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Efficacy of Chinese Pepper *Zanthoxyllum xanthoxyloides* (Lam) Watern against the Cowpea Bruchid *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae)

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ABSTRACT

The efficacy of acetone extract of the Chinese pepper, *Zanthoxylum xanthozyloides* (Lam) in the bruchids control was investigated under laboratory storage-conditions of 27-30^{o}C, 60-70% RH. Different concentrations of *Z. xanthozyloides* were applied against the cowpea bruchid, *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) at 0.5%, 1.5% and 2.5% (w/v) and 0.0% (control). Results showed that the extract achieved 100% adult mortality at 2.5% concentration within 96 h of treatment. 2.5% (w/v) *Z. zanthozyloides* reduced larvae development and adult emergence significantly after six weeks of treatment in storage, while control, 0.50 and 1.50% achieved 0.0, 83.11 and 93.25% reduction during the same period. Increase in Weevil Perforations Index of 1.03, 5.04, 12.13 and 50.00 were recorded for beans treated with 2.5%, 1.5%, 0.5% (w/v) and control after 6 weeks of storage, respectively. This study has revealed *Z. zanthoxyloides* as a potential bio-insecticide applicable in the control of stored products.

Keywords: *Zanthoxylum xanthoxyloides, Callosobruchus maculatus,* Weevil mortality, Weevil Perforation Index, Biocontrol, Plant extracts.

INTRODUCTION

Cowpea, *Vigna unguiculata* L. (Walp), is an important food crop and a major source of dietary phytoprotein in West Africa. The dry seed consists of about 25% protein and 67% carbohydrate among others (Onioume, 1978). In a developing country like Nigeria, cowpeas' relative abundance and cost vary with season. Available facilities for long term storage of cowpea are consistently threatened by the cowpea seed bruchid, *Callosobruchus maculates* (F) (Coleoptera: Bruchidae), which is regarded as cosmopolitan field-to-store pests, and also recognized as a constraint to food security in Africa (Markham et al., 1994). Efficient and effective control of storage insect pests had mainly centered on the use of synthetic insecticides (Jackai and Daoust, 1986), though, insecticides had offered consistent control of bruchids, the adoption and use of these chemical compounds in the traditional African setting is limited due largely to increase awareness of health risk associated withn usage, inadequate application skills by the local farmer, emergence of resistant pests and phytopathogenic microorganisms; environmental residual effects and pesticide adulteration.

Plant-derived seed-protectants were, therefore, considered as a suitable alternative for grain preservation in rural environment. Natural products have been used for thousands of years for the benefit of mankind as

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important sources of medicines and crop protection agents (Newman and Cragg, 2007). Plant products have made enormous contributions to human health through compounds such as quinine, morphine, aspirin, digitoxin and many others (Newman and Cragg, 2007). The search for new phytosanitary agents to control plant pests are becoming more numerous (Don-Pedro 1990; Ogunwolu et al., 1998; Arannilewa, 2002; Adedire and Akinneye, 2004; Akinkurolere, 2006) to the point of getting about half of pharmaceuticals and pesticides from natural sources (Newman and Crag, 2007).

Z. xanthoxyloides seeds have been used as Condimen; leaves and bark have been used as antitussive, antibacterial, antiviral and antivenin (Kassim et al., 2009). Leaves alone are used as scaring, antiseptic, astringent and laxative, while roots have found application as antiseptic, anti-sickler, digestive aid and parasiticidal (Ngassoum et al., 2003); stem bark as antirheumatic, anti-odontalgic, diurectic, urinary antiseptic, digestive aid and parasticide (Ngane et al., 2000). Benzophenanthridines are the most frequently reported type of alkaloid in the genus, *Zanthoxylum.* Aporphine alkaloids like N,N-dimethyllindicarpine 12 obtained from the root back of *Z. Xanthoxyloides* (Queiroz et al., 2006) have been reported to posses anti-helminthic properties. Ligans like diarylbutirolactones and 2,6-diaryl-3,7-dioxabicyclo [3.3.0] octanes have been reported in *Zanthoxylum* species. These ligans possessed numerous biological activities among which include the insecticidal and inhibitory effects on certain enzymes (Adesina, 2005). Amides such as Isobutyl amides have been reported in *Zanthoxylum* species and shown to possess insecticidal properties (Chaaib, 2004; Adesina, 2005). Isolated and purified alkaloids of the root bark of *Z. xanthoxyloides* have been reported to possess anti-prostaglandin synthetase activity, and this has been reported to inhibit prostaglandin production (Prempeh and Mensah-Attipoe, 2008). Azando et al. (2011) and Barnabas et al. (2011) have shown a promising antihelmitic activity of *Z. xanthoxyloides* leaves water, acetone and ethanol extract against *Asaris lumbricoides* (Ascaridida: Ascarididae), *Haemonchus contortus* (Rhaditidae: Haemonchinae), *Trichostrongylus colubriformis* (Rhabditida: Trichostrongylidae). The purpose of this research work was to evaluate the insecticidal activity of Acetone extract of *Z. xanthoxyloides* root bark at different dose-rates on *C. maculatus* adults.

MATERIALS AND METHODS

Insect Culture

A parent stock of *C. maculatus* was obtained from an established culture reared on disinfested cowpea grains at 28±2°C ambient temperature and 75±5% relative humidity in a Grain Storage Research Laboratory at the Federal University of Technology, Akure, Nigeria. The food medium (cowpea) used for bioassay was disinfested in a deep freezer for 96 h, and later air-dried in the Laboratory to prevent mouldiness. Adult stages of *C. maculatus* were then transferred onto the grains in 1-litre kilner Jars, and from this an established culture for the experiment was maintained as new generations emerged.

Plant Materials

Root back of *Z. xanthoxyloides* collected, was dried and pulverized into fine powder with the aid of a ball-mill machine. The powder was sieved and transferred into an airtight container. Twenty-five grams (25 g) of the plant materials were weighed into a thimble and extracted with 250 ml of acetone in a Soxhlet apparatus. After about 4-5 h of extraction, the thimble was removed from the unit and the acetone was recovered by redistilling the content in the extractor at 40-60°C. The resulting extract was air-dried to evaporate traces acetone. An aliquot of the extract was transferred into a sample bottle and kept in a refrigerator, in which the concentrations applied were prepared from this.

Application of Plant Extracts on Cowpea Bruchid

Twenty grams of the food medium were weighed into Petri-dishes (9 cm in diameter). Three different concentrations of 0.5%, 1.50% and 2.5% (w/v) of acetone extract were thoroughly mixed with the grains (food medium) to ensure uniform coating. The dishes were left open for 24 h to allow likely traces of acetone to dry off. After 24 h, 20 teneral adults (0-48h old) were confined in each Petri-dish and mortality was observed every 24 h for 96 h (e.i. 4 days). Grains that were treated with pure acetone served as a control treatment (0.0%).

All experiments were carried out in three replicates. Adults were considered dead where no response was observed after probing them with forceps.

Effect of Extracts on Cowpea Bruchid Emergence and Reduction

Both dead and live adult bruchids were removed from the treated grains after 96 h of treatment; and the grains were stored for 42 days (i.e. 6 weeks). At the end of the 42^{nd} day, the number of adults that emerged (F_1 generation) was counted, and the values obtained were used as criteria for evaluation of the percentage of reduction. The F_1 adult emergence in the treated sample compared to the F_1 adult emergence in the control is an index of effectiveness of the treated material in reducing infestation. The percentage of reduction of the extracts was then calculated.

$$\%Red = 100\left[\frac{Et}{Ec}\right] * 100$$

Where Et= No. of emerged adults from treated sample, and

E_C=No. of emerged adults from control.

Effect of Extracts on Grain Damage by Cowpea Bruchid

Damage done by *C. maculatus* to the cowpea grains was determined at the end of experiment by counting the insect-damaged kernels in the treated and control treatments. The Percentage Damage (PD) and Weevil Perforation Index (WPI) were adopted for the analysis of damage using the methods of Adedire and Ajayi (1996) and Fatope et al. (1995).

$$PD = \frac{Total\ number\ of\ treated\ grains\ perforated}{Total\ number\ of\ grains} * 100$$

$$WPI = \frac{Percentage\ of\ treated\ grains\ perforated}{Percentage\ of\ control\ grains\ perforated Percentage\ of\ treated\ grains\ perforated} * 100$$

Data Statistical Analysis

Data obtained were subjected to one-way analysis of variance and where significant difference ($P< 0.05$) existed, means were separated by New Duncan's Multiple Range Test. Analysis was carried out using SPSS version 11 (Hareware, 2000).

RESULTS

Contact Toxicity of *Zanthoxylum xanthoxyloides* on Adult Bruchids

Results of contact toxicity of *Z. xanthoxyloides* root bark on *C. maculatus* is presented in table 1. 80.0% mortality of bruchids was observed at 2.5% concentration of Z. *xanthoxyloides* treatment within day 1. This was significantly higher ($P <0.05$) than 46.70%, 30.0% and 0.0% mortalities that were recorded at 1.5%, 0.5% and the control, respectively. At day 2, mortalities of 60.0%, 80.0% and 100% were recorded at 0.5%, 1.5% and 2.5% concentrations, respectively. By the third day, 2.50% concentration gave 100% adult's mortality, while 1.5% had 93.30% and 0.50% concentration also evoked 83.0% mortality. No mortality was recorded in the control treatment. At day 4, 0.5%, 1.5% and 2.5% concentrations reported 90.0%, 96.7% and 100% adult mortality, respectively. However, no adult mortality was recorded in control treatment.

Table 1. Toxic effect of *Zanthoxylum xanthoxyloides* extract on the mortality of *Callosobruchus maculatus* adults in treated cowpea grains.

Conc. (w/v)	% mortality (±S.E) days post-treatment			
	1	2	4	4
Control (Solvent-treated)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.50	30.00±1.00^{b}	60.00±0.00^{b}	83.00±1.67^{b}	90.00±0.67^{b}
1.50	46.70±1.67^{bc}	80.00±2.65^{c}	93.30±1.67^{c}	96.70±0.67^{bc}
2.50	80.00±1.00^{d}	100.00±1.67^{d}	100.00±0.00^{d}	100.00±0.00^{d}

Each value is the mean of three replicates. Means followed by the same letter within each column are not significantly different (P<0.05) from each other, using New Duncan's Multiple Range Test.

Effect of Extract on Bruchids Emergence, Grain Damage and Percentage Reduction

The number of adult emergence and percentage reduction at the different concentrations of the extract after 6 weeks (42 days,) was presented in Table 2. The solvent-treated grains produced 98.67% adults and 0.0% reduction. While 0.5%, 1.5% and 2.5% concentrations reported adult emergence and % reduction of 16.67 and 83.11%, 6.67 and 93.25%, 1 and 98.99%, respectively.

Table 2. Emergence of Adult *C. maculatus* (after 6 weeks of storage) and Percentage of reduction.

Concentration (% w/v)	Mean no. of adults counted ± S.E	% Reduction (±S.E.)
Control (Solvent-Treated)	98.67±0.67^{d}	0.0±0.00^{a}
0.50	16.67±0.67^{c}	83.11±1.67^{b}
1.50	6.67±0.67^{b}	93.25±1.00^{bc}
2.50	1.00±0.00^{a}	98.99±0.00^{c}

Each value is the mean of three replicates. Means followed by the same letter are not significantly different (P<0.05) from each other, using New Duncan's Multiple Range Test.

Adult emergence and percentage reduction recorded for the varying extract concentrations and control exhibited a concentration dependent significant differences (Table 2). The damage inflicted on cowpea grains by *C. maculatus* after storage is shown in Table 3. The control (solvent-treated) recorded the highest level of damage (71.42%) and WPI 50.0, while 0.74% damage and 1.03 WPI were recorded for 2.5% extract concentration treated treatment.

Table 3. Damage by *C. maculatus* to the grains (after 6 weeks of storage)

Concentration (%w/v)	Total No. of grains	No. of grains damage	Grains undamaged	%damage	*WPI
Control (Solvent-Treated)	140	100	40	71.42	50.00
0.50	142	14	128	9.86	12.13
1.50	132	5	127	3.79	5.04
2.50	135	1	134	0.74	1.03

*Weevil Perforation Index (WPI). Value lower than 50 is an index of positive effect while WPI greater than 50 is an index of Negative protectant ability.

DISCUSSION

Result obtained from this study indicated that the root bark extract of *Z. xanthoxyloides* plant possesses an insecticidal potential (Adesina, 2005) on adult *C. maculatus*, thereby protecting stored cowpea grains from damaged by the bruchids. Fumigative or direct contact is usually the major mode of action of botanicals against adult insects in the laboratory tests (Rajapakse, 2006). Properties required in chemicals for controlling insects feeding on internal parts of stored products include: (i) Toxicity to adults; (ii) Reduction of oviposition; (iii) Ovicidal activity; and (iv) Toxicity to immature stages prior to or immediately following penetration of plant tissue (Ogunwolu and Odunlami, 1996). *Z. xanthoxyloides* was (i) toxic to adults through contact (ii) reduced oviposition, due to quick adult mortality within 4 days; and (iii) was also toxic to likely immature stages in the grains since only the larvae feed on the tissue (Southgate 1979), thereby reducing adult emergence and damage to grains. *Z. xanthoxyloides* contain isobutyl amides which has been shown to have strong insecticidal properties (Gregor, 1984; Yasuda et al., 1981) causing marked paralysis of mucous membranes. It was also reported to be about half as toxic as the pyrethrins to the house fly, *Musca domestica* L (Gregor, 1984; Yasuda et al., 1981).

Adult mortality increased significantly with days of exposure. The result showed that the concentration of *Z. xanthoxyloides* extract impact a dose-dependent mortality rate on exposed *C. maculates* while the efficacy of the extract also increased with exposure period. This is in agreement with Arannilewa et al., (2006) findings where *Sitophilus zeamais* showed a dose-dependent increase in adult mortality on exposure to *Aristolochia rigens* (Root bark), *Allium sativum* (Bulbs), *Ficus exasperate* (Leaves) and *Garcinia kola* (Seeds) extracts. The number of bruchids mortality reported by Arannilewa et al., (2006) also increased significant with days of exposure; meanwhile, no mortality was recorded for control treatment within four days of exposure.

The highest concentration (2.5% w/v) offerred effective control of *C. maculates* within 48 h on application by causing 100% mortality to bruchids as was observed. Previous studies on *Z. xanthoxyloides* have revealed its potency of evoking 100% mortality on *Sitophilus zeamais* and *C. maculatus* within 24 h of application (Arannilewa and Odeyemi, 2007). This further established Adesina (2005) statement that the plant possesses bioactive constituents (such as N-isobutylocta2,4- dienamide) which are potent insecticides.

The percentage reduction observed is an index of the activities of the plant materials in suppressing larval growth and adult emergence that invariably resulted into less grain damages, low WPI and good grain protection.

Zanthoxylum species occur in Central, North and South America, Africa, India, China, Japan and most other tropical areas of the Hemispheres (Altschul, 1973; Harrison et al., 1982; Sofowora, 1982). Hence, the plant can be exploited for possible mass production of bio-insecticide in various contries. A related species, like *Hawaiian Zanthoxylum*, has been confirmed by Marr and Tang (1992) to possess some active metabolites such as isobutylamides, aliphatic ketones, mono- and sesquiterpenes. Sofowora (1982) also found out that *Zanthoxylum spp.* root barks contained berberine, chelerythrine, canthine-6-one, P,-hydroxybenzoic acid, 2-hydroxymethyl benzoic acid, amillic acid and other phenolic acid derivatives. Some of these constituents may be responsible for the high toxicity observed in *Z. xanthoxyloides* plant. Constituents from the plant have been reported as active in reverting sickle-cell anaemia in vitro, and are active against some microorganisms (El-Said et al., 1971).

CONCLUSION

It is evident that *Z. xanthoxyloides* which has found a wide application as antitussive, antibacterial, antiviral and antivenin in human medicine, also possesses insecticidal properties. This therefore implied that it application as insecticide will present no or low health risk to man as against the conventional synthetic insecticide which is raising increasing public health concern. However, further investigation into determination of specific bioactive conponent, application dosage and modes of action of the bioactive components of the plant is recommended.

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كفاءة الفلفل الصيني *Zanthoxyllum xanthoxyloides* (Lam) Watern في مكافحة سوس اللوبياء *Callosobruhus maculatus* (F) (غمدية الأجنحة: Bruchidae)

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ملخص

تمّ اختبار كفاءة مستخلص اسيتون للفلفل الصيني *Xanthoxyloides Xanthoxyloides (Lam)* في مكافحة السوس في ظروف التخزين في المختبر على درجة حرارة 27 – 30 درجة مئوية، ورطوبة نسبية 60 – 70%. تمّ اختبار عدة تركيزات من الفلفل الصيني على سوس اللوبياء، وكانت التركيزات المُختبَرة 0.5%، 1.5%، 2.5% (وزن/حجم)، صفر بالمائة (شاهد). أظهرت النتائج أن المستخلص بنسبة 2.5% أعطى نتيجة موت بنسبة 100% خلال 96 ساعة من المعاملة. أدى تركيز المستخلص 2.5% من الفلفل الصيني إلى تخفيض معنوي في تطور معاملات الشاهد، 0.5%، 1.5% التي أدت إلى تخفيض نسبته صفر بالمائة، بنسبة 1.03، 5.04، 12.12، 50.00% والشاهد بعدسته أسابيع من التخزين، على التوالي. وكشفت هذه الدراسة أن الفلفل الصيني له مقدرة أن يكون مبيدا حيويا لاستعماله في مكافحة حشرات الحبوب المخزونة.

الكلمات الدالة: *Callosbruchus maculatus* و *Zanthoxylum xan thoxyloide*s، موت السوس، معامل عدد ثقوب السوس، مبيد حيوي، مستخلص نباتي.

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