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Evaluation of Plant Powders and Cooking Oils against Maize Weevil, *Sitophilus zeamais* M. (Coleopteran: Curculionidae) under Laboratory Conditions

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Abstract In storage, maize grains are severely destroyed by insects and other storage pests. One of the primary causes of grain loss in stored maize is the damage caused by maize weevil, *Sitophilus zeamais*. A study was conducted to evaluate selected locally available botanical powders and cooking oils for their effectiveness as grain protectants against maize weevils at JUCAVM in year 2011. The plant powders and cooking oils were compared with untreated control and Malathion super dust as standard check. The experiment was laid-out in Completely Randomized Design with 13 treatments each replicated thrice. Different dependent variables such as cumulative adult mortality, F₁ progeny emergency, grain damage were assessed. The results revealed that, among the botanical powders, there was an increased cumulative adult *S. zeamais* mortality, i.e. higher mortality, from powders of *Chenopodium ambrosoides* (70% with LT₅₀ of 6.50 days), *Azadiricta indica* leaf (70% with LT₅₀ of 6.00 days), *A. indica* bark (70% with LT₅₀ of 8.40 days) and *Tagetes erecta* (70% with LT₅₀ of 9.20 days) after 20 days of exposure periods. Apart from the untreated control, low mortality of *S. zeamais* adult was recorded from *Allium sativum* (50% with LT₅₀ of 18.01 days), *C. citratus* (55% with LT₅₀ of 18.30 days), *Maesa lanceolata* (55% with LT₅₀ of 16.20 days) and *Echinops kebericho* (55% with LT₅₀ of 14.50 days) after 20 days of exposure periods. Highest *S. zeamais* adult mortality of 95%, 100% and 100% was recorded from *Brassica carinata* oil, *Gossypium hirsutum* oil and the standard chemical Malathion, respectively with LT₅₀ of less than one day in all cases. Maximum number of progeny was emerged from untreated check from 25th~40th days of exposures with cumulative increase (2 to 14 adults). There was no F₁ progeny emergency from the three treatments (oils and Malathion) over the exposure periods reassuring the potency of the cooking oils against *S. zeamais*. As a result there was no perforated seed; no weight loss and maximum germination percentage of 94.60% were registered from the two cooking oils on par with the Malathion (95.50%). In conclusion, the two cooking oils were found to be the most potent bio-insecticides on par with standard check, Malathion and they can be used in integrated management of maize weevil, *S. zeamais*.

Keywords Cooking oils; Cumulative mortality; Exposure time; Grain damage; LT₅₀; Plant powders; *Sitophilus zeamais*

Introduction

Cereals are the main source of nutrition for one-third of the world's poorest population in Sub-Saharan Africa and South-East Asia. Out of the three major cereals (rice, wheat and maize), maize (*Zea mays* L.) is an important cereal crop grows in different parts of the world including in Africa serving as source of food and industrial raw materials (Abdurahman, 2009). Maize provides 20% of the world's food calories and 15% of all food crop protein (Meseret, 2011). According to the report from CIMMYT and IITA (2011), maize production is projected to double in the developing countries by the year 2050 and its global production surpasses all crops by 2025. In Ethiopia,

maize is second in the area coverage among cereal crops, accounting for about 1.8 million hectares with average yield of 2.2 ton/ha (Alemu, 2011).

Despite the worldwide continuous increase in the land coverage, production and demand for maize; there are multi-faceted production bottlenecks recognized as bio-physical factors in the world (Sahaf et al., 2008) and in Ethiopia too with emphasis on poor utilization of hybrid seeds (Negeri and Adisu, 2001). Food grain yield losses due to insect pests and diseases are estimated to be within the range of 5%~10% (temperate zone) and 50%~100% (tropical) regions (Van Wyk et al., 2009). Global postharvest grain and pulse crops loss, mainly due to insect pests,

was estimated to be 10% (Boxall et al., 2002). FAO (1985) estimated 200 million tons of grain loss every year due to damage caused by storage pests and improper storage practices by poor farmers of the developing countries. Among the major constraints of maize production and productivity, the primary causes of yield losses in storage is the damage caused by storage insect pests in different parts of Ethiopia (Tadesse, 1991, 1996, 1997; Khosravi et al., 2007; Eman and Tsedeke, 1999; Sori and Ayana, 2012). The serious pests of maize in the field and storage are stalk borers and weevils, respectively in Ethiopia (Eman and Tsedeke, 1999).

S. zeamais causes characteristics damage to maize by making holes on the grain that is about 1 mm in size in which the adult female deposit eggs. The holes are sealed with a gelatinous waxy secretion. The eggs, larval and pupal stages of the insect take place within the grain after which the emerging adult weevil comes out of the grain via the holes, leaving visible hole on the grain (Rees, 2004; Sahaf et al., 2008) which invites secondary infection. Maize weevil damage results directly in lost food ready for consumption or lost cash from farmers' pocket ready to buy other valuable resources for the family. Maize weevils damage caused to maize grain also reduce the viability of the seeds as the larvae and adult consumes the embryo affecting maize production of farmers who plant saved grain (estimated to be 70% of all maize planted in eastern and southern Africa) as seed (Boxall et al., 2002).

The use of synthetic chemical insecticides is an old age practices to control pests in general and stored product pests in particular. However, the indiscriminate use of many synthetic insecticides is associated with manifold human, technical, environmental, non-target organisms and even insect pest management problems such as resistance of insect pests, food and food product contamination with toxic residues, increased cost of application, handling hazards, environmental contamination, biodiversity erosion and other negative impacts incredible magnitude of human health's (Dubey et al., 2007; Kumar et al., 2007). In addition, the increased public awareness and concern for environmental safety, increased regulatory constraints, unavailability of insecticides to countryside farmers and ventilation

restrictions in storage are the negative effects of the synthetic chemicals (Oppert et al., 2010). Thus, the search for eco-friendly, cost effective, easily available insect pest management options is intensified worldwide to reduce the use of synthetic chemicals in pest management. Among the benign alternative insect pest management options is the use of plant based products to curtail the menace caused by insect pests in storage. Broadly speaking plant based products are an effective and benign tools in wide range of insect pest management with broad spectrum of action (Muhammad, 2009). Therefore, this study was aimed to determine the cumulative toxicity, reproduction inhibiting capacity and maize grain protection ability of different botanical powders and cooking oils against maize weevil, *S. zeamais* under laboratory conditions to contribute to the protection of maize grains in storage by generating eco-friendly effective weevil management tools.

2 Results

2.1 Cumulative adult *S. zeamais* mortality (%)

The cumulative mortality of *S. zeamais* from 1st~20th days after application of the botanicals and cooking oils was significantly different ($P < 0.05$) (Table 1). Among the botanicals used *C. ambrosoid*, *T. erecta*, *A. indica* leaf and bark powder registered higher adult mortality (70%) followed by *A. indica* kernel (65%), *C. citratus*, *M. lanceolata* and *E. kebericho* (55%) and *A. sativum* (50%). Greater adult weevils mortality due to the application of the botanicals was observed as the exposure time of the pest to the treatment increased. As exposure time proceeds, there was a progressive increase in the toxicity of the botanicals to the test insect registering appreciable mortality of *S. zeamais*. The two cooking oils had potent biocidal effects on *S. zeamais* with more or less the same efficacy with Malathion super dust inducing more than 95% cumulative toxicity to the test insect over 20 days exposure time. The efficacy of the treatments is in the order of Malathion and *G. hirusutum* > *B. carinata* > *A. indica* leaf, *A. indica* bark, *T. erecta* and *C. ambrosoides* > *A. indica* kernel > *C. citrates*, *M. lanceolata* and *E. Kebericho* > *A. sativum*.

The treatments were found significant ($P < 0.05$) with respect to the median lethal time (LT_{50}) (Table 1).

Among the treatments the time required to kill 50% of the test insect was less than one day for cotton and Ethiopian mustard oil statistically on par with Malathion super dust. Thus the most potent botanicals against maize weevil in storage are the two cooking oils. On the other hand, *C. citratus* required longer time (18.30 days) to kill 50% of *S. zeamais* which was statistically on par with *A. sativum* (18.0 days), *M. lanceolata* (16.2 days) and *E. kebericho* root (14.5 days) indicating their less

effectiveness against *S. zeamais*, *A. indica*, *T. erecta* and *C. ambrosoid* with LT_{50} values ranging from 6.0~9.2 days are considered as moderately effective botanicals on *S. zeamais*.

Maize weevil is an internal feeder and the different life stages normally develop successfully inside the grain. However; the three treatments, viz., Malathion, *G. hirsutum* and *B. carinata* completely inhibited growth of the weevils leading to no emergency of weevils.

Table 1 Cumulative adult mortality (%) and Median Lethal Time (days) (LT_{50}) of *S. zeamais* due to botanical powders and cooking oils

Treatment descriptions	Mortality (%)	LT_{50} (days)	Confidence interval		Slope[±SE]
			Lower	Upper	
Control	25	40.8 ^a	23.7	382.8	2.33±0.77
Malathion dust	100	<1.0 ^c	—*	—	—
<i>A. indica</i> leaf	70	6.0 ^b	3.2	13.4	0.82±0.25
<i>A. indica</i> bark	70	8.4 ^b	6.2	12.4	1.67±0.29
<i>A. indica</i> kernel	65	8.8 ^b	6.0	15.5	1.3±0.27
<i>C. citratus</i> leaf	55	18.3 ^{ab}	10.3	77.5	1.01±0.27
<i>T. erecta</i> leaf	70	9.2 ^b	7.02	12.9	1.95±0.31
<i>A. sativum</i> stem	50	18.0 ^{ab}	11.65	42.9	1.42±0.31
<i>M. lanceolata</i> seed	55	16.2 ^{ab}	9.2	70.0	0.95±0.26
<i>C. ambrosoides</i> leaf	70	6.5 ^b	4.7	9.6	1.49±0.27
<i>G. hirsutum</i> oil	100	<1.0 ^c	—	—	—
<i>B. carinata</i> seed oil	95	<1.0 ^c	—	—	—
<i>E. kebericho</i> root	55	14.5 ^{ab}	8.3	58.1	0.93±0.26

Note: No confidence interval for the oils and Malathion, because of the very low LT_{50} obtained which are beyond the computing capacity of the soft ware (USEPA probit analysis program)

2.2 *S. zeamais* F1 progeny emergency

There were no progenies emerged from the first day to fourth days of introduced weevils removal from the experimental jars. The botanicals and cooking oils were significant ($P < 0.05$) in terms of maize weevil F₁ progeny emergency on 25th, 30th, 35th and 40th days of weevils introduction to the experimental jars (Table 2).

Maximum mean numbers of progenies were emerged, on 25th days, from the jars that received no treatment, *A. sativum*, *M. lanceolata* (2 adults). But, there were no F₁ progeny emerged from the grains treated with Malathion super dust, *A. indica* kernel powder, *G. hirsutum* oil, *B. carinata* oil and *E. kebericho* root powder on the same day after adult weevils' introduction to experimental jars.

Table 2 *S. zeamais* F₁ progeny emergency from maize grains treated with botanical powders and cooking oils at different time intervals (days)

Treatments	Time interval after exposure (days)			
	25	30	35	40
Control	2 (1.53) ^{b*}	6 (2.03) ^{a*}	10 (3.1) ^{a*}	14 (3.3) ^{a*}
Malathion dust	0 (0.93) ^c	0 (0.7) ^c	0 (0.7) ^e	0 (0.7) ^d
<i>A. indica</i> leaf	1 (1.03) ^c	3 (1.47) ^{ab}	5 (1.5) ^{bc}	6 (1.47) ^b
<i>A. indica</i> bark	1 (1.2) ^{abc}	2 (1.4) ^b	3 (1.27) ^{bcd}	4 (1.3) ^{bc}
<i>A. indica</i> kernel	0 (0.87) ^c	0 (0.7) ^c	1 (0.87) ^{de}	2 (0.87) ^{cd}
<i>C. citratus</i> leaf	1 (1.03) ^c	2 (1.4) ^b	4 (1.6) ^{bc}	5 (1.2) ^{bcd}
<i>T. erecta</i> leaf	1 (1.13) ^{abc}	2 (1.2) ^{bc}	3 (1.2) ^{cd}	4 (1.27) ^{bc}
<i>A. sativum</i> stem	2 (1.67) ^a	4 (1.47) ^{ab}	6 (1.73) ^{ab}	7 (0.87) ^{cd}
<i>M. lanceolata</i> seed	2 (1.53) ^{ab}	3 (1.2) ^{bc}	5 (1.6) ^{bc}	6 (1.3) ^{bc}
<i>C. ambrosoid</i> leaf	1 (1.27) ^{abc}	2 (1.27) ^{bc}	3 (1.03) ^{de}	4 (1.03) ^{bcd}
<i>G. hirsutum</i> seed oil	0 (0.7) ^c	0 (0.7) ^c	0 (0.7) ^e	0 (0.7) ^d
<i>B. carinata</i> seed oil	0 (0.7) ^c	0 (0.7) ^c	0 (0.7) ^e	0 (0.7) ^d
<i>E. kebericho</i> root	0 (0.87) ^c	1 (1.3) ^b	2 (1.03) ^{de}	3 (1.03) ^{bcd}
P value	0.000 1	0.000 1	0.000 1	0.000 1
HSD	0.612	0.576	0.554	0.54
CV (%)	18.5	15.64	16.13	16.1

Note: The numbers inside parentheses are the transformed data ($\sqrt{x+0.5}$) and means with the same letters within the columns are not significantly different (P<0.05)

Generally, similar trends but with an increase in weevils emergency were observed on 30th, 35th and 40th days after adult weevils introduction. On the 40th day of adult weevils introduction (20 days from adult removal), maximum and significantly different number of progenies, 14 adults, were emerged from the jars that received no treatment. On the other hand, there was no progeny emergency from the jars that received Malathion, cotton and Ethiopian mustard oils. This indicates the efficacy of the two cooking oils against *S. zeamais* in preventing laying of eggs (reproduction) on maize seeds. The total number of *S. zeamais* adult

emerged from untreated control progressively increased with time of exposure compared to the other treatments. All the botanical powder treatments induced significant reduction in F₁ adult emergence of *S. zeamais* compared to the untreated check although the plant materials vary among themselves. Accordingly, *C. ambrosoid*, *T. erecta*, *E. kebericho*, *A. indica* kernel and bark were superior in reducing the production of F₁ progeny among the plant powders. However; *A. sativum*, *A. indica* leaf, *M. lanceolata* and *C. citratus* powder were less effective compared to other plant powder treatments.

2.3 Maize grain damage assessment

Grain damage by *S. zeamais* was assessed in terms of counting perforated holes on single seed; percent weight loss and viability loss of seeds caused by adult weevils and larvae feeding inside the seeds. The treatments 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 (average of 2.1 out of 10 seeds) and significantly different from untreated check which was on par with jars that received *A. indica* kernels and *C. citratus* (Av., 1.8 holed seeds out of 10) leaf powder.

Table 3 Grain holes, weight loss and germination percentage of maize grains infested with *S. zeamais* as influenced by different botanical powders and two cooking oils

Treatments	Hole number/10 seeds	Weight loss (%)	Germination (%)
Control	2.1(1.6) ^{a*}	4.6 (2.1) ^{a*}	86.5 (9.8) ^{c*}
Malathion dust	0.0 (0.7) ^f	0 (0.7) ^f	95.5 (10.3) ^a
<i>A.indica</i> leaf	1.1(1.3) ^{cd}	0.8 (1.7) ^{bc}	90.1 (10) ^{bc}
<i>A.indica</i> bark	1.3 (1.3) ^{cd}	1.2 (1.8) ^{abc}	90.1 (10) ^{bc}
<i>A.indica</i> kernel	1.6 (1.4) ^{ab}	0.8 (1.47) ^{cde}	90.1 (10) ^{bc}
<i>C.citratus</i> leaf	1.8 (1.5) ^a	0.8 (1.5) ^{abc}	90.1 (10) ^{bc}
<i>Teracta</i> leaf	1.1(1.3) ^{cd}	0.4 (1.3) ^{de}	90.1 (10) ^{bc}
<i>A.sativum</i> stem	1.1(1.3) ^{cd}	1.2 (1.8) ^{abc}	88.3 (9.9) ^c
<i>M.lanceolata</i> seed	1.1(1.3) ^{cd}	1.2 (1.9) ^{ab}	90.1 (10) ^{bc}
<i>C.ambrosoid</i> leaf	0.3 (0.9) ^e	0.4(1.2) ^e	91 (10.1) ^a
<i>G. hirsutum</i> seed oil	0.0 (0.7) ^f	0 (0.7) ^f	94.6 (10.3) ^a
<i>B.carinata</i> seed oil	0.0 (0.7) ^f	0 (0.7) ^f	94.6 (10.3) ^a
<i>E.kebericho</i> root	0.9 (1.2) ^d	0.8 (1.6) ^{bcd}	90.1 (10) ^{bc}
P value	0.0001	0.0001	0.0001
HSD	0.748	0.36	0.14
CV (%)	6.64	12.57	0.48

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

Percentage grains weight loss was highest from untreated check (4.6%) followed by grains treated with *A. indica* bark, *A. sativum* stem and *M. lanceolata* seed powder each with 1.2% weight loss. No grain weight loss was recorded from the two cooking oils on par with the standard check (Malathion). Some treatment effects were significantly different from others after 45 days of grain storage

and the untreated grain suffered highly significantly greater grain damage as well as weight loss than grains treated with Malathion 5% dust and all other treatments, except *M. lanceolata*, *A. sativum*, *C. citratus* and *A. indica* bark powder.

Germination percentage of the grains ranged from 86.5% to 95.5% in the untreated and Malathion treated

jars, respectively. The effect of the botanicals in powder form on the viability/germination rate of the treated grains indicated that none of the plant powders mixed with the grains adversely affected the germination of the maize grains compared to the untreated control.

Most of the treated seeds were germinated. But, statistically significantly higher germination percentage of seeds were recorded from grains treated with Malathion (95.50%) which was statistically on par and followed by *G. hirsutum* and *B. carinata* seed oil (94.60%) and *C. ambrosoid* leaf powder (91.00%).

Simple linear correlation studies among the variables revealed that there existed an association between

percent adult weevils' mortality, mean F_1 progeny emergency, number of perforated holes on the seeds, percent weight loss and germination percentage of maize grains infested with the same number of *S. zeamais* (Table 4). Percent adult mortality was inversely and significantly correlated with mean progeny emergency ($r = -0.47^*$), number seeds perforated ($r = -0.71^{**}$), percentage weight loss ($r = -0.79^{**}$) but positively and significantly correlated with germination percentage ($r = 0.74^{**}$). With an increasing trend of weevil's adult mortality; there is a decreasing trend of progeny emergency, number of perforated holes on the grains, percent weight loss. On the other hand, an increasing adult mortality is associated with an increasing germination percentage.

Table 4 Pearson correlation coefficients among different variables of maize grains infested by *S. zeamais* as influenced by botanicals and cooking oils treatments

	Mortality (%)	Progeny emergency	Hole number	Weight loss (%)	Germination (%)
Mortality (%)	1	-0.47*	-0.71*	-0.79*	0.74*
Progeny emerged		1	0.66*	0.71*	-0.69*
Hole number			1	0.90*	-0.84*
Weight loss (%)				1	-0.91*
Germination (%)					1

*Significant at 5% and 1% level of probability respectively

3 Discussions

The present laboratory study on the impact of different botanical powders and two cooking oils indicated that most of the botanical powders tested gave less control of *S. zeamais* when compared with that of the standard insecticide, Malathion super dust 5%. However, the two cooking oils gave, in most cases, similar results of effectiveness with the standard chemical, Malathion. This investigation is in disagreement with the report of Mekuria (1995) who stated that *C. ambrosoides* leaf and inflorescent parts applied at the rate of 4% w/w induced 100% mortality against maize weevil. The probable reason of less effectiveness of *C. ambrosoides* (70%) in the present study is the reduced dosage used which is only 0.20% w/w when compared to 4% W/W of Mekuria's study. Selase and Getu (2009) reported that *C. ambrosoides* leaf powder at higher rate (15 g/150 g) application to haricot bean weevil's resulted in 100% mortality of bean weevil. Ishii et al (2010) reported weak attractant effect of *C. ambrosoides* against rice weevil. The current findings are in agreement with the previous works, but because

of the very much reduced dosage used, suggesting that the efficacy of botanical powders (*C. ambrosoides*) could serve as alternative maize weevil controlling material especially when applied at a higher rate. The botanical powders and cooking oils had insecticidal properties which are broad, variable and dependent on different factors like dosage, the presence of bioactive chemicals which need to be identified, isolated and manufactured in the factory for insect pest management. The presence of insecticidal properties that is used as a fumigant in storage insect pest management in different botanicals have been reported (Shaaya et al., 1997; Bamaiyi et al., 2007) The plant powders may act as fumigant, repellent, stomach poison and physical barrier (Mulungu et al., 2007; Law-Ogbomo and Enobakhare, 2007) against various insects.

Similarly, Odeyemi (1993) found that *C. citratus* essential oil applied at 0.7 mL/50 g of maize increased the mortality of maize weevil compared to the untreated control. Solvent extracts of different lemon grass parts were reported to have toxicant, repellent



and fumigant activities against storage pests. Araya (2007) found that fresh *C. citratus* essential oil exhibited high (85%~100% mortality) acaricidal activity.

The reduction in F_1 progeny emergence in the treated grains might be due to increased adult mortality, ovicidal and larvicidal properties of the tested plant powders and cooking oils confirming the findings of Selase and Getu (2009) and Bamaiyi et al (2007). Tapondjou et al (2002) noted that, all concentrations of dry ground leaves of *C. ambrosoides* resulted in complete (100%) inhibition of oviposition and subsequent progeny production by *C. chinensis*, *C. maculatus* and *A. obtectus* and may kill the larvae hatching from eggs laid on grains, preventing feeding and damage. Likewise, it was reported that Chenopodium leaf powder mixed with maize and sorghum grains at the rates of 2% and 4% w/w caused complete reduction in F_1 progeny production by maize weevil (Mekuria, 1995; Dejen, 2002). The act of weakening of adults by botanical powders may make them lay fewer eggs than the normal leading to less hatchability to larvae and final metamorphosis to adults. Different botanicals effectiveness at higher dosage to various storage insect pests have been reported by several authors (Huang et al., 2000; Tripathi et al., 2000; Adedire and Lajide, 2003; Akinkurolele et al., 2006; Mbailao et al., 2006; Wang et al., 2006; Negahban et al., 2007; Oni and Ileke, 2008; Shahaf 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).

More numbers of weevils from the untreated check and most of the botanical plants were emerged on the 40th days of adult introduction. This indicates that the total development period (TDP) of the maize weevil, *S. zeamais* is estimated to be 40 days on an average under Jimma condition. This finding coincides with the work of Parugrug and Roxas (2008) who reported 39 days of TDP for *S. zeamais*. Based on the result of the study, powdered form of the test plants might not be effective in inhibiting growth as such. This might be because of the fact that the active compound of the plants with insecticidal characteristics could not penetrate well inside the grains, thus, did not affect the development of the weevil inside the seeds. Moreover, the dosage used may not be sufficient enough to cause

the needed mortality, less progeny emergency and damage to the grains suggesting subsequent need for effective dosage determination for the best botanicals before recommendation is made for resource poor farmers.

The germination test demonstrated that the plant materials tested against *S. zeamais* did not show any visible adverse effects on germination capacity of the grains. In contrary to the current findings, study conducted by Paranagama et al (2003) pointed out that *C. citratus* oil treatment reduced the germination capacity of rice paddy as compared to the control. Dejen (2002) showed that powders of *D. stramonium*, *J. curcas*, *P. dodecondra* and *A. indica* used in the control of *S. zeamais* did not show any significant effect on the germination capacity of sorghum.

The two cooking oils, *G. hirsutum* and *B. carinata* seed oils; 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 check. The toxicity of these cooking oils may be due to their active components responsible for the insecticidal properties against the insect pests including weevils. The effectiveness of these oils may be through their impact on the breathing system of the insect through blockage of spiracles preventing oxygen inhalation leading to deaths of weevils. Death of storage insects due to asphyxia when oils are applied to grains has been reported (Cooping and Menn, 2000).

4 Conclusions

The current findings demonstrated that most of the botanical plant powders and cooking oils tested against maize weevil, *S. zeamais* shows insecticidal activity. Insecticidal activity was confirmed in all the tested plant species, although the results showed variation in their effectiveness against *S. zeamais*. Moreover, some of these botanical extracts 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. Also, work in this regards should continue to obtain information regarding its practical effectiveness under natural conditions to protect the stored products without adverse side effects. Hence, there is a scientific



rationale for the incorporation of these botanical powders and cooking oils into the grain protection practice of resource-poor farmer.

5 Materials and Methods

5.1 Description of the study area

The experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) Western Ethiopia from September to December 2011. JUCAVM is located 354 km south west of Addis Ababa at an approximate geographical coordinates of latitude 06°36' N and longitude of 37°12' E at an altitude of 1 710 meters above sea level. The mean maximum and minimum temperatures are 26.8°C and 11.4°C, respectively and the mean maximum and minimum relative humidity are 91.40% and 39.92%, respectively. The experiment was conducted under room temperature in entomology laboratory.

5.2 Maize variety used for the experiment

Clean and well sieved maize grain of variety 'BH-660' was used, which was obtained from Nekemte Cereal Division and Distribution Enterprise. The seeds were frozen at -6°C for seven days to kill any live insects on and in it. This variety of maize is the most commonly grown maize hybrid developed by the National Maize Research Program based at Bako, Western Ethiopia and it is considered as one of the susceptible maize varieties to insect pests (Tadesse and Basedow, 2004). Maize grain not previously treated with any insecticide was adequately dried and used to rear the insects for several generations to obtain uniform population of the test insect, *S. zeamais*. The grains were graded manually and almost only larger grains were used for the study. The grains were cleaned of broken kernels and debris by hand and by using a 4.76 mm round holed sieve.

5.3 Collection and preparation of botanicals and cooking oils

Fresh and matured leaves, kernels and barks and roots of botanicals were gathered and brought immediately to the laboratory from different localities. *Tagetes erecta* and *Cymbopogon citratus* were harvested from Jimma University campus, while *Azadiricta indica* (leaf, bark and kernel) was collected from Melka Werer Research Centre in Afar regional state, Ethiopia. *Chenopodium ambrosoides*, *Maesa lanceolata* and Echinops kebericho were gathered from the natural habitat of eastern Wollega zone of Oromia regional state, Ethiopia and *Allium sativum* was purchased

from Merkato super market in Jimma city. The purified cooking oils of *Brassica carinata* and unrefined cooking oils of *Gossypium hirsutum* were brought from Hararghe Fadis and Addis Mojo oil industry of eastern showa zone of Oromia regional state, Ethiopia respectively. The leaves and plant materials were air and shade dried for 14 days in the entomology laboratory of JUCAVM. The dried leaves, kernels and roots were pulverized using a micro pulverizer machine and were sieved through a 0.25 mm pore size mesh sieve to obtain uniform particle size which is similar with the procedures followed by Araya (2007) and Parugrug and Roxas (2008). The resulting powders were kept separately in glass containers with screw cap and stored at room temperature in dark place until when needed. The amounts of powder mixed with the maize grain were calculated on weight by weight basis, i.e., weight of powder/weight of grain (w/w) (0.5 g of botanicals to 250 g of maize grains). For the cooking oils, 0.5 mL of oil mixed with 2 mL acetone (95%) to 250 g of maize grains was used. The synthetic chemical Malathion super dust (5%) was used as standard check chemical in this study. The details of the treatments are indicated in Table 5.

5.4 Rearing of *S. zeamais*

The initial generations of *S. zeamais* was obtained from maize store in Jimma town with maize grains and allowed to reproduce further in an incubator at 27°C and 50%~70% r. h. in JUCAVM Parasitological laboratory in rearing jars. Collections of about two thousand male and female adult maize weevils were secured from the storage which is free from pesticides. These unsexed weevils were introduced in to two separate rearing jars with 500 g of maize grains for further multiplication to obtain uniform population of test insects. These jars were with holes at top for ventilation purposes.

5.5 Treatments and experimental designs

There were 13 treatments (Table 5) replicated thrice and arranged in completely randomized design. There were two controls in the treatments (the untreated control and the standard control-Malathion super dust) for comparison. The treatments used were in powder form for the botanicals and oil form for the cooking oils. Each treatment was measured and introduced (mixed with maize grain) in to 250 g of maize grains in each jar. The same day emerging adult insects, 20 for each replication, were collected from mass rearing

Table 5 Description of botanicals and cooking oils used in the experiments

Descriptions	Dosage	Local name	Common name	Form used
Control	–	–	–	–
Malathion dust (5%)	0.125 g	–	–	Dust formulation
<i>Azadirachta indica</i> leaf	0.500 g	Mimi Zaf / Nimia	Neem	Leaf powder
<i>Azadirachta indica</i> bark	0.500 g	Mimi Zaf / Nimia	Neem	Bark powder
<i>Azadirachta indica</i> seed	0.500 g	Mimi Zaf / Neem	Neem	Kernel powder
<i>Cymbopogon citratus</i>	0.500 g	Lomi sar	Lemon grass	Leaf powder
<i>Tagetes erecta</i>	0.500 g	Yeferenji Adey	Mery gold	Leaf powder
<i>Allium sativum</i>	0.500 g	Nech Shinkurt	Garlic	Leaf powder
<i>Maesa lanceolata</i>	0.500 g	Kelewa/Abayi	–	seed powder
<i>Chenopodium ambrosoides</i>	0.500 g	Gime/Ajaye	–	Leaf powder
<i>Gossypium hirsutum</i>	0.5 mL	Tit/ Jirbi	Cotton	Seed oil
<i>Brassica carinata</i>	0.5 mL	Gomenzer	Ethiopian mustard	Seed oil
<i>Echinops kebericho</i>	0.500 g	Kebericho	–	Root powder

jars and introduced to each test jars at the same time to maintain uniformity in age as described by Parugrug and Roxas (2008). The jars were arranged 5~10 cm apart on a flat table and left undisturbed at 20~25 °C for oviposition of adult moths.

5.6 Bio-assay procedures

Two hundred fifty grams of healthy disinfected maize grain seeds was put in one-liter glass jars and admixed with 0.5 g powdered leaves, bark and/or root of each test plant. Oils were separately dissolved with 2 mL acetone before mixing it with the maize seed and allowed to evaporate for 2 hrs. The jar contents (treatments and maize grain) were shaken thoroughly for about five minutes to ensure uniform distribution of the botanical powders and the oils. Then, 20 early emerged adult weevils of the same age were collected from the previously reared culture of insects in the laboratory and introduced in to each jars. After introduction of the predetermined adult insects in to each experimental jar; adult mortality, F₁ progeny emergency, seed damage and germination percentage was inspected as described below.

5.6.1 Adult mortality test

Maize weevil adult mortality was assessed on first, second, third, fourth, fifth, 10th, 15th and 20th days after exposure of the weevils to the treatments. Adult weevils were considered dead when gently probed with sharp objects and there were no responses. The

data on each assessment day was summed up and considered as a cumulative adult weevil's mortality. Percent adult mortality was determined as per the method described by Parugrug and Roxas (2008) using the following formula:

$$\text{Germinations (\%)} = (\text{No. of seed germinated}) / (\text{Total grain sampled}) \times 100\%$$

5.6.2 F₁ progeny emergence test

Twenty days after the introduction of insects to each experimental jar, all dead and alive insects were removed from each container and the seeds were returned to their respective containers to further assess F₁ progeny emergency. F₁ progeny count was made on 21th, 22th, 23th, 24th, 25th, 30th, 35th and 40th days after adult weevil's introduction. Inspection of the progenies was made on each assessment day by displaying the seeds on paper and sieving the contents of the jars.

5.6.3 Damaged seeds (seeds with holes)

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

5.6.4 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 (%) = (Initial weight – Final weight) / (Initial weight) × 100%

5.6.5 Germination percentage (viability index) test

Germination test was carried out on 111 (18.5% of the total grain) seed samples randomly taken from each treatments replication wise. The seeds were placed in Petri dishes containing moistened filter paper (Whatman No.1) and arranged in an incubator at 30°C at JUCAVM School of Veterinary parasitological laboratory. The number of germinated seedlings from each Petri dish was counted and recorded from 7~10 days after start. The percent germination was computed (Ogendo et al., 2004) as follows:

Germinations (%) = (No. of seed germinated) / (Total grain sampled) × 100%

5.7 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) using SAS version 9.2 Software packages. United State Environmental Protection Agency (USEPA) probit analysis version 1.5 was used for analyzing percent mortality and median lethal time. Mean separations were conducted using Turkey's 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.

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