

## Insecticidal activity of the *Mentha arvensis* L. essential oil on stored grain pest *Callosobruchus maculatus* F.

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### ABSTRACT

Stored grain insect pests are becoming the foremost threat for reducing nutritional quality and quantity of grains in the storages worldwide. The pulse beetle, *Callosobruchus maculatus* F. is one the most serious post-harvest pest threat to stored legumes in tropical and subtropical regions. Today, for their control using eco-friendly and sustainable methods over the traditional chemical control are very crucial. In our study, an attempt was made to find the potency of the essential oil by two physical methods to control the beetle by using a common pulse grain – *Vigna radiata* L. (green gram). The repellency, fumigant and contact toxicity of the essential oil extracted from *Mentha arvensis* L. were checked by direct and indirect methods. Indirect method showed better results as the oil had prevented the pest from feeding on the grains. The *Mentha arvensis* essential oil was further screened for its chemical constituents by the technique of Gas Chromatography. The essential oil had high content of Menthol along with other constituents such as l-Menthone, Isomenthone, Menthol acetate, D-Limonene. Menthol which could all be responsible for the essential oil's significant insecticidal property against *Callosobruchus maculatus*.

**Keywords:** Stored grain, eco friendly, essential oil, menthol, insecticidal, toxicity

### INTRODUCTION

The economic loss and damage caused to the grains and food products by stored grain pests is a very serious issue. The damage caused by pests during grain harvest ranges from 10% to 40% (Lazzari and Lazzari, 2012). The infestation caused by pests affects the pace of seed germination, viability and also enhances the microbial infections (Lazzari and Lazzari, 2012; Johnson, 2013 and Yaseen *et al.*, 2019). Generally synthetic pesticides are used to control stored grain pests (Wijayarathne *et al.*, 2018 and Agrafioti *et al.*, 2019). Usage of synthetic pesticides causes range of undesirable effects leading towards adverse impact on the environment. In this regard, there is an urgent need to formulate eco-friendly bioinsecticides. In this scenario, there is an urge to evaluate the botanical extracts which could be developed into biopesticides to control the insect pests (Satti and Elamin, 2012; Rahim and Iqbal, 2019 and Mendoza Garcia *et al.*, 2019). Plants are the best sources to provide such ecofriendly insecticides as they are rich in bioactive molecules. Aromatic plants are most efficient sources of essential oils having insecticidal properties which also constitute the bioactive element of plant extracts (Khani and Rahdari,

2012). *Vigna radiata* stands at 7<sup>th</sup> place in production among pulses in the world. Under storage conditions, severe post – harvest losses are noted due to the pulse beetle, *Callosobruchus maculatus* attacks resulting in about 10% damage and rendering grains unfit for human consumption as it reduces the nutritional status of the grains. *Callosobruchus* species belonging to the family, Chrysomelidae – the leaf beetle family, and is considered to be the serious causes for the commercial loss for the stored legume grains (Shakil *et al.*, 2009). Predominantly 15 genera among the Leguminosae were affected by this insect including green gram, cow pea, bean, pea, soybean and many (Costa *et al.*, 2007). The major reason for the deterioration of the quality of legume pulses were because of infestation of insect population into the grains. Grain protectants and fumigants are being used since a long time for controlling stored grain pests but their injudicious and long –term use resulting in developing resistance to those pesticides. The grains stored for six months usually results in 70% of seed infestation with pests resulting in 30% weight loss and make the grain not suitable for consumption. The quantification of green gram losses through *Callosobruchus maculatus* is very desirable. The detrimental effect on the

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economy of the country is mainly due to the infestation by these insects. Not only on the economy, has it also caused a major danger to the food industry. From immemorable time, farmers depend on synthetic (chemical) fumigants, pesticides and insecticides, which results in causing damage / hazards to the environment and mankind. Methyl bromide is still used as synthetic fumigants which have been observed to have carcinogenic effects in the rats (Dansie *et al.*, 1984). These chemicals undoubtedly protect the grains but their excessive and inadvertent use created serious health hazards, environmental pollution, the ozone depletion and also resistant insects. These problems require the need for restriction of such chemicals to ensure that the food products should be free from pesticides. As a result of this there is environmentally safe and a convenient method such as the plant extracts, leaf, roots can be used to perform the same function. Insecticidal activities of the plant had been intensively investigated and observed that the pests attacking field crops and stored grains can be controlled better rather than spraying the chemical pesticides which have adverse effect on the environment. Plant derived chemicals are hazard free and it possess bitter substances which may show toxic, repellent, anti-feedant, growth and progeny inhibition activity against grain pests. Plant extracts and oils are a rich source of the bioactive chemicals, which reveal toxic effect and produce odours that repels adult beetles. *Mentha* species is an excellent store of medicine and possess toxic chemicals, but little investigations have been done on their use in pest management. The present study was designed to extract the essential oil from *Mentha arvensis* L. and its effect on *Vigna radiata* (green gram) to evaluate their effectiveness on mortality and adult emergence inhibition of *Callosobruchus maculatus* that is reared among the green gram. In addition, an assessment of the grain damage inhibition with the extract was also determined.

## MATERIALS AND METHODS

### Extraction of the essential oil

*Mentha arvensis* L. plant was collected from Lal Bagh Botanical Garden, Bengaluru, Karnataka, India. The plants were grown for one

month in Botanical Garden of Mount Carmel College, Autonomous, Bengaluru under normal conditions. After 45 days of growth, the leaves were collected and the fresh leaves were used for the oil extraction. The essential oil was extracted using Clevenger apparatus by hydro distillation method. Fresh leaves (200 g of each sample in 500 mL of distilled water) were taken in a Clevenger apparatus and was subjected to distillation for 3 hours. The oil thus obtained were dried over anhydrous sodium sulfate and stored in sealed glass vials in refrigerator. The yield of the oil was 5%. The different concentrations of the essential oil were prepared from the extracted oil like 100ppm, 150ppm and 200ppm using hexane as a solvent. Gas chromatography was carried out to find the composition of the *Mentha* essential oil. The GC instrument used for the analysis was Varian 450 GC. The column used was CP-SIL C18 with a dimension of 30m x 0.25mm x 0.25 µm. The detector used was Flame ionization detector. Nitrogen gas was used as the mobile phase. The flow rate was 1.0ml/minute. The injector temperature was 220°C and the detector temperature was 250°C. Oven temperature was 90°C for 3 minutes, then raised to 180°C at the rate of 6°C/min, held for 5 minutes. Components were identified according to databases and quantified by the comparison with the certified standards.

### Collection of the grains - *Vigna radiata* L.

Locally available green gram grains without any infestation were screened and selected to study insecticidal activity of essential oil on bruchids.

### Repellency Bioassay

Repellency assay was done according to the experimental method used by Jilani and Saxena (1990). Whatmann filter paper was cut into 5cm radius size and was divided into two equal halves and was demarcated with the help of adhesive tapes on either side of the paper in order to prevent the fusion of the two solvents. One side of the filter paper was applied with petroleum ether and the other half with the different concentration of essential oil, like 100ppm, 150ppm and 200ppm. 10 adult bruchids were released into the petri plates in the centre and it was sealed using a parafilm.

The movement of the number of bruchids was observed in equal interval of time of 30, 60 and 90 minutes. Percentage repellency was calculated by the formula (Nerio *et al.*, 2009).

$$PR = [(Nc - Nt/Nc + Nt)] \times 100$$

Where Nc = number of insects on the untreated area after exposure interval

Nt = number of insects on the treated area after exposure interval

### Contact toxicity

Whatmann filter was cut at 5cm radius size and the paper was wetted with the respective concentration. 10 adult bruchids were released in the centre of the plates and it was sealed using parafilm tapes. The mortality of the bruchids was observed in equal interval of time of 30 minutes. The rate of mortality was calculated using Abbott's formula (Abbott, 1925).

$$\text{Corrected Mortality} = \left(1 - \frac{n \text{ in } T \text{ after treatment}}{n \text{ in } Co \text{ after treatment}}\right) \times 100$$

Where, n = insect numbers

T = treated, Co = control

### Fumigant toxicity

*Mentha arvensis L.* oil was applied on the Whatmann filter paper discs. Treated filter paper were placed in the petri plates. 10 adult bruchids were placed in glass tubes, with open ends covered with cloth. The tubes were hung in the geometrical centre of the glass bell jar in such a way that they faced the petri plates containing the essential oil, it was then sealed with air tight lids. In the control groups filter paper was treated with the solvent petroleum ether. Mortality was observed after 30, 60 and 90 minutes.

**Insecticidal Activity:** Two different types of physical methods direct and indirect methods were followed to observe the insecticidal activity of the essential oil extracted from *Mentha arvensis L.*

### Direct Method:

100gms of the *Vigna radiata L.* was weighed and placed in four different grain storing jars. Then the respective concentrations of 100ppm, 150ppm and 200ppm of the essential oil were applied directly to the green gram and it was manually agitated for 2mins. The fourth jar

which was not treated with any essential oil, served as the control and the other jars were the treated jars. All the four jars were placed with other grains for 45 days and were stored as in the households.

### Indirect Method:

100gms of the *Vigna radiata L.* was weighed and was placed in four different grain storing jars. Then the respective concentrations of 100ppm, 150ppm and 200ppm of the essential oil was applied on to the strips of the Whatman filter paper No.1 of the size 5 cm x 1 cm and was allowed to dry for 10 secs, then these treated strips were placed in to the jars respectively and was manually agitated for 2 mins. The fourth jar, with a plain strip served as the control. All the four jars were placed with other grains for 45 days and were stored as in the households.

**Calculation of damage inhibition:** the damage inhibition caused by the pest to the grains were calculated by the formula

$$\% D = \frac{\text{No. of damaged grains in control} - \text{No. of damaged grains in treatment}}{\text{No. of damaged grains in control}} \times 100$$

The mortality percentage of insects was observed at LC<sub>50</sub>. Concentrations of oil were tested on bruchids. Data obtained were subjected to Probit analysis. LC<sub>50</sub> values were estimated by Probit analysis using MS excel 2019 version.

## RESULTS AND DISCUSSION

The chemical configuration of the extracted essential oil is depicted in the Table 1. From the GC analysis indicated that menthol was the predominant component in *Mentha* oil. The essential oil of *Mentha arvensis* was 0.5% (w/v). A total of 72 components were present in the essential oil. The chief compounds were menthol (66.73%), isomenthane (11.44%), l-menthone (6.66%) and D-limonene (4.61%) (Table 1).

Table 1: Chemical composition of oil

Compound name	Area (%)	Retention time (min)
Menthol	66.73%	16.84
Isomenthane	11.44%	16.06
l-menthone	6.66%	16.33
D-limonene	4.61%	12.1

The repellent effect of essential oils was observed in *Adhatoda vasica* against *S. oryzae* and *B. chinensis*. Similar repellent activities have been reported in *Ocimum suave* against *S. zeamais* and *Acorus calamus* repelled *T. castaneum* (Regnault – Roger, 1997).

Observations on the percentage of repellency by using the essential oil of *Mentha arvensis* L. against *Callosobruchus maculatus* F. (Bruchid) varied. The 100ppm concentration of the essential oil showed 63.33% of repellency while the highest concentration of 200ppm showed 100% repellency. This activity is majorly dependent on the exposure time of the essential oil to bruchids (Fig 1.). The activity of repellency was dependent upon the concentration and exposure time (Anju Viswan *et al.*, 2014). The knowledge on the understanding of physiological actions of EOs on insects is very little. The chemical compounds present in the oil could be responsible for insecticidal activity against the stored grain pests (Zhou *et al.*, 2012). But, when the insects were treated with the various constituents of the essential oil shown effect on the nervous system giving knowledge on the neurotoxic effect of the compound (Coats *et al.*, 1991 and Kostyukovsky *et al.*, 2002).

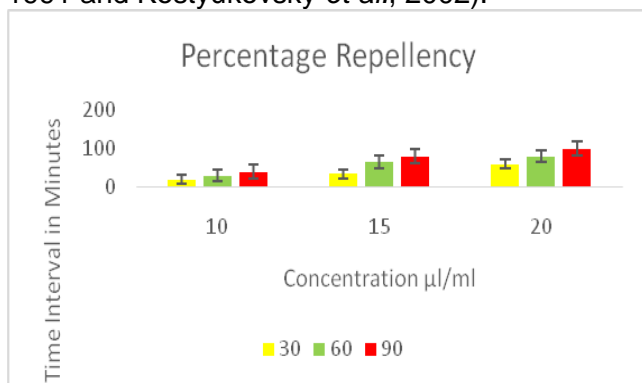


Fig. 1. Percentage repellency

The essential oil of *M. arvensis* showed a strong repellency towards the Bruchids. After 90 minutes of exposure, the PR of the EO reached 100% at the highest concentration 200ppm. As the concentrations of EO decreased, the PR values were also decreased. Similar kind of results were observed by Xiao-Meng *et al.*, (2018) in their study against two stored product insects using *Bupleurum bicaule* Helm. EO. The results of Yunho F Yang *et al.* (2020) are also in agreement with the current study where they studied 28 different essential oil insecticidal activity. The essential oils of *Mentha microphylla*

act as fumigant and in contact against the adults of *T. castaneum* and *Sitophilus oryzae* (Mokhtar *et al.*, 2020). The percentage of repellency was observed more as the concentration of the EO increases. The PR was 40%, 80% and 100% for the concentrations 100ppm, 150ppm and 200ppm respectively. Insecticidal effects of EO on adult Bruchids was studied in many plants. In this particular species studied for the first time and it was observed that the menthol present in this species has significant toxic effects with LC<sub>50</sub> value of 10.71 mg/cm<sup>2</sup> essential oil against *C. maculatus*. It was observed that 90% of the insects showed percent mortality when 25µl/l concentration on *E. kuehniella* essential oil applied (Ayyaz *et al.*, 2010). In the present study, the EO showed a good contact toxicity against Bruchids with a 50% mortality in 100ppm concentration and 100% mortality in 200ppm. This mortality may be due to the compounds present in EO. The insects when treated with essential oils, it shows the neurotoxic activity like seizures, tremors, hyperactivity followed by paralysis.

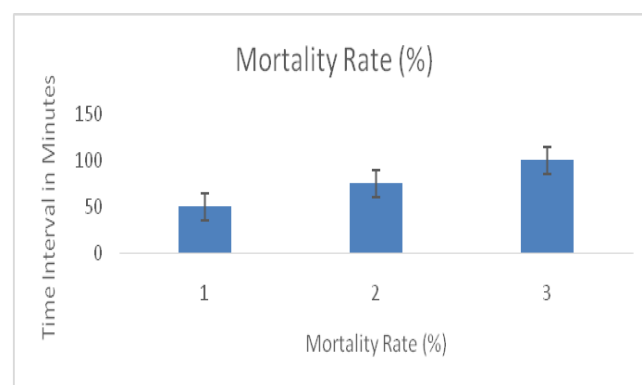


Fig. 2 Essential oil showing mortality rate

### Fumigant toxicity

The rate of mortality was observed between the time interval from 30 to 90 minutes. When the movements in the insect legs and antenna were not observed, then the insect was considered as dead.

*Callosobruchus maculatus* L. had been infested in the controlled jar and treated jars were uninfested by *Callosobruchus maculatus* L. Generally, adults of *Rhyzopertha dominica*, *Callosobruchus* sp. were more susceptible to essential oils or their components than those of other insect species (Tripathi *et al.*, 2003, Lee *et al.*, 2004 and Ahmed and Eapen, 1986).

Essential oil extracted from *Artemisia* species showed a high potency towards *Callosobruchus maculatus*, *Sitophilus oryzae* and also in *Tribolium castaneum* (Negahban *et al.*, 2006 and Ahmed and El-Salam. 2010). The toxicity of EOs on stored product insects can be described mainly depend on the chemical configuration of the essential oil which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and part of the plant used (Lee *et al.*, 2001). It was observed that 90% percent mortality at the concentration of 25µl/l showed significant increased insecticidal activity on myrtle of *E. kuehniella* essential oil (Ayaz *et al.*, 2010). The predominant chemical compounds of the essential oils are monoterpenes which are volatile in nature which helps in detecting the insects as a fumigant. These chemicals also act as signaling molecules behaving as pheromones in repelling the insects (Morgan and Wilson, 1999). The biological functions of the terpenoids are repellency, reduced palatability, growth and enzyme inhibition and also toxicity (Harborne, 1993). These essential oils which also are extracted from the plants can be used in

integrated pest management (Rozman *et al.*, 2007). In place of toxic insecticides, it will be beneficial to use the essential oil as insecticide. EOs are also used as an topical application which can be used to protect the environment from harmful insects (Oyedele *et al.*, 2000).

From the present study it was concluded that the essential oil extracted from *Mentha arvensis* showed a positive insecticidal activity towards the *Callosobruchus masculatus* which is a major pest which attack major stored products. The chemical constituents predominantly menthol could be the reason to control the insecticidal activity. From the scientific studies it can be concluded that instead of using chemical pesticides which causes damage to the environment the natural biopesticides can be used to control the pests.

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