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BIOLOGICAL EFFECT OF METHANOL EXTRACTS OF CANDLEWOOD *Zanthoxylum xanthoxyloides* (Lam.) AGAINST INFESTATION OF STORED MAIZE AND COWPEA BY THREE STORED PRODUCT BETTLES

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ABSTRACT

Methanol extract of dried leaf (DLE), dried bark (DBE), dried root (DRE), fresh bark (FBE) and fresh root (FRE) of *Zanthoxylum xanthoxyloides* (Lam.) was assessed in the laboratory against infestation of *Sitophilus zeamais, Callosobruchus maculatus* and *Tribolium castaneum* in stored maize and cowpea. One hundred grams each of maize and cowpea were treated with 2 ml of each extract to test for contact toxicity, damage assessment, progeny production, effect on immature and developmental stages of *S. zeamais* and *C. maculatus*. Furthermore, contact toxicity by topical application and repellency test using a choice bioassay method were carried out on the three insect species. Results obtained showed that FBE and DBE applied topically caused significant ($P<0.001$) mortality of the three insects. Fresh bark extract also significantly ($P<0.001$) reduced grain damage and completely inhibited progeny production and development of eggs within grain kernels. All the extracts evoked a strong repellent action against *T. castaneum* but were moderately repellent to *S. zeamais* and *C. maculatus*. The potentials of using extracts of *Z. xanthoxyloides* as grain protectant against infestation by storage pests is being discussed.

KEYWORDS: Zanthoxylum, Extract, Stored product, Beetles, infestation.

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INTRODUCTION

Grains in storage are attacked by several species of insects and in Africa losses averaging over 30% have been recorded (IITA, 1995). This threatens food security and warrants urgent control measures.

Presently, insect control in stored food products relies heavily on the use of gaseous fumigants and residual chemical insecticides, which are toxic to the consumer (Zettler and Cuperus, 1990), constitute health hazards to grain handlers and induce widespread development of resistance in insect pests. The development of insecticide-based techniques for protecting grains in small traditional farm stores in Africa has only been partially successful because of high cost of synthetic insecticides and erratic supply due to foreign exchange constraints (Obeng-Ofori *et al.*, 1997). These problems therefore, call for new alternative control measures. Presently, attention has been turned to botanicals because most of them are broad spectrum, safe to the environment and cause few hazards to man and other animals. This gave impetus to the screening of candlewood for insecticidal properties against storage pests.

Zanthoxylum xanthoxyloides Lam. (Rutaceae) is a shrub growing to a small tree of up to 1.25 m high and 0.13 m girth. Its medicinal values have long been known as various parts of the shrub have been employed in treating many ailments such as ulcers, syphilitic sores, purulent conjunctivitis and others. The leaves are fed to sheep in certain Ga villages in Ghana (Irvine, 1961). This work investigated the insecticidal activity of methanol extract of candlewood, *Z. xanthoxyloides* against infestation by *S. zeamais, T. castaneum* and *C. maculatus*.

MATERIALS AND METHODS

Insects

S. zeamais and *C. maculatus* were collected from infested stock of grains at the Madina market, Accra and reared on 500 g each of sterilized whole maize and cowpea grains while *T. castaneum* was obtained from a stock culture maintained at the Zoology Department, University of Ghana and reared on pulverized groundnut in the laboratory. After two weeks of oviposition, the parent adults were removed by sieving using an Impact Test Sieve with mesh size of 710 micron. Progeny that emerged were re-cultured and used for the various experiments. Culture conditions

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Table 1. Toxicity of extracts applied topically to the three insect species.

Treatment	Mean (± S.E.) % mortality after 48h		
	S. zeamais	*C. maculatus*	*T. castaneum*
DLE	$8^{c} \pm 0.25$	$25^{b} \pm 0.75$	$53^{bc} \pm 0.86$
DBE	$98^{a} \pm 0.25$	$100^{a} \pm 0.00$	$83^{a} \pm 1.03$
DRE	$25^{c} \pm 1.33$	$81^{a} \pm 0.29$	$65^{ab} \pm 0.87$
FBE	$98^{a} \pm 0.25$	$100^{a} \pm 0.00$	$83^{a} \pm 1.48$
FRE	$53^{b} \pm 1.03$	$79^{a} \pm 0.48$	$33^{c} \pm 1.29$
Control	$0^{c} \pm 0.00$	$0^{c} \pm 0.00$	$0^{d} \pm 0.00$

Mean of four replicates of 10 insects each. Means in the same column for each species followed by different letter(s) are significantly different at (P<0.001), LSD test.

DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

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

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

Mean of four replicates of 20 insects each. Means in the same column for each species followed by different letter(s) are significantly different at (P<0.001), LSD test.

DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

were 28 +- 2 °C, 65 – 70% relative humidity and 12hL: 12hD light regime and all experiments were carried out under same conditions.

Collection of plant materials and preparation of extracts

Leaves, bark and root of *Z. xanthoxyloides* were collected from the University farm, Legon – Accra and methanol extracts of fresh and air – dried plant materials were prepared. Two hundred and fifty grams of plant materials were mixed with 70% methanol (by volume) in glass jars and allowed to stand in the dark for three days. The extracts were filtered into round bottom flasks of 500 ml capacity. Methanol was completely evaporated from the extract using a rotary evaporator at 30 – 40°C with rotary speed of 3 – 6 rpm for 8 hours (Godefroot *et al.*, 1981). The

residues obtained after evaporation were insoluble in acetone but soluble in water.

Contact toxicity by topical application

Forty adult unsexed insects in batches of 10 each of *S. zeamais*, *C. maculatus* and *T. castaneum* were chilled for three minutes to reduce their activity (mobility) and transferred into petri dishes (11.0 cm diameter) containing food. One microlitre of extract of dry leaf, dry bark and dry root as well as fresh bark and fresh root was applied separately using a micro-apllicator to the

Table 3 Toxicity of fresh bark extract against *S. zeamais* and *C. maculatus*

Dosage (ml / 50 g of grains)	Mean percent mortality, hours after treatment			
	24	48	72	96
S. zeamais				
0	0^{c}	0^{c}	0^{d}	0^{b}
0.5	3^{c}	20^{c}	36^{c}	81
1.0	8^{c}	53^{b}	61^{b}	85^{a}
1.5	25^{b}	58^{b}	66^{b}	96^{a}
2.0	39^{a}	91^{a}	100^{a}	100^{a}
C. maculatus				
0	0^{c}	0^{b}	0^{b}	0^{b}
0.5	18^{b}	71^{a}	95^{a}	98^{a}
1.0	15^{b}	80^{a}	96^{a}	100^{a}
1.5	36^{b}	86^{a}	100^{a}	100^{a}
2.0	56^{a}	96^{a}	100^{a}	100^{a}

Means in the same column for each species followed by different letter(s) are significantly different (P<0.001), LSD test.

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

Treatments	Mean % weight loss	
	S. zeamais	*C. maculatus*
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	$16^{a} \pm 2.86$	$25^{a} \pm 11.38$

Mean of four replicates of 20 insects each. Means in the same column for each species followed by different letter(s) are significantly different at (P<0.001), LSD test.

DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

dorsal surface of the thorax of each insect taken individually. Distilled water was applied to the control insects and each treatment was replicated four times. Mortality was recorded after one hour for 48 hours.

Toxicity of the extracts in grains

The toxicity of methanol extracts against *S. zeamais*, and *C. maculatus* on maize and cowpea grains was tested in the laboratory. Two millilitre of each extract of dry leaf, dry bark and dry root as well as fresh bark and fresh root was applied to 50 g of pre-equilibrated grains in 200 ml glass jars and covered with white muslin cloth, held in place with rubber bands. Twenty adults of *S. zeamais* (7 – 14 days old) and 20 adults of *C. maculates* (3 – 7 days old) were introduced into treated and control grains and left in the controlled environment room. There were four replicates and mortality was recorded daily for three days. Insects were assumed dead if they did not respond to three probings with a blunt probe. Fresh bark extract showed higher biological activity and was tested further at dosages of 0.5, 1.0, 1.5 and 2.0 ml per 50 g of grains against *S. zeamais*, and *C. maculatus*.

Damage assessment

One hundred grams of maize and cowpea grains were treated with 2 ml of each extract and 20 adults of *S. zeamais* (7 – 14 days old) and 20 adults of *C. maculatus* (3 – 7 days old) were introduced into the treated and control grains. Each treatment was replicated four times and left to stand undisturbed for four weeks. Control grains were treated with distilled water. Samples of 100 grains were taken from each jar and the number of damaged grains (grains with

Table 5 Mean number of *S. zeamais* and *C. maculatus* adults produced in extract treated grains at different times after oviposition period

Extract	Time after adult removal (days)		
	1	7	14
S. zeamais			
Control	$18^{a} \pm 6.94$	$18^{a} \pm 7.68$	$23^{a} \pm 8.23$
DLE	$14^{ab} \pm 5.33$	$15^{a} \pm 7.01$	$18^{ab} \pm 7.94$
DBE	$14^{ab} \pm 6.09$	$10^{a} \pm 3.03$	$17^{b} \pm 8.14$
DRE	$13^{b} \pm 6.71$	$15^{a} \pm 4.69$	$14^{b} \pm 6.09$
FBE	$4^{c} \pm 2.06$	$4^{b} \pm 2.02$	$7^{c} \pm 2.85$
FRE	$16^{a} \pm 5.86$	$12^{a} \pm 3.83$	$17^{ab} \pm 7.73$
C. maculatus			
Control	$52^{a} \pm 9.32$	$21^{a} \pm 7.89$	$18^{a} \pm 8.06$
DLE	$25^{ab} \pm 23.19$	$1^{b} \pm 0.89$	$11^{a} \pm 9.60$
DBE	$0^{b} \pm 0.00$	$0^{b} \pm 0.00$	$0^{b} \pm 0.00$
DRE	$1^{b} \pm 0.45$	$1^{b} \pm 0.81$	$7^{ab} \pm 5.92$
FBE	$0^{b} \pm 0.00$	$0^{b} \pm 0.00$	$0^{b} \pm 0.00$
FRE	$8^{b} \pm 4.38$	$1^{b} \pm 0.59$	$11^{a} \pm 5.84$

Means in the same column for each species followed by different letter(s) are significantly different ($P<0.001$), LSD test.

DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

Table 6 Effect of plant extracts on the number of F1 progeny produced by *S. zeamais* and *C. maculatus*

Extracts (2 ml / 25 g of grains)	Mean number of F1 progeny treatment	
	S. zeamais	*C. maculatus*
DLE	$99^{a} \pm 11.71$	$56^{ab} \pm 11.66$
DBE	$96^{a} \pm 4.09$	$36^{bc} \pm 3.67$
DRE	$99^{a} \pm 19.00$	$30^{bc} \pm 16.32$
FBE	$0^{b} \pm 0.00$	$3^{c} \pm 2.52$
FRE	$82^{a} \pm 13.85$	$18^{bc} \pm 2.78$
Control	$103^{a} \pm 23.78$	$87^{a} \pm 22.29$

Mean of four replicates of 20 insects each. Means in the same column for each species followed by different letter(s) are significantly different at ($P<0.001$), LSD test.

DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

characteristic holes) and undamaged grains were counted and weighed. The percent weight loss was computed using the method of FAO (1985):
% 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 extracts on eggs and immature stages

Batches of 200 g of equilibrated maize and cowpea in 500 ml glass jars were infested with 100 adults each of *S. zeamais*, and *C. maculatus*, respectively to allow egg laying. The parent adults were removed after seven days. One day after removal of adults, four batches of 25 g each of maize and cowpea grains were treated with 1 ml of extract of dry leaves, dry bark, dry root, fresh bark and fresh root to test their effect on the eggs and immature stages. This was repeated one and two weeks after adult removal. Control was treated with distilled water and adults emerging subsequently were counted weekly following the removal of parent adults (Su, 1977).

Progeny production

One hundred grams of pre-equilibrated maize and cowpea grains were treated with 2 ml each of dry leaves, dry bark, dry root, fresh bark and fresh root extracts and allowed to stand for three hours after which, 20 adults each of *S. zeamais*, and *C. maculatus* were introduced into the grains while the control was treated with distilled water. The containers were covered with white muslin cloth and held in place with rubber bands. The experiment was replicated four times and left undisturbed for five weeks and number of insects emerging was counted.

Repellency test

Repellency of the extracts was assessed in a choice bioassay method using baked wheat cakes. Wheat flour was mixed with water to form a paste and rolled into thin bars of about 5 cm diameter. The cakes were then cut using a knife into circular pieces and baked in an oven at 40°C for 48 hours. The cakes were treated with 0.5 ml of extracts of dry leaves, dry bark, dry root, fresh bark and fresh root and left to dry for one hour. This was followed by the introduction of 10 adults of *S. zeamais*, *C. maculatus and T. castaneum* into petri dishes (11.0 cm diameter) containing two treated and two untreated cakes placed at opposite ends with a space separating them in the centre. Each treatment was replicated four times and the control was treated with distilled water. The number of insects present on control (N_c) and treated (N_t) cakes were recorded after one hour for 48 hours. Percent repellency (PR) was computed as $PR = (N_c - N_t) / (N_c + N_t) \times 100$ and data was analysed using ANOVA after transformation into arcsine values. All negative PR values were treated as zero (Obeng-Ofori *et al.*, 1997).

RESULTS

Contact toxicity by topical application

Toxicity of the various methanol extracts

Table 7 Mean % repellency (pr) values for the extracts against the three insect species in the choice test

Extract	Mean % repellency (PR)		
	S. zeamais	*T. castaneum*	*C. maculatus*
DLE	$5^{b} \pm 0.3$	$56^{b} \pm 1.2$	$36^{b} \pm 0.6$
DBE	$2^{b} \pm 0.1$	$66^{ab} \pm 1.1$	$68^{a} \pm 1.2$
DRE	$14^{a} \pm 0.8$	$49^{c} \pm 0.9$	$9^{c} \pm 0.1$
FBE	$14^{a} \pm 0.8$	$79^{a} \pm 1.3$	$66^{a} \pm 1.1$
FRE	$13^{a} \pm 0.6$	$64^{b} \pm 1.1$	$48^{b} \pm 0.9$
Control	$0^{b} \pm 0.00$	$0^{d} \pm 0.00$	$0^{c} \pm 0.00$
Overall PR	10	63	45

DLE = Dry leaf extract, DBE = Dry bark extract, DRE = Dry root extract, FBE = Fresh bark extract, FRE = Fresh root extract.

applied topically to *S. zeamais*, *C. maculatus* and *T. castaneum* is summarized in Table 1 there was a significant (P<0.001) difference amongst the treatments with fresh bark and dry bark extracts inducing the highest mortality in the three insect species within 48 hours after treatment. Dry leaf extract showed the least toxicity to the three insect species.

Toxicity of methanol extracts

Methanol extracts of the various plant parts showed different levels of toxicity against *S. zeamais* and *C. maculatus* (Table 2). Fresh bark extract was most toxic, causing 93% and 86% mortality in *S. zeamais* and *C. maculatus*, respectively. All the dosages of fresh bark extract tested further at doses of 0.5, 1.0, 1.5, and 2.0 ml caused significant (P<0.001) mortality of the beetles compared to the control. For example, the 1.5 and 2.0 ml dosages killed all *C. maculatus* exposed after 72 hours (Table 3).

Damage assessment

There were significant differences (P<0.001) amongst the extracts in reducing damage caused by the beetles. Fresh bark extract provided 100% protection to both maize and cowpea against infestation by *S. zeamais* and *C. maculatus*, respectively (Table 4). Extracts of dry bark, dry root and fresh root also caused highly significant reduction in grain damage compared to control treatment (Table 4).

Effect of extracts on eggs, immature stages and Progeny production

Maize and cowpea grains treated with the extracts significantly (P<0.001) affected the immature stages of *S. zeamais* and *C. maculaus* (Table 5). Dry bark and fresh bark extracts completely inhibited the development of *C. maculatus* while complete mortality was observed for emerging insects on grains treated with fresh bark extract. The extracts applied to maize and cowpea reduced the F1 generation of *S. zeamais* and *C. maculatus* compared to the control (Table 6). Fresh bark extract completely inhibited the development of *S. zeamais* and recording the least number of progeny emergence in *C. maculatus*.

Repellency bioassay

The various extracts showed different levels of repellency to the three insect species (Table 7). All the extracts tested induced low repellency to *S. zeamais*. Extracts from fresh and dry bark invoked high repellency against both *T. castaneum* and *C. maculatus*. Fresh bark extract recorded the highest repellency of 79% against *T. castaneum*. The overall percent repellency values indicate *T. castaneum* recording the highest value of 63%.

DISCUSSION

Fresh bark extract caused significant mortality in *S. zeamais* and *C. maculatus* and this could be attributed to the presence of highly pungent phenolic secondary metabolites in *Z. xanthoxyloides* while other constituents include fagarol and zanthoxylol (Adesina, 1986). Beetles killed in treated grains had their metathoracic wings unfolded and stretched outside the elytra

(Obeng-Ofori *et al.*, 1997), suggesting that toxicity was not due to ingestion of treated grains. Also, the significant reduction in damage shows tha the plant contains antifeedant properties (Niber, 1994).

C. maculatus was highly susceptible to the extracts applied topically probably because of the absence of hard and highly sclerotized thoracic cuticle (Talukder and Howse, 1994). The complete inhibition of the development of eggs and immature stages within grain kernels suggest the presence of ovicidal properties in the plant (Ogunwolu and Idowu, 1994). This increases the protectant potential of *Z. xanthoxyloides* against insect damage in storage. The high mortality induced by fresh and dry bark extracts in *C. maculatus* is noteworthy since these preparations can be used in severely infested grain bulks for immediate control of this insect pest.

Fresh bark extract gave the highest repellent effect of 79% against *T. Castaneum* and this can be explained by the fact that *Tribolium* spp. react more strongly to antifeedants than other stored product pests (Nawrot *et al.*, 1986).

The results obtained from the study suggest good potential for the use of *Z. xanthoxyloides* in storage pest management systems, in view of the relative safety of the plant which is used in treating various ailments like ulcer, sores and drunk as bitters while the leaves are fed to animals. This also demonstrates that many botanicals are broad spectrum in action and safe to the environment with fewer hazards to man and other mammals (Talukder and Howse, 1994).

REFERENCES

Adesina, S. K., 1986. Further novel constituents of *Zanthoxylum zanthoxyloides* root and pericarp. Journal of Natural Products. 49: 715 – 716

FAO, 1985. Prevention of Post harvest food losses. Training series No. 10:122. Rome, F.A.O. 120 pp.

Godefroot, M., Sandra, P. and Verzele, M., 1981. New method for quantitative essential oil analysis. Journal of Chromatography. 203: 325 – 335.

IITA, 1995. Plant Health Management Division. Annual Report. 43 p.

Irvine, F. R., 1961. Woody plants of Ghana with special reference to their uses. Oxford University Press, London. 868 pp.

Nawrot, J., Bloszvk, E., Harmatha, J., Novotny, L. and Drozdz, B., 1986. Action of antifeedants of plant origin on beetles infesting stored products. *Acta Entomologica Bohemoslovacao*. 83: 327 – 335

Niber, T. B., 1994. The ability of powders and slurries from ten plant species to protect stored grain from attack by *Prostphanus truncatus* Horn (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coloeptera: Curculionidae). Journal of Stored Products Research, 30: 297 – 301

Obeng-Ofori, D., Reichmuth, C. H., Bekele, A.J. and Hassanali, A., 1997. Biological activity of 1,8 cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored product beetles. Journal of Applied Entomology 121: 237 – 243.

Ogunwolu, O. and Idowu, O., 1994. Potential of powdered *Zanthoxylum zanthoxyloides* (Rutaceae) root bark and *Azadirachta indica* (Meliaceae) seed for control of the cowpea seed bruchid, *Callosobruchus maculatus* (Bruchidae) in Nigeria. Journal of African Zoology. 108: 521 –528

Su, H. C. F., 1977. Insecticidal properties of black pepper to rice weevils and cowpea weevils. Journal of Economic Entomology. 70: 18 – 21.

Talukder, F. A. and Howse, R. E., 1994. Laboratory evaluation of toxic and repellent properties of the pithraj tree, *Aphanamixis polystachya* Wall and Parker, against *Sitophilus oryzae* (L). Journal of Pest Management. 40: 274 – 279.

Zettler, J. L. and Cuperus, G. W., 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. Journal of Economic Entomology. 83: 1677 – 1681.