

Effects of *Azadirachta indica* oil volatiles on adult stage of *Corcyra cephalonica* and characterization of active ingredients by GC-MS

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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 *Azadirachta indica* (Meliaceae) local name neem plant in India is a medicinal plant 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 *Azadirachta indica* 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 *Azadirachta indica* oil volatiles, during rearing control and experimental set up 12h , 24h, 48h and 72h of time duration. It was also recorded that when parent adults were exposed to 20, 40, 80, 160 µl volume of selected non host plant of *Azadirachta indica* 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 *Azadirachta indica* oils. With the help of (GC-MS) test *Azadirachta indica* volatile oil contained maximum number of It was recorded that oil volatile contained maximum number of Oleic acid percentage than Hexadecanoic acid and Sulphur components etc., which are responsible for the Sulphury odour. The result of this study offers a platform for using *Azadirachta indica* leaf oils in management of rice moth population in godowns and warehouses around the world.

Key words: *Corcyra cephalonica*, Clevenger's apparatus, Gas-chromatographic and Mass- Spectrometry (GC- MS), Phyto-chemicals, Hydro-distillation, Active ingredient

Introduction

India is one of the world's largest producers of rice, accounting for 20% of all world rice production. The post-harvest losses in India amount 12 to 16 million metric tons of food grains each year. There was evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities by Highland H. A. (1978). This amount damaged by insect infestation could feed one-third of India's population. The monetary value of these losses amounts to more than Rs 50,000 cores per year report on insect infestation of Cacao. However, the production becomes wasteful if it is destroyed before it reaches the consumers. Million tons of food grains are either damaged or lost by various pests during storage. During recent years much attention has been paid to ways and means to increase the food production but little emphasis has been given for their healthy storage. The need of time , therefore is not only to produce more but to reduce the losses in food commodities that occur between harvest and consumption. Effect of Azadirachtin an active ingredient on hormone treats during the gonadotrophic cycle of *locusta migratoria* and natural pesticides from neem tree and other tropical plants as reported by Rembold, H., Uhl M. and Muller T. (1987) have valuable significance. Laboratory observations on the development of the rice moth, *Corcyra cephalonica* (Stainton) on millet and sorghum at 28°C. by Krishi *et. al.* (1985);. Allotey, J. And W. Azalekor (2000); and Zhang, Y. Z., Cheng, M. Z., Zhou, W. R. and Wang, C. X., (1991) contributed important studies on the efficiency of rearing rice moth, *Corcyra cephalonica* (Stainton). Young *Corcyra* larvae hatched out from the egg within 4 -5 days and the larvae feed on the broken grains. Tiny larva after hatching is creamy-white, with a prominent head or brownish head. It moves about actively and feeds on broken grains for some time and then starts spinning web to join grains. The larval development was inside the grain cluster. Feeds on broken grains and develop, punctuated by 4 moults covering 5 instars, into silken cocoons. Full grown larva is pale whitish in colour, 15 mm long with short scattered hairs and no markings on body. Total Larval period is 15-20 days (depending upon temperature and humidity) in summer and may be extended in winter. There is a conspicuous seta above each spiracle and on the eighth abdominal segment (Haines and Hodges, 1991). The spiracles of the larvae of this species are thickened on the posterior rim. This differentiates them from the larvae of other stored product moths. Only caterpillars cause the damage stored material by feeding. Rice moth, *Corcyra cephalonica* (Stainton) is probably one of the most catholic feeders among the storage pests which feed on a wide variety of dried vegetable materials, dried fruits like almonds, date palm, nuts, chocolates, biscuits, oilcakes etc. (Adeyemi, 1968; Hodges, 1979). It is major pest of rice but also feeds on grams, sorghum, maize and some pulses. In addition, the larvae also cause extensive indirect quantitative and qualitative damage by making durable silk webs fecal material and leaving threads like silk when they shift and the stored grains are contaminated by excreta and pupal cocoons (Allotey and Azalekor, 2000; Hill, 2002). Effect of *Azadirachta indica* leaves oil volatiles on egg Hatchability of *Corcyra cephalonica* was reported by Vikas Chandra Verma and P.H. Pathak (2014). Therefore it was thought desirable to record the

volatiles action of neem leaf oil volatiles on adult stages of *C. cephalonica* and their subsequent GC-MS analysis.

Material Methods

A rich standard culture of *Corcyra cephalonica* was maintained in the laboratory, on coarsely ground Jowar (*Sorghum vulgare* (L.) Moench) containing 5% powdered yeast as per methodology of (Mishra and Krishna, 1979). The general layout of the experiments, the methodology adopted to treat the eggs with vapour action of the selected oils of *Azadirachta indica* and the parameters chosen to assess their impact on reproductive potential of the pest was similar as are outlined by Pathak and Sangita Pandey (2011); Kumari Kiran and Pathak P.H. (2015, 2016).

EGG EXPOSURE TO OIL:

Freshly laid eggs (<24 h) were taken. To estimate hatchability laid 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 160µl of Neem oils separately. This experimental setup was kept in a glass chamber having 30 cm diameter and 13cm height from inside. For each experimental regimen five replicates were kept. In first experiment after 12h, in second experiment after 24h, in third experiment after 48h and in fourth experiment after 72h, the impregnated paper discs were removed and eggs were shifted from odorous to normal environment, wherein their hatchability was monitored daily as per Pathak and Krishna (1992).

EXTRACTION OF NEEM LEAF OIL BY CLEVINGER'S APPARATUS:

Fresh leaves of *Azadirachta indica* plant were taken in two liter of oval flask of Clevenger's Apparatus. For hydro-distillation clean distilled water was used for heating 6-8h at 40 to 80°C in the laboratory. The volatile material is carried away in the steam through tubes and then cooled in condensation chamber. The volatile oil is then removed from the top of the hydrosol by separating funnel. In this process compounds are not destroyed by heat. Hydro distillation needs large amount of plant material and the time for extraction (process take around 3-4h) was similar as are outlined by (K. Satish Kumar *et.al.*, 2010), (Figure No. 1) then the oils sample was either used for hatchability experiment and was send for characterization of active ingredient by Gas-chromatographic and mass-spectrometry (GC-MS) test at N.B.R.I. Lucknow (GC-MS for sample of essential oils 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, 0.25µm film; Oven having temperature 50°C (5 min) to 250°C at 4°C/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 leaf oil of *Azadirachta indica* (Meliaceae) which was extracted by Clevenger's apparatus with hydro-distillation in laboratory conditions).

Result

Changes in reproductive potential of adult stage in *Corcyra cephalonica* by the action of *Azadirachta indica* leaves oil volatiles. All the experiments were performed with 20, 40, 80 and 160 µl volume of selected *Azadirachta indica* leaves oil volatiles. In (Fig. no. 1) selected new born adult males and females were exposed/ unexposed continuously to the volatiles action at different time durations. Details of methodology are already described in 'Materials and Methods' section of this thesis. When *Corcyra cephalonica* adult stage was exposed to 20, 40, 80 and 160 µl volume of *Azadirachta indica* leaves oil volatiles, i) Unexposed (control), ii) 12h exposure, iii) 24h exposure, iv) 48h exposure, v) 72h exposure. 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 (Table:1,2) at different time durations, maximum at 160 and 80µl volumes. All experiments were accompanied by appropriate controls, where males and females were always in an environment devoid of any of the odours of the test materials. The means of the data, pooled from five independently run tests, were subjected to Least Significant Difference (LSD) analysis (Paterson,1939). Sharp reductions in egg hatchability was noticed at varying degrees of exposure period of volatiles. Most severe reduction in egg hatchability was noticed at 48 and 72h exposure of the Neem oil volatile in comparison to 12 and 24h exposure period. Presumably, the volatiles liberated from these oils/extract diffused into the eggs, like air, Chapman, 1982, through the shell or they entered into them via aeropyles - tiny holes in the chorion connected with respiration of embryos, Sehnl, 1985., Mill, 1985. Later, these volatiles through their vapour action succeeded in terminating the entire gamut of vital physiological and biochemical processes associated with embryogenesis, only in those eggs genetically programmed to be weak leading to their death and there by their non - hatchability. A similar result was obtained when eggs of *Earias vitella* - another, though taxonomically unrelated, insect were likewise interacted with volatile of eucalyptus oil. Total inhibition of hatchability, however, occurred if eggs of *Earias vitella* were uninterrupted exposed to eucalyptus oil vapour for 4 days (Pathak and Krishna, 1992). All such marked adverse consequences did not happen with eggs affected by cedar wood oil, unlike in *C. cephalonica*. Component identification was carried out by the Non-Host plant volatile components of the leaf of *Azadirachta indica* (Meliaceae). Thus, generally the oil was found to contain variable constituents of aromatics it mainly consisted of D-Limonene- 5.81% ; 1,8 Cineol-1.21%; Tetradecanol-0.22%; Hexa-hydro- farnesol-0.02%; DPG-0.33%; Hexadecane-1.67%; Beta-himachalene-0.37%; Pentacosane-1.61%; Docosane- 1.33%; Diethyl Phthalate-4.23%; Corymbolone-0.59%; Pentadecanoic acid-0.28%; 11-Octadecenal-0.03%; Hexadecanoic acid-12.42%; Octadecanoic acid-0.96%; Oleic acid-63.02%; Isochiapin-0.24%; Dotriacontane- 0.03%. The oil also contained a series of unidentified organosulphur compounds. (Figure No.1, Table No.1). Applied significance of this investigation lies in the formulation of appropriate technology from where the desirable quantity of oil / active ingredients can be used for management of the pest population.

Table No. 1: Estimate of egg laid and their egg hatchability of <i>Corcyra cephalonica</i> after their programmed exposure with 20, 40, 80, 160µl concentration of <i>Azadirachta indica</i> leaf 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)	288.600 ±2.821	288.600 ±2.821	290.800 ±3.322	290.800 ±3.322	297.200 ±2.154	297.200 ±2.154	295.600 ±2.158	295.600 ±2.158	
12h	287.000 ±3.146	285.400 ±3.203	288.200 ±2.817	287.600 ±2.580	279.800 ±6.800	278.200 ±6.880	279.200 ±6.829	277.800 ±6.575	
24h	285.600 ±3.075	283.600 ±3.264	282.600 ±2.181	280.800 ±2.416	245.200 ±12.030	239.600 ±13.347	243.000 ±14.024	236.200 ±13.039	
48h	284.000 ±3.646	282.000 ±3.563	278.800 ±3.056	275.600 ±4.154	207.400 ±9.724	212.000 ±11.575	207.200 ±10.011	191.000 ±4.012	
72h	279.800 ±3.720	275.400 ±3.572	269.600 ±2.501	265.200 ±1.655	180.400 ±7.580	123.000 ±5.205	175.000 ±7.286	129.400 ±7.736	
Mean	285.000 ±1.505	283.000 ±2.193	282.000 ±3.743	280.000 ±4.546	242.000 ±21.769	230.000 ±30.581	240.000 ±22.291	226.000 ±30.156	
LSD 1%	13.270	13.260	11.280	11.870	33.540	35.590	36.040	30.840	
LSD 5%	9.730	9.720	8.270	8.700	24.590	26.090	26.430	22.610	

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

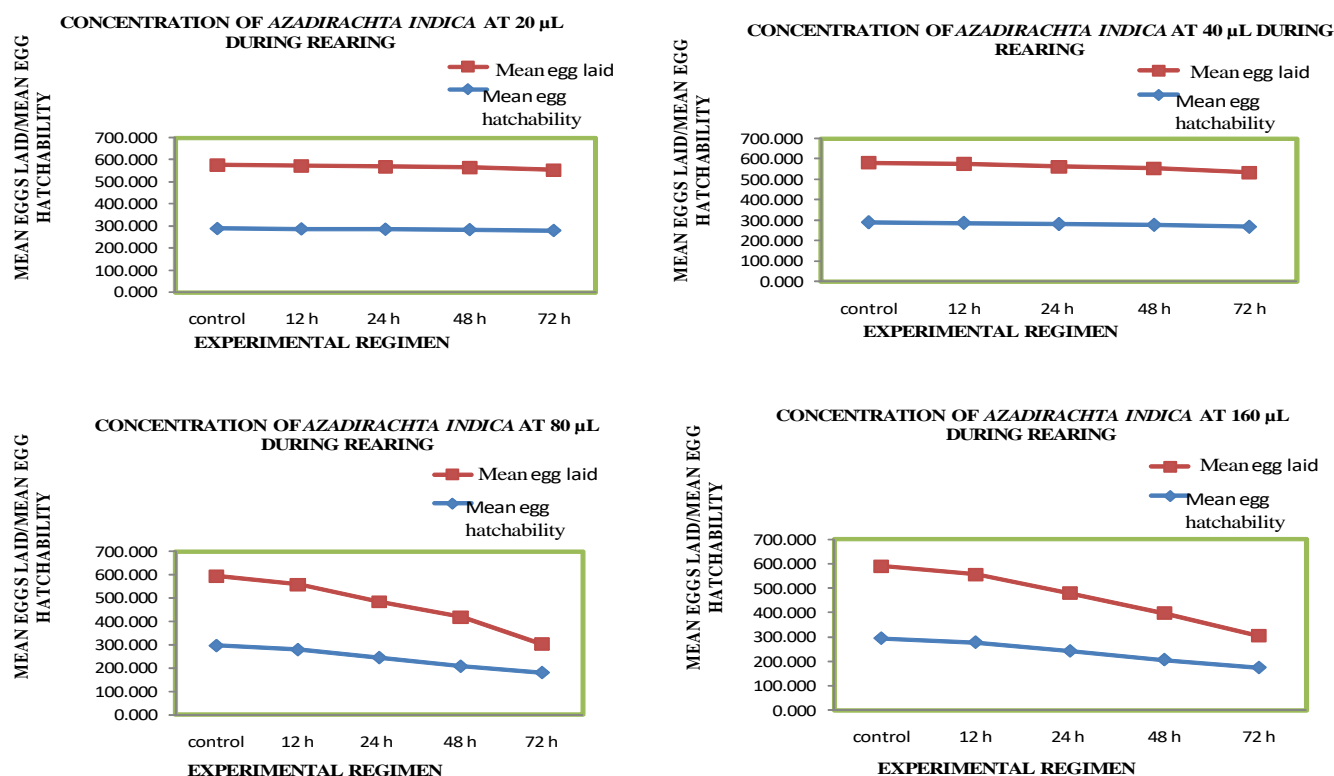


Figure No. 1 : Line graphs showing Estimation of eggs laid and egg hatchability in *C. cephalonica* after the programmed exposure during their adult stage at 20,40,80,160 µl oil concentration and different time duration with action of *Azadirachta indica* oil during rearing.

Discussion

The breeding programme of *Corcyra cephalonica* following their interaction with the odorous environment maintained by laboratory extracted leaves oil from *Azadirachta indica* A. Juss (Meliaceae), studied. Information's on the effect of such treatment of non-host plants oil volatiles on (a) Egg hatchability, (b) Immature 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 *Eariasfabia*, Krishna *et.al.*, (1977) but distinct from that a certain others such as *Solenobiasp.* Sauter, (1956), *Bombyx mori* Austaurav, (1967) and confirms the earlier findings published by Krishna and Narain (1976).

This thesis considers alternative which could be used as storage grain protectants, concentrating particularly on plants which have found other uses as food species or for medicinal application. Four non host-plants were selected for extraction of oils which was used in laboratory experiments i.e. leaves of *Azadirachta indica* because of their availability in abundance in and around the locality of university area and in Eastern Uttar Pradesh. When freshly emerged males and females were exposed to 20, 40, 80 and 160 µl volume of *Azadirachta indica* leaves oil 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 exposure for 12, 24, 48 or 72h time duration. Maximum effect of *Azadirachta indica* leaves oil volatile was recorded with 160 and 80µl volumes. This feature was reported in another, though taxonomically unrelated, lepidopteron pest following their exposure to different volatiles alone, Pathak and Krishna, (1986), (1987). Similar type of results were reported by Pathak and Krishna (1985) and Mani *et.al.*, (1993) as a effect of neem oil and certain volatile chemicals and other factors on the reproductive Biology of *Corcyra cephalonica* (Stainton), respectively. These observation are in a broad sense, akin to those reported for *Earias vitella* treated with *Blumeaeriantha* oil, Dongre and Rahalkar, (1982). Presumably, vapours from such an oil, dose deleteriously affects the delicate and sensitive mechanisms associated with mating a decisive factor influencing the total number of eggs a species lays, Engelmann, (1970) as well as those concerning fertilization in

the reproductive physiology of this insect. The outcome of such fall in the reproductive potential is possibly due to some kind of spermicidal effect of the vapour action of oils, specially neem oil leading to less number of eggs getting fertilized in the females, Pathak and Krishna, (1986). Knowledge emphasizing the significance of odours from plant products in regulating ovipositional behaviour of lepidopterans is still limited Ansari and Krishna, (1987); Tabashnik, (1987); Rembold, (1984), (1987); Krishna, (1988); Pathak *et.al.*, (1994). The *modus operandi* of such control linked with olfaction needs deeper understanding according to Feeny *et.al.*, (1983). 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.*, (1983).

GC-MS of *Azadirachta indica* leaves oil contained maximum number as in (figure 4,3,2) i.e., Oleic acid percentage than Hexadecanoic acid and sulphur components i.e., D-Limonene- 5.81% ; 1,8 Cineol-1.21%; Tetradecanol-0.22%; Hexa-hydrofarnesol- 0.02%; DPG-0.33%; Hexadecane-1.67%; Beta-himachalene- 0.37%; Pentacosane-1.61%; Docosane 1.33%; Diethyl Phthalate-4.23%; Corymbolone-0.59%; Pentadecanoic acid-0.28%; 11-Octadecenal-0.03%; Hexadecanoic acid-12.42%; Octadecanoic acid-0.96%; Oleic acid-63.02%; Isochiapin-0.24%; Dotriacontane- 0.03%. The oil also contained a series of unidentified organosulphur compounds as given in result section which are responsible for the sulphury odour. Component identification was carried out by the Non-Host plant volatile components of the leaves of *Azadirachta indica* (*Meliaceae*). *Azadirachtin*, one of the important active ingredient of neem was not recorded in GC-MS analysis of neem leaves oil volatiles due to fact that *Azadirachtin* is non-volatile in nature for better understanding and utilization of active ingredients of *Azadirachta indica* leaves oil in IPM programme the chemical structures were drawn.

Table No. 2 TABLE OF AZADIRACHTA INDICA OIL ACTIVE INGREDIENTS PERCENTAGE

S./No.	Compound Name	Area %
1	D-Limonene	5.81
2	1,8 Cineol	1.21
3	Tetradecanol	0.22
4	Hexa-hydro-farnesol	0.02
5	DPG	0.33
6	Hexadecane	1.67
7	Beta-himachalene	0.37
8	Pentacosane	1.61
9	Docosane	1.33
10	Diethyl Phthalate	4.23
11	Corymbolone	0.59
12	Pentadecanoic acid	0.28
13	11-Octadecenal	0.03
14	Hexadecanoic acid	12.42
15	Octadecanoic acid	0.96
16	Oleic acid	63.02

17	Isochiapin	0.24
18	Dotriacontane	0.03

Figure No. 2 GRAPH SHOWING AZADIRACHTA INDICA OIL ACTIVE INGREDIENTS PEAKS

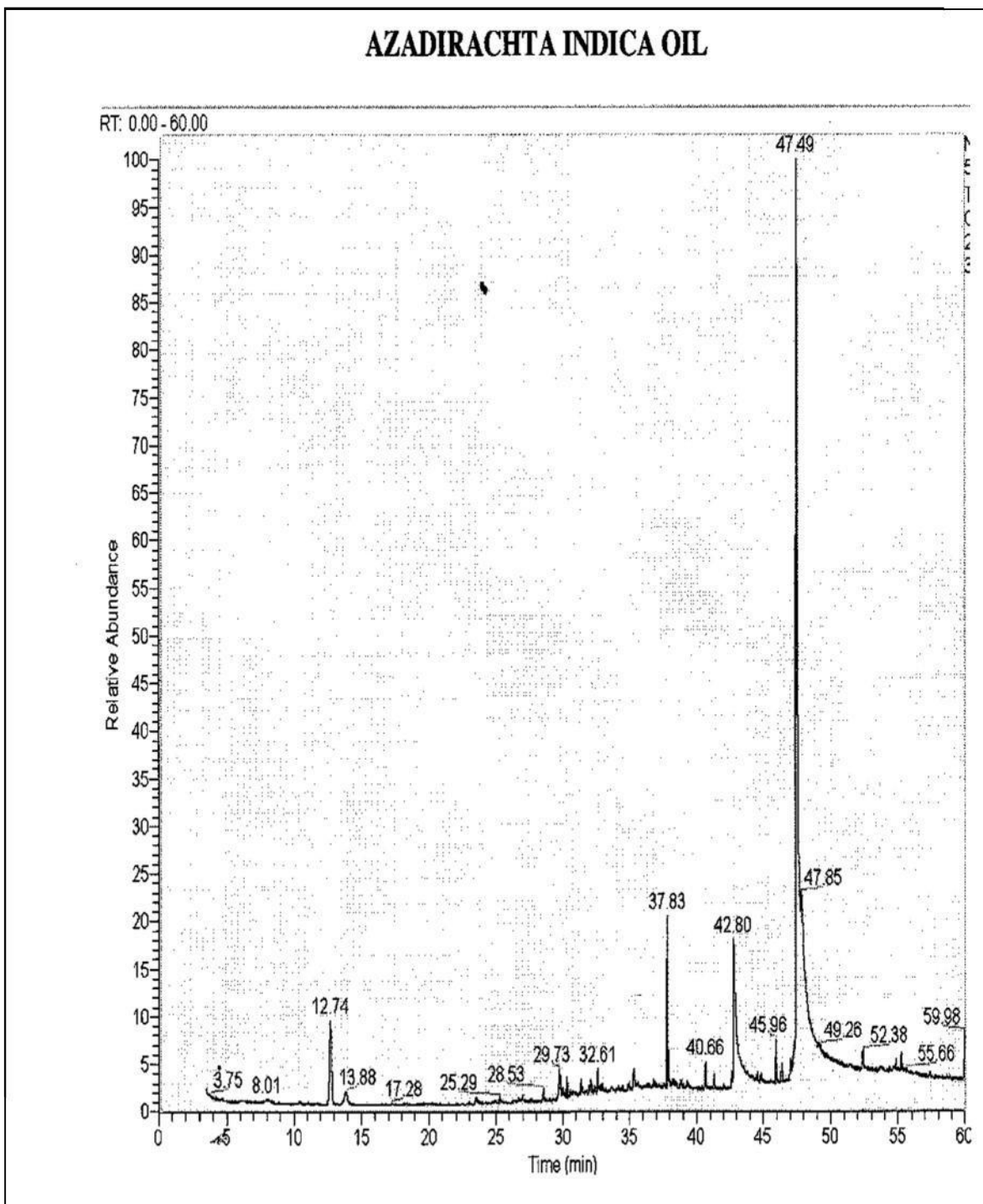


Figure No. 3 CHEMICAL STRUCTURE OF AZADIRACHTA INDICA OIL ACTIVE INGREDIENTS

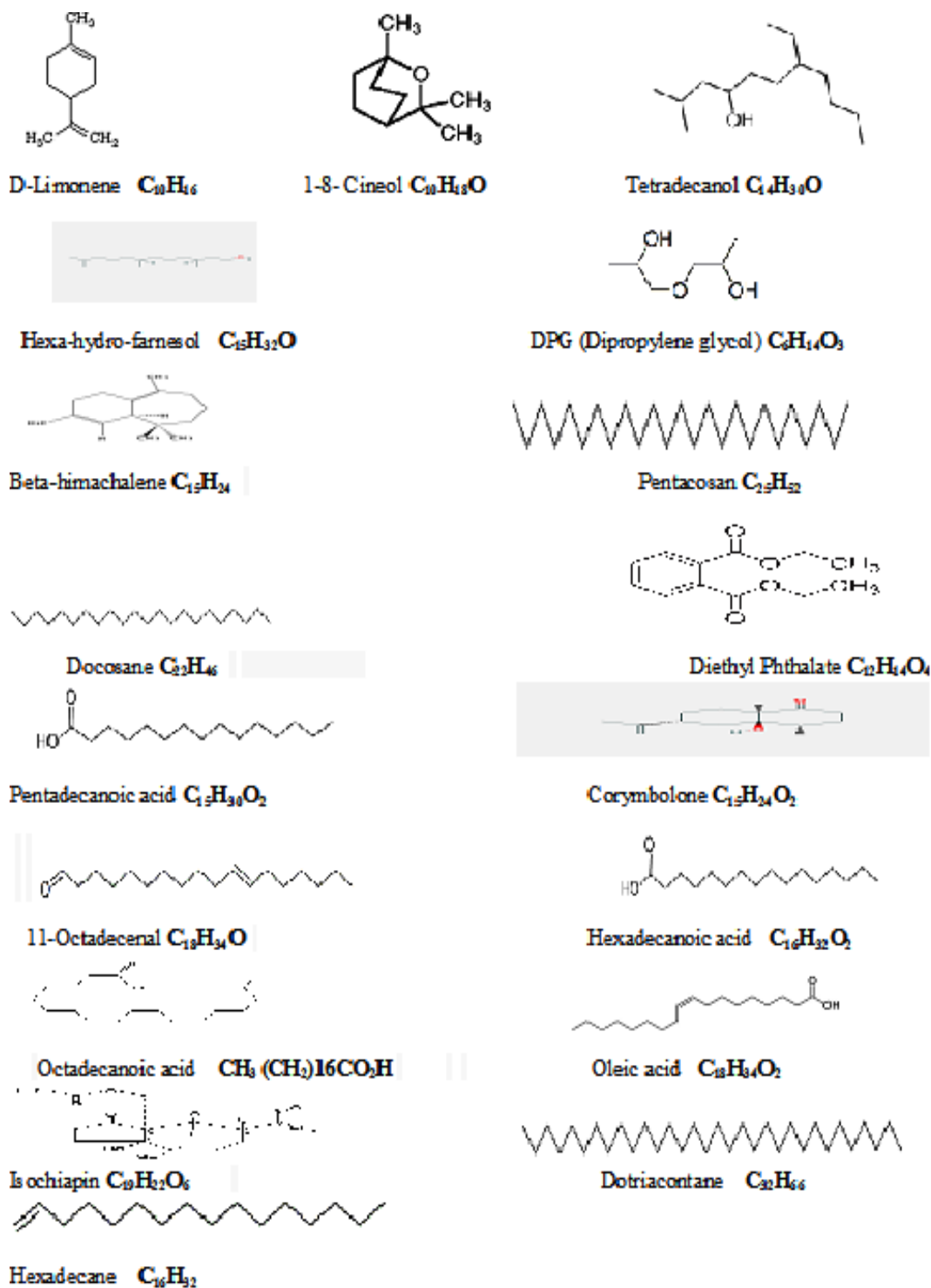


Figure No. 4

IMAGE OF CLAVENGER'S APPARATUS



Conclusion

Most effective oil volatile was *Azadirachta indica* with 160 μ l exposure at 12, 24, 48 or 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. 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.). With the help of (GC-MS) test *Azadirachta indica* volatile oil contained maximum number of Oleic acid percentage than Hexadecanoic acid and Sulphur components etc., as we can see in (Table 2) which are responsible for the Sulphury odour. The result of this study offers a platform for using *Azadirachta indica* leaf oils in management of rice moth population in godowns and warehouses around the world.

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