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RESEARCH ARTICLE

INSECTICIDAL ACTIVITY OF THE LEAF EXTRACT OF *Hyptis spicigera* AGAINST *Callosobruchus maculatus* ON GRAINSHARVESTED IN ZARIA, NORTHERN NIGERIA

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ABSTRACT

The insecticidal properties of *H.spicigera* leaf extracts (hexane, ethylacetate and methanol) tested against *Callosobruchus maculatus* on cowpea was carried out using Azadirachtin as a standard. Ethylacetate extract and standard azadirachtin showed the highest percentage mortality of 98% each at 72hr, while methanol, hexane and control gave percentage mortality of 75%,87% and 21% respectively. Percentage emergence of Azadirachtin standard and ethylacetate extract showed 6% and 8% while methanol, hexane and control gave percentages emergence of 18%,13% and 87% at 72hr respectively. Ethylacetate and methanol extracts showed oviposition deterrent at 3.16% and 5.56% while hexane, and control gave 11.11%, and 76.39% respectively. These results showed that ethylacetate and methanol extracts were active in controlling *C.maculatus* eggs laid relative to azadirachtin (2.78%) used as standard in this study. At 16wk, hexane extract showed the least percentage (2%) seed damaged while ethylacetate, methanol and control gave 5%, 23%,88% relative to standard azadirachtin (10%).The data showed ethylacetate leaf extract of *H. spicigera* with 3.16% oviposition deterrent,98% mortality and 6% emergences having potentials for use as a bio-control agent in post- harvest storage on grains susceptible to insect attack/infestation than methanol and hexane extracts.

INTRODUCTION

Hyptis spicigera whichbelongs to the family Lamiaceae is commonly known as Black beniseed, or Black sesame (Burkill, 1995). The plant is an annual weed which grows in damp and waste dump sites. It is an erect aromatic herb, up to 1m in height, with a terminal inflorescence in which the seeds are packed in quadruplets or more in the flowers. The plant is found around Senegal to western Cameroon, possibly native to Brazil, now widely naturalized in tropical Africa and Asia as well as northern Nigeria. Generally, the whole plant is used in traditional stores to protect cowpea against damage by *Callosobruchus* species (Lambert *et al*., 1985) and locally used as mosquito repellant by burning of the whole plant (Dalziel, 1937) in mosquito infested parts of Nigeria.

The use of synthetic chemicals for post- harvest storage must be discouraged due to the attendant negative environmental consequences associated with the constant use of these chemicals on grains meant for human consumption. The rising incidence of health related problems such as cancers, tumors, organ failures in both the old and the young in developing and developed countries associated with the constant use of synthetic chemicals in the preservation of grains has prompted research into the development of new substances with insecticidal properties from plants and clay materials as postharvest storage agent. The storage of grains and other food products with respect to insect infestation is a serious problem throughout the world.

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In 1989, 9% post-harvest losses, due to insect and termite infestation were reported worldwide suggesting a need to make an all-out effort to combat these post-harvest losses. Maize and cowpeas among other grains, constitutes 80% of the staple food of Nigerians and are highly sensitive to the attack by insects. Currently, worldwide attention is focused on screening and development of less hazardous and cheap material as natural products such as powdered vegetable/fruit peels as grain protectants (Singh *et al*., 1997). The search for deriving effective insecticides from natural material has become highly imperative. The use of synthetic chemicals as a method of controlling pests in postharvest storage is effective but expensive, dangerous to human health. It may create other health related problems in post-harvest industry, thus, the use of botanical insecticides which are indigenous, effective and with low mammalian toxicity favors the industry. This is because botanical insecticides provide safe, environment-friendly and cheap source of preventive stored product pests like the maize weevil, S. *zeamais* Motsch during post-harvest storage.

Plants provide phytochemicals which are useful in pharmaceuticals, cosmetics, agrochemicals, flavor and fragrance amongst other uses. The most diffused utilization of species belonging to the *Hyptis* genus is related to their essential oils, obtained in industrial scale, from several species. Such oils are largely used in cosmetics, flavouring agents and as insecticides. Dried whole plants are used for pest control while the oils, parts of the plants and extracts find diffused application in ethno pharmacology in tropical countries. About the biological activities and related pharmacology, a lot of information is reported in a review (Falcao and Menezes, 2003) and the references therein.

However, there is the need to exploit more plants to solve these challenges faced by the African population who rely mostly on the use of synthetic/chemical insecticides for post-harvest storage. This study report the insecticidal activity of the leaf extracts of *H.spicigera* found in Zaria, Nigeria and its potential use as a soft natural bio-insecticide in post-harvest storage.

EXPERIMENTALS

Plant collection

The plant was collected in Basawa Village, Zaria environs and taxonomically identified and authenticated by Mr. U.S. Gallah at the Herbarium Section, Department of Biological Science, Ahmadu Bello University, Zaria, and a sample deposited at the herbarium with Voucher No.528.

Extraction and isolation

The plant was dried in the shade for 14 days and pulverized to powder using pestle and mortar in the Laboratory. Approximately 200g of the powdered plant material was macerated sequentially with hexane, ethyl acetate and methanol at room temperature and concentrated *in vacuo* to afford the various crude extracts which were stored in the refrigerator (4^oC) until needed for further analysis.

Insecticidal evaluation

The cowpea was fumigated with phostoxin to clear any initial infestation like eggs and larvae. After the fumigation, the grains were dried for 72hrs to remove any residue of the phostoxin on the grains. In the experimental design, there were sixty-six (66) treatments and three replicates. Concentration of 1000ppm each of the crude extracts and standard check (Azadirachtin) namely: hexane, ethylacetate and methanol were prepared. About 0.5ml, 1.0ml and 1.5ml were carefully sprayed on the grains and mixed properly using a shaker to ensure good coating.The sprayed seeds were exposed to allow for dryness and complete removal of the solvents. Ten pairs of *Callosobruchus maculatus* were infested with 100g of grains in each set of the experiments. The set-up was left for F1 progeny emergence. Mortality count was carried out at 24, 48 and 72hrs respectively while oviposition count was taken after one week (7days).Progeny emergence was taken at eight weeks (2 months).

RESULTS AND DISCUSSION

The various yields of the crude extracts for the leaf parts of *Hyptis spicigera* are shown in Table 1.Yields of ethylacetate and methanol extracts for the leaf (16.77%; 19.54%) were the highest with methanol being the best extracting solvent amongst the solvents used. Generally, methanol being a polar solvent extracted (19.54%) the highest quantity of the plant material followed by ethyl acetate (16.77%) while hexane being a non-polar solvent extracted the lowest quantity (7.44%) in the leaf, Table 1.The difference in the extracts showed the affinity of solvents on different types of plant constituents and this is due to their different polarities. This feature enables the extraction of plant constituents to be more selective with optimal results.

Table 1. Percentage extraction of the leaf part of the plant material

Sample	Extracts	% Yield	Texture/Color
Leaf	Hexane	7.44	Viscous/Light yellow
	Ethylacetate	16.77	Solid/greenish
	Methanol	19.54	Solid/greenish

The percentage mortality of the leaf extracts (hexane, ethylacetate and methanol) of *H.spicigera* tested against *Callosobruchus maculatus* on cowpea (Table 2) was carried out using Azadirachtin as a standard. Ethylacetate extract and standard azadirachtin showed the highest percentage mortality of 98% each at 72hr, while methanol, hexane and control gave percentage mortality of 75%, 87% and 21% respectively.

Table 2. Percent Mortality and adult emergence of *C. maculatus* treated with extracts of *Hyptis spicigera*

Treatments	24hrs	48hrs	72hrs
(a) Mortality:			
Hexane	(40%)	(55%)	(87%)
Ethylacetate	(60%)	(80%)	(98%)
Methanol	(35%)	(47%)	(75%)
Azadirachtin Std.	(70%)	(88%)	(98%)
Control	(8%)	(15%)	(21%)
(b) Emergence:			
Hexane	(30%)	(18%)	(13%)
Ethylacetate	(20%)	(16%)	(8%)
Methanol	(25%)	(28%)	(18%)
Azadirachtin Std.	(15%)	(10%)	(6%)
Control	(55%)	(70%)	(87%)

Some workers have shown the essential oils from *Hyptis spicigera* to possess high mortality on the first larval instars and on young adults of some insects with doses 436.04 and 548.18ppb (Kouninki *et al*., 2007). They found that the fifth larval instars were resistant at all the doses tested, and no important mortality was observed. From the present study it showed that the essential oils of *H.spicigera* as reported by some of these workers has more insecticidal potency than the leaf extracts used for similar purpose. This could probably be due to the presence of various components in the volatile oils which work in synergy to boost the potency in controlling pests unlike the phytochemicals from the leaf extracts. *Hyptis* is widely used against stored pests, other pest insects and against mosquitoes (Palsson and Jaeson, 1999). Some of the leaves of the *Hyptis* species especially *Hyptis suaveolens* and *H.spicigera* are largely used as potent insect repellents, by native populations of many parts of the world (Ayeard *et al.*, 1993; Pereda-Miranda and Delgado, 1990). The bioactivity of the essential oils from *Hyptis suaveolens* against storage mycoflora have been carried out on stored food grains (Sharma, 2002). This study showed that the volatile oils of *H.spicigera* contain 1,8-cineole which possessed fungitoxic activity and can be exploited for the management of spoilage of stored commodities. The insecticidal activity of Hyptis species using its volatile oils is well documented (Othira *et al*, 2009; Sanon *et al*., 2006, Raja *et al.*, 2001, Sharma, 2002). The genus *Hyptis* is a very large (400 species), and many of its reported species are known for their medicinal and insecticidal use by local herbal practitioners and have been evaluated by scientists (Almtorp *et al*., 1991; Kuhnt *et al*., 1995; Raja *et al.,* 2001).

Essential oils are secondary metabolites abundant in aromatic plants families such as Lamiaceae and Annonaceae, and contain a large number of compounds such as monoterpenes and sesquiterpenes. Essential oils of aromatic plants have been tested for their potential as protective agents for human and/or livestock feeds. Many researchers have pointed out the use of essential oils from aromatic plants as the best way to control pests since it does not lead to human and animal toxicity (Isman, 2000; Shaaya and Kostyukovysky, 2006). Many aromatic plants are known to possess insecticidal activity, ovi-positing deterrent, and reduce the progeny. Essential oils are known to exhibit low toxicity to mammals, and the most important constituents are terpenoids and phenols found in plant essential oils which have minimal toxicity and have even been approved as flavouring agents in food (Isman, 2000; Shaaya and Kostyukovysky, 2006). Previous reports have shown that a lot of attention has been focused on the pesticidal properties of the *Hyptis* and other plant species with the sole purpose of developing soft natural biodegradable natural insecticide that will replace chemical insecticides (Kouninki *et al*., 2007; Othira *et al*, 2009; Sanon *et al*, 2006; Raja *et al*, 2005; Sharma, 2002). The insecticidal potency of the leaf extracts of *Hyptis spicigera* is shown (Tables 2-4). All the extracts tested at 1000ppm using different volumes of 0.5ml, 1.0ml and 1.5ml showed insecticidal activity relative to azadirachtin standard and control. A decrease trend was observed in the percentage emergence of *C.maculatus* after 72hr, with azadirachtin standard (6%), ethylacetate (8%), hexane (13%) and methanol (18%) while the control (87%) gave the highest percentage emergence (Table 2). Azadirachtin standard showed the strongest

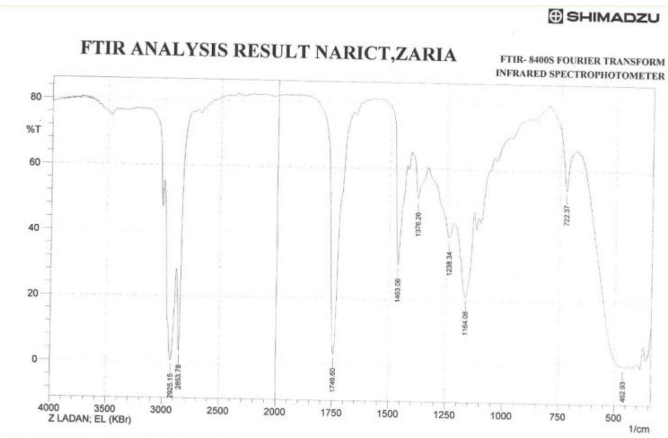
Table 3. Oviposition deterrent of extracts of *H.spicigera* on *C.maculatus*

Treatments	No. of eggs laid	% Eggs laid
(c)Oviposition deterrent:		
Hexane	8.0	11.11
Ethylacetate	3.0	3.16
Methanol	4.0	5.56
Azadirachtin Std.	2.0	2.78
Control	55.0	76.39

Table 4. Percent seed damage to cowpea treated with extracts of *H.spicigera* infested with *C.maculatus*

Treatments	No. of weeks		
	8	12	16
Hexane	0.00%	1.00%	2.00
Ethylacetate	0.00%	3.00%	5.00%
Methanol	9.00%	15.00%	23.00%
Azadirachtin Std.	0.00%	2.00%	10.00%
Control	40.00%	60.00%	88.00%

suppression of *C.maculatus* emergence possibly due to the presence of limonoids which are known to possess potent insecticidal properties. These limonoids in neem acts as insect repellents, feeding deterrents and disrupts the metamorphic stages of insect larvae. Neem leaves have been used as an effective postharvest protectant for some grains prone to insecticidal attack. Village farmers usually mix powdered neem leaves with their grains in bags and keep in barn and this has been found to be effective in the control of cowpea weevil (*Callosobruchus maculates,* F.), a major pest of cowpeas. Some workers (Jenkins *et al*., 2003) have found that neem kernel extract has reduced infestations of *C. maculatus*, after the neem kernel extract had been stored at high temperatures for 2 weeks at 50°C followed by 5 months storage at 28°C. However, the number of neem kernel extract-treated eggs that survive to become adults was significantly reduced even when the neem kernel extract was exposed to 50°C for 2 weeks. This was attributed to the mortality observed and the presence of other limonoids other than azadirachtin in neem kernel extract (Schmutterer, 1985). In the present study, the extracts of the leaves of *Hyptis spicigera* was found to possess insecticidal activity in controlling *Callosobuchus maculatus* Fab. infesting stored cowpea



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	462.93	0.561	1.272	675.11	459.07	181.224	1.053
2	722.37	54.144	17.379	800.49	676.07	20.352	3.296
3	1164.08	21.035	24.551	1215.19	1129.36	43.233	13.704
4	1238.34	39.243	7.883	1331.89	1215.19	36.157	2.555
5	1376.26	51.061	12.315	1405.19	1331.89	17.517	2.74
6	1463.06	30.748	38.156	1518.99	1424.48	22.219	7.592
7	1746.6	3.042	75.971	1855.58	1665.59	56.665	37.521
8	2853.78	3.159	34.681	2880.78	2745.76	52.157	16.513
9	2925.15	0.428	38.565	2990.73	2881.75	112.529	68.42

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Fig. 1. FT-IR spectrum of hexane extract

seeds (*Vigna unguiculata*).This study has shown that *Hyptis spicigera* extracts can be formulated to soft natural botanicals for post-harvest storage thereby replacing chemical insecticide used for the same purpose. Ethylacetate and methanol extracts showed oviposition deterrent at 3.16% and 5.56% while hexane, and control gave 11.11%, and 76.39% respectively. The results showed that ethylacetate extract contained bioactive constituents relative to the azadirachtin standard that can hinder rapid multiplication of the studied pest in stored grains thereby controlling its population. The results of percentage seed damage to cowpea treated with extracts of *H.spicigera* infested with *C.maculatus* is shown in Table 4. At 16wk, hexane extract showed the least percentage (2%) seed damaged while ethylacetate, methanol and control gave 5%, 23%, 88% relative to standard azadirachtin (10%).Hexane preserved the grains from *C.maculatus* and other pest damage probably due to the outer coatings of the extract on the grains. The extract revealed the presence of a long chain fatty acid as the main constituent by FT-IR (Fig.1). In the FT-IR spectrum, the major peaks are $C{=}O_{str}$, $O\text{-}H_{str}$,and $C\text{-}H_{str}$ stretching vibrations (Fig.1) which showed signals at $1740cm^{-1}$, $3320cm^{-1}$ and $2863cm^{-1}$,$2925cm^{-1}$ respectively corresponding to a long chain fatty acid.

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