Bioefficacy of *Zanthoxylum xanthoxyloides* and *Securidaca longependuncata* against *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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ABSTRACT

Prostephanus truncatus Horn (Coleoptera: Bostrichidae) and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) are two important stored product insect pests found in most storage warehouses. Their activities tend to reduce the viability and the nutritive values of seeds and grains. In this study the bio-efficacy of *Zanthoxylum xanthoxyloides* and *Securidaca longependuncata* against *P. truncatus* and *T. castaneum* was investigated. The biological effects of methanol extracts of dry roots and leaves of *Z. xanthoxyloides* and the roots of *S. longependuncata* at 0.25 g mL^{-1}, 0.5 g mL^{-1}, 1.0 g mL^{-1} and 2.0 g mL^{-1} on the two beetle species were determined in the laboratory using dipping, grain treatment, progeny emergence, repellency and grain damage assessment assays. Adult mortality by dipping ranged between 80% and 100% in both insects for the plants extracts. There was no survival recorded at 2.0 g mL^{-1} of *Z. xanthoxyloides* roots and leaves and *S. longepedunculata* roots for both insects in treated grains. Also, no adult emerged in grains treated with 2.0 g mL^{-1} of the *Z. xanthoxyloides* roots while the same concentration of *S. longepedunculata* resulted in only 2% adult emergence for both insects. The methanol extracts of the two plants yielded various levels of repellency against the insects. 2.0 g/mL^{-1} of the *Z. xanthxyloides* roots yielded the highest repellency of 90.0 ± 9.5 % and 100 ± 0.0% against *P. truncatus* and *T. castaneum* respectively. Grains treated with plant extracts significantly reduced damage caused by *P. truncatus* and *T. castaneum* compared with the untreated grains.

Key words: Bioefficacy, *Prostephanus truncatus,* repellency, *Tribolium castaneum*, *Securidaca longependuncata, Zanthoxylum xanthoxyloides*

INTRODUCTION

Maize (*Zea mays*) play a predominant role in the diets of people all over the world. Cereals are the cheapest sources of food energy and contribute a high percentage of calories and proteins in the diets of Ghanaian population (ISSER, 2006). Stored-product arthropods can cause serious postharvest losses, estimated from up to 9% in developed countries to 20% or more in developing countries (Obeng-Ofori, 2008). The Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a worldwide stored product pest which attacks stored grain products like cereals, pasta, biscuits, beans, nuts, etc. and causing considerable quantitative and qualitative losses (Obeng-Ofori, 2008). Introduced into Africa and for that matter Ghana in the 1980's, the larger grain borer *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) continues to establish and threaten the production of grains and dried cassava. Under many circumstances the easiest, most rapid and economical method of controlling insects is the use of insecticides (Boateng and Obeng-Ofori, 2008). However, most of the contact insecticides used in the stored product insect pest management are lipophilic and accumulate in areas of high fat content such as the germ and bran of cereals (Mensah *et al.,* 1979). These toxic residues tend to persist in the treated products which may be detrimental to the consumer, affect non-target insect pests (natural enemies), lead to insecticide resistance and may have an effect on the applicator.

The use of natural products has been found to be effective against stored product insect pests (Owusu *et al.,* 2007). These are considered to be cheap, easily biodegradable and readily available for stored product protection. *Securidaca longepedunculata* Fress (Polygalaceae) a semi-deciduous shrub or small tree that grows to 12 m tall, with an often flattened or slightly fluted bole. It is spiny and much branched, with an open, rather straggly looking crown. Research shows that the bark contains methyl salicylate which possesses repellent effects against insects. The presence of saponins in the bark and crushed seeds give a soapy solution in water and are used as soap for washing or bleaching clothes (Orwa *et al*., 2009). *Zanthoxylum xanthoxyloides* (Rutaceae) a shrub that can grow to a small tree of up to 1.25 m high and 0.13 m girth (Irvine, 1961). The medicinal property of the plant has been established and has been shown to possess insecticidal activity against some stored product pests (Udo, 2000, 2011). This paper investigated the effect of methanol extracts of leaves and roots of these two plants against *P. truncatus* and *T. castaneum* in the laboratory.

MATERIALS AND METHODS

Insect cultures

Prostephanus truncatus and *T. castaneum* were cultured in the laboratory on whole maize grains wheat flour, respectively in glass jar bottles. The setup was maintained at a temperature of 30 ± 2 ^{0}C, 70 % relative humidity and 12: 12 (L: D) photo regime. The adult insects were removed a week after oviposition to allow new progenies to emerge which were then used for the experiments.

Preparation of Plant Extracts

Zanthoxylum xanthoxyloides and *S. longepedunculata* were obtained from the wild around University of Ghana, Legon campus. The roots and leaves of these plants were air-dried under room temperature for 14 days and were pulverized into powder with traditional mortar and pestle. Methanol (100%) was used as the solvent for the crude extraction. Four sets of the powders of roots and leaves (500 g each) soaked in methanol (750 mL) were prepared for each plant. These were left to stand for two days in the laboratory and were filtered and concentrated using the Rotary evaporator. Concentrations of 2.0 g mL^{-1}, 1.0 g mL^{-1} 0.5 g mL^{-1} and 0.25 g mL^{-1} of each plant extract was prepared in methanol and used for the bioassays.

Toxicity of Methanol Extracts on the Adult Insects

Twenty (0-3 day-old) adult insects each was dipped singly into the different concentrations of the plants extract (2.0 g mL^{-1}, 1.0 g mL^{-1}, 0.5 g mL^{-1} and 0.25 g mL^{-1}) for 2 seconds These were transferred into clean petri dishes lined with filter paper containing maize and groundnuts separately for the *P. truncatus* and *T. castaneum* respectively. Methanol (100%) only was used as the control. There were four replications of each treatment and these were kept under laboratory conditions as mentioned above. Mortality was recorded after 72 hrs. Insect were considered dead if they did not respond to prodding with a blunt probe.

Survivorship of adult insects in treated maize grains and damage assessment

Whole maize (1 kg each) was sterilized in the oven at 60^{O}C for 3 h to ensure there was no hidden infestation. Maize (100 g each) was put into glass jars and treated with the different concentrations (2.0 g mL^{-1}, 1.0 g mL^{-1}, 0.5 g mL^{-1} and 0.25 g mL^{-}) of the plants extracts. The control was treated with Methanol only. The treated grains were air-dried for 1 h and 20 cohort of adults each of *P. truncatus* and *T. casteneum* were introduced into the treated maize. There were four replications for each treatment and maintained under laboratory conditions. Grains were sieved daily for 7 days and dead and live insects were recorded. Dead insects included those which could not respond to prodding with a blunt probe. Live insects were returned into the jars on each occasion until after the 7th day when all adults were removed and the culture left to stand for 40 days after which damage was assessed. The damage caused by insects was determined using Count and Weigh method as follows: Weight loss (%) = [(Und – Dnu) / U(Nd + Nu)] x 100%

where U = Weight of undamaged grains, D = Weight of damaged grains, Nd = Number of damaged grains, Nu = Number of undamaged grains

Effects of Extracts on progeny development and adult emergence

The effect of the extracts on progeny development and adult emergence was determined by modifying the method employed by Udo (2000). Grains (100 g each) were put into separate glass jars and treated with 2.0 g mL^{-1}, 1.0 g mL^{-1}, 0.5 g mL^{-1} and 0.25 g $mL^{-1}$of the plants extracts . These were then infested with 50 unsexed adults of *P. truncatus* and *T. casteneum.* The adults were sieved out after two weeks and the set up was monitored for 20 days for larval and pupal development (Hodges, 1986; Obeng-Ofori, 2008). Methanol (100%) only was used as the control. There were four replications of each treatment. The number of adults that emerged after the 20 day period were counted and recorded.

Repellent Effect of the Plant Extracts

The repellent effect of the extracts was determined using the method adopted by Sheehu (2009). Concentrations (2.0 g mL^{-1}, 1.0 g mL^{-1}, 0.5 g mL^{-1} and 0.25 g mL^{-}) of the plants extracts were applied to half filter paper disc; the other half was treated with Methanol (100%) only which served as the control. The treated filter paper was air dried for 1 hr after which the two halves were joined together and placed in a Petri dish. Twenty adult insects which had been immobilized in the refrigerator at 4^{O}C for 2 min were then placed in the middle of the joined filter papers and covered. There were four replications of each

Table1. Contact toxicity (%), mean adult emergence (± SE), mean % repellency (± SE) and root and leaves on damage in terms of mean weight loss (± SE) of methanol extracts of *Z. xanthoxyloides* root and leaves against *P. truncatus* and *T. castaneum* mortality (± SE) by dipping

Parameters	Treatment (g/ml)	Root		Leaves	
		P. truncatus	*T. castaneum*	*P. truncatus*	*T. castaneum*
		Z. xanthoxyloides			
Contact toxicity	2.0	100.0±0.0	90.0 ±1.0	100.0 ± 1.5	80.0± 1.7
	1.0	95.0±0.5	90.0 ±1.0	30.0 ± 2.8	30.0±2.7
	0.5	73.0± 2.5	75.0 ±2.4	27.0 ±2.5	25.00±2.4
	0.25	70.0±2.8	70.0±2.7	5.0±0.5	15.00 ±1.2
	0	0.0	0.0	0.0	20.00± 1.4
Mean adult emergence	2.0	0.00±0.	0.00±0.00	0.25 ±0.01	0.00±0.00
	1.0	13.00±2.30	12.00±2.00	1.75 ±0.25	2.75±0.05
	0.5	19.25±4.10	16.50 ±2.55	11.0 ±2.10	4.75±2.05
	0.25	23.50±2.50	20.00 ±2.50	21.25 ±2.25	13.25± 1.45
	0	26.25±2.30	26.25 ±2.25	26.25 ±3.25	26.25±3.35
Mean % repellency	2.0	90.0±9.5	100.0 ± 10.0	60.0 ±3.5	100.0± 10.0
	1.0	90.0±9.5	100.0 ± 10.0	40.0 ±2.8	80.0±8.2
	0.5	80.0±7.5	25.0 ±2.8	40.0 ±2.9	80.0±8.2
	0.25	70.0±6.5	10.0 ±0.8	25.0 ±0.7	80.0±8.2
Weight loss	2.0	0.47±0.01	0.80 ±0.04	0.0 ±0.00	0.00±0.00
	1.0	1.60±0.08	1.37 ±0.05	0.17 ±0.05	0.65±0.08
	0.5	8.50±2.10	5.75 ±0.52	0.17 ±0.05	0.65±0.08
	0.25	9.60±2.30	6.00 ±2.00	0.90 ±0.05	2.50±0.65
	0.0	44.50±5.3	17.50 ±3.50	44.50 ±3.25	17.50±1.25

treatment and the percentage repellency was determined using the formula adopted by Obeng- Ofori *et al*. (1997)

PR = [(Nc - Nt) / Nc + Nt)] X 100%

where Nc: Number of insects on control, Nt: Number of insects on the extract. All negative PR were treated as zero.

Data Analysis

Data involving counts and percentages were square root and arcsine transformed, respectively before analyses using Analysis of Variance at 0.05 probability level using GenStat Statistical Package 9.2 (9th Edition). Where significant differences existed means were separated using the Least Significant Difference (LSD)

RESULTS

Contact toxicity

There was significant ($P < 0.05$) difference in the mortalities of insects for all plants parts used for the treatment. In all the treatments percentage mortalities of the insects increased as the concentration increased (Table 1). The root and leaf extracts (2 g mL^{-1}) of *Z. xanthoxyloides* induced 100% mortality in *P. truncates,* and 90% and 80%, respectively in *T. castaneum* after 72 hrs. These values compared favourably with the mortality observed in the root extract of the reference plant (*S. longepedunculata*) extracts where 100% and 95% mortalities were observed against *P. truncatus* and *T. castaneum.*

Survivorship of adult insects in treated grains

The percentage survival of *P. truncatus* on treated grains was higher at lower concentrations (< 1 g mL^{-1}) for both root and leaf extracts (Fig. 1, 2). The leaf extract treatment appeared to have a more deleterious effect on the insects as there was only 10% survivorship in the grains treated with 2 g mL^{-1} after one day and this reduced to 0% after three days while the same concentration of root extract showed about 60% survivorship after one dayand reduced to 0% after six days

Table 2. Contact toxicity (%), mean adult emergence (± SE), mean % repellency (± SE) and root and leaves on damage in terms of mean weight loss (± SE) of methanol extracts of *S. longepedunculata* root against *P. truncatus* and *T. castaneum* mortality (± SE) by dipping

Parameters	Treatment (g/ml)	*P. truncatus*	*T. castaneum*
contact toxicity	2.0	100.0±0.0	95.0±1.0
	1.0	100.0±0.0	95.0±1.0
	0.5	95.00±1.3	93.0±3.5
	0.25	90.00±3.4	83.0±2.5
	0	0.00±0.0	0.00±0.0
Mean adult emergence	2.0	2.00±0.50	3.00±0.60
	1.0	8.00±2.50	10.00±2.40
	0.5	16.25±2.40	17.25±2.30
	0.25	23.00±3.50	25.00±3.40
	0	26.25±2.30	26.25±2.30
Mean % repellency	2.0	60.0±3.5	80.0±8.2
	1.0	60.0±3.5	80.0±8.2
	0.5	60.0±3.5	40.0±2.5
	0.25	25.0±0.7	40.0±2.5
Weight loss	2.0	3.08±1.18	0.00±0.00
	1.0	8.17±3.25	1.09±0.02
	0.5	9.33±3.30	1.64±0.01
	0.25	17.17±4.50	5.45±2.15
	0.0	44.50±8.35	17.50±4.00

(Fig. 1, 2). The survival rates of *P. truncatus* was higher for the various concentrations of the root extracts of *S. longepedunculata* which was used as reference than the same concentration of the roots and leaves extracts of *Z. xanthoxyloides* for the same storage period of seven days. The response of *T. castaneum* to the treated grains followed similar pattern with the leaf extract of *Z. xanthoxyloides* showing higher efficacy (Fig. 3, 4). Grains treated with 2 g mL^{-1} killed all the beetles after one day while there was more than 30% survivorship of the insect in grains treated with concentrations less than 2 g mL^{-1}. The same concentration of root extract (2 g mL^{-1}) of the plant showed 30% survivorship of the insect after five days while over 60% survivorship was observed in grains treated with lower (< 2 g mL^{-1}) concentrations. This same trend was observed in grains treated with root extracts of the reference plant (Fig. 5, 6). All the control treatments showed 100% survivorship.

Effect of Extracts on Adult Emergence

The root and leaf extracts (2 g mL^{-1}) of *Z. xanthoxyloides* completely suppressed adult emergence in both *P. truncatus* and *T. castaneum* (Table 2). Only 11.0% adult emergence of *P. truncatus* was observed in grains treated with 0.5 g mL^{-1} *Z. xanthoxyloides* leaf extract which differed significantly ($P < 0.05$) with grains treated with 0.25 g mL^{-1} leaf extracts. The leaf extract at the same concentrations had significantly ($P < 0.05$) greater effect on *T. castaneum* as 4.75% and 13.25% adult emergence were observed in grains treated with 0.5 g mL^{-1} and 0.25 g mL^{-1} *Z. xanthoxyloides* leaf extract, respectively (Table 2). The root extracts at 0.5 g mL^{-1} of *Z. xanthoxyloides* and *S. longepedunculata* showed between 16% to 19% adult emergence in both insects (Tables 2). As much as 26.5% adult emergence was observed in the control for both insects and this differed significantly ($P < 0.05$) from both root and leaf extract at 0.5 g mL^{-1} for both plant extracts.

Repellent Effect of Extracts on the Insects

Both plants were repellent against all the insects; however repellency varied with plant part and insect species (Table 3). Root extract (0.25 – 2.0 g mL^{-1}) of Z. *xanthoxyloides* repelled between 70–90% in *P. truncatus* while 100% repellency was recorded in 1.0–2.0 g mL^{-1} treatment in *T. castaneum*.

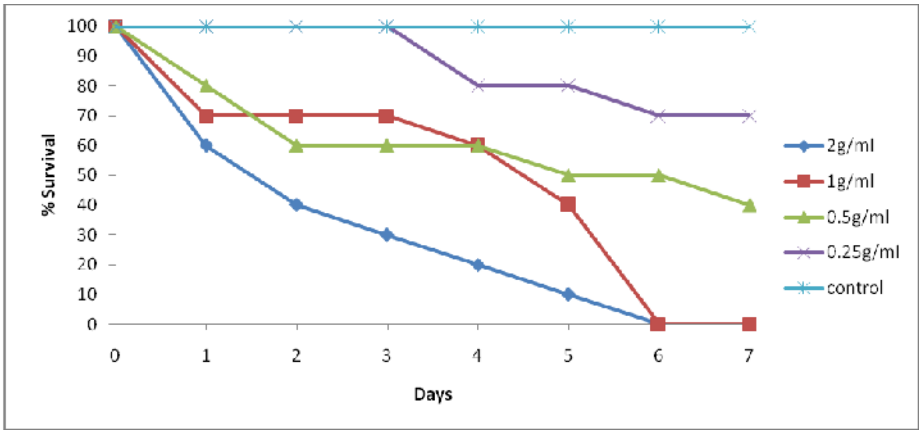

Figure 1. Survivorship of *P. truncatus* on grains treated with different concentrations of Methanol extracts of the roots of *Z. xanthoxyloides*.

Concentration < 0.5 g mL^{-1} repelled less than 25% adult *T. castaneum*. The leaf extract (0.25–2.0 g mL$^{-1)}$ of Z. *xanthoxyloides* was more repellent against *T. castaneum* (80–100%) than *P. truncatus* where concentrations (0.5 – 2.0 g mL^{-1}) repelled 40 -60% of the adults (Table 3). Root extracts of *S. longepedunculata* showed similar results against the test insects (Table.3)

Damage Assessment

Damaged assessment in terms of percentage weight loss due to the feeding activities of the insects is summarized in Table 2. Grains treated with root extracts (0.5 g mL^{-1} and 1 g mL^{-1}) of Z. *xanthoxyloides* showed significant ($P < 0.05$) reduction in grain damage (8.5% and 1.6%) caused by *P. truncatus* compared to 44.5% in the control. Similar trends were observed in *T castaneum* where 5.95% and 1.37% damage respectively was observed (Table 2). Similar trends were observed in S. *longepedunculata* treatment (Table 4).

DISCUSSION

The methanol extracts of the leaves and roots of *Z. xanthoxyloides* and the roots extracts of *S. longependuculata* were bioactive against *P. truncatus* and *T. castaneum*. The high mortality levels observed as the concentration of the extracts increased may be attributed to the higher levels of a secondary metabolite, zanthoxylol, a phenolic compound noted for its insecticidal activity present in the extracts (Wongo, 1998). Owusu *et al.* (2007) showed that methanol extract of *Z. xanthoxyloides* induced 100% mortality in *S. zeamais* and *C. maculatus* in laboratory studies while Udo *et al.* (2004) reported 53% and 79% mortality in *S. zeamais* and *Callosobruchus maculatus*, respectively when fresh root methanol extract of *Z. xanthoxyloides* was topically applied

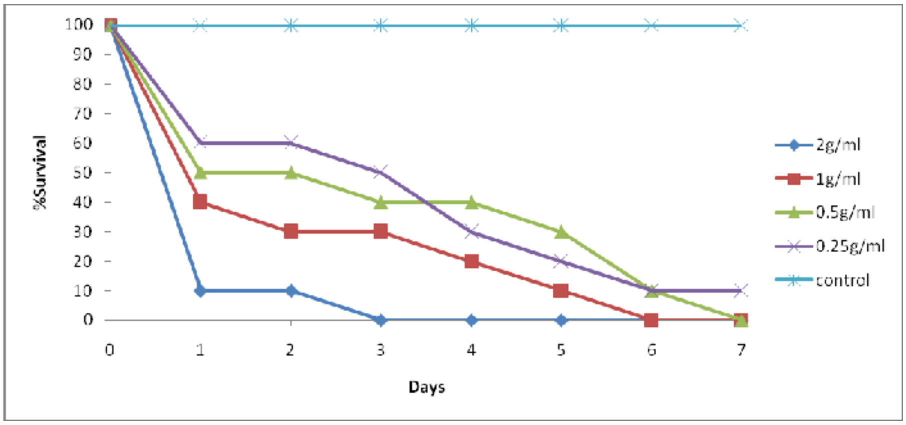

Figure 2. Survivorship of *P. truncatus* on grains treated with different concentrations of methanol extracts of the leaves of *Z. xanthoxyloides*.

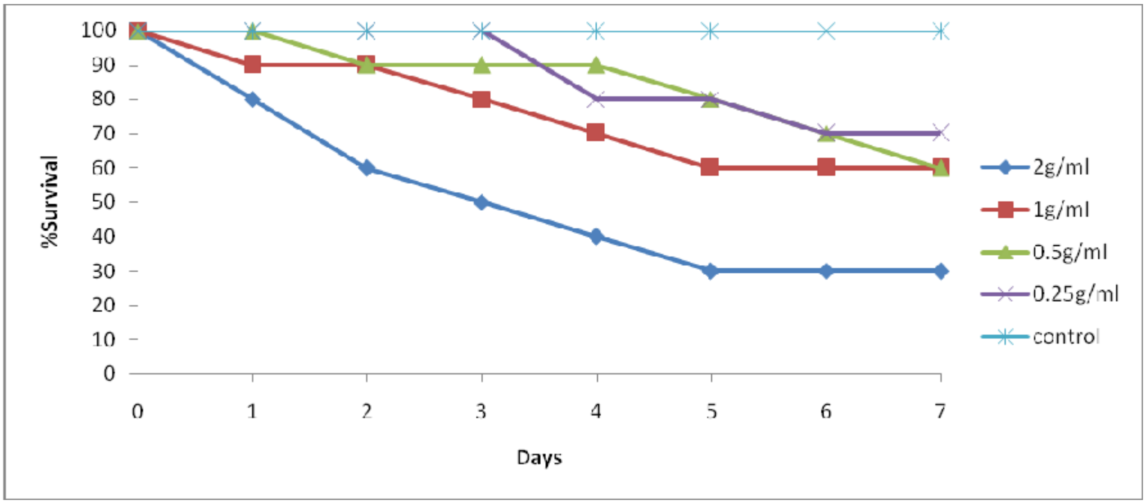

Figure 3. Survivorship of *T. castaneum* on grains treated with different concentrations of Methanol extracts of the roots of *Z. xanthoxyloides*

to the insects. Belmain (2002) reported in the work done in parts of Northern Ghana that Methanol extract of roots of *S. longepedunculata* at 100,000 ppm induced close to 100% deterrence against *S. zeamais* in stored maize. This was attributed to the toxicant, repellent and antifeedant effect of methyl salicylate present in the roots and bark of the plant (Talukder, 2006).

There was complete suppression of adult emergence of the two insects from grains treated with 2 g mL^{-1} extracts of the plant species. There was only 11% adult emergence in *P. truncatus* and 13% in *T. castaneum* when grains were treated with leaf extract of *Z. xanthoxyloides* (0.5 g mL^{-1}). The inhibition of adult emergence in the treated grains at higher concentration further confirms the activity of the secondary metabolites which suppress oviposition and progeny development in the insects. Udo *et al.* (2004) reported complete inhibition in progeny production when adults of *S. zeamais* and C. *maculatus* were exposed to methanol extracts of the *Z. xanthoxyloides*. Stevenson *et al.* (2009) indicated that the methanol extracts of *S. longepedunculata* roots contained compounds which reduced progeny emergence of beetles. It was established that the methyl salicylate and

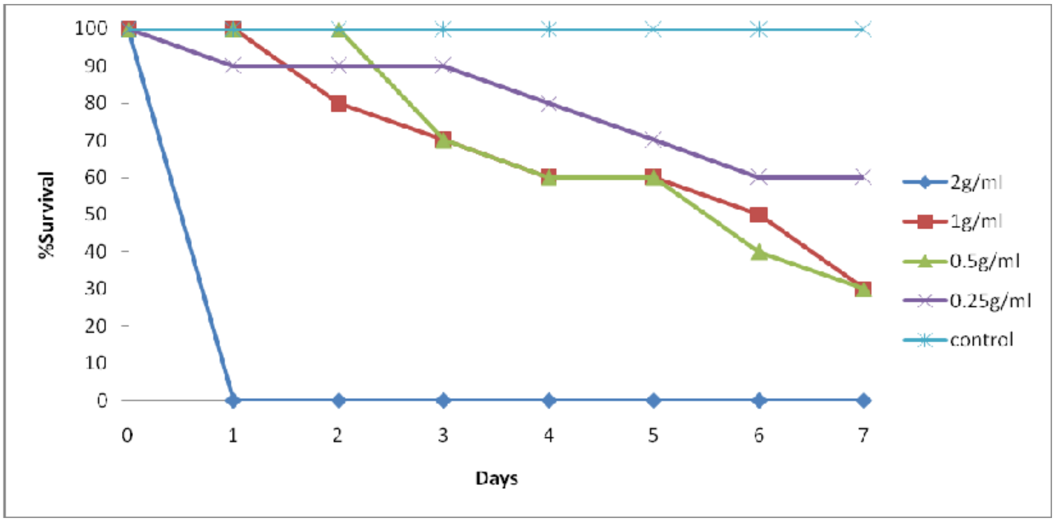

Figure 4. Survivorship of *T. castaneum* on grains treated with different concentrations of Methanol extracts of the leaves of *Z. xanthoxyloides*.

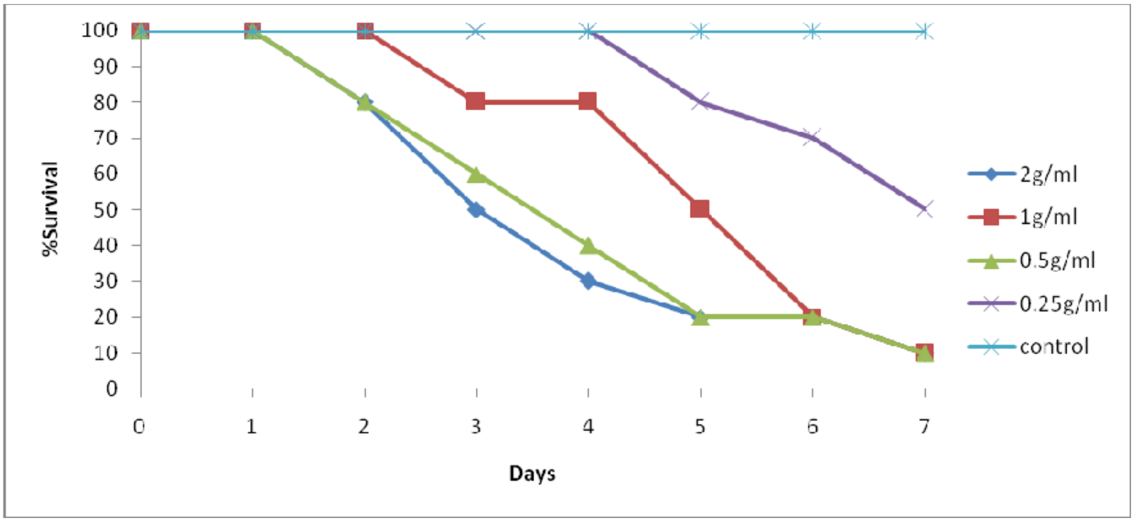

Figure 5. Survivorship of *P. truncatus* on grains treated with different concentrations of Methanol extracts of *S. longepedunculata* roots.

securidacasidae acted as oviposition deterrent and were toxic to the progenies of the insects. Hubrect *et al*. (1989) reported that *S. longepedunculata* contains saponnins that cause high larval and nymphal mortality in *S. ferugiperda*.

The general repellent action of the extracts against the insects is an indication of the presence of chemicals in the plants that stimulate or cause the insects to make oriented movement away from the source stimulus (Dethier *et al*., 1960). A high insect repellency was recorded at higher concentrations for both plant species. However, Udo *et al.* (2004) reported of moderate repellency against *S. zeamais* and *C. maculatus* with *S. zeamais* showing only 13% of root and bark extracts of *Z. xanthoxyloides* while as much as 48% repellency was observed in *C. maculatus*. The differences in repellency might be due to the different orientation of different insects to stimuli. The roots of *S. longepeduculata* are composed of over 90% methyl salicylate (Jayasekra *et al.,* 2002) which is an ubiquitous aromatic ester with a well known insect repellent properties (Hardie *et al.,* 1994).

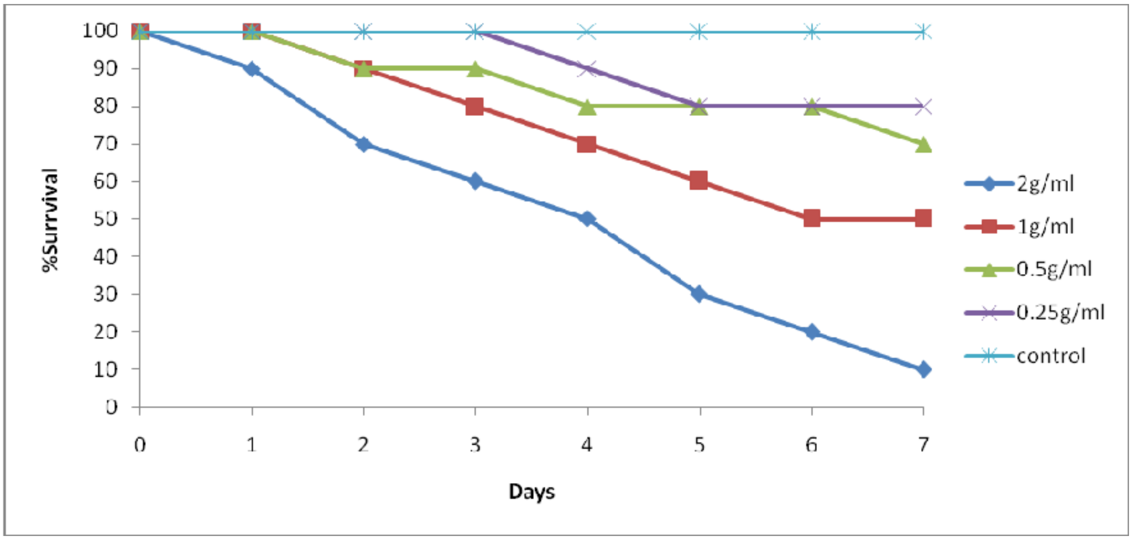

Figure 6. Survivorship of *T. castaneum* on grains treated with different concentrations of Methanol extracts of *S. longepedunculata* roots.

Owusu *et al*. (2007) showed that methanol extracts of *Z. xanthoxyloides* was an effective repellent to stored product beetles. Methyl selicylate which is found in the *S. longepedunculata* roots coupled with the alkanoids and flavinoids together with other secondary metabolites found in the roots and leaves of *Z. xanthoxyloides* (Adesina, 1986) have been found to be good repellents to several insects. The repellent action therefore increases the protecting potential of *Z. xanthoxyloides* and *S. longepedunculata* against storage insect pests since the treatments with high repellency reduced damage caused by the insects in the stored grains.

Grain damage by *P. truncatus* and *T. csateneum* was significantly reduced in all treatments. At higher concentrations of the methanol extracts of the plants, damage caused was significantly reduced. Udo (2011) showed that damage to maize by *S. zeamais* and cowpea by *C. maculatus* was reduced with treatments which exhibited high repellency to the insects. Owusu *et al*. (2007) also observed that methanol extracts of *Z. xanthoxyloides* roots and barks also offered protection to stored grains against *S. zeamais* and *C. maculatus*. This establishes the fact that the active compounds in these plants acted as feeding deterrents and repellents to the insects. These inhibited feeding on the grains by rendering them unattractive or unpalatable to the insects (Saxena *et al*., 1988). It is recommended that a further work be carried out on the field to validate the results so that the products can be used in the field for stored product protection.

ACKNOWLEDGEMENT

The authors are grateful to The Carnegie Corporation through The University of Ghana (UG-CCNY 'Next Generation Project'), Office of Research, Innovation and Development (ORID), who funded this work.

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Received: 8.3.2013 Revised: 26.5.2013 Accepted: 31.5.2013