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PLANTS ESSENTIAL OILS AS STORED GRAIN PRORTECTANTS: AN ECO-FRIENDLY APPROACH AGAINST *SITOPHILUS* ZEAMAIS (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Maize weevil is a serious pest of stored grains. Laboratory studies were conducted to evaluate the efficacy of eight plant essential oils viz. lemon grass *(Cymbopogon flexousus)*, turmeric *(Curcuma longa)*, basil *(Ocimum basilicum)*, harmal *(Peganum harmala)*, heeng *(Ferula assa-foetida)*, neem *(Azadirachta indica)*, tumma *(Citullus colocynthis)* and mint *(Mentha arrensis)* against maize weevil, *Sitophilus zeamais* at three different concentrations (5,10 and 15%) and at three different time intervals (2, 4 and 6 days) in the laboratory of the Stored Grain Management Cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. The results revealed that the maize weevil mortality increased with increase in concentration and time intervial. Maximum mortality of the pest was observed in *A. indica* essential oil at 15% concentration after 6 days of application i.e. 90.52% followed by *C. colocynthis* oil (82.62%). Minimum mortality was observed in *M. arvensis* oil i.e. 44.44 percent at 5% concentration after 6 days. As far as adult emergence was concerned the minimum number of mean adult was emerged in *A. indica* i.e. 44.94% and maximum was in *M. arvensis* (90.78). *A. indica* also showed minimum number of infested grains i.e. 9.22%, mean percent infested grains 6.07% and

3.35% weight loss while *M. arvensis* showed maximum mean number of infested grains percent and weight loss for *S. zeamais*, i.e. 24.33%, 15.94% and 8.80%, respestively.

Keywords: maize weevil, plant essential oil, stored maize, mortality, weight loss, adult emergence

INTRODUCTION

Cereals serve as most vital sources of food for both humans and livestock all over the world. After wheat and rice, maize (*Zea mays* L.) is main cereal crop, which is grown in different regions of the world. In Pakistan storage facilities available in the country are insufficient and/or in-appropriate at various levels **[1]**. The situation is further aggravated when storage is done in open areas where insect pests, rodents and birds cause lots of damage **[2]**. It is estimated that 50% grain production is lost due to improper storage and attack of insect pests in tropical countries including Pakistan **[3, 4]**. According to a conservative estimate, population growth rate in Pakistan is 1.6 percent/annum which will result in 250 million people by 2050. This ever-increasing population needs regular and consistent supply of food for consumption **[5]**. With limited resources it is impossible to increase the agriculture land use to accommodate the consistent food supply to future population increase. Post-harvest losses are also a reality which adversely affects the economy of a country. The maize weevil, *Sitophlilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a primary pest that affect the maize grain in field as well as in storage. Besides a principal pest of maize, it also plays the role of secondary pest for many crop grains and their products like rice, sorghum, cassava flour and yam products **[6]**. 30-40% weight loss can occur due to its heavy infestations **[7]**. Healthy grains are reduced to powder form as a result of juveniles and adult weevils feeding **[8]**. Eggs, larval and pupal stages are not normally visible as all are found within stored grain kernels. Adult emergence leaves a visible hole on the grain **[9]** facilitates disease and secondary fungal infection. Viability of the seeds is also reduced due to damage caused by maize weevil larvae and adult consuming the embryo affecting maize production **[3, 10-12]**.

Recent increased in public awareness regarding ill effect of insecticides, concerns about environment and ever raising regulatory constraints invited scientist's attention to devise some eco-friendly management tactics complying with international phytosanitary regulatory framework **[13]**. Plant derivatives are effective and benign tools in wide range of insect pest management with broad spectrum of action **[14]**. Botanical insecticides are more readily biodegradable, cheap, affordable and easy to use technology that minimizes post-harvest losses of maize during storage. A number of studies have shown that integration of different plant parts i.e. powder, oil and extracts with grain minimizes insect fecundity, egg hatching, postembryonic development and progeny development **[15-18]**. The present study was designed to determine the bio efficacy, cumulative toxicity, reproduction inhibiting capacity and maize grain protection of botanical essential oils of indigenous plants against maize weevil under laboratory conditions.

MATERIALS AND METHODS

The experiment was conducted to assess the toxicity of different plants' essential oil viz. Lemon grass (*Cymbopogon flexousus*), Turmeric (*Curcuma longa*), Neem (*Azadirachta indica*), Basil (*Ocimum basilicum*), Harmal (*Peganum harmala*), Heeng (*Ferula assa-foetida*), Mint (*Mentha arvensis*) and Tumma (*Citullus colocynthis*) against maize weevil in Stored Grain Management Cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. The culture of adult maize weevils was maintained in transparent plastic jars on sterilized maize grains. To ensure the proper ventilation, polyester mesh was used on one side. Initial culture was collected from the laboratory of Entomology, Agricultural Research Institute, Dera Ismail Khan, and further multiplied. Insect culture was raised under controlled conditions at temperature of 27°C ±3°C at 70%±5% RH under 12:12 h day length (L: D) in laboratory.

Essential oils of plants' materials were prepared from the fresh leaves, rhizomes and seeds. The plants' parts were cleaned by using fresh and clean water. After shade drying the plants' parts were ground in electrical grinder and then sieve it to make fine powder form. The oil extraction was done with Soxhlet's extraction apparatus. Fifty (50) grams of plants' powders were put in the thimble after rap up in a filter paper and 250 ml acetone was taken in the flask as solvent. Heat from the burner boil the acetone and then it evaporates and drop on the thimble containing plant powder, after condensation due to running of fresh water on the upper portion of the apparatus. This hot acetone took oil from thimble to the flask on completion of cycle. The essential oil with acetone was removed from the apparatus. After evaporating acetone, obtained essential oils were put in clean and air tight lid bottles.

Finally, samples were stored in the refrigerator (at 5°C) before use **[19]**. The experiment was laid out in a completely randomized design replicated 6 times. The conditions for experiment were adjusted at 27°C ± 3°C and 70% ± 5% relative humidity (RH) and a photoperiod of 12:12 hours (L: D). Maize variety Azam was used in all trials. There were 9 treatments and each treatment was consisting of 20g of sterilized maize seed treated with three concentrations (5, 10 and 15%) of plant powders. After treatment seeds were kept in plastic jars and were vigorously shaken before release of weevils for complete mixing of plant powders. Ten pairs of newly emerged adult weevils were released in the tested arena. An interval of 1 h was observed between the mixing of oil and release of weevils to ensure complete drying of maize seeds. The data on insect mortality was recorded 2, 4 and 6 d after treatment and then converted to percent mortality. The dead weevils were removed from the petri dishes on daily basis.

PT = (Po Pc) / (100 – Pc) Abbot [20] formula.

Where: PT = corrected mortality (%), Po = observed mortality (%), Pc = control mortality (%)

The growth regulatory effect of plants' essential oil on maize weevil was also determined. The experiment was laid out in a completely randomized design using six replications. The conditions for experiment were adjusted at 27±3°C and 70±5% relative humidity (R.H.) and a photoperiod of 12:12 hours (L: D). Sterilized maize grains (50g) were treated separately with each concentration of plants' essential oil and were kept in transparent plastic jars. A total of 10 pairs of newly emerged adult weevils were released in each plastic jars and then the jars were sealed with a piece of muslin cloth, fastened with rubber band to restrict the escape or entry of the beetles. The beetles were kept in jars for 45 days. The adult beetles were removed from all jars after 45 days of release and total number of F_1 adults emerged in each treatment was counted to see the effect of tested plant materials. Percent inhibition rate (%IR) was calculated by using the following formula Lu *et al.*, **[13]**:

% IR = [(Cn - Tn) / Cn] ×100

Where: Cn is the number of newly emerged adults in the control and Tn the number of newly emerged adults in the treated petri dish.

The bio-active effect of plants' essential oil on weight loss of maize grains was also determined. The experimental conditions were the same as previous. The beetles were kept in jars for 45 days. After 45 days of the release of beetles, the adult beetles were removed from all jars and data on number of infested grains, weight of infested grains and weight loss of grains were recorded.

The percent loss in weight was determined as follow Lleke and Oni, **[21]**:

$$\textbf{\% weight loss} = \frac{\textbf{Initial weight – final weight}}{\textbf{Initial weight}} \times \textbf{100}$$

Statistical analysis was based on a completely randomized design. Analyses of variance (ANOVA) was performed using GenStat-8 (GenStat, 2005. VSN International Ltd. Oxford, UK). Least significance difference test (LSD) was used to determine statistical difference between the treatment means at $P = 0.05$.

RESULTS

The results **(Table 1)** showed effectiveness of eight plant essential oils with three concentrations (5, 10 and 15%) at different time intervals against maize weevil. The results exhibited that all the treatments showed significant difference (P > 0.05) in mean percent mortality of the maize weevil. At 5 % concentration, maximum mortality (55.08%) was recorded with *A. indica* oil. Interaction effect showed that maximum mortality (73.51%) was recorded in *A. indica* oil after six days of application followed by *C. colocynthis* (70.09%) and these were statistically at par with each other. The results showed that at 10% concentration, mean percent mortality of maize weevil ranged from 62.85 to 33.15 percent. Among them maximum mortality (62.85%) was recorded treated with *A. indica* oil followed by *C. colocynthis* (58.08%). Interaction effect showed that maximum mortality (80.51%) was recorded in *A. indica* oil after six days of application and minimum mortality (15.83%) was observed in *M. arvensis* after 2 days of application. The results also disclosed that mortality of maize weevil increased with increase in concentration. The results revealed that at 15% concentration; mean percent mortality of maize weevil ranged from 70.48 to 39.59 percent. Interaction effect indicated that maximum mortality (90.52%) was recorded in *A. indica* oil after six days of application followed by *C. colocynthis* (83.62%) and *P. harmala* oil (81.04) and these both were statistically at par with each other. While minimum mortality (12.50%) was observed in *M. arvensis*oil after 2 days of application.

The results **(Table 2)** of present study explained the growth regulatory effect of different plant essential oils with three concentrations (5, 10 and 15%) against maize weevil in respect of adult emergence. Significant variations (P > 0.05) were found among different plant essential oils in term of adult emergence of the maize weevil. Mean total number of adult emerged ranged from

140.50 to 44.94 adults. The maximum adult emergence (140.50) was recorded in control treatment followed by *M. arvensis*. Interaction effects showed that adult emergence ranged from 142.17 to 23.67 adults. The highest adult emergence (142.17) was recorded in control treatment followed by *M. arvensis* with 107.00 adults at 5% concentration. While minimum adult emergence (23.67) was observed in *A. indica*oil at 15% concentration. In case of percentage reduction in adult emergence, highest reduction (67.89%) was recorded in *A. indica* oil followed by *C. colocynthis* (64.97%). While lowest reduction (35.29%) was recorded in *M. arvensis* oil. Interaction effect showed that percentage reduction of adult emergence ranged from 83.36 (in *A. indica* at 15%) to 22.93% (in *M. arvensis*at 5%). The maximum reduction (83.36%) was recorded in *A. indica* oil.

The results **(Table 3)** depicted bio-active effects of eight plant essential oils used with three concentrations (5, 10 and 15%) against maize weevil. The findings showed significant variation ($P > 0.05$) among different treatments of plant essential oils in term of number of infested grains, %age of infested grains and %age weight loss of grains caused by maize weevil. The mean number of infested grains in 50 grams maize at different concentration ranged from 46.94 to 9.22 grains. Minimum number of infested grains (9.22 grains) was recorded in *A. indica* oil. While, maximum numbers of infested grains were observed in control treatment i.e. 46.94 grains. Interactional effect of treatments and concentrations showed that minimum numbers of infested grains were recorded in *A. indica* at 15% concentration. While maximum numbers of infested grains were observed in *M. arvensis* oils and control i.e. 27.83 grains at 5% concentration and 47.33 grains. The trend regarding number of infested grains showed that infestation level decreased continuously with the increase in concentration in all the treatments.

The results **(Table 3)** regarding mean percentage of infested grains at different concentration of treatments showed ranged from 30.81 to 6.07 percent. Minimum infested grains percentage (6.07%) was recorded with *A. indica* oil. While, maximum percentage of infested grains was observed in control treatment i.e. 30.81 percent. Interactional effect of treatments and concentrations showed that minimum percentage of infested grains were recorded in *A. indica*, *C. colocynthis* and *P. harmala* oil with 3.05, 5.55 and 6.23% infested grains at 15% concentration. While maximum numbers of infested grains were observed in

M. arvensis oils and control treatment i.e. 18.35 percent at 5% concentration and 31.04 percent. The trend regarding percentage of infested grains showed that infestation level decreased continuously with the increase in concentration in all the treatments.

The finding **(Table 3)** regarding mean percentage weight losses at different concentration of treatments after 45 days of application showed ranged from 17.06 to 3.35 percent. The results revealed that minimum weight loss (3.35%) were observed in *A. indica* oil. Interactional effect of treatments and concentrations showed that minimum percentage of weight losses were observed in *A. indica*, *C. colocynthis* and *P. harmala* oil with 1.70, 3.09 and 3.45 percent at 15% concentration. While maximum weight losses were recorded in *M. arvensis* oils and control i.e. 9.99 at 5% concentration and 17.20 percent. The trend regarding percentage weight losses showed that weight losses decreased continuously with the increase in concentration in all the treatments.

Table 1: Comparison of mean percentage mortality of *S. zeamais* against three concentrations of plants essential oils after three exposure times

Treatments	5% Concentration				10% Concentration				15% Concentration			
	2 days	4 days	6 days	Mean	2 days	4 days	6 days	Mean	2 days	4 days	6 days	Mean
Neem (*A. aindica*)	38.33 fg	53.39 c	73.51 a	55.08 a	44.17 h	63.87 d	80.51 a	62.85 a	50.83 ij	70.09 cd	90.52 a	70.48 a
Basil (*O. basilicum*)	20.00 k	33.05 h	54.70 c	35.92 e	25.83 lm	42.86 h	58.47 e	42.39 e	32.50 n	50.43 ij	69.83 cde	50.92 e
Heeng (*F. assa-foetida*)	14.17 l	27.96 ij	47.01 d	29.71 f	20.83 mn	36.98 ij	53.39 efg	37.07 f	26.67 o	42.74 kl	64.66 ef	44.69 f
Mint (*M. arrensis*)	8.33 m	24.58 jk	44.44 de	25.79 g	15.83 n	32.78 jk	50.85 g	33.15 g	20.83 p	39.32 lm	58.62 gh	39.59 g
Tumma (*C. colocynthis*)	34.17 gh	48.30 d	70.09 a	50.85 b	40.83 hi	57.98 ef	75.42 ab	58.08 b	45.83 jk	64.96 def	83.62 b	64.80 b
Turmeric (*C. longa*)	25.83 ij	39.83 ef	59.83 b	41.83 d	30.83 kl	50.42 g	66.10 cd	49.12 d	36.67 mn	54.70 hi	72.42 c	54.60 d
Harmal (*P. harmala*)	30.83 hi	44.92 d	62.39 b	46.05 c	36.67 ij	52.94 fg	70.34 bc	53.32 c	41.67 klm	60.68 fg	81.04 b	61.13 c
Lemongrass (*C. flexousus*)	13.33 lm	27.12 ij	45.31 d	28.59 f	17.50 n	36.14 ij	53.39 efg	35.67 fg	23.33 op	41.88 klm	62.07 fg	42.43 f
LSD value @ 5%	5.0444			2.3667	5.1019			2.6837	5.3058			2.7064

Means sharing similar letter in columns are not significantly different by LSD Test

Table 2: Comparison of mean number of adult emergence and percentage reduction in of *S. zeamais* against three concentrations of plants essential oils

Treatments	Number of adult emergence			Mean	Percentage reduction in adult emergence			Mean
	5%	10%	15%		5%	10%	15%	
Neem (*A. indica)*	64.67 k	46.50 o	23.67 r	44.94 i	53.42 i	66.90 e	83.36 a	67.89 a
Basil (*O. basilicum)*	91.17 e	70.50 hi	56.17 m	72.61 e	34.33 n	49.82 jk	60.49 g	48.21 e
Heeng (*F. assa-foetida)*	96.50 d	77.33 g	59.83 l	77.89 d	30.49 o	44.96 l	57.91 h	44.45 f
Mint (*M. arrensis)*	107.00 c	91.67 e	73.67 h	90.78 b	22.93 p	34.76 n	48.19 k	35.29 h
Tumma (*C. colocynthis)*	68.17 ij	51.50 n	27.50 q	49.06 h	50.90 j	63.35 f	80.66 b	64.97 b
Turmeric (*C. longa)*	80.33 fg	61.17 l	43.67 o	61.72 f	42.14 m	56.47 h	69.29 d	55.96 d
Harmal (*P. harmala)*	72.33 h	54.17 mn	36.00 p	54.17 g	47.90 k	61.45 fg	74.68 c	61.34 c
Lemongrass (*C. flexousus)*	98.67 d	81.67 f	66.17 jk	82.17 c	28.93 o	41.88 m	53.46 i	41.42 g
Control	138.83 b	140.50 ab	142.17 a	140.50 a	0.00 q	0.00 q	0.00 q	0.00 i
LSD value @ 5%	3.3491			1.6357	2.2996			1.1115

Means sharing similar letter in columns are not significantly different by LSD Test

Table 3: Bio-active effects of plants essential oils at three different concentrations on number of infested grains, % infested grains and % weight losses of maize grains

Treatments	Number of infested grains			Mean	% Infested grains			Mean	Weight loss %			Mean
	5%	10%	15%		5%	10%	15%		5%	10%	15%	
Neem (*A. indica*)	13.17 mn	9.83 o	4.67 p	9.22 h	8.67 lm	6.50 no	3.05 p	6.07 h	4.78 mn	3.57 o	1.70 P	3.35 h
Basil (*O. basilicum*)	22.83 ef	18.17 ij	14.33 mn	18.44 d	15.09 def	11.91 hi	9.36 lm	12.12 d	8.30 ef	6.60 ij	5.21 mn	6.70 d
Heeng (*F. assa-foetida*)	25.33 cd	22.17 efg	17.50 jk	21.67 c	16.56 cd	14.55 efg	11.47 ij	14.19 c	9.21 bcd	8.05 efg	6.36 jk	7.87 c
Mint (*M. arrensis*)	27.83 b	24.50 cde	20.67 fgh	24.33 b	18.35 b	16.02 de	13.46 fgh	15.94 b	9.99 b	8.90 cde	7.51 fgh	8.80 b
Tumma (*C. colocynthis*)	15.17 klm	12.33 n	8.50 o	12.00 g	9.97 jkl	8.10 mn	5.55 o	7.87 g	5.51 klm	4.48 n	3.09 O	4.36 g
Turmeric (*C. longa*)	20.17 ghi	16.83 jkl	13.50 mn	16.83 e	13.31 gh	11.03 ijk	8.84 lm	11.06 e	7.33 ghi	6.12 jkl	4.91 mn	6.12 e
Harmal (*P. harmala*)	18.83 hij	14.67 lmn	9.50 o	14.33 f	12.37 hi	9.61 klm	6.23 o	9.40 f	6.84 hij	5.33 lmn	3.45 O	5.21 f
Lemongrass (*C. flexousus*)	26.83 bc	23.50 de	19.17 hij	23.17 b	17.72 bc	15.31 de	12.56 hi	15.19 b	9.75 bc	8.54 de	6.97 hij	8.42 b
Control	46.83 a	47.33 a	46.67 a	46.94 a	30.82 a	31.04 a	30.57 a	30.81 a	17.02 a	17.20 a	16.96 a	17.06 a
LSD value @ 5 %	2.4172			1.3164	1.6374			0.8671	0.8910			0.4934

Means sharing similar letter in columns are not significantly different by LSD Test

DISCUSSION

Present investigations displayed that mortality of maize weevil increased with the increase in concentration and time intervals in all treatment. These finding were parallel to the findings of previous studies **[22-25]** they found that mortality increased with the passage of time and increased in concentration. The findings regarding neem oil were parallel to the findings of previous studies **[26, 27]** they concluded that neem seed oil gave 90 to 100% mortality of maize weevil. Moreover, after neem *C. colocynthis* gave the best results in oil formulation. Similar kind of results was also confirmed by Akpotu et al. **[28]**, they evaluated *C. colocynthis* oil against *D. maculatus* and reported that *C. colocynthis* has a potential to control the insects and gave 81.00 to 91.11% mortality. These findings were also confirmed by Sarwar et al. **[29]** they illustrated that *C. colocynthis* depicted 89.11% mortality at 10% concentration after 10 days of application. The results regarding toxicity of *P. harmala*, *C. longa*, *O. basilicum*, *F. assafoetida*, *C. flexousus* and *M. arrensis* revealed that they also have potential to control this insectpest. These findings were confirmed by Aryani and Auamcharoen **[30]** they investigated the toxicity of turmeric, cassumunar ginger and peacock ginger rhizomes extracts against maize weevils. From present investigation, it was concluded that oil formulations of plant materials have potential to control this pest very effectively and these tested materials are cheaper, eco-friendly and safer for human health. In the current study adult emergence decreased with the increase in concentration. These findings were confirmed by various scientists **[22, 24]**. Current investigations displayed that among different treatment *A. indica* proved to be the best plant material which gave minimum adult emergence of 23.67 adults at 15% concentration. These findings stand parallel with the findings of previous studies **[31-36]** they reported that neem in all formulations and concentration gave significant result regarding adult emergence.

In the current study after neem *C. colocynthis* gave the best results in all oil formulation (27.50 adults at 15% concentration) as compared with control. The results of present findings supported by Chukwunonso et al. **[37]**, they evaluated *C. colocynthis* oil against *C. maculatus*and reported that *C. colocynthis* significantly reduced the adult emergence and has a potential to control the insects. Present investigations displayed that neem oil was found very effective with minimum grain infestation (3.05%) and weight losses (1.70%). These finding were parallel to the findings of previous studies **[36, 38-41]** they reported that neem found very effective against this pest with respect to minimum weight losses and grain damage. After neem *C. colocynthis* was found very effective plant material against maize weevil with 5.55 to 9.91% grain infestation and 3.09 to 5.51% weight losses at 15% concentration. No relevant research work about tumma, harmal has been reported regarding this pest However, Chukwunonso and Nnaemeka **[37]** worked on effectiveness of tumma against *Callosobruchus maculatus* and reported that oil formulation of tumma showed 4.58% grain infestation in cowpea. Present studies demonstrated that *P. harmala* also proved very effective against maize weevil with 6.03 to 11.90% grain infestation and 3.45 to 6.66% weight losses at 15% concentration as compared with control. From present investigation, it was concluded that among different plant materials neem, tumma and harmal have potential to reduce the grain infestation and weight loss in maize.

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