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Efficacy of eucalyptus oil alone and in combination with phosphine against pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae)

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Abstract
The experiment was designed to study the toxic effect of locally available volatile eucalyptus oil, *Eucalyptus globulus* (5, 10 and 15 per cent) alone and in combination with phosphine gas (4 ppm) against pulse beetle, *Callosobruchus chinensis* L. The research work was carried out during 2017- 2018 at the Regional Research Centre, Amravati (M.S.). Observations on per cent mortality were recorded at 20 minutes and 24 hours after exposure to the treatment. It was observed that per cent mortality increased with increase in concentration of volatile oil and exposure period. 20 minutes after exposure to treatment, no mortality was observed in eucalyptus oil having 5 per cent concentration but mortality increased up to 30.00 per cent after 24 hours of exposure in this treatment. 83.33 per cent adult mortality was observed in the treatment, eucalyptus oil 15 per cent + phosphine 4 ppm at 24 hours of exposure.

Keywords: Eucalyptus oil, phosphine gas, test insect, per cent mortality, *Callosobruchus chinensis*

1. Introduction
All over the world, there are about 30 species of bruchids which are serious pests of stored legumes [1]. Infestation of bruchids starts in the field but it is difficult to detect it at the time of harvest [2]. Infestation is generally noticed in storage when seeds are stored for longer period. During 2014-15, India produced 257.07 Mt food grains including pulses and grains were stored as bag- stacks in warehouses [3]. Bruchid population increased rapidly in storage and infested grains could not fetch price in the market. Hence it is necessary to control this pest in storage. Generally, the insecticides *viz.* Malathion, pyrethrins, cyfluthrin, diatomaceous earth, etc. and fumigants like phosphine, methyl bromide are used to control insect pests in storage. As the use of fumigants in warehouses is easy and economical, phosphine gas is most commonly used to control storage pests. But due to indiscriminate use of phosphine in storage structures, the problem of resistance development to phosphine has raised. In 1985, Dyte, C.E. & Halliday, D. [4] published a paper on the global survey conducted by FAO for pesticide sensitivity of stored grain pests in 1972–73. The results showed that phosphine resistance was observed in about 9.7% insect strains from 82 countries, especially in *Tribolium* spp. and *Rhyzopertha dominica*. Due to worldwide use of phosphine, resistance has occurred at higher level in insect pests like *Cryptolestes ferrugineus, Oryzaephilus surinamensis*.
Hence, to overcome the problem of phosphine resistance, use of botanicals is one of the best options. It is emerging as one of the important ways to protect grains/ seeds in storage organically. Many essential oils from different plants are in use to manage stored grain pests. Oils of thuja, peppermint, nishinda (*Vitex negundo* L.), eucalyptus (*Eucalyptus globulus*), bankalmi (*Ipomoea sepiaria*), neem (*Azadirachta indica*), safflower (*Carthamus tinctorius*), sesame (*Sesamum indicum*), bablah (*Acacia arabica*), rosemary (*Rosemarinus officinalis*), juniper (*Juniperus communis*) etc. are used by scientists to control stored grain pests [5, 6, 7, 8].
In this experiment, we have evaluated insecticidal activity of eucalyptus oil (*Eucalyptus globulus*) against pulse beetle, *Callosobruchus chinensis* L. Eucalyptus oil is extracted from *Eucalyptus globulus* leaves. The oil is used as an insect repellent and biopesticide. First time in 1948, eucalyptus oil was registered as an insecticide and miticide in United States of America [9]. The insecticidal potency of essential oil of *Eucalyptus floribundi* on the adult of *Rhyzopertha dominica* and *Oryzaephilus surinamensis* was studied by Aref, *et al.*, 2016 [10].

The present work is carried out to determine the fumigant toxicity of essential oil of the leaves of *Eucalyptus globulus* and also the synergistic effect of eucalyptus oil with phosphine against pulse beetle, *Callosobruchus chinensis* L.

2. Materials and Methods

The experiment was carried out in the laboratory of Entomology section at Regional Research Centre, Amaravati (M.S.) during 2017-18. The materials were comprised of adults of pulse beetle, *Callosobruchus chinensis* L., eucalyptus volatile oil, aluminium phosphide sachet 10 gms, 5% H_2SO_4, filter papers, jars, glass gas chambers of 125 ml volume, syringe, falcon tubes, pipette, rubber bands, muslin cloth, healthy, Uninfested green gram grains, etc.

2.1 Test insect

Nucleus culture of Pulse beetle, *Callosobruchus chinensis* L. was collected from the Department of Post-Harvest Technology, College of Agril. Engineering, Dr. Panjabrao Deshmukh Krishi Viyapeeth, Akola (M.S.). The insect culture was maintained in sterilized plastic jars at 29+2^{0}C temperature and 80+5 % relative humidity. The culture medium comprises of green gram seeds. Initially 1-2 days old adults were released on healthy, uninfested green gram seeds in plastic jars. The jars were covered with the muslin cloth for better aeration. The jars were kept on iron racks for mating and oviposition.

Emergence of adults from infested grains started from 22-25 days after release. These adults were further reared for multiplication up to nine generations. The adults from tenth susceptible generation were used as test insect for conducting the experiment.

2.2 Test material

Crude volatile eucalyptus oil was obtained from local market to carry out the experiment. The oil was diluted in acetone at different concentrations *i.e.* 5%, 10% and 15%. To prepare these concentrations, first 10 ml acetone was taken in falcon tube. Then 2 ml eucalyptus oil was added in that to make 20 per cent stock solution.

Through this stock solution, some amount of solution was taken out and diluted in the acetone to form further concentrations by following formula [11]:

$$V = \frac{C \times A}{a.i.}$$

Where

V= Quantity of essential oil to be collected from stock solution

C= Required concentration of essential oil

A= Total volume of the solution

a.i.= Stock solution *i.e.* 20 per cent By using this formula, 15 per cent eucalyptus oil was prepared by taking 3.75 ml eucalyptus oil from 20 per cent stock solution and then 1.25 ml acetone was added in that to prepare 5 ml solution of 15 per cent eucalyptus oil. Similarly, 10 per cent and 5 per cent eucalyptus oil solution was prepared. The filter papers were cut in circular shape according to the bottom diameter of the glass canister.

Phosphine gas was generated in the collection tube using 56 per cent aluminium phosphide (10 gram) in acidified water (5% H_2SO_4). From collection tube, known quantity of gas was suck out with the help of an air tight syringe and was injected into a sealed desiccator of known volume to make required concentration of phosphine [12].

2.4 Toxicological impact of essential oil against *Callosobruchus chinensis* L.

There were eight treatments and three replications in this experiment. Ten adult bruchids were used as test insect per replication. The oil of different concentrations was applied on filter papers. Solvent *i.e.* acetone was allowed to evaporate for 10-15 min. prior to introduction of insects. Each filter paper was then placed at the bottom of a glass gas chamber. Test insects were released in each replicated glass gas chamber and then covered with rubber cork. As the test insects should not stay on the lid, the inner side of the cork was coated with vasaline. In the treatments T4 to T6, 4 ppm phosphine gas was released in the glass gas chamber in addition to the essential oils. In treatment T7, only phosphine gas was released to observe the efficacy of poisonous gas. Number of dead insects were observed 20 minutes after exposure to oils and phosphine gas and thereafter 24 hours. The data thus obtained were analysed statistically by Completely Randomized Design test. The treatment details are as follows.

Table 1: Treatment details

Sr. No.	Treatments	Treatments
1.	T1	Eucalyptus oil 5 %
2.	T2	Eucalyptus oil 10 %
3.	T3	Eucalyptus oil 15 %
4.	T4	Eucalyptus oil 5 % + phosphine 4 ppm
5.	T5	Eucalyptus oil 10 % + phosphine 4 ppm
6	T6	Eucalyptus oil 15 % + phosphine 4 ppm
7.	T7	Phosphine 4 ppm
8.	T8	Untreated control / acetone only

3. Results

The insecticidal activity of eucalyptus oil (*Eucalyptus globulus*) against pulse beetle *Callosobruchus chinensis* L. was evaluated by fumigation assay. The volatile oil was diluted using acetone at different concentrations i.e. 5%, 10% and 15%. The results are presented in table 2 and Fig.1.

Table 2: Efficacy of eucalyptus oil with phosphine against pulse beetle, *Callosobruchus chinensis.*

Treatments	Per cent Adult mortality after exposure period of	
	20 min	24 hours
T1 -Eucalyptus oil 5 %	0.00 (1.00) *	30.00 (5.57)
T2 -Eucalyptus oil 10 %	10.00 (3.32)	36.67 (6.13)
T3 -Eucalyptus oil 15 %	16.67 (4.16)	43.33 (6.65)
T4 -Eucalyptus oil 5 % + phosphine 4ppm	20.00 (4.58)	70.00 (8.41)
T5 -Eucalyptus oil 10 % + phosphine 4ppm	30.00 (5.52)	76.67 (8.81)
T6 -Eucalyptus oil 15 % + phosphine 4ppm	36.67 (6.13)	83.33 (9.18)
T7 -Phosphine 4ppm	23.33 (4.91)	60.00 (7.79)
T8 - Untreated control/ Acetone only	0.00 (1.00)	3.33 (1.77)

SE(m) +	0.28	0.36
C.D. at 5%	0.86	1.10

*Figures in parantheses are square root values.

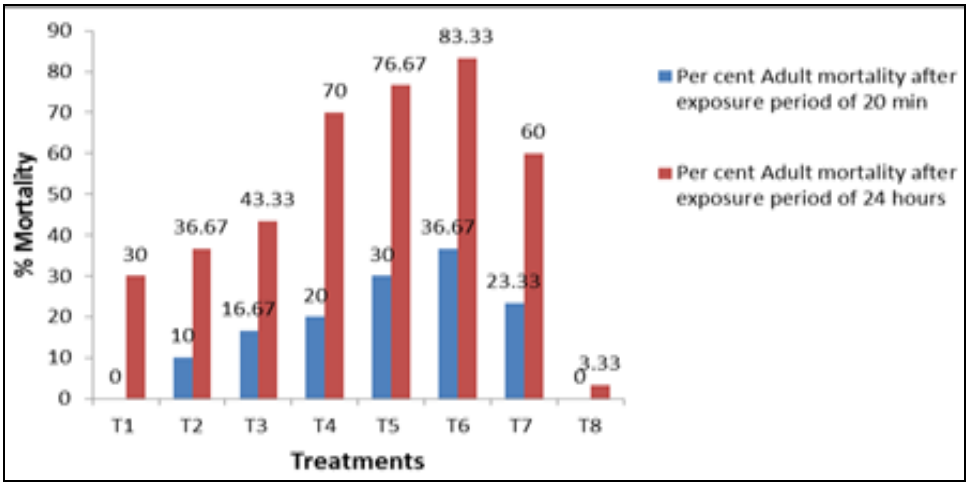

Fig 1: Graph showing the per cent adult mortality after exposure period of 20 min. and 24 hours.

From table 2, it was revealed that eucalyptus oil was found to be less effective with no mortality at 5 per cent concentration of oil at 20 minutes after exposure to fumigant. The treatment T6 (Eucalyptus oil 15% + phosphine 4ppm) was found to be significantly superior (causing 36.67 per cent adult mortality) over all other treatments except treatment T5 (Eucalyptus oil 10% + phosphine 4ppm) having mortality 30.00 per cent. Treatment T6 was at par with the treatment T5. Treatment T6 was followed by T7 (Phosphine 4 ppm) with 23.33 per cent mortality.

24 hours after exposure to fumigants *i.e.* eucalyptus oil and phosphine gas, 83.33 per cent mortality was observed in the treatment T6 (Eucalyptus oil 15% + phosphine 4ppm). This treatment was found at par with the treatment T5 (Eucalyptus oil 10% + phosphine 4ppm) and T4 (Eucalyptus oil 5% + phosphine 4ppm) causing adult mortality 76.67 per cent and 70.00 per cent respectively. Mortality increased from 0.00 per cent to 30.00 per cent in treatment T1 (Eucalyptus oil 5%) as the exposure period to fumigants increased from 20 minutes to 24 hours.

4. Discussion

In this experiment, we observed the synergistic effect of eucalyptus oil and phosphine gas showing 83.33 per cent mortality in *C. chinensis* at 24 hours after exposure to fumigants. It was also observed that mortality of test insect increased with increase in concentration of essential oil and exposure period.

Results obtained in this research work, showed similarity with the results of some the researchers who worked on efficacy of eucalyptus oil on some stored grain pests. Rahman, A and Talukder, F. A. [13] evaluated direct toxicity of acetone extract of *Eucalyptus globulus* leaves on *C. maculatus* at 2,4 and 6 per cent concentration and observed that insect mortality increased with increase in time (24, 48 and 72 hours after treatment).

The present results are in accordance with the results of Mahfuz, I. and Khalequzzaman, M. [14] who investigated toxic effect of eucalyptus and other oils against *Callosobruchus maculatus* adults through contact and fumigation bioassay. In the contact bioassay, eucalyptus oil was most effective in causing mortality both after 24 and 48 h of treatments while in fumigation bioassay reverse results were obtained. Pathipati, U.R. [15] observed that in fumigation assay, eucalyptus oil at 130µg/cm^2, caused 100% toxicity to *C. chinensis* within 24 hrs of treatment. Izakmehri K, *et.al*.[16] evaluated lethal and sublethal effects of essential oils from *Eucalyptus Canadensis* and *Heracleum persicum* against adults of *C. maculatus* and results showed that low lethal concentration (LC_{20}) of essential oils negatively affected longevity, fecundity, and fertility of female adults. Olotuah, O.F. [17], have recorded that *Eucalyptus globulus* at 1 per cent concentration was least effective against *Callosobruchus maculatus*. Siddique, S. *et al*. [18] reported that *Eucalyptus rudis* was most potent against *T. castaneum.* Fouad, H.A. [19] reported that *Eucalyptus globulus* at 1% concentration repelled the adult *C. maculatus.*

The results of this experiment are on the same line with the results of Hamza, A.F., *et al*., 2015 [5] who observed that volatile oil of eucalyptus was effective against one-week-old adults of *Sitophilus granarius* (L.) when exposed for 24,48 and 72 hours. It was also observed that mortality increased with increase in concentration and exposure period.

Aref, S. P. *et al*., 2016 [10] studied lethal and sub-lethal effects of essential oil of *Eucalyptus floribundi* on the adult of *Rhyzopertha dominica* and *Oryzaephilus surinamensis* and observed that with increase in the concentration of essential oil and the exposure time, mortality increased. The essential oil of Eucalyptus *floribundi* was found to be effective against both the pests. Fattah, A., *et.al*. [20] reported that *Callosobruchus maculatus* adults were susceptible to essential oil of *Eucalyptus globulus*. The fumigant toxicity of oil on test insects increased with increase in oil concentration.

5. Conclusion

From the results, it is concluded that volatile eucalyptus oil in combination with phosphine gas was effective against pulse beetle showing synergistic effect. Application of eucalyptus oil alone may not be that much effective to control storage pests immediately, but applying this volatile oil along with very less concentration of phosphine gas against *C. chinensis* is effective having complete mortality. These results also showed the importance of insecticides of plant origin in the management of *C. chinensis*.

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