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Efficacy of Essential Oils against Cowpea Weevil, *Callosobruchus maculatus* (F.) under the Laboratory Conditions in Chitwan, Nepal

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ABSTRACT

The lentil (*Lens culinaris* Medikus subsp. *Culinaris*), a legume, is commonly grown and consumed in Nepal. The cowpea weevil, *Callosobruchus maculatus* Fabricius, is the major insect pest that threatens effective legume storage in Nepal. A laboratory experiment was conducted to assess the efficacy of six different essential oils (soybean oil, sunflower oil, olive oil, clove oil, rapeseed oil, and sesame oil) against damage by cowpea weevil. All the essential oils were applied at 5 mL/kg of lentil, and all substantially-reduced the number of damaged seeds and weight loss compared to the untreated control. The lowest grain damage and weight loss (measured by mean damaged grains and percentage weight loss per kilogram, respectively) were observed in the lentils treated with clove oil (5.00, 0.10), and the effects were statistically similar to those of soybean (7.33, 0.16), sunflower (7.66, 0.16), mustard (8.00, 0.22), olive (8.33, 0.17), and sesame (8.66, 0.13) oil. The smallest number of mean live beetles per kilogram was observed in the lentils treated with clove oil (0.00) and olive oil (0.00), followed by those treated with mustard oil (0.33), soybean oil (0.33), and sunflower oil (1). All essential oils effectively controlled cowpea weevils in stored lentils, but the clove oil produced the best results in reducing cowpea weevil damage under storage conditions.

Key words: cowpea weevil, lentil, essential oils, grain damage, weight loss

Introduction

Lentils (*Lens culinaris* Medikus subsp. *Culinaris*) are a major legume, with harvests of 251,185 Mt over a 208,766 ha area reported in Nepal, and they have emerged as a potential export commodity for this nation (MOAC, 2020). The cowpea weevil (*Callosobruchus maculates* Fabricius) is a major cosmopolitan pest that causes damage to various legumes in storage conditions (Mahmoud and Mohamed, 2015).

Numerous synthetic chemicals are

employed to control insect pests in agriculture. The repeated use of those chemicals resulted in numerous problems, including environmental pollution, side effects in humans, and adverse residual effects in foods. Owing to the drawbacks of using such chemicals in pest control, many experts have encouraged the pursuit of healthier alternatives. Various botanicals used to manage grain storage pests are effective and eco-friendly (Kafle and Shih, 2013; Paneru and Shivakoti, 2001). Isman (2006) contended that botanical pesticides are the best option for managing agricultural pests in organic food production in developing countries. Compared with synthetic chemicals, plant-derived materials are more environmentally friendly, less toxic to humans and animals, and more readily biodegradable (Rajashekar *et al.,* 2012). Essential oils are considered efficient pest control materials due to their fumigant and topical toxicity and their antifeedant or repellant effects (Regnault-Roger, 1997). Since the 1970s, thousands of plants have been screened as potential sources of repellents and toxicants (Sukumar *et al.,* 1991). Oils of mustard, sunflower, safflower, castor, and cotton have been employed as surface protectants against cowpea weevils to control insect population growth (including the number of offspring exposed to the protectant) and reduce seed damage rates (Rahman and Talukder, 2006). According to Ramzan (1994), sunflower, mustard, cotton, groundnut, and soybean oils mixed in cowpea completely suppressed the emergence of adult cowpea weevil. Used as a seed protectant, neem and sesame oils completely inhibited the adult emergence of *Callosobruchus chinensis* (Ahmed *et al.,* 1999). According to Jumbo *et al.* (2018), the application of clove oils controlled cowpea weevil damage to crops in storage conditions and was capable of reducing the oviposition and population growth of cowpea weevil even at sublethal dosages. Therefore, this study assessed the long-term efficacy of six commonly available essential oils (soybean, sunflower, olive, clove, mustard, and sesame) in preventing cowpea weevil damage to lentils under laboratory conditions in Chitwan, Nepal.

Materials and Methods

Rearing of cowpea weevil

Adult cowpea weevils (*C. maculatus*) were collected from a local farmer's lentil seed storage facility. Beetle offspring were reared in a container (4 kg), storing fresh, undamaged, and untreated lentil seeds. Approximately 300 pairs of cowpea weevils were released into the container and reared under room conditions (27 ± 3ºC, 65 ± 5% RH, and in natural light and dark conditions) at the Entomology Laboratory, Nepal Polytechnic Institute, Chitwan, Nepal. The beetles were cultured for 25 days, and newly emerged adults were used for further studies.

Essential oils and bioassay

The six essential oils (soybean, sunflower, olive, clove, mustard, and sesame) were obtained from a local market (Table 1).

The experiment was conducted according to a completely randomized design involving seven treatments and three replications. Simal variety of lentil was obtained from Shree Lumbini Seed Company, Bhairahawa, Nepal. Lentil seeds were sun-dried until they maintained 12% of moisture content. In total, 21 jars that were to store 0.5 kg of lentil seeds each were treated with 2.5 mL essential oils per container. After that, seven male-female pairs of newly emerged adult cowpea weevil were introduced into each jar. The tops of the containers were covered with muslin cloths to prevent cowpea weevils from escaping, and each plastic container was labeled appropriately. The study was conducted under laboratory conditions (27 ± 3°C, 65 ± 5% RH, and natural light and dark conditions).

Data analysis

A random sample of 25 g of lentil seeds was selected from each jar at 30, 60, 120, and 180 days after treatment (DAT), respectively, for data collection. The number of seeds, numbers of damaged and undamaged seeds, weights of damaged and undamaged seeds, number of beetles, and weight loss percentage were recorded from each sample. The grain loss weight was calculated as a percentage using the following equation:

$$\text{Percentage weight loss} = \frac{(\text{UNd} - \text{DNu})}{\text{U(Nd+Nu)}} \times 100$$

U = weight of undamaged seeds in the sample
D = weight of damaged seeds in the sample
Nu= number of undamaged seeds in the sample

Table 1. Treatment and source details for essential oils used for the control of *C. maculatus*

SN	Essential oils applied (5 mL/kg)	Manufacturer's name and address
1	Soybean (*Glycine max*) oil	Shree Shiva Shakti Ghiu Udyog Pr. Ltd. Bara, Nepal
2	Sunflower (*Helianthus annuus*) oil	Swastik Oil Industries Ltd. Morang, Nepal
3	Olive (*Olea europaea*) oil	Olitalia Srl, Forli, Italy
4	Clove (*Syzygium aromaticum*) oil	Sisla Laboratories, Narela, Delhi, India
5	Rapeseed (*Brassica napus*) oil	Self-Produced, Ilam, Nepal
6	Sesame (*Sesamum indicum*) oil	Dynasty sesame seed oil, JFC International, Japan
7	Control (untreated)	-

Table 2. Effects of six essential oils (5 mL/kg) on the lentil grain damage caused by *C. maculatus* under laboratory conditions

Essential oils applied (5 mL/kg)	Number of damage grain (Mean ± SD)*			
	30 DAT	60 DAT	120 DAT	180 DAT
Soybean oil	12.66 ± 3.06a	19.66 ± 11.23b	10.33 ± 2.08b	7.33 ± 0.58b
Sunflower oil	21.66 ± 8.14a	17.66 ± 5.77b	10.00 ± 1.73b	7.66 ± 0.58b
Olive oil	14.33 ± 2.08a	19.00 ± 1.73b	10.33 ± 2.08b	8.33 ± 1.53b
Clove oil	15.00 ± 3.46a	20.66 ± 2.30b	7.33 ± 2.51b	5.00 ± 1.00c
Mustard oil	14.00 ± 3.46a	17.33 ± 2.51b	10.00 ± 1.00b	8.00 ± 1.00b
Sesame oil	13.00 ± 4.00a	13.33 ± 2.08b	9.33 ± 4.16b	8.66 ± 0.58b
Control	20.33 ± 1.52a	33.66 ± 3.51a	16.66 ± 1.53a	12.66 ± 1.53a

* Means within the same column followed by different letters represent significant differences at the 5% level according to Duncan's multiple range test.

Nd= number of damaged seeds in the sample

Data were analyzed using R-STAT (The R Foundation for Statistical Computing, Vienna, Austria, 2018), and means were compared using Duncan's multiple range test at a significance level of 0.05.

Results

Effect of different treatments on lentil seed damage

At 30 DAT, the numbers of lentil seeds damaged by cowpea weevil under all the treatment conditions were statistically similar to those under the control condition ($F = 2.03$, $P = 0.14$). Furthermore, at 60 and 120 DAT, the number of damaged lentil seeds in the oil-treated samples was significantly (60 DAT: $F = 4.31$, $P = 0.02$ and 120 DAT: $F = 5.39$, $P = 0.01$) lower than that in the control condition, and the numbers of damaged lentil seeds in the other treated samples did not differ significantly. Similarly, at 180 DAT, the clove oil-treated sample had significantly less damaged seeds than the untreated (control) sample ($F = 12.40$, $P = 0.001$), which had the largest number of damaged seeds. In samples treated with soybean, sunflower, olive, mustard, and sesame oil, the number of damaged seeds was not significantly different from that in the clove oil-treated sample (Table 2).

Effects of treatments on lentil seed weight loss

The loss of weight in lentil seeds induced by cowpea weevils did not differ significantly at 30, 60, and 120 DAT (30 DAT: $F = 0.54$, $P = 0.76$; 60 DAT: $F = 1.39$, $P = 0.29$; and 120 DAT: $F = 2.38$, $P = 0.09$) among the treated samples compared with the untreated control sample. However, at

Table 3. Effects of six different essential oils (5 mL/kg) on the weight loss percentages of lentil seeds damaged by the cowpea weevil, *C. maculatus,* under laboratory conditions

Essential oils applied (5 mL/kg)	Weight loss percent (Mean ± SD)*			
	30 DAT	60 DAT	120 DAT	180 DAT
Soybean oil	0.31 ± 0.16a	0.25 ± 0.24a	0.09 ± 0.05a	0.16 ± 0.03b
Sunflower oil	0.28 ± 0.25a	0.30 ± 0.17a	0.16 ± 0.02a	0.16 ± 0.09b
Olive oil	0.55 ± 0.60a	0.18 ± 0.03a	0.18 ± 0.10a	0.17 ± 0.08b
Clove oil	0.19 ± 0.11a	0.26 ± 0.21a	0.11 ± 0.13a	0.10 ± 0.04b
Mustard oil	0.17 ± 0.17a	0.16 ± 0.09a	0.15 ± 0.06a	0.22 ± 0.07b
Sesame oil	0.27 ± 0.06a	0.21 ± 0.11a	0.16 ± 0.09a	0.13 ± 0.05b
Control	0.59 ± 0.71a	0.50 ± 0.20a	0.30 ± 0.04a	0.34 ± 0.05a

* Means within the same column followed by different letters represent significant differences at the 5% significance level according to Duncan's multiple range test.

Table 4. Effects of essential oil treatments on the number of live cowpea weevils, *C. maculutes,* 180 days after treatment under laboratory conditions

Essential oils applied (5 mL/kg)	Number of live beetle (Mean ± SD)*
Soybean oil	0.33 ± 0.58b
Sunflower oil	1.00 ± 1.00b
Olive oil	0 ± 0b
Clove oil	0 ± 0b
Mustard oil	0.33 ± 0.58b
Sesame oil	2.00 ± 1.00b
Control (Untreated)	39.33 ± 3.06a

* Means within the same column followed by different letters represent significant differences at the 5% level according to Duncan's multiple range test.

180 DAT, the loss of weight in all treated lentil seeds was significantly lower (F = 4.67, P = 0.01) than the untreated control. The differences in lentil seed weight loss were not significant regardless of the treatment type (Table 3).

Effects of treatments on live cowpea weevil numbers

At 180 DAT, the numbers of live cowpea weevils in lentil seeds treated with soybean, sunflower, olive, mustard, clove, or sesame oil were significantly lower (F = 391.01, P = 0.001) than in the untreated control seeds. However, no significant difference was observed between the numbers of live cowpea weevils in lentil seeds treated with soybean, sunflower, olive, mustard, clove, or sesame oil (Table 4).

Discussion

This study demonstrates that essential oils have an insecticidal function against cowpea weevil under grain storage conditions. According to Lale and Mustapha (2000), the cowpea weevil cannot lay eggs on grain treated with oil due to the surface's smoothness; cowpea weevils prefer not to lay eggs on legume surfaces dabbed with oil. The essential oils of different plants are rich in various insecticidal, antimicrobial, and bioregulatory components (Holley and Patel, 2005). According to Credland (1992), the funnel structure of bruchid eggs may be the common respiratory tract of these developing insects; therefore, the application of oil to bruchid eggs might obstruct the funnel, leading to the death of developing insects through suffocation. When oil was used as a contact treatment, an oil film was produced that obstructed the breathing holes or spiracles of adult beetles, which caused insect suffocation (Aider *et al.,* 2016). Similarly, Weinzeirl (1998) reported that fatty acid toxicity negatively affected cell membrane and insect exocuticle function and oxidative phosphorylation.

Sabbour and Shadia (2010) reported that clove oil and mustard oil exhibited stronger repellent activity (up to 89%) against bruchids. Jumbo *et al.* (2014) also reported that clove oil adequately controlled bean weevil (*Acanthoscelides obtectus*) damage to common beans by delaying insect emergence. Mahdi and Rahman (2008) discovered that clove oil treatment effectively controlled pulse beetle proliferation and ensuing black gram weight loss. Eugenol is the main constituent of clove oil and is neurotoxic against arthropods (Kafle and Shih, 2013).

Wang *et al.* (2016) reported that bioactive compounds of clove oil (eugenol, aceteugenol, caryophyllene, humulene, and viridiflorol) are toxic to the cowpea bruchid *C. chinensis*. Kafle and Shih (2013) noted that eugenol and eugenol acetate are lipophilic and that these compounds were absorbed through the cuticular lipids and then gradually entered into the hemocoels and nervous systems of insects. This process may have caused the deaths of cowpea weevils in the present study as well.

Seed treatment with soybean oil reduced the oviposition of cowpea weevils and inhibited adult weevil emergence (Pacheco *et al.,* 1995). Bhatnagar *et al.* (2001) revealed that soybean and mustard oil treatment at 10 mL per kilogram of cowpea was an effective deterrent against cowpea weevil. Neupane *et al.* (2016) also reported a lower weight loss percentage of green gram and a reduced cowpea bruchid eclosion rate when soybean, sesame, and mustard oil treatments were used compared with an untreated control sample. The rates of cowpea bruchid (*C. chinensis*) egg-laying and adult emergence on green gram was lowest in soybean oil-treated seeds followed by in sesame oil-treated seeds (Akter *et al.,* 2019). Soybean oil consists of triglycerides of linoleic acid, oleic acid, linolenic acid, and saturated acid, which has a non-toxic mode of action but functions as a pesticide through inducing suffocation in small, soft-bodied insects (Baker *et al.*, 2018).

Paneru and Shivakoti (2001) reported a significant mortality rate in cowpea weevils caused by mustard oil treatment over a week. Mustard oil treatment at 5 mL/kg of chickpea seeds reduced the number of eggs laid, adult emergence, and seed damage percentage associated with *C. chinensis* (Singal and Singh, 1990; Khaire *et al.,* 1992). Allyl isothiocyanate is the principal constituent of mustard oil; it disrupts the mitochondrial electron transport chain, which hampers mitochondrial gene expression (Bakkali *et al.,* 2008; Cardiet *et al.,* 2012) and ultimately interrupts insects' physiological activities and leads to their death.

Uvah and Ishaya (1992) reported that olive oil reduces the adult longevity of cowpea weevils. Biological parameters such as adult longevity, fecundity, and the amount of hatched eggs and emerged adult cowpea weevils were significantly reduced by treating olive oil and the major fatty acids oleic acid and linoleic acid (Aider *et al.,* 2016). These treatments induced toxicity in adult insects; they caused the death of such insects and thus prevented adult emergence and egg-laying. According to Raccaud-Schoeller (1980), olive oil, oleic acid, and linoleic acid produce white polyester on contact with chitin, a nitrogenous polysaccharide of the major constituents of insect cuticle. The reaction between polysaccharide hydroxide OH and fatty acid (R-COOH) may cause suffocation in insects.

Das and Karim (1986) ascertained that all cowpea bruchid adults died within 4 days of release and that insect oviposition was completely inhibited when the chickpea seeds were treated with sesame oil; furthermore, only a few eggs were found on the seeds treated with soybean and mustard oils. Significantly higher adult mortality, a lower rate of adult emergence of *C. chinensis,* and reduced seed damage were observed when chickpea seeds were treated with sesame oil (Regmi and Dhoj, 2011). Similarly, 1% sesame oil application completely prevented egg-laying and increased the adult mortality of cowpea weevil, *C. chinensis,* causing a significant reduction in seed damage (Ali *et al.,* 1983; Choudhary, 1990). Lignins, sesamin, and sesamolin are the active components of sesame oil, which induces suffocation in insects and has a synergistic action when used with other insecticides (Baker and Grant, 2018).

Sunflower oil treatment of 10 mL/kg of seed reduced the life span of adult cowpea weevils and cowpea bruchids (Rajapakse and Van Emden, 1997) by suffocating them. Similarly, the oviposition rate of the cowpea bruchid, *C. chinensis*, was significantly reduced by sunflower oil treatment (Srinivasan, 2008).

In conclusion, the cowpea weevil, *C. maculatus,* is one major pest of legumes in storage conditions. All essential oils tested in this study effectively reduced grain damage, grain weight loss percentage, and the number of surviving adult beetles. Therefore, those oils are recommended for the eco-friendly management of cowpea weevils at lentil seed storage facilities in Nepal.

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精油在實驗室環境下對四紋豆象（*Callosobruchus maculatus* (F.)）的防治效力

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摘　　要

小扁豆 (*Lens culinaris*) 在尼泊爾是被普遍種植和食用的豆類之一。四紋豆象 (*Callosobruchus maculatus*) 在尼泊爾是豆類儲倉中的主要害蟲。本實驗評估六種不同的精油 (丁香、橄欖、芥子、黃豆、葵花籽和芝麻) 對抗四紋豆象的功效。與對照組比較，所有精油以 5 mL/kg 的施用率處理小扁豆可明顯減少小扁豆的受損數量和其重量損失。被丁香油處理的小扁豆之受損數量與重量損失的情況最低 (5.00, 0.10)，此結果與大豆 (7.33, 0.16)、葵花籽 (7.66, 0.16)、芥子 (8.00, 0.22)、橄欖 (8.33, 0.17) 和芝麻 (8.66, 0.13) 等精油的數據在統計上相似。而在被丁香油處理的小扁豆中，存活的四紋豆象之數量最少 (0.00)，此結果亦與橄欖 (0.00)、芥子 (0.33)、大豆 (0.33) 和葵花籽油 (1) 的數據在統計上相似。本研究證明，精油能有效防治四紋豆象在貯藏條件下對小扁豆之危害，而丁香油對該害蟲的防治效力最佳。

關鍵詞：四紋豆象、小扁豆、精油、穀物損傷、重量損失