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The Evaluation of Toxicity Effects of Synthetic Volatile Compounds and Essential Oils on *Callosbruchus maculatus* L. and its Biology

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The biology studies and contact toxicity of synthetic volatile compounds, namely Propionic acid and Benzaldehyde, and essential oils namely, *Ocimum basilicum* and *Mentha piperita,* and their different possible combinations evaluated against *Callosobruchus maculatus* and were carried out at the Department of Entomology, Annamalai University to develop an economically viable and effective alternative to chemical insecticides to manage the stored product insect pests. In the biology study, the egg period was 5±0.07 days. The larval and pupal period together was 21.24±0.67. The total development period recorded from the day of oviposition to adult emergence was 35.03±0.35 days. The maximum contact toxicity was observed in Propionic acid + Benzaldehyde with 8.73 µl LC_{50}. Therefore, in addition to being incorporated into the storage pest management to prevent the development of resistance, combinations of essential oils and synthetic volatiles, such as propionic acid and benzaldehyde, could be used as an alternative to the chemical fumigants that are mostly used to control pests of stored products.

Keywords: Ocimum basilicum; Mentha piperita; Callosobruchus maculatus; biology studies and contact toxicity.

ABBREVIATIONS

OC: Ocimum basilicum
PM: Mentha piperita
PA: Propionic acid
B: Benzaldehyde

1. INTRODUCTION

The most significant pests of stored grains and pulses in the Indian subcontinent can be broadly classified into two categories: primary pests, which can pierce and infest grain kernels and have juvenile stages that develop within the grain kernel, and secondary pests, which are unable to infest whole grains but feed on debris, high moisture seeds, broken pieces of grain, and grain damaged by primary pests. The pulse beetle, *Callosobruchus maculatus* (L.) (Coleoptera: Bruchidae), is a significant primary pest. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the rust-red flour beetle, is a secondary pest [1]. The prevention of this insect in harvested crops, and stored grain has drawn significant attention. Multiple techniques are employed to safeguard post-harvest crops and stored grains from this pest [2]. Chemical pesticides are frequently used in agricultural areas and warehouses to prevent and control pests. Methyl bromide and phosphine pesticides are typically employed to suppress coleopteran pests in stored grains [3]. Excessive consumption of these chemical pesticides causes several adverse consequences, including pest resistance, toxicity to non-target species, and harm to the ecosystem [4]. However, [5] noted that this substance was deemed hazardous to the human heart, blood vessels, and lungs. These substances' irrational usage may be linked to several dangerous issues. These chemical pesticides are hazardous to the environment and public health in addition to their persistence, improper application by farmers, insect recurrence, and genetic resistance [6]. According to [7], bioinsecticides generated from plants could offer a good, safe substitute for chemical insecticides. With a record of over 2000 plant species, numerous studies have demonstrated the pesticidal qualities of botanical products against a variety of stored grain pests [8]. The primary applications for these plants are oils, volatiles, slurries, powders, and aqueous/solvent extracts. [9] Observed that the essential oils from rhizomes of *Alpiniacon chigera* Griff, *Zingiber zerumbet* Smitt, and *Curcuma zedoaria* (Berg.) were proven for contact toxicity, feeding reduction and repellent activity against *S. zeamais,* and *T. castaneum* adults.

Based on the literature survey, Plant-based essential oils like *O. basilicum* and *M. piperita* were used in this study. The combination of volatile substances released by barley grains contains propionic acid [10]. On the other hand, the food sector frequently uses it as a preservative in a variety of food products. Insect control and fungal growth prevention are its main functions, particularly when it comes to storing moist food grains. To control the pests of stored products, one alternative to the chemical fumigants that are typically utilized is benzoaldehyde and its derivatives [11]. The selected essential oils and synthetic volatile compounds were procured and tested individually and as well as in different combinations and are selected from the previous fumigant toxicity study as follows (Kathirvelu *et al.,* 2020): (i) Propionic acid (ii) Benzaldehyde (iii) *Ocimum basilicum* (OC) (iv) *Mentha piperita* (PM) (v) Propionic acid + Benzaldehyde (1:1) (vi) Benzaldehyde + M. piperita (1:1) (vii) Benzaldehyde + *O. basilicum* (1:1) and (viii) Benzaldehyde + *M. piperita* + *O. basilicum* (1:1:1).

2. MATERIALS AND METHODS

2.1 Mass Culturing of *C. maculatus*

The test insects namely Pulse Beetle, and *C.maculatus* were cultured at the Department of Entomology and these beetles were reared on healthy and clean grains/flour in glass jars. The beetles were raised in glass jars on healthy, clean grains. The test insects were mass cultivated in a glass jar with a 1kg capacity, measuring 15 x 10 cm, and included 500 g of black grams as a nutritional source. The temperature was kept between 30 and 35ºC and the relative humidity was between 60 and 70%. Half of the fully-infested grains were replaced with an equal amount of uninfested materials after a two-generation gap [12]. For this investigation, the synthetic volatile chemicals propionic acid and benzaldehyde, as well as the essential oils *M. piperita* (peppermint) and *O.basilicum* (Ocimum), were chosen and acquired from Allins Exports Private Limited, Noida, and Uttar Pradesh.

2.2 Biology of *Callasobruchus maculatus*

A pair of 1-2day old *C. maculatus* was transferred from the rearing colony to a petri plate containing 10 undamaged black gram seeds maintained at room temperature of 22°C to 25°C and 50 to 65% RH. It was replicated three times and the seeds were changed daily. The removed seeds were kept in separate petri plate for the eggs to develop. After allowing the eggs to grow, the petri plates was inspected, and the freshly emerged adults were counted, mated, and removed as soon as the adult emergence started. For every adult, the number of days from the beginning of egg-laying to the adult's emergence was noted. The length of oviposition was calculated using the dates of egg-laying onset and termination as well as adult mortality [13] (Fig. 1).

2.3 Contact Toxicity against *C. maculatus*

The contact toxicity of selected synthetic volatile compounds and essential oils, and their combinations was determined against freshly emerged beetles using the dry film technique. The synthetic volatile compounds, essential oils, and their combinations of different concentrations were prepared by diluting with acetone. The different combinations were applied on the whole inner surface of the petri plates and dried for five minutes to evaporate the solvent. Twenty seeds were taken from each petri plate and ten adults were introduced into it and kept at room temperature. Two controls were set, one was a standard check (*i.e.*) treatment was made with acetone only and another one was an untreated check. The mortality of the test insects was recorded after 24 h of treatment [14].

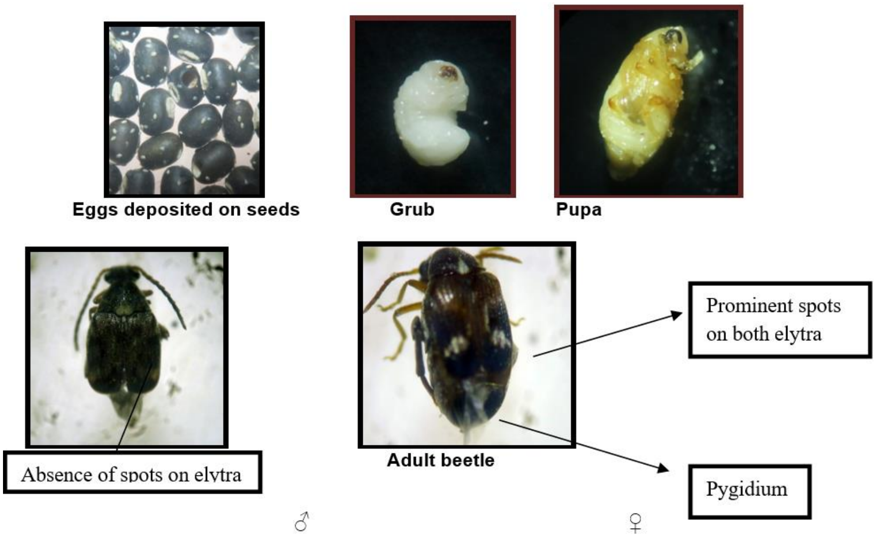

Fig. 1. Biology of pulse beetle, *Callosobruchus maculatus* on black gram

3. RESULTS AND DISCUSSION

In the laboratory conditions, *Callosobruchus maculatus* were found to mate between 50-60 minutes after their emergence. The duration of egg deposition continued for about five days with a peak of oviposition on the third day after the initial laying. Eggs were attached singly to the seed coat, often with several eggs. Individual eggs were oval or spindle-shaped, clear, shiny, and firmly glued to the seed surface. The daily egg laying was gradually decreased and stopped on the fifth day. The egg period was 5±0.07 days. The larva that hatches from the egg burrows the seed coat and enters into the endosperm without moving outside. Once the larva burrows into the seed, the remaining egg (shell) becomes opaque white or mottled as it fills with frass from the larva. The larva feeds on the content of grains molts four times inside the seed and pupates inside. The larva passed through at least four instars which were creamy coloured 'C' shaped and scrabeiform. Pupa was obtect and also cream coloured. The larval and pupal stages were completed inside the seed. The larval and pupal period together was 21.24±0.67. The total development period recorded from the day of oviposition to adult emergence was 35.03±0.35 days. Male and female beetles are easily distinguished from one another by general appearance. The elytra of females had strong markings, consisting of two large marginal dark patches while the males were less distinctly marked. The antennae of both sexes were slightly serrated. The most distinguishing characteristic was the coloration of the plate covering the end of the abdomen. In the female, the plate was enlarged and darkly coloured on both sides whereas in the male, the plate was smaller and lacked stripes. Females were larger than males. The longevity of adult males was 9.31±0.13 days and for females was 8.23±0.12 days (Table 1).

On black gram, [15] discovered that *C. maculatus* required 3.0 days to incubate. The grub and pupal duration was determined to be 21.24±0.67 days in the current study. Similar reports of the pulse beetle's 18.20-day larval and pupal periods were made [16]. The mature male and female had respective lifespans of 9.31±0.13 days and 8.13±0.12 days. However, [17] noted that the average lifespan for men and women was 7.83 to 11.75 days and 6.0 to 10.33 days, respectively. In the current study, *C. maculatus* has undergone a total of 35.03±0.35 days of

Table 1. Biology of pulse beetle, *Callosobruchus maculatus* in black gram

Developmental period of stages	Mean (days)
Egg stage	5±0.07
Grub and pupal stage	21±0.67
Adult	
a) longevity (Male)	9±0.13
b) longevity (Female)	8±0.12
Total development period	35.03±0.35

**Mean of three replications*
**Mean values followed by standard error*

Table 2. LC_{50} of selected synthetic volatiles compounds and essential oils and their combinations at 24 h exposure periods against adults of *Callosobruchus maculatus* (contact toxicity)

Treatments no.	Treatments	LC_{50}^{a} (µl/l air)	UCL[b]	LCL[b]
1.	Propionic acid (PA)	13.37	6.25	16.62
2.	Benzaldehyde (B)	11.31	5.89	15.28
3.	*Ocimum basilicum* (OC)	120.79	110.56	143.54
4.	*Mentha piperita* (PM)	102.92	90.32	120.10
5.	PA +B	8.73	4.76	12.92
6.	B + OC	14.48	7.45	18.70
7.	B+ PM	18.37	9.67	23.12
8.	B+PM+OC	16.57	10.67	20.82

[a] LC_{50} represents lethal concentrations that cause 50% mortality
[b] UCL and LCL represents upper and lower confidence levels

development. According to [18], *C. maculatus* typically takes 30±0.50 to 37.44±0.11 days to fully mature on black grams. Similar findings were reported by [15], who studied the biology of *C. maculatus* on black gram. They discovered that the species had a 3-day incubation period, a 96.50 egg hatching percentage, a 23-day total larval and pupal period, a 25-day total life span, a 63.5% adult emergence rate, and 108.5 eggs deposited.

The insecticidal activities of the synthetic volatile compounds and essential oils and their combinations against *C. maculatus* adults were examined by direct application method. The contact toxicity effect of synthetic volatile compounds and essential oils and their combinations at 24-h exposure periods revealed that the maximum toxicity was observed in Propionic acid + Benzaldehyde with 8.73 µl LC_{50}. It was followed by Benzaldehyde, Propionic acid, and Benzaldehyde + *O. basilicum* with LC_{50} values of 11.31 µl, 13.37 µl and 18.37 µl respectively. The last contact toxicity was evidenced in Benzaldehyde + *M. piperita* with LC_{50} of 18.37 µl followed by Benzaldehyde + *M. piperita* + *O. basilicum* (16.57 µl) against *C. maculatus* (Table 2) [19]. Found the insecticidal activities of pine, eucalyptus, and coriander oils against *S. oryzae*, *C. chinensis,* and *Corcyra cephalonica* adults by direct contact application method. Which, coriander oil showed greater efficacy compared with that eucalyptus and pine oil. [14] Also reported the contact toxicity of *Allium sativum* essential oils against the adults of *C. chinensis*.

4. CONCLUSION

Based on the current study's findings, it is clear that synthetic volatile compounds like benzaldehyde and propionic acid were effective against *C. maculatus*. This suggests that combining essential oils with synthetic volatiles can enhance their activity. To investigate the mechanism of action against target pests and the safety concerns with synthetic volatiles and essential oils against non-target organisms, more research is necessary. Additionally, a thorough understanding of the pesticidal activity and the creation of readily applied formulations against pests of stored products will be facilitated by the isolation and characterization of the chemical constituents of the essential oil.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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