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Effects of *Azadirachta Indica* Leaf Oil Volatiles on Immature Stages of *Corcyra Cephalonica* and Characterization of Active Ingredients by Gas Chromatography-Mass Spectrometry

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ABSTRACT: *Azadirachta indica* (Meliaceae) local name neem plant in India is a medicinal plant, claimed for the management and control of rice moth *Corcyra cephalonica* an important pest stored commodities. The action of *Azadirachta indica* leaf oil volatiles affects the immature stage of *Corcyra cephalonica* when they were exposed to these volatiles for time duration 12, 24, 48, and 72h with 20, 40, 80 or 160 µl of oils concentrations. A significant reduction in the reproductive potential of breeding pairs in this pest was recorded after exposure of 48 and 72h time duration. When laboratory extracted neem leaf oil was subjected to Gas chromatographic and mass spectrometry (GC-MS) analysis it indicates the presence of eighteen Phyto-chemicals and a series of unidentified organosulphur compounds. 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**);** Noorma Osman. (1986). 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 immature stages of *C. cephalonica* and their subsequent GC- MS analysis.

MATERIALS AND METHODS: A rich standard culture of *Corcyra cephalonica* was maintained in the laboratory, on coarsely ground Jowar (Sorghum vulgar (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 CLAVENGER'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. 2) 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-Accreditated, 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 50^0C (5 min) to 250^0C at 4^0C/min, 250^0C (5min); Inlet is taken of 0.5 µl, 250^0C, 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 AND DISCUSSION: Action of *Azadirachta indica* (Meliaceae), leaf oil volatiles effect percent egg hatchability in rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: pyralidae), when freshly laid eggs were exposed to these volatiles for time duration (12, 24, 48, and 72h) with different oils concentrations a marked decline in egg hatchability in *Corcyra cephalonica* is seen after exposure of 48 and 72h with 80µL and 160µL of *Azadirachta indica* leaf oils. 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. (Figure No. 3) 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 , Sehnul, 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.

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(Table No. 1) 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

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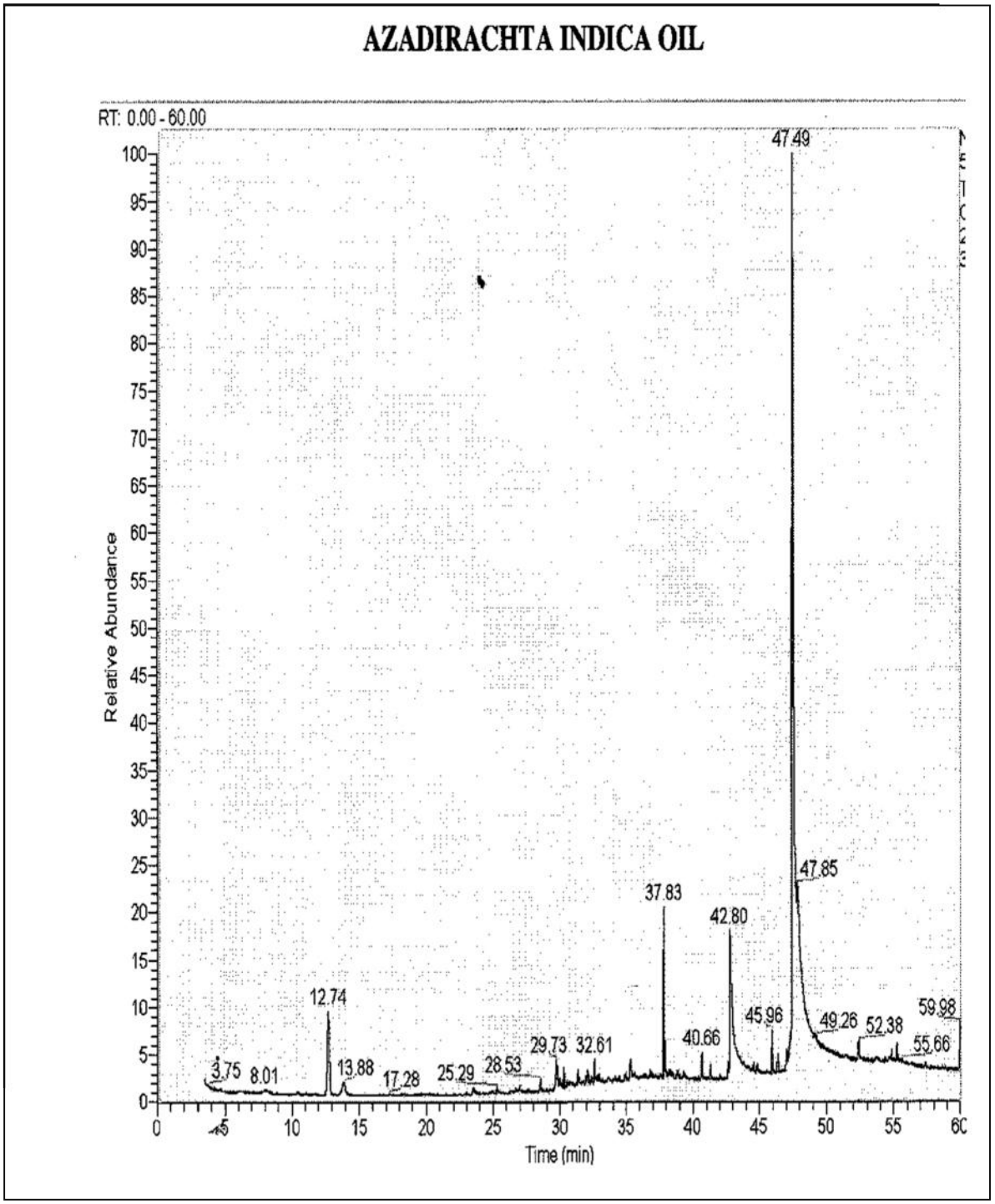
(Figure No. 1) GRAPH SHOWING *AZADIRACHTA INDICA* OIL ACTIVE INGREDIENT PEAKS



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(Figure No. 2) **IMAGE OF CLAVENGER'S APPARATUS**

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(Figure No. 3) Graph showing *Azadirachta indica* oil volatiles effect on mean egg laid / mean egg hatchability of breeding pairs who immature stages were exposed to neem leaf oil volatiles in *Corcyra cephalonica*