

Effectiveness and activity of essential oils as bio-insecticides against the pulse beetle, *Callosobruchus chinensis* (L.) in different pulses.

Abstract :

Callosobruchus chinensis L. is considered as the most destructive pest that attacks stored grains. The use of synthetic chemicals has many adverse effects on human health and results in environmental contamination. Essential oils can effectively combat this pest as a natural alternative to synthetic pesticides. The most frequently utilized techniques for evaluating insecticidal activity were the tests for contact toxicity, inhalation/fumigant toxicity, and repellency. As its primary mechanism of action, Acetyl cholinesterase, GABA receptors, and octopaminergic receptors are all impacted by essential oils, which also inhibit acetyl cholinesterase and play a part in GABA receptor regulation. The present review offers an overview on already published research and reports with regard to the utility of Essentials oils to combat the pulse beetle.

Keywords: antifeedents, *botanicals*, *chemicals*, *essential oils*, *insecticides*, *pulse beetle*, *repellents*.

Abbreviations: AChE = Acetyl cholinesterase, EOs = Essential oils, PVOC = Plant volatile organic compounds, FDI = Feeding deterrent index, PR = Percentage repellency.

Introduction:

Pulses are members of the subfamily Papilionaceae of the legume family Fabaceae or Leguminosae. For low-income groups of people and vegetarians, pulses are a significant part of their daily diet since they are a good supply of nutrients such as proteins, carbohydrates, minerals, and vitamins without any cholesterol or fat. Additionally, they are a wonderful source of minerals like iron, magnesium, phosphorus, zinc and others that are vital for optimum health. They have 20–25 per cent protein content by weight, which is twice as much as wheat and rice (Yunus *et al.*, 2015). With 35% of the world's land planted to pulses, India tops the production, importation and consumption of pulses, contributing 25% to worldwide production. In India,

they are grown on 128.88 Lha of area and produce an average of 76.49 Lt. (**Directorate of Economics and Statistics, DAC and FW, 2020**).

Pulses require additional care when being stored since microorganisms and insect infestations cause them to suffer significant harm. Storage pests greatly diminish the number and quality of seeds by severely damaging the grains that are kept in storage. (**Bashir et al., 2015**).

Insect infestation causes a staggering loss of 25–30 per cent of the world's agricultural productivity each year (**Hajjar et al., 2023**). In India, annual storage losses have been estimated 14 –million tons worth of Rs. 7,000 crores in which insects alone account for nearly Rs. 1,300 crores. Out of these post-harvest losses storage -insects alone account for 2.0 to 4.2 percent. (**Achary et al., 2021**). The biggest problem with storage is losses from pest infestation, especially in the villages and cities of tropical and subtropical regions due to humid weather, substandard hygiene and inadequate storage facilities. A reduction in the food grain's nutritional value results from pests in storage that not only consume the food grain but also alter its organic composition. Along with numerous other alterations, they also cause the grains to ferment and become acidic. The insect attack damages the grains, making them vulnerable to numerous bacteria. The chemical compound known as "Aflotoxin," which is said to be linked to human liver cancer, is produced by moulds growing in contaminated grains. (**Yadav et al., 2018**).

There are about 1700 species of bruchids belonging to 62 genera worldwide (**Romero and Johnson, 2004**). *Callosobruchus chinensis*, *C.analis* and *C. maculates* are among the primary species of pulse beetles (**Jadhav et al., 2012**). About 200 different types of pest insects exist in India, and they can harm grains and stored grain products. There have been reports of the genus *Callosobruchus* being a major pest in India, Middle and Far East Africa. *C.chinensis* (L.) (Coleoptera: Chrysomelidae) which is commonly known as “gram dhora” was first identified in China in 1758, where the species name of beetle was given to it (**Firth et al., 1973**). Almost all types of pulses are severely damaged by *Callosobruchus chinensis* L., including chick peas, lentils, black grams, cowpea, pigeon peas, mung beans, grass peas, garden peas, etc. In India and many other nations, it has reportedly caused major harm to pulse crops. It is widely distributed around the world in areas with tropical and subtropical climates. According to reports, it is a severe insect pest that damages stored goods in temperate zones, costing an estimated 0.21 million tons of ₹ 315 million annually (**Varma and Anandi, 2010**). It has the capability to

infest not only cultivated host plants in the field but also in storage (**Fahad, 2011**). It is recorded that 55- 60% loss in seed weight and 45.50 to 66.30% loss in protein content of pulses due to infestation caused by this beetle under normal conditions and severity of damage increases with the duration of storage (**Faruk et al., 2011**). Pulses become unfit for human consumption due to the infestation induced by *C. chinensis* L. Infested seeds become non-viable and sometimes unusual sprouting compels re-sowing. The germination rate of the pulse seeds is also reduced to a great extent. The severity of the damage varies depending on the kind of legume, the period of exposure, the location of storage, and other elements related to the seeds (**Tauthong and Wanleelag, 1983**).

Pulse beetles are destructive pests that attack stored pulses like chickpea, pigeon pea, green gram and black gram. Beginning in the field, the infestation spreads to the store. The pulse grains are not harmed by the adult insects. Although they mate and lay their eggs on grains, they do not consume the seed. The harm is entirely caused by the larva. The grub creates a hole, pierces the grain, and consumes seeds within, rendering them unfit for human ingestion. This causes the nutritional reserves of the embryo to be depleted, which results in low germination rates and weak seedlings. This lowers both the market prices and the quality. The potency of the pulse beetle infestation is greater in the summer than in the winter, primarily because of the high temperatures and high relative humidity, however this pest reproduces throughout the year in the stored pulses. When conditions are favourable, such as an ideal temperature of 30°C and relative humidity of 70%, the amount of damage is greatest (**Srinivasan, 2008**).

In the storage, pulse beetle can be managed using a variety of techniques. These include mechanical, cultural, physical, biological, and chemical methods, as well as the use of botanical pesticides (**Katiyar and Khare, 1989**). The utilization of synthetic chemicals to combat storage pests is one of these, farmers and stockholders frequently employ this technique. The chemical approach, which poses health risks to users and grain consumers, is now widely acknowledged to have a number of drawbacks. It results in residual toxicity, environmental contamination and the emergence of bruchid-specific pesticide resistance.

Sometimes persistent pesticides build up in the higher levels of both the wildlife and human food chains and concentrate through biomagnification (**Metcalf, 1975**). Contrarily, the

conventional approach of sun-drying is less hazardous to both environment and health of the human beings. However, this approach is time-intensive, exorbitant and needs sufficient drying yard where a huge volume of stored grain is engaged. Additionally, it depends on favourable atmospheric phenomenon.

The majority of the success thus far in protecting pulses in storage from the infestation of beetle pivoted on synthetic chemicals like phosphine fumigation and methyl bromide. Despite the fact that these chemicals unavoidably and excessively protect the grains, their usage pollutes the environment, creates major health risks, ozone depletion, and insect resistance. (**Kim *et al.*, 2003**). To ensure that foods are free of pesticides, these issues need the regulation of these substances (**Daglish, 2008**). These circumstances necessitate the requirement for pest control materials that are secure, affordable and easily accessible. Therefore, there is increased interest in using practical and environmentally friendly ways to substitute synthetic pesticides, such as the use of plant extracts, leaf powders, oils, pressurised carbon dioxide and temperature management approaches. (**Yuya *et al.*, 2009**). There are promising studies about the usage of specific native plant products as grain protectants (**Bhargava and Meena, 2002**). Plants' insecticidal activities have been thoroughly studied and have shown promise for controlling pests in the field and storage. Plant-derived compounds are risk-free and contain bitter components that have the potential to be poisonous, repulsive, antifeedant, and impede growth and offspring in insect pests (**Roy *et al.*, 2014**). Numerous plant-derived substances have been shown to be poisonous and growth inhibitors against insects that feed on stored-products (**Cosimi *et al.*, 2009**). Plant lectins are biodegradable insecticides that have detrimental effects on the survival, development, oviposition and reproduction of insects that attack stored grain (**Oliveira *et al.*, 2011**). According to **Onu *et al.* (2015)**, organic plant extracts give protection with less negative effects on the ecosystem and keep insect pests away from the treated grains by activating their olfactory receptors.

Bioactive compounds found in plant powders, extracts and oils have harmful effects and emit odor that deter adult beetles. Less research has been done on using weed plants to manage insect pests, despite the fact that they are a valuable source of medicine and contain toxic compounds.

Only 10% of the essential oils—which are derived from the flowers, leaves, bark of decorative, aromatic, medicinal, and other plants—are used for scent, flavor and smell. Due to their wide range of insecticidal abilities, eco-friendly nature and possibility for commercial application against stored grain pests, essential oils will be regarded as alternatives to chemical pesticides. Oils can kill insects by causing anoxia or by interfering with their regular respiration, which can lead to asphyxia. The oils could also act as anti-feedants or modify the storage micro-environment, thereby discouraging insect penetration in the grain and feeding **Haghtalab *et al.* (2009)**. Essential oils contain a complex blend of phytochemicals with insecticidal effects on pests that attack the stored grains (monoterpenes, sesquiterpenes, etc.). The commercially available essential oil-based treatments to manage pests in stored grains are not yet accessible. Based on the foregoing context, the primary objectives of the current work are to assess the fumigant, antifeedent, chemosterilant, repellent and ovicidal effects of the essential oils, against the pulse beetle.

Methodology:

The analysis of published studies examining the insecticidal activity of essential oils against pulse beetle was done. The following search terms were entered into several databases, including Web of Science, Scopus, Google Scholar, and Science Direct: essential oils, pulse beetle, stored grains, insecticidal activity and pests.

Fumigant bioassay

In 30 ml glass vials, *C. chinensis* were tested both with and without food to estimate the fumigant toxicity of essential oils. The Whatman No. 1 Filter paper sheets (5 1 cm) were immediately impregnated with essential oils at concentrations of 5 L, 10 L, 15 L, 20 L, and 25 L, and left to air dry for 2 min. To resist the test insects from coming into direct contact with the filter paper containing essential oils, the essential oils-impregnated filter papers were folded into L shape at 0.5 cm length and adhered to the inner top surface of the screw cap with double-sided adhesive tape. To each vial, ten *C.chinensis* adult insects were introduced. To establish an airtight condition, the tubes were sealed with parafilm and closed with a cover containing filter paper. The experimental setup described above was used for the food condition, with the minor modification of adding insects to vials (10 g) containing green gram. The filter paper in the

control lacked any essential oil. Each treatment was replicated thrice. In the without food condition, the insect mortality was observed for 4, 8, 12 and 24 h, whereas in with food condition the mortality was recorded for 4, 8 and 12 h. (Bincy *et al.*, 2023).

The mortality is approved when there is no sign of movement. Mortality percentages (% M) were calculated using the Abbott's (Abbott, 1925).

$$\% \text{Mortality} = \frac{\% \text{Me} - \% \text{Mo}}{100 - \% \text{Mo}} \times 100$$

Where Me = mortality in the tested EOs and

Mo = mortality in the negative control.

Contact assay:

Essential oils are prepared in a solvent (acetone) and then poured out into a filter paper disc (9cm in diameter). After the solvent is evaporated the disc is placed in a petri dish. Each Petri dish is closed with parafilm after placing adult insects. The mortality is calculated every 24h for 6 days (Alilou and Akssira, 2021).

Oviposition deterrent:

Oviposition deterrents are substances that stop or hinder insects from depositing eggs. Oviposition deterrents can serve as the primary defense against insect pests and have great potential for preventing insect infestation. Ovipositing females typically employ plant volatiles as cues to find host plants or substrates. Many specialists have claimed that PVOC plays a role in partially or totally suppressing the deposition of eggs as well as the development of different species of insect pests. It was discovered that the volatile component of 1, 8 cineole, which was extracted from essential oils of various plants in the Lamiaceae family, influences the pace at which various insects lay their eggs. The use of garlic oil as an oviposition deterrent is also

familiar. The essential oils of *Eucalyptus citriodora*, *E.globulus* and *E.Staigeriana* have severe effect on the oviposition, thereby reducing the viability of eggs and insect's emergence of *C.maculatus*. (Singh *et.al.*, 2021).

Repellency bioassay:

Repellency assays of essential oils were carried out using the preferred area method on filter paper according to the experimental protocol described by Mediouni Ben Jemaa and his team (Jemaa *et al.*, 2012). As a result, the 9 cm diameter filter paper discs used for the purpose of this work was divided into 2 equal pieces, each with a surface area of 31.80 cm². By dilution in acetone, four dosages of the essential oils were prepared: 5, 10, 40, and 160 ml. Then, 0.25mL of each solution—corresponding to dosages of 0.157, 0.314, 1.258, and 5.031L/cm²—was evenly distributed over one half of the disc while the other half obtained only 0.25mL of acetone. The two sides of the discs were re-welded with the help of adhesive tape after fifteen minutes. A batch of 10 adults, that were up to ten days old were kept in the centre of each reconstituted filter paper disc, which was then placed on a petri dish. Three replications were performed for each dose. The number of insects on the filter paper treated with the essential oils (Nt) and the number of insects on the untreated region (Nc) were counted after two hours.

The percentage repellency (PR) was calculated using the following formula: (Yakhlef *et al.*, 2020)

$$PR = \frac{Nc - Nt}{Nc + Nt} \times 100$$

Antifeedant activity :

“Antifeedants” or “feeding deterrents” are the chemical substances that disrupt the feeding behavior of insect by making the treated food unappealing or unpalatable . Antifeedants can potentially cause a temporary or permanent halt of feeding. As a result of particular compounds in plants

preventing insects from feeding on them, the insects eventually starve to death. Antifeedants are easily recognized by the way they affect insects when they come into touch with them. Antifeedant makes an effort to get rid of insects without ever messing with the ecology. Antifeedant donot kill the target insect, instead it allows them to be accessible for their natural enemies. The deleterious effects could be activated to serve a novel role in the management of stored grain insect pest. **(Singh et.al., 2021)**. The activity of essential oils on the nutritional behavior of insects was described in the study of **Shukla et al. (2011)**. In this test, the antifeeding ability of the essential oils is evaluated using a 1L jar. First, the EO needs to be placed on the inner surface of the jar after being individually soaked in a cotton swab. The jar that exclusively receives acetone serves as the negative control. Then, 500g of uncontaminated grain with a moisture percentage of 12–13% should be added to the fumigant jars. Finally, the insects (100 insects) should be released into the jars and the effectiveness of EOs is determined after 6 months of storage by evaluating the mortality, weight of the jars, and the weight of insects which are alive. **(Mssillou et al., 2022)**.

Weight loss is calculated using Equation.

$$\text{Per cent weight loss} = \frac{w-f}{w} \times 100 \quad \text{(Kobir et al. 2019)}$$

Where, w = Initial weight of seeds,

f = Final weight of seeds.

The EO activity as antifeedant is observed by calculating the feeding deterrent index (FDI, %).**(Rajkumar et al., 2019)**:

$$W_0 - T$$

$$\text{FDI} = \frac{\text{-----}}{\text{W0}} \times 100$$

W0 = weight loss in control sample.

T= weight loss in treated samples.

Chemosterilants:

Chemosterilants are chemicals that prevent insects from reproducing. Such chemicals simply stop the generation of F1 offspring, they do not impact their mating behavior or lifespan. Instead, they cause irreversible sterility. In some instances, eggs may not be laid, may not hatch, may not pupate or may not fully develop into pupae. Chemosterilants harm the ovaries, preventing the development of eggs. As a result, ovarian development is inhibited, which results in sterility. Chemosterilants have been proven to be a crucial component of integrated pest control plans because they lower the incidence of pest-resistant organisms. According to **(Saxena *et al.*, 1977)** asarone, a compound derived from the rhizomes of *A. calamus*, has insect chemosterilant characteristics and inhibits the activity of interstitial cells in insects. The active principle, 1,3,7-trimethylxanthine isolate from seed extract proved effective as chemosterilant for *C. chinensis*. **(Singh *et al.*, 2021)**.

Mechanism and efficacy of essential oils against pulse beetle:

The complete reduction of the oviposition rate in pulse beetles treated with plant oils could be as a result of disrupting the mating and sexual communications as well as deterring females from laying eggs. The effectiveness of the plant oils in reducing oviposition could be attributed to the presence of active ingredients such as Tetranortriterpenoids, ricinine and karanjin which have anti fertility, repellent and ovipositional deterrent effects on stored products **(Chaudhary *et al.*, 2017)**. **Sharanabasappa and Kulkarni (2008)** reported that neem oil, castor oil and karanj oil recorded lowest number of eggs per 50 seeds. **Bhatnagar *et al.*, 2001** also observed that the number of eggs laid (8.9) were lowest in seeds treated with neem oil. **Biswas and Biswas (2005)** also reported that karanj oil and neem oil effectively controlled pulse beetle population by reducing oviposition rate. **Sharma *et al.* (2013)** and **Bhatnagar *et al.* (2001)** reported that neem

oil was found to be most effective oviposition deterrent against *C. chinensis* in chickpea and cowpea, respectively. **Rashmi et al. (2014)** also reported that botanicals viz., neem oil, castor oil and mentha oil were found effective against pulse beetle in terms of ovicidal effect. **Miah et al. (2013)** reported that there is no development of eggs, larvae and pupae *C. maculatus* in green gram treated with neem oil, castor oil and camphor oil due to the toxic effect. The egg mortality and failure to hatch on the seed treated with oil has been attributed to the toxic component of the oil and also to the physical properties which cause changes in the surface tension and oxygen tension within the eggs (**Singh 1978**). Oil vapors likely entered eggs through diffusion and impacted the physiological and biochemical processes involved in embryonic development. (**Marimuthu et al., 1997**).

Essential oils showed the highest percent adult mortality, due to their high lipophilic nature and therefore have the ability to penetrate the cuticle of insects (**Abdullahi et al., 2011**). **Raghvani and Kapadia (2003)**, reported 100 per cent adult mortality with neem oil at 10 ml/kg seed in pigeon pea against *C. maculatus* up to six months of storage.

The low grain damage in oil treatment might be, due to the decrease in number of adult emergence (**Raja and Williams, 2008**). **Ramangoudar et al. (2000)** observed no seed damage by *C. chinensis* in seeds of horse gram when treated with neem oil at 5 ml/kg seed. **Singh (2003)** reported that pigeon pea seeds coated with neem and castor oil gave significant protection against pulse beetle compared to the untreated control.

Keita et al. (2001) reported that seeds treated with botanical oils did not lose their viability and the powders made from essential oils of different basil species provided complete protection against *C. maculatus*, and also did not show significant effect on the seed germination rate.

Yalamanchilli and Pudukollu (2000) observed that the volatile oil from the leaves of *Curcuma domestica* could effectively protect the seeds against *C. chinensis*, at a low concentration. **Mulatu and Gebremedhin (2000)** showed that the oils of *A. indica*, *Milletia ferruginea* and *Chrysanthemum cinerariaefolium* were the most effective in partially or completely preventing egg laying and pulse beetle emergence from the laid eggs. **Paneru and Shivakoti (2001)** reported that mustard oil caused significant mortality of *C. maculatus* after seven days when used 2% formulations and neem oil caused 100% mortality in *C. maculatus*.

Compared to purified and non-edible oils, crude and non-edible oils provided superior protection for grain. These oil coatings possess ovicidal qualities. Without influencing seed germination or cooking quality, it results in poor oviposition, decreases egg production and increases larval and adult mortality (**Wanderley et al., 2020**). To avoid infestation, several culinary and non-edible oils have occasionally been used to combat insect pests. The oils were also tested for their effectiveness on egg laying and adult longevity at various concentrations. According to **Chelav and Khashaveh (2013)**, after three days of exposure, a higher mortality rate was seen with 10 ml of poppy oil per kg of grains. According to **Wahedi et al. (2015)** neem seed oil reduces capacity of adult females of *C. maculatus* to lay eggs. According to **Abd El-Aziz, (2001)** clove oil and eucalyptus oil prevent *C. maculatus* from laying eggs. In his research, grains that had been exposed to neem oil showed the lowest levels of oviposition, undamaged seeds and weight loss. **Ketker (1986)** evaluated the effects of neem, castor and coconut oils on green gram against pulse bruchid and declared neem oil to be the best surface protector. Neem oil prevented egg production by controlling insects.

Conclusion:

Plant-derived pesticides have long been a key tool in farmers' arsenals for controlling pest insects that are stored in crops. The insecticidal properties of essential oils, their primary chemical constituents and their mode of action against the pulse beetle have been discussed in this review. Since plant volatile organic compounds are affordable, easily accessible at the farmer level, eco-friendly and have low mammalian toxicity, they may be suggested as a potential alternative to synthetic insecticides. Plant volatile organic compounds may provide a solution to issues with resistance, cost, availability and health hazards.

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