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Full Length Research Paper

Bioactivity of *Securidaca longepedunculata* Fres. against *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) and *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae)

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The powders of the roots of *S. longepedunculata* with the roots of *Zanthoxylum xanthoxyloides* acting as reference product at two concentrations (1 and 5% wt/wt) were screened in the laboratory against *S. zeamais* and *C. maculatus*. The root powder of *S. longepedunculata* proved effective at 1 and 5% against *S. zeamais* and *C. maculatus* due to the presence 2-hydroxy-benzoic acid methyl ester (methyl salicylate, I) which is responsible of their biocide effect against the stored grain insects. Methanol extracts of root powders of *S. longepedunculata* and *Z. xanthoxyloides* were effective at 0.0, 0.04, 0.06 0.08 and 0.1 g/ml, significantly reducing (P<0.05) the development of eggs and immature stages and emergence of *C. maculatus* and *S. zeamais* progenies. The number of grains damaged by *C. maculatus* and *S. zeamais* were significantly reduced (P<0.05) by the extract of *S. longepedunculata* and *Z. xanthoxyloides*. These products also invoked 30-70% repellency against the beetles. The potential use of *S. longepedunculata* for the protection of stored product is discussed.

Key words: *Securidaca longepedunculata, Callosobruchus maculatus, Sitophilus zeamais*, 2-hydroxy-benzoic acid methyl ester (methyl salicylate, I), methanol extract, root powder.

INTRODUCTION

Cowpea and maize constitute very important staples for growing population in most parts of the world and are usually stored to provide food reserves as well as seeds for planting (Niber, 1994). Stored grains are attacked by several species of insects and in Africa losses could exceed 30% thus threatening food security (IITA, 1995). According to IITA (1989), *C. maculatus* consumes 50-90% of cowpea in storage annually throughout tropical Africa and can cause up to 100% loss of stored cowpea with an estimated value of over 30 million U.S. dollars in Nigeria (Jackai and Daoust, 1986). In Ghana out of an estimated total annual harvest of 250,000-300,000 tonnes of maize about 20% is lost to *Sitophilus zeamais* (Obeng-Ofori and Amiteye, 2005). Currently, insect control in stored food products relies heavily on the use of gaseous fumigants and residual chemical insecticides. These synthetic insecticides have made a tremendous impact over the years in stored product protection. However, constant misuse of these synthetic chemicals have promoted faster evolution of resistant forms of pests, destroyed natural enemies, turned formerly innocuous species into pests, harmed other non-target species and contaminated food (Obeng-Ofori et al., 1997). These problems have necessitated the search for alternative eco-friendly insect pest control methods such as the use of botanicals (Owusu, 2001; Obeng-Ofori,

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2007). *Securidaca longepedunculata* Fres is a semi deciduous shrub used as a traditional medicine in many parts of Africa against a number of invertebrate pests, including insects infesting stored grain (Burkill, 1997). *Securidaca longepedunculata* Fres roots is characterized by the occurrence of 2-hydroxy-benzoic acid methyl ester (methyl salicylate, I) which accounts for about 90% of the plant material and a saponin which contains presenegenine (Cmelik and Ley, 1984). The study was therefore aimed at evaluating the biological activity of *S. longepedunculata* against *S. zeamais* and *C. maculatus*.

MATERIALS AND METHODS

Culturing of insects

Sitophilus zeamais and *C. maculatus* were collected from infested stock of grains at the Madina market, Accra and reared on whole maize and cowpea grains in controlled environment room maintained at 28 ± 2°C, 65% relative humidity and 12 L: 12D photoregime (Osafo, 1998; Weaver et al., 1998; Udo et al., 2009) at the Department of Animal Biology and Conservation Science laboratory University of Ghana. Grains were sterilized in an oven at 60°C for three hours. One hundred adult weevils of mixed sex were placed in jars containing 500 g of sterilized grains to allow oviposition. The parent adults were removed after three weeks to enable the emergence of same age progeny that were used to establish the experimental cultures (Udo et al., 2009).

Preparation of plant materials

Roots of *S. longepedunculata* and *Zanthoxylum xanthoxyloides* obtained from Bole and Nyankpala in the Northern region of Ghana and the University of Ghana farms respectively were air-dried at room conditions for 10 days. The plant materials were pounded and sieved using impact Test Sieve with a mesh size of 700µ to obtain fine powder. Hundred grams of the fine powders were extracted with methanol (100%). The solvent was evaporated using a rotary evaporator and the residues redissolved in distilled water for the various bioassays.

Effect of plant powders on adult insects

One hundred grams of cowpea and maize grains from the sterilized grains were put into plastic jars and the powders of the test plant *S. longepedunculata* and the reference plant *Zanthoxylum xanthoxyloides* admixed to the grains in a proportion of 1 and 5% wt/wt. five replications were made for each treatment with the control treatment having no plant powder. One hour after the addition of the plant powders, twenty adult *S. zeamais* (5 to 10 days old) and 20 adult *C. maculatus* (3-7 days old) were introduced into the treated and untreated maize and cowpea. Dead insects were recorded daily for one week. Insects were considered dead if they did not respond to three probing of a blunt probe.

Contact toxicity by topical application

One micro-litre of five different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 g/ ml), of the methanol extract of *S. longepedunculata* were applied topically to the dorsal surface of the thorax of 3 to 5 day old unsexed *C. maculatus* adults and 5 to 10 day *S. zeamais* adults using a micro-applicator. Four replications of this were made with water acting as a control whiles the methanol extract of *Z. xanthoxyloides* (0.08 g/ml) served as the reference product. Insects that failed to respond to three probings of a blunt probe were considered dead. Mortality was recorded after 72 h.

Effect of Extracts on adults, eggs and immature stages

Twenty adult *S. zeamais* (5-10 days old) and 20 *C. maculatus* adults (3 to 5 days old) were introduced into fifty grams of sterilized grains and pre-equilibrated at 0.02, 0.04, 0.06, 0.08 and 0.1 g/ml, of the methanol extract of *S. Longepedunculata* and replicated four times. The control was treated with water and 0.08 g/ml of the methanol extract of *Z. xanthoxyloides* acting as the reference product. The extracts applied to the grains were dried and allowed to stand for one hour. Mortality was recorded daily for one week (Owusu, 2001).

The effect of the methanol extract of *S. longepedunculata* on the eggs, larvae and pupa of *S. zeamais* and *C. maculatus* was assessed using the method adopted by Udo (2000). Four replications were made and the number of adults that emerged were counted and recorded weekly for five weeks.

Effect of extracts on progeny development

One hundred grams of maize and cowpea grains were measured into separate plastic jars and the methanol extract of *S. longepedunculata* applied at five concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 g/ml) with 0.08 g/ml of methanol extract of *Z. xanthoxyloides* as reference product. Ten pairs of *S. zeamais* and *C. maculatus* adults of different sex were introduced into treated and control jars which were covered with white muslin cloth and held in place with rubber bands. Adult insects were removed after one week. Each treatment was replicated four times and the experiment left to stand undisturbed for five weeks. The number of insects emerging was counted daily for one week and recorded (Udo, 2000).

Repellency

The repellent action of the methanol extract of *S. longepedunculata* against *C. maculatus* and *S. zeamais* was assessed using the method adopted by Shehu

Table 1. Contact Toxicity of the methanol extracts of *S. longepedunculata* and *Z. xanthoxyloides* by topical application to *S. zeamais* and *C. maculatus.*

Treatment	Mean % mortality (± S.E) after 72 h	
	S. zeamais	*C. maculatus*
S. longepedunculata		
0.02	47.88±1.18	49.33±0.83
0.04	50.77±1.83	53.06±2.24
0.06	51.51±0.74	53.09±2.59
0.08	68.30±1.09	69.39±1.26
0.10	81.07±5.53	83.54±3.37
***Z. xanthoxyloides*(0.08)**	78.93±3.91	77.55±4.35
Control	0.00±0.00	0.00±0.00
LSD (P<0.05)	8.44	7.61

(2009). Five concentrations (0.02g/ml, 0.04, 0.06, 0.08 and 0.1 g/ml) of the methanol extract of *S. longepedunculata* and 0.08g/ml of the methanol extract of *Z. xanthoxyloides* acting as reference product were applied to half filter paper disc as uniform as possible with a pipette. The other halves of the filter paper were treated water only. The treated filter papers were air dried under shade for 3 hour to evaporate the water (Obeng-Ofori and Reichmuth, 1997). Full discs were remade by attaching treated halves to untreated halves with cellotape. Each full filter paper was placed in a petri dish and 10 (3-5 days old) *C. maculatus* and 10 (5-10 days old) *S. zeamais* of mixed sexes were released at the centre of each filter paper and covered. Each treatment was replicated four times. The number of insect present on control (Nc) and treated sides (Nt) were recorded after 30 minutes. Percent repellency (PR) values were computed as PR= [(Nc- Nt)/(Nc + Nt)] X 100.

Damage assessment

Damage assessment was done using the method adopted by Udo (2000). One hundred grams of grains were treated with five concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 g/ml) of the methanol extract of *S. longepedunculata* with 0.08g/ml of methanol extract of *Z. xanthoxyloides* as reference product and the jars were left to stand for four weeks. Ten pairs of adult *S. zeamais* (5 to 10 days old) and twenty *C. maculatus* unsexed adults (3 to 5 days old) were introduced into the treated grains and each treatment was replicated four times. The controls were treated with water only. Samples of 100 grains of maize or cowpea were taken from each jar and the number of damaged grains (grains with characteristic holes) and undamaged grains were countered and weighed. Weight loss was computed using the method of FAO (1985) as modified by Udo et al., (2004) as follows:

%Weight loss =[(Und- Dnu) / U(Nd +Nu)] × 100
Where U = weight of undamaged grains
D = weight of damaged grains
Nd = number of damaged grains
Nu = number of undamaged grain

Data analysis

Analysis of variance (ANOVA) using SPSS Statistics 17 and Genstat Statistical Package 9.2 (9^{th} Edition) was carried out on the data collected. Data involving percentages were transformed using arcsine transformation before analysis. In all cases, mean separation was done using LSD at 95% confidence level after ANOVA had indicated a significant difference.

RESULTS AND DISCUSSION

Contact toxicity by topical application

The effect of the methanol extract of *S. longepedunculata* applied topically on the two insects is summarized in Table 1. There was no significant difference (P > 0.05) in the mortality of the beetles treated with the methanol extract of *S. longepedunculata* and *Z. xanthoxyloides.* However, there was a significant difference (P < 0.05) between beetles treated with the methanol extract of both plants and the control. The highest mortality of 81.07% ± 5.53 and 83.54% ± 3.73 was recorded after 72 h at a concentration of 0.1 g/ml for *S. zeamais* and *C. maculatus*, respectively.

Toxicity of extracts to the insects in treated grain

The toxicity of the methanol extract of *S. longepedunculata* and *Z. xanthoxyloides* on the adult insects in grains is summarized in Figures 1 and 2. As the concentration of the methanol extract of *S. longepedunculata* increased the survival of S*. zeamais and C. maculatus* decreased. The highest concentration of the methanol extract of *S. longepedunculata* after day 7 caused less than 5% survival of *C. maculatus* and less

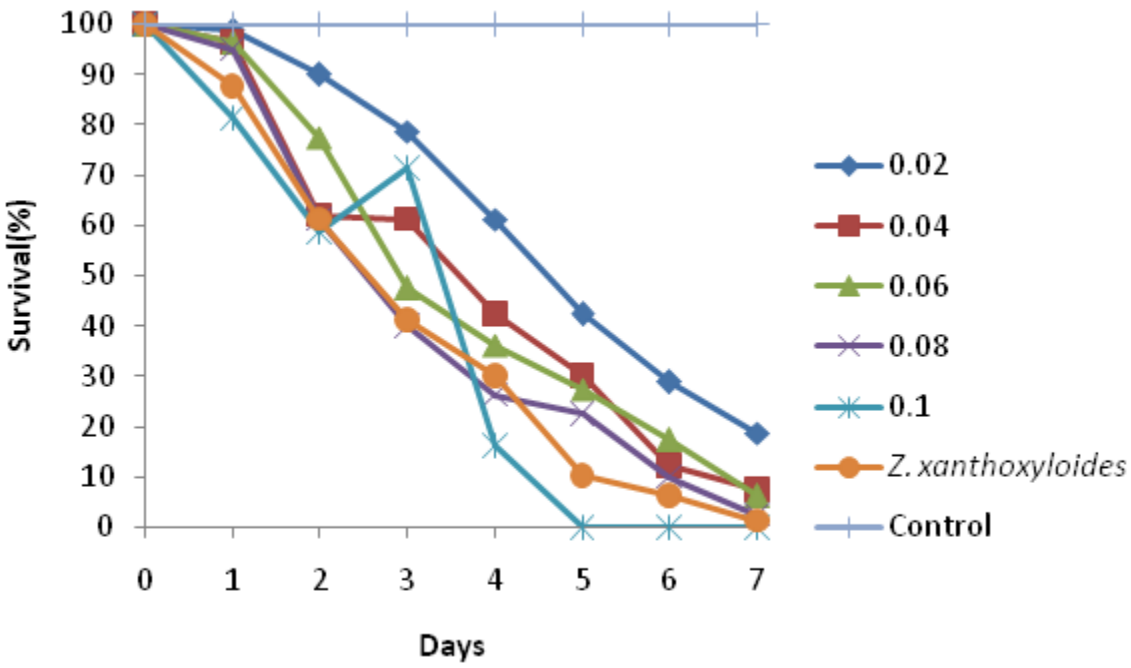

Figure 1. Effect of *S. longepedunculata* and *Z. xanthoxyloides* extracts on adult *C. maculatus* survival.

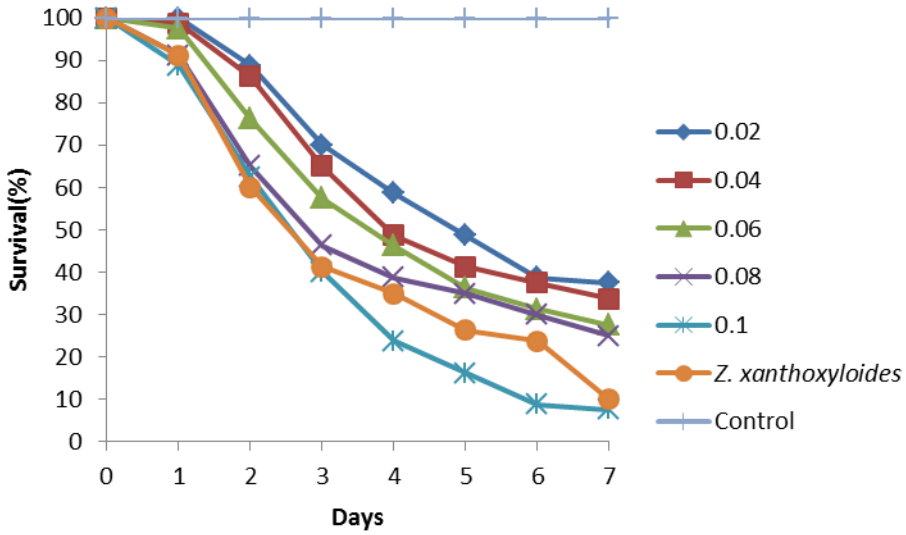

Figure 2. Effect of *S. longepedunculata* and *Z. xanthoxyloides* extracts on adult *S. zeamais* survival.

than 10% survival of *S. zeamais*. This survival rate was higher than the reference product *Z. xanthoxyloides* for the same storage period.

Effect on eggs

The *S. longepedunculata* and *Z. xanthoxyloides* extracts reduced the emergence of *S. zeamais* and *C. maculatus* when grains containing the eggs of the insects were treated (Table 2). There was a significant difference ($P<0.05$) between the extract treated grains and the control. The highest concentration (0.1 g/ml) of *S. longepedunculata* extract and *Z. xanthoxyloides* caused the least emergence in both insect species. There was no significant difference between the highest concentration (0.1 g/ml) of *S. longepedunculata* extract and *Z.*

Table 2. Effect of the methanol extract of *S. longepedunculata* and *Z. xanthoxyloides* on eggs of *C. maculatus* and *S. zeamais.*

Treatment(g/ml)	Mean adult emergence ± S.E	
	C. maculatus	*S. zeamais*
S. longepedunculata		
0.02	36.0 ± 2.5	24.0 ± 1.7
0.04	28.0 ± 0.7	20.0 ± 1.1
0.06	18.0 ± 0.9	14.0 ± 0.6
0.08	11.0 ± 0.6	10.0 ± 0.5
0.10	2.0 ± 1.3	1.0 ± 0.8
Z. xanthoxyloides(0.08)	3.0 ± 1.7	2.0 ± 0.4
Control	78.0 ± 0.6	43.0 ± 1.5
LSD (P<0.05)	4.04	3.16

Table 3. Effect *S. longepedunculata* and *Z. xanthoxyloides* extract on larvae of *C. maculatus* and *S. zeamais.*

Treatment(g/ml)	Mean adult emergence ± S.E	
	C. maculatus	*S. zeamais*
S. longepedunculata		
0.02	28.0 ± 0.8	22.0 ± 1.2
0.04	22.0 ± 1.4	13.0 ± 0.7
0.06	17.0 ± 1.5	9.0 ± 0.7
0.08	11.0 ± 0.6	7.0 ± 1.4
0.10	1.0 ± 0.7	1.0 ± 0.5
Z. xanthoxyloides(0.08)	1.0 ± 0.3	1.0 ± 0.4
Control	52.0 ± 1.5	34.0 ± 2.7
LSD (P<0.05)	3.19	3.88

xanthoxyloides (0.08g/ml).

Effect on larvae

The effect of the methanol extract of *S. longepedunculata* and *Z. xanthoxyloides* on grains containing larvae of *C. maculatus* and *S. zeamais* is summarized in Table 3. The highest concentration (0.1 g/ml) of *S. longepedunculata* and *Z. xanthoxyloides* extract caused the least emergence of *C. maculatus* and *S. zeamais* larvae with no significant difference between the two (P > 0.05). There was a significant difference (P < 0.05) between extracts treated grain and the control.

Effect of pupae

The *S. longepedunculata* and *Z. xanthoxyloides* extracts significantly reduced the emergence of *S. zeamais* and *C. maculatus* pupae when grains containing the eggs of the insects were treated compared to the control (Table 4). The highest concentration (0.1 g/ml) of *S. longepedunculata* and *Z. xanthoxyloides* extract caused the least emergence of *S. zeamais* whiles a complete inhibition was observed in *C. maculatus*. There was no significant difference between the highest concentration of *S. longepedunculata* and *Z. xanthoxyloides* extracts (P>0.05).

Progeny production

Extracts of *S. longepedunculata* and *Z. xanthoxyloides* significantly reduced (P<0.05) the number of progeny produced by *S. zeamais and C. maculatus* compared to the control (Table 5). The effect of the extracts was concentration dependent. Grains treated with 0.1g/ml of either *S. longepedunculata* and *Z. xanthoxyloides* completely suppressed progeny production of both beetle species.

Damage assessment

Grains treated with the methanol extract of *S. longepedunculata* and *Z. xanthoxyloides* significantly (P<0.05) reduced the damage caused by *C .maculatus* and *S. zeamais* compared to the control (Table 6). As the concentration of the extracts increased the damage caused by *S. zeamais* and *C. maculatus* reduced. The highest concentration (0.1 g/ml) of *S. longepedunculata* and *Z. xanthoxyloides* extracts was significantly reduced by *C. maculatus* and *S. zeamais.*

Table 4. Effect *S. longepedunculata* and *Z. xanthoxyloides* extract on pupae of *C. maculatus* and *S. zeamais.*

Treatment(g/ml)	Mean adult emergence ± S.E	
	C. maculatus	***S. zeamais***
S. longepedunculata		
0.02	17.0 ± 1.3	14.0 ± 1.0
0.04	17.0 ± 1.8	10.0 ± 0.5
0.06	12.0 ± 1.1	9.0 ± 1.1
0.08	9.0 ± 0.5	8.0 ± 1.2
0.10	1.0 ± 0.5	0.0 ± 0.2
Z. xanthoxyloides(0.08)	1.0 ± 1.1	1.0 ± 0.8
Control	49.0 ± 0.6	31.0 ± 2.3
LSD	3.18	3.49

Table 5. Mean number of *C. maculatus* and *S. zeamais* progeny in grains treated with methanol extract of *S. longepedunculata* and *Z. Xanthoxyloides*.

Treatment(g/ml)	Mean number of adult emergence ± S.E	
	C .maculatus	***S. zeamais***
S. longepedunculata		
0.02	42.0 ± 1.2	31.0 ± 2.2
0.04	21.0 ± 1.3	19.0 ± 0.5
0.06	12.0 ± 1.1	12.0 ± 0.8
0.08	8.0 ± 1.7	5.0 ± 0.2
0.10	0.0 ± 0.0	0.0 ± 0.0
Z. xanthoxyloides(0.08)	1.0 ± 0.5	1.0 ± 0.7
Control	88.0 ± 1.2	56.0 ± 2.1
LSD (P<0.05)	3.43	3.57

Table 6. Effect of the methanol extract of *S. longepedunculata* and *Z. xanthoxyloides* on damage caused by *C. maculatus* and *S. zeamais* on stored grains.

Treatment(g/ml)	Mean % weight loss ± S.E	
	C. maculatus	***S. zeamais***
S. longepedunculata		
0.02	2.90 ± 0.09	2.82 ± 0.06
0.04	2.00 ± 0.21	2.45 ± 0.10
0.06	1.52 ± 0.24	1.77 ± 0.10
0.08	0.35 ± 0.10	1.20 ± 0.07
0.10	0.02 ± 0.05	0.10 ± 0.07
Z. xanthoxyloides(0.08)	1.22 ± 0.27	1.20 ± 0.35
Control	9.95 ± 0.28	10.12 ± 0.34
LSD (P<0.05)	0.58	0.58

Repellency

The repellent action of the *S. longepedunculata* and *Z. xanthoxyloides* extracts is summarized in Table 7 Both extracts were more repellent to *S. zeamais* than *C. maculatus*.

The highest repellency was invoked by 0.1g/ml of *S. longepedunculata* and *Z. xanthoxyloides* extract to *S. zeamais* and *C. maculatus*.

Toxicity of extracts applied topically to the insects

The methanol extract of *S. longepedunculata* applied topically were highly toxic to *S. zeamais* and *C.*

Table 7. Mean % repellency of methanol extract of *S. longepedunculata* and *Z. xanthoxyloides on C. maculatus* and *S. zeamais*.

Treatment(g/ml)	Mean repellency (%) ± S.E	
	C .maculatus	***S. zeamais***
S. longepedunculata		
0.02	45.00 ± 3.33	29.73 ± 3.17
0.04	47.89 ± 2.88	42.12 ± 2.88
0.06	53.94 ± 3.16	47.89 ± 2.88
0.08	60.27 ± 3.16	54.21 ± 5.82
0.10	70.07 ± 6.64	60.27 ± 3.16
Z. xanthoxyloides (0.08)	45.00 ± 3.33	41.83 ± 5.77
LSD (P<0.05)	11.80	12.36

maculatus compared to the control treatments after 72 h. *Callosobruchus maculatus* was more susceptible to the extracts than *S. zeamais*. This may be attributed to the absence of hard and highly sclerotized thoracic cuticle found in *S. zeamais* which may have reduced the physical sorption of the active constituents of the extracts on the cuticle (Talukder and Howse, 1995). The effectiveness of the extracts indicates a possible contact action of the active constituents which is 2-hydroxy-benzoic acid methyl ester (methyl salicylate, I) (Cmelik and Ley, 1984).

Taura et al. (2004) found that the aqueous extract of *S. longepedunculata* was active against Culex mosquito larva by direct contact.

Effect of methanol extracts of *S. longepedunculata* on adults in treated grain

The methanol extract of *S. longepedunculata* and *Z. xanthoxyloides* on the adult insects in grains after the one week period reduced the survival of *S. zeamais and C. maculatus* compared to the control. This could be due to the presence of the methyl salicylate and other saponins found in the root of this plant which might act as antifeedant. Stevenson et al. (2009) confirmed the antifeedant properties of the compound found in the methanol extract of *S. longepedunculata* and this might have caused the reduction in damage of grains treated with the methanol extract of *S. longepedunculata* in this study.

Effect of methanol extracts of *S. longepedunculata* on eggs and immature stages

All the concentrations of the methanol extracts of *S. longepedunculata* and *Z. xanthoxyloides* reduced the number of eggs and inhibited the development of larvae and pupae of *C. maculatus* and *S. zeamais* into adults. The highest concentration (0.1 g/ml) caused complete inhibition of emergence larvae of *C. maculatus*. Stevenson et al. (2009) showed that aside methyl salicylate (the main constituent of *S. longepedunculata*) and securidacaside (a secondary component) acted as oviposition deterrent and were toxic to *C. maculatus* larvae. Also, Hubrecht et al., (1989) reported that *S. longepedunculata* contains saponins that cause high larval and nymphal mortality in *S. frugiperda* (Smith). The presence of these compounds might have caused similar inhibition in *S. zeamais*. The complete inhibition of the development of eggs and immature stages within grain kernels increases the protectant potential of *S. longepedunculata* against insect damage in storage.

Effect of methanol extracts of *S. longepedunculata* on progeny development

The number of *S. zeamais* and *C. maculatus* adults emerging in the F1 generation from maize and cowpea treated with the methanol extract of *S. longepedunculata* and *Z. xanthoxyloides* were significantly lower than the control. The highest concentration (0.1 g/ml) of the extract of *S. longepedunculata* caused complete inhibition of the first filial generation for *C. maculatus and S. zeamais*. Boeke et al. (2004) reported that *S. longepedunculata* significantly reduced progeny production of *C. maculatus* whiles Stevenson et al. (2009) indicated that the methanol extract of *S. longepedunculata* roots contained compounds which reduced the F1 emergence of *C. maculatus* and *S. zeamais*. This indicates the high presence of ovicidal and larvicidal properties in this part of the plant which inhibited the development of eggs and larvae of *C. maculatus* and *S. zeamais* in the present study.

Repellency of methanol extract of *S. longepedunculata*

The extract of *S. longepedunculata* extract was repellent to *S. zeamais* and *C. maculatus* with repellency range of 30 to 70% compared to *Z. xanthoxyloides* extract which had 41% on *S. zeamais* and 45% on *C. maculatus*. The highest concentration of 0.1 g/ml of *S. longepedunculata* gave the highest repellency of 70% and 60% to *C. maculatus* and *S. zeamais*, respectively. This repellency is due to the methyl salicylate which comprises more than 90% of the volatile components found in the roots of *S*.

longepedunculata (Jayasekara et al., 2002). This suggests that the insects were able to detect methyl salicylate through olfaction and avoided it when given the choice (Jayasekara et al., 2005). This could explain, at least in part, why the application of roots of *S. longepedunculata* protects grain from insect infestation and increase its practical value as a grain protectant.

Damage assessment

All concentrations of *S. longepedunculata* extracts significantly reduced *C. maculatus* and *S. zeamais* damage compared to the control. The number of damaged seeds in the maize treated with the highest concentration was significantly lower than that of the *Z. xanthoxyloides* extracts. Stevenson et al. (2009) found that securidacaside A (a secondary component found in the root of S*. longepedunculata*) was deterrent or toxic to *S. zeamais* and *C. maculatus* and helped reduce the damage caused by the insects.

The powder and methanol extract of *S. longepedunculata* has been shown from this study to contain insecticidal, anti-ovipositant, ovicidal and repellent properties. Maize and cowpea treated with the methanol extract of *S. longepedunculata* induced over 80% mortality on *S. zeamais* and *C. maculatus* when applied topically. The damage caused by *S. zeamais* and *C. maculatus* were significantly reduced by the methanol extract of *S. longepedunculata*. The results obtained suggest good potential for the use of *S. longepedunculata* in stored product pest management system. The results of this study have established the scientific bases of the practice by farmers in northern Ghana in which the roots of *S. longepedunculata* are pounded and mixed into stored grain. The powder and methanol extract of *S. longepedunculata* can be used as a component of Integrated Pest Management in stored product protection.

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