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RESEARCH ARTICLE

LABORATORY EVALUATION OF COTTON SEED, (*GOSSYPIUM HIRSUTUM*) AND ETHIOPIAN MUSTARD, (*BRASSICA CARINATA*) SEED OILS AGAINST ANGOMOIS GRAIN MOTH, *SITOTROGA CEREALLELA* (LEPIDOPTERA: *GELECHIIDAE*) OLIVER IN DILLA CONDITIONS

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ABSTRACT

As maize is the second most widely grown cereal crop in Ethiopia, effective protection is needed against storage insect pests including grain moth, *Sitotroga cereallela*. In an effort to develop a non-synthetic pesticide control approach, a study was conducted to determine the best dose of two cooking oils, Ethiopian mustard (*Brassica carinata*) and cotton (*Gossypium hirsutum*), to control *S. cereallela* 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}), moth progeny emergence, seed hole's number, weight loss and germination rate. The results showed that the oils caused 50 to 100% mortality at the different concentrations used. Both oils, *G. hirsutmn* and *B. carinata* had LT_{50} of 2.6 and 1.8 days, respectively when applied at the concentration of 0.2 ml. At concentration of 0.5 ml, both oils caused zero moth progeny emergence, minimum seed damage, zero grain weight loss and 88.3 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 in stored maize grain and can be used as the components of grain moth protectant in an integrated pest management option.

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INTRODUCTION

One of the most important challenges confronting the grain-handling agencies and stored product entomologists of the world are safety of food grains, in their combat against hunger, today. Cereals are the only source of nutrition for one-third of the world's population especially in developing and underdeveloped nations of Sub-Saharan Africa and South-east Asia (Muhammad Anwar, 2009). In Ethiopia, maize (*Zea mays* L.) is one of the major cereal crops grown for their food and feeding values. It is one of the most important staple food and cash crops providing calories for the consumers and income for the traders. The production and productivity of maize has increased since the development of high yielding hybrid varieties by the Ethiopian Institute of Agricultural Research and ranks first in production and productivity since the release of these hybrid varieties (CSA (Central Statistical Agency), 2007). However, these hybrid varieties are reported to be highly susceptible to insect pest attacks both in the field and storage (Girma *et al*., 2008). Hence, farmers are not as such the beneficiaries of this increased production and productivity potential of new varieties. Traditionally, maize grain is stored by Ethiopian farmers, both in and outdoors for consumption and sell in the later months of the year depending on the quantity produced per household. The most important insect pests that cause damage to maize in the field and storage are lepidopteran and coleopterans (Abraham, 1997; Emana, 1993).

Storage insect pests such as maize weevil (*Sitophilus zeamais)* followed by grain moth (*Sitotroga cereallela*), rice weevil (*Sitophilus oryzae)* and flour beetle (*Tribolium confusum)* were the major pests of stored maize in south western Ethiopia. Even though farmers store maize grain for food security, seed, and to sell when the prices are high enough (Deng *et al*., 2009; Waktole Sori1 and Amsalu Ayana, 2012; Fekadu Gemechu *et al*., 2013) lack of suitable storage structures and the absence

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of storage management technologies often force the small holder farmers to sell their produce immediately after harvest (Tadele *et al*., 2011).

This study was conducted to evaluate the efficacy of the different concentrations of the two cooking oils, *G. hirsutmn* and *B. carinata* 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 grain moth on stored maize grains.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted in Dilla University which is in the administrative center of Gedeo zone in the south nation, nationalities and people's regions. Dilla university is located 361.3 km on the southern direction of Addis Ababa on the main road which takes to Nairobi Kenya and has longitude and latitude of 6^0 24' 30'' N and 38^0 18' 30'' E coordinates, respectively with an elevation of 1570 above sea level.

Maize Grain Used for the Experiment

In all experiments, clean and well sieved maize grain of the variety 'BH-660' was used which was obtained from Ethiopian Seed Enterprise, Wollega branch and frozen at -6°C for seven days to kill any live insects. It is the most commonly grown maize hybrid developed by the National Maize Research Program based at Bako, Western Ethiopia and now days is considered as one of the susceptible maize verities to insect pests (Abraham, 1997). Then it was adequately dried in ultra violate light for six hours and not previously treated with pesticides. The grains were manually graded and almost only larger grains were used in the study. The grains were cleaned of broken kernels and debris removed by hand and by using a 4.76-mm round holed sieve.

Preparation of Cooking Oils

The purified cooking oil of *Brassica carinata* and unrefined cooking oil of *Gossypium hirsutmn* were gathered and brought immediately to the laboratory. The purified cooking oil, *B. carinata was* purchased from market and that of *G. hirsutmn* was brought from Addis Mojo oil factory of eastern showa zone, respectively following the procedure used by (Fekadu Gemechu *et al*., 2013).

Rearing of the Experimental Insects

The initial generations of *Sitotroga cereallela* was obtained from maize store culture of Hawassa town with maize grains and allowed to then further rear on 500g maize grains in four larger jars of approximately 2 litre volume capacity in an incubator at 27 ^{0}C and 60-70 r. h. This was for 35 days in Dilla University microbiology laboratory with experimental jars. Then after, another protocol was prepared for the experimental jar which was screwed plastic jar pinned with an electric pining machine and hole was sealed with cotton cloth to prevent the escaping of insects and entry of mites and other insects. It also allows the exchange of gases in and out of the container. The early emerged moths were transferred to the experimental jars using smaller test tubes, insect net and locally prepared aspirators.

Experimental Designs and Procedures

The experimental design was completely randomized design (CRD) replicated by three and percentage data were transformed using *Square-root transformation* (*x* + 0.5). Each prepared concentrations of oils were measured (from 0.2ml to 0.5ml) and applied to 250g of maize grains in each jar. Jars were arranged 5-10 cm apart on a flat table and left undisturbed after the introduction of 10 adult moths of almost similar age group at 25-30°C for oviposition.

The oil was dissolved with 2ml acetone per all concentration levels and allowed to evaporate for 2hrs before mixing with the maize seed. The jar contents were shaken thoroughly after mixed for about two minutes to ensure uniform distribution of the solution over grain surface. Jar with no treatment was used as a negative control. Malathion was applied in another jar as standard check at the rate of (0.125g/250g) to maize grain. After releasing of the adult insects, the toxicity effect of each dose was inspected from one to twenty days. After 20 days of adult introduction, all the live and dead insects were removed from each jar to monitor F1 progeny emergency until the next 40 days. On 45th days onward, samples of grains were taken from each experimental jar to check for number of seeds perforated (number of holed grains), the weights loss and percent germination.

Adult Mortality Test

Adult mortality by the oils was assessed throughout each jar on an interval basis (from one to five daily, and by five days interval to the next 20 days) after introduction as used by (Fekadu Gemechu *et al*., 2013) and percentage mortality was computed by the following formula:

$$Percent\,Mortality = \frac{Number\,of\;dead\,insects}{Total\,number\,of\;insects}\,x\,100$$

Progeny Emergence Test

After 20 days onward, all dead and live insects were removed from each container and the seeds were returned to their respective containers for the further assessment of F1 progeny emergency. Inspection was conducted similar to the 1st intervals used for the adult mortality till fourteen days.

Damaged seeds (seeds with holes)

Damaged seeds were assessed on 45th day after adult introduction by randomly taking 10 seeds from the total seeds in each jar and counting wholesome and bored or seed with insect emergent holes. The damaged seeds were expressed in number out of ten seeds.

Grain weight loss

Percentage weight loss was of assessed for both insects by measuring the initial and final weight of the grain as described by (Ileke and Oni, 2011).

$$Percentage\ weight\ loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight} x\,100$$

Germination percentage (viability index) test

Germination test was carried out up on 30 randomly selected seed out of the total seed grains from each jar. The seeds were placed in Petri dishes containing moistened filter paper (What man No.1) and arranged in an incubator at 30^0C in microbiological laboratory. The number of emerged seedlings from each Petri dish was counted and recorded from 7-10 days. The percent germination was computed according to (Ogendo *et al*., 2004) using the following formula:

$$Germination\ Percentage = \frac{Number of\ seed\ germinated}{Total\ grain sampled} x\,100$$

Data Analysis

All data were transformed using square root transformation to homogenize the variance (Gomez and Gomez, 1984) before analysis. Data were analyzed using one-way Analysis Of Variance (ANOVA) by SAS version 9.2 Software packages. USEPA Probit analysis version 1.5 was used for analyzing percent mortality and median lethal time. Mean separations were conducted using Tukeys' Studentized (HSD) test at 5% level of significance when treatments were found significant. All variables recorded were analyzed according to one-way ANOVA statistical model, i.e., $Y_{ij} = \mu + T_i + E_{ij}$, Where; Y_{ij} = is the response, μ = is the general mean effect, T_i = is the i^{th} treatment effect and E^{ij} = is the experimental error.

Table 1. Results revealed that, *G. hirsutmn* was seen the most promising growth inhibitors which cause maximum mortality at 0.5ml of 100% and minimum toxicity at 0.2ml with 66.7%. The other cooking oil *B. carinata* recorded 100% mortality at 0.5ml concentration and minimum mortality (50%) at 0.2ml at 20th days of exposures. But, highly significance differences were observed comparatively with that of untreated control that records (11.1 %). For the 20 days exposures, the median lethal time (LT_{50}) was <0.5 for the highest(100%) mortality revealed from fraction-1 in both cases that completely inhibited the growth of *S. cereallela* which is similar with that of Malathion super dust formulations (100%) and still highly significant difference was observed compared with that of control treatment (Table 1).

Progeny Emergence of *Sitotroga cereallela* after Grains Treated *G. hirsutmn* and *B. carinata* Seed Oils

Mean number of *S.cereallela* adult progeny emerged from maize grains treated with *G. hirsutmn* and *B. carinata* seed oils at different application rates were presented in Table 2. The result discloses that no adult was emerged from the first through 10-days from the days of adult removal i.e. the different rates of cooking oils significantly (P<0.05) reduced *S.cereallela* progeny emergency. However, starting from the next 15^{th} days onward, few progenies were started to be seen and significant difference in emerged progeny number between all concentrations were recorded.

Treatments with highest ratios resulted with no progeny emerged which was with significantly different number of progeny from the untreated control treatment. The reduction in F1 progeny emergence in the treated grains might be due to increased adult mortality, ovicidal and larvicidal properties of the tested cooking oils. But, still higher dosages and longer exposure periods are needed to achieve appreciable control as been reported by several authors.

Table 1. Adult mortality (Cumulative) and Median Lethal Time (LT_{50}) of *Sitotroga cereallela* by different concentrations of *Gossypium hirsutmn* and *Brassica carinata* seed oils

Treatment	Treatment Levels	Mortality (%)	Median Lethal Time (LT_{50}) in days	Confidence Interval		Slope
				Lower	Upper	
G. hirsutmn oil	0.2ml	66.7	1.8^c	0.3	3.0	1.60±0.68
	0.3ml	77.8	0.5^b	-*	-*	1.57±0.93
	0.4ml	83.3	0.6^b	-	-	1.57±0.96
	0.5ml	100	$<0.5^a$	-	-	-
B. carnata oil	0.2ml	50	2.6^b	-	-	1.67±0.91
	0.3ml	61.1	1.6^b	-	-	0.5±0.46
	0.4ml	94.4	0.6^{cd}	0.0	1.0	2.40±0.20
	0.5ml	100	$<0.5^a$	-	-	-
Malathion	0.125g	100	$<0.5^a$	-	-	-
Control	-	11.1	68^d	-	-	1.17±0.66

*The confidence interval for the oils and malathion could not provide, because of the very low LT_{50} obtained which are beyond the computing capacity of the soft ware (USEPA probit analisis program)

RESULTS AND DISCUSSION

Toxicity of (Cumulative) *G. hirsutmn* and *B. carinata* Seed Oils against *Sitotroga Cereallela*

Results regarding mortality effects of cooking oils (*G. hirsutmn* and *B. carinata* seed oils) against *S. cereallela* are presented in

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But, still higher dosages and longer exposure periods are needed to achieve appreciable control as been reported by several authors.

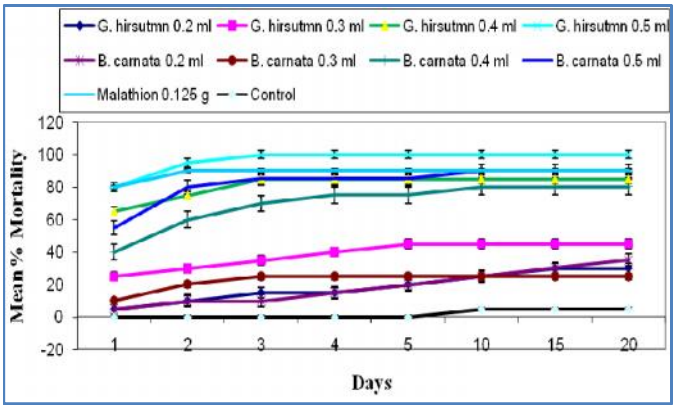

Figure 1. Percent adult mortality (Cumulative) of *Sitotroga cereallela* by different concentrations of *G. hirsutmn* and *B. carinata* seed oils at different time intervals (days)

The number of grain moth progeny emergence was significantly reduced from the grain treated with *G. hirsutmn* and *B.carinata* oils. Practically, plant oils coating can be effective in reducing progeny production by storage insect pests which is similar with (Javed Iqbal *et al*., 2010), that he reported the possible cause for reduction of F1 progeny production of *S. cereallela* in treated grains with cooking oils. This was likely that immature stages of the insects were killed physically by oil coating and impairing respiration through blockage of spiracles thereby resulting in inhibiting immature stages survival or reduced longevity of adult females.

Table 2. *Sitotroga cereallela* progeny emergence from maize grains treated with different concentrations of cooking oils at different time intervals (days)

Treatment	Levels	Progeny emerged after 40th days
G. hirsutmn oil	0.2ml	2 (1.6)b*
	0.3ml	1 (1.2)bc
	0.4ml	0 (0.7)c
	0.5ml	0 (0.7)c
B. carinata oil	0.2ml	2 1.6)b
	0.3ml	0 (0.7)c
	0.4ml	0 (0.7)c
	0.5ml	0 (0.7)c
	0.125g	0 (0.7)c
Control	-	4 (2.1)a
P value		0.0047
HSD		0.37
CV (%)		13.5

*Figures with similar letters are not significantly different along the columns

Maize Grain Damage by *Sitotroga cereallela* after Treated with *G. hirsutmn* and *B.carinata* Seed Oils

Grain damage by *S. cereallela* was assessed in terms of counting perforated holes, percent weight loss and percent germination reduction caused by adult moth and larvae feeding inside the seeds 45th days from the day of introduction. The treatment levels were significantly different ($P<0.05$) with respect to the number of perforated seeds, percent weight loss and grain viability (Table 3). Mean numbers of perforated seeds were maximum (0.2) from untreated check which was significantly different from jars that received higher rates (with no hole) on par with Malathion used.

Maximum weight loss was recorded from untreated grains i.e. (0.63%) and the higher concentration (0.5ml) scored 0.5% weight loss was from grains treated with least concentration (0.2ml). No weight loss was recorded from the higher rates of the two cooking oils on par with the standard check (Malathion). Similar trends were followed for the percent germination assessed. That means, higher germination percentages were recorded from the two promising cooking oils with higher ratios i.e. (95.5%) *G. hirsutmn* and (93.7%) *B. carinata* at 0.5ml and (88.3%) at 0.2ml level for both, respectively this is on par with standard check chemical, Malathion (95.5%). However, significantly different results were obtained compared to the untreated control with (85.6%) (Table 3).

The cooking oils at all dosage rates nevertheless offered better protection than the control. The reduced damage recorded by the bio-pesticide (cooking oils) is an indication of their efficacy against grain moth infestation. Hence, the damage seems to follow the trend of potency of the trial insecticides on insect mortality. The result observed on the toxicity of *S.cereallela* is in agreement with the study of (Javed Iqbal *et al*., 2010), that extracts of *Acorus calamus*, sweet flag, *Azadrachta indica* and *Curcuma longa* (turmeric) prepared in petroleum ether, acetone and ethanol evaluated as growth inhibitor against *Sitotroga cereallela* resulted promising result.

Table 3. Hole number counted, percent weight loss and percent germination of maize grains treated with different concentrations of *G. hirsutmn* and *Brassica carinata* Seed Oils by *Sitotroga cereallela*

Treatment	Levels	Hole Number	Weight Loss %	Germination %
G. hirsutmn oil	0.2ml	0.1 (0.8) ab*	0.5 (1.27) ab*	88.3 (9.9) dc*
	0.3ml	0.1(0.8) bc	0.41(1.03) bc	90.1(10) cd
	0.4ml	0.0(0.8) c	0.31(0.77) c	93.7(10.2) a
	0.5ml	0.0(0.8) c	0.28(0.7) c	95.5(10.3) a
B. carinata oil	0.2ml	l0.1(0.8) ab	0.52(1.3) ab	88.3(9.9) de
	0.3ml	0.1(0.87) bc	0.41(1.03) ab	89.2(9.97) cd
	0.4ml	0.0(0.7) c	0.37(0.93) bc	91.9(10.1) ab
	0.5ml	0.0(0.7) c	0.31(0.9) c	93.7(10.2) ab
	0.125g	0.0(0.7) c	0.31(0.9) c	95.5(10.3) a
Control	-	0.2 (0.8) a	0.63 (1.1) a	85.6 (9.77) c
P value		0.0001	0.0001	0.0001
HSD		0.41	0.495	0.14
CV (%)		14.79	16.7	0.5

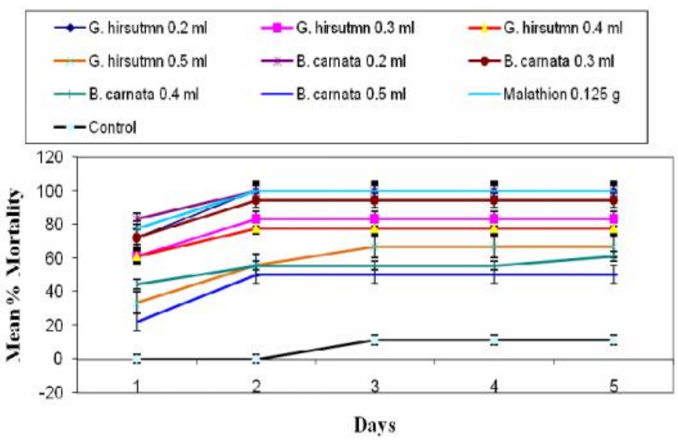

Figure 2. Percent toxicity of different concentrations of *G. hirsutmn* and *B.carinata* oils against *Sitotroga cereallela*

Petroleum ether extract of sweet flag at application rates of 1000, 500 and 250µg/g and its acetone extract at 1000 and 500µg/g completely inhibited emergence of moth adults. (Zaidi *et al*., 2003), compared extracts of 'neem', turmeric and sweet flag as insect repellents against *Sitotroga cereallela,* under laboratory conditions and found that the acetone-extract of neem was the most effective botanical insecticide.

When the grain moth, *Sitotroga cereallela* and rice meal moth, *Corcyra cephalonica* were controlled by *T. chilonis,* it was found that percentage parasitism and adult longevity of *T. chilonis* was the highest on *S. cereallela* as compared to *C. cephalonica* eggs. It means *S. cereallela* was more affected than *C. cephalonica*. Maximum parasitism and adult emergence in *T. chilonis* were observed on *S. cereallela* and *C. cephalonica* at 28 °C, respectively (Perveen and Sultan, 2012). The results showed that *T. chilonis* preferred young eggs when offered older eggs, simultaneously (Perveen *et al*., 2012).

Conclusion

This study demonstrated that cotton and Ethiopian mustard seed oils exhibit strong toxic activity against grain moth at concentration levels less than or equal to 0.2% (v/w) up on applied to grain storage. 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. However, further study is recommended on the detail impact of these and similar oils to determine the repeated efficacy, technical usage and economic feasibility of the oils in line with the resistance formation of moths and still other insects.

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