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Phytotoxic Efficacy of Rosemary Oil Against Tribolium Castaneum and Callasobruchus Maculatus

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Abstract- The quantitative and qualitative food loses and feed commodities is mainly done by insect pests which are prolific in nature and causes development of hot spots as a result of metabolic heat by developing insect populations, thereby create favorable conditions to various pathogens. Thus they cause two way spoilage of food commodities resulting into economic loss as well as loss to public health. *Tribolium castaneum* and *Callasobruchus maculates* are important pests on household material like wheat flour and Indian chick pea respectively. The essential oil of Rosemary Rosemarinus officinalis L. (Lamiales: Lamiaceae) has been investigated on toxicity of these pests under controlled conditions. The major compounds of the oil were analyzed by GC were as 1, 8 Cineol (20.021%), Borneol (7.17%), Camphor (6.541%), Geraniol (6.281%), Camphene (5.623%), Linalool (4.993%) Alpha fenchyl acetate (4.222%) and Verbenone (4.147%). Efficiency of Rosemary Oil was evaluated by various toxicity assays like Fumigant, Repellency, Contact and Ovicidal on both the pests simultaneously. The oil marked different activity against both of them.

Keywords: essential oil, callasobruchus maculates, tribolium castaneum, GC, fumigant, repellency, contact and ovicidal.

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Strictly as per the compliance and regulations of :

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I. Introduction

Awareness of the environmental health hazards posed by synthetic pesticides, development of resistance to these chemicals leading to recurrent pest outbreaks, danger of misuse and presence of toxic residues in food, has led to a search for safe and environmentally-friendly alternatives (1);(2). Plants are a rich source of natural products. Many species synthesise their own chemicals in defence against attack by herbivores, pests and pathogens. Some are well-known for their insecticidal properties, e.g. Ocimum viridi, Piper mullesua., neem (Azadirachta indica) and tobacco (Nicotiana tabacum L.)(3); (4), (5); (6); (7); (8). Phytochemicals such as rotenone, nicotine and pyrethrum have been used as pesticides by man before the advent of synthetic insecticides (3);(4). Various members of the families Annonaceae, Asteraceae, Meliaceae, Myrtaceae and Piperaceae produce chemical compounds which act as antifeedants, repellents, biocides or growth inhibitors detrimental to many insect species (6).These insecticides of plant origin are commonly used in the form of aqueous/ solvent extracts, powders, slurries, volatiles and oils, or as shredded segments (9) ;(6);(10) . The protection of stored agricultural products using plant materials is an age-old practice in India.

Pest-repelling plants includes plants known for their ability to repel insects, nematodes, and other pests. The essential oils of many plants are also well known for their pest-repellent properties. Oils from families Lamiaceae (mints), Poaceae (true grasses), and Pinaceae (pines) are common insect repellents worldwide. Rosmarinus officinalis L. (Family Lamiaceae) popularly named rosemary, is a common household plant grown around the world. Natural products or eco-friendly pesticides are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment.

The currently used fumigants, phosphine and methyl bromide, are still the most effective means for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation. However, repeated use of those fumigants for decades has disrupted biological control by natural enemies and led to resurgence of stored-product insect pests, sometimes resulted in the development of resistance, and had undesirable effects on non-target organisms (11). Plant essential oils and their components have been shown to possess potential to be developed as new fumigants and they may have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (12, 13). The move toward green chemistry processes and the continuing need for developing new crop protection tools with novel modes of action makes discovery and commercialization of natural products as green pesticides, an attractive and profitable pursuit that is commanding attention. The concept of "Green Pesticides" refers to all types of nature-oriented and beneficial pest control materials that can contribute to reduce the pest population and increase food production. Green pesticides are safe, eco-friendly and are more compatible with the environmental components than synthetic pesticide. The advantages of using pesticide oil-in-water micro emulsions for improving the biological efficacy and reducing the

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dosage of pesticides would be a useful strategy in green pesticide technology. Rosemary aerial parts are used as flavoring agent in foods, beverages, and cosmetic preparations and have various traditional uses in ethnomedicine.

II. Materials and Methods

a) Plant material and essential oil extraction

Rosemary Oil was obtained from farm of CIMAP. After weighing 200 gms of raw material the essential oil was obtained by steam distillation. The oil was fractionated in a Clevenger apparatus at 100̊ C for 3 hrs. Water was kept on through the entire time period to emphasize effective cooling and condensation of the oils. All the oils were analyzed by Gas chromatograph for detection of constituents.

b) Analysis of essential oil

i. Characterization

One micro liter of the essential oil was injected to GC-Ms with Varian CP3800 equipped with selective detector (Varian, Mexico) and a DB-5 capillary column (30mx0.25mm,thickness 0.25μm). Column temperature rose from 55 to 65° C at a rate of 1°C/min and held 3 min, then the temperature rose from 60 to 240° C at a rate of 31°C/min and was held at this final temperature for 10 min . Hydrogen was the carrier gas , at a flow at a flow rate of 2 ml/min. Identification of the compounds was based on the comparison of their mass spectra and retention time with the standard spectra data of the GC-MS system. Composition: The chemical composition of Rosemary oil was studied by gas chromatography mass spectrometry (GCMS).About 40 compounds were identified out of which the major compounds were Camphor (29.3%), 1-8 cineol (21.15%), α-Pinene (10.90%), camphene (5.85%), pcymene (4.874%), Berbenone (4.00%), Limonene(3.94%), Caryophyllene Oxide (1.33%), α-Thujene (0.173%).

Table 1 : Chemical constituents of the essential oil of R. officinalis

Compound	Retention Time (Min)	Area/Composition(%)
α-Thujene	5.48	0.173
α-Pinene	5.76	10.904
Camphene	6.13	5.854
p-cymene	8.27	4.874
Limonene	8.43	3.949
1,8 cineol	8.54	21.157
Camphor	12.59	29.339
Berbenone	15.14	4.005
Caryophyllene Oxide	29.80	1.331
Humulus Oxide.	30.76	1.023

c) Insect rearing and evaluation of insecticidal efficiency of essential oil

Parent adults of Chickpea beetle (Callasobruchus *maculates*) and Tribolium *castaneum*, were obtained from laboratory stock cultures. They were reared in climatic chamber at 25 ± 2 °C, 70 ± 10% relative humidity (RH) and a photoperiod of 12: 12 L/D hrs . The food used was whole Chick pea grains and wheat flour respectively. Adults of both Callasobruchus maculates and Tribolium castaneum were placed in separate jars with adequate raw material for culturing.

i. Fumigant Toxicity Assay

Exposure time in all the tests was kept the same that is 24 hrs. In order to test the toxicity of essential Oil vapors to the adults of T. castaneum and C. maculatus, gas tight glass bottles of 300ml volume with plastic screw caps were used as exposure chambers(Fig 1). A small piece (6x8) cms filter paper strips were kept inside the glass bottle to serve as an oil diffuser after the appropriate amount of pure essential oil has been applied on it. Doses were calculated based on nominal concentrations and assumed 100% volatilization of the oils in the exposure vessels / glass bottles. In each bottle 5 insects/replication were used and kept inside plastic vials fitted with 40 copper wire net on both the ends. This arrangement with the insects was suspended into the 300 ml glass bottle and then sealed with its cap. This whole set was considered as one replication. 3 such Replns for each concentration of oil was taken. After 24 hrs of exposure to essential oil vapors the dead insects were counted.

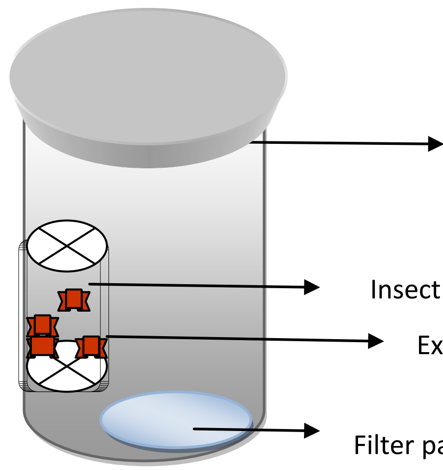


Fig 1

ii. *Repellency Bioassay*

Repellency was arranged in 9 cm test arenas. Whatman filter paper No.1 was cut into half. Test solutions were prepared by dissolving 0.5, 1.6, 2.4, 3.2 and 4 μl (.05,0 .16,0.24,0.32,0.40 % respectively) in 1 ml acetone. The paper disc was cut into 2 equal halves and then joined to a full disc with a sticking tape. Each prepared conc was applied to one half of a filter of the filter paper disc as uniformly as possible with a micropippete. The other half of the filter paper disc was treated with acetone alone and termed as untreated. This dried disc was kept inside the petridishes. Ten adults of mixed sexes of each beetle species were released separately at the centre of the filter disc and the petridish was covered. 10 replicates/conc was prepared. Observation on the no. of insects on the treated and untreated halves was recorded after 3hrs. % repellency was computed using the formula-

$$PR= \{ Nc\text{-}Nt\}/ \{Nc+Nt\}X100$$

Where Nc – No. of insects on the control half

Nt – No. of insects on the treated half

iii. *Contact Toxicity assay*

The insecticidal activity of various essential oils against the adults of T.*castaneum* was evaluated by direct contact application assay. (Qui and Burkholder 1981, Broussali's et al 1999). 20 ,40, 60 80 and 100 μl /ml (2,4,6 and 8% solutions) in acetone were prepared). Males and females of T. castaneum were transferred into petridishes and chilled for 2-5 min to reduce their mobility. One μl of the test solution was applied to the dorsal surface of the insect insects with the micropippete. Ten insects were treated /conc of the test solution and this was termed as one replication. Ten such replications for each dose were done. After treatment, insects were transferred into empty 12 cms diameter glass petridishes. Insects were examined after 24hrs of treatment.

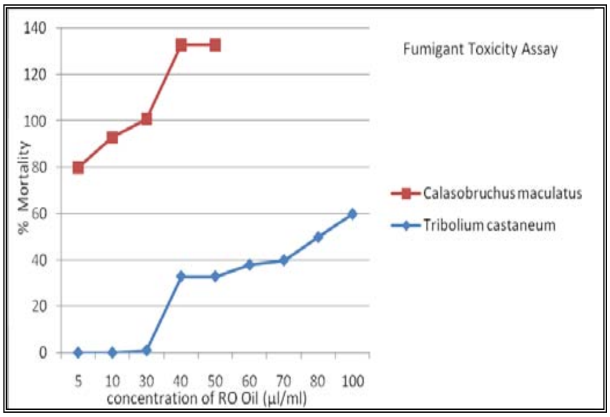
iv. *Ovicidal activity*

Fresh, intact chickpea seeds were palced in plastic jars into which 20 pairs (10Mand 10F) of pulse beetle /CM were released for egg laying. After 7 days the chick pea seeds containing the eggs were sorted. 3,6,9,12 μl essential oil of rosemary officinalis was dissolved in 1ml acetone to make (0.3%, 0.6, 0.9, 1.2%) solutions. Total 50 viable eggs /Repln were mixed thoroughly with the test solution and air dried and considered as one replicate. 5 replicates for each concentration were used . Treated chick pea seeds were placed in 300 ml glass bottles and their mouth covered with muslin cloth and left as it is for 1 month for egg hatching and adult emergence. Data on egg hatching was recorded. Ovicidal assay was not possible to be performed for Tribolium castaneum.

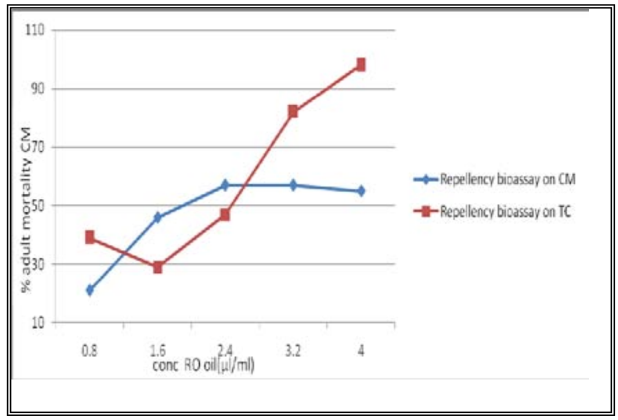
III. Results

In fumigant toxicity assays dose of 10μl/ml caused 100% mortality of *Callasobruchus maculates and no* mortality to Tribolium *castaneum*. LC50 value for *Callasobruchus maculates* was 5μl/ml for fumigant toxicity assays (Fig 2). On the contrary, the oil proved to be very promising for *Tribolium castaneum* in repellency assay with 95% mortality at 4μl/ml (LC50-2.40 μl/ml) and 58% mortality of *Callasobruchus maculates* at the same conc. (Fig 3). In Contact Toxicity assay, dose of 60 μl/ml caused 100% mortality of *Callasobruchus maculates.* LC50 value was 35μl/ml whereas only 50% mortality was achieved with the highest concentration to Tribolium *castaneum* giving 22% mortality to Tribolium castaneum at the LC50 Conc. (Fig 4). In Ovicidal assay for *Callasobruchus maculates,* LC50 value was 6 μl/ml (Fig 5). Rosemary oil was found to be more toxic towards *Callasobruchus maculates* as compared to *Tribolium castaneum.*

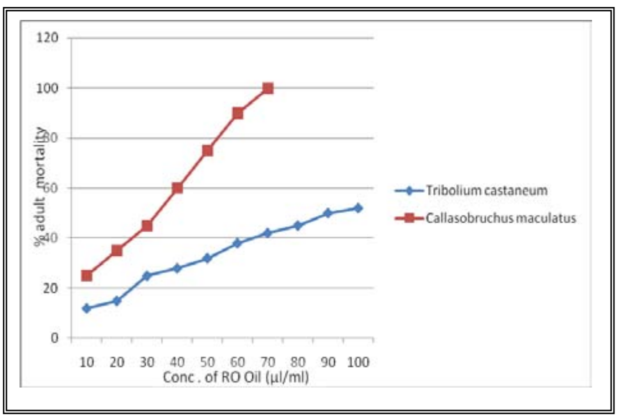
Fumigant Toxicity (Fig2)



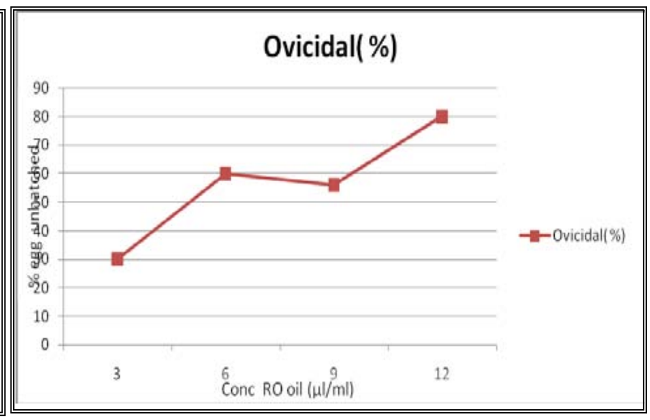
Repellency Bioassay Fig(3)



Contact toxicity Fig(4)



Ovicidal Bioassay Fig(5)



IV. Discussion

This study showed that the essential oil has significant and good toxicity against *Callasobruchus maculatus* as compared to *Tribolium castaneum.* Varying activity of different pests to essential oil of *Rosemary officinalis* indicated that the pest controlling and repelling factors were not uniformly present in this aromatic plant. Essential oil of rosemary was also found to be an effective insecticide against larvae and adults of *sycamore lace bug*. (Helena rojht *et al*, 2009) Therefore, this essential oil may be recommended at farmer level, as it is eco-friendly with low mammalian toxicity and works as a good alternative to synthetic insecticide. It could further reduce the use of synthetic insecticide. According to (Odeyemi et al 2009), Specific protocols could be developed for certain insect groups, which may be modified when required to avoid alteration of results with the same insect species due to variations at morphological and chronological level. Presence of high level of 1,8 cineole may be one of the reasons for differing activity of the oil with different pests.

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