun, M.A.; Houenon, J.G.; Sossou, P.V.; Menut, C.; Lamaty, G. and Bressuere, J.M. (1997). Aromatic plants of tropical West Africa. V. Major components of the leaf and fruit essential oils of *Clausena anisata* (Willd.) J.D. Hook from Benin. Journal of Essential Oil Research 9: 247 – 248.
5. Reimer, B.; Hofer, O. and Greger, H. (1997). Tryptamine derived amide from *Clausena anisata*. Phytochemistry 45: 337 – 341.
6. Adesina, S.K. and Adewunmi, C.O. (1981). The isolation of molluscidal agents from the root of *Clausena anisata* (Willd.)Oliv. Abstract of the 4th International Symposium of Medicinal Plants, pp. 44 – 45, Ile-Ife, Nigeria.
7. Watt, J.M. and Breyer-brandwijck, M.G. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd edition (Edinburgh: Longmans).
8. Adebayo, S.A. (1989). Phytochemistry of *Clausena anisata* leaves. B.Sc. Chemistry research Project Report. Nigerian Defence Academy, Kaduna, Nigeria.
9. Ayensu, E.S. (1978). Medicinal Plants of West Tropical Africa, Alcognac Publication, Michigan, 330pp.
10. Hill, D.S. (1983). Agricultural Insect Pest of the Tropics and Their Control, 2nd edition (London, Cambridge University Press).
11. Adebayo, T.A. and Gbolade, A.A. (1994). Protection of stored cowpea from *Callobruchus maculatus* using plant products. Insect Science and Its Application. 15: 185 – 189.
12. Ogunwolu, E.O. and Odunlami, A.T. (1996). Suppression of seed bruchid (*Callosobruchus maculatus* F.) development and damage on cowpea [*Vigna unguiculata* (l.) Walp.] with *Zanthoxylum zanthoxyloides* (Lam.) Waterm. (Rutaceae) root bark powder when compared to neem seed and pirimiphos-methyl. Crop Protection 15: 603 – 607.
13. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265 – 267.
14. Anarson, J.T.; Mackinnon, S.; Durst, A.; Philogene, B.J.R.; Hasbun, C.; Sanchez, P.; Poveda, I.; Sanroman, L.; Isman, M.B.; Satasook, C.; Towers, G.H.N.; Wiriyachitra, P. and McLaughlin, J.L. (1993). Insecticides in tropical plants with non-neurotoxic modes of action. Recent Advances in Phytochemistry 27: 107 – 131.
15. Champagne, D.E.; Koul, O.; Isman, M.B.; Scudder, G.G.E. and Towers, G.H.N. (1992). Biological activity of limonoids from the Rutales. Phytochemistry 31: 377 – 394.
16. Jacobson, M. (1989). Botanical Pesticides: Past, present and future. American Chemical Society Symposium Series 387: 1 – 10.
17. Ogunwolu, E.O. and Idowu, O. (1994). Potential of powdered *Zanthoxylum zanthoxyloides* (Rutaceae) root bark powder and *Azadirachta indica* (Meliaceae) seed for control of the cowpea seed bruchid, *Callosobruchus maculatus* (Bruchidea) in Nigeria. Journal of African Zoology 108: 521 – 528.
18. Harborne, J.B. (1990). Role of secondary metabolites in chemical defense mechanisms in plants. In: Bioactive Compounds from Plants (Chadwick, D.J. and Mash, J. eds.). CIBA Foundation Symposium 154, pp. 128-139, (Chichester: Wiley).
19. Waterman, P.G. and Mole, S. (1989). Extrinsic factors influencing production of secondary metabolites in plants. In: Insect-Plant Interactions, vol. 1, pp. 1107 – 1134. (Bernays, E.A. ed.), Boca Baton, Florida: CRC Press.
20. Bushland, R.C. (1939). Volatile oils as ovicidesfor the screw worm, *Cochliomyia americana*. Journal of Economic Entomology 32: 430 – 431.
21. Iwuala, M.O.E. and Osisiogu, I.U.W. and Agbakwuru, E.O.P. (1981). Dennettia oil, a potential new insecticide: tests with adults and nymphs of *Periplaneta americanum* and *Zonocerus variegatus*. Journal of Economic Entomology 74: 249 – 252.
22. Isman, M.B. (1994). Botanical insecticides. Pesticide Outlook (June), 76 – 81.
23. Isman, M.B. (1994). Botanical insecticides and antifeedants: New sources and perspectives. Pesticide Research Journal 6: 11 – 19.