



Repellent effects of *Rosmarinus officinalis*, *ocimum tenuiflorum*, *Cinnamomum verum* essential oil against *Callosobruchus maculatus* in Black gram

V. Sushmetha, Muthuvel, Gowshik bala, Priyavashini
School of Agriculture,
Bharath institute of higher education and research

Abstract

The main aim of the presented study is to assess the potential repellent effect of selected essential oils (EOs) against *Callosobruchus maculatus*, which can cause economic losses in storage. Due to the development of pesticide resistance in *Callosobruchus maculatus* populations, as well as an attempt to limit extensive use of potentially harmful pesticides in food-related industries, there is a strong need for the development of alternative methods of dealing with *Callosobruchus maculatus* infestations. Because of their cost-effectiveness, availability, and low vertebrate toxicity, EOs are promising agents in pest management. In this test insect is reared under laboratory condition Grains were kept in jars at 28 ± 2 °C and $65 \pm 5\%$ Relative humidity (RH) and covered with muslin cloth to supply adequate humidity to the grains. To establish the stock culture of *C. maculatus* black gram was used as a host. Grains of black gram were kept in the jars and the jars were covered with muslin cloth and placed at 28.5 °C and 67.25 RH. Essential oil *Rosmarinus officinalis*, *ocimum tenuiflorum*, *Cinnamomum verum* were tested as potential repellents. Moreover, a preference assay, providing an extended analysis of the preference and the locomotor response, was used. The most effective EOs were: *ocimum tenuiflorum*, *Cinnamomum verum*. A few of the tested EOs caused significant alterations to the locomotor activity, although no direct relation was observed. In conclusion, EOs can be potentially used as repellent agents in *Callosobruchus maculatus* management. Thus, *Cinnamomum verum* oil can be used as an effective option of commercial Repellent for the storage of black gram seeds against *Callosobruchus maculatus*.

Introduction

Pulses are the ancient food crops with evidence of their cultivation for over 8000 years. They are biologically rich source of protein and essential minerals and complement well with cereal-based diet because of high amount of lysine. Indian Council of Medical Research has recommended an average daily consumption of 40 grams of pulses. *Callosobruchus maculatus*, is



a key pest that infests both pods in the field and seeds in storage (Stoll, 1988). According to Singh et al. (1978), 100% of pulses seeds are infested after 3 ± 5 months of storage. Tanzubil (1991) found that this insect can damage 100% of stored seeds causing weight losses of up to 60%. After six months of storage, losses in terms of perforated seeds can reach 90% (Seck et al., 1991). The pulse beetle *Callosobruchus maculatus* is reported to be the major pest infesting all types of pulses both in the field and in storage. The differential rate of damage infected by *C. maculatus* in different pulses was reported to be 68, 56, 49 and 52 per cent in cowpea, bengal gram, red gram and green gram respectively over a storage period of 6 months. The grain damage was as high as 69.93% under storage condition (Seck et al., 1991). This pest is a serious problem at small farmers' level, village traders and average households where storage conditions are poor and inadequate. To control the pulse beetle in storage, a number of synthetic organic insecticides such as malathion and dichlorvos have been recommended. The admixture synthetic insecticide with food grains has more recently been banned in many countries. There are also reports that the pulse beetle is developing resistance to malathion. In contrast, Essential oils used against pulse beetle appear to be quite safe and promising. Several authors have reported the insecticidal action and growth inhibiting effects of plant products on *C. maculatus*.

Essential oils can be used to keep the stored pulse free from pulse beetle attack. Stored products meet heavy loss in both quality and quantity due to pest attack. The Pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), a cosmopolitan pest of legumes, is a common throughout the tropics and subtropics of the world. Plants may provide potential alternative to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Aromatic plants are among the most efficient insecticides of botanical origin and essential oils often constitute the bioactive fraction of plant extracts. Plant essential oils have many useful applications beyond those in the fragrance and flavouring industries, among which are their uses as pesticides and insect repellents. An emerging body of scientific literature reports the efficacy of various essential oils for use against pests of public health, stored product pests, and agricultural pests (Isman 2000, 2004; Isman & Machial, 2006). Now a days essential oil is commercial developed Rosemary *Rosmarinus officinalis*, Clove *Syzygium aromaticum*, Thyme *Thymus wvulgaris*, Peppermint *Mentha piperita*, Holy basil (*ocimum tenuiflorum*) and Cinnamon *Cinnamomum verum*. In recent years research is increasing to use plant secondary metabolites, particularly essential oils, as natural pesticides for crop protection and storage, because of their



low toxicity to human beings and minimal environmental impact, in contrast to some synthetic pesticides. Some plants have received global attention and their secondary metabolites have been formulated as botanical pesticides in plant protection. The essential oil composition of *O. basilicum* varies depending on the environment and the chemotype. Essential oils of *Cinnamomum* plants are widely used as promising antibacterial agents and the essential oils of *C. camphora* and *C. cassia* reverse antibiotic resistance and biofilm formation. Essential oils are volatile secondary metabolites that plants produce for their own needs other than nutrition (i.e., protectant or attractant). In general, they are complex mixtures of organic compounds that give characteristic odour and flavour to the plants.

The evolutionary role of EOs in plants is mainly to be a protective agent against pests, including insects and fungi. *R. officinalis* EO is known to be an effective fumigant agent against various insect pests, such as confused flour beetle, *Tribolium confusum* (du Val.) (Coleoptera: Tenebrionidae) (Isikber, A.A et al., 2006), red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), almond moth, *Cadra cautella* (Walker) (Lepidoptera: Pyralidae) (Sim, M.J et al., 2009), and pulse beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Chrysomelidae). Thus, *R. officinalis* is a promising bioinsecticide agent; especially valuable when considering the widely spreading resistance to conventional insecticides (Athanassiou, C.G et al., 2018). Although there are relatively numerous studies on the insecticidal effectiveness of the EO against cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) most of them focus exclusively on mortality assessment omitting behavioral and biochemical parameters (Dayaram, L et al., 2016). The insecticidal effects of EOs are multimodal affecting a broad range of the physiological processes.

The potential uses of *O. basilicum*, *O. canum*, *O. gratissimum* and *O. sanctum* essential oils, particularly as antioxidant and antimicrobial agents have also been explored (AI Hussain et al., 2008) (B Bozin, N et al., 2006). Recently, Mondal et al. reviewed the antimicrobial, adaptogenic, antidiabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radioprotective, immunomodulatory, neuro-protective, cardio-protective and mosquito repellent properties of *O. sanctum* (S Mondal et al., 2009).

Among the essential oils that have been shown to adequately control insect pests, the oils extracted from cinnamon, *Cinnamomum zeylanicum* (L.), plants have drawn particular interest



because of their promising insecticidal activities against various pests of stored products such as the maize weevil *Sitophilus Zeamais* and the red flour beetle *Tribolium castaneum* (Regnault-Roger C. et al., 2012) (Oliveira NN. et al., 2017) In insects, these essential oils have neurotoxic action both as fumigants and or contact insecticides and their metabolites can act upon variety of molecular targets including inhibition of acetylcholinesterase or disturbing the functions of GABAergic and aminergic systems (Jankowska M. et al., 2017).

Hence, the present study was undertaken to investigate the repellent effect *Rosmarinus officinalis*, *ocimum tenuiflorum*, *Cinnamomum verum* of Essential oil against *C. maculatus* on black gram.

Materials and methods:

Insect Rearing:

Starting new cultures requires no more than containers to contain black gram and beetles. Virtually any closable containers will work successfully with lidded plastic Petri dishes, screen covered glass jars, snap lid vials, and cotton plugged shell vials are all suitable containers. We prefer to use disposable plastic containers in our teaching laboratories to minimize breakage and to keep cultures relatively small but replicated. Although beetles are the easiest of insects to successfully culture, sometimes a culture will fail if adults were very old when introduced to the new culture container or too few adults were introduced for adequate numbers of eggs to be laid. It is always a good practice to check a new culture a few days after it is started to see if numerous eggs have been laid. Plastic Petri dishes 150 x 25 mm are ideal containers can easily view cultures and remove selected adults. Although the lids fit loosely on the plates, Petri dishes will confine adults and permit adequate ventilation without any modification. Covering the bottom of a Petri dish with a single layer of black gram (approximately 50 ml volume) and introducing 10 adults males and 10 adult females is sufficient to 7 produce a dense culture. Cultures established in this manner will typically sustain two or three sequential generations without adding additional black gram and without inducing the production of dispersal morph adults. Cultures older than three generations on the same set of black grams should be discarded. We also have had good results using plastic snap-lid containers with pin-holes punched in the lid for ventilation. As with the Petri dishes, we use only 50 ml of black grams in each 300 ml snap-lid container. Raising beetles on black gram is best done in 150 mm petri dishes, which minimizes mould growth on the black gram. We prefer to use organically grown black gram to minimize pesticide problems in our cultures, but it is not essential for successful culturing of beetles. Black gram and adult beetles in a container



that keeps the beetles from escaping are all that you need. Keep the cultures at temperatures between 22° - 30°C (not in direct sunlight and away from radiators).

Essential Oils:

The essential oils used in the experiment were obtained from local vendors *Rosmarinus officinalis*, *ocimum tenuiflorum*, *Cinnamomum verum*. The oils were tested separately and as mixtures. The mixtures were formulated by mixing equal parts of the tested EOs. For each oil and mixture, a series of dilutions was prepared in ultrapure deionised water of the following concentrations: 8, 16, 24 and 32 µ. The obtained suspensions were stirred until emulsified and poured into the respective bubbler before phase separation occurred. The bubbler, controlling the airstream, was filled with an equal volume of ultrapure water.

Repellency bioassay with choice chamber method

Repellency of selected essential oil on test insect *C. maculatus* was tested by using area preference test described by Mc Donald et al. (1970). The entire tests of repellency were carried out in a 'choice chamber'. It consists of Whatman No.1 filter paper placed in the petri dish, cut in to 2 halves. Different test solutions of 8, 16, 24, and 32µl from 1% of essential oil preparation were applied on the half portion of filter paper disc as uniformly as possible with micropipette. Other half is treated with acetone as control. Each half is dried to evaporate the solvents and placed in the petri dish of experimental set up. Lower part of filter paper disc was pasted in the petri dish in order to prevent the escape of insects to underneath the filter paper. Then 10 adults of mixed sex of *C. maculatus* were released at the center of the filter paper disc and made a closed set up by using another petri dish, where the insects are allowed to move on to any direction of test half or control half. Then number of insects present in test half and control half were counted and recorded periodically after 1,2,4,8 and 16 hours to find out the percentage of repellency. Each dose of treatment was replicated for 4 times. The mean value of 4 replications of each dose at above-described intervals were taken and percentage of repellency (PR) was calculated using the following formula adopted by Obeng-Ofori (1995)

Percentage Repellency:

$$(PR) = [(NC - NT) / (NC+NT)] \times 100$$

(Where, NC is number of insects present in control half and NT is number of insects present in test half)



Repellency classes assigned according to scale described by Mc Govern et al. (1977),

Class I - range of % repellency 0.1-20

Class II - range of % repellency 20.1- 40

Class III = range of % repellency 40.1 - 60

Class IV - range of % repellency 60.1- 80

Class V- range of repellency 80.1-100

Result and Discussion:

Repellent effects of different doses 8, 16, 24 and 32 μ l of Rosemary, Holy basil and Cinnamon essential oil against *C. maculatus* in varying duration of exposure. Essential oils exhibiting insecticidal activities are extensively used as botanical pesticides as an alternative to chemical pesticides. As an attempt to assess the pesticidal properties of Rosemary, Holy basil and Cinnamon essential oils, present study tried to elucidate the repellent activity of on *Callosobruchus maculatus*. In repellency bioassay essential oil of Rosemary showed its potency the control of *C. maculatus* Essential oil imparts higher rate of repellency from 36% -86% with 8 - 32 μ l of concentration. The highest repellency rate of 86% was observed for the concentration of 32 μ l at 4 hours of duration. Essential oil of Holy basil showed it impart higher rate of repellency from 43%-98% with 8-32 μ l of concentration and highest repellency rate of cinnamon oil against *C. maculatus* is from 55% to 100% with same 8-32 μ l of concentration. The highest repellency rate 100% was observed for the concentration of 32 μ l.

In the Repellency bioassay, the higher rate of repellency was found to increase with an increase in concentration of essential oils indicating that the response was concentration dependent and the time of exposure of the insect. Compare with rosemary, Holy basil and cinnamon essential oil though higher concentration of each essential oil show's significant repellent effect the highest 100% repellency was observed in 32 μ l of cinnamon essential oil and 98% repellency observed in 32 μ l holy basil essential oil and comparative less in rosemary essential oil 86% was observed



Table: 1 Repellent activity of *Rosmarinus officinalis* Essential oil

Percentage Repellence of varying duration of exposure (hrs)/Dose	1	2	4	8	16	Overall average percentage Repellence	Repellence class
16 μ l	28 $\pm$ 6.12	33 $\pm$ 10.33	38 $\pm$ 10.22	55 $\pm$ 10.23	47 $\pm$ 6.33	40.2	Class III
24 μ l	44 $\pm$ 5.2	58 $\pm$ 6.41	62 $\pm$ 10.42	74 $\pm$ 6.32	59 $\pm$ 4.22	59.4	Class III
36 μ l	48 $\pm$ 10.3	60 $\pm$ 8.32	86 $\pm$ 10.33	76 $\pm$ 6.21	58 $\pm$ 8.33	65.6	Class IV

Table: 2 Repellent activity of *ocimum tenuiflorum* Essential oil

Percentage Repellence of varying duration of exposure (hrs)/Dose	1	2	4	8	16	Overall average percentage Repellence	Repellence class
16 μ l	52 $\pm$ 6.32	67 $\pm$ 9.66	75 $\pm$ 4.32	65 $\pm$ 10.32	46 $\pm$ 10.32	61	Class IV
24 μ l	74 $\pm$ 4.32	84 $\pm$ 9.66	89 $\pm$ 10.32	84 $\pm$ 6.32	65 $\pm$ 6.32	79.2	Class IV
36 μ l	84 $\pm$ 5.33	89 $\pm$ 9.32	98 $\pm$ 1.41	79 $\pm$ 9.66	72 $\pm$ 10.32	84.4	Class V

Table: 3 Repellent activity of *Cinnamomum verum* Essential oil

Percentage Repellence of varying duration of exposure (hrs)/Dose	1	2	4	8	16	Overall average percentage Repellence	Repellence class
16 μ l	61 $\pm$ 6.33	78 $\pm$ 10.32	75 $\pm$ 4.32	87 $\pm$ 6.45	55 $\pm$ 8.61	71.2	Class IV
24 μ l	80 $\pm$ 8.53	89 $\pm$ 6.36	62 $\pm$ 10.23	84 $\pm$ 6.32	100 $\pm$ 0.00	83	Class V
36 μ l	89 $\pm$ 10.11	95 $\pm$ 4.22	65 $\pm$ 10.81	79 $\pm$ 9.66	100 $\pm$ 0.00	85.6	Class V

The present findings were in accordance with the findings of N Subekti, et al., (2019) described that those essential oils produced a significant range of biological effect on *Tribolium castaneum* and *C. maculatus*. However, cinnamon oil was the most effective making it suitable botanical extract to develop repellency *Tribolium castaneum* and *C. maculatus* with less environmental hazards.



Sushmita, *et al.*, (2019) stated that volatile oils of *Cinnamomum zeylanicum*, *Pogostemon cablin* and *Zingiber officinale* caused fumigant and contact toxicity against bruchid adults. In the toxicity assay, the mortality rate was found to increase with an increase in concentration of plant volatile oils indicating that the response was concentration dependent.

Mohammad Mahmoudvand, *et al.*, (2011) explained that percentage of insect mortality was increased with the concentration and the time of exposure. In the other hand, toxicity depends on concentration, type of essential oil and exposure time. Dadang, *et al.*, (2016) described that those essential oils produced a significant range of biological effect on *Tribolium castaneum* and *C. maculatus*. However, cinnamon oil was the most effective making it suitable botanical extract to develop repellency *Tribolium castaneum* and *C. maculatus* with less environmental hazards. Mehrdad Ahmadi and Saeid Moharramipour, *et al.*, (2016) stated that there was a significant difference in the mortality of the adults at different levels of concentration of essential oils. Mortality was increased with the increasing concentration. The results indicated that the doses higher than 300 caused complete mortality (100%)

Conclusion:

The present experimental results suggested that *ocimum tenuiflorum*, *Cinnamomum verum* oil are effective in all the immature stages and have the efficiency to reduce the pulse beetle. It is therefore required to study about Cinnamon and Zinger oil as it reduce environmental problems, chemical pesticides and maintain a balance in agro ecosystem.

Reference:

- Athanassiou, C.G.; Arthur, F.H. *Recent Advances in Stored Product Protection*; Springer: Berlin/Heidelberg, Germany, 2018; ISBN 9783662561256.
- Dayaram, L.; Khan, A. Repellent, Fumigant and Contact Toxicity of *Salvia Officinalis*, *Rosmarinus Officinalis* and *Coriandrum Sativum* Against *Callosobruchus Maculatus* (Fab.) (Coleoptera: Bruchidae). *Int. J. Trop. Agric.* **2016**, *34*, 893–902.
- Isikber, A.A.; Alma, M.H.; Kanat, M.; Karci, A. Fumigant toxicity of essential oils from *Laurus nobilis* and *Rosmarinus officinalis* against all life stages of *Tribolium confusum*. *Phytoparasitica* **2006**, *34*, 167–177.
- Mehrdad Ahmadi¹, Saeid Moharramipour², 2016, *5* (4): 519-527; Interaction between essential oil of *Rosmarinus officinalis* and gamma radiation against *Callosobruchus maculatus*
- N Subekti, H Nur², A Fanidya¹, S Susanti¹, R Saputri¹, P Indrawati¹, 1402 (2019); Chlorpyrifos organophosphate and essential oils activities against *Callosobruchus maculatus* (F.) warehouse pests.
- Sim, M.-J.; Choi, D.-R.; Ahn, Y.-J. Vapor Phase Toxicity of Plant Essential Oils to *Cadra cautella* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **2009**, *99*, 593–598



- Singh, S.R., Luse, R.A., Leuschner, K., Nangju, D., 1978. Groundnut oil treatment for the control of *Callosobruchus maculatus* (F.) during cowpea storage. *Journal of Stored Products Research* 14, 77±80.
- Stoll, G. 1988. Protection naturelle des végétaux en zones tropicales. CTA; AGRECOL, 177 pp
- Sushmita TH, MK Gupta, KI Singh, Karthik S, 2019; 7(1): 597-599; Contact toxicity of some plant volatile oils against pulse beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae).