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Effect of essential oils on mortality and repellency of maize weevil (*Sitophilus zeamais*) (Motschulsky) under laboratory condition of Lamjung campus

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Abstract
Maize (*Zea mays*) is a crucial staple food crop and consumption of maize products has increased around the world due to the expansion in the global population. Maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), has been an increasing concern during the storage of the grain. Although insecticides can curtail the jeopardy of maize weevil, development of toxic residue and resistance by pests pose a constant threat in its adoption. The essential oils could provide a viable and safer alternatives to control the pest. Hence, an experiment was carried out at the Entomology Laboratory of the Institute of Agriculture and Animal Science to assess the fumigation and repellent effects of seven different essential oils *viz.* Mentha (*Mentha arvensis*), Lemon grass (*Cymbopogon citratus*), Fresh basil (*Ocimum basilicum*), Eucalyptus (*Eucalyptus globule*), Palmarosa (*Cymbopogon martini*), Citronella (*Cymbopogon winterianus*) and Juniper berry (*Juniperus recurva*) at 50 µl and 100 µl for fumigation mortality and 0.125% and 0.25% for repellency against maize weevil. The effects of the oils were dosage-dependent, and there was a progressive increase in insect death as concentrations increased. Furthermore, eucalyptus oil resulted in the highest mortality at 100 µl and repellency at 0.25%.

Keywords: *Maize weevil*, *Eucalyptus*, essential oils, repellency, mortality

Introduction
Maize (*Zea mays* L.), also known as queen of cereal, is one of the widely grown cereal crops and is produced in a variety of agro-climatic areas for several purposes. In addition to being a staple diet, maize is also an important source of feed and fodder for farm animals. In Nepal, maize is second only to rice in terms of area and production. In fiscal year 2020/21, maize covered an area of 979,776 ha with a production of 2,997,733 metric tons (MOALD, 2022) [16]. The quality and quantity of maize in its post-harvest are deteriorated by several organisms, including rodents, birds, and insects. All *Sitophilus* species have the potential to infect wheat, greatly increasing storage losses. Among the insects, *Sitophilus zeamais* is one of the major pests of maize in storage conditions.

The maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera, Curculionidae), also known as the greater rice weevil, is a serious pest of wheat and maize and is ubiquitous across tropical regions of the world (Dobie, 1974) [8]. It is a major pest of the grains that not only attacks the stored cereal grains including rice, sorghum, wheat, oats, barley, rye, buckwheat, peas, and cottonseed but also targets standing crops. Even fruits like apples and pears, in storage conditions, have been reported to be attacked by them. Furthermore, the maize weevil infests a variety of coarse, milling grains, pasta, and other goods made from refined, stored cereals.

Both the adult and larvae are responsible for the damage to the grain and possess a strong potential for rapid population growth, which can seriously harm the grain that has been stored (Cosmos *et al*. 2012) [7]. The maize weevil bore a small hole in the seed and the female lays the egg in the resulting cavity. Zakladnoi and Ratanova (1987) [23] reported that during the course of development, larvae ingest about half of the total grain weight. The physical and chemical characteristics of the product determine the severity of damage. Being an internal feeder, their activities include excretion in grain, molting and the contamination of their dead bodies, uric acid production causing an unpleasant odor, decreased weight, quality, diminished germination percentage, lowered aesthetic value, decreased nutritional value, as well as loss in market price. As the damage in the grain persists, moisture content rises and total or partial blackening of the grain, bad odor, heating, and even the production of mycotoxins by molds may occur on the grains at elevated humidity (Magan *et al*, 2003) [14]. If the grains contaminated with aflatoxins (produced by *Aspergillus flavus*) are intended for animal or human consumption, they can cause illness when consumed in low amounts while eventually causing death when consumed in large quantities (Massayuki and Gomi, 2010; Agrios, 2005) [15, 1].

Pingali and Pandey (2001) [19] estimated a loss of about 20-80% in tropical countries by the maize weevil. In a country like Nepal, where subsistence food grain production supports the livelihood of a majority of the population, there is a major economic loss of stored grains due to insect pest damage. Post-harvest losses have been identified as a significant barrier to maize and wheat storage in Nepal. Boxall and Gillet (1984) [4] recorded that a weevil attack in Nepal's eastern hills caused an average grain weight loss of 5.5%. Moreover, Paneru *et al*. (1996) [18] reported a weight loss of 31% due to severe maize weevil infestation in stored corn. The control of insect pests is heavily dependent on the use of synthetic chemical pesticides, but their continuous use had led to problems like pest resistance, pest resurgence, environmental and health hazards due to pesticide residues found in food, and their carcinogenicity. *Sitophilus zeamais* have been resistant to DDT as reported by Sayaboe and Aceda (1990) [2]. High cost, non-availability of chemical pesticides to a resource-poor farmer, lack of technical expertise, and its toxic effect on non-target organisms are also imposing constant threats to the use of chemical pesticides. Moreover, there are limited chemicals registered for use on grains and in storage structures. Fumigation provides a better solution by heating the grain to 60ºC, which can kill the larvae but hampers the germination and baking quality of the grain. The fumigants like phosphine have progressive restrictions on their use due to their ozone depletion potential (EPA 2001) [9]. Therefore, it is necessary to develop new alternative methods for controlling the maize weevil that is safe for human health and do not threaten biodiversity.

Natural substances, such as essential oil control agents, are gaining more attention as a substitute since they are widely available, and non-toxic to humans and the environment. Apart from being widely available, they are relatively safer because of their biodegradable nature, and have the potential for commercial usage (Ngamo *et al*., 2007) [17]. These essential oils contain fumigant, antifeedant, repellent, larvicidal, and/or adulticidal against maize weevil. The essential oils have a lipophilic chemical structure that can penetrate an insect and result in metabolic malfunction and death. Insects may absorb the common essential oils through their skin, inhalation, or ingestion. It is still possible to employ plants growing in Nepal, which are known to contain high concentrations of essential oils, to effectively control insect pests of stored agricultural products. Gyawali (1993) [10] reported the possibility of using 50 plant species of Nepal as a viable option to control the insect pests in storage grains. Genera Mentha, Cympogon, Ocimum, Eucalyptus, and Juniperus all contain these kinds of plants. Hence, these plant oils were tested in the experiment. The study was carried out to explore the efficacy of different essential oils on the mortality and repellency of maize weevil.

Materials and Methods

The experiment was carried out in Entomology Lab of IAAS, Lamjung, which is located at an altitude of about 650 masl. The research was conducted in a Completely Randomized Design (CRD) with three replications and seven treatments. Each treatment with two concentrations was used for mortality and repellency. The required essential oils were procured from the Jadibuti Association of Nepal (JABAN), Kathmandu. Airtight conditions were maintained until the oils had been ready for application.

Irrespective of sex, live *Sitophilus zeamais* specimens were gathered from the Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur. To raise insects, a local weevil-prone maize variety was employed. One hundred pairs of S. Zeamais were added to 2 kg of maize grains in 6.5 kg Kilner jars with mesh tops. After 20 days, the adults were separated into another jar. The eggs laid by the female were reared into the jar. The 7-14 days-old adult weevils were used for the experiment.

Table 1: List of treatment oils

Treatment	Essential oils	Scientific Name
T_1	Mentha	*Mentha arvensis*
T_2	Lemon grass	*Cymbopogon citratus*
T_3	Fresh basil	*Ocimum basilicum*
T_4	Eucalyptus	*Eucalyptus globule*
T_5	Palmarosa	*Cymbopogon martini*
T_6	Citronella	*Cymbopogon winterianus*
T_7	Juniper berry	*Juniperus recurva*

Fumigant toxicity assay

Five grams of maize grains were used to test the toxicity of seven different essential oils on the maize weevil. The oil extract was then applied to filter paper (Whatman No. 1, 70-mm diameter) and put in glass Petri plates (7 cm diameter). Various oil extract amounts were employed, including 0 (control), 50, and 100 l diluted in acetone. Ten unsexed adults were placed in each dish after the acetone had been allowed to evaporate for 10 minutes.

This bioassay consists of applying 0 (control), 50, and 100 µl of the seven essential oils diluted in acetone on filter paper (What man No. 1, 70-mm diameter). Filter papers were impregnated with a series of concentrations of each essential oil. The petri dish (7 cm diameter) was filled with 5gm of maize flour. The same procedure was used for the control with filter paper and placed on the petri dish. Ten unsexed adults were placed in each dish after the acetone had been allowed to evaporate for 10 minutes. Four separate replications of each treatment were done. Assessments of mortality were made at 24, 48, 72, and 96 hours of exposure by using the formula:

$$\text{Corrected Mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

Repellent activity of essential oils on Maize weevil adults

The percentages of concentration of the essential oils in the repellency assay were 0.125% and 0.250%. Glass Petri dishes were used for the repellency assay. The following essential oils were created as test solutions of serial dilution in acetone: Mentha, Lemongrass, French basil, Eucalyptus, Palmarosa, Citronella, and Juniper berries. With a micropipette, each essential oil solution was evenly applied to one half of Whatman No. 1 filter paper (70 mm in diameter), which was divided into two equal halves. The other half of the filter paper was treated by acetone. The untreated halves were taped together with cellophane and placed at the bottom of Petri plates after both halves were dried until the acetone evaporated. At the middle of the filter paper disc, ten adult maize weevils were released, and the Petri dishes were then covered and kept in the dark. For each concentration of the essential oil solution, three duplicates were predetermined. The number of insects on the treated and untreated halves were counted after 3 and 6 hours in moderate light, and the repellency percentages were determined as follows:

$$\text{Repellency percentage (RP)} = \frac{\%\text{ control halves} - \%\text{ treated halves}}{\text{Control halves} + \%\text{treated halves}} \times 100$$

Mean RP values were classified using the method of Juliana and Su (1983) [11].

Statistical Analysis

Analysis of variance (ANOVA) of the recorded parameters was performed in a two-factorial complete randomized design. Duncan's Multiple Range Test (DMRT) was used to separate the means at a 0.05 level of significance. Microsoft word 2007 was used for word processing, MS Excels for data tabulation, and R-Stat version 3.6.1 for running statistical analysis.

Results and Discussion

Table 2: Effect of treatments on mortality of the maize weevil in the laboratory of IAAS Lamjung Campus, Sundarbazar Lamjung

Treatment	Concentration	24 hrs	48 hrs	72 hrs	96 hrs
Citronella	100 µl	20b	33.33b	46.67d	56.67d
Citronella	50 µl	10e	23.33d	33.33f	40e
Eucalyptus	100 µl	23.33a	46.67a	73.33a	90a
Eucalyptus	50 µl	13.33d	30c	63.33b	76.67b
Fresh basil	100 µl	13.33d	20e	40e	56.67d
Fresh basil	50 µl	0g	10g	20j	36.67d
Juniper Berry	100 µl	13.33d	33.33b	53.33c	70c
Juniper Berry	50 µl	13.33d	20e	26.67h	40e
Lemon grass	100 µl	10e	13.33f	23.33j	36.67f
Lemon grass	50 µl	6.67f	6.67h	10l	13.33h
Mentha	100 µl	20b	30c	46.67d	56.67d
Mentha	50 µl	16.67c	20e	30g	36.67f
Palmarosa	100 µl	6.67f	10g	26.67h	36.67f
Palmarosa	50 µl	0g	6.67i	13.33k	20g
CV		5.01	2.75	1.65	1.25
F-lue (0.01)		0.00980**	0.0098**	0.00017***	0.00023***
Mean		11.90	21.66	36.19	47.61
LSD		0.999	0.999	0.999	0.99

There were significant differences (p<0.05%) among the essential oils on the extent of mortality of maize weevil (table 2). The average mortality in 24 hours was 11.9%, which gradually increased and reached maximum (47.61%) at 96 hours of exposure.

Eucalyptus oil at 100 µl recorded significantly higher mortality (23.33%) at 24 hrs followed by mentha (20%) and citronella (20%) at 100 µl. The lowest mortality at 24 hrs was recorded in fresh basil. Similarly, significantly higher mortality of the maize weevil was recorded in eucalyptus at 100 µl at 48, 72 and 96 hrs. At 48 hrs, lowest mortality was observed in fresh basil at 50 µl and palmarosa at 50 µl, which were statistically at par between each other, but significantly inferior to all other treatments. Significantly lower mortality rate was obtained in lemon grass at 50 µl at 72 hrs and 96 hrs. At 96 hrs, significantly higher mortality rate was recorded in eucalyptus at 100 µl (90%) followed by eucalyptus at 50 µl. The mortality of maize weevil increases with higher concentration in each essential oil, and duration of exposure. Hence, a high concentration is more effective than a low concentration.

Repellency of Maize weevil

Table 3: Effect of treatments on the repellency of the maize weevil in the laboratory of IAAS Lamjung campus, Sundarbazar Lamjung

Treatment	Conc (%)	Rep 3hr	Rep 6 hr
Citronella	0.25	25.44b	49.49d
Citronella	0.125	6.67f	33.33g
Eucalyptus	0.25	33.33a	69.54a
Eucalyptus	0.125	12.45e	38.89f
Fresh basil	0.25	25.44b	57.14c
Fresh basil	0.125	6.67f	33.33g
Juniper Berry	0.25	23.44c	57.14c
Juniper Berry	0.125	6.67f	33.33g
Lemon grass	0.25	33.33a	63.47b
Lemon grass	0.125	12.45e	42.07e
Mentha	0.25	23.44c	49.49d
Mentha	0.125	6.67f	33.33g
Palmarosa	0.25	16.64d	49.49d
Palmarosa	0.125	6.67f	22.62h

The treatments were significantly different (*p*<0.05%) based on maize weevil repellency. After 3 hours, the highest repellency was seen in eucalyptus (33.33%) and lemon grass (33.33%) at 0.25% concentration. After 6 hours, significantly higher repellency was shown by eucalyptus at 0.25% concentration (69.54%) followed by lemon grass at 0.25% conentration. Significantly lower repellency rate was observed in palmarosa at 0.125% which was statistically inferior to all other treatments. Moreover, repellency of the maize weevil was elevated with increment in time of exposure and increasing the concentration of the essential oils.

Table 4: Mean RP classification

Treatment	Conc (%)	Rep 6 hr	Repellency Class
Citronella	0.25	49.5	III
Citronella	0.125	33.3	II
Eucalyptus	0.25	69.5	IV
Eucalyptus	0.125	38.9	II
Fresh basil	0.25	57.1	III
Fresh basil	0.125	33.3	II
Juniper Berry	0.25	57.1	III
Juniper Berry	0.125	33.3	II
Lemon grass	0.25	63.5	IV
Lemon grass	0.125	42.1	III
Mentha	0.25	49.5	III
Mentha	0.125	33.3	II
Palmarosa	0.25	49.5	III
Palmarosa	0.125	22.6	II

Repellency class: 0 = <0.1 RP, I = 0.1 -20 RP, II = 20.1-40 RP, III = 40.1-60 RP, IV = 60.1-80 RP and V = 80.1-100 RP (Jiliana and Su, 1983) [11]

Discussion

Efficacy of essential oils on mortality of maize weevil

Monoterpenes, aromatic phenols, sesquiterpenes, alcohols, ethers, esters, oxides, aldehydes, and ketones are some of the many compounds that make up eucalyptus oil (Brookerand Kleinig, 1983) [5]. It also contains some insecticidal ingredients, which trigger stomach poisoning. Citronella, citronellyl acetate, citronellol, 1,8-cineole, eucamalol, linalool, limonene, a-pinene, a-terpineol, allocimene, aromadendrene, and g-terpinene are some of the compounds that have been attributed to the pesticide action of eucalyptus oils (Su *et al*., 2006; Liu *et al*., 2008). Eucalyptus has carminative, insecticidal, and antimicrobial properties and may also be utilized to protect grains, because eucalyptus oils may kill insect pests and inhibit oviposition (Ogunwande *et al*, 2003) [12].

Efficacy of essential oils on repellency of maize weevil

The chemical- 1,8-cineole, which had strong repellent action when tested separately against *Sitophilus zeamais*, was the chief constituent of the essential oils extracted from various species of *Eucalyptus viz. E. camaldulensis*, *E. citriodora* and *E. globulus*. Moreover, the chemical largely contributed to the high repellent activity of the oils. However, the higher level of the repulsiveness of essential oils extracted from *E. citriodora* and *E. camaldulensis* may have also been brought on by "minor" elements, because each compound's specific function depends on the interactions between the components of the combination and is mixture-specific (Akhtar *et al*., 2012) [2].

The insects were observed to be turning away and settling on the untreated filter paper. The choice for the untreated filter paper was because of the repellent chemicals inherent in the essential oils. Insect repellents are secondary metabolites that have been identified to be alcohols, alkaloids, phenolics, flavonoids, and terpenes. Eucalyptus oil showed excellent repellency activity than other treatments. Again, these reactions might be explained by variations in the chemical makeup of oil extracts and the ways in which these chemical components work, notably as insect repellents (Casida, 1990) [6]. It is worth noting that volatile oils are not very harmful to consumers, because they're washed away and quickly evaporate during cooking. (Bauer *et al*., 1990; Tapondjou *et al*., 2005) [3, 22].

Summary and Conclusion

Among the tested essential oils, eucalyptus was found very effective in terms of mortality and repellency of *Sitophilus zeamais*. The research also revealed that there was a direct correlation between the dosage of essential oils and their impact on insect pests, meaning that a higher dosage would result in a higher insect pest mortality rate. The Eucalyptus of higher concentration (100 µl) showed a higher mortality (90%) than the lower concentration (50 µl) showed a lower mortality (76.67%). Hence, increasing the amount of eucalyptus essential oil might result in greater mortality rates. Therefore, in order to boost beneficial outcomes, farmers should raise the dosages of eucalyptus essential oil. This study strongly encourages further investigation into the appropriate dosage to provide the greatest outcomes for optimal grain protection.

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