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BIOACTIVITIES OF CANDLEWOOD, *ZANTHOXYLUM XANTHOXYLOIDES* (LAM.) SOLVENT EXTRACTS AGAINST TWO STORED-PRODUCT INSECT PESTS

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ABSTRACT:- *Bioactivities of candlewood, Zanthoxylum xanthoxyloides (Lam.) solvent extracts on control of Sitophilus zeamais Mots. (Coleoptera: Curculionidae) and Callosobruchus maculatus F. (Coleoptera: Bruchidae) were assessed on maize and cowpea respectively. Of the four solvent extracts investigated, methanol (MeOH) extract gave the highest yield of 75 ± 10.89 %, and caused significant (p<0.001) mortalities in both insects. It also gave hundred percent protections to maize and cowpea against damage by S. zeamais and C. maculatus respectively, while causing complete inhibition of F1 progeny production and egg development within grains. All solvent extracts studied however, evoked strong to moderate repellent actions against both insect pests, with MeOH extract being the most bioactive (%R > 95; Fr = 0).*

INTRODUCTION

Post harvest losses to storage insect pests is recognized as an increasing constraint in Africa with reports of losses averaging 30% of grain dry weight in stored maize due to a complex of insect pests (IITA, 1995).

Insect pest control in stored food products relies heavily on the use of gaseous fumigants and residual chemical insecticides. The development of insecticide-based techniques for grain protection in traditional farm stores in Africa however, has been partially successful due to the high cost of synthetic insecticides (Obeng-Ofori *et al*., 1997). Consequently, due to the misuse of such chemicals and the accompanying undesired effects, the need to revisit traditional pest control options to look for alternatives is very pressing (Owusu, 2001). The grain protectant potential of Candlewood, *Z. xanthoxyloides* is under critical investigation by the Department of Zoology, University of Ghana, with bark and roots proving highly bioactive against stored-product insect pests (Osafo, 1999, Nutsukpui, 2001, Udo, 2001, Koomson, 2004, Udo *et al*., 2004). Consequently, further works to purify, isolate, and chemically determine the bioactive components of the plants are still on-going. This paper reports on the bioactivity of some solvent extracts of candlewood against infestation by *S. zeamais* and *C. maculatus*.

MATERIALS AND METHODS

Insect cultures

S. zeamais and *C. maculates* were cultured on whole maize and cowpea grains respectively. Cultures were maintained in 0.5 liter glass jars held in a controlled temperature chamber at 28±1ºC, relative humidity (r.h.) of 65 – 75ºC and photoperiod of LD 16:8 (hours light:dark) (Owusu, 2001). After 3 weeks of oviposition, all adult insects were sieved out to enable emergence of new individuals that could be classified as same-age progeny. This system of re-culturing was repeated every 2 weeks.

Plant materials and preparation of solvent extracts

Barks of *Z. xanthoxyloides* were collected from various locations within the Greater Accra and Central regions of Ghana and sent to the laboratory where they were air-dried at room temperature for 5 days. The plant parts were chopped up into pieces and 250 g each extracted respectively with methanol, hexane + isopropyl alcohol (4 + 1 by volume), hexane, isopropyl alcohol, and ethanol. The extracts were made to stand in the dark for 3 days after which they were filtered. Solvents were evaporated using a rotary evaporator and obtained residues were re-dissolved in water for bioassay.

Effect of extracts on adult mortality and grain damage

Hundred grammes of maize or cowpea grains were placed in 200-ml plastic cups and mixed with 5 ml of 50 g ml^{-1} concentration of previously prepared plant extracts in water for 1 hr. The control was treated with water alone. Twenty adults of *S. zeamais* (10 male and 10 female; 7-14 days old) and mixed sexes of *C. maculatus* (3-7 days old) were introduced into treated and untreated grains (control). The cups were covered with white muslin cloth, held in place with rubber bands and placed in the controlled environment room (Owusu, 2001). Mortality was recorded daily and up to one week. Insects were considered dead on failure to respond to three probing with blunt probe.

After the feeding period of 30 days, grains were reweighed and a feeding ration (*Fr*) was calculated as follows : *Fr* = 1 – FW/100, where FW represents the final grain weight (Owusu, 2001).

For grain damage assessment, samples of 100 grains of maize or cowpea were taken from each cup and the number of damaged grains (grains with characteristic holes) and undamaged grains were countered and weighed. Pest damage was computed using the method of FAO (1985) as modified by Udo *et al*., (2004) as follows:

% Weight loss = [UaN- (U+D)]/UaN x 100

Where:
U = weight of undamaged fraction in sample
N = total number of grains in the sample
Ua = average weight of one undamaged grain
D = weight of damaged fraction in the sample

Effect of solvent extracts on progeny production

Batches of 200g each of equilibrated maize and cowpea in 500 ml glass jars were infested respectively with 50 adult insects each of *S. zeamais* and *C. maculatus* to allow for egg laying. The containers were covered with white muslin cloth and held in place with rubber bands. The control was treated with water alone. The experiment was replicated five times and left undisturbed for 4 weeks to allow for maximum oviposition. The parent adults were removed after 1 week. One day after removal of adults, 25 g each of maize and cowpea grains were treated with 1 ml (50g ml^{-1}) of each solvent extract to assess their effect on egg viability. Application of the solvent extracts was repeated 2 weeks after adult removal, while emerging adults were counted weekly.

Repellency test

Fifty microliters each of water-dissolved plant extracts were pipetted onto filter paper disks (d = 12 mm) (treated). For each solvent extract, two treated disks and two disks treated with water alone (control) were arranged alternatively 5 cm from each other in Petri dishes. Four grains of either maize or cowpea were deposited evenly on each of the filter paper disks. Ten previously starved adult insects (either *S. zeamais* or *C. maculatus*) were released into the middle part of each respective Petri dish and covered. Dishes were placed either in dark or light (28 ±1ºC; 75% rh) and the numbers of insects that settled after 1 hr were countered. There were four replicates for each solvent extract, and positions of treated and control were changed in each replicate. Mean counts were expressed in percentage repulsion (*%R*) as *R* = 2 x (*X*-50), where *X* = percentage of insects on either maize or cowpea (McDonald et al., 1970) as modified (Owusu, 2001).

RESULTS

The most suitable organic solvent in terms of yield and appropriateness for further purification and isolation of bioactive components is depicted in Table 1. Of the five solvents studied, MeOH gave the highest yield of 75%, followed by Hexane:Isopropyl alcohol mixture (4:1), i.e. 55%. Ethanol (EtOH) and hexane however, gave the least yields of 32 and 42% respectively.

Table 1: Percent recovery of solvent extracts

Solvent	Percent (%) Yield (± SE)
Methanol	75 ± 10.89
Hexane	42 ± 12.96
Isopropyl alcohol	45 ± 9.78
Ethanol	32 ± 13.40
Hexane: Isopropyl (4:1)	55 ± 11.55

Bioactivity effects of *Z. xanthoxyloides* solvent extracts are shown in Table 2. MeOH extract proved highly efficacious recording mortalities of 100% in both *S. zeamais* and *C. maculatus*, while EtOH extract gave the lowest mortality value of 15.7% even though significantly different from the 0% value recorded for control insects.

Complimentarily, grain damage was completely non-existent (0%) in both maize and cowpea treated with MeOH extracts of *Z. xanthoxyloides* (Table 3). Grain protections offered by all solvent extracts however, were significantly higher than that of the control, even though notable significant differences existed among solvent extracts (Table 3).

Table 2: Effect of solvent extracts on survival of *S. zeamais* and *C. Maculatus*

	Mean % mortality (± SE)	
Solvent	*S. zeamais*	*C. maculatus*
Methanol	100.0 ± 0.00	100.0 ± 0.00
Hexane	20.5 ± 0.76	15.2 ± 0.19
Isopropyl alcohol	28.9 ± 0.96	35.4 ± 2.15
Ethanol	15.7 ± 1.17	10.2 ± 0.92
Hexane: Isopropyl (4:1)	45.9 ± 2.34	52.2 ± 3.12
Control	0.0 ± 0.00	0.0 ± 0.00

Table 3: Effect of solvent extracts on grain damage caused by *S. zeamais* and *C. maculatus*

	Mean % weight loss (± SE)	
Solvent	*S. zeamais*	*C. maculatus*
Methanol	0.0 ± 0.00	0.0 ± 0.00
Hexane	18.4 ± 1.79	16.9 ± 1.21
Isopropyl alcohol	15.7 ± 2.28	17.4 ± 2.19
Ethanol	19.2 ± 1.21	18.8 ± 0.96
Hexane: Isopropyl (4:1)	12.4 ± 1.15	9.6 ± 1.79
Control	25.9 ± 0.94	20.6 ± 1.04

Effects of solvent extracts on egg viability were studied using F1 progenies produced by both *S. zeamais* and *C. maculatus* (Table 4). While hexane, isopropyl alcohol and ethanol extracts exerted little inhibitory effects on egg viability (as compared to the control), MeOH and hexane:isopropyl alcohol (4:1) extracts reduced F1 progeny succession significantly with mean progeny numbers of 0 and 46 individuals respectively. As in all cases noted above, MeOH extract proved highly bioactive and either interfered with oviposition by adult insects or rendered oviposited eggs non-viable.

Table 4: Solvent extracts effect on produced F1 progeny by *S. zeamais* and *C. Maculatus*

	Mean No. of F1 Progeny (± SE)	
Solvent	*S. zeamais*	*C. maculatus*
Methanol	0.0 ± 0.00	0.0 ± 0.00
Hexane	102.9 ± 25.21	98.7 ± 20.55
Isopropyl alcohol	95.7 ± 15.21	88.6 ± 18.72
Ethanol	109.6 ± 24.21	100.9 ± 26.71
Hexane: Isopropyl (4:1)	45.8 ± 16.71	38.6 ± 17.51
Control	148.4 ± 16.92	135.8 ± 20.21

The effects of solvent extracts on feeding behaviours of *S. zeamais* and *C. maculatus* are shown in Table 5. Generally, MeOH extract gave better results for control of both insects. This is because it was highly repellent to both insects (> 95% R), as well as completely preventing feeding (Fr = 0).

Table 5: Solvent extracts effect on feeding of *S. zeamais* and *C. Maculatus*

Solvent	Percent repulsion (% R) (± SE)		Feeding ratio (Fr)	
	S. zeamais	*C. maculatus*	*S. zeamais*	*C. maculatus*
Methanol	98.5 ± 7.5	96.9 ± 3.2	0.00	0.00
Hexane	43.2 ± 3.8	35.2 ± 6.1	0.42	0.51
Isopropyl alcohol	44.9 ± 8.4	38.4 ± 3.9	0.31	0.29
Ethanol	35.2 ± 5.6	42.9 ± 6.3	0.51	0.49
Hexane: Isopropyl (4:1)	52.9 ± 8.1	61.4 ± 4.1	0.14	0.16
Control	-	-	0.72	0.65

DISCUSSION

Candlewood, *Z. xanthoxyloides* is relatively safe and has many uses such as, food flavouring and animal feed (Irvine, 1961), treatment for various ailments, as well as post-delivery pain relieving (Abbiw, 1990).

The general use of methanol in the extraction of plant-mediated chemicals is widely appreciated. Throughout the study, the MeOH extracts proved highly efficacious, causing significant mortalities and preventing significant build-ups of *S. zeamais* and *C. maculatus* populations. Of significance is the fact that, solvent extracts, especially from MeOH were not soluble in acetone, but rather water, hinting on the possible involvement of a hydrophilic compound.

The bioactivity of *Z. xanthoxyloides* against *S. zeamais* and *C. maculatus* may depend on chemical composition, insects' susceptibility and variation in insect behaviour. The significant mortalities and oviposition deterrencies caused in the two insects could be attributed to the presence of highly pungent phenolic secondary metabolites (Adesina, 1986), or other compounds such as alkaloids, flavonoids, etc. These possibly repel the insects and influence their locomotion, oviposition, feeding behaviour, developmental and physiological processes, as well as general behavioural pattern. Some of the secondary plant materials may also likely act as both insecticides and antifeedants or phagostimulants.

A significant observation was that, insects killed in treated grains had their metathoracic wings unfolded and stretched outside the elytra, suggesting that toxicity was not due to ingestion of treated grains. This coupled with the significant reduction in grain damage (both maize and cowpea), especially by MeOH extracts suggest the presence of high levels of antifeedant (s), hence the extremely high percent repulsion and zero feeding ratio values obtained for both insects.

The rich potential of Ghanaian traditional plants in the management of stored-product and other insect pests cannot be over-emphasised (Owusu, 2001). A thorough and systematic study is therefore needed to transform these bioactive plant parts into suitable formulations for commercial use. The results obtained from this and other studies (Osafo, 1999, Udo, 2000, Nutsukpui, 2000, Koomson, 2003, Udo *et al*., 2004) indicate unequivocally the enormous potential of *Z. xanthoxyloides* in insect storage pest management systems.

CONCLUSION

The efficacy of candlewood in control of stored-product weevils is truly established, with methanol extracts of the plant proving the most potent and reliable for further work on isolation and identification of bioactive compounds.

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