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RESEARCH ARTICLE

BIO-EFFICACY OF *CROTON HIRTUS* LEAF AND STEM POWDERS AGAINST *CALLOSOBRUCHUS MACULATUS* INFESTATION ON STORED COWPEA

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ABSTRACT

Background and aim: *Croton hirtus* L'Her (Euphorbiaceae), known as hairy croton, is popular in traditional medicine for the treatment of hypertension, fever, inflammation, diabetes, amongst others.

Methods: Leaf and stem powders at concentration range of 0.2 – 2.0 g per 50 cowpea seeds, and standard insecticide, permethrin at 3.0 mg were used to dress cowpea seeds using standard method to determine their effectiveness in protecting cowpeas from attack by weevils, *Callosobruchus maculatus*. Oviposition deterrency at 15 days after treatment (DAT) and inhibition of adult insect emergence at 50 DAT were assessed. Other parameters of seed damage were also investigated.

Result: Study indicated differences in the protective ability of the leaf and stem powders on stored cowpeas. Both powders produced oviposition deterrency (greater for leaf, 73.6% at peak concentration) and adult insect emergence inhibition (greater for leaf, 84.61%) in a concentration-dependent manner. Efficiency of *C. hirtus* powders was comparable to that of permethrin. All indices of seed damage witnessed diminution in a concentration-dependent manner, and the leaf powder elicited greater percentage protective ability (48.7%) which was comparable with permethrin.

Conclusion: Leaf and stem powders of *C. hirtus* examined for the first time, proved to be effective in protecting stored cowpea seeds from weevil attack.

Key words: *Croton hirtus,* leaf powder, stem powder, *Callosobruchus maculatus,* cowpea seeds, oviposition deterrency, adult emergence inhibition

INTRODUCTION

Croton hirtus L'Hèr (Euphorbiaceae) is commonly known as hairy croton. It is an erect annual herbaceous plant growing to about 90 cm in height mostly found in waste places and cultivated soils in most environment [1]. Stem is covered in rigid star-shaped hairs and leaves are star-shaped-hairy on both sides with toothed margins. The plant originated from tropical America, but has become naturalized pan-tropical and widespread in the Guinea savannah zone of West Africa, particularly in Ivory coast and Sierra Leone [1]. Pharmacological assays such as anti-inflammatory, antioxidant, antimicrobial, hypolipidemic, hypoglycemic, antiestrogen and anticancer studies of *C. hirtus* [1,2,3] have been used to corroborate the traditional uses of *Croton* species. Tans-dehydrocrotonin clerodane and other diterpenoids were reported as bioactive compounds.

Aside from the mosquito larvicidal activity [3] and insecticidal activity of *C. hirtus* against *Amitermes evuncifer* [4], literature is devoid of any reported toxicity on *Callosobruchus maculatus* in stored cowpea. In view of the growing concern to develop natural products-based pesticides, we investigated the suitability of the leaf and stem powders of *C. hirtus* for the control of cowpea weevils.

MATERIALS and METHODS

***Plant collection and preparation*:** Fresh aerial parts of *C. hirtus* were obtained from the Crown Estate, Igbinedion University Okada (IUO), Ovia Northeast Local Government Area, Edo State in November 2022. Authentication was done at the herbarium of Department of Pharmacognosy, College of Pharmacy, IUO where a voucher specimen (IUO/19/290) was deposited. Plucked leaves and stem were separately air-dried on concrete floor in the shade for a week at ambient condition, ground separately using a local milling machine, and kept in air-tight container.

***Insect culture and bioassay procedure*:** Adult weevils (*C. maculatus*) were obtained from infested cowpea seeds purchased in November 2022 from Okada market in Okada community, Edo State, Nigeria. Rearing method of Ayinde *et al.* [5] was adopted. Insects were removed and transferred into a transparent plastic jar (2.5L) containing sufficient wholesome un-infested cowpea seeds (brown variety), covered with a clean muslin cloth for aeration and kept in the laboratory at room temperature (26 - 28°C; 75±5% RH) for oviposition. The parent stocks were removed after 15 days, discarded and jar incubated for further 15 days. The emerging F_1 progenies obtained from the culture were used for bioassay.

Bioassay was done at room temperature (26 - 28°C; 75±5% RH) as previously published by Ayinde *et al* [5]. Fifty wholesome, clean cowpea seeds (14.8-16.4 g) were transferred into air-tight transparent bioassay plastic beakers (450 ml) containing 0.2, 0.4, 0.6, 1.0 and 2.0 g *C. hirtus* leaf or stem powder. The powder was spread over the seeds by gentle shaking. Positive control jar contained 0.5g Rambo® insecticidal powder (Gongoni Company Limited, Kano, Nigeria; MFD 06/2022; EXP 08/2025) (containing 0.60% permethrin) while the negative control beakers had no plant powder. A pair of newly emerged five males and five females *C. maculatus* (F_1 progenies) were then introduced into each jar and covered. Plastic beakers were incubated in wooden cabinets in the laboratory for 15-20 days and allowed to oviposit. Fifteen days after treatment (DAT), the weevils were removed to prevent them from mixing with the first generation

(F_1) offspring as they later emerged, and numbers of eggs laid (appearing as white spots) were counted and recorded. Bioassay beakers were further incubated and the number of adults that emerged at three days' interval were counted, removed and discarded. This procedure continued until 50 DAT when there was no evidence of further emergence. Total adult emergence up to 50 DAT was summed up and recorded. Both controls were similarly treated. Means of all triplicate determinations (±SEM) were recorded together with statistical analyses of results. Furthermore, extent of damage to seeds at 50 DAT was evaluated by counting the number of holes on seeds, weight loss and other indices of seed damage for every bioassay beaker were determined.

The following formulae [7] were used for determinations:

Mean adult emergence (by physical counting)
= Total number of live weevils in the 3 beakers / 3

$$PI = \frac{\text{C-emergence} - \text{T-emergence}}{\text{C-emergence}} \times 100$$

PI = percentage inhibition, C = control, T = Test

$$PWL = \frac{\text{Weight of IS} - \text{Weight at 50 DAT}}{\text{Weight of IS}} \times 100$$

PWL = percentage weight loss, IS = initial seeds

Statistical analysis

Results of assays were expressed as a mean±standard error of mean. The differences between the negative control and tested agents were determined by analysis of variance (one-way ANOVA). Difference in means were considered significant* at P<0.05.

RESULTS AND DISCUSSION

From this study, mean egg count at 15 DAT decreased with concentration (Table 1). Values of 39.33 - 12.67 for leaf powder and 34.30 - 18.50 for stem powder were observed in cowpea seeds treated with concentration range of 0.2 - 2.0 g/ 50 seeds (Table 1). Using mean egg count as an index of weevil control, leaf powder could be adjudged a better bio-pesticide. As expected, mean egg count in untreated negative control (48.00) was higher than in the powder treatments, and this suggests the need for weevil control measures. This finding is consistent with literature reports [6,7,8]. In addition, increased oviposition deterrency with increasing concentration was noted for both powders (Table 1).

At lower concentrations (0.2-0.4 g), stem powder (28.54-35.20%) gave better oviposition deterrency than leaf powder (18.06-27.08%). However, leaf (60.73-73.60%) was more potent than stem (42.92-61.46%) as oviposition deterrent at higher concentrations (0.6-2.0 g). Likewise, stem powder was comparable with permethrin in oviposition deterrency (61.46vs59.73%) at the peak concentration, but was less effective than leaf powder (73.60%). Nevertheless, leaf gave comparable deterrency with permethrin at a lower concentration, 0.6 g. The phenomenon of toxicity of *C. hirtus* powders to weevils during cowpea storage is in agreement with previous reports on different plants [9,10,11.

According to this study, adult insect emergence also decreased with concentration (leaf powder 30.8-1.95; stem 33.33-9.33). Leaf has demonstrated better efficacy in curbing emergence. Stem (9.33) was found to compare with permethrin at 0.2 g whereas, leaf gave comparable emergence at 0.6 g. With the more reliable parameter, percentage inhibition of emergence, concentration-dependent increases were observed for both treatments (Table 1). At 50 DAT, *C. hirtus* leaf powder gave higher inhibitions (26.00 - 84.61%) than stem powder (4.63 - 49.57%), indicating a two-fold efficacy over stem at the peak concentration.

Table 1: Effect of *Croton hirtus* powders on oviposition and adult emergence of C*allosobruchus maculatus* on stored cowpea seeds

Concentration (g/50 seeds)	**Mean egg count±SEM (at 15 DAT)/ % oviposition deterrency#**		**Mean adult emergence ±SEM (at 50 DAT)**		**Percentage inhibition of adult emergence**	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
0.2 g	39.33±7.81 (18.06)	34.30±11.43 (28.54)	30.80±2.19	33.33±3.56	21.68	2.83
0.4 g	35.00±8.21 (27.08)	31.10±10.36 (35.20)	25.90±1.58	29.66±0.50	26.00	4.63
0.6 g	18.85±2.91* (60.73)	27.40±9.13 (42.92)	9.00±1.33	18.33±3.46	52.25	33.10
1.0 g	15.33±1.02* (68.06)	23.25±7.70* (51.56)	2.42±1.15	12.66±2.16	84.21	45.55
2.0 g	12.67±6.55* (73.60)	18.50±6.10* (61.46)	1.95±2.04	9.33±0.19	84.61	49.57
Permethrin, 3 mg	19.33±6.40* (59.73)		10.66±0.19		44.85	
Untreated negative control	48.00±2.65		42.67±9.01		11.10	

Values above are mean of three replicates. n=3 (±SEM). Values with superscripts * indicate significant difference at P<0.05 when compared to negative control using ordinary One –way analysis (ANOVA). # Relative to untreated negative control

While leaf at 1.0 g/ 50 seeds, was twice as potent as permethrin (44.85%) in inhibiting emergence, the stem gave comparable result. This concentration of leaf powder which elicited highest inhibition of insect emergence may be recommended for use in commercial preservation of stored cowpeas. Hence, leaf powder could possess better bio-efficacy than stem powder. This present study is in consonance with recent publications by Gbolade *et al.* [8], Esang *et al.* [11] and Shunmugadevi and Radhika [12] on potential of plant products in controlling *Callosobruchus maculatus* in stored cowpea. It also represents an addendum to the compendium of natural bio-pesticides for weevil control. The bioactive constituents such as flavonoids and diterpenoids previously reported for *C. hirtus* [1,13] may be responsible for the observed toxicity of these powders to *Callosobruchus maculatus*.

Moreover, seed damage parameters such as mean number of holes, percentage damage (PD), weevil perforation index (WPI), and percentage weight loss (PWL) were found to decrease with concentration (Table 2). Mean number of holes was 35.30-15.25 for leaf, and 29.80-13.50 for stem were recorded over the entire concentration range. Meanwhile, percentage reduction in holes increased with concentration for both powders, with the stem and leaf giving comparable protection (70.86vs67.08%) at peak concentration. These powders also compared with permethrin (14.3 holes) at peak concentration as regards number of holes.

Table 2: Effect of *Croton hirtus* powders on damage to stored cowpea seeds

Conc (g/ 50 seeds)	Mean holes±SEM / % reduction[#]		Damage (PD, %)		Weevil perforation index (WPI, %)		Weight loss (PWL, %)		Protectant Ability (%)	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
0.2	35.30±3.00 (23.80)	29.8±1.38 (35.67)	94.0	79.3	71.6	79.3	33.1	16.4**	28.4	20.7
0.4	28.60±1.73 (38.26)	28.1±0.50 (39.34)	76.0	69.3	67.1	77.0	29.4	15.2*	32.9	22.9
0.6	26.65±5.77* (42.47)	23.9±3.68* (48.41)	70.7	63.3	65.4	75.3	28.7	8.9*	34.6*	24.6
1.0	21.75±3.06* (53.05)	17.0±3.16* (63.3)	57.0*	44.7*	60.6	68.4	27.1	7.0*	39.4*	31.6
2.0	15.25±8.62* (67.08)	13.5±1.34* (70.86)	39.3*	34.6*	51.3	62.7	25.1	6.1*	48.7*	37.3*
PMT	14.3±1.17* (69.13)		20.7*		50.0		6.0*		50.0	
NC	46.33 ± 4.04		92.7%		71.3		39.6		17.9	

Values above are mean of three replicates. n=3 (±SEM). Values with superscripts * indicate significant difference at P<0.05 when compared to negative control using ordinary One –way analysis (ANOVA). Conc = concentration, PMT = positive control (Permethrin, (3.0 mg), NC = untreated negative control, [#]Relative to untreated negative control

In addition, stem powder was more effective in lowering PD (34.6%) and PWL (6.1%) at peak concentration when compared with leaf powder. WPI for both treatments (62.7vs51.3%) was greater than the recommended value, 50% [9,14]. Based on the parameters of seed damage, *C. hirtus* stem powder produced comparatively reduced damage, and would be a suitable natural bio-pesticide for weevil control in stored cowpea.

With respect to PPA which increased with concentration, only the leaf powder (48.7%) was comparable to permethrin (50%) at the highest concentration tested. Our current finding is in agreement with previously published reports for other plants on reduction in cowpea damage [8,9,10,11].

CONCLUSION

The results obtained in this study revealed that *C. hirtus* leaf powder exhibited greater deterrence of oviposition, minimal reduction in seed damage and better percentage protective ability than the stem powder. Hence, it has better potential as a bio-pesticide against *Callosobruchus maculatus* during cowpea storage.

Conflict of interest: The authors declare no conflict of interest in this work.

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