

## Evaluation of pesticidal efficacy of methanolic extracts of *Eucalyptus camaldulensis* leaves and *Eugenia caryophyllata* fruit buds against the rice weevil, *Sitophilus oryzae*

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### Abstract

This study explores the pesticidal efficacy of methanolic extracts from *Eucalyptus camaldulensis* leaves and *Eugenia caryophyllata* dry fruit buds against the rice weevil, *Sitophilus oryzae*. The extracts were prepared by soaking dried, powdered plant parts in methanol, followed by filtration and evaporation. Various concentrations of the extracts were tested in fumigant bioassays, with mortality rates recorded over four days. *E. camaldulensis* extracts demonstrated a dose-dependent effect, achieving an 87.11% mortality rate at 500 ppm. The LC<sub>50</sub> value for *E. camaldulensis* was calculated to be 225.04 ppm, with a regression equation of  $y = 0.1565x + 14.781$  and an R<sup>2</sup> value of 0.902, indicating a strong correlation between concentration and mortality.

In comparison, *E. caryophyllata* extracts exhibited a higher efficacy, achieving complete mortality at 500 ppm. The LC<sub>50</sub> value for *E. caryophyllata* was determined to be 45.65 ppm, with a regression equation of  $y = 0.1142x + 44.787$  and an R<sup>2</sup> value of 0.9656, indicating an even stronger correlation. The lower LC<sub>50</sub> value of *E. caryophyllata* highlights its superior pesticidal properties compared to *E. camaldulensis*. These findings suggest that both *E. camaldulensis* and *E. caryophyllata* extracts have significant potential as alternatives to synthetic pesticides for managing *S. oryzae* infestations.

**Keywords:** *Eugenia caryophyllata*, *Eucalyptus camaldulensis*, *Sitophilus oryzae*, methanolic extracts, natural pesticide, LC<sub>50</sub>

### Introduction

*Oryza sativa* L., commonly known as rice, is the primary food source for over half of the global population. This crop plays a crucial economic role by providing nutritional diversity and aiding in poverty reduction (Lakra, *et al.*, 2014) [1]. Rice is a crucial crop in the Asia-Pacific region, where more than 90 percent of it is cultivated and consumed (Segal & Nguyet, 1998). The global demand for rice is projected to increase significantly, rising from 723 million tons in 2015 to 852 million tons by 2035 (Brar & Khush, 2018) [3].

*O. sativa* is susceptible to various arthropod infestations, leading to significant quantitative and qualitative losses. Insect contamination of food presents a major issue for food industries and export commodities (Rajendran, 2002) [4]. Among the most destructive pests of rice grains, the rice weevil (*Sitophilus oryzae* L.) (Figure 1) is one (Howe, 1965; Bell & Wilson, 1995) [5]. In India, approximately 10% of total food grain production is lost during storage at the farm level (Lal, 1988) [8].

Control of these pests primarily relies on synthetic insecticides (Matthews, 1993) [6]. However, the extensive use of these chemicals poses significant drawbacks, including increased risks of neurotoxic, carcinogenic, teratogenic, and mutagenic effects on non-target organisms, as well as the development of cross- and multi-resistance in insect populations (Pimentel *et al.*, 2009; Dyte and Halliday, 1985; Sharaby, 1988) [7, 9, 10].

Consequently, there is growing interest in developing alternative and sustainable pest management strategies, including the re-evaluation of plant-derived substances (Erler *et al.*, 2010; Rajendran & Sriranjini, 2008; Saroukolai *et al.*, 2010; Yang *et al.*, 2010; Aliakbarpour *et al.*, 2011). Several phytochemicals were tested in previous studies for their pesticidal properties against *S. oryzae*. *Agave americana* leaf extracts insecticidal potential was tested through contact and repellent bioassays, revealing LD<sub>50</sub>,

LC<sub>50</sub>, and RC<sub>50</sub> values of 10.55 µg/insect, 8.99 µg/cm<sup>2</sup>, and 0.055 µg/cm<sup>2</sup>, respectively (Maazoun, *et al.*, 2019) [11]. *Mentha pulegium* L. oil exhibited significant fumigant effects against *S. oryzae* (L.) adults, with the effect varying based on the doses and exposure periods tested (Zekri, *et al.*, 2013) [12].

Isopropyl alcohol extracts from the leaves of *Nerium oleander* and *Datura stramonium* were tested against the rice weevil, *S. oryzae* at 2%, 4%, 6%, 8%, and 10% concentrations and found that mortality increased with concentration. Among the two extracts, *D. stramonium* exhibited the highest biocontrol activity (Yuvanandhini & Dhivya, 2021) [13].

The *Eucalyptus* plant is known for its therapeutic benefits, including improving respiratory health, reducing inflammation, boosting immune function, lowering blood sugar, protecting skin health, alleviating tension and anxiety, and combating infections. Various *Eucalyptus species* are renowned for their rich content of bioactive compounds, including phenolics, flavonoids, terpenoids, tannins, phloroglucinol, and cardiac glycosides, all of which exhibit potential antimicrobial and pesticidal properties (Bahadar, *et al.*, 2016; Bouharb, *et al.*, 2014) [16, 17].

Methanolic extracts of *E. camaldulensis* leaves were found to have significant antibacterial activity (Elansary, *et al.*, 2017) [18]. Additionally, phytochemical analysis of the leaves and bark of *E. camaldulensis* revealed a high concentration of bioactive compounds such as flavonoids, saponins, tannins, and glycosides (Ayepola & Adeniyi, 2008; Ibrahim, *et al.*, 2016) [19, 20].

*Eugenia caryophyllata* essential oils (EOs), rich in phenolic and flavonoid derivatives (Abou Baker *et al.*, 2020) [14], are renowned for their diverse pharmacological and biological activities. These include antioxidant, anticancer, anti-inflammatory, antibiofilm, and antimicrobial properties (Jugreet *et al.*, 2020) [15].



**Fig 1:** Adult rice weevil, *Sitophilus oryzae*

**Material and methods**

The study on *Sitophilus oryzae* (rice weevil) aimed to explore the efficacy of methanolic extracts of *Eucalyptus camaldulensis* leaves and *Eugenia caryophyllata* dry fruit buds as alternatives to synthetic pesticides.

To prepare the extracts, the selected plant parts were washed under running water thoroughly to remove dust. After shade drying them for 15 days, they were finely ground to make them into powder form. 200 grams of powders were soaked in 500 ml of methanol for 3 days with frequent shakings. Later the liquids were filtered on Whatman filter paper no. 1. The filtrates were allowed to evaporate to get the semi-solid extracts which were stored in the refrigerator until usage.

Stock solutions were prepared by soaking 1 gram of the extract in 10 ml of methanol and 990 ml of distilled water. Test solutions of 62.5, 125, 250, and 500 ppm were prepared by serial dilution method. The control solution was prepared by mixing 10 ml of methanol in 990 ml of distilled water.

For conducting fumigant bioassays, clean plastic bottles were used. 40 grams of rice grains were taken into each bottle along with 50 adults of *S. oryzae*. Filter papers of 5 cm diameter were soaked in the test solutions separately and airdried before they were fixed to the inside of the test bottle caps. Insect mortality was observed for 4 days. Insects that did not respond to touch with a brush were recorded as dead. The experimental design was a randomized setup with four replications. Statistical analysis was performed using MS Excel software. Regression analysis was performed.

**Results & discussion**

The results of the present study are given in Table No. 1 and Figure No. 2. The extracts of *E. caryophyllata*, at 0 PPM, caused a mortality rate of  $0.00 \pm 0.46\%$ . When the concentration was increased to 62.5 PPM, the mortality rate rose to  $46.94 \pm 1.96\%$ . Further increasing the concentration

to 125 PPM resulted in a mortality rate of  $62.76 \pm 2.29\%$ . At 250 PPM, the mortality rate reached  $76.53 \pm 3.43\%$ ; at the highest concentration of 500 PPM, the mortality rate was  $100 \pm 3.66\%$ . These findings indicate that higher concentrations of the extract correlate with higher mortality rates, demonstrating a dose-dependent effect. The extracts of *E. camaldulensis* also demonstrated a significant effect on the mortality of the test insects at varying concentrations. At the control concentration of 0 PPM, the observed mortality rate was  $0.00 \pm 0.5\%$ . When the concentration was increased to 62.5 PPM, the mortality rate rose to  $29.38 \pm 1.53\%$ . At 125 PPM, the mortality further increased to  $44.85 \pm 2.76\%$ . A concentration of 250 PPM resulted in a mortality rate of  $59.28 \pm 2.22\%$ . The highest concentration tested, 500 PPM, showed a mortality rate of  $87.11 \pm 4.20\%$ . The results were subjected to regression analysis (Figures 3 and 4). The LC50 value of *E. caryophyllata* was determined to be 45.65 PPM. This value highlights the potent pesticidal properties of the *E. caryophyllata* extract. The resulting regression equation was:  $y = 0.1142x + 44.787$ . The positive slope (0.1142) signifies an increase in mortality percentage with increasing concentration of the extract. The R<sup>2</sup> value was 0.9656. The LC50 value of *E. camaldulensis* extracts was calculated to be 225.04 PPM. The relationship between concentration and mortality was described by the regression equation  $y = 0.1565x + 14.781$ , with a coefficient of determination (R<sup>2</sup>) value of 0.902. This high R<sup>2</sup> value indicates a strong correlation between the concentration of the extract and the observed mortality rates.

The results of the present study reveal that *E. caryophyllata* extract exhibited significant pesticidal activity, achieving complete mortality at 500 PPM. In Contrast, the extracts of *E. camaldulensis* achieved a mortality rate of  $87.11 \pm 4.20\%$  at the highest test concentration. The low LC50 value (45.65 PPM) of *E. caryophyllata* underscores its superiority over the extracts of *E. camaldulensis* whose LC 50 value was higher (225.04 PPM).

**Table 1:** Results of Fumigant Bioassays of *E. caryophyllata* & *E. camaldulensis* against the adults of *S. oryzae*

Tested plant extracts	Mortality % against test Conc. in PPM					LC 50 in PPM	Regression equation	R <sup>2</sup> Value
	0	62.5	125	250	500			
<i>E. caryophyllata</i>	$0.00 \pm 0.46$	$46.94 \pm 1.96$	$62.76 \pm 2.29$	$76.53 \pm 3.43$	$100 \pm 3.66$	45.65	$y = 0.1142x + 44.787$	0.9656
<i>E. camaldulensis</i>	$0.00 \pm 0.5$	$29.38 \pm 1.53$	$44.85 \pm 2.76$	$59.28 \pm 2.22$	$87.11 \pm 4.20$	225.04	$y = 0.1565x + 14.781$	0.902

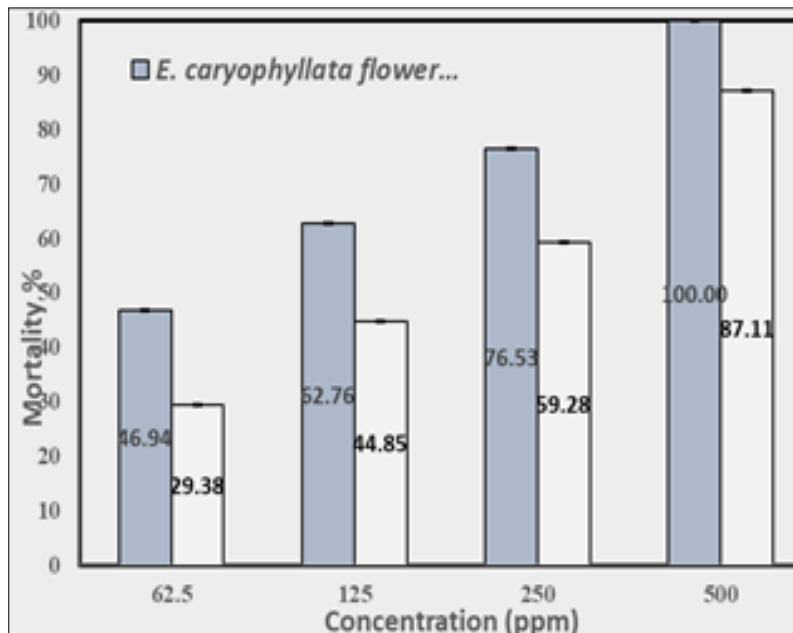


Fig 2: Results of Fumigant Bioassays of *E. caryophyllata* & *E. camaldulensis* against the adults of *S. oryzae*

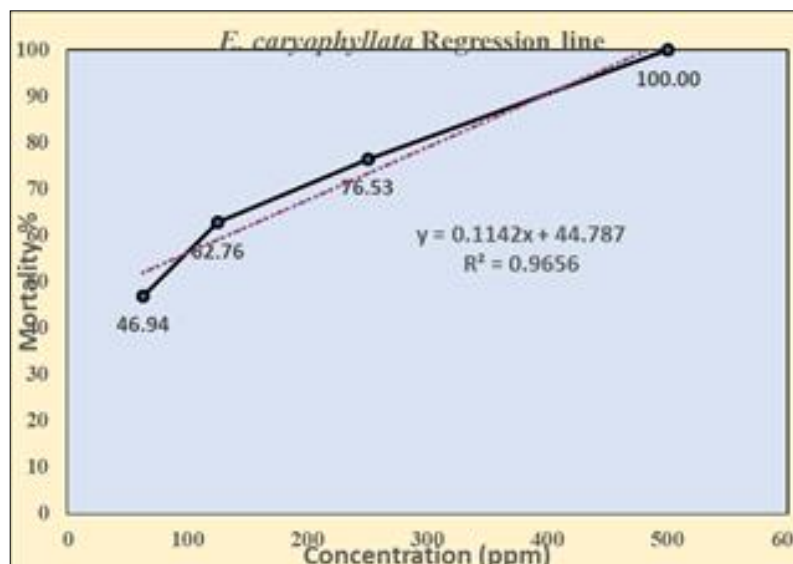


Fig 3: Regression analysis of Fumigant bioassay of *E. caryophyllata* flower bud extracts against the adults of *S. oryzae*

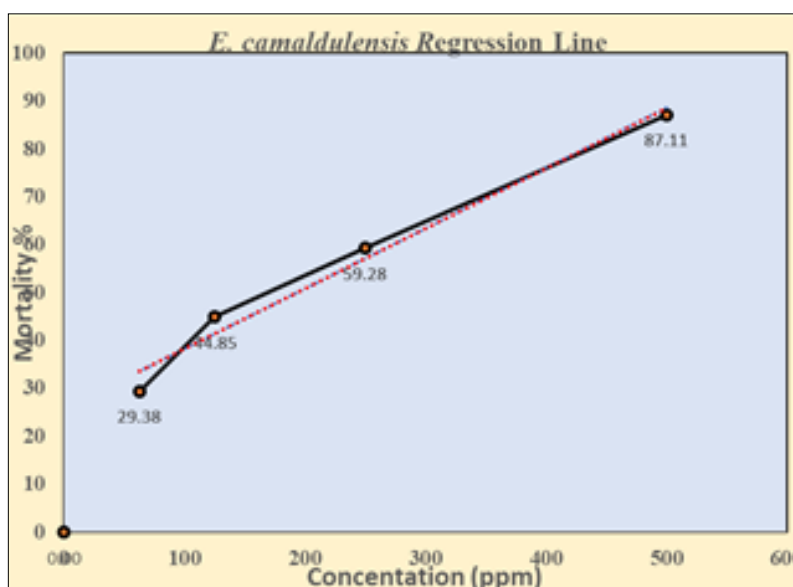


Fig 4: Regression analysis of Fumigant bioassay of *E. camaldulensis* leaf extracts against the adults of *S. oryzae*

## Conclusion

The study demonstrates that methanolic extracts from *Eugenia caryophyllata* and *Eucalyptus camaldulensis* possess significant pesticidal properties against *Sitophilus oryzae*. *E. caryophyllata* extracts exhibited superior efficacy, achieving complete mortality at 500 ppm and an LC50 value of 45.65 ppm, compared to *E. camaldulensis* extracts, which reached an 87.11% mortality rate at the same concentration and had an LC50 value of 225.04 ppm. These results highlight the potential of these plant extracts as sustainable alternatives to synthetic pesticides for controlling rice weevil infestations. Further research is recommended to optimize extraction methods and application techniques for practical use in pest management strategies.

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