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RESEARCH ARTICLE

TOXICITY OF *MONOON LONGIFOLIUM* FABACEAE LEAF EXTRACTS ON *CALLOSOBRUCHUS MACULATUS* DURING COWPEA STORAGE

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ABSTRACT

Background and aim: *Monoon longifolium* (Sonn.) B. Xue & R.M.K. Saunders (Fabaceae) (syn. *Polyalthia longifolia*), known as false Ashoka and masquerade tree, is popular in traditional medicine for the treatment of fever, helminthiasis, diabetes and cardiac problems amongst others.
Methods: Protectant activities of *M. longifolium* leaf hexane and acetone extracts (0.1 - 0.8 g per 50 cowpea seeds) and standard insecticide, permethrin (1.5 and 3.0 mg), as seed dressings, were assessed. Oviposition deterrence and inhibition of adult insect emergence were determined at 15 days after treatment (DAT) and 50 DAT, respectively, in addition to parameters of seed damage.
Results: The result indicated differences in protectant effects between the different concentrations of the extracts used. Hexane and acetone extracts produced oviposition deterrence and adult insect emergence inhibition in a concentration-dependent manner, with hexane extract exhibiting greater efficiency. Efficiency of the extracts exceeded that of permethrin at 3 mg. Peak concentration (0.8 g/ 50 seeds) of both extracts gave highest protectant effect for oviposition deterrency (hexane: acetone, 94.12vs83.33%), adult emergence inhibition (100vs93.93%), mean hole reduction (100vs92.60%), seed damage (0vs1.40%), mean weight loss (0vs18.20%) and weevil perforation index (0vs7.33%).
Conclusion: *M. longifolium* leaf extracts examined for the first time, proved to be an effective protectant for stored cowpea seeds from weevil attack. Hexane extract was more effective than standard permethrin in eliciting weevil toxicity and protecting stored cowpeas.

Keywords: *Monoon longifolium* leaf, hexane extract, acetone extract, *Callosobruchus maculatus*, cowpea seeds, oviposition deterrency, adult emergence inhibition

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) (Fabaceae) is an important legume species, both for its consumption as food and as animal feed worldwide, especially in semi-arid tropical and desert regions [1]. It is an excellent source of vitamins, antioxidants, fiber, trace elements and other nutrients and plays an important role in malnutrition avoidance in the least developed countries where it is mainly cultivated [2]. Almost all its aerial parts are consumed. In addition to its mature dry seeds, its leaves, green pods and green seeds are consumed in various countries. It is also used for flour, as its seeds contain a high protein content (23–32%) compared to many other legume species [2].

Cowpea farming forms the major economic mainstay in the west African sub-region which enables farmers to buy other cereal grains and farm inputs such as fertilizers [2]. The full economic potential of cowpea will only be realized if other value added products especially those targeted at the ever growing urban population, are introduced. It is an important weaning food in many communities in Africa and Asia. Its storage is often threatened by infestation by the cowpea weevil or cowpea seed beetle, *Callosobruchus maculatus* F. This pest has a cosmopolitan distribution, occurring on every continent except Antarctica causing extensive damage to cowpeas in storage leading to great economic loss. Various measures for curbing seed damage during storage include use of synthetic chemicals which tend to lose their persistence or effectiveness with high humidity, high temperature, time, and sunlight [3]. Involvement of natural products for cowpea preservation is a new globally-accepted tool of improving farmers' economic gains. Reviews of plant products from Nigeria [4] and elsewhere [5,6] with protectant properties on stored cowpeas from bruchid infestation have been published. Furthermore, extracts of many plants have been similarly reported to protect cowpeas from weevil attack [7-10].

The search for other potent plant-based protectants for stored cowpeas is a growing research endeavour encompassing investigating the potential of *Monoon longifolium* (Sonn.) B. Xue & R.M.K. Saunders (*Polyalthia longifolia*). The plant is an evergreen tree growing up to 15-20 metres, originally cultivated in India but now found in Africa, Southeast Asia, Australia and New Zealand [11]. It is commonly called false Ashoka, Street tree because of its effectiveness in combating noise pollution. It is employed in traditional medicine for the treatment of diabetes, cardiac problems fever, helminthiasis, among other diseases [12,13]. Aside from the various pharmacological activities such as antibacterial, anti-tumour, antidiabetic and antioxidant documented for *M. longifolium*, about 100 compounds typified by steroids, flavonoids, alkaloids and clerodane diterpenes have also been isolated from its leaf, bark and root [11,13]. Sesquiterpene derivatives have been reported to dominate the plant essential oils [14].

Limited insecticidal properties of *M. longifolium* such as antifeedant action of its extracts against lepidopteran and sucking insect pests [15] and larvicidal effect of aqueous pericarp extracts on *Aedes aegypti* [16] are known. No mention has been recorded on any other insecticidal activity or stored grain preservation potential, and this formed the basis of this present study which screened hexane and acetone extracts of *M. longifolium* in controlling damage to stored cowpea by *C. maculatus*.

MATERIALS AND METHODS

***Plant collection and extraction*:** Fresh leaves of *M. longifolium* were obtained from various locations at Crown Estate and within the College of Pharmacy premises, Igbinedion University Okada (IUO) in November 2022. It was authentication was done in the Department of Pharmacognosy herbarium, IUO (voucher number: IUO /16/102). Leaves were air dried for a week on a concrete floor and ground into fine powder (300 g) using a mechanical grinding machine. Powder plant sample was subjected to exhaustive gradient maceration with n-hexane and acetone, each extract concentrated to dryness over stem bath (45^{o}C), weighed and refrigerated until required.

***Preparation of cowpea seeds*:** Wholesome, clean and un-infested cowpea seeds (brown variety) used in this experiment were purchased from Aduwawa market, Benin city, Edo State. The seeds were sun dried for 2 days to remove pesticides that may have been used for preservation, and later stored in a 500 ml air-tight, transparent plastic container until required.

***Source and maintenance of insect culture*:** Infested cowpeas were purchased from Okada market in Okada community, Edo State, Nigeria. Insect culture was maintained according to Fotso *et al.* [17]. Adult bruchids (*Callosobruchus maculatus*) obtained from the infested seeds were put in a 500 ml transparent plastic jar containing sufficient number of wholesome un-infested cowpea seeds. The jar was covered with a clean muslin cloth to allow aeration for respiratory purpose and kept in the laboratory at ambient conditions (26 - 28°C; 75±5% RH). The parent stocks of bruchids were removed after 15 days when adequate oviposition was evident and discarded. Further incubation continued for up to 50 days when sufficient F_1 progenies were realised and were used for the experiment.

Bioassay procedure: This experiment was done as previously published by Fotso *et al.* [17] in triplicate under ambient conditions (26 - 28°C; 75±5% RH). Fifty wholesome, clean cowpea seeds were transferred into a 300 ml air-tight transparent plastic jars. Varying amounts of *M. longifolium* leaf hexane and acetone extracts (0.1, 0.2, 0.4, and 0.8 g in 4 ml of each solvent) were added separately, and jars swirled for uniform seed coating and left open for 24h. for solvent to evaporate. Rambo® insecticidal powder (Gongoni Company Limited, Kano, Nigeria; MFD. 05/2021, EXP. 06/2023 and containing 0.60% permethrin) (1.5 mg and 3.0 mg) was put separately into another jar of seeds as positive control while negative control was prepared without either solvent. Five pairs of newly emerged 15 days old adults of males and females were introduced into each experimental jar and then incubated at prevailing conditions for bruchids to oviposit. Bruchids were removed fifteen days after (DAT) and the numbers of eggs laid (white spots) counted and recorded. The number of adults that subsequently emerged were counted at three days' interval till 50 DAT, and discarded, followed by determination of inhibition of emergence [17]. Extent of damage to seeds was evaluated by counting the number of holes as well as determining the percentage weight loss, seed damage [17] per every concentration after day 50.

***Statistical analysis*:** Results of assays were expressed as a mean ± standard deviation. 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 Table 1, concentration-dependent decreases in egg count at 15 DAT (12 - 13 eggs at 0.1 g/ 50 seeds to 1 – 3 eggs at 0.8 g/ 50 seeds) was observed for cowpea seeds dressed with both hexane and acetone extracts. Hexane extract (0.2 - 0.8 g/ 50 seeds) and acetone extract (0.4 - 0.8 g/50 seeds), significantly ($P<0.05$) reduced egg count compared with their respective negative controls. Oviposition deterrency, which increased with concentration, was generally greater in hexane extract-treated seeds and peaked (94.12% vs 83.33%) at the highest concentration of 0.8 g/ 50 seeds. The standard insecticide, permethrin, at 3.0 mg/ 50 seeds, demonstrated comparable oviposition deterrency (80.41%) as acetone extract at 0.8 g/ 50 seeds but was less effective than hexane extract (94.12%). Insecticidal potency of some plant extracts against cowpea weevils characterized by weevil mortality, oviposition deterrency and adult emergence inhibition, and reduced seed damage has been previously published [8,18].

Table 1: Effect of *Monoon longifolium* leaf hexane and acetone extracts on adult emergence of *Callosobruchus maculatus* on stored cowpea seeds

Concentration (g/ 50 seeds)	**Mean egg count±SEM at 15 DAT /oviposition deterrency#**		**Percentage (%) mean adult emergence at 50 DAT**		**Percentage (%) inhibition of adult emergence**	
	Hexane extract	Acetone extract	Hexane extract	Acetone extract	Hexane extract	Acetone extract
0.1 g	12.00±1.15 (29.41)	13.00±1.15 (27.78)	77.8	82.1	34.89	31.90
0.2 g	9.66*±0.88 (43.18)	12.00±1.15 (33.33)	75.9	77.8	48.84	40.45
0.4 g	4.33*±0.88 (74.53)	7.33*±0.88 (59.28)	38.6	33.3	88.34	84.42
0.8 g	1.00*±0.56 (94.12)	3.00*±1.15 (83.33)	0	31.8	100	93.93
Negative Control (4 ml)	17.00±1.50	18.00±1.52	84.3	87.1	-	-
Permethrin 1.5 mg	4.33*±0.88 (74.53)		53.8		83.74	
Permethrin 3 mg	3.33*±0.88 (80.41)		50.2		88.34	

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 negative control.

Furthermore, percentage adult emergence at 50 DAT in both treatments followed concentration-dependent decreases for hexane extract- (77.8 – 0%) and acetone extract-treated seeds (82.1 – 31.8%) (Table 1). None of the two extracts was comparable with permethrin in adult emergence. However, both extracts showed concentration-dependent increases in the more reliable percentage inhibition of emergence parameter, with complete inhibition evident in hexane extract-treated seeds at 0.8 g/ 50 seeds, and 93.93% inhibition for acetone extract-treated seeds. With reference to permethrin, 0.4 g/ 50 seeds of both hexane and acetone extracts gave comparable inhibition (88.34 vs 84.42%). Hexane extract is therefore more effective than acetone extract in inhibiting weevil emergence. This is agreement with the report of Fotso *et al.* [17] and Oladipo *et al.* [19] which documented better efficacy for hexane extract in weevil control for the Cameroonian *Hemizygia welwitschii* and *Xylopia aethiopica*, respectively. These authors have also reported the non-polar solvent, hexane, as more efficient for extracting bioactive compounds in cowpea weevil toxicity studies compared with acetone. This possibly suggests presence of non-polar constituents in the hexane extract as being responsible for weevil toxicity. Katkar *et al*. [12] and Firdous *et al* [13] have reviewed the predominant presence of diterpenoids and alkaloids of *M. longifolium*. It is plausible to attribute cowpea weevil toxicity described in this study to the diterpenoids previously associated with insecticidal (antifeedant) activity [12].

The biochemical mechanisms underlying toxicity of plant extracts to *Callosobruchus* species have been linked to reduction in activity of glutathione reductase and glutathione peroxidase implicated in protein levels of the adult insects [19]. Furthermore, Shunmugadevi and Radhika [9] and Vishala *et al*. [11] have reviewed the potential of plant products including extracts, in controlling *C. maculatus* attack in stored cowpea.

Table 2: Effect of *Monoon longifolium* leaf hexane and acetone extracts on damage to stored cowpea

Concentration (g/ 50 seeds)	**Mean no of holes ± SEM/ % reduction[#]**		**Percentage damage (PD, %)**		**Weevil perforation index (WPI, %)**		**Percentage weight loss (PWL, %)**	
	HXN	ACT	HXN	ACT	HXN	ACT	HXN	ACT
0.1 g	13.3±0.9 (-)	17.7±0.9 (-)	25.12	24.76	55	62.00	66.0	58.5
0.2 g	10.0*±0.6 (24.8)	10.0*±0.6 (43.5)	14.77	14.02	43	41.00	59.1	57.6
0.4 g	6.7*±0.9 (49.6)	6.7*±0.9 (62.14)	9.85	9.35	33.67	33.67	46.9	45.3
0.8 g	0±0 (100)	1.3*±0.7 (92.6)	0	1.40	0	7.33	0	18.2
Negative Control (4 ml)	20.0±0.6	30.3±0.9	42.86	42.52	86.67	85.97	72.8	62.5
Permethrin, 1.5 mg	4.0*±0.6 (69.9)	4.0*±0.6 (77.4)	4.43	4.21	18.33	17.43	19.1	21.6
Permethrin, 3 mg	2.3*±0.3 (82.7)	2.7*±0.3 (84.7)	2.96	3.72	16.70	16.67	17.9	19.8

HXN = hexane extract, ACT = acetone extract. 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 0.1 g powder/ 50 seeds

This recent study is therefore an addendum to the compendium of bioactive natural products for weevil control.

Table 2 represents effects of *M. longifolium* leaf extracts tested at 0.1 - 0.8 g, on cowpea seed damage due to infestation by the cowpea weevil, *C. maculatus*. With hexane and acetone extracts, comparable percentage damage (25.12-9.85% vs 24.76-9.35%), weevil perforated index (55-33.67% vs 62-33.67%), percentage weight loss (66-46.90% vs 58.5-45.30%) at concentration of 0.1 - 0.4 g/50 seeds were recorded. With respect to percentage reduction in holes, values of 24.8-100% (hexane extract) vs 43.5-92.6% (acetone extract) were recorded at 0.2-0.8 g/50 seeds. At the peak concentration (0.8 g) however, hexane extract was more effective (100% inhibition) in reducing seed damage. Weevil perforation index is a pointer to the effectiveness of a plant product in protecting cowpea seeds, and values above 50% imply ineffectiveness [18].

Consequently, both extracts of *M. longifolium* leaf can be considered as effective cowpea protectants at 0.2 - 0.8 g. In all instances, both extracts are less effective than the standard insecticide, permethrin (1.0 and 3.0 mg) in preserving stored cowpea seeds. Reduction of stored seed damage in the Callosobruchus maculatus habitat has been frequently reported for plant-based natural products. [6,8,17].

Judging from all indices of seed protection using *M. longifolium* hexane and acetone extracts, it would be reasonable to recommend concentration range of 0.4 – 0.8 g/ 50 seeds of either extract for commercial preservation of stored cowpea against weevil damage.

CONCLUSION

Monoon longifolium leaf, investigated for the first time, could be a suitable protectant for stored cowpea seeds against *C. maculatus* infestation. The leaf extracts, especially hexane extract, reasonably deterred oviposition, adult emergence and all indices of seed damage better than the standard insecticide, permethrin. It is suggested that resource-limited farmers adopt this natural alternative rather than synthetic insecticides for preserving high-quality food sources such as cowpea seeds. Hexane extract of *M. longifolium* leaf, may therefore be included in pest management strategies for cowpea weevil in cowpea seed stored on the farm.

Conflict of Interest

The authors declare no conflict of interest in this work.

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