Efficacy of Ethanol Extracted Selected Botanicals and Diatomaceous Earth against Maize Weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) in the Laboratory

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Abstract

Ethanol extracted three botanicals (*Azadirachta indica*, *Chenopodium ambrosoides* & *Maesa lanceolata*) and diatomaceous earth (DA) were tested to evaluate their effectiveness against maize weevil, *Sitophilus zeamais* M. They were applied at three rates of 0.3, 0.4 and 0.5 ml/g per 250 g of grain and compared with untreated control, ethanol treated control and Malathion super dust as a standard check. The study was laid-out in completely randomized design with three replications. Data were collected on cumulative adult mortality, median lethal concentration, median lethal time, F_1 progeny emergency, and grain damage. The results revealed that, higher mortality of *S. zeamais* was observed from ethanol extracts of *C. ambrosoides* (98.3% with LT_{50} of 1.13 days), *A. indica* (97.5% with LT_{50} of 1.26 days), and diatomaceous earth (100% with LT_{50} of 1.33 days) at 0.5ml/g after 21 days of exposure periods. Apart from the untreated and ethanol treated controls, the lowest cumulative adult mortality of *S. zeamais* was recorded from extracts of *M. lanceolata* (58.4% with LT_{50} of 17.62 days) at 0.3ml. The LC_{50} indicated that *C. ambrosoides* extracts (0.23g/250g of maize) was the most toxic at minimum concentration while *M. lanceolata* extracts and diatomaceous earth (0.27g) was the least potent to weevil at lower concentration. No F_1 progeny emerged from the grains treated with the former three treatments similar to the standard chemical leading to no seed with hole, no weight loss and maximum germination percentage (93.50- 95.2%). The study demonstrated that the extracts and DE at their higher concentration/dosage can be used as components of maize weevil integrated management option.

Keywords: Botanical extracts, diatomaceous earth, cumulative mortality, stored grain, *Sitophilus zeamais*.

Introduction

Maize (*Zea mays* L.) is one of the major food crops of the world and it has the first position in terms of production worldwide (Emily and Sherry 2010). It is the most important cereal food crop in Sub-Saharan Africa, particularly in eastern and southern Africa accounting for 53% of the total area covered by cereals (FAO 2010) and 30-70% of the total caloric consumption (Langyintuo *et al.* 2010). In Ethiopia, maize is the staple food and one of the main sources of calories particularly

in the major maize producing regions of the country (Girma *et al.* 2008). It is Ethiopia's leading cereal in terms of production, with 6 million tons produced in 2012 by 9 million farmers on 2 million hectares of land (CSA 2012). In 2012/13, the maize production in Ethiopia was on 2,013,044.93 ha, and productivity was 3059 kg/ha (CSA 2013). Hence food security and welfare of the farming population in Ethiopia are dependent on the productive capacity of maize farmers (Wekesa *et al.* 2003).

Despite the worldwide increase in the demand, production and land coverage for maize, the use value of maize is hampered due to many biotic and abiotic factors both in the field and storage. Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is one of the most serious insect pests of stored cereal grains, especially of maize, in tropical and subtropical regions (Throne, 1994). Infestation by this weevil begins in the field (Demissie *et al.* 2008), but significant damage happens during storage. The maize weevil together with other storage insect pests cause an estimated 24.5% loss of maize, and damaged grains have reduced nutritional value and weight, low germination percentage, and low or no market value (Demissie *et al.* 2008; Napoleao *et al.* 2013). To overcome the losses caused by maize weevil, synthetic chemical insecticides have been recommended for use, but indiscriminate use of chemical insecticides from time to time created a number of problems (high cost, toxicity to non-target organisms and human being, inherent environmental hazards and the development of resistance by insect pests) that have limited their effective use for maize storage (Al-Moajel 2006). Furthermore, the majorities of farmers in Ethiopia are resource-poor and have neither the means nor the skills to obtain and handle pesticides appropriately (Esayas 2014). Therefore, chemical inert materials, such as diatomaceous earth or plant derived insecticide products that are environmentally safe and economically feasible weevil management practices needs to be developed (Asawalam *et al.* 2008). Different authors have reported the effectiveness of different plant powders by testing against maize weevil, *S. zeamais* (Sharma and Gubta 2009; Defago *et al.* 2011; Fekadu *et al.* 2012). However, the publications on botanicals used in storage against different storage insect pests are dominated by powdered materials with very little on solvent extracts of botanicals especially in Ethiopia. Similarly, research outputs are limited on the efficacy of diatomaceous earth in Africa (Stathers *et al.* 2002; Dimessie *et al.* 2008) including Ethiopia. Therefore, this study was initiated with the objective to evaluate the potential activity of ethanol extracted botanicals and diatomaceous earth against maize weevil, *S. zeamais* at different concentrations under laboratory conditions.

Materials and Methods

Description of the study area and laboratory conditions

The experiments were conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) Postharvest Laboratory under room temperature in 2014/15. JUCAVM is located in southwestern part of Ethiopia at 356 km from Addis Ababa at 7°33"N latitude and 36°57"E longitude with an elevation of 1710 meters above sea level. The experiment was repeated to ensure the

validity of the results of the experiments and the final results were averaged. The laboratory room temperature and relative humidity were 24.5^{0}C to 27.4 ^{0}C and 52.3-59.4% from the start to end of the experiment respectively.

Maize grain used for the experiment

Maize variety BH-660, which is the most commonly grown hybrid maize variety in the country and considered to be the most susceptible to storage insect infestation (Abraham and Basedow, 2004) was used as a sample grain. This variety, developed by the National Maize Research Program, Bako, Western Ethiopia, was obtained from Nekempte Cereal Division and Distribution Enterprise. The grains were cleared of broken kernels and debris and then graded manually according to size and were kept in a deep freezer at -18°C for three days before the commencement of the experiment to kill any live insect pests and their eggs that might be present.

Rearing of *S. zeamais*

The initial generation of *S. zeamais* was obtained from maize store in Jimma town with maize grains and allowed to reproduce further at room temperature in JUCAVM Postharvest laboratory to obtain uniform population of weevils reared under the same condition/environment feeding the same produce for the study. Maize grain not previously treated with any insecticide was used to rear the insects.

Collection, preparation and extraction of botanicals

Fresh and matured seeds of *Azadirachta indica* (Neem), *Maesa lanceolata* (False assegai) and fresh leaves of *Chenopodium ambrosoides* (Mexican tea) were collected from different localities and brought immediately to the laboratory. *A. indica* was collected from Melka Werer Agricultural Research Centre, while *M. lanceolata was* collected from the natural habitat of southwestern Ethiopia, Jimma zone of Oromia Regional State; Mana Woreda and *C. ambrosoides* was gathered from Jimma Agricultural Research Center and Jimma town along road sides.

The collected seeds and leaves were air dried under shade for 14 days in the laboratory at JUCAVM. The dried leaves (*C. ambrosoides*) and seeds (*A. indica* and *M. lanceolata*) were pulverized using a micro pulverizer machine and were sieved through a 0.25 mm pore size mesh sieve to obtain uniform particle size as indicated in Araya (2007) and Parugrug and Roxas (2008). Oil was extracted from the powder of each botanical with 95% ethanol by soxhlet extraction (model EV-16, Germany) for 6 hrs at above 78°C (Evans and Raj, 2008). At the end of extraction, the liquid extract was filtered through filter paper and evaporated to separate the solvent from the extract under vacuum at the same temperature using a rotary-evaporator apparatus. The extracts obtained from each plant species were stored in volumetric flasks and maintained in a refrigerator (4°C) to reduce evaporative loss until further use. Malathion super dust 5% was purchased from farm inputs store (market). Diatomaceous earth was obtained from Europe, Belgium.

Experimental designs and treatments details

The experiment was arranged in completely randomized design with three replications (Table 1) Three control treatments were included in the experiment (the untreated control, maize treated with ethanol alone and maize

treated with Malathion super (5% dust formulation as a standard check) for comparison. The treatments were formulated in the form of oil for the botanicals and powder for the diatomaceous earth. Each treatment was measured and mixed with 250g of maize grain in one-liter high density polyethylene plastic container. Treatments were arranged 10 cm apart on a laboratory bench for oviposition of adult weevils.

Bioassay procedures

Prior to treatment application, the extracts were dissolved with 2 ml of 95% ethanol before mixing it with the maize seed and shaken thoroughly to ensure uniform distribution over grain surface. Treated grains were kept for 2 hours to allow the ethanol to evaporate completely before bioassays were conducted. Ethanol solution which served as a control was also left for 2 hrs before the introduction of 20 adult weevils. The container contents (treatments and maize grain) were shaken thoroughly for about five minutes to ensure uniform distribution of the extracts and diatomaceous earth. Then, 20 newly emerged adult weevils were collected from the rearing jar and introduced into the treatments jars at the same time. After introduction of the predetermined adult insects; adult mortality, F_1 progeny emergency, seed damage, weight loss, and germination percentage were determined at prescribed period.

Table 1: Description of treatments used in the experiments

List	Treatments	Plant part used	Dosage	Local name	Common name	Formulation
T1	*A. indica*	Seed	0.3 ml	Mimi zaf	Neem	Oil Extracts
T2	*A. indica*	Seed	0.4 ml	Mimi zaf	Neem	Oil extracts
T3	*A. indica*	Seed	0.5 ml	Mimi zaf	Neem	Oil extracts
T4	*C. ambrosoides*	Leaf	0.3 ml	Tirign	Mexican tea	Oil extracts
T5	*C. ambrosoides*	Leaf	0.4 ml	Tirign	Mexican tea	Oil extracts
T6	*C. ambrosoides*	Leaf	0.5 ml	Tirign	Mexican tea	Oil extracts
T7	*M. lanceolata*	Seed	0.3 ml	Abayi	-	Oil extracts
T8	*M. lanceolata*	Seed	0.4 ml	Abayi	-	Oil extracts
T9	*M. lanceolata*	Seed	0.5 ml	Abayi	-	Oil extracts
T10	Diatomaceous earth	-	0.3g	-	Inert dust	Powder
T11	Diatomaceous earth	-	0.4g	-	Inert dust	Powder
T12	Diatomaceous earth	-	0.5g	-	Inert dust	Powder
T13	Untreated maize control	-	-	-	-	-
T14	Ethanol treated control	-	2 ml	-	-	Liquid
T15	Malathion dust (5%)	-	0.125 g	-	-	Dust

Adult mortality test

Maize weevil adult mortality was assessed on 1st, 3rd, 7th, 14th and 21st days after exposure of the weevils to the treatments as described in Yankanchi and Gadache (2010). Insects were counted dead when they failed to move any part of their body in response to gentle probing of the exposed abdomen with sharp objects. Those dead insects were removed from the sample once the data were collected. In subsequent day counting's, the data on each assessment day was summed up and considered as a cumulative adult weevils' mortality. Percent adult mortality was determined as per the method described in Parugrug and Roxas (2008).

$$Mortality\ (\%) = \frac{Dead\ weevils}{Total\ weevils} x\ 100$$

Median lethal concentration (LC_{50}) and median lethal time (LT_{50})

Median lethal concentration (LC_{50}) is the concentration required to kill 50% of adult *S. zeamais*, while median lethal time (LT_{50}) is the time required to kill 50% due to botanical extracts and diatomaceous earth. They were determined by taking in to account, respectively the concentration and time in which the extracts and diatomaceous earth caused mortality of 50% of *S. zeamais* population. Probit analysis (Finney 1971) was used to determine median lethal concentration and lethal time.

F_1 progeny emergence test

Twenty one days after the introduction of the weevils to each experimental plastic box, all dead and live insects were removed from each container and the seeds were returned to their respective containers for an additional period of 24 days for further F_1 progeny emergence assessment. F_1 progeny count was made on 25th, 30th, 35th, 40th and 45th days after adult weevils' introduction. The number of F_1 progeny emerged from each assessment day was summed up. Inspection of the progenies was made on each assessment day by displaying the seeds on paper and sieving the contents of the container with test sieve that has aperture of 2.36 mm.

Damaged seeds

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

Grain weight loss

Percentage weight loss was assessed by measuring the initial and final weight of the grain as described by Ileke and Oni (2011):

$$Weight\ loss(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} X100\%$$

Germination percentage (viability index) test

Germination test was carried out on randomly taken 50 seeds from each treatment. The seeds were placed in Petri dishes containing moistened soft paper and kept at 30°C in an incubator. The number of germinated seedlings from each Petri dish was counted and recorded from 7 to 9 days after putting in the Petri dishes. The percent germination was computed as described in Ogendo *et al.* (2004):

$$Germination(\%) = \frac{No\ of\ seed\ germinated}{Total\ grain\ sampled\ (50)} X\ 100\%$$

Data analysis

All data were analyzed using one-way Analysis of Variance (ANOVA) model by SAS software, version 9.2 packages (SAS 2008). Mortality data were corrected using Abbott's formula (Abbot 1925). The median lethal concentration and median lethal time were determined using the United State Environmental Protection Agency Probit analysis program version 1.5 (Finney 1971). Prior to analysis, data on progeny emergency, seed damage, and weight loss were square root-transformed to reduce variance heterogeneity (Gomez and Gomez 1984). Mean separations were conducted using Tukey's Honestly Significant Difference (HSD) test at 5% level of significance.

Results

Cumulative mortality

Cumulative mortality of *S. zeamais* from 1st to 21st days after application of the botanical extracts and diatomaceous earth was found significantly different ($P<0.05$) (Table 2). On day one after exposure, the highest adult mortality .(65%) was recorded from the jar that received malathion dust which was on par with the jar that received highest concentration (0.5ml) of extracts of *C. ambrosoides* (55%). On the other hand, extracts from *M. lanceolata* at lowest concentration (0.3ml) resulted the lowest mortality (4.2%) among materials tested on par with the negative control, Ethanol, *C. ambrosoides* and *A. indica* at lowest concentration. Similar trends but with an increase in adult mortality were observed on 3rd, 7th, 14th and 21st days after exposures to the botanical extracts and diatomaceous earth. On 21st days after introduction, diatomaceous earth at 0.5g and malathion registered maximum adult mortality (100%) followed by *A. indica* and *C. ambrosoides* at 0.5ml (97.5 & 98.3%, respectively), and diatomaceous earth at 0.4g (90.9%) all of which are statistically similar to each other. In general, all treatments except untreated and ethanol treated controls (9.2 & 15.9%, respectively) showed appreciable toxicity which is above 58% death of maize weevils.

Table 2 Cumulative adult mortality (%) of *S. zeamais* due to oil extracts of three botanicals and diatomaceous earth across different time exposure

		Mean mortality (%) over days				
Treatment	Dosage	1st day	3rd days	7th days	14th days	21st days
A. indica	0.3ml	8.3^{efg}	24.2^{d}	35.9^{fg}	54.2^{fg}	74.2^{d}
	0.4ml	17.5^{cde}	40.0^{bc}	54.2^{de}	67.5^{de}	84.2^{bc}
	0.5ml	50.8^{b}	73.3^{a}	80.9^{ab}	90.9^{b}	97.5^{a}
C. ambrosoide	0.3ml	10.0^{efg}	25.9^{d}	37.5^{fg}	49.2^{fg}	77.5^{cd}
	0.4ml	23.9^{cd}	45.9^{bc}	64.2^{d}	75.9^{cd}	85.9^{bc}
	0.5ml	55.0^{ab}	72.5^{a}	81.7^{ab}	89.2^{b}	98.3^{a}
M. lanceolata	0.3ml	4.2^{fg}	9.3^{ed}	31.7^{g}	44.2^{gh}	58.4^{e}
	0.4ml	10.8^{efg}	34.2^{cd}	53.4^{de}	65.9^{def}	70.9^{d}
	0.5ml	22.5^{cd}	54.2^{b}	64.2^{d}	70.9^{de}	79.2^{cd}
DE	0.3g	14.2^{def}	35.9^{cd}	49.2^{ef}	60.0^{ef}	72.5^{d}
	0.4g	25.8^{cd}	42.5^{bc}	65.9^{cd}	81.7^{bc}	90.9^{ab}
	0.5g	47.5^{b}	75.0^{a}	94.2^{a}	99.2^{a}	100.0^{a}
Control	-	0.0^{g}	0.0^{f}	0.9^{i}	4.2^{h}	9.2^{g}
Ethanol	2ml	3.3^{f}	7.5^{e}	9.2^{h}	14.2^{g}	15.9^{f}
Malathion dust	0.125g	65.0^{a}	80.9^{a}	94.2^{a}	100.0^{a}	100.0^{a}
CV (%)		17.3	11.3	8.7	5.5	4.8
$HSD_{5\%}$		12.3	14.2	14.2	12.8	10.8

Means within a column followed by the same letter (s) are not significantly different, $P<0.05\%$, Tukey's Honestly significance test (HSD)

Median lethal concentration (LC_{50})

The median lethal concentrations (LC_{50}) after 21 days of treatment application on the mortality of the weevils were presented in (Table 3). The minimum concentration required to kill 50% of *S. zeamais* was determined for each botanical extracts and diatomaceous earth. The LC_{50} values for *A. indica, C. ambrosoides, M. lanceolata* and diatomaceous earth indicated that *C. ambrosoides* extracts at 0.23ml concentration was sufficient to cause death of 50 % of the weevils. While a concentration of 0.27ml and the same amount was recorded for the LC_{50} values of *M. lanceolata* extract and diatomaceous earth.

Table 3. Median Lethal Concentration (LC_{50}) of extracts of three botanicals and diatomaceous earth on *S. zeamais*, after 21 days of exposure

		95% CI limit		
Treatments	LC_{50}	Lower	Upper	Slope[±SE]
A. indica	0.24ml	0.15	0.29	5.17±1.45
C. ambrosoides	0.23ml	0.12	0.28	5.08±1.51
M. lanceolata	0.27ml	0.07	0.34	2.88±1.17
DE*	0.27g	0.21	0.30	7.88±1.79

* DE: Diatomaceous Earth

Median lethal time (LT_{50})

The median lethal time, the time required to kill 50% of *S. zeamais* due to botanical extracts and diatomaceous earth at different concentrations were indicated in Table 4. The least time was required for the standard check, malathion (0.82 day) followed by extracts of *C. ambrosoides* (1.13 days), A. *indica* (1.26 days) and diatomaceous earth (1.33 days) at their higher concentration, while ethanol treated control took significantly the longest time (107.15 days) followed by *M. lanceolata* at 0.3mL (17.62 days) to kill 50% of the target insect pest, maize weevils.

F_1 progeny emergency

Botanical extracts and diatomaceous earth showed significant difference ($P<0.05$) in terms of maize weevil F_1 progeny emergency on 25th,-30th, 35th, 40th and 45th days of weevils introduction to the experimental jars (Table 5). Maximum mean numbers of progenies were emerged, on 25th days, from the jars that received no treatment (1.7 adults), followed by the jar that received ethanol (1.3 adults). But, there were no F1 progeny emerged from the grains treated with Malathion super dust and all other treatments on the same day after adult weevils' introduction to experimental jars. Generally, similar trends but with an increase in weevils emergency were observed on 30th, 35th, 40th and 45th days after adult weevils introduction. On the 45th days of adult weevils' introduction, maximum and significantly the same number of progenies, 12.4 and 8.4 adults, were emerged from the samples that received no treatment and ethanol respectively. This was followed with the lowest dosage of diatomaceous earth and the lowest concentration of *M. lanceolata* extracts, 3.4 and 3.2, respectively.

Table 4. Median Lethal Time (days) (LT_{50}) of *S. zeamais* due to botanical extracts and diatomaceous earth insecticidal effect

			95% CI limit		
Treatments	conc./dosage	LT_{50}	Lower	Upper	Slope[±SE]
A. indica	0.3ml	12.12	9.01	16.05	1.90±0.40
	0.4ml	6.49	4.42	8.81	1.53±0.25
	0.5ml	1.26	0.64	1.97	1.28±0.20
C. ambrosoides	0.3ml	12.19	8.88	16.42	1.83±0.38
	0.4ml	4.29	2.93	5.72	1.82±0.26
	0.5ml	1.13	0.52	1.81	1.22±0.20
M. lanceolata	0.3ml	17.62	13.22	26.67	1.81±0.41
	0.4ml	8.52	5.89	11.71	1.50±0.26
	0.5ml	4.63	2.86	6.73	1.19±0.20
DE	0.3g	9.21	6.21	13.22	1.37±0.25
	0.4g	4.28	2.89	5.77	1.66±0.24
	0.5g	1.33	0.88	1.78	2.07±0.28
Control	-	NA	NA	NA	NA
Ethanol	2ml	107.15	-	-	1.40±0.81
Malathion dust	0.125g	0.82	0.43	1.21	1.76±0.28

Note: No confidence interval for ethanol, because of the LT_{50} obtained is beyond the computing capacity of the software (USEPA probit analysis program),
Key: NA: not applicable; CI: confidence interval

Maize grain damage assessment

Significant differences ($P<0.05$) were recorded among treatments with respect to the number of perforated seeds, percent weight loss and grain viability (Table 6). Maximum mean and significant numbers of perforated seeds (average of 0.5, 0.4 and 0.2 out of 10 seeds) were obtained from the untreated control, ethanol treated control, *M. lanceolata* and diatomaceous earth at their lowest concentration respectively. On the contrary, none of the seeds was perforated when maximum concentration of *A. indica, C. ambrosoides*, diatomaceous earth and Malathion super dust were applied. The average numbers of holes when lower dosages of extracts and diatomaceous earth applied were 0.2, 0.1, 0.1 and 0.2 for *M. lanceolata, A. indica, C. ambrosoides* and diatomaceous earth, respectively. At maximum dosage (0.5mL/g), the grains exhibited no holes at all, indicating that the weevils were effectively prevented from laying eggs on the grains.

Regarding percentage grain weight loss, the maximum and significant number of percentage grains weight loss was from untreated check (1.1%) which is on par with grains treated with ethanol (0.9%). No grain weight loss was recorded from the highest concentration of *A. indica, C. ambrosoides,* diatomaceous earth and standard check (Malathion). All treatments significantly reduced weight loss compared to the untreated and ethanol treated controls 45 days after introduction of *S. zeamais* in to the grains.

Table 5: F_1 progeny emergency from maize grains treated with botanical extracts and diatomaceous earth at different time intervals (days)

Treatment	Dosage	Time interval after exposure (days)				
		25	30	35	40	45
A. indica	0.3ml	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0.4(0.9)^{d}	1.6(1.3)^{bc}
	0.4ml	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0(0.7)^{de}	1.4(1.2)^{bc}
	0.5ml	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0(0.7)^{de}	0(0.7)^{d}
C. ambrosoide	0.3ml	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0.5(1)^{d}	1(1.2)^{bc}
	0.4ml	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0(0.7)^{de}	0.7(1.1)^{bc}
	0.5ml	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0(0.7)^{de}	0(0.7)^{d}
M. lanceolata	0.3ml	0(0.7)^{c}	0.4(0.9)^{b}	1(1)^{c}	1.9(1.6)^{c}	3.2(1.9)^{b}
	0.4ml	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	1(1.1)^{d}	1.7(1.5)^{b}
	0.5ml	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0(0.7)^{de}	0.7(1.1)^{bc}
DE	0.3g	0(0.7)^{c}	0.5(1)^{b}	1.3(1)^{c}	2.5(1.8)^{c}	3.4(2.0)^{b}
	0.4g	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0(0.7)^{de}	1(1.2)^{bc}
	0.5g	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0(0.7)^{de}	0(0.7)^{d}
Control	-	1.7(1.5)^{a}	3.9(2.1)^{a}	6.2(2.6)^{a}	8.3(3.0)^{a}	12.4(3.6)^{a}
Ethanol	2ml	1.3(1.2)^{b}	3(1.9)^{a}	4.7(2.3)^{b}	6.3(2.6)^{b}	8.4(3.0)^{a}
Malathion dust	0.125g	0(0.7)^{c}	0(0.7)^{b}	0(0.7)^{d}	0(0.7)^{de}	0(0.7)^{d}
CV (%)		9.46	10.91	7.44	9.93	12.22
$HSD_{5\%}$		0.23	0.30	0.23	0.35	0.54

Note: Means with the same letters within the same columns are not significantly different ($P<0.05$);

The result on viability (germination percentage) of maize seeds indicated that none of the plant extracts and diatomaceous earth mixed with the grains adversely affected the germination of maize grains compared to the untreated and ethanol treated controls. Significantly minimum germination percentage of 83 % was recorded from the untreated seeds which was statistically on par and followed by maize grains treated with ethanol (84.7%) and *M. lanceolata* extracts (85.6%), respectively. On the other hand, the highest germination percentage was recorded from grains treated with Malathion (95.2%) which was statistically on par and followed by highest dosages of diatomaceous earth (94.3%), *A. indica* (93.6%) and *C. ambrosoides* extracts (93.5%).

The result of simple linear correlation studies among the variables revealed that there is an association between percent adult weevils' mortality, mean F1 progeny emergency, number of seed perforated, percent weight loss and germination percentage of maize grains infested (Table 7). Percent adult mortality was inversely and highly significantly correlated with mean progeny emergency ($r = -0.94^{**}$), number of seeds perforated ($r = -0.89^{**}$), percentage weight loss ($r = -0.85^{**}$) but positively and highly significantly correlated with germination percentage ($r = 0.91^{**}$). Besides, weevil's progeny emergency, maize grain weight loss and seed holes (damage) are positively correlated

Table 6. Grain holes, weight loss and germination percentage of maize grains infested with *S. zeamais* as influenced by botanical extracts and diatomaceous earth

Treatments	Dosage	Hole number/10 seeds	WL (%)	GP (%)
A.indica	0.3ml	0.1(0.8)^{ab}	0.1(0.8)^{c}	88.7^{bc}
	0.4 ml	0.1(0.8)^{ab}	0.0(0.7)^{c}	91.2^{b}
	0.5 ml	0.0(0.7)^{bc}	0(0.7)^{c}	93.6^{a}
C.ambrosoide	0.3 ml	0.1(0.8)^{ab}	0.1(0.8)^{c}	88.4^{bc}
	0.4 ml	0.1(0.8)^{ab}	0.1(0.8)^{c}	90.9^{b}
	0.5 ml	0.0(0.7)^{bc}	0(0.7)^{c}	93.5^{a}
M.lanceolata	0.3 ml	0.2(0.8)^{ab}	0.5(1.2)^{a}	85.6^{d}
	0.4 ml	0.1(0.8)^{ab}	0.1(0.8)^{c}	88.7^{bc}
	0.5 ml	0.1(0.8)^{ab}	0.1(0.8)^{c}	91.1^{b}
DE	0.3g	0.2(0.9)^{a}	0.5(1)^{b}	88.5^{bc}
	0.4g	0.1(0.8)^{ab}	0.2(0 8)^{c}	91.4^{b}
	0.5g	0.0(0.7)^{bc}	0(0.7)^{c}	94.3^{a}
Control	-	0.5(1.0)^{a}	1.1(1.3)^{a}	83.0^{d}
Ethanol	2ml	0.4(0.9)^{a}	0.9(1.2)^{a}	84.7^{d}
Malathion dust	0.125g	0.0(0.7)^{bc}	0(0.7)^{c}	95.2^{a}
CV (%)		3.69	5.69	1.0
$HSD_{5\%}$		0.12	0.15	2.6

Key: WL: weight loss; GP: germination percentage

Table 7. Pearson simple correlation coefficients among different variables of maize grains infested by *S. zeamais* as influenced by botanical extracts and diatomaceous earth treatments

	Mortality	Progeny emergency	Seed hole	Weight loss (%)	Germination (%)
Mortality	-				
Progeny emergency	−0.94**	-			
Seed hole	−0.89**	0.92**	-		
Weight loss (%)	−0.85**	0.92**	0.87**	-	
Germination (%)	0.91**	−0.82**	−0.86**	−0.84**	-

**Simple linear correlation is highly significant

Discussion

Ethanol extracted botanicals and diatomaceous earth at the highest concentration/dosage (0.5ml/g) gave a comparable result with the standard insecticide, Malathion (5%) super dust. The potency of DE, extracts of *A. indica* and *C. ambrosoides* is due to the presence of high bioactive chemicals within these concentrations to kill the target insect pest (Isman 2006). The present investigation concurs with the report of Fekadu *et al.* (2013) who stated that *Brassica carinata* and *Gossypium hirsutum* seed oils caused appreciable mortality of *S. zeamais* at differem concentrations of 0.3, 0.4, and 0.5mL/250g of maize grain and caused mortality of 45%, 80% and 100% for *G. hirsutmn)* and (35%, 80% and 90% for *B. carinata*) after 20 days of exposure, respectively. The levels of extracts found effective against the maize weevil in the present study likewise are compared favorably with a dosages used by the above authors against *S. zeamais*.

Likewise, diatomaceous earth caused high mortality of *S. zeamais* at all dosage used and resulted in 91.7 and 100% adult mortality at 0.4g and 0.5g, respectively after 21 days of exposure weevils to the inert material. Demissie *et al.* (2008) also reported that diatomaceous earth caused

99 and 100% mortality of *S. zeamais* within 3 and 7 days of exposure periods, respectively, at the rate of 2%. Marsaro *et al.* (2006) observed that, in the dosages of 400, 600, 800 and 1,000 g/t of DE, the accumulated mortality of *S. zeamais* was superior of 95 % in 14 days of exposure time. In the present study, it was observed that there was an increase in the mortality of *S. zeamais* as the exposure time of diatomaceous earth increased. Arthur (2001; 2002) also verified the same pattern when studying the effect of diatomaceous earth on mortality of *Oryzaephilus surinamensis*. This fact is related to the mode of action of diatomaceous earth in the insect. According to Subramanyam and Roesli (2000) the death of the insects by diatomaceous earth is attributed to the dehydration provoked by the abrasiveness of the small particles of this inert dust and by adsorption of oils in the body of the insect, which breaks the layer of wax on the epicuticle, causing loss of water and death. It also limits free movement of insects inside the container by filling the gap spaces and causes the insects to loss availability of sufficient oxygen and leads to death.

The result of median lethal concentration indicated that though all the applied botanical extracts and diatomaceous earth were effective to control *S. zeamais*, the degree of toxicity depends on the concentration applied. From the above result, it is clear that all the tested treatments were more or less effective for controlling the weevil, *S. zeamais*, but *C. ambrosoides* extracts was the most effective followed by *A. indica* extracts with minimum concentration. Nukenine *et al.* (2010) determined the seven-day LC_{50}-values for diatomaceous earth and obtained 0.56 g/kg to kill 50% *S. zeamais* in the exposed time. The potency of the treatments, in terms of the time needed to kill 50% *S. zeamais* population is also concentration dependent. As the concentration of extracts and diatomaceous earth decreases, the time taken to kill 50% of the test insect (weevils) increases and vice versa. Though the botanical extracts and diatomaceous earth were found to be promising bio-pesticide, the minimum time was taken by the standard check, Malathion super dust (5%). It appears therefore that the botanical extracts and diatomaceous earth screened have insecticidal properties and effectiveness against *S. zeamais* and could be of use in weevil management in storage.

The reduction in F1 progeny emergence in the treated grains might be due to increased adult mortality, ovicidal and larvicidal properties of the tested extracts of the botanicals (Araya and Emana 2009). The current findings are similar to the results of Cotton and Ethiopian mustard cooking oils at the same concentration levels which also resulted to the same percentage reduction of F1 progeny emergency (Fekadu *et al.* 2013). Generally, the present study showed that ethanol extracted oils and diatomaceous earth have strong oviposition effect against *S. zeamais* as the standard insecticide, Malathion, by significantly reducing F1 progeny emergence. Large numbers of weevils were emerged from the untreated check, ethanol treated and most of the other treatments at lower concentration/dosage after 40^{th} days of adult introduction. This indicates that the total development period (TDP) of the maize weevil, *S. zeamais* is about 40 days on an average under Jimma condition. This means that the tested botanical extracts and diatomaceous earth did not affect the growth and development of maize weevil at their lower concentration

inside the grain. This finding coincides with the work of Fekadu *et al.* (2012) and Parugrug and Roxas (2008) who reported 40 and 39 days, respectively for the same insect in other countries.

At higher dosage of botanical extracts and diatomaceous earth, the grains exhibited no holes at all, indicating that the weevils were effectively prevented from laying eggs on the grains. This finding coincides with the work of Fekadu *et al.* (2013) who reported no grain holes when maize was treated with mustard and cotton cooking oils at different rates. Nukenine *et al.* (2010) also reported the same by testing diatomaceous earth against maize weevil.

The present finding showed that extracts of all three botanicals and diatomaceous earth at higher doses effectively reduced the grain damage and its weight loss was comparable to the standard check, Malathion super dust. Similarly, there was no significant weight loss in the grains treated with the highest dose of essential oil from *Ocimum gratissimum* (Hassanali 2008). Maize grains treated with cotton and mustard seed oils also indicated no weight loss even at lower dosage (Fekadu *et al.* 2013).

The germination test demonstrated that the botanical extracts and diatomaceous earth tested against *S. zeamais* did not show any visible adverse effects on germination capacity of the grains. Dejen (2002) also showed that powders of *Datura stramonium*, *Jatropha curcas*, *Phytoloca dodecondra* and *A. indica* applied for the control of *S. zeamais* did not show any significant effect on the germination capacity of stored sorghum grains. Fekadu *et al.* (2012) also reported that *A. indica, C. ambrosoides, M. lanceolata,* and cooking oils from cotton and mustard seed did not show any effect on germination capacity of maize. Similarly, Stathers *et al.* (2000; 2002) and Nukenine *et al.* (2010) reported that diatomaceous earth products did not have negative effects on seed germination. Some of the treatments were infected by moulds of fungal pathogens grown on the soft paper kept on the underside of the petridishes, which resulted in a reduced germination percentage (Fekadu *et al.* 2012; 2013; Araya and Emana 2009).

The simple linear correlation studies indicated that there was strong association between percent adult weevils' mortality, mean F1 progeny emergency, number of perforated holes on the seeds, percent weight loss and germination percentage of maize grains. Fekadu *et al.* (2012; 2013) reported the same pattern concerning the association between adult weevils' mortality and these four variables.

Conclusions

The current findings demonstrated that the three botanical extracts and diatomaceous earth tested have insecticidal activity against maize weevil, *S. zeamais*. Insecticidal activity was confirmed in all tested materials, although the results showed variation in their effectiveness against *S. zeamais*. Among the botanical extracts, *A. indica* and *C. ambrosoides* were observed to be the highest potent botanicals and *M. lanceolata* revealed to be moderately toxic, while diatomaceous earth at higher dosage was the most promising potent among all types of materials used against maize weevil over 21 days of exposure. *A. indica, C. ambrosoides* extracts and diatomaceous earth at their highest concentration caused mortality ranging from 98.3-100% to *S. zeamais* after 21 days of exposures. Generally, the results revealed that

botanical extracts and diatomaceous earth have considerable potential as storage insect pest management options. Moreover, some of these botanical extracts and diatomaceous earth could find a place in IPM strategies of *S. zeamais*, which needs to be investigated, especially where the emphasis is on environmental, food safety and on replacing the more dangerous toxic insecticides with more eco-friendly insecticides. Traditional pest control methods, especially the use of indigenous pesticide plants and diatomaceous earth, offer a safer, low cost and more dependable method of stored produce protection. Then, issues of propagation and cultivation of these botanicals as well as proper storage facility on post-harvest pest control will be looked at closely in order to enhance crop productivity and food security.

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