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RESEARCH PAPER

EVALUATION OF ETHANOLIC EXTRACT OF *ZANTHOXYLUM XANTHOXYLOIDES* (LAM) AS SEED PROTECTANT AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) AND *SITOPHILUS ZEAMAIS* MOTSCH. ON STORED COWPEA AND MAIZE UNDER TROPICAL CONDITIONS

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ABSTRACT
The efficacy of Zanthoxylum xanthoxyloides stem bark and root bark ethanol extracts as grain protectant against Sitophilus zeamais and Callosobruchus maculatus was investigated in the laboratory under ambient conditions. The root extract was more effective in controlling both insects, and it was more toxic to C. maculatus than S. zeamais. Insect mortality was dose-dependent. After 24 hours, the highest dose of 50% stem extract evoked 55% and 85% mortalities in S. zeamais and C. maculatus, respectively; while 50% root extract evoked 97.5% and 100% mortalities in S. zeamais and C. maculatus, respectively. Development of eggs and immature stages within the treated seeds as well as progeny emergence significantly decreased with increasing dosage of both extracts. Emergence of C. maculatus was completely inhibited by 30% treatment of both extracts, while for S. zeamais inhibition was achieved at 40% treatment. The effect of the secondary metabolites present in Z. xanthoxyloides responsible for its efficacy is investigated and discussed. The potential use of Z. xanthoxyloides as insecticidal, feeding and oviposition deterrent to protect the stored product is discussed.

Keywords: *Sitophilus zeamais, Callosobruchus maculatus, Zanthoxylum xanthoxyloides, secondary metabolites, mortality*

INTRODUCTION
Cowpea, *Vigna unguiculata* (L.) Walp, is a major source of dietary protein in tropical and subtropical regions of the world especially where availability and consumption of animal protein is low (Oparaeke *et al*., 1998). *Callosbruchus maculatus* (F.) (Coleoptera: Chysomelidae) damage 50-90% of cowpea in storage annually throughout tropical Africa and can cause up to 100% loss with an estimated value of over 30 million U.S. dollars in Nigeria (Caswell and Akibu, 1981; Jackai and Daoust, 1986; IITA, 1989).

Maize, *Zea mays* (L.), is an important staple food for more than 1.2 billion people in sub-Saharan Africa and Latin America (IITA, 2007). It is rich in dietary fiber and calories which are sources of energy. Maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae), is a major pest of stored maize. In Ghana about 20% out of an estimated total annual harvest of 250,000-300,000 tonnes of maize is lost to *Sitophilus zeamais* (Obeng-Ofori and Amiteye, 2005).

Small scale and peasant farmers in third world countries, especially Africa, commonly admix their stored produce with different kinds of plant materials against pests damage. This is because the cost of synthetic insecticides is beyond their reach. The precise strategy differs between communities and place depending on the availability of the plant product and partly on the type of efficacy it evokes (Hassanali *et al*., 1990).

Plant materials have been suggested as important approach in insect pest management because the constant misuse of synthetic chemicals/ insecticides have promoted faster evolution of resistant forms of pests, destroyed natural enemies, turned formerly innocuous species into pests, and have harmed other non-target species and contaminated food (Obeng-Ofori *et al.*,1997; Rajapaske and van Emden 1997; Allotey and Azalekor, 2000).

Plant products such as candlewood, *Zanthoxylum xanthoxyloides* Lam. has shown to have a promising prospect to control insect pests. In Africa, especially Ghana, the root and stem extract are used to prepare aqueous/alcoholic beverages and the leaves are fed to animals.

MATERIALS AND METHODS

Culturing of insects

Clear uninfested local white variety of cowpea seeds and yellow variety of maize seeds obtained from Bodija market, Ibadan, Nigeria, were sterilized by freezing for seven days. About 1 kg each of the seeds was separately put into two cylindrical plastic containers, measuring 60 cm diameter x 22 cm high. Several unsexed adults of *C. maculatus* and *S. zeamais* obtained from a laboratory stock of insects from the Entomology Laboratory of the Crop Protection and Environmental Biology were introduced into the respective containers. All the adult insects were sieved out after two weeks and the culture left undisturbed till newly emerged adults evolved. All cultures and experimental set-ups were maintained at ambient laboratory conditions in the Entomology Laboratory of the Crop Protection and Environmental Biology, Ibadan, Nigeria.

Preparation of plant materials

Bark and roots of *Z. xanthoxyloides* were collected from the Ede, Osun State, Nigeria. The root and stem barks were cut into small pieces and air-dried at the ambient laboratory temperature for two weeks. The materials were separately ground into fine powder using an electric grinder. A simple photochemical screening revealed the presence of the following compounds (Table 1).

Ethanolic extraction of the essential oils from both root and stem bark was done using the Soxhlet extractor. Excess ethanol was evaporated using a rotary evaporator at 30 to 40^{o}C for two hours. Serial dilution of 10, 20, 30, 40 and 50% of the extracts were prepared using distilled water for the bioassay plus a control of 0% (only distilled water).

All test containers measured 7.8 cm diameter x 3.6 cm high, unless otherwise stated.

Effect of extracts on adults

In cylindrical plastic containers, 50 g of clean, sterilized maize were admixed thoroughly with 2 ml each of the stem bark extract serial dilution. In a similar set-up, root bark serial dilution was applied and a control. After 1 hour, five pairs of young newly emerged adults of *S. zeamais* were introduced.

In a similar set-up, five pairs of newly emerged adults of *C. maculatus* were introduced after 1 hour. Each treatment of all set-ups was replicated four times and mortality was recorded at an interval of 24 hours up to 72 hours.

Table 1: Phytochemicals present in the stem bark and root bark powders of *Z. xanthoxyloides*

Compound	Root Extract	Stem Extract
Terpenoids	+	−
Flavonoids	+	−
Alkaloids	+	+
Glycosides	+	+
Resins	+	+

+ indicates presence
− indicates absence

Effect of extracts on oviposition, immature stages and emergence

Batches of 50 g each of clean, sterilized maize seeds were admixed thoroughly with 2 ml each of 0, 10, 20, 30, 40 and 50% of stem extract in cylindrical plastic containers. Batches of 50 g each of clean, sterilized cowpea seeds were admixed thoroughly with 2 ml each of 0, 10, 20, 30, 40 and 50% of stem extract in cylindrical plastic containers. A similar batch of set-up was admixed with root extract. After 1 hour, five pairs of newly emerged adults of *C. maculatus* and *S. zeamais* were introduced separately into each container. Each treatment was replicated four times. After a 2-week oviposition period, all the parent adults were removed with a fine forceps. Insects subsequently emerging were counted daily for 2 weeks.

Seed damage

The effect of the plant extracts was assessed from the proportion of seeds with *C. maculatus* and *S. zeamais* emergent holes. The data was collected 5 weeks after the set-up of experiment. Seeds with emergent holes irrespective of the number of holes on a seed were counted as damaged. The percentage grain damage (PGD) was calculated using the formula in the equation:

$$PGD = \frac{G_1}{G_2} \times 100$$

G_1 = number of grains with exit holes; G_2 = total number of grain seeds.

Data analysis

The experimental design used was the completely randomized design. All the data were analysed using one-way ANOVA and means separated using LSD.

RESULTS

Adult mortality

After 24 hours treatment with the stem extract, the highest adult mortality of 55% and 85% was recorded for 50% treatment for *S. zeamais* and *C. maculatus*, respectively. The highest cumulative mortality occurred at the 50% treatment of application after 72 hours. It was 65% and 100% for *S. zeamais* and *C. maculatus*, respectively (Tables 2 and 3).

At 50% root extract treatment, 97.5% and 100% mortality was recorded after 24 hours for *S. zeamais* and *C. maculatus*, respectively. However, for *C. maculatus*, 97.50% and 100% mortality was recorded at 40% treatment application after 48 and 72 hours, respectively (Tables 4 and 5).

Oviposition and Progeny production

The number of eggs laid by both insects and subsequent emergence decreased with increasing concentration for both extracts. Complete inhibition was only achieved for *S. zeamias* at treatment 50% (Table 6) whereas in *C. maculatus* complete inhibition was from 30% to 50% treatment (Table 7).

Table 2: Percentage cumulative mortality of *S. zeamais* on maize grains treated with stem extract of *Z. xanthoxyloides*

Treatments (%)	24 hours	48 hours	72 hours
0	$2.50^{a} \pm 2.50$	$2.50^{a} \pm 2.50$	$5.00^{a} \pm 5.00$
10	$7.50^{a} \pm 2.50$	$7.50^{a} \pm 2.50$	$7.50^{a} \pm 2.50$
20	$2.50^{a} \pm 2.50$	$5.00^{a} \pm 5.00$	$7.50^{a} \pm 4.79$
30	$2.50^{a} \pm 2.50$	$2.50^{a} \pm 2.50$	$2.50^{a} \pm 2.50$
40	$27.50^{b} \pm 4.79$	$27.50^{b} \pm 4.79$	$30.00^{b} \pm 4.08$
50	$55.00^{c} \pm 11.90$	$57.50^{c} \pm 11.09$	$65.00^{c} \pm 8.66$
LSD (<0.05)	**16.70**	**16.70**	**14.96**

n = 10

Table 3: Percentage cumulative mortality of *C. maculatus* on cowpea seeds treated with stem extract of *Z. xanthoxyloides*

Treatments (%)	24 hours	48 hours	72 hours
0	$12.50^{a} \pm 4.79$	$25.00^{a} \pm 2.89$	$25.00^{a} \pm 2.89$
10	$20.00^{a} \pm 4.08$	$22.50^{a} \pm 4.79$	$42.50^{ab} \pm 7.50$
20	$12.50^{a} \pm 7.50$	$17.50^{a} \pm 4.79$	$50.00^{b} \pm 10.00$
30	$52.50^{b} \pm 4.79$	$60.00^{b} \pm 4.08$	$95.00^{c} \pm 5.00$
40	$55.00^{b} \pm 8.66$	$80.00^{c} \pm 8.16$	$87.50^{c} \pm 4.79$
50	$85.00^{c} \pm 8.66$	$95.00^{d} \pm 2.89$	$100.00^{c} \pm 0.00$
LSD (<0.05)	**19.88**	**14.65**	**17.68**

n = 10

Means followed by the same alphabet in each column are not significantly different from each other.

Table 4: Percentage cumulative mortality of *S. zeamais* on maize grains treated with root extract of *Z. xanthoxyloides*

Treatments (%)	24 hours	48 hours	72 hours
0	$2.50^{a} \pm 2.50$	$2.50^{a} \pm 2.50$	$2.50^{a} \pm 2.50$
10	$0.00^{a} \pm 0.00$	$2.50^{a} \pm 2.50$	$5.00^{a} \pm 2.89$
20	$2.50^{a} \pm 2.50$	$5.00^{a} \pm 5.00$	$5.00^{a} \pm 5.00$
30	$10.00^{a} \pm 4.08$	$10.00^{a} \pm 4.08$	$17.50^{a} \pm 6.29$
40	$40.00^{b} \pm 9.13$	$40.00^{b} \pm 9.13$	$55.00^{b} \pm 10.41$
50	$97.50^{c} \pm 2.50$	$97.50^{c} \pm 2.50$	$97.50^{c} \pm 2.50$
LSD (<0.05)	**13.22**	**14.54**	**16.88**

n = 10

Table 5: Percentage cumulative mortality of *C. maculatus* on cowpea seeds treated with root extract of *Z. xanthoxyloides*

Treatments (%)	24 hours	48 hours	72 hours
0	$5.00^{a} \pm 2.89$	$10.00^{a} \pm 4.08$	$10.00^{a} \pm 4.08$
10	$12.50^{a} \pm 4.79$	$15.00^{a} \pm 5.00$	$30.00^{b} \pm 9.13$
20	$40.00^{b} \pm 8.16$	$50.00^{b} \pm 12.91$	$55.00^{c} \pm 10.41$
30	$85.00^{c} \pm 2.89$	$97.50^{c} \pm 2.50$	$100.00^{d} \pm 0.00$
40	$87.50^{c} \pm 7.50$	$97.50^{c} \pm 2.50$	$100.00^{d} \pm 0.00$
50	$100.00^{c} \pm 0.00$	$100.00^{c} \pm 0.00$	$100.00^{d} \pm 0.00$
LSD (<0.05)	**15.46**	**18.03**	**17.51**

n = 10

Table 6: Effect of *Z. xanthoxyloides* stem bark and root bark extracts on emergence of *S. zeamais* in treated maize grains

Treatments (%)	Stem Extract	Root Extract
0	$83.50^{d} \pm 4.99$	$89.25^{d} \pm 3.01$
10	$41.50^{c} \pm 4.97$	$36.00^{c} \pm 2.61$
20	$44.50^{c} \pm 8.62$	$37.25^{c} \pm 5.17$
30	$23.25^{b} \pm 3.04$	$16.25^{b} \pm 1.11$
40	$9.00^{ab} \pm 3.58$	$5.25^{a} \pm 2.46$
50	$0.25^{a} \pm 0.25$	$0.00^{a} \pm 0.00$
LSD (<0.05)	**14.66**	**8.57**

n = 4

Means followed by the same alphabet in each column are not significantly different from each other.

Table 7: Effect of *Z. xanthoxyloides* stem bark and root bark extracts on emergence of *C. maculatus* in treated cowpea

Treatments (%)	Stem Extract	Root Extract
0	$22.75^{c} \pm 3.59$	$17.25^{c} \pm 2.29$
10	$9.50^{b} \pm 1.26$	$12.75^{b} \pm 1.18$
20	$5.25^{b} \pm 0.75$	$10.00^{b} \pm 0.41$
30	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
40	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
50	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
LSD (<0.05)	**4.70**	**3.16**

n = 4

Means followed by the same alphabet in each column are not significantly different from each other.

Damage assessment

The percentage seed damaged significantly reduced with increasing treatment concentration for both stem and root extract for both *S. zeamais* and *C. maculatus*. The percentage maize seed damaged at 40% treatment for both extracts was less than 5%, and the damage was not significantly different from 50% treatment where there was no damage (Table 8). There were no emergent holes on both cowpea treatments from 30% - 50% concentration, implying, no damage (Table 9).

Table 8: Percentage seed damage of *S. zeamais* on maize grains treated with *Z. xanthoxyloides* extracts

Treatments (%)	Stem Extract	Root Extract
0	$44.38^{d} \pm 2.36$	$46.59^{d} \pm 1.33$
10	$22.11^{c} \pm 2.65$	$19.06^{c} \pm 1.54$
20	$23.62^{c} \pm 4.51$	$19.71^{c} \pm 2.94$
30	$12.40^{b} \pm 1.67$	$8.70^{b} \pm 0.67$
40	$4.76^{a} \pm 1.87$	$2.79^{a} \pm 1.30$
50	$0.13^{a} \pm 0.13$	$0.00^{a} \pm 0.00$
LSD (<0.05)	**7.60**	**4.69**

n = 10

Means followed by the same alphabet in each column are not significantly different from each other.

Table 9: Percentage seed damage of *C. maculatus* on cowpea treated with *Z. xanthoxyloides* extracts

Treatments (%)	Stem Extract	Root Extract
0	$5.95^{c} \pm 1.18$	$4.06^{c} \pm 0.32$
10	$1.99^{b} \pm 0.27$	$3.86^{c} \pm 0.30$
20	$1.^{44ab} \pm 0.15$	$2.94^{b} \pm 0.21$
30	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
40	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
50	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
LSD (<0.05)	**1.48**	**0.59**

n = 10

Means followed by the same alphabet in each column are not significantly different from each other.

DISCUSSION

The effectiveness of the extracts was dose dependent. The root extract was more potent than the stem and it evoked higher mortality in *C. maculatus* than *S. zeamais*. High mortalities were recorded just after 24 hours or at least 48 hours of exposure to both extracts. The root extract induced 100 and 97% mortalities in *C. maculatus* and *S. zeamais* just after 24 hours when seeds were treated with 50% dosage (Tables 4 and 5) as compared to 85 and 55% for the stem extract for *C. maculatus* and *S. zeamais* (Tables 2 and 3). Toxicity is attributed to terpenoids (Ryan and Byrne, 1988; Turlings *et al*., 1995) and alkaloids and both have insecticidal properties. As well as glycosides, a defensive product that functions as toxins and herbivore repellents (Lewis and Elvin-Lewis, 1977). Most of these secondary metabolites are volatile (Ördög and Molnár, 2011), thus their potency reduced considerably with time and this also accounted for the higher mortality just after 24 hours.

Although both extracts suppressed oviposition with increasing concentration, the root extract was more effective. Terpenoids and glycosides are repellent chemicals which discourage insects from feeding or laying eggs (Rosenthal and Berenbaum, 1991), it disturb/modify the behaviour of the insects, making them restless, thus mating is disrupted and oviposition is inhibited (Kumbhar and Dewang, 2001). Flavonoids present only in the roots are known to modulate oviposition behavior in insects (Morimoto *et al*., 2000; Simmonds, 2001). Higher doses in the root explain why the root extracts were better suppressants. Consequently, emergence of progeny was effectively reduced. Suppressed emergence may be due to terpenoids that disrupts molting and larval development and increased insect mortality (Heftmann, 1975; Slama, 1979, 1980). Alkaloids, flavonoids, terpenoids and glycosides are insecticidal, feeding deterrents and toxic, together, they accounted for the suppressed emergence.

CONCLUSION

Higher doses of secondary metabolites in the root extract accounted for its better potency. *Callosobruchus maculatus* was more susceptible to the *Zanthoxylum xanthoxyloides* than *Sitophuilus zeamais*. The present study shows that 40 ml to 1 kg of 30% root extract can effectively protect stored local white cowpea from *C. maculatus* attack for over 3 month period. In the case of stored local yellow maize, 40 ml to 1 kg of 40% root extract is needed to protect it from *S. zeamais* attack. *Z. xanthoxyloides* has insecticidal, feeding deterrent and repellent action properties. It suppresses oviposition and offer complete inhibition of the development of immature stages of insects. This makes it one of the best potential botanical to consider as a component of Integrated Pest Management in stored product protection.

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