

Effect of oil volatiles in reproductive potential of adult stage in *Corcyra cephalonica* by the action of *Citrus sinensis* oil and its (GC-MS) analysis

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Abstract- The present study was under taken 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 reproductive potential of rice- moth (*Corcyra cephalonica*) which is a serious pest of stored commodities. A sharp reduction by the action of *Citrus Sinensis* (*Rutaceae*) oil volatiles effect when the adult stage mean number of eggs laid / hatchability in *C. cephalonica* following their programmed exposure during their adult stage to 20 µl action of *Citrus sinensis* volatiles, during rearing control and experimental set up 12h, 24h, 48h and 72h of exposures. It was also recorded that when parent adults were exposed to 20, 40, 80, 160 µl volume of selected non host plant of *Citrus sinensis* oil that causes significant reduction ($P < 0.05$ or < 0.01) in their reproductive potential (in terms of eggs output and their hatchability) when compared to control. 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.

Index Terms- Hydro-Distillation, Clevenger's Apparatus, *Corcyra cephalonica*, *Citrus Sinensis*

I. INTRODUCTION

Host plant selection by phytophagous insect in a complex phenomenon involving a series of behavioural events, wherein chemosensory system of the insect respond differently to the plant chemicals enabling the insect to discriminate between host and non host plants. An understanding of *modus operandi* involved in the discrimination is vital for a better appreciation of the degree of oligophagy and polyphagy and to draw adequate conclusions regarding the evolution of monophagy. At the same time involvement of such behavioural as well as metabolic changes in insects to cope with the physical and chemical characteristics of plant to which phytophagous insects become adopted in evolutionary time also become equally significant. In recent year quite some efforts have been made to established the causes for the host selection behaviour through an in-depth assessment to the sensory physiology of an insect, enabling a better understanding of the casual factors responsible for host plant susceptibility as well as host plant resistance.

In this context the integration of what has come to be known as basic and directed approaches to the study of Insect - Plant interaction appears very relevant as well as challenging. While an understanding of the nature of plant defences and the insects' potential to overcome them enabling the identification of vital links in the sequence of events leading to successful exploitation of the host are essentially basic in approach, the tactics for manipulating these interactions and developing practices aimed at protecting crops lend themselves to an applied or directed approach. Very little is known of the dynamics of Insect - Plant interaction and in inspite of the isolation and identification of stimulatory, toxic and deterrent substances from several plants our understanding of the behaviour of these components in the interactions process leaves much to be desired.

CITRUS SINENSIS



[Scientific classification](#)

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae
Genus	<i>Citrus</i> L.
Species	<i>Citrus sinensis</i> (L.)-sweet orange

Fig. 1 Classification of *Citrus sinensis* (Orange),

The genus *Citrus*, belonging to the Rutaceae or Rue family (Figure 1), comprises of about 140 genera and 1,300 species. *Citrus sinensis* (Orange), *Citrus paradise* (Grapefruit), *Citrus limon* (Lemon), *Citrus reticulata* (tangerine), *Citrus grandis* (shaddock), *Citrus aurantium* (sourorange), *Citrus medica* (Citron) and *Citrus aurantifolia* (lime) are some important fruits of genus *Citrus* Singh, U., *et. al.*, (1983) , Anwar, F.R *et. al.*, (2008). The Rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) is a major pest of stored grain commodities in the tropics Pathak, P.H. and K. Kiran, (2014), Piltz, H. *et. al.*, (1977). 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 Ayyar, P.N.K., (1934),

Carmona, M.M. (1958), Hodges, R.J., (1979), Preveit, P.F.(1964) Some aspects of the Biology and control using botanicals of the rice moth, *C. cephalonica* (Stainton), on some pulses Allotey, J. and W. Azalekor, (1999) *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 flavanones which are very rare in other plants Ahmad, M.M., *et. al.*, (2006). Information is available pertaining to specified plant components effect Pathak, P.H. and S.S. Krishna (1985), Pathak, P.H. and S.S. Krishna (1991 and 1992), Mani, H.C. *et. al.*, (1993), Pathak, P.H.(1994). Ansari, A.A. and S.S. Krishna (1987) on the insect reproductive 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 Anon (1980). 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 Singh, A.K., A.*et. al.*,(1983). Bishop, C.D. and I.B. Thornton, (1997). Shaaya, E.,M. *et. al.*,(1997). Tiwari, R. and V. Dixit, (1995), Shukla, A.C. *et. al.*, (2000). Plant product as a fumigant for the management of stored-product pests Isman, M.B.,(2000). The most attractive feature of using essential oils or their constituents as crop protectants is due to their low 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 Abbas, S.K., *et al.*, (2012) Insecticidal and Growth Inhibition Activities of *Citrus paradisi* and *Citrus reticulata* essential oils against lesser grain borer Mishra, S.N. and S.S. Krishna (1979). Further more work is needed to understand the delicate and sensitive mechanism associated with trans-generational effect of oil volatiles, active ingredients to be used for proper management of this insect pest. we hope that in future combined action of these oils will show more better results to be utilized in insect pest management programme (IPM). The applied significance of these findings lies in the formulation of appropriate technology from which quantity of these volatile can be maintained in population areas, particularly in households.

II. MATERIAL METHOD

Technique to determine the action of *Citrus sinensis* oil volatiles on the reproductive potential of adult rice moths:

A rich standard culture of *C. cephalonica* was maintained in the laboratory (Figure 2), on coarsely ground Jowar (*Sorghum vulgar* (L.) Moench) containing 5% powdered yeast as per methodology of Saqi Kosar Abbas *et al.*, (1991). 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 by Mishra, S. N. and S. S. Krishna and Pathak P.H., G. Gurusubramanian, SS. Krishna (2010,1994).



Culture of *Corcyra cephalonica* inside BOD



Single Culture Jar



Larva of *Corcyra cephalonica* Adults of *Corcyra cephalonica*

Fig. 2 CULTURE OF CORCYRA CEPHALONICA IN (DIFFERENT STAGES)

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 Pathak, P.H. and Sangita Pandey (2011). A clear light yellow colour oily layer was obtained on the top of the aqueous distillate, separated and collected in collecting tube. Newly emerged (<24 hours old) adults of both sexes were employed at the outset of all tests included in this investigation and were individually reared from the egg stage on *sorghum vulgare* and yeast. A 250 ml glass beaker, internally divided into a lower and upper compartment by a wire-mesh partition (0.2 mm thickness; 200 meshes/cm²) and tightly covered at the top with a muslin cloth fastened by elastic bands, served as a specially designed experimental chamber in which the odours of the different plant oils (*Citrus sinensis*) were placed.

The amount and duration of presentation of these materials within the chamber, however, varied with each experiments as outline below. To ascertain the effect of the odours of oils / extract on the insects breeding potential, male or female moth confined to the upper compartment of the experimental chamber, was exposed for a period, of 12, 24, 48 and 72 h, to the oil vapours emanating from filter paper discs impregnated with 0, 20, 40, 80, or 160 µl of the oils and placed in the lower section of the chamber. Such an arrangement prevented contact of the moths with the untreated or treated paper discs. At the end of exposure time this male and female moth kept in another

clean and fresh beaker of 250 ml, covered at the top with a muslin cloth fastened by elastic band for mating and subsequent egg laying. Experiments were replicated five times for each experimental regimen. All experiments were accompanied by appropriate controls wherein males and females coupled for breeding were always in an environment devoid of any of the odours of the test materials. The total number of eggs laid by females associated with the different regimens were monitored daily after 24h during the first four days of oviposition (The period when they deposited most of their eggs during their life time). These eggs were arranged in linear fashion on the surface of a glass petridish and hatchability of these eggs was monitored daily according to Pathak and Sangita Pandey (2011). All experiments were replicated five times for each experimental regimen and were accompanied by appropriate controls, wherein males and females were always in an environment devoid of any of the odours of the test materials.

In adverse condition we use B.O.D. incubator to maintained oxygen demand and temperature required by an insect during experiment in laboratory condition. All tests, performed at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $85 \pm 5\%$ 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 according to Pathak and Sangita Pandey (2011).

GC/MS analysis of *Citrus sinensis* oil volatiles:

The details method of GC-MS analysis was done from the sample of essential oils peels (Epicarp) of *Citrus Sinensis* fruits of plant were taken in two liter of oval flask of Clevenger's Apparatus with hydro-distillation in laboratory conditions for extraction of oil volatiles. Analytical conditions test, which was done at National Botanical Research Institute (Council of Scientific and Industrial Research) Post Box No: 436, Rana Pratap Marg; Lucknow-226001, India.

NABL-Accredited, Central Instrumentation Facility; Ref. No: NBRI/CIF/288/2012; 24.07.2012- Instrument name GCMS-DSQ-II (Thermo Scientific); Column was taken of TR-50 MS, 30m x 0.25mm ID, 0.25 μm film; Oven having temperature 50°C (5 min) to 250°C at $4^{\circ}\text{C}/\text{min}$, 250°C (5min); Inlet is taken of 0.5 μl , 250°C , split 50:1; the Carrier was taken i.e. Helium to analyze the four above mention oils.

Details of GCMS conditions for sample of essential oils

Analytical Conditions

Instrument	:	GCMS-DSQ-II (Thermo Scientific)
Column	:	TR-50MS, 30 m x 0.25 mm ID, 0.25 μm film
Oven	:	50°C (5 min) to 250°C at $4^{\circ}\text{C}/\text{min}$, 250°C (5 min)
Inlet	:	0.5 μl , 250°C , split 50:1
Carrier	:	Helium

III. RESULTS

When freshly laid eggs of *C. cephalonica* were exposed to the action of *C. sinensis* oil volatiles liberated from these oils diffused into the eggs, like air Chapman, R.F. (1982), Engelmann, F. (1970), through the shell or they entered into them via aeropyles-tiny holes in the chorion connected with respiration of embryos Krishna, S.S. (1988). In this investigation we estimated the number of egg laid and their hatchability of *Corcyra cephalonica* after their programmed exposure with 20, 40, 80, 160 μl concentration of *Citrus sinensis* oil volatiles as shown in (Table 1)

Line graphs depicted estimation of eggs laid and egg hatchability in *C. cephalonica* after the programmed exposure during their adult stage at 20,40,80,160 μl oil concentration and different time duration with action of *Citrus sinensis* oil volatiles, during rearing (Figure 3). (Figure 4) showing the relative abundance of *Citrus sinensis* oil graph peak through GC/MS study. (Figure 5) Shows chemical structures of active ingredients present in *Citrus sinensis* oil volatiles.

Knowledge emphasizing the significance of odours from plant products, in regulating ovipositional behavior of lepidopterans is still limited according to Feeny, F., L. Rosenbery and M. Carter, (1983), Harrow, I.D (1983). The *modus operandi* of such control linked with olfaction, needs deeper understanding according to Feeny *et. al.*, 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 Beckett, A.H., E.J. Triggs, (1996), Berger, B.M., *et. al.*, (1997), on the basis of their preliminary physiological recording experiments, in *Menduca sexta* (tobacco hornworm)-a plant feeding lepidopteran.

Identification of the active ingredients of *Citrus sinensis* oils:

Citrus sinensis oils when subjected to GC-MS analysis 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 dieneol-1.04%; p-menth 1-en 8-ol-3.37%; carveol-3.24%; carvone-4.80%; durohydroquinone-0.41%; caryophyllene oxide-0.43%; dodecanoic acid-0.05%; limonene 6-ol pivalate-0.12%. The oil also contained a series of compounds (Fig.: 19 and 20). It was recorded that oil volatile contained maximum number of limonene-67.47%; while carvone-4.80%.

Table :1 Estimate of egg laid and their egg hatchability of *Corcyra cephalonica* after their programmed exposure with 20, 40, 80, 160µl concentration of *Citrus sinensis* oil volatiles

Duration of oil exposure	Percent eggs laid and their egg hatchability							
	20µl		40µl		80µl		160µl	
	Egg laid	Egg hatchability	Egg laid	Egg hatchability	Egg laid	Egg hatchability	Egg laid	Egg hatchability
Normal male paired with normal female (control)	303.000 ±2.000	303.000 ±2.000	291.400 ±2.441	291.400 ±2.441	298.400 ±0.927	298.400 ±0.927	303.000 ±2.000	303.000 ±2.000
12h	289.400 ±2.803	285.600 ±2.204	284.400 ±3.558	282.000 ±3.646	275.800 ±3.277	274.200 ±3.411	289.400 ±2.803	289.400 ±6.268
24h	260.000 ±6.156	255.600 ±7.131	257.200 ±5.571	255.400 ±5.723	250.600 ±12.048	247.600 ±11.817	260.000 ±6.156	260.000 ±
48h	228.200 ±4.608	220.400 ±5.844	222.000 ±8.000	218.400 ±7.473	210.200 ±6.621	207.000 ±5.549	228.200 ±4.608	184.200 ±5.489
72h	204.400 ±2.785	195.400 ±4.118	195.000 ±5.000	187.800 ±3.638	190.000 ±6.488	182.800 ±4.235	204.400 ±2.785	088.400 ±1.536
Mean	257.000 ±18.899	252.000 ±19.931	250.000 ±18.382	247.000 ±19.480	245.000 ±20.084	242.000 ±21.188	257.000 ±18.399	225.000 ±39.862
LSD 1%	15.960	18.940	21.180	19.800	28.030	25.500	15.960	16.310
LSD 5%	11.700	13.890	15.530	14.520	20.550	18.700	11.700	11.960

Mean followed by different letters differs significantly with control at 5% or 1% by Least Significant Differences (LSD) test

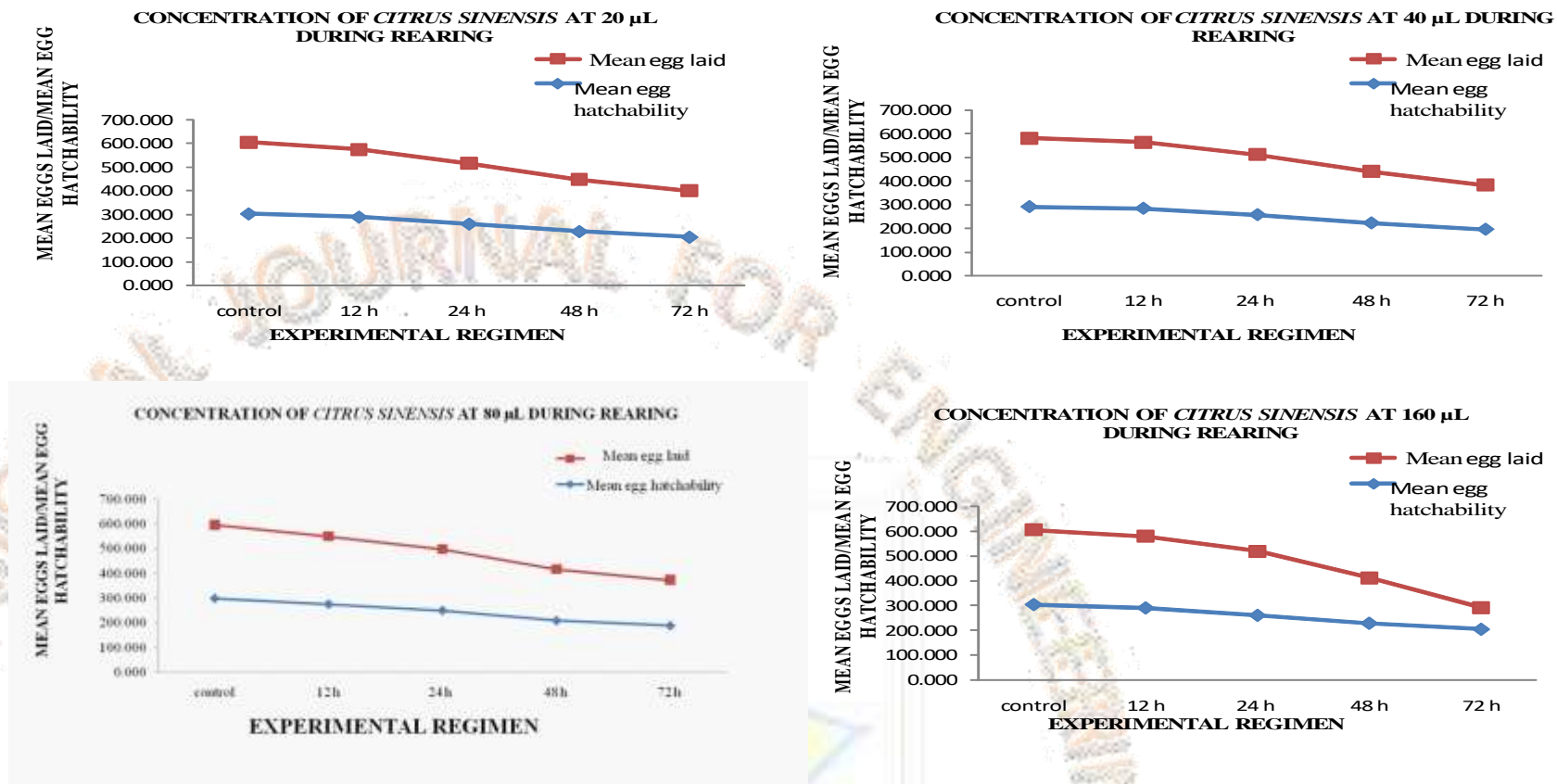


Fig. 3 Line graphs showing Estimation of eggs laid and egg hatchability in *C. cephalonica* after the programmed exposure during their adult stage at 20,40,80,160 µl oil concentration and different time duration with action of *Citrus sinensis* oil volatiles, during rearing.

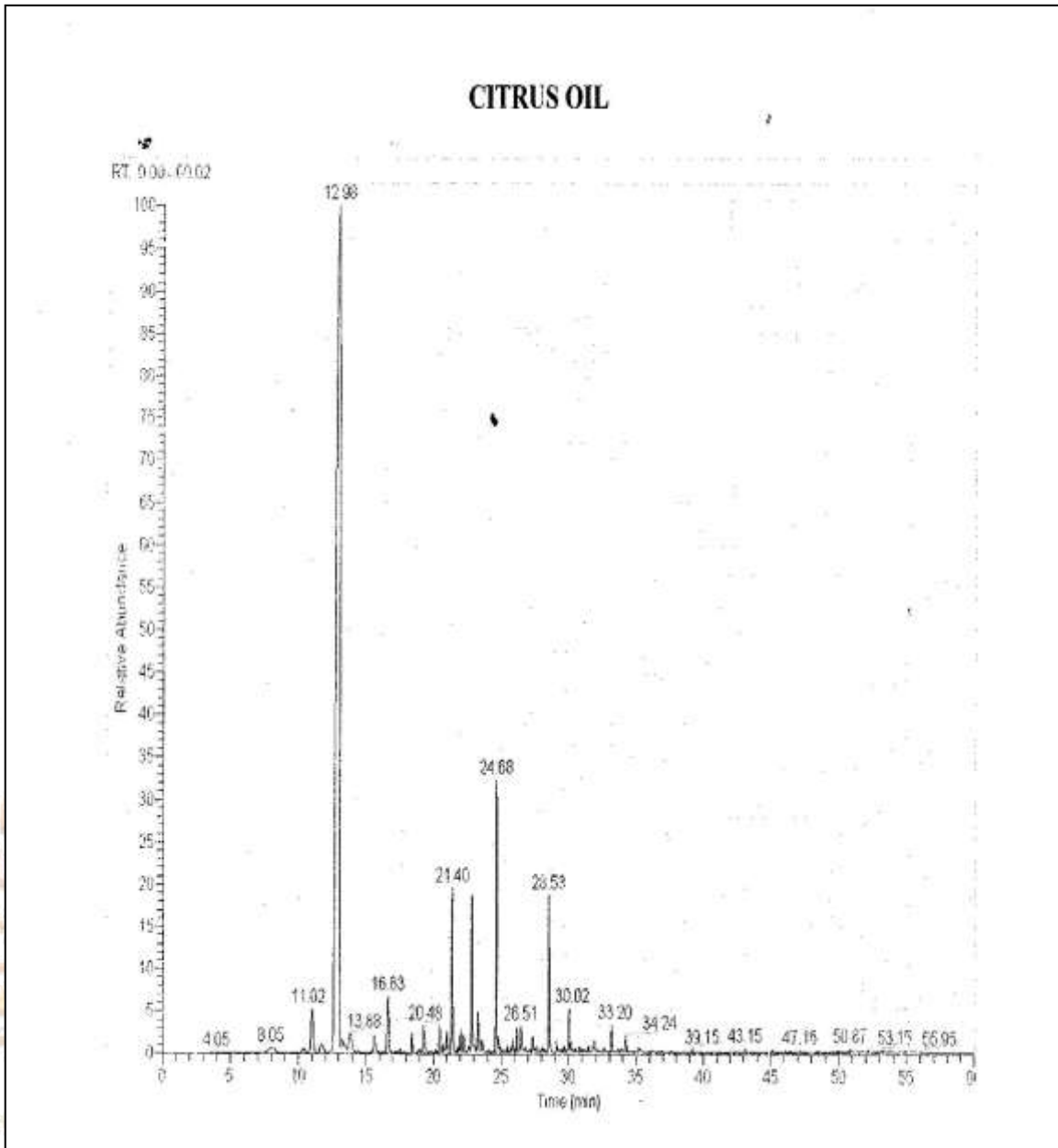


Fig. 4 Graph showing Relative abundance with respect to time

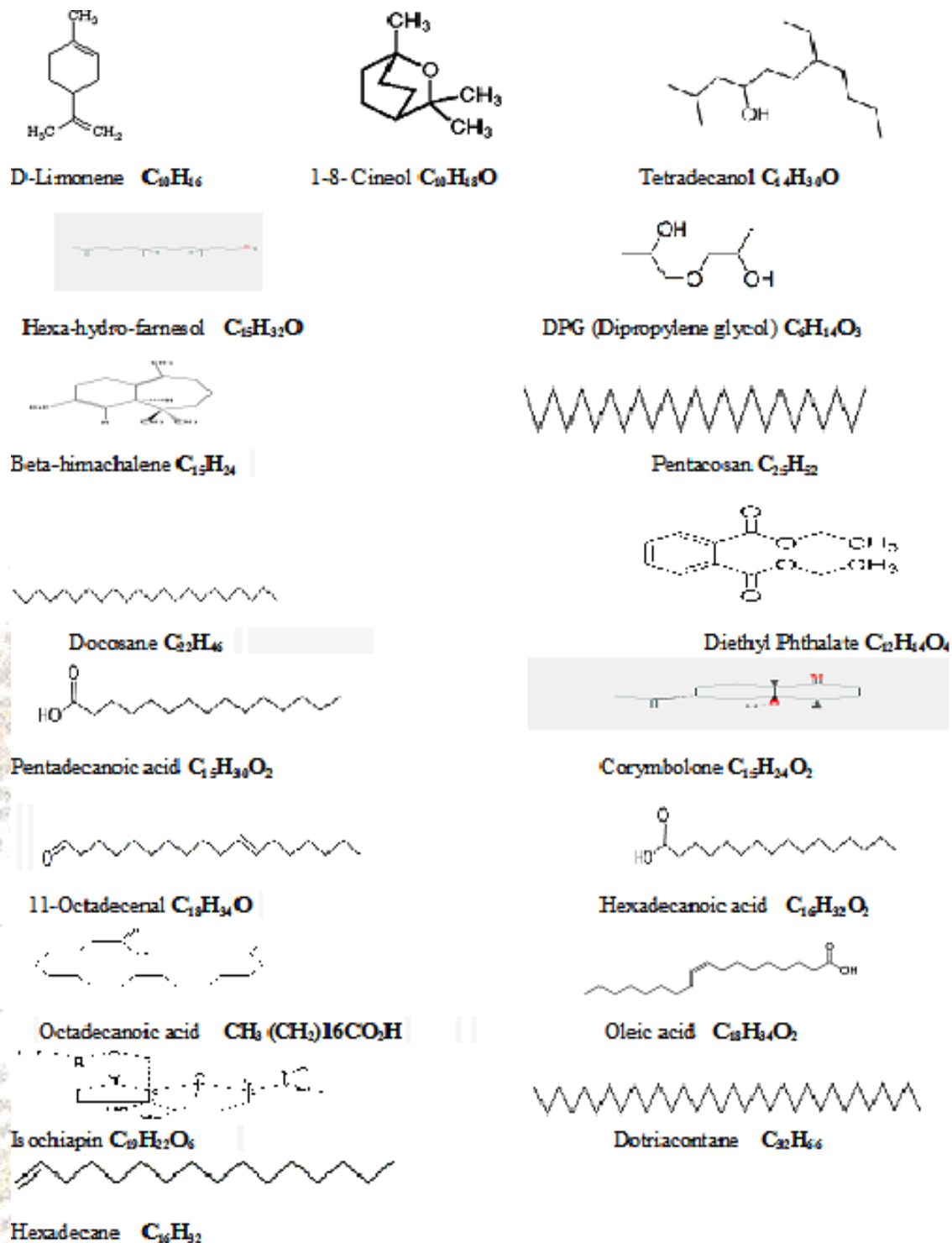


Fig. 5 Chemical structure of active ingredients in *Citrus sinensis*

IV. DISCUSSION

The breeding programme of *Corcyra cephalonica* following their interaction with the odorous environment maintained by laboratory extracted oil from *Citrus sinensis* (Rutaceae) (from fruit epicarp (peels) oil volatiles) were studied. Information's on the effect of such treatment of non-host plants oil volatiles on (a) Egg hatchability, (b) Adult stages, (c) Adult moths exposure, and (d) Characterization of active ingredients in this investigation are now considered at length here to arrive at valid conclusions for a more comprehensive appreciation of the problem associated with the developmental and reproductive biology of this pyralid in such a manipulated chemical environment. The absence of males in the life of these females, right from the time of the emergence did not postpone the process of oviposition and occurred, as in mated, between 24 and 48 hours of the adulthood of females. In this respect *Corcyra cephalonica* virgins differ from those of many other diverse lepidopteran species Rau and Rau, (1914); Schulze, (1926); Eidmann, (1931); Mokia, (1941);

Hillyer and Thorsteinson, (1971); Krishna *et al.*, (1977) all of which possessed a tendency to withhold most of their eggs for eventual deposition, if not mated, with “reluctance” shortly before death Engelmann, (1970). The fact that all the eggs laid by the unmated females of *Corcyra cephalonica* were non-viable indicates the absence of parthenogenesis in this insect, reproductive features resembling that reported in another moth species *Earias fabia*, Krishna *et al.*, (1977) but distinct from that of certain others such as *Solenobiasp.* Sauter, (1956), *Bombyx mori* Austaurav, (1967) and confirms the earlier findings published by Krishna and Narain (1976). When freshly laid eggs of *C. cephalonica* were exposed to the action of *Citrus sinensis* (Rutaceae), epicarp of fruit (peels) oil volatiles, When freshly emerged males and females were exposed to 20, 40, 80 and 160 µl volume of *Citrus sinensis* leaves volatiles in a programmed manner it causes significant reduction ($P < 0.05$ or < 0.01) in their reproductive potential (in terms of eggs output and their hatchability) when compared to control. A significant reduction ($P < 0.01$) in eggs output and eggs hatchability of breeding pairs was observed when adults were continuously exposed for 12, 24, 48 or 72h time duration. Maximum effect of oil volatile was recorded with 20 and 160µl volumes.

The retention time and chemical composition of essential oils of *C. sinensis* (Rutaceae) was recorded with the help of Gas Chromatography and Mass Spectrometry (GC-MS), It revealed Seventeen volatile constituents 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 diene-1.04; p-menth 1-en 8-ol-3.37; carveol-3.24; carvone-4.80; durohydroquinone-0.41; caryophyllene oxide-0.43; dodecanoic acid-0.05; limonene 6-ol pivalate-0.12. The oil also contained a series of compounds. Gas-chromatographic and mass-spectrometry (GC-MS) was done for the characterization of active ingredients from the *Citrus sinensis* oil extracted in laboratory was analyzed by GC-MS test. It was recorded that oil volatile contained maximum number of limonene- 67.47; percentage than carvone-4.80. All these findings are reported in the form of a research papers and reported in Academic Journal of Entomology, 2015; 8(2): 45-51. (Vikas Chandra Verma and P. H. Pathak). Adult Exposure: *Citrus sinensis* (Rutaceae), oil volatiles are First most effective oil volatile – (When we compare the general mean values of adult exposure table) among four above mentioned oil volatiles (Student-t test) the best effect was observed in *Citrus sinensis* with 160µl exposure with 12, 24, 48 and 72h time durations, Second most effective oil volatile. It can be conclude that the Non- host plant oil volatiles are not only very useful in conservation of food grains in house hold, godowns or worldwide but they are also degradable and non toxic in nature. Further more work is needed to understand the delicate and sensitive mechanism associated with transgenerational effect of oil volatiles active ingredients uses for proper management of this insect pest and we hope combined action of these oils in future will show more better response in insect pest (*C. cephalonica*) management programme. Further more work is needed to understand the delicate and sensitive mechanism associated with transgenerational effect of oil volatiles active ingredients to be used for proper management of this insect pest and we hope that in future combined action of these oils will show more better results to be utilized in insect pest management programme (IPM.). The applied significance of these findings lies in the formulation of appropriate technology from which quantity of these volatile can be maintained in population areas, particularly in house – holds.

V. CONCLUSION

This study shows that the *C. sinensis* volatile oils affects 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%. 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. *Citrus sinensis* (Rutaceae) oil volatiles have capable ingredient to check adult rice moth reproduction at the level of egg hatchability and number of eggs laid, which provide us tool that can be used at appropriate time for the management of this pest population below threshold level. Adult exposure first most effective oil volatile with the best effect was *Citrus sinensis* with 160µl exposure for 12, 24, 48 and 72h time durations, It can be concluded that the Non- host plant oil volatiles are not only very useful in conservation of food grains in house hold, godowns or worldwide but they are also degradable and non toxic in nature.

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