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Full Length Research Paper

Integrating varietal resistance with *Xylopia aethiopica* (Dunal) A. Richard seed extract for the management of *Sitophilus zeamais* Motschulsky in stored maize

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In search for alternatives to synthetic pesticides in maize (*Zea mays*, L.) storage, an integrated weevil management scheme involving varietal resistance and *Xylopia aethiopica* (Dunal) A. Richard seed extract was evaluated in the laboratory. Varying rates of *X. aethiopica* extract were assessed on three maize varieties (Pajo White, Tsolo Yellow, and DMR-LSR-Y) with differing susceptibility to *Sitophilus zeamais.* The different rates of *X. aethiopica* extract significantly interacted with maize varietal resistance and reduced fecundity of *S. zeamais* and maize seed weight loss due to insect's feeding. Treatment of seeds with extract at 1.0 ml/20 g seed caused significant (above 50%) mortality at 24 h post treatment in all varieties whereas no mortality was recorded with the control and 0.2 ml/20 g seed. DMR-LSR-Y was better protected than the remaining two varieties in the integrated weevil management scheme. A repellency bioassay of the extract also revealed that the extract was strongly repellent to *S. zeamais* with 73% (Class IV) repellency recorded in 0.4 ml/30 cm^2.

Key words: Ethiopian pepper, fecundity, mortality, maize varieties, maize weevil, integrated weevil management.

INTRODUCTION

Maize (*Zea mays,* L.) is a major cereal being cultivated in the rain forest and the derived savannah zones of Nigeria (Iken and Amusa, 2004). It is an important source of carbohydrate in the tropics (Baba, 1994). Although it is increasingly being utilized for livestock feed, it is still a very important staple food for millions of Nigerians. In order to satisfy specific consumer preferences, the varieties developed vary in grain colour. Yellow grain varieties are increasingly being requested for producing livestock feed in order to impart yellow colour in the egg yolk, while white grain varieties are preferred for food dishes (Iken and Amusa, 2004).

The maize weevil, *Sitophilus zeamais* Motschulsky (*Coleoptera: Curculionidae*) is a serious cosmopolitan field-to-store pest of maize in tropical and sub-tropical regions (Throne, 1995; Obeng-Ofori and Amiteye, 2005). Since its infestation starts from the field, its management is rather difficult (Kitaw et al., 2001).

West African "Pepper tree" [*Xylopia aethiopica* (Dunal) A. Rich, Annonaceae] is a slim, tall tree of about 60 – 70 cm in diameter and up to 15 – 30 m high with straight stem and a slightly stripped or smooth bark. It is widely distributed in the humid forest zones of West Africa especially along rivers in the dry country sides (Irvine, 1961). *X. aethiopica* has a wide variety of application; the very odorous roots of the plant are employed in West Africa in tinctures, administered orally to expel worms and other parasitic animals from the intestines, or in teeth-rinsing and mouth-wash extracts against tooth-aches. The fruits are also used in various forms and exhibit repulsive properties, especially when mashed with grains. These properties are used advantageously in the external treatment of rheumatism. Crushed powdered fruits can also be mixed with shea butter fat and coconut oil and used as creams, cosmetic products, and per-

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fumes (Burkill, 1985) and the dried fruits are also used as spices in the preparation of two special local soups named "obe ata" and "isi-ewu" taken widely in the southwest and southeastern parts of Nigeria (Tairu et al., 1999).

Insecticidal natural products from locally available plants are gaining ground recently as alternatives to synthetic pesticides in storage. The insecticidal properties of *X. aethiopica* have been reported (Blaske and Hertel, 2001; Adewoyin et al., 2003; Udo, 2005; Omotoso and Ogunleye, 2006). Nevertheless, total reliance on its application for the control of weevils may result in emergence of resistant strains of weevil through selection. Serratos et al. (1987) suggested identification of resistant lines in maize gene pool, determination of the mechanism(s) of resistance and focusing on stable heritable characters for breeding as a promising solution to storage problem due to *S. zeamais.* However, resistance in most pest problems does not last for a long time because of the development of virulent biotypes (Adane, 1995) that are tolerant of the resistant mechanism(s) of the host plant. Dick and Credland (1986) reported that TVu 2027, a resistant cowpea variety, had become ineffective against certain biotypes of *Callosobruchus maculatus,* Fabricius.

Considering the highlighted problems and the need for an integrated scheme, the incorporation of resistant varieties (complete or partial) into the scheme for the protection of maize should have been a long-term goal. The most attractive feature of using varietal resistance is that the grower needs no extra skill or cash investment. Therefore, the objective of this study was to evaluate the potential of combining *X. aethiopica* seed extract with maize varietal resistance for the management of *S. zeamais* in Nigeria.

MATERIALS AND METHOD

Insect rearing

S. zeamais were obtained from the Entomology Unit of Agronomy Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The insect stock was maintained on Pajo White, a local maize cultivar, under prevailing storage conditions (29 ± 3°C temperature and 69 - 75% RH) and photoperiod of Ld 12:12 (hours light: dark). Experiments were conducted under these conditions.

Maize source

Two (Pajo White and Tsolo Yellow) of the three varieties were obtained from Sabo Market in Ogbomoso while DMR-LSR-Y was obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Preparation of seed extract

Fruits of *X. aethiopica* were obtained from Jagun Market, Ogbomoso and split opened to remove a large quantity of its oily seeds. The seeds were then air-dried and milled into powder using a hammer mill. A 100 g of the seed powder were boiled in 100 ml (1:1 w/v) 95% ethanol at 60°C for 30 min using a regulated heat source. After cooling for 3 h, the seed extract was separated from the residue using a Whatman No.1 filter paper. The extract was stored in a glass jar at room temperature as the stock solution throughout the period of the study.

Fecundity test

The three maize varieties (Pajo White, Tsolo Yellow and DMR-LSR-Y) weighing 20 g per batch were introduced into 150 ml glass jars. They were treated with 0.0, 0.2, 0.4 and 0.6 ml of *X. aethiopica* extract which corresponded to 0, 1, 2 and 3% extract content (v/w) respectively. The ethanol was allowed to completely evaporate for 1 h after which twenty 5-to 10-day old *S. zeamais* were introduced into each batch. The glass jars were covered with mesh to prevent insects' escape. After 5 day oviposition period, the insects were removed and the batch kept for 49 days. Data on emerging F_1 adults were taken. Each treatment was replicated three times.

Feeding deterrence test

The method used for feeding deterrence was based on Owusu (2001) with some modifications. Varying concentrations (0.0, 0.2, 0.4, 0.6 and 0.8 ml) of *X. aethiopica* extract corresponding to 0, 1, 2, 3 and 4% extract content (v/w) were introduced into 20 g of each maize variety using 150 ml glass jar similar in all respects to those used in the first bioassay. Twenty previously starved adult insects were allowed to feed for 30 days. After the feeding period, weight loss was determined with the aid of a sensitive top loading weighing machine (Gibertini® TM 1600, Italy) according to Ta-poundjou et al. (2005). The experiment was replicated three times.

Mortality test

The three maize varieties (Pajo White, Tsolo Yellow, and DMR-LSR-Y) weighing 20 g per variety were introduced into 150 ml glass jars. Varying concentrations of *X. aethiopica* extract (0.0, 0.25, 0.5, 0.75 and 1.0 ml) corresponding to 0.0, 1.25, 2.25, 3.75 and 5% (v/w) were uniformly introduced into the 20 g maize seed. The ethanol was allowed to evaporate completely for 1 h after which twenty 5 to 10 day old adults were introduced into each 150 ml experimental unit. The glass jar was covered as done for previous bioassays. Mortality data were taken 24 h after the intro-duction of the adults. The experiment was replicated three times.

Repellency test

The method used for testing repellency of *X. aethiopica* seed extract to *S. zeamais* was based on the area preference method described by McDonald et al. (1970) and modified to varying degrees by other workers (Obeng-Ofori and Reichmuth, 1997; Lale and Alaga, 2001). Test area consisted of 9 cm diameter Whatman No.1 filter paper cut in half. Four treatments were prepared by dissolving 0.1, 0.2, 0.3, and 0.4 ml of stock solution separately in 0.2 ml of ethanol. Each dosage was applied to a half papers disc of an area of 30 cm^2 as uniformly as possible with the aid of a hypodermic syringe, Menoject®, China. The other half of the filter paper was left untreated. In the control experiments, one paper half was treated with pure ethanol (0.2 ml) and the other halves were left untreated. The treated and the untreated halves discs were rejoined using clear adhesive tape and placed in 9 cm diameter Petri dish. The filter paper was air dried to evaporate the solvent (ethanol) completely. Twenty adults *S. zeamais* similar in all respects to those used in previous bioassays were released at the

Table 1. Mean numbers (± S.E.) of *S. zeamais* progenies emergence from maize varieties protected with *X. aethiopica* seed extracts.

Maize variety	Dosage of extract (ml/20 g seed)			
	0.0	0.2	0.4	0.6
DMR-LSR-Y	10.33 ± 1.45	6.00 ± 1.52	5.00 ± 1.15	3.33 ± 0.66
Tsolo Yellow	18.60 ± 2.40	9.67 ± 1.76	13.62 ± 0.88	8.67 ± 1.73
Pajo White	16.67 ± 1.28	11.67 ± 3.50	11.00 ± 0.58	9.33 ± 1.45

SED = 1.94, LSD (p ≤ 0.05) = 3.88 (maize variety).
SED = 1.42, LSD (p ≤ 0.05) = 2.85 (dosage of extract).
SED = 2.47, LSD (p ≤ 0.05) = 4.94 (interaction).

Table 2. Mean percentage weight loss (± S.E) of maize varieties protected by *X. aethiopica* seed extract due to *S. zeamais* feeding.

Maize variety	Dosage of extract (ml / 20 g seed)				
	0.0	0.2	0.4	0.6	0.8
DMR-LRS-Y	16 .53 ±0.28	14.18 ±6.40	6.61 ±0.03	6.12 ±0.08	6.01 ± 0.23
TsoloYellow	15.62 ±0.06	11.56 ±0.05	11.21 ±0.06	10.95±0.13	10.12 ± 0.09
PajoWhite	14 .02 ±0.29	12.90 ±0.32	12.23 ±0.24	12.60 ±0.9	11.90 ± 0.20

SED = 0.31, LSD (p ≤ 0.05) = 0.62 (maize variety).
SED = 0.13 LSD (P ≤ 0.05) = 0.26 (dosage of extract).
SED = 0.23, LSD = (p ≤ 0.05) = 0.45 (interaction).

centre of each repellency chamber and then covered. Each treatment was replicated three times and the numbers of insects present on the control (N_c) and treated (N_t) areas of the discs were recorded after 30 min (Obeng-Ofori and Reichmuth, 1997; LaLe and Alaga, 200l). Percentage repellency (PR) values were computed as follows:

$$PR = [(N_c - N_t) / (N_c + N_t)] \times 100$$

Data analysis

Mortality data were expressed as percentage of the total number of insects introduced. Weight loss due to feeding was expressed as a percentage of the initial seed weight. Percentage data were transformed to arcsine after which they were subjected to analysis of variance (ANOVA). Fecundity test data were transformed to square root, $\sqrt{(n + 0.5)}$, and were afterwards also subjected to ANOVA. Repellency data were also subjected to ANOVA and later assigned to repellency classes (Juliana and Su, 1983). Class 0 (PR < 0.1%), Class I (PR = 0.1 - 20%), Class II (PR =20.1- 40%), Class III (40.1 – 60%), Class IV (60.1 - 80%), Class V (80.1 – 100%). For each ANOVA, least significant difference (LSD) statistic at 5% level of probability was used to indicate significant difference between means (SAS Institute, 1985).

RESULTS

Fecundity test

Table 1 shows that the interaction of *X. aethiopica* extract and the resistance status of the maize varieties had significant negative effect on numbers of emerging progenies on the three maize varieties. The effect of *X. aethiopica* extract on emerging progenies was significant (P ≤ 0.05) in all varieties. Significant higher number of progenies was observed in the control than all treatments in all varieties, with DMR-LSR-Y having significantly lowest number of progenies at any dosage of *X. aethiopica* extract.

Feeding deterence test

The interaction of varietal resistance and the rate of *X. aethiopica* extract application also caused a significant (p ≤ 0.05) reduction in weight loss due to weevil feeding (Table 2). In each of the varieties, untreated seeds (control) were significantly more damaged than treated seeds. In all the varieties, efficacy of extract increased with increase in dosage with Tsolo Yellow better protected than Pajo White at extract dosage of 0.2 to 0.8 ml. However, treatments of DMR-LSR-Y seeds with 0.4, 0.6, and 0.8 ml of *X. aethiopica* seed extract reduced weight loss from 16.53% to less than 7%.

Mortality test

No mortality was recorded in untreated maize seed (control) and at the lowest dosage of 0.25 ml *X. aethopica* extract. Mortality was dose-dependent increasing with increasing dosage of *X. aethiopica* (Table 3). Although varietal resistance has no significant effect on mortality the highest mortality was observed in DMR-

Table 3. Mean percentage mortality (± S.E.) of *S. zeamais* on seeds of maize varieties protected with *X. aethiopica* seed extract.

Maize variety	Dosage of extract (ml/20 g seed)				
	0.0	**0.25**	**0.5**	**0.75**	**1.0**
DMR-LSR-Y	0.00 ± 0.00	0.00 ± 0.00	16.67 ± 4.4	36.67 ± 3.33	58.33 ± 1.66
Tsolo Yellow	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 2.8	35.00 ± 5.77	51.67 ± 7.2
Pajo White	0.00 ± 0.00	0.00 ± 0.00	8.33 ± 1.6	36.67 ± 3.33	55.00 ± 2.89

SED = 2.56, LSD ($P > 0.05$); not significant (maize variety).
SED = 2.74, LSD ($p \leq 0.05$) = 5.49 (dosage of extract).
SED = 4.84, LSD ($p \leq 0.05$) = 9.67 (interaction).

Table 4. Mean percentage repellency of *X. aethiopica* seed extract to *S. zeamais*.

Dosage of extracts (ml/30cm^2)	% Repellency	Repellency class
0.0	24.33	II
0.1	33.33	II
0.2	50.00	III
0.3	70.00	IV
0.4	73.00	IV
SED	9.26	
LSD (0.05)	18.54	

Class II = 20.1 – 40%, Class III = 40.1 – 60%, Class IV = 60.1 – 80%.

LSR-Y, with an occasional exception at 0.75 ml /20 g seed dosage when the percentage mortality (36.67%) was also observed in Pajo White.

Repellency test

The percentage of weevils that were repelled from paper discs treated with different dosages of *X. aethiopica* seed extract was significant ($P \leq 0.05$). Repellency was dose-dependent as 0.3 ml/30 cm^2 evoked Class IV repellency in *S. zeamais*, while 0.2 ml/30 cm^2 evoked Class III and 0.1 ml/30 cm^2 evoked Class II repellency, respectively, in *S. zeamais* adults (Table 4).

DISCUSSION

The study has shown that integrating *X. aethiopica* seed extract and partial varietal resistance significantly increased percentage of seeds of different maize varieties protected against damage by *S. zeamais.* This finding agrees with Ashamo (2005), Ajayi and Lale (2001) and Lale and Mustapha (2000) who reported potentials of integrating botanicals with varietal resistance in protecting stored products against insect pests. DMR-LSR-Y was more protected than the other two varieties (Pajo White and Tsolo Yellow). Varietal resistance significantly interacted with *X. aethiopica* extract in reducing the number of F_1 progenies. This suggests that DMR-LSR-Y probably has antibiosis properties against *S. zeamais*. This is evident as the least number of F_1 adult obtained at any dosage of *X. aethiopica* extract was observed in the cultivar and significantly lowest number of F_1 progenies emerged in the cultivar at 0.0 ml/20 g seed. The antibiosis mechanism could be in reduced number of eggs laid or presence of growth inhibitors or even deleterious compound(s) against the immature insect (Semple, 1992). As well, DMR-LSR-Y successfully integrated with *X. aethiopica* extract to reduce weight loss due to insect feeding. This implies that the interaction induced some anti-nutritional components of the variety to be released against *S. zeamais*.

Dick and Credland (1986) showed that the number of adult *C. maculatus* which can emerge from cowpea seeds depends, among other things, on the numbers of eggs initially present. Successful infestation is, however, determined by the number of eggs that hatched as well as the number of first instar larvae that are able to penetrate the cotyledons (Lale and Mustapha, 2000). Any of these processes can lead to reduction in degree of seed damage. Odeyemi and Daramola (2000) attribu-ted antibiosis in seeds of plants to chemical substances unacceptable to insects, or nutritional properties of seeds such as a vitreous endosperm. Since mortality was dosage-dependent and the highest percentage was recorded in DMR-LSR-Y, we opined that the deleterious compound(s) that reduced fecundity in DMR-LSR-Y could also have effect on adult mortality.

The integration of *X. aethiopica* seed extract with varietal resistance appears to have great potential for the management of weevil in stored maize. In DMR-LRS-Y, their joint use resulted in a possible synergism that may provide a sustainable control of *S. zeamais* in stored maize. In addition, their joint use in weevil control would be likely to delay the emergence of biotypes of the weevil that are capable of breaking down resistance in maize varieties or strains of *S. zeamais* with resis-tance to *X. aethiopica* seed extract. Several authors (Obeng-ofori et al., 1997; Owusu, 2001; Tapondjou et al., 2005; Udo, 2005) have reported that *S. zeamais* adults were significantly repelled by botanicals. The strong repellent property of *X. aethiopica* seed extract implies that stored maize may be significantly protected against non-resident population of *S. zeamais* thus reducing the tendency of cross infestation.

Since *X. aethiopica* is readily available (Irvine, 1961), ecologically friendly and economical, its adoption by resource-poor farmers in the tropics would be easy. Iwu et al. (1999) and Tairu et al. (1999) had documented the medicinal uses of *X. aethiopica* as a carminative, as a cough remedy, and as a post partum tonic and lactation aid. Other uses are stomachache, bronchitis, biliousness and dysentery. It is also used externally as a poultice for headache and neuralgia. It is used with lemon grass for female hygiene. Hence its incorporation into maize stored for human consumption and animal feeds are not likely to pose any health hazards.

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