academicJournals

Vol. 8(32), pp. 4374-4379, 22 August 2013
DOI: 10.5897/AJAR2013.6932
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Laboratory Evaluation of Cotton (*Gossypium hirsutum*) and Ethiopian Mustard (*Brassica cariata*) Seed Oils as Grain Protectants against Maize Weevil, *Sitophilus zeamais* M. (Coleoptera:Curculionidae)

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Accepted 13 August, 2013

Maize is the second most widely grown cereal crop in Ethiopia. In storage, maize is severely destroyed by storage insect pests, mainly maize weevil *(Sitophilus zeamais).* In an effort to develop a non-synthetic pesticide control approach, a study was conducted to determine the efficacy of two cooking oils, Ethiopian mustard (*Brassica carinata*) and cotton (*Gossypium hirsutum*), to control *S. zeamais* under laboratory conditions. The oils were applied at the rate of 0.2 to 0.5 ml per 250 g of grain and compared with untreated control and malathion super dust as standard check. The study was laid-out in completely randomized design (CRD) with three replications for each treatment. The efficacy of the oils was assessed on the basis of total insect mortality, median lethal time (LT_{50}), weevil progeny emergence, seed hole's number, weight loss and germination rate. The results showed that the oils caused 25 to 100% mortality at the different concentrations used. Both oils had LT_{50} of 0.5 day when applied at the concentration of 0.5 ml. At concentration of 0.3 to 0.5 ml, both oils caused zero weevil progeny emergence, minimum seed damage, zero grain weight loss and 89.2 to 95.5% seed germination rate which were similar to those of malathion (Diethyl succinate) and significantly different from those of the untreated control. The tests demonstrated that the two oils are effective stored maize grain protectants and can be used as components of maize weevil integrated pest management option.

Key words: Cooking oils, maize weevil*, Sitophilus zeamais,* mortality, stored grain.

INTRODUCTION

In Ethiopia, maize is the second widely grown cereal crop with annual land coverage of 1.8 million ha and average yield of 2.2 tons/ha (CSA, 2007). The importance of maize for Ethiopia is underscored by its use as food and as raw material for various industries (Meseret, 2011). A major constraint in the storage of maize is the damage caused by the maize weevil, *Sitophilus zeamais* (Abraham, 1991, 1995; Emana and Tsedeke, 1999; Negeri and Adisu, 2001; Sori and Ayana, 2012). The insect bores a hole of 1 mm in diameter into the grain, deposits the eggs and seals the opening with a gelatinous waxy secretion. The post-embryonic immature stages develop in the grain and emerge as adults from the grain feeding on the content that leads to total grain loss (Boxall et al., 2002;

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Table 1. Treatments (concentrations) of the chemicals applied on maize for the control of *S. zeamais*.

Oil	Dosage (Treatments)	Local name	Common name
Untreated control	-	-	-
Malathion dust (5%)	0.125 g	-	-
G. hirsutum	0.20 ml 0.30 ml 0.40 ml 0.50 ml	Tit/ Jirbi	Cotton
B. carinata	0.20 ml 0.30 ml 0.40 ml 0.50 ml	Gomenzer	Ethiopian mustard

Rees, 2004).

At present, the use of synthetic pesticides is the only recourse for farmers in protecting their maize stored grains. However, synthetic pesticides have a number of unwanted side-effects such as poisoning among handlers, toxic residues in food and feedstuff, ecological disruption as well as chronic and genetic maladies (Dubey et al., 2007; Kumar et al., 2007). This situation calls for the development of alternative methods of maize grain protection that are safer, health and eco-friendly. One attractive approach is the use of cooking and essential oils as seed protectants. Their modes of action include fumigant effect, topical toxicity, antifeedant or repellent activity, among others (Muhammad, 2009).

In a previous study, Fekadu et al. (2012) tested several botanical powders and two cooking oils, cotton (*Gossypium hirsutum*) and Ethiopian mustard (*Brassica carinata*) seed oils, for their activity against *S. zeamais*. The results indicated that the two oils were effective in killing the weevil and preventing damage to maize grains. This study was conducted to evaluate the efficacy of the two cooking oils at different application rates under laboratory conditions. The goal was the generation of a new technology for safe, low-cost, easy and efficacious control of maize weevil on stored maize grains.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at room temperature in entomology laboratory of Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Southwestern Ethiopia from July to December, 2011. JUCAVM is located 354 km Southwest of Addis Ababa (capital city of Ethiopia) at an approximate geographical coordinates of latitude (06°36' N) and longitude (37°12' E) at an altitude of 1710 m above sea level. The mean maximum and minimum temperatures are 26.8 and 11.4°C, respectively and the mean maximum and minimum relative humidity are 91.4 and 39.92%, respectively.

Sitophilus rearing conditions

S. zeamais adults were collected from stored cereal grains from shops in Jimma town market and brought to the laboratory of the College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia. The weevils were reared on maize grains in jars covered with perforated caps for gas exchange at 27°C and 70% RH in an incubator (heating incubator made in Spain called T.P. Selecta s.a). Newly emerged F_1 adults (1:1 male-female ratio) were used as the test insects.

Maize grains

Maize variety BH-660 grains were obtained from Nekempte Cereal Division and Distribution Enterprise. This variety, developed by the National Maize Research Program, Bako, Western Ethiopia, is the most commonly grown hybrid in the country and considered to be susceptible to insect infestation (Abraham and Basedow, 2004). The grains were kept at -6°C for 7 days to kill any infesting insects, cleared of broken kernels and debris and then graded manually according to size. Similar sized grains were selected for the experiment.

Cooking oils and bioassay procedure

Refined Ethiopian mustard (*B. carinata*) and unrefined cotton (*G. hirsutum*) seed oils were obtained respectively from Haranghe Fadis and Addis Mojo oil refineries, Eastern Ethiopia and stored in the laboratory at room temperature until needed.

The oils were separately mixed by handshaking with 2 ml of acetone in such a way that the final dosages were 0.2 to 0.5 ml per 250 g maize grains. Malathion super dust (5%) formulation was used as the positive control at a dosage of 0.125 g/ 250 g maize grain and maize grains treated with acetone alone served as the negative control. Treatments (Table 1) were replicated three times and laid out using completely randomized design (CRD). In all cases, 12 × 16 × 7 cm^3 plastic microwave boxes with perforated covers (for aeration) were used as containers (experimental jars). After thorough mixing of the chemicals with the maize grains by shaking for 5 min, the boxes were uncovered to allow the solvent to evaporate for 2 h.

To each box were added 20 adult weevils and the insects, maintained at 27°C and 70% RH in incubator. Mortality of the

Table 2. The Effect of different concentrations of *the chemicals used* on *S. zeamais*.

Treatment	Dosage	Mortality (%)	Median lethal time, LT_{50} (day)	Confidence Interval		Slope
				Lower	Upper	
G. hirsutmn oil	0.2 ml	30	25.8^{b}	13.9	94.9	1.43 ± 0.29
	0.3 ml	45	8.4^{c}	-	-	0.78 ± 0.54
	0.4 ml	85	0.6^{d}	-	-	1.18 ± 0.69
	0.5 ml	100	0.5^{d}	0.0	0.92	3.33 ± 1.56
B.carinata oil	0.2 ml	25	50.9^{a}	13.9	94.9	1.43 ± 0.23
	0.3 ml	35	15.5^{c}	-	-	0.78 ± 0.80
	0.4 ml	80	1.3^{d}	0.18	2.2	1.18 ± 0.40
	0.5 ml	90	0.6^{d}	0.01	0.23	3.33 ± 0.44
Malathion	0.125 g	90	0.14^{d}	-	-	1.01 ± 1.03
Control	-	5	NA	NA	NA	-

-, The confidence intervals are not provided because of the very low LT_{50} values are beyond the computing capacity of the soft ware (USEPA probit analysis program); NA**,** not applicable.

weevils was recorded daily from day 1 to 5 and at days 10, 15 and 20 (Parugrug and Roxas, 2008). At the end of the observation period, all adult weevils were removed from the boxes and the grain seeds were then returned to the incubator. From days 20 to 40 of adult weevil's introduction to experimental jars, the grains from each box were sieved to determine the number of progenies that emerged. On day 45, samples of the grains were taken for the determination of grain damage holes, weight loss and germination rate.

For grain damage determination, 10 seeds were taken randomly from each replication and examined for exit holes and occurrence of larvae within the seeds, if any. Weight loss was obtained using the formula described by Ileke and Oni (2011) which is given by:

$$Percentage\ Weight\ Loss = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} x\,100$$

The percentage seed germination (viability index) was determined from randomly selected 110 grains placed in Petri dishes containing moist filter papers which were incubated at 30°C for 7 to 10 days (Ogendo et al., 2004).

Data analysis

The insect percentage mortality and treatment median lethal time were determined using the US Environmental Protection Agency probit analysis program version 1.5 (Finney, 1971). Mortality rate were corrected using Abbott (1925) formula. Prior to analysis, data on progeny emergency, seed damage, weight loss and germination were square root-transformed to reduce variance heterogeneity (Gomez and Gomez, 1984). One-way analysis of variance and Tukey's honestly significant difference post hoc test at 5% significance level were performed using SAS version 9.2 software.

RESULTS

Fatal effects of oils on *Sitophilus zeamais*

The efficacy of the oils as killing agents on *Sitophilus* is presented in Table 2. Both cotton and mustard seed oils caused appreciable mortality of the weevils at all dosages used. Maximum total mortality (100%) of *S. zeamais* was caused when unrefined cotton oil was applied at 0.5 ml/250 g of maize grain followed by mustard oil at 0.5 ml and malathion, both inducing 90% total mortality. On the other hand, the lowest dosage of cotton and mustard oils (0.2 ml per 250 g of grain) respectively resulted in minimum total mortality among the concentration used, 30 and 25% in 20 days. The killing efficacy of the oils increased with increasing dosage. At a dosage of merely 0.4 ml/ 250 g (1.6 ml/kg), both cotton and mustard seed oils killed respectively 85 and 80% or more of the weevils and maximum insect fatality (90 and 100% mortality) was achieved at a dosage of 0.5 ml/250 g (2 ml/kg) in 1 day. The percentage mortality caused to weevils due to the toxicity of the oils were comparable with that of the positive check, malathion at 0.125 g/250 g (90%) and significantly different from that of the untreated check (5% total mortality). Consequently, the median lethal time for both oils at 0.5 ml/250 g was less than 1 day (0.5 and 0.6 days for both cotton and mustard, respectively), an indicative of the high efficacy of both oils against *Sitophilus*. As the concentration of the oils decreases, the time taken to kill 50% of the test insect (weevils) was longer and vice versa.

Effects of oils on weevil progeny emergence

Both oils inhibited the reproduction of the weevils at all dosages significantly ($P \leq 0.05$) (Table 3). Maximum and significantly more number of progenies (8 F_1 progenies) were emerged from the maize grain untreated with the oils. This was followed with the lowest concentration of both cooking oils. At the lowest dosage (0.2 ml/250 g) of

Table 3. Number of progenies of *S. zeamais* emergency from maize grains treated with different concentrations of the chemicals used.

Treatment	Dosage	Progeny number
G. hirsutmn oil	0.2 ml	3 (1.9)[b]*
	0.3 ml	0 (0.7)[d]
	0.4 ml	0 (0.7)[d]
	0.5 ml	0 (0.7)[d]
B. carinata oil	0.2 ml	1 (1.2)[c]
	0.3 ml	0 (0.7)[d]
	0.4 ml	0 (0.7)[d]
	0.5 ml	0 (0.7)[d]
Malathion	0.125 g	0 (0.7)[d]
Control	-	8 (2.9)[a]
P value		0.0001
HSD		0.37
CV(%)		15.3

***The numbers inside parentheses are the transformed data (√x+0.5) and means with the same letters within the column are not significantly different (P < 0.05)

both oils, insect progeny production was less than half (1 to 3 progenies) of that of the untreated control (8 progenies) and at higher oil dosages, as with the positive check, reproduction was completely inhibited, that is, no progeny emerged. These results are of course related to the relatively high fatal effects of the oils on the introduced weevils.

Effects of oils on weevil damaging activity

Except for cotton oil at a dosage of 0.2 ml/250 g, both oils at all dosages resulted in significantly less (P < 0.0001) damaged grains than did the untreated control (Table 4). Maximum and significant number of bored grains (0.54 out of 10 seeds), percentage weight loss (0.8%), and minimum percentage germination (84.8%) were obtained from the untreated control. On the contrary, significantly less number of bored seeds, weight loss and more percentage germinated seeds were obtained from the maximum concentration of the two cooking oils and the positive control. The number of holes of grains treated with lower dosages (0.2 and 0.3 ml/250 g) of the oil were less than half of that of the untreated check (0.2 versus 0.54 holes per 10 grains). At the dosage of 0.5 ml/250 g of the oils, the grains exhibited no holes at all, indicating that the weevils were effectively prevented from laying eggs on the grains. Similarly, weight loss of grains treated with low dosages of oil was half that of the untreated check (0.4 versus 0.8%) and those grains that received the highest dosage showed no loss in weight. The percentage germination data for grains treated with the oils followed similar trend; the oil-treated grains had germination rates of 88.3 to 95.5% which were significantly higher (P < 0.0001) than 84.8% for the untreated control.

DISCUSSION

Fekadu et al. (2012) found that both cotton and Ethiopian mustard seed oils caused, respectively, 100 and 95% mortality of *S. zeamais* with corresponding median lethal time of less than 1 day. In an effort to fine tune the use of these oils at lower dosages of 0.2 to 0.4 ml/250 g of maize grain were tested in addition to the dosage of 0.5 ml/250 g previously tested. The results of the test, using such variables as median lethal time, progeny emergence, seed hole number, weight loss and percentage germination, indicate that the dosage of 0.4 ml/250 g (1.6 ml/kg) is statistically comparable with the slightly higher dosage of 0.5 ml/250 g (2 ml/kg). These levels of treatment correspond respectively to 0.16 and 0.20% (v/w) of the oils. Demissie et al. (2008) obtained 100% mortality of maize weevil with a much higher dosage of 4.0% (w/w) cooking oil. The levels of oil found effective against the weevil in the present study likewise is compared favorably with a dosage of 0.7 ml *Cymbopogon citratus* essential oil per 50 g maize, equivalent to 1.4% (v/w), used by Odeyemi (1993) against *S. zeamais*. Essential oils are highly volatile and do pose fumigant activity leading to stored-product insects mortality (Ahn et al., 1998). These authors tested essential oils obtained from savory, oregano and myrtle and with varying degree of toxicity oils from the three plants have showed insecticidal activity against three species of adult insects namely: *Ephestia kuehniella*, *Plodia interpunctella* and *Acanthoscelides obtectus*.

There was a significant reduction in progeny emergence as a result of the efficacy of the two cooking oils. This might be due to increased adult mortality, ovicidal and/or larvicidal properties of the cooking oils confirming the findings of Selase and Getu (2009) and Bamaiyi et al. (2007). Different concentrations of dry ground leaves of *Chenopodium ambrosoides* resulted in complete (100%) inhibition of oviposition, progeny emergency and mortality of larvae of *Callosobruchus chinensis*, *Callosobruchus maculatus* and *A. obtectus* preventing feeding and damage (Tapondjou et al., 2002). In a separate study, *Chenopodium* leaf powder mixed with maize and sorghum grains at the rates of 2 and 4% w/w caused complete reduction in maize weevil F_1 progeny production (Mekuria, 1995; Dejen, 2002). Plant based products weaken adults weevils leading to laying of fewer eggs ultimately even with reduced hatchability of the eggs to larvae and final metamorphosis to adults. The effectiveness of different non-synthetic chemical products to various storage insect pests of stored products have been reported by several authors (Huang et al., 2000; Tripathi et al., 2000; Mbailao et al., 2006; Negahban et

Table 4. Effect of different concentrations of the chemicals used on maize seed grain percentage weight loss and germination percentage of infested maize by *S. zeamais*.

Treatment	Dosage	Hole number/10 seeds	Weight loss (%)	Germination (%)
G. hirsutum oil	0.2 ml	0.5(1.0)[a]*	0.4(1.2)[b]*	86.5(9.8)[fg]*
	0.3 ml	0.2(0.8)[b]	0.0(0.7)[c]	89.2 (10)[de]
	0.4 ml	0.1(0.8)[c]	0.0(0.7)[c]	92.8 (10.2)[bc]
	0.5 ml	0.0(0.7)[d]	0.0(0.7)[c]	95.5 (10.3)[a]
B. carinata oil	0.2 ml	0.3(0.9)[b]	0.4(1.1)[bc]	88.3 (9.9)[ef]
	0.3 ml	0.2(0.8)[b]	0.4(0.9)[bc]	89.2 (9.97)[de]
	0.4 ml	0.1(0.8)[c]	0.0(0.7)[c]	91.9 (10.1)[cd]
	0.5 ml	0.0(0.7)[d]	0.0(0.7)[c]	93.7 (10.2)[ab]
Malathion	0.125 g	0.0(0.7)[d]	0.0(0.7)[c]	95.5 (10.3)[a]
Control	-	0.54(1.0)[a]	0.8(1.6)[a]	84.8 (9.7)[g]
P value		0.0001	0.0001	0.0001
HSD		0.20	0.37	0.11
CV(%)		6.75	13.9	0.4

***The numbers inside parentheses are the transformed data (√x+0.5) and means with the same letters within the columns are not significantly different ($P < 0.05$)

al., 2007; Oni and Ileke, 2008; Sahaf et al., 2008; Ayvaz et al., 2010; Bachrouch et al., 2010; Sivakumar et al., 2010; Adedire et al., 2011; Ileke and Oni, 2011; Mahmoudvand et al., 2011). In other studies, it was reported that plant based products may act as fumigant, repellent, stomach poison and physical barrier against various insects (Mulungu et al., 2007; Law-Ogbomo and Enobakhare, 2007). Also, Araya (2007) found that fresh *Cymbopogon citratus* essential oil exhibited high mortality (85 to 100%) on mites showing acaricidal activity of the essential oils.

The study of Paranagama et al. (2003) showed that rough rice treated with *C. citratus* essential oil exhibited reduced germination rate in comparison with the control. In contrast, the cotton and mustard seed oils used in the present study did not show any adverse effect on maize seed germination. In agreement with the present study, the investigation of Dejen (2002) showed that powders of *Datura stramonium*, *Jatropha curcas*, *Phytoloca dodecondra* and *Azadrachta indica* applied for the control of *S. zeamais* did not show any significant effect on the germination capacity of stored sorghum grains.

The two cooking oils, *G. hirsutum* and *B. carinata* exhibited toxicity to adult weevils, inhibition of progeny emergency and as a result no damage to the grains throughout the storage period similar to the standard chemical. The toxicity of these cooking oils may be due to their active components responsible for the insecticidal properties against the insect pests including weevils. Oils are known to have toxic effects on insects involving their spiracular system (Cooping and Menn, 2000). Blockage of the spiracles by oils severely limits breathing leading to asphyxiation and death of the insect. The fatty acid composition seemed to be responsible for this acute toxicity of oils.

Conclusion

The study demonstrated that cotton and Ethiopian mustard seed oils exhibit toxic activity to maize weevil at treatment rates of less than or equal to 0.2% (v/w) when applied on stored maize. These oils are used for cooking and thus are safe for treating maize grains. They pose no danger to humans or animals even when the grains are used for food or feedstuff. It is recommended that studies be conducted to determine the efficacy, technical and economic feasibility of the oils against the maize weevil in pilot scale involving 50 kg of grains and eventually in the scale of tons of grain.

ACKNOWLEDGMENT

The authors thank Jimma University College of Agriculture and Veterinary Medicine for the financial and materials support and provision of laboratory facilities.

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