Biological Activities of Hexane Extract of *Piper cubeba* Against Rice Weevils and Cowpea Weevils (Coleoptera: Curculionidae)[1,2]

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J. Entomol. Sci. 25(1): 16-20 (January 1990)

ABSTRACT *Piper cubeba* fruit was evaluated for its biological activites to adults of *Sitophilus oryzae* (L.) and *Callosobrunchus maculatus* (F.). The hexane extract of berries was toxic (>50% mortality) to both species of insects when applied topically at ≥ 30 µg/insect to *S. oryzae* and at ≥ 40 µg/insect to *C. maculatus*. The pulverized berries and its hexane extract, when applied to wheat, were repellent to *S. oryzae* with the berry powder having greater repellency than the extract. When applied to wheat or black-eyed peas at dosages of ≥ 500 ppm, the hexane extract reduced infestation of *S. oyrzae* and *C. maculatus* in these commodities.

KEY WORDS *Piper cubeba*, cubeb berry, toxicity, repellency, Rice weevil, *Sitophilus oryzae*, cowpea weevil, *Callosobruchus maculatus*.

Piper cubeba L. f., cubeb or tailed pepper, is an evergreen shrub or climbing vine in the Piperaceae family. The economically useful part of the plant is the dried, full grown but unripe fruit, commonly called cubeb berry. When the crushed fruits are steam distilled, cubeb oil is obtained. Cubeb berry and oil have been used in household medicine as a diuretic-urinary antiseptic for genito-urinary disorders (Hartwell 1970, Dasgupta and Datta 1980). The oil is used as a flavoring ingredient in most major categories of food products and as a fragrance component of detergent and cosmetic lotions and perfumes (Opdyke 1976); and it also has been reported to exhibit antiviral, antiseptic and antibacterial actvities (George et al. 1947).

The essential oil of cubeb was reported to be ovicidal to the screwworm, *Cochliomyia hominivorax* (Coquerel) (=*americana* C. & P.) (Bushland 1939). Larvicidal activity of the acetone extract to the southern house mosquito, *Culex quinquefasciatus* Say, was reported by Hartzell (1944). In this study, the biological activities of powdered cubeb berries and its hexane extract to rice weevils, *Sitophilus oryzae* (L.) and cowpea weevils, *Callosobruchus maculatus* (F.) are presented.

Materials And Methods

Preparation of *P. cubeba* Extract. Pulverized *P. cubeba* fruit, a pure herbal tea, was purchased from the Indiana Botanic Gardens, Inc., Hammond, IN. The

[1] Accepted for publication 6 July 1989.

[2] This paper reports the results of research only. Mention of a proprietary product does not constitute a recommendation or an endorsement by the USDA.

powder (142 g) in hexane (400 ml) at 40-50°C was stirred for 30 min and filtered. The extraction process was repeated with the residue for four more times using 200 ml of hexane each time. The combined filtrate was concentrated under reduced pressure (100 - 200 mm Hg) to a small volume, and lyophilized to provide the hexane extract.

Test Insects. Adults of rice weevil, *S. oryzae* (24 - 48 h old), and cowpea weevil, *C. maculatus* (< 5 h old) reared at 27 ± 1°C and 60 ± 5% RH from wheat or black-eyed peas, respectively at the Stored-Product Insects Research and Development Laboratory, Savannah, GA, were used in this study.

Toxicity by Topical Treatment of Insects. A stock solution of 100 mg/ml was obtained by dissolving 100 mg of cubeb hexane extract in 1 ml acetone (Reagent grade). Lower concentrations of 80, 60, 40, and 20 mg/ml were obtained by dilution of the stock solution with acetone.

Insects were anesthetized briefly with CO_2 and then picked up individually with a vacuum needle. Using a microapplicator, 0.5 µl of a solution was applied to the dorsum of the thorax of each insect. Twenty insects of each species (unsexed for rice weevils, and 10♂ and 10♀ for cowpea weevils) were treated with each dose. After treatment, the insects were placed in 100-mm dia. petri dishes, 10 insects/dish (10 kernels of undamaged soft winter wheat were placed in each dish together with rice weevils), and kept in a room maintained at 27 ± 1°C and 60 ± 5% RH under alternating 12-h light and dark cycles. The insects were examined daily for one week. Those that did not move or respond to the gentle touch of a small probe were considered dead.

Repellency to Rice Weevil by Food Preference Method. Required amounts of cubeb hexane extract were dissolved in 1 to 2 ml of acetone and applied to wheat at concentrations of 2000, 1000, and 500 ppm. The cubeb powder was also used to surface treat wheat at concentrations of 20000, 10000, and 5000 ppm, 10-× that of the extract, by thoroughly mixing the powder and wheat.

These treated samples were evaluated using the modified Loschiavo food preference apparatus as described by Laudani and Swank (1954). The apparatus consisted of a circular platform 50 cm in diameter, with a 5-cm metal rim. The platform had 12 holes, 8.75 cm in diameter, equally spaced along the outer edge to accommodate paper cups which were filled to the rim (about 60 g each) with treated or untreated grain samples. The center of the wheel cover had an opening 1.25 cm in diameter, fitted with a plastic tube through which 120 adult rice weevils were introduced for a 24 h exposure period. For each experiment, three cups were filled with untreated wheat, and three were each filled with wheat whose surface was treated with the extract or with the powder at each of the dosages. The cups were arranged in different repeated sequence for each experiment (in A-B-C-D- for expt. 1, A-C-B-D- for expt. 2, and A-B-D-C- for expt. 3). At the end of each exposure period, the insects in each cup were collected and counted to determine their distribution. For each material, the experiment was repeated three times.

Evaluation of Cubeb Hexane Extract as Protectant for Wheat and Black-Eyed Peas. The wheat or black-eyed peas were surface treated at dosages of 2000, 1000, and 500 ppm (w/w). Required amounts of cubeb hexane extract for the indicated concentrations were dissolved in 2-3 ml of acetone and were each applied to wheat or black-eyed peas. Treated samples were air-dried and kept in glass jars overnight in a room maintained at 27 ± 1°C and 60 ± 5% RH before use. Acetone-treated wheat or acetone-treated black-eyed peas were used as controls.

A plastic ring (90 mm dia × 5 mm high) was placed in the center of a glass crystallizing dish (160 mm dia × 100 mm high). A 20-g lot of test sample was placed inside the ring which acted as a barrier to prevent the wheat or peas from scattering. Ten pairs of adult rice weevils or 5 pairs of adult cowpea weevils were placed on each sample. The dish was covered with a plexiglass lid which had two 35 mm dia. holes covered with 40-mesh metallic cloth, opposite to each other, 20 mm from the edge. Dishes were kept in a room at $27 \pm 1°C$ and $60 \pm 5\%$ RH. After 7 days of exposure, insects inside and outside the ring were removed and examined for mortality and repellency. Each sample was kept in a 0.237-liter glass jar and stored in the exposure room. Four weeks after exposure, samples were examined every 2 or 3 days for F_1 emergence. When emergence started, insects were counted every 2 or 3 days and removed. Readings were stopped 8 weeks after exposure for rice weevils and 7 weeks for cowpea weevils in order to avoid including the F_2 insects.

The number of F_1 adults was used as a criterion for evaluation. F_1 emergence in the treated sample compared to F_1 emergence in the control is an index of effectiveness of the treated material in preventing infestation. Only the experiments of same date and with the same insects colony were used in the comparison.

Data were analyzed by using analysis of variance and Duncan's multiple range test (Duncan 1955).

Results and Discussion

The hexane extraction of cubeb powder produced a 5.6% yield as a dark brown mass. The contact toxicity from topical application of this extract to rice weevils and cowpea weevils is shown in Table 1. The extract was quite toxic at concentrations of $\geq$ 30μg/insect for the rice weevils, and $\geq$ 40 μg/insect for the cowpea weevils. The toxic effect almost reached its maximum at 3 days with a slight increase thereafter. However, at dosages of 20 and 30μg/insect the toxic effect decreased on cowpea weevils. This was due to the eventual recovery of some insects from the moribund state at these lower dosages. This moribundity phenomenon, due to leg paralysis, existed only in cowpea weevils. The hexane extract of cubeb powder was not topically toxic to cigarette beetle, *Lasioderma serricorne* (F.) (<3 days old), and confused flour beetle, *Tribolium confusum* Jacquelin du Val (<7 days old), (Su, unpublished data).

The repellency of cubeb powder and its hexane extract to adult rice weevils is summarized in Table 2. When rice weevils were exposed to both cubeb powder or its hexane extract on wheat by the food preference method, both materials strongly repelled the insects at all doses tested. The powder was slightly more repellent than the extract. This could be due to loss of volatile components during lyophilization of the concentrated extract.

Protection by the extract on wheat or black-eyed peas against infestation by rice weevils or cowpea weevils is presented in Table 3. When wheat was treated at dosages of 2000, 1000, and 500 ppm, all wheat samples showed significant

Table 1. Toxicity of hexane extract of cubeb berry applied topically to adult rice weevils and cowpea weevils.

Dose (μg/insect)	Avg. % DM* and SEM† after application at days indicated 3 days	7 days
	Rice Weevils	
50	67.5 ± 4.3 a	72.5± 2.5 a
40	60.0 ± 7.4 a	63.8± 8.5 ab
30	48.8 ± 8.3 ab	51.3± 7.7 bc
20	35.0 ± 5.4 b	42.5± 4.3 c
10	6.3 ± 2.4 c	15.0± 4.6 d
Acetone Only	0	0
	Cowpea Weevils	
50	83.8 ± 2.4 a	87.5± 3.2 a
40	68.8 ± 5.2 b	70.0± 5.8 b
30	38.8 ± 4.3 c	37.5± 3.2 c
20	23.8 ± 5.2 d	10.0± 2.0 c
10	3.8 ± 1.3 e	3.8± 1.3 d
Acetone Only	0	0

* DM = dead and moribund insects; averge of four replicates, 20 insects per replicate.

† SEM = standard error of the mean. Values followed by the same letter within a column per species of insect are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table 2. Distribution of rice weevil adults in treated and untreated wheat with cubeb berry powder or its hexane extract after a 24-h exposure in a repellency wheel.

Dose (ppm)	Overall Avg. (%) of insects ± SEM*,†
	Cubeb Berry Powder
20000	6.1 ± 2.4a
10000	10.0 ± 2.4a
5000	15.3 ± 3.5a
Control	68.1 ± 3.2b
	Cubeb Berry Hexane Extract
2000	2.0 ± 1.2a
1000	16.9 ± 2.4b
500	22.8 ± 2.3b
Control	58.3 ± 3.5c

* Distribution of 120 insects with three samples of each treatment in each experiment, average of three experiments per dose. SEM = standard error of the mean.

† Values followed by the same letter within a column are not significantly different at the 0.05 level according to Duncan's multiple range test.

reduction of F_1 rice weevil adults when compared to the control (Table 3). Fewest F_1 insects emerged at the 2000 ppm dosage. When the same treatments were used on black-eyed peas, the extract, at 2000 and 1000 ppm also gave a significant reduction of F_1 cowpea weevil progeny (Table 3). At a dosage of 500 ppm, the number of F_1 progeny was still slightly fewer than that of the control, although it was not significantly different.

Table 3. Effect of cubeb berry hexane extract applied to wheat against adult rice weevils and to black-eyed peas against adult cowpea weevils.

Dose (ppm)	Avg. No. of F_1 adults ± SEM*,†	% Reduction
	Rice Weevils	
2000	48.7 ± 8.8a	70.6
1000	79.9 ± 15.5b	51.8
500	129.8 ± 5.9c	21.5
Control	165.3 ± 4.6d	0
	Cowpea Weevils	
2000	146.0 ± 18.5a	42.5
1000	177.0 ± 22.2ab	30.3
500	236.3 ± 20.4bc	7.0
Control	254.0 ± 15.3c	0

* Ten pairs (10 ♂ and 10♀) of rice weevils in each wheat sample; and five pairs (5 ♂ and 5♀) of cowpea weevil in each black-eyed pea sample; six replicates in each test dose. SEM = Standard error of the mean.

† Values followed by the same letter within a column are not significantly different at the 0.05 level according to Duncan's multiple range test.

The toxicity of *P. cubeba* to rice weevils and cowpea weevils, the repellency to rice weevils, and the reduction of F_1 adults in treated wheat and black-eyed peas were demonstrated in this study. Further investigation of the active components of the *P. cubeba* as a potential source of a new insecticide for these insects is warranted.

References Cited

Bushland, B. C. 1939. Volatile oils as ovicides for the screwworm, *Cochliomyia americana* C. & P. J. Econ. Entomol. 32: 430-431.

Dasgupta, A. and Datta, P. C. 1980. Medicinal species of *Piper,* pharmacognestic delimitation. Quart. J. Crude Drug Res. 18: 17-25.

Duncan, D. M. 1955. Multiple range and multiple F tests. Biometrics. 11: 1-42.

George, M., Venkataraaman, F. R., and Pandalai, K. H. 1947. Investigations on plant antibiotics. II. A search for antibiotic substances in some Indian medicinal plants. J. Sci. Ind. Res. 6B: 42.

Hartwell, J. L. 1970. Plants used against cancer. A survey. Lloydia. 33: 288-392.

Hartzell, A. 1944. Tests on plant products for insecticidal properties. Contributions from Boyce Thompson Inst. 13: 243-252.

Laudani, H. and Swank, G. R. 1954. A laboratory apparatus for determining repellency of pyrethrum when applied to grain. J. Econ. Entomol. 47: 1104-1107.

Opdyke, D. L. J. 1976. Food and cosmetics toxicology. Vol 14 Suppl. page 729.