Effect of *Citrus sinensis* Oil Volatiles on Egg Hatchability of Rice Moth (*Corcyra cephalonica*) and its (GC-MS) Analysis

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Abstract: The present study was undertaken in a view to explore the possibilities of using volatiles, emanating from oils extracted from the epicarp of *Citrus sinensis* by hydro-distillation through Clevenger's apparatus in the laboratory, on egg hatchability and reproductive potential of rice-moth, *Corcyra cephalonica*, is important pest of stored commodities. A sharp reduction by the action of *Citrus Sinensis* (*Rutaceae*) oil volatiles effect when freshly laid eggs were exposed to these volatiles for time duration (12hrs, 24hrs, 48hrs, 72hrs) with different oils concentrations (control, 20µL, 40µL, 80µL, 160µL) a marked decline in egg hatchability is seen after exposure the *Citrus Sinensis* oils. With the help of Gas chromatography-Mass spectrometry (GC-MS) test *Citrus species* volatile oil contained maximum number of Limonene 67.47% than Carvone 4.80%. The findings are helpful in the management of population of *Corcyra cephalonica* in store houses holds, godowns and warehouses.

Key words: Hydro-Distillation · Oil volatiles · Clevenger’s Apparatus

INTRODUCTION

The genus *Citrus*, belonging to the Rutaceae or Rue family, comprises of about 140 genera and 1,300 species. *Citrus sinensis* (Orange), *Citrus paradise* (Grapefruit), *Citrus limon* (Lemon), *Citrus reticulate* (tangerine), *Citrus grandis* (shaddock), *Citrus aurantium* (shaddock) *Citrus aurantifolia* (lime) are some important fruits of genus *Citrus* [1, 2]. The Rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) is a major pest of stored grain commodities in the tropics [3, 4]. The larvae of *C. cephalonica* cause considerable damage to stored food commodities while feeding, leaving silken threads wherever they move. The webbing formed is noticeably dense and tough adding to the damage caused [5-8]. Some aspects of the Biology and control using botanicals of the rice moth, *C. cephalonica* (Stainton), on some pulses [9] *C. sinensis* fruits are mainly used by juice processing industries, while the peels are generally wasted. The peel of *C. sinensis* fruit is a rich source of commercial application [19-24]. Plant product as a fumigant for the management of stored-product pests [25]. The most attractive feature of using essential oils or their potential and egg hatchability. However, nothing is known about the changes that are likely to occur in the reproductive biology of this insect, by the action of *C. sinensis* oil volatiles during rearing or breeding. Therefore, it was thought desirable to ascertain the impact of oil volatiles from the peels of *C. sinensis* on embryonic development, egg hatchability and reproductive potential of this pest. Effective pest control is no longer a matter of heavy application of pesticides, partly because of the rising cost of petroleum derived products but largely because excessive pesticide use promotes speedier evolution of resistance in insect pests, destroys natural enemies, turns formerly innocuous species into pests, harms other non-target species and contaminates food [18]. Concern about the widespread use of broad-spectrum pesticides has led to a surge of research into alternative pest control technologies. The pesticide formulations based on herbal products have attracted particular attention because of their specificity to insect pests, their biodegradable nature and their potential for commercial application [19-24]. Plant product as a fumigant for the management of stored-product pests [25]. The most attractive feature of using essential oils or their constituents as crop protectants is due to their low

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mammalian toxicity. Certain plant essential oils and/or their constituents have a broad spectrum of activity against insect and mite pests, plant pathogenic and other fungi and nematodes. Recent information indicates that they are safe to the user and the environment [26].

Insecticidal and Growth Inhibition Activities of Citrus paradisi and Citrus reticulata essential oils against lesser grain borer [27].

MATERIALS AND METHODS

A rich standard culture of C. cephalonica was maintained in the laboratory, on coarsely ground Jowar (Sorghum vulgär (L.) Moench) containing 5% powdered yeast as per methodology of Saqi Kosar Abbas et al. [28]. The general layout of the experiments, the methodology adopted to treat the eggs with vapour action of the selected oils of C. sinensis and the parameters chosen to assess their impact on reproductive potential of the pest was similar as are outlined Mishra, S. N. and S. S. Krishna and Pathak P.H., G. Gurushubramanian, SS. Krishna, by [29] and [16].

Plant material collection and isolation of the essential oil by Clevenger’s Apparatus: The peels of C. sinensis were collected from the fruit juice shops near the campus of D.D.U. Gorakhpur University Gorakhpur, Uttar Pradesh. The volatiles oil was extracted from peel of C. sinensis fruits. After cleaning with water peels were subjected to oils extraction, obtained at 0.001% yield by Hydro-distillation for 3-12 h using a Clevenger apparatus in laboratory Fig. 3. The samples of fresh C. sinensis peels were extracted to obtain oil [30]. A clear light yellow colour oily layer was obtained on the top of the aqueous distillate, separated and collected in collecting tube.

Eggs exposure to the oils: In this experiment freshly laid eggs (<24 hours) were taken. To estimate percent hatchability 100 eggs were arranged singly in a linear fashion on the floor of a glass petridish (10 cm diameter). One filter paper discs of 3.5 cm. diameter were kept in another petridish of same diameter, impregnated with 20, 40, 80 or 160 µl of Citrus oil. This experimental setup was kept in a glass chamber having 30 cm. diameter and 13 cm. height from inside. In first experiment after 12 hours, in second experiment after 24 hours, in third experiment after 48 hours, in fourth experiment after 72 hours, the impregnated paper discs were removed and eggs were shifted from odorous to normal environment, where in their hatchability was monitored daily till 7 days [31] and [32]. All tests, performed at 27°C ± 2°C and 85±5% RH, were accompanied by appropriately designed controls, wherein the insects were not exposed to the oil volatiles. The data procured from adequately replicated experiments, were then subjected to Student’s t-test used in statistical analysis [33].

Non-host plant volatile oils of C. sinensis (Rutaceae) oil was tested in N.B.R.I. Lucknow, Uttar Pradesh, India; by Gas Chromatography and Mass Spectrometry (GC-MS) (Analytical conditions Instrument: GCMS-DSQ-II (Thermo Scientific); Column:TR-50 MS, 30m x 0.25mm ID, 0.25µm film; Oven: 50°C (5 min) to 250°C at 4°C/min, 250°C (5min); Inlet:0.5 µl, 250°C, split 50:1;Carrier:Helium).

RESULTS AND DISCUSSION

When freshly laid eggs of C. cephalonica were exposed to the action of C. sinensis oil volatiles, for 12 hour duration, a significant reduction in percent egg hatchability was observed at 40, 80, 160µl volume only (P<0.05) out of 20, 40, 80 or 160 µl volume of oil. However, if the exposure period was increased for 24 hours duration a significant reduction in percent egg hatchability was observed at 40, 80 and 160µl volume (P<0.05 or <0.01). When exposure period was increased 48 hours a significant decline were noticed at 20, 80 and 160 µl volume (P<0.05 or < 0.01) while, severe reduction in percent hatchability was noticed at 40, 80 and 160 µl volume, exposure period was increased for 72 hours exposure (P<0.05 or < 0.01) Table no. 2. Reduction in hatchability was observed [34], when eggs of C. cephalonica was exposed to the combined action of Garlic extracts and Mint or Neem and Eucalyptus oil volatiles for 3, 6, 12 or 24 hours exposure at 40, 80 or 160 µl volume of oils. Presumably, the volatiles liberated from these oils diffused into the eggs, like air [35], through the shell or they entered into them via aeropyles-tiny holes in the chorion connected with respiration of embryos [36]. Knowledge emphasizing the significance of odours from plant products, in regulating ovipositional behavior of lepidopterans is still limited [37, 38]. The modus operandi of such control linked with olfaction, needs deeper understanding according to Feeny et al [39]. However, involvement of receptors of a labial-pit organ associated with an “accessory” olfactory pathway and responding to volatiles such as odours have been reported by Harrow et al [40], on the basis of their preliminary physiological recording experiments, in Manduca sexta (tobacco hornworm)-a plant feeding lepidopteran.
Gas Chromatography and Mass Spectrometry (GC-MS): The retention time and chemical composition of essential oils of *C. sinensis* (*Rutaceae*) are presented. Seventeen volatile constituents, representing the total composition were identified in the *C. sinensis* oils Table 1 and Fig.1, 2. The most abundant components found in *C. sinensis* oil were limonene 67.47%, followed by Carvone 4.80%. Several chromatographic techniques are being applied to describe for the determination of active ingredients in various plants extract, like- beta-pinene, limonene, cineole, linalool oxide, beta-terpineol, limonene oxide, p-mentha, carveol, carvone, durohydroquinone, caryophyllene oxide, limonene 6-ol etc. [41-52].

**Identification of Citrus Sinensis (Rutaceae) Active Ingredients:** *C. sinensis* mainly consisted of alpha-pinene-0.63; sabinene-0.27; beta-pinene-2.16; 3-carene-0.61; limonene- 67.47; 1,8 cineole-1.00; linalool oxide-0.76;...
beta-terpineol-1.84; limonene oxide-0.52; trans-p-mentha 2,8 dienol-1.04; p-mentha 1-en 8-ol-3.37; carveol-3.24; carvone-4.80; durohydroquinone-0.43; caryophyllene oxide-0.43; dodecanoic acid-0.05; limonene 6-ol pivalate-0.12. The oil also contained a series of compounds. This can be seen in Fig. 1, 2 and Table 1.

**CONCLUSION**

This study shows that the *C. sinensis* volatile oils effect egg hatchability of *C. cephalonica*. The applied significance of these findings lies in the formulation of appropriate technology from which quantity of these volatiles can be maintained in population areas, particularly in house-holds. Volatile oil of the *C. sinensis* species contained maximum number of Limonene 67.47% than Carvone 4.80%. When *Coreyra cephalonica* freshly lay eggs were exposed to the volatiles for time duration
(12, 24, 48 and 72 h) and on different oils concentrations (control, 20µL, 40µL, 80µL, 160µL) a marked decline in egg hatchability is seen after exposure of *C. sinensis* oils. Highly selective and sensitive GC-MS systems permit a dilution approach to be taken when analyzing *C. sinensis* oils which brings synergetic and fast result to the laboratory.

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