Original Paper   http://indexmedicus.afro.who.int

Bioactivities of crude extracts of the candlewood *Zanthoxylum xanthoxyloides* Lam. (Rutaceae) against the cowpea beetle *Callosobruchus maculatus* (Walp)

B.D.H. BISSELEUA [1*], D. OBENG-OFORI [2] and W.S.K. GBEWONYO [3]

[1] *Laboratory of Entomology, IRAD, P.O. Box 2067, Yaoundé, Cameroon.*
[2] *Crop Sciences Department, University of Ghana, P.O. Box 82 Legon, Accra, Ghana.*
[3] *Department of Biochemistry, University of Ghana, P.O. Box 52 Legon, Accra, Ghana.*
[*] *Corresponding author, E-mail: hbissel@gwdg.de , Tel: +237 77689627; +237 96944497.*

ABSTRACT

The efficacy of petroleum-ether crude extracts of the candlewood, *Zanthoxylum xanthoxyloides* Lam. was assessed for their contact toxicity, feeding and oviposition deterrence to the cowpea beetle *Callosobruchus maculatus* (Walp). Percent mortality, number of progeny produced and damage caused to were dose–dependent. Root extracts was the most effective as toxicant to the beetles. The LD_{50} in 24 h topical application of root extracts was 4.98 µg. The LD_{50} (96 h toxicity in grain) suggests that root extract was highly toxic to *C. maculatus*. Development of eggs and larvae within grain kernels, as well as progeny emergence were significantly inhibited in treated grains. There were no progeny produced by *C. maculatus* in grains treated with dosage ≥ 0.4 g per 100 g of grain. Root extracts provided the greatest protection of cowpea against feeding by *C. maculatus* with no observable feeding damage to grains treated with the highest dosages of the three materials. Extracts were repelled *C. maculatus* but with considerable variation in their repellent action. *Z. xanthoxyloides* contains phytochemicals, and crude extracts could be used as a botanical insecticide in alternative control strategies against *C. maculatus*.
© 2008 International Formulae Group. All rights reserved.

Keywords: Antifeedant, Bruchids, Botanical insecticides, Reproduction retardant, Toxicity.

INTRODUCTION

The constant growth of the world's population and the soaring of food prices require substantial resources for the production of food. Post-harvest protection has an important role to play in achieving this objective. Post-harvest losses to storage insect pests such as *Callosobruchus maculatus* (F.) are recognized as an increasing constraint in Africa causing a significant threat to food security and household incomes particularly when losses are severe (Tapondjou et al., 2002; Owusu et al., 2007).

Control measures applied against *C. maculatus* are the heavy use of gaseous fumigants and residual chemical insecticides. However, the appearance of insect populations resistant to conventional insecticides and the high cost of synthetic insecticides, together with the growing environmental and health concern over the use of many synthetic insecticides (Obeng-Ofori et al., 1997; Belmain et al., 2001) have stimulated interest in the development of alternative control strategies such as botanical insecticides (Owusu, 2001; Huang et al., 2002; Liu et al., 2002; Tapondjou et al., 2005; Kestenholz et al., 2007; Isman, 2008). The use of these plant materials has recently induced active research to establish the scientific basis for their continued use regarding their efficacy, active constituents and effective application technology (Huang et al., 2000; Belmain et al., 2001; Boeke et al., 2004a; Rajendran and Sriranjini, 2008).

The grain protecting potential of Candlewood, *Zanthoxylum xanthoxyloides*, traditionally use as food flavoring, animal feed (Irwine, 1961) or in the treatment of various ailments and in post-delivery pain

relieving (Abbiw, 1990) has been reported by Koomson (2003) and Udo et al. (2004).

This study investigated the scientific basis for the use of leaves, stems, and roots of the candlewood *Z. xanthoxyloides* for grains protection in Africa. The bioactivity of dry and ground leaves, stem and root extracts against *C. maculatus* in stored cowpea was evaluated.

MATERIALS AND METHODS
Culturing of insects

The parent stocks of *C. maculatus* were reared on black-eyed cowpea sterilized by heat disinfestations for three hours at 60 °C. One hundred randomly selected unsexed adults of *C. maculatus* were introduced into 500 g of sterilized cowpea in rearing jars and kept in the laboratory. The laboratory was maintained at 27 ± 2 °C, 65-70% r.h. and 12:12 LD. The seeds were sieved to remove the parent adults after 14 days. Progeny emergence began after 21 days and the emerging F1 adults were used for the various assays.

Plant materials

Two kilograms of fresh leaves and five kilograms of fresh stem, and roots from *Z. xanthoxyloides* were collected in October 2000 in Accra. The collected plant parts were oven dried at 40 °C for 5 days and milled into powder using a Fritsch (TÜV- CERT, Germany) milling machine. The powdered materials were kept at 4 °C. The plant was identified by the University Herbarium, Botany Department, University of Ghana, Legon, where a voucher specimen is deposited.

A 1:10 weight of powdered plant material to volume of extracting solvent (i.e. 30 g of powder from plant material to 300 ml of petroleum ether [Chimtex, Ghana] extract was prepared. The extraction was done at room temperature. The mixture of solvent and various powders was stirred with a magnetic stirrer for eight hours. The extracts were then filtered with a Buchner filtration system using a vacuum pump (Edwards High Vacuum Ltd., Crawley, England). The filtrates were collected in labeled flat bottom flasks and concentrated in vacuum and the concentration of crude extracts was determined. To prepare the concentrations for topical applications, the crude extracts were further concentrated by measuring 200 µg of each extract into 2 ml vial, drying with N_2 gas (obtained from Air Liquide, Ghana) and redissolving in acetone. Fresh leaves were collected and subjected to hydrodistillation using a Clevenger–type apparatus.

Contact toxicity by topical application

A hand microapplicator (Burkard Manufacturing Co, Ltd.) was used to topically apply acetone [Chimtex, Ghana] solutions of the crude extracts to adult beetles. One microlitre of the test solution was applied on the thorax of four-day-old adult *C. maculatus* of mixed sexes selected randomly. Ten insects were used for each treatment and treatments were replicated four times. The doses vary geometrically from 0.625 to 10 µg/insect. The treatment series included four groups of *C. maculatus* treated with acetone alone to serve as controls. The beetles were anaesthetized with CO_2 (obtained from Accra Brewery Ltd., Ghana) and treated at the rate of 10 per minute. Each group of beetles was held in a Petri dish (8.5 cm diameter) for 24 hours after treatment and the number of dead insects was recorded.

Effect of extracts on *C. maculatus* in grain

Toxicity of petroleum-ether extracts against beetles in cowpea grains was tested in the laboratory by applying extracts (leaves, stems and roots) at rates of 0.016, 0.08, 0.4, 2, and 10 g of extracts/100 g of grains. One hundred grams of sterilized and pre-equilibrated grains were measured into 200 ml plastic containers covered with muslin cloth held in place with rubber bands. The control consisted of grains treated with the solvent alone and then dried. Unsexed adult insects were introduced into the treated and control grains and left in a controlled environment room at 28 ± 2 °C, 65 % r. h and 12:12 LD. Twenty unsexed adults of *C. maculatus* (3 - 4 days old) were used. Each treatment was replicated four times. The number of dead insects in each plastic container was counted after 24, 48, 72, and 96 hours to estimate mortality.

Effect of extracts on eggs and immature stages

The effect of *Z. xanthoxyloides* on the

development of eggs and larvae of *C. maculatus* in grains was investigated. Five hundred grams of equilibrated cowpea placed in 1-litre glass jars were infested with 250 adults of *C. maculatus* to allow for egg laying. The parent adults were removed after seven days. One day after adult removal, 25 g of infested cowpea were treated with 2 g of extract (in 2 ml of petroleum ether) of dry and ground leaves, stems, and roots. Thereafter, these treatments were repeated at one and two weeks after adult removal to determine the effect of the extracts on the early and late instars larvae of *C. maculatus*. Each treatment was replicated four times. The control was treated with petroleum-ether alone. Adults emerging subsequently were counted for a period of 5 weeks following the removal of adults.

Progeny production and damage assessment

The effect of crude extracts of *Z. xanthoxyloides* on F_1 progeny produced by *C. maculatus* was investigated in cowpea treated with 0.016, 0.08, 0.4, 2, and 10g/100 g of grains. One hundred grams of pre-equilibrated cowpea grains were treated with the above concentrations of each extract dissolved in 2 ml petroleum-ether. The solvent was allowed to completely evaporate within 3 hours after application and twenty adults of *C. maculatus* were introduced onto the grains. The containers were covered with white muslin cloth held in place with rubber bands. Control treatments consisted of grains mixed only with petroleum-ether. After a week oviposition period, the parent adults were removed and insects subsequently emerging were counted daily for 4 weeks. Thereafter, the damage caused to the grains by *C. maculatus* was assessed.

To assess the level of damage, samples (100 grains) were taken from each jar and the number of damaged grains (with characteristic hole(s)) and undamaged grains were counted and weighed. Percent weight loss was calculated using the method of Udo et al. (2004) as follows:

% WL = [(UNd - DNu) / U(Nd + Nu)] x 100

Where, WL = Weight loss; U = weight of undamaged grains; D = weight of damaged grains; Nd = number of damaged grains; Nu = number of undamaged grains

Repellency

The area preference test described by Obeng-Ofori et al. (1997) was used to evaluate the repellent action of extracts (dry leaves, dry stems and dry roots) against *C. maculatus*. Test arenas consisted of 9 cm Whatman's No. 42 filter papers cut in half. Different doses of the test materials were prepared (0.625 to 10 µg cm^{-2}). Each solution was applied to a half filter paper disc as uniformly as possible with a pasture pipette. The other half of the filter paper was treated with acetone alone. The extract-treated and acetone–treated (control) half discs were air-dried to evaporate the solvent completely. Full discs were then remade by re-attaching two halves (extract/acetone treated) of the same dimensions with sellotape. Each filter paper was placed in a Petri dish and 10 adults' *C. maculatus* (2 - 4 days old) of mixed sex were released at the center of each filter paper disc and covered. Each treatment was replicated four times. The number of insects present on control (Nc) and treated (Nt) strip were recorded after 1 hour exposure.

Percent repellency (PR) values were computed as follows: PR = [(Nc - Nt) / (Nc + Nt)] x 100

PR data were analyzed using ANOVA after transformation into arcsine values. All negative PR values were treated as zero (Obeng-Ofori et al., 1997).

Statistical analysis

Data involving counts were transformed using square root (y = $\sqrt{x+0.5}$) transformation while those involving percentages were transformed using arcsine (y = $\sin^{-1}\sqrt{x/100}$) transformation before analysis. Means were separated using LSD. Probit analysis for the determination of LD_{50} was based on the methods of Finney (1971) and analyzed using SPSS for Windows. Correction of natural mortality in control treatment was done using Abbott (1925) formula.

RESULTS
Contact toxicity by topical application

For all extracts, the percentage mortality increased with increasing dose of extracts. The root extract was more toxic than the extracts from other parts of the plant, based on LD_{50} values (Table 1).

Table 1: Lethal dose toxicity (LD_{50}) of crude extracts of *Z. xanthoxyloides* applied topically to *C. maculatus* after 24 hours.

Plant species	Extracts	LD_{50} (µg/insect)	95 % Fiducial limits		χ^2	Slope
			Upper	Lower		
Z. xanthoxyloides	Leaves	9.98	12.95	7.01	14.36	0.96
	Stem	8.00	10.83	5.17	15.03	1.29
	Roots	4.98	7.46	2.50	14.98	3.42

Table 2: Lethal dose toxicity (LD_{50}) of crude extracts of *Z. xanthoxyloides* on *C. maculatus* in grains after 96 hours.

Plant species	Extracts	LD_{50} (g/100g of grain)	95 % Fiducial limits		χ^2	Slope
			Upper	Lower		
Z. xanthoxyloides	Leaves	0.10	0.15	0.00	7.35	1.09
	Stem	0.12	0.16	0.00	21.28	1.19
	Roots	0.18	0.28	0.01	17.62	3.16

Effect of extracts on *C. maculatus* in grain

The extracts showed different levels of toxicity to *C. maculatus* in grain. Toxicity to *C. maculatus* was dose–dependent and no mortality was observed in untreated grains. The extracts significantly (P < 0.05) induced high mortality of *C. maculatus* in treated grains compared to untreated controls, with stem and root extracts inducing 100% mortality of *C. maculatus* after 96 hours. The Extracts were not that active within 24, 48 and 72 hours period of exposure on the beetles. The LD_{50} of these dose-response curves at 96 hours are given in Table 2.

Effect on eggs and immature stages

The effect of different plant extracts of *Z. xanthoxyloides* on the development of eggs and larvae of *C. maculatus* inside grains are presented in figure 1. Cowpea grain treated with crude extracts of *Z. xanthoxyloides* was significantly (P < 0.05) toxic to the eggs and larvae of *C. maculatus* compared to control. Root extracts of *Z. xanthoxyloides* significantly (P < 0.05) reduced progeny emergence of *C. maculatus* during the trial period and completely inhibited the development of *C. maculatus* when applied one day after oviposition.

Progeny production and damage assessment

Crude extracts from the different parts of *Z. xanthoxyloides* significantly (P < 0.05) reduced the number of F_1 progeny produced by *C. maculatus*. All the materials applied at 0.4 g extracts per 100 g of grains completely (P < 0.001) inhibited progeny emergence of *C. maculatus* compared to the control (Figure 2). At lower concentrations the materials were less effective in reducing the number of F_1 progeny produced by *C. macularus*. The action of the materials was thus dosage–dependent. The extracts caused a significant reduction in the damage caused by *C. maculatus* to treated cowpea grains compared to the control. More than 50% damage was observed in grains treated with lower concentrations of crude extracts and the control.

Repellency

The various extracts showed different levels of repellency to *C. maculatus* (Figure 3). Analysis of variance indicated significant differences (P < 0.001) between the responses of *C. maculatus* to the plant materials of *Z. xanthoxyloides*. Except for stem extracts of *Z. xanthoxyloides*, applied at 0.625 and 1.25 µg.cm^{-2}, all the plant materials were repellent to *C. maculatus*. However, repellency was not dose–dependent.
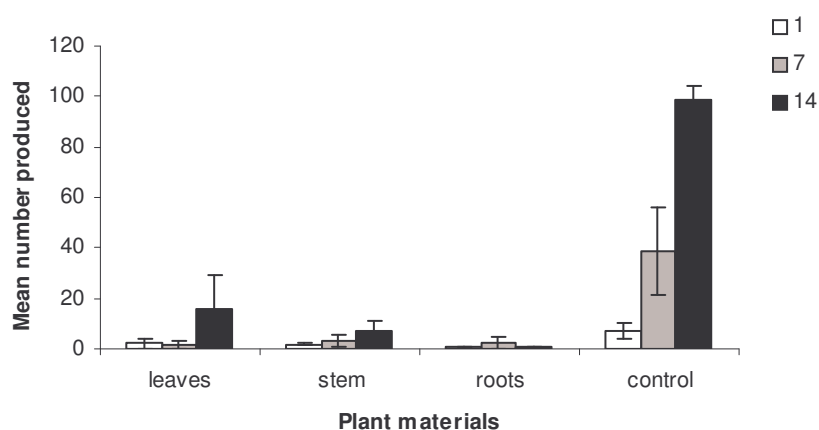
Figure 1: Mean number of *C. maculatus* adults produced in cowpea treated with *Z. xanthoxyloides* extracts at different times after oviposition period.
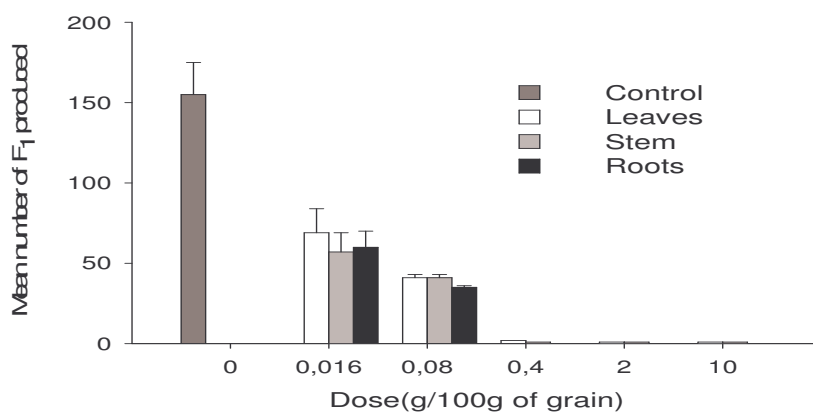

Figure 2: Effect of crude extracts of *Z. xanthoxyloides* on the number of F_1 progeny produced by *C. maculatus*.
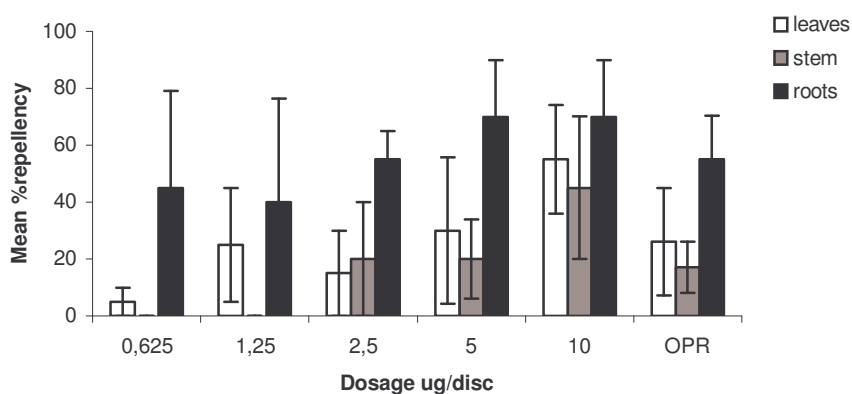

Figure 3: Mean percent repellency values of different plant parts from *Z. xanthoxyloides* to *C. maculatus*. OPR: Overall percent repellency.

DISCUSSION

Crude extracts from the various parts of *Z. xanthoxyloides* applied topically to insects or on grains were toxic to *C. maculatus*, although activity varied with plant parts. Significant insecticidal activity (> 80% mortality) was obtained with root extracts. The basis for toxicity by topical application of plant extracts to stored product pests has been fairly documented (Tripathi et al. 2002). However, many studies have drawn the attention of plant extracts on adult mortality (Boeke et al., 2004b; Tapondjou et al., 2005; Kestenholz et al., 2007; Rajendran and Sriranjini, 2008). Stem and root extracts applied to cowpea grains at doses ≥ 0.4 g per 100 g of grains, provided the greatest protection to cowpea against beetle attack. Beetles killed in treated grains appeared paralyzed with their metathoraxic wings unfolded and stretched outside the elytra (Obeng–Ofori et al., 1997), suggesting that toxicity was not due only to ingestion of treated grains but also through contact. This also suggests that the bioactivity of the different parts against insects may depend on several factors, including chemical composition, species susceptibility and variation in insect behaviour (Owusu et al., 2007). Further research is needed in order to establish precise mode(s) of action.

Udo et al. (2004) reported that dry plant parts (leaves, bark and roots) of *Z. xanthoxyloides* tested at a concentration of 5 % (wt/wt) and methanol extracts of fresh and dry plant parts of *Z. xanthoxyloides* were toxic to *C. maculatus*. Our results suggest that the active ingredient present in *Z. xanthoxyloides* plant parts acts more by contact than through ingestion. However, as phytochemicals information about this plant is unavailable, it not known how much variability of secondary metabolites has biased our results.

Crude extracts of different plant parts of *Z. xanthoxyloides* caused significant reduction in feeding damage, number of F_1 progeny produced, and inhibition of the development of eggs, and larvae of *C. maculatus*. Crude extracts from *Z. xanthoxyloides* has been documented as suppressing the hatching and reducing the subsequent survival rate of larvae of *Sitophilus zeamais* (Owusu et al., 2007). Ogunwolu and Odunlami (1996)

reported the reproduction suppression properties of root bark powder of *Z. xanthoxyloides* against *C. maculatus* at doses of 0.15 – 3 g per 20 g of stored cowpea. Udo et al. (2004) observed that fresh bark methanol extracts of *Z. xanthoxyloides* caused complete inhibition of the development of eggs, larvae and pupae of *C. maculatus* within 14 days after oviposition and complete inhibition of the development of the first filial generation of *C. maculatus* or completely protected grains against damage caused by *C. maculatus*. However, in their studies extracts from dry materials of *Z. xanthoxyloides* were found to be less toxic to the beetles.

Our results indicate the higher protecting potential of these materials against insect damage in storage. The complete protection of grains by some extracts of *Z. xanthoxyloides* applied at dosage ≥ 0.4 g per 100 g of grain, may suggest the presence of antifeedant, growth regulatory and ovicidal properties in the plants (Akhtar and Isman, 2004; Omar et al., 2007). This indicates a rationale for the incorporation of the roots, stems and leaves of *Z. xanthoxyloides* into grain protection practices of certain communities in Ghana and Africa (Abbiw, 1990).

Extracts from *Z. xanthoxyloides* were repellent to the beetles. There was considerable variation in the repellent action of the various plant materials and this may reflect the complexity of the chemical composition of the materials. Although it is more likely that the repellent property of *Z. xanthoxyloides* is what is employed when leafy branches of these plants are layered with grain to protect them from storage pests, or leaves are burned in houses to repel mosquitoes and related biting flies, there is also the possibility of volatile oils playing an important role. Essential oils could not however be obtained in substantial quantities from the plants for biossay. Tropical plant species might form a new source of compounds for protection of storage pests.

In summary, at lower concentrations, the extracts did not affect progeny production of *C. maculatus*. The toxic effect coupled with the repellent action of this tropical plant increase the protection potential of these plant materials against grain damages. A combination of the toxic and repellent

properties are however, probably evoked when the fresh leaves of the plants are burned in homes. However, under practical storage conditions, relatively higher doses may be required as compared to doses applied in the laboratory experiment. Further work is in progress to isolate, identify and exploit the active constituent(s) in leaves and root of *Z. xanthoxyloides* as promising stored grain protectant(s).

ACKNOWLEDGMENTS

REFERENCES

Abbiw DK. 1990. *Useful Plants of Ghana*. Kew Intermediate Technology Publication and the Royal Botanic Gardens.

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J. Entomol.*, **18**: 265 - 267.

Akhtar Y, Isman MB. 2004. Comparative growth inhibitory and Antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. *J. Appl. Ent.*, **128**: 32 – 38.

Boeke SJ, Kossou DK, Van Huis A, Van Loon JJA, Dicke M. 2004a. Field trials with plant products to protect stored cowpea insect damage. *Inter. J. Pest Manag.*, **50**: 1 – 9.

Boeke SJ, Baumgart IR, Van Loon JJA, Van Huis A, Dicke M, Kossou DK. 2004b. Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against *Callosobruchus maculatus*. *J. Stored Prod. Res.*, **40**(4): 423 – 438.

Belmain SR, Neal GE, Ray D, Golob P. 2001. Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. *Food Chem. Toxicol.*, **39**: 287 – 291.

Finney DJ. 1971. *Statistical Method in Biological Assay* (3rd edn). Charles Griffin and Company Ltd: London.

Huang Y, Lam SL, Ho SH. 2000. Bioactivities of essential oil from *Elletaria cardamomum* (L.) Manton to Sitophilus zeamais Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst). *J. Stored Prod. Res.*, **36**: 107-117.

Huang Y, Ho SH, Lee HC, Yap YL. 2002. Insecticidal properties of eugenol, isoeugenol and methyl eugenol and their effects on nutrition of Sitophilus zeamais Motsch. (Coleoptera: Curculionidae) and Tribolium castaneu,m (Herbst) (Coleoptera : Tenebrionidae). *J. Stored Prod. Res*, **38**: 403-412.

Irvine FR. 1961. *Woody Plants of Ghana with Special Reference to their Uses*. Oxford University Press: London.

Isman MB. 2008. Perspective botanical insecticides: For richer, for poorer. *Pest Manag. Sci.*, **64**: 8– 11.

Kestenholz C, Stevenson PC, Belmain SR. 2007. Comparative study of field and laboratory evaluations of the ethnobotanical *Cassia sophera* L. (Leguminosae) for bioactivity against the storage pests *Callosobruchus maculatus* (F.) (Coleoptera : Bruchidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *J. Stored Prod. Res.*, **43**: 79 – 86.

Koomson CK. 2003. On-farm evaluation of candlewood *Zanthoxylum xanthoxyloides* (Lam) on two stored product pests. M.Phil Thesis, University of Ghana, Ghana, p. 86.

Liu ZL, Xu YJ, Wu J, Goh SH, Ho SH. 2002. Feeding deterrents from Dictamnus dasycarpus Turcz against two stored-product insects. *J. Agric. Food Chem.*, **50**: 1447-1450.

Obeng-Ofori D, Reichmuth CH, Bekele J, Hassanali A. 1997. Biological activity of 1,8 cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored product pests. *J. Appl. Entomol.*, **121**: 237 - 243.

Omar S, Marcotte M, Fields P, Sanchez PE, Poveda L, Mata R, Jimenez A, Durst T, Zhang J, MacKinnon S, Leaman D, Arnason JT, Philogène BJR. 2007. Antifeedant activities of terpenoids isolated from tropical Rutales. *J. Stored Prod. Res.*, **43**(1): 92 – 96.

Owusu EO. 2001. Effect of some Ghanaian plant components on control of two

stored-product insect pests of cereals. *J. Stored Prod. Res*., **37**: 85-91.

Owusu EO, Osafo WK, Nutsukpui ER. 2007. Bioactivities of candlewood *Zanthoxulum xanthoxiloides* (Lam.) solvent extracts against two stored-product insect pests. *African J. Sci. Tech*., **8**(1): 17- 21.

Rajendran S, Sriranjini V. 2008. Plant products as fumigants for stored-product insect control. *J. Stored Prod. Res*., **44**: 126 – 135.

Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth Ch. 2005. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. *J. Stored Prod. Res*., **41**: 91–102

Tapondjou LA, Adler C, Bouda H, Fontem

DA. 2002. Efficacy of powder and essential oil from Chenopodium ambrosioides leaves as post-harvest grain protectants against six-stored product beetles. *J. Stored Prod. Res.*, **38**: 395- 402

Tripathi AK, Prajapati V, Verma N, Bahl JR, Bansal RP, Khanuja SPS. 2002. Bioactivities of the leaf essential oil of *Curcuma longa* (Var. Ch-66) on three species of stored-product beetles (Coleoptera). *J. Econ. Entomol.*, **95**: 183– 189.

Udo IO, Owusu EO, Obeng-Ofori D. 2004. Efficacy of candlewood *Zanthoxylum xanthoxyloides* (Lam) for the control of *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae) and *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *Global J. Agric. Sci*., **3**: 19-23.