



Fumigant toxicity of synthetic volatile compounds and essential oils against coleopteran pests of stored produce

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Abstract

The current study was carried out at the Department of Entomology, Annamalai University to find out the fumigant action of two synthetic volatile compounds, namely Propionic acid and Benzaldehyde and two essential oils namely, *Ocimum basilicum* and *Mentha piperita* and their different possible combinations against *Callosobruchus maculatus* and *Tribolium castaneum* using fumigant toxicity assay under laboratory conditions on adults and larval stages of target insects. Among the selected synthetic volatile compounds and essential oils and their combinations evaluated for fumigant toxicity against target pests, Propionic acid + Benzaldehyde was effective by causing a Lethal Concentration (LC₅₀) of 6.76, 25.67 and 70.83 µl/l air in 24-h period and found promising against adults of all the two test insects viz., *C. maculatus*, *T. castaneum* and larvae of *T. castaneum* respectively. Benzaldehyde and Propionic acid manifested their effectiveness against the target insects of coleopteran pest of stored produce namely, *C. maculatus* and *T. castaneum* and the activity of essential oils might be improved by mixing with synthetic volatiles.

Keywords: alkaloids, coleoptera, essential oils, formulations, fumigant, stored, product insects, volatiles

1. Introduction

India is the second biggest manufacturer of food grains after China and the total food grain production is 277.49 million tonnes in 2017-18. Among the total grains produced 65-70 per cent of the food grains are retained by the farmers and stored in the household storage structures for consumption, seed and feed purpose. The surplus grains are procured from farmers and stored by Food Corporation of India (FCI), a government-based grain storage agency. It stores majority of the procured grains on their own warehouses, but also utilize the facility and services of allied partners, Central Warehousing Corporation (CWC) and State Civil Supplies Corporation (SCSC) [1]. In India, annual loss of storage have been estimated as 14 million tonnes of food grain and worth of Rs.7,000 crore in that insects alone report to nearly Rs.1,300 crores. There are many reasons for the destruction of stored food grains, including insect attacks, leakages in stored godowns, procurement of poor-quality materials, exposure to rains, floods, and negligence on the part of the persons worried in taking precautionary measures. The loss of food grains in the farmer holdings in Tamil Nadu was estimated in paddy as 12.9 %, sorghum (16.0%), bajra (14 %) and maize (12.7%). Out of post-harvest losses, storage alone account to 6.58 %, in which insects cause 2.0 to 4.2 % followed by rodents 2.50 %, birds 0.85 % and moisture 0.68 %.

Callosobruchus maculatus (Fabricius) (pulse beetle), *Tribolium castaneum* (Herbst) (red flour beetle), *Rhyzopertha dominica* (Fabricius) (lesser grain borer), *Oryzaephilus surinamensis* (Linnaeus) (saw toothed grain beetle), *Sitophilus oryzae* (Linnaeus) (rice weevil), *Trogoderma granarium* (Everts) (khapra beetle) and *Plodia*

interpunctella (Hubner) (Indian meal moth) are considered as serious threats for long term storage of food grains. Among these storage pests, the pulse beetle is the notorious pests of common legumes and pulses grown in Asia, Africa, Central and South America. It lays eggs on the seed coat, the grub hollow out the grains and cause huge yield loss [2]. The red flour beetle *T. castaneum* is a problematic insect in milled products worldwide in particular tropical regions [3]. Several methods were reported to manage the stored grain insect pests in bulk storage including physical, behavioural and chemical control.

Since 1950s, inorganic chemical insecticides have been widely used in stored grain to control insect pests. The fumigants likes methyl bromide, phosphine, cyanogens, ethyl formate or sulfuryl fluoride which are rapidly used to kill all the stages of insect pests of stored products in storage. In Australia and India, resistance to phosphine is so high and causes failures in management. The nonstop and blanket use of these chemicals not only has led to develop resistant strains but also for the accumulation of toxic residues on food grains that became used for human consumption and which has led to many health problems. In sight of all these problems, numerous insecticides have either been excluded or limited in their use. This awareness has developed a interest in the development of alternate strategies, including the innovation of newer insecticides. Hence, there is in need of plant materials which may produce possible alternatives to the presently used chemical agents as they comprised rich source of bioactive compounds [4]. The plant extracts obtained from *A. vulgaris*, *E.globulus* and *M. piperita* were found as promising plant spices showed fumigant action to minimize the insect

population in the grains during storage. Exploiting these biofumigants may lead to no fear of poisoning, easy handling and safer to the environment [5].

Some volatile oils and volatile compounds have been performed better as attractants, repellents, insecticides, growth inhibitors and oviposition deterrents against many stored product insect pests including *T. castaneum* and are considered as a hopeful pest control agent [3]. Propionic acid found in the mix of volatile compounds which were released by barley grains [6]. On the other hand, it is also commonly used by the food industry as a preservative agent in several food products. Propionic acid acts as preventing the fungal growth as well as controlling the insects especially in storage of food grains in moist conditions. According to [7], the stored product pests might be controlled by using Benzaldehyde and its derivatives as alternate agents to the regularly used chemical fumigants. It is derived from many nuts, seeds and leaves. In the United States, Benzaldehyde and Propionic acid is generally reported as safe (GRAS) food additive and preservatives and is established as a flavoring agent in European Union. Hence, both the Benzaldehyde and Propionic acid was regarded as organic substance and easily biodegradable in nature.

Many plant essential oils showed as a broad range of activity against insect pests and fungal pathogens. These oils have been long traditionally used to protect the pest of stored produce. Keeping the above in view, an attempt was made to develop economically feasible and also effective alternate to chemical insecticides to manage the stored product insect pests, an attempt was made to find out the fumigant toxicity of selected synthetic volatile compounds and essential oils against *C. maculatus* and *T. castaneum*.

2. Materials and Methods

The test insects namely Pulse Beetle, *C. maculatus* and Red flour beetle *T. castaneum* were reared for the present study. The adult insects were obtained from the storage insect culture from Department of Entomology and these beetles were cultured on healthy and clean grains/flour in glass jars. The test insects were mass cultured in 1kg capacity glass jar of size 15x10 cm containing respective food materials such as black gram for *C. maculatus* and wheat flour for *T. castaneum* each 500 g as a nutritional source at 60-70 % relative humidity and temperature ranged from 30-35°C. Maximum of seven days were permitted for copulation and egg laying. Then the parent stocks were replaced and food materials with eggs were incubated in the temperature and humidity as mentioned above in darkness to obtain similar aged insects. With the interval of two generations, half of the completely infested grains/flour was replaced with the same quantity of uninfested materials [8]. The synthetic volatile compounds and essential oils used for this study were purchased from the Sisco Research Laboratories Private Limited, Mumbai and Allins Exports Private Limited, Noida, Uttar Pradesh respectively. The synthetic volatile compounds and essential oils were tested individually as well as in different combinations. Besides a set of control, 12 different treatments were used as follows: (i) Propionic acid (ii) Benzaldehyde (iii) *M. piperita* (iv) *O. basilicum* (v) Propionic acid + Benzaldehyde (1:1); (vi) *M. piperita* + *O. basilicum* (1:1); (vii) Propionic acid + *M. piperita* (1:1); (viii) Propionic acid + *O. basilicum* (1:1); (ix) Benzaldehyde + *M. piperita* (1:1); (x) Benzaldehyde + *O. basilicum* (1:1); (xi) Propionic acid + *M. piperita* + *O.*

basilicum (1:1:1) and (xii) Benzaldehyde + *M. piperita* + *O. basilicum* (1:1:1).

The toxicity of synthetic volatile compounds (Propionic acid and Benzaldehyde), essential oils (Peppermint and Ocimum) and their various combinations was tested against target insects by filter paper dip method. Whatman No. 1 filter paper discs (2 cm diameter) were immersed with various concentrations of synthetic volatile compounds and essential oils and their combinations prepared in acetone and were fixed to the inner portion of the screw cap of glass vials (10 cm long) separately as described by [9]. The treated filter paper strips was air dried for 15 minutes for evaporating the solvents and then ten grams of respective food materials were placed in each vial and 10 adults were released in each vials and the open end of the vials was closed by cap so that the treated filter paper left inside the vial. For each synthetic volatile compounds, essential oils and their combinations, five different concentrations and for each concentration three replicates were used. The vials were kept at 30±2°C, 75±5 % RH and a photoperiod of 10:14 (L:D) hours. Mortality of adults was observed after 24-h. Two controls were set one is standard check (*i.e.*) filter paper strip was treated only with acetone and another one was untreated check.

The LC₅₀ values for selected synthetic volatile compounds and essential oils and their combinations were calculated by using POLO programme analysis (Probit Analysis software).

3. Results and Discussion

The fumigant effect of synthetic volatile compounds and essential oils and their combinations at 24-h exposure period was tested against the adult beetles of *C. maculatus*, *T. castaneum* and larvae of *T. castaneum* and the Lethal Concentration (LC₅₀) along with Upper and Lower Confidence Limit (UCL and LCL) were obtained and the results are presented in Table 1. Among the treatments tested against *C. maculatus*, Propionic acid + Benzaldehyde performed better at 24-h of exposure period followed by Benzaldehyde and Propionic acid and least performance was noticed with peppermint oil, their LC₅₀ values were 6.76, 7.53, 9.86 and 17.02 µl/l air respectively. The LC₅₀ values of Benzaldehyde + Peppermint and Ocimum oil were found almost similar during the exposure period. The results revealed that the individual treatments of Benzaldehyde and Propionic acid were more effective than essential oils. However, Benzaldehyde + Ocimum oil combination showed the LC₅₀ value of 10.75 µl/l air compared to individual Ocimum oil treatment with a LC₅₀ value of 13.89 µl/l air. For *T. castaneum*, Propionic acid + Benzaldehyde excelled other treatments at 24-h of exposure period followed by Benzaldehyde and Propionic acid with the LC₅₀ values of 25.67, 26.89 and 27.79 µl/l air respectively. The least LC₅₀ value was noticed with the treatment peppermint oil registered 56.03 µl/l air. However, Benzaldehyde + ocimum oil combination was witnessed with a LC₅₀ value of 29.30 µl/l air compared to individual ocimum oil treatment which recorded with a LC₅₀ of 34.67 µl/l air. It was noticed that the mixture of Benzaldehyde and Propionic acid found to be effective than others.

These synthetic volatiles in combinations with essential oils had the capability to emit the strong pungent fumigant odour that acts against the target insects. Due to this insecticidal action, the insect became susceptible and showed promising

lethal effects. The pepper mint oil and ocimum oil obtained from plants was reported as a potential toxicant against many stored product insects. In the present studies, peppermint and ocimum oils showed fumigant toxicity against adult stages of *C. maculatus* and both larvae and adults of *T. castaneum*. But the effect of peppermint and ocimum oils was less than Benzaldehyde and Propionic acid or their combination treatments. The insecticidal activities of pepper mint and ocimum oil is due to the presence of chemical composition. The pepper mint oil comprises metabolic compositions such as menthone and menthol compositions and include toxic effects against stored product pests. The *O. basilicum* oil was found to contain 36 components with methyl eugenol (71.3 %), α -cubebene (6.4 %), linalool (4.1 %) and epi-amurolol (3.6 %) are the major volatiles [10]. Even the tablet formulation of plant materials found effective against stored product pests, the efficacy was not long lasting [11] as the fumes or smell was not released prominently as like the essential volatile or synthetic oils.

Among the synthetic volatile compounds, essential oils and their combinations, Propionic acid + Benzaldehyde was found to be better and caused a LC_{50} of 70.83 $\mu\text{l/l}$ air followed by Benzaldehyde (89.07 $\mu\text{l/l}$ air) and Propionic acid with 97.64 $\mu\text{l/l}$ air at 24-h of exposure period. The least LC_{50} values were noticed with the treatment peppermint oil registered 140.79 $\mu\text{l/l}$ air. However, Benzaldehyde + *O. basilicum* combination recorded as high LC_{50} of 103.78 $\mu\text{l/l}$ air compared to individual *O. basilicum* treatment which was found with a LC_{50} of 119.92 $\mu\text{l/l}$ air. The results indicated that the mixture of Benzaldehyde and Propionic acid were established to be more efficient than other treatments using fumigant toxicity assay in the laboratory.

It was found that the combination of the synthetic compounds, Benzaldehyde and Propionic acid was caused the maximum toxicity against the *C. maculatus* and *T. castaneum* than individual synthetic compounds. However, the statistical analysis noticeably showed that the effect of Benzaldehyde and Propionic acid treatment was slightly high when compared to their own individual treatment. The

results of the current study is supported by [12] who found that the propionic acid was more lethal to larvae and eggs of *Trogoderma variabile* Ballion and *Attagenus megatoma* (F) when added to insect's food at (2%) concentration. Propionic acid found in the mix of volatile compounds which were emitted by barley grains [6].

Further, the results are well supported by [13] who reported that synthetic volatile compounds (Benzaldehyde and Propionic acid) and volatile oils (camphor and eucalyptus) were observed individually and in combination against various life stages of *T. castaneum*. They reported that the Benzaldehyde - Propionic acid combination noted for the maximum larvicidal ($LC_{50} = 78.03 \mu\text{l/l}$) and adulticidal ($LC_{50} = 30.60 \mu\text{l/l}$) activities and this treatment was also efficient in controlling oviposition, egg hatchability and adult emergence of *T. castaneum*. The individual treatment of Benzaldehyde was established to be more toxic to adults ($LC_{50} = 34.07 \mu\text{l/l}$) and larvae (94.15 $\mu\text{l/l}$). The individual treatment of camphor and eucalyptus oil was less effective, but combination of Benzaldehyde - camphor oil was found to be effective. Benzaldehyde - Propionic acid combination reported 99.30 % adult mortality. Further, they recorded the high larvicidal effects in Benzaldehyde + Propionic acid combination and the LC_{50} value was 78.03 $\mu\text{l/l}$ after 24-h exposure against *T. castaneum*. Similar findings were made by [14] who reported that the Propionic acid (99.5 % purity) was added to samples of 150 g of wheat at the doses of 0.5, 0.7 and 1 % by weight and observed the absence of progeny in the treatment with 1 % by weight against *Sitophilus granarius*. According to [15] who reported that the fumigant toxicity of propionic acid against *Sitophilus granarius* and *S. oryzae* adults, Propionic acid was found to successful in killing adult weevils in fumigant assay. The LC_{50} of Propionic acid against *S. granarius* and *S. oryzae* varied between 5 and 10 mg/l air at 23 and 30°C respectively. In the current experiment, the LC_{50} values of Propionic acid and Benzaldehyde against the adults of *C. maculatus* were 9.86 and 7.53 $\mu\text{l/l}$ air and the adults of *T. castaneum* with 51.05 and 28.35 $\mu\text{l/l}$ air and larvae of *T. castaneum* with 89.07 $\mu\text{l/l}$ and 97.64 $\mu\text{l/l}$ air.

Table 1: LC₅₀ of selected synthetic volatile compounds and essential oils and their combinations at 24-h exposure period against target insects using fumigant toxicity assay

| Sl. No. | Treatments | Adults of <i>Callosobruchus maculatus</i> | | | Adults of <i>Tribolium castaneum</i> | | | Larvae of <i>Tribolium castaneum</i> | | |
|---------|-----------------------------------------------------------|-------------------------------------------|------------------|------------------|------------------------------------------|------------------|------------------|------------------------------------------|------------------|------------------|
| | | LC ₅₀ ^a (µl/l air) | UCL ^b | LCL ^b | LC ₅₀ ^a (µl/l air) | UCL ^b | LCL ^b | LC ₅₀ ^a (µl/l air) | UCL ^b | LCL ^b |
| 1. | Propionic acid | 9.86 | 6.48 | 14.80 | 27.79 | 24.20 | 36.07 | 97.64 | 83.23 | 103.99 |
| 2. | Benzaldehyde | 7.53 | 3.93 | 14.42 | 26.89 | 21.40 | 39.58 | 89.07 | 71.90 | 95.34 |
| 3. | <i>M. piperita</i> | 17.02 | 4.67 | 25.25 | 56.03 | 43.16 | 73.44 | 140.79 | 136.56 | 153.54 |
| 4. | <i>O. basilicum</i> | 13.89 | 3.90 | 20.85 | 34.67 | 21.09 | 48.29 | 119.92 | 111.32 | 131.10 |
| 5. | Propionic acid + Benzaldehyde | 6.76 | 5.30 | 8.61 | 25.67 | 21.20 | 31.07 | 70.83 | 60.16 | 91.43 |
| 6. | <i>M. piperita</i> + <i>O. basilicum</i> | 16.07 | 9.52 | 24.25 | 52.30 | 40.53 | 67.48 | 137.03 | 132.67 | 148.79 |
| 7. | Propionic acid + <i>M. piperita</i> | 14.14 | 4.12 | 19.05 | 46.49 | 39.64 | 54.52 | 130.48 | 123.23 | 141.96 |
| 8. | Propionic acid + <i>O. basilicum</i> | 14.08 | 5.05 | 25.25 | 38.92 | 31.55 | 48.02 | 121.23 | 119.54 | 135.02 |
| 9. | Benzaldehyde + <i>M. piperita</i> | 13.67 | 5.95 | 21.53 | 32.91 | 24.30 | 50.69 | 117.32 | 105.66 | 128.85 |
| 10. | Benzaldehyde + <i>O. basilicum</i> | 10.75 | 5.03 | 20.37 | 29.30 | 21.20 | 36.07 | 103.78 | 99.68 | 110.43 |
| 11. | Propionic acid + <i>M. piperita</i> + <i>O. basilicum</i> | 15.02 | 4.71 | 22.93 | 48.56 | 39.64 | 54.52 | 134.12 | 130.16 | 143.79 |
| 12. | Benzaldehyde + <i>M. piperita</i> + <i>O. basilicum</i> | 13.23 | 5.75 | 28.43 | 30.78 | 27.30 | 34.69 | 113.95 | 101.79 | 121.95 |

4. Conclusion

From the results of the present study, it is understandable that the synthetic volatile compounds, Benzaldehyde and Propionic acid manifested their effectiveness against the target insects of coleopteran pest of stored produce namely *C. maculatus* and *T. castaneum* and the activity of essential oils might be improved by mixing with synthetic volatiles. However, further studies are required on the safety issues of synthetic volatiles and essential oils against non-target organisms and to explore the mechanism of action against target pests. Furthermore, isolation and characterization of chemical compounds of the essential oil will provide complete insight into the pesticidal activity and will help in the preparation of easily usable formulations against stored produce pests. Hence, the use of naturally occurring synthetic volatile compounds and essential oils of plant species may also be incorporated in the storage pest management programme as an alternative so as to avoid the usage of harmful synthetic chemicals and can avoid resistant development.

5. Acknowledgements

The authors are thankful to the authorities of Annamalai University for their permission to carry out the research work.

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