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Comparison of Toxic and Repellent Effects of *Lantana camara* L. with *Tephrosia vogelii* Hook and a Synthetic Pesticide Against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in Stored Maize Grain

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Abstract—The insecticidal and repellent properties of *Lantana camara* and *Tephrosia vogelii* were evaluated against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in stored maize grain. Five treatment rates (1.0, 2.5, 5.0, 7.5 and 10.0% w/w) of each powdered plant material, an untreated control and a synthetic insecticide (Actellic Super™ 2% dust) were used to investigate treatment efficacy on mortality of the adult insect (five to eight days old), F_1 progeny emergence and repellency against *S. zeamais* adults. After 21 days, *L. camara* and *T. vogelii* caused 82.7–90.0% and 85.0–93.7% insect mortality, respectively. The mean lethal exposure times (LT_{50}) to achieve 50% mortality varied from five to six days (7.5–10.0% w/w) to seven to eight days (2.5–5.0% w/w) for both plants. Probit regression analysis showed a significant relationship between plant powder concentration and insect mortality. The plant powders and synthetic insecticide reduced adult F_1 insects by more than 75% compared to the untreated control. *Tephrosia vogelii* was most repellent to *S. zeamais* at 7.5–10.0% (w/w), repelling 87.5% of the insects, followed by *T. vogelii* at 2.5% w/w and *L. camara* at 10% w/w which repelled 65.0 and 62.5% of insects respectively. The implications of these results are discussed in the context of smallscale farmer usage of these plants for stored product protection.

Key Words: *Zea mays*, botanical insecticides, grain storage, stored product protection

Résumé—Les propriétés insecticides et répulsives de *Lantana camara* et *Tephrosia vogelii* ont été évaluées contre *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) sur du maïs stocké en grain. Le matériel végétal de chaque espèce, réduit en poudre, a été appliqué à cinq concentrations (1,0, 2,5, 5,0, 7,5 et 10,0%), et comparé à un insecticide synthétique (Actellic Super™ 2% en poudre) et à un témoin non-traité. L'efficacité des traitements a été évaluée en mesurant la mortalité des adultes (agés de cinq à huit jours), l'émergence de la descendance des F_1, et l'effet répulsif sur les adultes. Après 21 jours, la mortalité provoquée par *L. camara* est comprise entre 82,7 et 90,0%, celle causée par *T. vogelii* entre 85,0 et 93,7%. Pour les deux plantes, les temps moyens d'exposition léthale, entrainant une mortalité de 50% (LT_{50}), varient de cinq à six jours (7,5 et 10,0% m/m) et de 7 à 8 jours (2,5 et 5,0% m/m). Une analyse de régression Probit a démontré une relation significative entre la mortalité des insectes et la concentration des poudres végétales utilisées. L'application de ces poudres végétales, comme l'insecticide synthétique, entraine une réduction de plus de 75% de la descendance en F_1, par rapport au témoin non-traité. *Tephrosia vogelii* s'avère être le plus répulsif contre *S. zeamais*,

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aux concentrations de 7,5 et 10,0% (m/m), repoussant 87,5% des insectes. Puis viennent *T. vogelii* à 2,5% m/m, repoussant 65,0% des insectes et *L. camara* à 10,0% m/m, repoussant 62,5%. La signification et les conséquences de ces résultats sont présentées dans le contexte de l'utilisation de ces plantes pour la protection des denrées stockées par les petits agriculteurs, dans les pays en voie développement.

Mots Clés: *Zea mays*, insecticide botanique, stockage de grain, protection des denrées stockées

Introduction

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) is one of the most serious pests of stored maize in the tropics (Victor and Ojuarega, 1993). Although it can be effectively controlled by synthetic insecticides (Labreque, 1983; Golob, 1988; Pierce and Schmidt, 1992; Bekele et al., 1996), the majority of farmers in Africa are resource-poor and have neither the means nor the skills to obtain and handle pesticides appropriately (Saxena et al., 1990). The prohibitive costs of commercial synthetics, the increasing development of insect resistance to pesticides, toxicity concerns and the often erratic supply of insecticides have given impetus to the search for alternative insect control methods (Tembo and Murfitt, 1995).

Most of the grain produced in sub-Saharan Africa comes from smallscale farmers, many of whom use different kinds of plant products for pest control (Poswal and Akpa, 1991; Bekele et al., 1996). In recent years, research has focused on the bioactivity, application methods, cost-effectiveness and sustainable use of botanical pesticides against insect pests (Regnault-Roger and Hamroui, 1993; Talukder and Howse, 1993; 1994; Fatope et al., 1995). *Lantana camara* L. (Verbenaceae) has been used as a tonic and stimulant in folk medicine (Getahun, 1976; Siddiqui et al., 1995; Masinde, 1996) and is reported to have anti-insect properties against certain field and storage insect pests (Reddy et al., 1990; Sharma et al., 1992; Babu et al., 1994; Facknath, 1994; Deka et al., 1998a, b). *Tephrosia vogelii* Hook (Fabaceae) has been used as a source of contact insecticides, fish and arrow poisons (Allen and Allen, 1981; Oliver-Bever, 1986; Lambert et al., 1993; Ayoub, 1999; Soko, 1999). The objective of the current study was to investigate the potential of these two plant species as sources of sustainable alternatives to synthetic insecticides for use in stored product protection using methods compatible with smallscale farmer practices in Africa.

Materials and Methods

Toxicity bioassay

Fresh (mixture of leaves, succulent stems, inflorescence and fruits) samples of *Lantana camara* L. and *Tephrosia vogelii* Hook were collected from the Kasipul division of Rachuonyo district in Kenya during September 1999. The plant materials were dried under shade at ambient temperatures of 27–30 °C for 3 days and further oven-dried at 35 °C for 48 hours. The dried plants were ground to a fine powder using an electric mill.

Sitophilus zeamais were reared on maize (*Zea mays* L.) under laboratory conditions (17–28 °C, 38–69% RH, 12:12 light: dark). The maize had been previously disinfested in an oven at 40 °C for four hours (Bekele et al., 1996). Two hundred unsexed adults were placed in one-litre plastic jars containing 500 g of maize. The top of each jar was covered with nylon mesh fastened tightly with elastic bands. The insects were allowed a seven-day egg laying period before all adults were removed. After 25 days, the emerging adults were removed daily and kept in separate jars according to age for experimental use.

Ground powders of *L. camara* and *T. vogelii* were admixed with 250 g of maize in plastic jars at five different dosages (1.0, 2.5, 5.0, 7.5 and 10.0% w/w). Two control trials were used: (1) maize treated with Actellic Super™ 2% dust at 0.05% w/w and (2) untreated maize. Forty unsexed five- to eight-day-old *S. zeamais* adults were placed into the jars arranged in a completely randomised design (CRD) with four replicates. The top of each jar was covered with nylon mesh held tightly with elastic bands. A 2-cm-wide band of fluon (polytetrafluoroethylene) was smeared around the inside near the top of the jar to deter the insects from climbing out. The number of dead insects in each jar was counted every day for the first 10 days and then on the 14th and 21st day. The percent mortality was calculated by expressing the number of dead as a percent of the total number of adult insects introduced into the jar at the start of the experiment. All adult insects were removed from the jars after 21 days. Cumulative counts of emerging F_1 insects commenced on the 28th day (Bekele et al., 1996) and continued up to the 56th day.

Repellency bioassay

The repellency of *L. camara* and *T. vogelii* against *S. zeamais* was evaluated in a choice bioassay consisting of a circular flat-bottomed plastic basin (45 cm in diameter by 30 cm high) whose base was divided into four equal portions. Alternate treated and untreated maize (100 g) was placed equidistant from the centre of the circular base. This was repeated for all the treatments including a 'no-choice' control with untreated maize in all four portions. A 2-cm fluon band was smeared at 15 cm height all around the basin. The treatments were arranged in a CRD with four replicates. Forty unsexed five- to eight-day-old adult *S. zeamais* were placed at the centre of each basin, and the top of the basin was covered with nylon mesh. The total number of insects that settled on the control and the treated grain was recorded after 3, 4 and 24 hours of exposure. The percent repulsion (PR) was calculated and assigned to repellency classes (0–V) according to Talukder and Howse (1993) as follows:

$$PR = 2 \times (C-50)$$

where C = the percentage of insects that settled on the untreated grain.

Trials showing a positive (+) PR value demonstrate repellency.

Data from toxicity and repellency trials were log-transformed to correct for heterogeneity of treatment variances (Gomez and Gomez, 1984) before being subjected to ANOVA and repeated measures analysis. The transformed means were separated by Tukey's studentised (HSD) test (Gomez and Gomez, 1984; Scheiner and Guvevitch, 1993). For purposes of final presentation, the treatment means were converted to the original scale with the transformed means in parentheses. The relationship between the plant concentration applied and insect mortality was investigated using probit regression analysis of log-transformed concentrations. The regression lines for $\log_{10}$ concentration and insect mortality were then computed (Finney, 1964; Talukder and Howse, 1994). Any two regression lines were considered significantly different if their standard errors did not overlap (Talukder and Howse, 1994).

Results

There were significant ($P < 0.01$) treatment, exposure time and treatment by exposure time interaction effects on the adult insect mortality (Table 1, Fig. 1) (repeated measures analysis, $P < 0.01$ for each of the three effects; critical HSD value (logmort) = 4.55575). No mortality was recorded from untreated control maize, whereas maize treated with Actellic Super™ 2% dust achieved a 100% kill within 2 days. The two test plants induced insect mortality over a much longer exposure period. Both plant materials registered less than 50% mortality during the first five days and 82–95% mortality at 21 days. The lethal mean exposure times (LT_{50}) varied from five to six days for higher dosages (7.5 and 10% w/w) to seven to eight days for the lower dosages (2.5 and 5% w/w). Probit regression analysis showed a significant linear relationship between weevil mortality and the concentration of plant powder applied (Table 2).

Table 1. Effect of treating maize with *Lantana camara* and *Tephrosia vogelii* on the mean (cumulative %) adult mortality of *Sitophilus zeamais*

Treatment	Dose (% w/w)	Percent mortality after 21 days, n = 4, (Mean ± SEM)	
Untreated control	0.0	0.00 ± 0.00	(–) a
Actellic Super™ 2% dust	0.05	100.00 ± 0.00	(2.303) c
L. camara	1.0	88.75 ± 6.30	(2.242) bc
L. camara	2.5	82.75 ± 9.60	(2.204) b
L. camara	5.0	95.00 ± 4.10	(2.277) bc
L. camara	7.5	87.50 ± 8.70	(2.234) bc
L. camara	10.0	90.00 ± 7.10	(2.249) bc
T. vogelii	1.0	92.50 ± 8.70	(2.262) bc
T. vogelii	2.5	85.00 ± 7.10	(2.220) bc
T. vogelii	5.0	93.75 ± 4.80	(2.270) bc
T. vogelii	7.5	90.00 ± 9.10	(2.248) bc
T. vogelii	10.0	93.75 ± 9.50	(2.269) bc

Means in a column followed by different letters are significantly different at $\alpha = 0.05$ by Tukey's studentised range (HSD) test. Figures in parentheses are the log-transformed values.

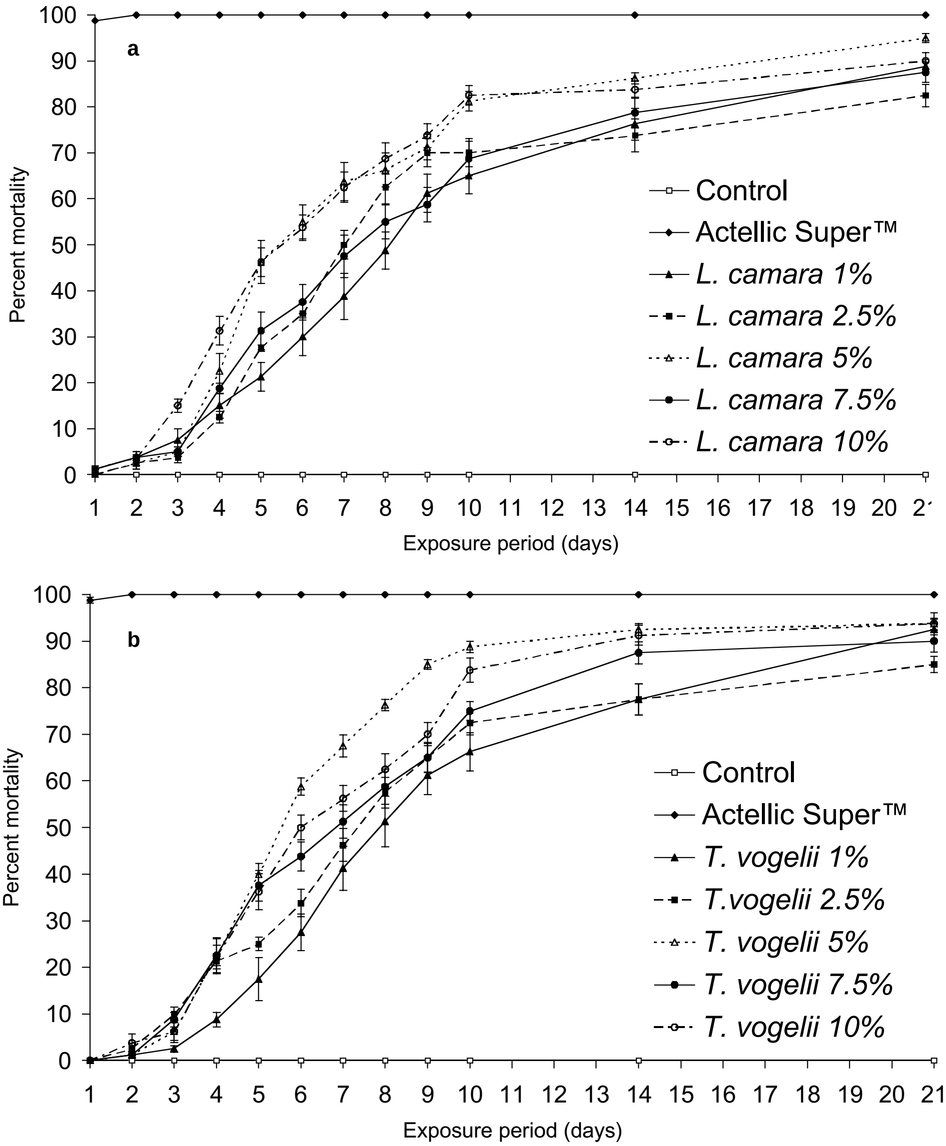

Fig. 1. Cumulative mean (± SEM) percent adult mortality of *Sitophilus zeamais* over 21 days when exposed to maize admixed with different concentrations of ground (a) *Lantana camara* and (b) *Tephrosia vogelii*

Results showed significant differences among treatments and over time in the cumulative number of emerging adult F_1 insects (Fig. 2) (Tukey's HSD minimum significant difference = 6.7, $P < 0.01$). All the botanical treatments and the synthetic insecticide effectively reduced the adult F_1 progeny by more than 75% when compared to the untreated control (Table 3). Interestingly, the concentration of plant powder applied showed a variable effect on F_1 emergence, and neither plant showed a consistent decreasing F_1 emergence with increasing botanical concentration.

Results from the repellency bioassay showed significant (Tukey's studentised range (HSD) test, $P < 0.01$) differences in repellency between the untreated control and the treatments (Table 4). There were also differences between Actellic Super™ and the two plant materials at all rates except for *L. camara* at 1.0% (w/w). Maize treated with *T. vogelii* had higher percent repulsion (PR) values than maize treated with *L. camara*. Maize treated with Actellic Super™ registered a negative PR value, indicating an arrestment of *S. zeamais* by the chemical. The PR values for all plant treatments significantly increased with increasing exposure time (Fig. 3).

Table 2. Relationship between botanical concentration and the mortality (%) of adult *Sitophilus zeamais* 3–21 days after the commencement of the trial demonstrated by probit regression analysis

Day	Pearson χ^2 value	Prob.	Regression line (± SEM)
Lantana camara			
3	26.780 ns	0.083	Y= 0.299 (± 0.27) X- 1.049
4	26.155 ns	0.096	Y=0.399 (± 0.203) X- 0.277
5	32.868 *	0.017	Y=0.443 + 0.612 (± 0.187) X
6	30.111 *	0.036	Y=0.527 + 0.523 (± 0.179) X
7	40.060 **	0.002	Y=0.733 + 0.484 (± 0.177) X
8	32.586 *	0.019	Y=0.767 + 0.365 (± 0.178) X
9	29.261 *	0.045	Y=0.661 + 0.159 (± 0.181) X
10	29.680 *	0.041	Y=1.184 + 0.397 (± 0.186) X
14	27.313 ns	0.073	Y=1.198 + 0.261 (± 0.197) X
21	22.749 ns	0.200	Y=1.385 + 0.123 (± 0.230) X
Tephrosia vogelii			
3	23.873 ns	0.159	Y=0.309 (± 0.287) X- 1.074
4	24.442 ns	0.141	Y=0.519 (± 0.214) X- 0.153
5	29.959 *	0.038	Y=0.401 + 0.649 (± 0.269) X
6	26.828 ns	0.082	Y=0.689 + 0.631 (± 0.180) X
7	31.879 *	0.023	Y=0.619 + 0.401 (± 0.177) X
8	35.502 **	0.008	Y=0.719 + 0.312 (± 0.255) X
9	30.812 *	0.030	Y=0.862 + 0.258 (± 0.182) X
10	26.536 ns	0.088	Y=1.470 + 0.514 (± 0.191) X
14	30.704 *	0.031	Y=1.923 + 0.615 (± 0.210) X
21	28.799 *	0.050	Y=1.465 + 0.089 (± 0.248) X

The X-axis refers to the treatment concentration and the Y-axis refers to insect mortality. *, ** and ns refer to significant ($P < 0.05$), highly significant ($P < 0.01$) and insignificant ($P > 0.05$), respectively. n = 400, based on five concentrations with four replicates of 20 weevils each.

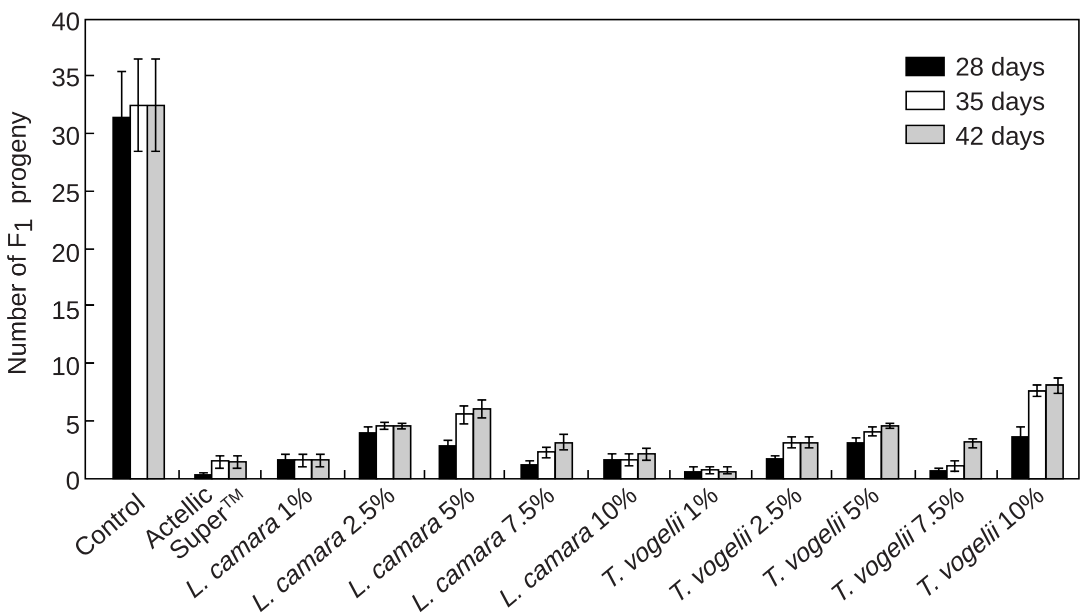

Fig. 2. Effect of admixing maize with *Lantana camara* or *Tephrosia vogelii* powder on the cumulative mean (± SEM) number of *Sitophilus zeamais* F_1 progeny emerging over time

Table 3. Effect of treating maize with *Lantana camara* and *Tephrosia vogelii* on the mean adult F_1 emergence of *Sitophilus zeamais*

Treatment	Dose (% w/w)	F_1 progeny[+]
Untreated control	0.0	32.20 ± 7.93 (1.4620) a
Actellic Super™ 2% dust	0.05	1.10 ± 0.72 (0.2211) f
L. camara	1.0	1.50 ± 0.96 (0.2940) ef
L. camara	2.5	4.30 ± 0.61 (0.7170) bc
L. camara	5.0	4.70 ± 1.29 (0.6929) bcd
L. camara	7.5	2.08 ± 0.97 (0.3923) cdef
L. camara	10.0	1.70 ± 0.91 (0.3338) def
T. vogelii	1.0	0.50 ± 0.50 (0.1193) f
T. vogelii	2.5	2.50 ± 0.88 (0.4645) bcdef
T. vogelii	5.0	3.80 ± 0.80 (0.6679) bcde
T. vogelii	7.5	1.50 ± 0.69 (0.2946) ef
T. vogelii	10.0	6.30 ± 1.32 (0.7910) b

[+]Mean (± SEM) number of F_1 progeny produced by 40 unsexed *S. zeamais*, n = 4. The number of F_1 progeny was recorded for 42 days. Means in a column followed by different letters are significantly different at $\alpha = 0.05$ by Tukey's studentised range (HSD) test. Figures in parentheses are the log-transformed values.

Table 4. Response of *Sitophilus zeamais* in a choice bioassay testing for repellent properties of *Lantana camara* and *Tephrosia vogelii* when mixed with maize

Treatment	Dose (% w/w)	PR (± SEM)[1]		RC[2]
Untreated control	0.0	0.00 ± 0.00	(0.7433) e	0
Actellic Super™ 2% dust	0.05	-7.50 ± 16.52	(2.1659) d	I
L. camara	1.0	40.00 ± 13.54	(2.3365) c	II
L. camara	2.5	35.00 ± 8.66	(3.3244) c	II
L. camara	5.0	55.00 ± 13.23	(3.4623) b	III
L. camara	7.5	52.50 ± 8.54	(3.0783) b	III
L. camara	10.0	62.50 ± 2.5	(3.3239) ab	IV
T. vogelii	1.0	55.00 ± 8.66	(3.5742) b	III
T. vogelii	2.5	65.00 ± 6.46	(3.3573) ab	IV
T. vogelii	5.0	57.50 ± 16.52	(3.5187) b	III
T. vogelii	7.5	87.50 ± 2.50	(3.8168) a	V
T. vogelii	10.0	87.50 ± 6.29	(3.4356) a	V

[1]Percent repellency after 24 hours, n=4; [2]Repellency class. Means in a column followed by different letters are significantly different at α= 0.05 by Tukey's studentised range (HSD) test. Figures in parentheses are the log-transformed values.

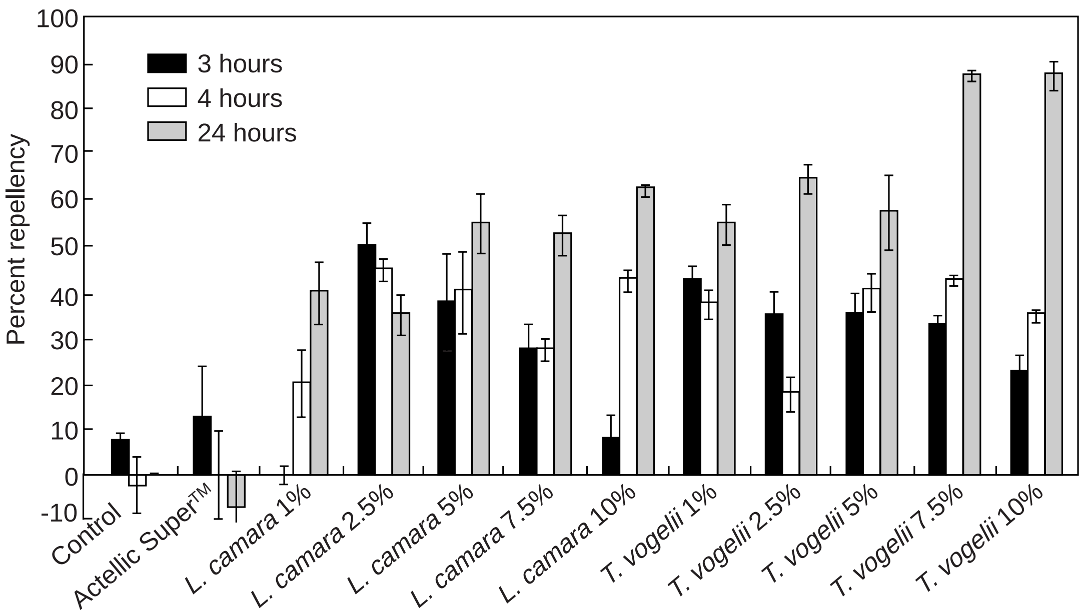

Fig. 3. Effect of exposure time on the repellent properties (mean percent ± SEM) of *Lantana camara* and *Tephrosia vogelii* against *Sitophilus zeamais* adults

Discussion

The botanicals showed two distinct types of effect on *S. zeamais* by reducing the adult mortality and the F_1 emergence. A slow but direct insecticidal activity was manifested in the cumulative adult mortality. Toxicity results in the current study compare favourably with the findings of Kasa and Tadese (1996) in which crude powders from eight plant species caused 58–88% mortality of *S. zeamais*. Similarly, Regnault-Roger and Hamraoui (1993) reported that plant materials produced a slow insecticidal effect on the bean beetle, *Acanthoscelides obtectus*, during a 12-day period. The linear relationship between the treatment concentration of *T. vogelii* and *L. camara* and the percent insect mortality of *S. zeamais* showed that a 100% kill could be achieved if the insects were exposed for greater than 21 days. The botanicals showed an effect on the reproductive cycle in which the F_1 progeny were reduced by more than 75%. Throughout the trials, the relative humidity and ambient conditions in the laboratory were conducive for oviposition and subsequent development to the adult stage (Haines, 1991; Victor and Ojuarega, 1993). This implies that the recorded reduction in F_1 progeny is due to factors such as the observed adult mortality and potential anti-oviposition, ovicidal and/or larvicidal effects caused by the botanicals. The presence of insecticidal properties in *T. vogelii* has been noted previously, even when dried *T. vogelii* had been stored for 22 years (Oliver-Bever, 1986; Lambert et al., 1993). Various bioactive isoflavanoids have been isolated from *T. vogelii* and are likely to be partly responsible for the insecticidal properties observed in the current study (Oliver-Bever, 1986; Lambert et al., 1993; Ibrahim et al., 2000). *Lantana camara* has been reported to have insecticidal, anti-oviposition and growth-regulating effects against field and storage insect pests (Saxena et al., 1992; Facknath, 1994). Compounds isolated from *L. camara* include triterpenoids, iridoid glycosides, furano-naphthoquinones, flavonoids and phenylethanoid glycosides, some of which could be responsible for the observed insecticidal properties (Morton, 1994; Siddiqui et al., 1995; Sharma et al., 2000; Yadav and Tripathi, 2000). However, for both plants, the bioactive constituents and their mode(s) of action against *S. zeamais* remain unknown.

Maize treated with *L. camara* and *T. vogelii* was significantly repellent against adult *S. zeamais*. The degree of repellency was greatly influenced by the plant species, dosage of powder applied and the exposure time. *Tephrosia vogelii* powder was more repellent (mean PR value = 70.5%) than *L. camara* (mean PR value = 49%). Although many studies on insecticidal botanicals have been conducted in the past (Bowry et al., 1984; Regnault-Roger and Hamroui, 1993; Niber, 1994; Talukder and Howse, 1993; 1994; Bekele et al., 1996), no data were available on the bioactivity of *L. camara* and *T. vogelii* as grain protectants against major stored product insects. The insect mortality and repellency results of the current study suggest that there exists good potential for the two local plant species, *L. camara* and *T. vogelii*, to be effectively used as grain protectants in the traditional resource-poor farming communities in sub-Saharan Africa. The use of botanical pesticides will boost food security in those environments where investment in synthetic pest control is uneconomical.

However, the safety of widely promoting and using botanicals for stored product protection must be established through further research. The rotenoid class of isoflavones are present in *T. vogelii*, explaining its traditional use as a fish poison as well as its efficacy against a range of insect pests. Rotenone can also be toxic to mammals, and its traditional use in stored product protection should be carefully assessed. Residue analysis of grain treated with *T. vogelii* or, indeed, any botanical treatment is required to ascertain the presence of potentially harmful compounds on grain that has been mixed with plant materials. Many farmers throughout Africa continue to use *T. vogelii* and other plant species for stored product protection, and this research can be used to help optimise indigenous practice by minimising the risks of potential toxicity.

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