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Fumigant toxicity of silica encapsulated essential oils against pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)

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ABSTRACT

India is the largest producer and consumer of pulses and shares 959.68 lakh ha area and 973.92 lakh tonnes of the global production. Among the insect pests infesting stored pulses, bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) is the most serious one and it attacks invariably all the pulses under storage conditions with mild infestation in the field as well. It is therefore, imperative to save the stored pulses from this serious pest. In many storage systems, fumigants are the most economical and convenient tool for managing stored grain insect pests. The increasing problem with today's fumigants makes it necessary for research to devise other control procedures and to identify new fumigants, which are eco-friendly and less expensive. Nano formulations of essential oils have been tested as alternatives to synthetic pesticides. Essential oil-based nano formulations have shown significant efficacy in the management of different storage pests over the past decade. The efficacy of silica encapsulated essential oils *viz.*, clove, citronella, cinnamon, sweet basil and geranium were investigated against pulse beetle *C. maculatus* through fumigant toxicity bioassay and LC_{50} values were 16.767, 13.483, 9.930, and 60.486 µg/ cm^3 respectively. The study showed that all the essential oils tested, except citronella, were toxic to *C. maculatus* adults, with varying degrees of toxicity. The LT_{50} values for silica encapsulated essential oils were as follows: sweet basil (80.479 hours), cinnamon (72.845 hours), clove (68.326 hours), and geranium (73.938 hours). In comparison, the LT_{50} values for the non encapsulated essential oils were: sweet basil (56.867 hours), cinnamon (61.263 hours), clove (44.424 hours), and geranium (59.808 hours). These results indicate that mesoporous silica nano particles (MSNs) encapsulated with essential oils are more effective than the non encapsulated essential oils.

Key Words: *C. maculatus, Essential oils, Mesoporous silica nanoparticles and Nano formulations.*

In India, Agricultural produce, mainly of cereals and legumes, is severely damaged by stored insect pests to the tune of 20 -22 per cent (Rajasekhar *et al.*, 2010), with an annual loss of 14 million tons, worth about seven thousand crores due to post harvest pest damage (https://igmri.dfpd.gov.in). Among them, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) is well known for its destructive effect on stored pulses (Singh and Jackai 1985). It is a significant, worldwide pest of peas, mung, cowpeas, and lentils. The pulses are very important source of vegetable protein for millions of people of tropical and subtropical regions. Many synthetic chemicals have been used to protect these grain products from pest attack while they are being stored. Currently, the most common method to prevent insect damage to grain that has been stored is to use synthetic insecticides. However, frequent or extensive usage of synthetic pesticides has led to major issues like direct toxicity to parasites, fish, man, pollinators, and predators. Additionally, it causes crop plants to become more susceptible to insect pests develop resistance to pesticides (Mahmud *et al.*, 2002) and have higher environmental and social costs. Therefore, environment needs some other alternatives of chemical pesticides. Nano formulations of essential oils have been tested as alternatives to synthetic pesticides. By using such formulations for pest control and treatment, it is possible to improve the solubility of active ingredients, bioavailability of agrochemicals, and stability and wettability properties during application in order to achieve better results (Martin - Belloso *et al.*, 2010). Therefore, these formulations are expected to be more productive than conventional botanicals and insecticides (Anjali *et al*., 2010, 2012). Essential oil-based nano formulations have shown significant efficacy in the management of different storage pests over the past decade (Pavoni *et al*., 2019). Different formulations of essential oils have been extensively used for pesticide delivery such as nano emulsions and nano capsules in recent years. Silica nanoparticles are also widely applied in other areas such as energy source, electronic, sensor, and catalysis purposes. In the present, investigation essential oils from cinnamon, citronella, clove, sweet basil, and geranium encapsulated with silica were studied for their fumigant effects on the *C. maculatus*.

MATERIAL AND METHODS

The test insects, Pulse beetle (*Callosobruchus maculatus)* culture was maintained in the Veterinary Laboratory of National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. They were mass cultured in plastic jars containing Black gram grains. In every generation, half of the completely infested grains were replaced with the same quantity of uninfected materials. Thus, a continuous culture was maintained throughout the study period and subsequent progenies of the insects were used for the experiments. The essential oils of sweet basil (*Ocimum basillicum),* clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), citronella (*Cymbopogon nardus*), geranium (*Pelargonium graveolens*), and mesoporous silica were procured from the same laboratory. Plastic containers (volume 138cm^3) were used testing the fumigant toxicity of test insects. The desired concentration of oils was treated on Whatman No. 1 filter paper (2x2 cm^2). The treated filter paper was then pasted on the underside of the screw cap and 10 adults were releasedin each container and the caps were tightly closed. Each treatment was replicated thrice. Mortality was determined after 24 hours from commencement of exposure.

Insects showing any movements were considered to be alive. The LC_{50} values were calculated by Probit analysis (Finney, 1971). To study the lethal time of each essential oil, it was mixed with dichloromethane (1:10) and 5 mg of mesoporous silica in a microcentrifuge tube.Then these tubes were kept in plastic containers and 10 adult insects were released in each container. Then that caps were sealed with parafilm and the observations were taken every 24 hours after the release.

RESULTS AND DISCUSSIONS

The results of toxic effect of different essential oils applied as fumigation method on pulse beetle are presented in Table 1. The essential oils of sweet basil, cinnamon, clove and geranium attained LC_{50} for *C. maculatus*, respectively at 16.767, 13.483, 9.930, and 60.486 µg/ cm^3. The fumigant toxicity assay indicated that all the essential oils tested were toxic to *C. maculatus* adults, with varying levels of toxicity except citronella.

The LT_{50} values of different concentrations of essential oils and mesoporous silica encapsulated with the essential was recorded against *C. maculatus* adults and presented in Table no.2. The highest LT_{50} values was observed in silica encapsulated with the different essential oils. The descending order of LT_{50} values (hours) of the silica encapsulated essential oils were sweet basil (80.479), Geranium (73.938),Cinnamon (72.845), Clove (68.326) and the LT_{50} values of the non -encapsulated essential oils followed the descending order as: Cinnamon (61.263), Geranium (59.808), sweet basil (56.867), , Clove (44.424) The result indicated that mesoporus silica nanoparticles (MSNs) were the most effective compared to non – encapsulated essential oils.

The efficacy of clove oil might be due to phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde. Thymol was superior to all the tested EOs and compounds with the lowest lethal dose. Oliveira *et al*. (2018) have reported thymol causing acute toxicity to adults from five populations of *Sitophilus oryzae* from different regions of Brazil by fumigant toxicity was evident in adult *T.castaneum* when exposed to thymol; the LC_{50} was 0.0012 mg/ cm3 (Kim *et al*., 2010). The most effective oil against *C. maculatus* eggs, larvae, and adults was cinnamon oil, according to Tarigan and Harshap (2016). Its LC_{50} values were 0.01%, 0.132%, and 0.186%, respectively. However, geranium oil has comparatively reduced toxicity, as seen by its higher LC_{50} values of over 30 ppm. Similarly, Sivakumar *et al*. (2010) reported that when tested against *C. maculatus*, geranium oil exhibited the lowest level of toxicity. Mesbah *et al*. (2017) reported a similar observation: silica nanoparticles were found to be significantly more biologically active than normal silica against the Chinese beetle *Callosobruchus chinensis* (Linnaeus). The LT_{50} values of the silica nanoparticles

Table 1. Fumigant toxicity of silica encapsulated essential oils against pulse beetle, *C.maculatus*

S.no	Essential oil	LC_{50} Values(µg/cm^3)	Fiducial limits		Chi-square(x^2)	Regression equation
			Lower	Upper		
1	Sweet basil	16.767	14.184	19.902	2.061	Y= 3.40 + 2.79
2	Cinnamon	13.483	11.198	15.87	5.281	Y= 3.27 + 2.95
3	Clove	9.93	7.844	11.839	3.536	Y= -3.00 + 3.04
4	Geranium	60.486	50.016	70.833	5.512	Y= 5.66 + 3.21

Table 2. Time mortality response of *C. maculatus* to different Silica encapsulated essential oils

S.no	Silica encapsulated with essential oils	LT_{50} Values(hours)	Fiducial limits		Chi-square(x^2)	Regression equation
			Lower	Upper		
1	Sweet basil	80.479	76.5	90.91	5.275	Y= - 3.89 + 7.34
2	Cinnamon	72.845	63.71	82.08	0.996	Y= - 4.14 + 7.68
3	Clove	68.326	57.8	78.93	7.427	Y= -3.19 + 5.78
4	Geranium	73.938	64.64	83.21	6.145	Y= -3.94 + 7.26

were analysed and found to be 67.52, 72.23, and 83.44 h, while the corresponding values for normal silica were 120.15, 131.44, and 140.00 h. Furthermore, Ebadollahi *et al*. (2022) found that the persistence of *Eucalyptus largiflorens* essential oil was extended to 19 and 17 days, respectively, by loading it in MCM-41 and Zeolite 3A. Encapsulating essential oils improved their fumigant toxicity against *C. maculatus,* even though pure and capsulated essential oil formulations caused up to 50% mortality after 5 days.

CONCLUSION

The present study indicated that among the five selected plant essential oils, clove oil was the most effective at the lower concentration. It also exhibited a significant toxic effect on *C. maculatus* under storage conditions of black gram seeds. Clove oil can be used as an alternative to synthetic insecticides for the management of *C. maculatus* in the storage. It demonstrates the potential of using plant essential oils and MSN-encapsulated essential oils as effective biopesticides against *C. maculatus*. The complete inhibition of fecundity on treated grains highlights their promise as alternatives to synthetic pesticides in protecting stored legumes. This information will be very useful for farmers in developing countries to control stored product pests, especially *C. maculatus*.

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