Journal of Stored Products and Postharvest Research Vol. 2(3) pp. 40-44, March 2011
Available online http://www.academicjournals.org/JSPPR
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Full Length Research Paper

Potentials of *Zanthoxylum xanthoxyloides* (LAM.) for the control of stored product insect pests

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Accepted 17 January, 2011

The biological activity of *Zanthoxylum xanthoxyloides* against *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Callosobruchus maculatus* (Coleoptera: Bruchidae) was investigated in the laboratory by admixing leaf, root and bark powders of *Z. xanthoxyloides.* Methanol extracts of the plant were applied at 2.0 ml per 50 g of grains to assess contact mortality, damage assessment, progeny development and repellent action. The root and bark powders caused 100% mortality of both *S. zeamais* and *C. maculatus* while complete protection of grains and progeny inhibition were achieved. Topical application of the extracts caused significant (P<0.01) insect mortality with fresh bark extract offering complete protection of grains and complete inhibition of progeny production by both *S. zeamais* and *C. maculatus*. The extracts also evoked moderate repellent effect against the two insect pests. The findings of this study lend credence to the usefulness of yet another biopesticide and its incorporation into traditional storage pest management system is recommended.

Key words: Coleoptera, extracts, pests, powders, progeny, *Zanthoxylum.*

INTRODUCTION

Grains in storage are damaged by several species of insect pests, the commonest being beetles and moths, leading to loss in weight and seed quality. In recent years, post harvest losses to storage insect pests such as the maize weevil, Sitophilus *zeamais* (Mots.) (Coleoptera: Curculionidae) and the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae) have been recognized as a constraint to food security in Africa (Markham et al., 1994). Reports from Africa estimate losses of stored grain at an average of 30% per annum (IITA, 1995). However, stored maize alone is attacked by about 20 different species of insects including *S. zeamais* and *Tribolium castaneum* while an estimated 20 to 25% of maize produced in Ghana is destroyed by weevils alone (Ayertey, 1982). Apart from contamination of grains by dead beetles, pupae and larval cocoons, the integument of Sitophilus *granaries* (L.) has been found to contain various carcinogenic compounds such as ethyl, methyl and methoxy quinines, which are heat resistant and cannot be destroyed by boiling or baking (Zehrer, 1980).

Presently, insect control in stored food products relies heavily on the use of gaseous fumigants and residual contact insecticides (Shaaya et al., 1991; White, 1995). The widespread use of synthetic chemicals has led to some serious problems including development of resistance, toxic residues on stored grains and health hazards to grain handlers (Obeng-Ofori et al., 1998). In recent years, there has been renewed effort internationally at developing new sources of insecticides from the vast store of chemical substances in plants, which are safe and biodegradable (Olaifa et al., 1997).

The candlewood, *Zanthoxylum xanthoxyloides* (Lam.) is a shrub which grows to a small tree of up to 1.25 m high and 0.13 m girth and belongs to the family Rutaceae. The medicinal value of the plant has long been known. Therefore, the plant is believed to be safer as a biopesticide for stored food protection (Shaaya et al., 1991; Olaifa et al., 1997). This led to the present study.

MATERIALS AND METHODS

Culturing of insects

S. zeamais and *C. maculatus* were collected from infested stock of maize and cowpea grains, respectively at the Madina market, Accra and reared on whole maize and cowpea grains, respectively in the laboratory at 28±2°C, 65% RH and 12L:12D photoregime (Olaifa et al., 1997). Insects were sieved using a 2 mm impact test sieve into a bowl covered with a nylon mesh and left under the sun for 3 h. By this process, insects infested with mites died while the surviving ones were collected and washed in 1% sodium hypochloride solution. Insects were dried by placing them on filter paper before transferring them into a glass jar containing 500 g of grains, which had previously been sterilized in an oven at 40°C for 6 h. After three weeks of oviposition, the parent adults were sieved out and killed by freezing. The emerging progeny of insects was used to establish the main culture which was used for the various bioassays.

Collection of plant materials and preparation of extracts

Leaves, bark and root of *Z. xanthoxyloides* were collected in the morning before flowering from the University farm, University of Ghana, Legon and air-dried in the laboratory for five days. The materials were cut into smaller pieces and powdered using electric blender. The powders were stored away in black polythene bags and kept in the dark. Methanol extracts of leaves, bark and root were prepared by soaking 250 g of plant material in 70% methanol and left to stand for 72 h. Filtration was carried out and methanol evaporated using a rotary evaporator at 30 to 40°Cwith rotary speed of 3 to 6 rpm for 8 h. The extracts were then dissolved in distilled water and used for the various bioassays.

Toxicity of plant powders

Leaf, bark and root powders were admixed with 100 g of maize and cowpea grains, respectively at 5% concentration while 10 pairs of adult unsexed insects (3 to 7 days old) were introduced into treated and untreated grains. Mortality was scored after 24 h and up to 96 h

Toxicity of extracts

Toxicity of methanol extracts against weevils on maize and cowpea grains was tested by applying 2 ml of each extract on fifty grams of sterilized and pre-equilibrated grains. After 3 h, *S. zeamais* and *C. maculatus* were introduced into treated and untreated grains. Each treatment was replicated four times while the control was treated with distilled water. Insect mortality was recorded up to 96 h after treatment.

Contact toxicity by topical application

Forty adult insects of each species in batches of 10 were chilled for three minutes to reduce their activity (mobility) and transferred into Petri dishes. One microlitre of each extract was applied using a micropipette to the dorsal surface of the thorax of each insect individually. Distilled water was applied to control insects and each treatment replicated four times while mortality was recorded after one hour and up to 48 h.

Damage assessment

Five percent of plant powders and 2 ml of each extract were added to the grains and ten pairs of each insect pest introduced into treated and untreated grains. The experiment was left to stand for four weeks with each treatment replicated four times. Samples of 100 grains were taken from each cup and number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed. Percent damage was computed using the method of FAO (1985).

Progeny production

Grains treated with both powder plant and the extracts were assessed for the emergence of the first filial generation. Maize and cowpea grains which had been kept in the freezer to prevent hidden infestation were used for the experiment. Twenty adults of each insect species were introduced into treated and untreated grains covered with white muslin cloth and left to stand undisturbed for five weeks; the number of insects emerging from each treatment was counted for one week.

Repellency test

Repellency was tested in a choice bioassay system using baked wheat flour cakes. The cakes were treated with 0.5 ml of the various extracts and left to dry off for one hour before introducing 10 adults of *S. zeamais* and *C. maculatus* into Petri dishes containing the cakes. The number of insects present on control (Nc) and treated (Nt) cakes were recorded after one hour and up to 48 h. Percent repellency (PR) was computed using the method of Obeng-Ofori et al. (1997). All negative PR values were treated as zero.

Data analysis

All data generated from the work were subjected to one-way analysis of variance after carrying out appropriate transformations and means were separated using Duncan's multiple range test (DMRT).

RESULTS

Effectiveness of plant powders

Leaf, bark and root powders tested at 5% level showed various bioactivity against *S. zeamais* and *C. maculatus* (Table 1). There was a significant difference ($p < 0.01$) in mortality of both insects amongst the treatments, with root and bark powders causing 100% mortality.

Toxicity of extracts

Significant mortality ($p < 0.01$) was observed for both *S. zeamais* and *C. maculatus* when the different extracts were applied against them (Table 2). Fresh bark extract induced over 90% mortality in *S. zeamais* and over 85% in *C. maculatus*. Dry bark extract caused about 50% mortality of *C. maculatus* after 96 h of treatment. Fresh root, dry root and dry leaf extracts caused over 35% mortality of *C. maculatus* and when compared with the control recorded a significant ($p < 0.01$) effect.

Contact toxicity by topical application

Toxicity of the various extracts applied topically to *S. zeamais* and *C. maculatus* is summarized in Table 3.

Table 1. Toxicity of leaf, bark and root powders of *Z. xanthoxyloides* tested at 5% against S. zeamais and C. maculatus

Plant powder	% mortality	
	S. zeamais	***C. maculatus***
Leaf	45^{b} ± 0.73	64b ± 0.37
Bark	100^{a} ± 0.00	100 a ± 0.00
Root	100 ^{a} ± 0.00	100 a ± 0.00
Control	0^{c} ± 0.00	0c ± 0.00

Means (± SE) represent four replicates of 20 insects each. Means for each species and in the same column followed by different letter are significantly different ($p < 0.01$).

Table 2. Toxicity of methanol extracts of *Z. xanthoxyloides* against *S. zeamais* and *C. maculatus* in stored grains.

Treatment	% mortality	
	S. zeamais	***C. maculatus***
DLE	15^{b} ± 1.56	36^{c} ± 1.11
DBE	6^{c} ± 1.23	50^{b} ± 0.63
DRE	1^{d} ± 0.41	38^{c} ± 1.44
FBE	93^{a} ± 0.87	86^{a} ± 0.29
FRE	15^{b} ± 1.66	38^{c} ± 0.75
Control	0^{d} ± 0.00	0^{d} ± 0.00

Means (± SE) represent four replicates of 20 insects each. Means for each species and in the same column followed by different letter are significantly different ($p < 0.01$). DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

Table 3. Toxicity of extracts of Z. xanthoxyloides applied topically to S.zeamais and C. maculatus.

Treatment	% mortality	
	S. zeamais	***C. maculatus***
DLE	8^{d} ± 0.25	25^{b} ± 0.75
DBE	98^{a} ± 0.25	100^{a} ± 0.00
DRE	25^{c} ± 1.33	81^{a} ± 0.29
FBE	98^{a} ± 0.25	100^{a} ± 0.00
FRE	53^{b} ±1.03	79^{a} ± 0.48
Control	0^{d} ± 0.00	0c ± 0.00

Means (± SE) represent four replicates of 20 insects each. Means for each species and in the same column followed by different letter are significantly different ($p < 0.01$). DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

There was a significant ($p < 0.01$) difference amongst the treatments with fresh bark and dry bark extracts inducing the highest mortality in the two insect species 48 h after treatment.

Damage assessment

Grains treated with plant powders showed significant difference ($p < 0.01$) in the reduction of damage caused by *S. zeamais* and *C. maculatus* (Table 4). Dry bark and dry root provided complete protection of the grains against infestation. The various extracts also significantly ($p < 0.01$) reduced damage caused by the insect pests with fresh bark extract providing 100% protection to maize and cowpea grains.

Progeny production

The different extracts significantly ($p < 0.01$) reduced the progeny of *S. zeamais* and *C. maculatus* while fresh bark extract completely inhibited the development of *S. zeamais* (Table 5). However, when the plant powders were applied, dry bark and dry root completely inhibited the F1 development of both insect species.

Repellency bioassay

The extracts under consideration showed different levels of repellency to the two insect species (Table 6). *S. zeamais* was least repelled by the extracts with the highest repellency of 14% observed in dry root and fresh bark extracts. *C. maculatus* was observed to be highly affected by the extracts with an overall repellency of 45%.

DISCUSSION

Leaf, bark and root powder of *Z. xanthoxyloides* were toxic to both *S. zeamais* and *C. maculatus* with the bark and root killing all the beetles exposed to treatment. This phenomenon suggests the presence of highly pungent secondary metabolites (Adesina, 1986). One of the constituent secondary metabolites, Zanthoxylol has been identified as a phenolic compound reputed for insecticidal activity (Elujoba and Nagels, 1985; Wongo, 1998). Some secondary plant metabolites may act both as insecticides and antifeedants thus influencing insect's locomotion, oviposition, feeding behavior, developmental and physiological processes as well as behavioral patterns. The plant powders tested at 5% concentration reduced damage caused by the two insect species with root and bark powders recording 100% protection thus indicating the presence of antifeedant and oviposition deterrent properties in the plant. This is further evidenced by the reproduction inhibition observed against both insects and confirms the reported reproduction suppression properties of root bark powder of *Z. xanthoxyloides* against *C. maculatus* due to high contact toxicity at concentrations of 0.125 to 3 g per 20 g of stored cowpea (Ogunwolu and Odunlami, 1996). The various extracts

Table 4. Effect of *Z. xanthoxyloides* on damage caused by *S. zeamais* and *C. maculatus* to stored grains.

Treatment	% grain damage	
	S. zeamais	***C. maculatus***
Plant powders		
Leaf	$10^{a} \pm 1.68$	$11^{a} \pm 1.98$
Bark	$0^{b} \pm 0.00$	$0^{b} \pm 0.00$
Root	$0^{b} \pm 0.00$	$0^{b} \pm 0.00$
Control	$13^{a} \pm 2.41$	$12^{a} \pm 1.13$
Extract		
DLE	$13^{ab} \pm 1.73$	$21^{a} \pm 8.04$
DBE	$11^{b} \pm 1.12$	$3^{b} \pm 1.62$
DRE	$12^{ab} \pm 2.39$	$3^{b} \pm 2.77$
FBE	$0^{c} \pm 0.00$	$0^{b} \pm 0.00$
FRE	$11^{b} \pm 0.57$	$3^{b} \pm 1.61$
Control	$16^{a} \pm 2.86$	$25^{a} \pm 1.38$

Means (± SE) represent four replicates of 20 insects each. Means for each species and in the same column followed by different letter are significantly different ($p < 0.01$). DLE = Dry leaf extract.

Table 5. Effect of *Z. xanthoxyloides* on the number of F1 progeny produced by *S. zeamais* and *C. maculates.*

Treatment	Mean number of F1 progeny	
	S. zeamais	***C. maculatus***
	Plant powders	
Leaf	135a ± 20.33	$62^{b} \pm 10.64$
Bark	0b ± 0.00	$0^{c} \pm 0.00$
Root	0b ± 0.00	$0^{c} \pm 0.00$
Control	154a ± 20.20	$125^{a} \pm 6.40$
Extract		
DLE	$99^{a} \pm 11.71$	$56^{ab} \pm 11.66$
DBE	$96^{a} \pm 4.09$	$36^{b} \pm 3.67$
DRE	$99^{a} \pm 19.00$	$30^{c} \pm 16.77$
FBE	$0^{b} \pm 0.00$	$3^{c} \pm 2.52$
FRE	$82^{a} \pm 13.85$	$18^{b} \pm 2.78$
Control	$103^{a} \pm 23.78$	$87^{a} \pm 22.29$

Means (± SE) represent four replicates of 20 insects each. Means for each species and in the same column followed by different letter are significantly different ($p < 0.01$). DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

Table 6. Mean % repellency (PR) values for the extracts of *Z. xanthoxyloides* against *S. zeamais* and *C. maculatus.*

Extract	% repellency (PR)	
	S. zeamais	***C. maculatus***
DLE	$5^{b} \pm 0.30$	$36^{b} \pm 0.60$
DBE	$2^{b} \pm 0.10$	$68^{a} \pm 1.20$
DRE	$14^{a} \pm 0.80$	$9^{c} \pm 0.10$
FBE	$14^{a} \pm 0.80$	$66^{a} \pm 1.10$
FRE	$13^{b} \pm 0.60$	$48^{b} \pm 0.90$
Overall PR	10	45

Means (± SE) represent four replicates of 10 insects each. Means for each species and in the same column followed by different letter are significantly different ($p < 0.01$). DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

evaluated showed fresh bark extract inducing a mortality of over 90% in both insect species. Beetles skilled in treated grains had their metathoracic wings unfolded and stretched outside the elytra thus suggesting that contact toxicity was not due to ingestion (Obeng-Ofori et al., 1997). Topical application of the extracts was most potent against *C. maculatus* probably because of the absence of hard and highly sclerotized thoracic cuticle as found in their *Sitophilus* counterparts. The effectiveness of the extracts indicates a possible contact action of the active constituents of *Z. xanthoxyloides.* The repellent effect against the insect pests indicates the presence of repellent principles in *Z. xanthoxyloides* thus confirming the findings of earlier researchers (Nawrot et al., 1986; Udo, 2005) that some insect species react more strongly to antifeedant secondary metabolites. The degree of repellency could also depend on the habitats of stored product environment. The repellent action therefore increases the protectant potential of *Z. xanthoxyloides* against storage insect pest. The effective utilization of *Z. xanthoxyloides* as a botanical pesticide could minimize the use of hazardous chemicals in stored product pest control. In the traditional post-harvest system in Africa, resource poor farmers could prepare the bark and root powders and use them locally at cheaper cost. Therefore, botanical pesticides represent an important component of integrated pest management systems in traditional grain storage as they are broad spectrum in action, based on local materials and potentially less expensive while many are safe to the environment and poses no danger to man and other mammals.

ACKNOWLEDGEMENTS

The author is grateful to Prof. J. N. Ayertey, the coordinator of ARPPIS programme, University of Ghana for his contributions and Prof. D. Obeng-Ofori and Dr. E. O. Owusu for reading through the manuscript. DAAD, Germany funded the work.

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