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Research Article

EFFECT OF *CALLISTEMON LANCEOLATUS* (MYRTACEAE) LEAF OIL VOLATILES ON EGG HATCHABILITY OF *CORCYRA CEPHALONICA* AND ITS GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

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ABSTRACT
Callistemon lanceolatus (Myrtaceae) plant claimed for the management and control of rice- moth *Corcyra cephalonica* an insect pest of stored commodities. When freshly laid eggs were exposed to *Callistemon lanceolatus* oil volatiles for 12, 24, 48, and 72 h with 20, 40, 80 or 160 µl of oils, a marked decline in egg hatchability in this pest was seen after exposure period. The oil having insecticidal properties which was extracted by Clevenger apparatus in the laboratory by hydro-distillation process, subjected to Gas chromatography-mass spectrometry technique. The presence of active ingredients of *Callistemon lanceolatus* oils obtained at 0.001 % yield by hydro-distillation mainly consisted of 1, 8 Cineole*62.25 %; Alpha-terpineol*8.73; D-limonene*8.40 %; Alpha Pinene*7.03 %; Alpha Phellendrene*2.85 %; Linalool*0.67 % etc. The result offers a platform for using *Callistemon lanceolatus* leaf oils for control and management of rice moth *Corcyra cephalonica* in godowns, warehouses around the world.
Keywords: Clevenger apparatus, (GC-MS) Gas chromatography-mass spectrometry, *Callistemon lanceolatus*, *Corcyra cephalonica.*

INTRODUCTION

With the help of gas chromatography-mass spectrometry on the leaf of *Callistemon lanceolatus* the large number of food/agricultural samples that must tested to control the pest. *Callistemon* is a genus of 34 species of shrubs in the family Myrtaceae, *Callistemon* species are commonly referred to as bottlebrushes because of their cylindrical, brush like flowers resembling a traditional bottle brush. They are found in the regions of India. The extraction of oil from different parts of *Callistemon lanceolatus*[6,12,16,21,22,34,37] plants is reported. So, the extraction of *Callistemon lanceolatus* oil from *Callistemon* leaf was done in laboratory conditions. We have taken *Callistemon lanceolatus* leaves from nearby region of D.D.U. Gorakhpur University Gorakhpur, Uttar Pradesh, India. Selected leaves were plucked out from plant and washed properly with water then oil extraction was done with the help of Clevenger Apparatus by hydro-distillation process. *Callistemon lanceolatus*[3,5,10,17,18] leaves and their products are biodegradable and beneficial for the society in many ways. We use the *Callistemon lanceolatus* leaves oil volatiles for *Corcyra cephalonica*, which act as pesticides as well as non toxic and safe for the environment. It actively reduced the egg hatchability larval development and adult emergence. It affects all stages in life cycle of this pest under the laboratory condition as well as in warehouses and households[27,35,36,39]. Information is available pertaining to specified plant components affect[1,7,8,24-26,28-33] on the insect reproductive potential and egg hatchability. The analysis of the leaf of *Callistemon lanceolatus* oil volatile carries some active ingredients which act as pesticides. The fact that during production of these oils there is a large amount of highly concentrated matrix produced that causes difficultly for pesticide analysis. This highly complex matrix is known for its high background during GC-MS analysis and especially for high boiling point components. Therefore, it was thought desirable to ascertain the impact of action of active ingredient of *Callistemon lanceolatus* oil volatiles on different stages of *Corcyra cephalonica*, for a stipulated period, in terms of eggs laid and their hatchability of such individuals are analyzed at laboratory conditions. Therefore, it was thought desirable to ascertain the impact of *Callistemon lanceolatus* (Myrtaceae) leaves oil volatiles on percent hatchability and its GC-MS analysis. GC-MS analysis was done at N. B. R. I. Lucknow, Uttar Pradesh, India as per[2,4,9,11,13-15,19,20,40,41] for identification of active ingredients of extracted oil. It is believed that the characterized active ingredient will be proved as an asset for the management of stored grain pests in households and warehouse.

MATERIAL AND METHODS

Culture of *Corcyra cephalonica* in laboratory condition

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 *Callistemon lanceolatus* and the parameters chosen to assess their impact on reproductive potential of the pest was similar as are outlined[27,33].

Extraction of *Callistemon lanceolatus* leaf oil by Clevenger apparatus

Fresh leaves of *Callistemon lanceolatus* plant were taken in two liter of oval flask of Clevenger Apparatus. For hydro-distillation clean distilled water was used for heating 6-7 h at 42 to 86˚C in the laboratory shown in Figure 3. The volatile material was carried away in the steam through tubes and then cooled in condensation chamber. The volatile oil was then removed from the top of the hydrosol by separating funnel. During this process compounds are not destroyed by heat. Hydro-distillation needs large amount of plant material and the time for extraction (process take around 3.5-4.5 hours) was similar as are outlined by[38] then the oils sample was used for hatchability experiment and subsequently send for characterization of active ingredient by Gas-chromatographic and mass-spectrometry (GC-MS) test at N.B.R.I. Lucknow, India.

Egg exposure to oil

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 *Callistemon lanceolatus* oils separately. This experimental setup was kept in a glass chamber having 30 cm diameter and 13 cm height from inside. For each experimental regimen five replicates were kept. In first experiment after 12 hours, in second experiment after 24 hours, in third experiment after 48 hours and in fourth experiment after 72 hours, the impregnated paper discs were removed and replaced after 24 h as per requirement, and then eggs were shifted from odorous to normal environment, wherein their hatchability was monitored daily[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 suitable statistical analysis[23].

GC/MS analysis of *Callistemon lanceolatus* oil

GC-MS for samples of essential oils of *Callistemon lanceolatus* was done at National Botanical Research Institute (Council of Scientific and Industrial Research) Post Box No: 436, Rana Pratap Marg; Lucknow, India. NABL-Accreditated, Central Instrumentation Facility; Reference No: NBRI/CIF/288/2012; 24.07.2012- Instrument name GCMSDSQ-II (Thermo Scientific); Column was taken of TR-50 MS, 30 m x 0.25 mm ID, 0.25 µm film; Oven having temperature 500°C (5 minutes) to 2500°C at 40°C/min, 2500°C (5 minutes); Inlet is taken of 0.5 µl, 2500°C, split 50:1; the Carrier was taken i.e. Helium to analyze the leaf oil of *Callistemon lanceolatus* which was extracted by Clevenger apparatus with hydro-distillation in laboratory conditions.

Table 1: Percent value of *Callistemon lanceolatus* oil Active ingredients by GC-MS

S. No.	Compound Name	Area %
1	Bezylicosanoate	0.03 %
2	Alpha Pinene	7.03 %
3	Beta-pinene	0.39 %
4	Alpha Phellendrene	2.85 %
5	D-limonene	8.40 %
6	1,8 Cineole	62.25 %
7	Linalool	0.67 %
8	Trans-Pinocarveol	0.18 %
9	Terpinen-4-ol	1.60 %
10	Alpha-terpineol	8.73 %
11	Nerol	0.36 %
12	Caryophyllene	0.54 %
13	Eugenol	0.42 %
14	Hexadecane	0.46 %
15	Durohydroquinone	0.65 %
16	Caryophyllene oxide	0.87 %
17	Gibberelic acid	0.04 %
18	Levomepromazine	0.03 %

Table 2: Mean value of eggs Hatchability of *Corcyra cephalonica* followed by their programmed exposure to the different concentration of *Callistemon lanceolatus* oil volatiles for 12 h, 24 h, 48 h, 72 h

Experimental regimen (oils in µl)	Time of egg Exposure			
	12 H	24 H	48 H	72 H
0(control)	92.800 ± 1.198	92.000 ± 0.835	89.800 ± 0.799	90.600 ± 1.206
20 µl	88.600NS ± 0.399	86.000* ± 2.254	78.000** ± 0.547	75.600** ± 2.416
40 µl	79.400** ± 1.658	75.600** ± 2.132	70.200** ± 1.390	68.800* ± 1.390
80 µl	68.200** ± 3.210	68.000** ± 2.276	62.000** ± 2.341	58.600** ± 2.223
160 µl	51.000** + 0.706	48.400** ± 0.245	45.000** ± 1.578	41.400** ± 1.433
Mean	76 ± 1.422	74 ± 1.548	69 ± 1.331	67 ± 1.734
LSD 1 %	7.015	7.111	5.935	7.260
LSD 5 %	5.144	5.214	4.352	5.323

Values are mean ± SE of five replicates, Values in parenthesis are percent change with control taken as 100 percent, NS not significant, *significant (P < 0.05) and **significant (P < 0.01) when treated groups were compared with controls, Data analyzed through Student's t-test

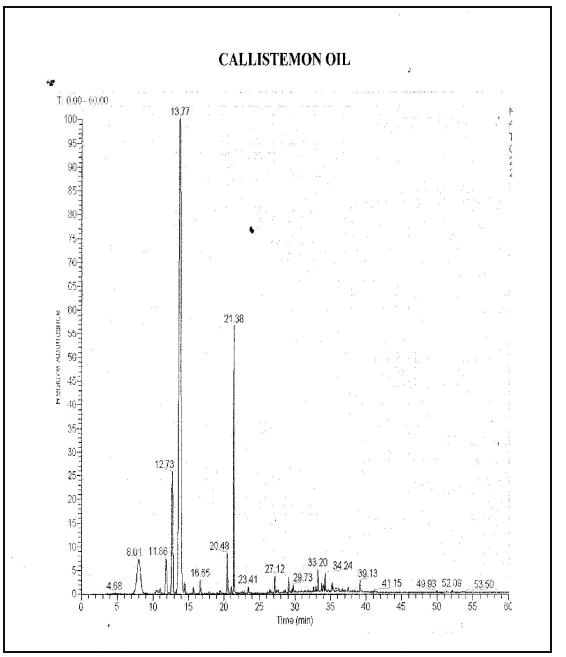
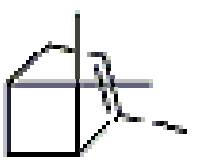
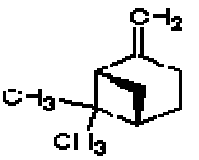
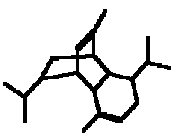
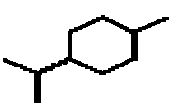


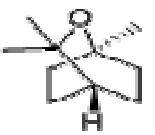
Figure 1: Chromatogram of *Callistemon lanceolatus* leaf oil

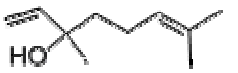

Alpha-pinene **$C_{10}H_{16}$**

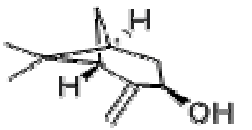

Beta-pinene **$C_{10}H_{16}$**

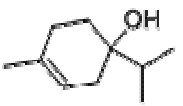

Alpha Phellendrene **$C_{20}H_{32}$**

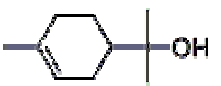

D-limonene **$C_{10}H_{16}$**

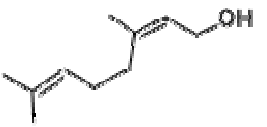

1-8 Cineole **$C_{10}H_{18}O$**

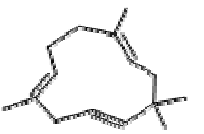

Linalool **$C_{10}H_{18}O$**

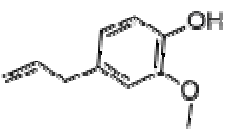

Trans-Pinocarveol **$C_{10}H_{16}O$**


Terpinen-4-ol **$C_{10}H_{18}O$**

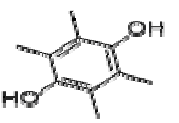

Alpha-terpineol **$C_{10}H_{18}O$**

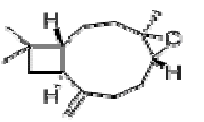

Nerol **$C_{10}H_{18}O$**

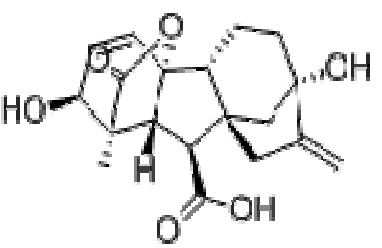

Caryophyllene **$C_{15}H_{24}$**


Eugenol **$C_{10}H_{12}O_2$**


Hexadecane **$C_{16}H_{32}$**


Durohydroquinone **$C_{10}H_{14}O_2$**


Caryophyllene oxide **$C_{15}H_{24}O$**


Gibberelic acid **$C_{19}H_{22}O_6$**

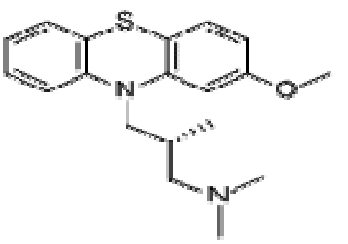

Levomepromazine **$C_{23}H_{28}N_2O_5S$**

Figure 2: Structure of Active ingredients of *Callistemon lanceolatus* Oil by GC-MS

Figure 3: Image of Clevenger Apparatus

RESULT AND DISCUSSION

Action of *Callistemon lanceolatus* leaf oil volatiles affect percent egg hatchability variably in rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: pyralidae)[36,5] when freshly laid eggs were exposed to these volatiles for a time duration of 12, 24, 48 and 72 h separately with different oils concentrations, a marked decline in egg hatchability was recorded after exposure of 24 h, 48 h and 72 h with 40, 80 or 160 µL of *Callistemon lanceolatus* leaf oils shown in Table 2. Although 20 µL exposure is not effective at any exposure time selected. Presumably, the volatiles liberated from this oils/extract diffused into the eggs, like air[5] through the shell or they entered into them via aeropyles – tiny holes in the chorion connected with respiration of embryos[17]. 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. Same types of result in percent egg hatchability was noticed at varying degrees of exposure period of volatiles[26,27]. A similar insect were likewise interacted with volatile of eucalyptus oil causes total inhibition of hatchability, however, occurred if eggs were uninterrupted exposed to eucalyptus oil vapour for 4 days. All such marked adverse consequences did not happen with eggs affected by cedar wood oil, unlike in *C. cephalonica*[29,32].

Gas chromatography-mass spectrometry (GC-MS)

Gas chromatographic and mass-spectrometry (GC-MS) is done for the characterization of active ingredients from the *Callistemon lanceolatus* leaf oil extracted in laboratory was analyzed by GC-MS test Column TR-50 MS. The retention time and chemical composition of essential oils of *Callistemon lanceolatus* are presented in Figure 1. Eighteen volatile constituents, representing the total composition, were identified in the leaves oils Table 1 and Figure 2. The most abundant components found in *Callistemon lanceolatus* leaf oil were 1, 8 Cineole*62.25 %; Alpha-terpineol*8.73; D-limonene*8.40 %; Alpha Pinene*7.03 %. Several chromatographic techniques are being applied to describe for the determination of active ingredients in various plants extract,[2,4,9,11,13-15,19,20,39,40.]

Identification of the components

Component identification was carried out by the Non Host plant volatile components of the leaf of *Callistemon lanceolatus.* It mainly consisted of Bezylicosanoate*0.03 %; Alpha Pinene*7.03 %; Beta-pinene*0.39 %; Alpha Phellendrene*2.85 %; D-limonene*8.40 %; 1,8 Cineole*62.25 %; Linalool*0.67 %; Trans-Pinocarveol*0.18 %; Terpinen-4-ol*1.60; Alpha-terpineol*8.73; Nerol*0.36 %; Caryophyllene*0.54 %; Eugenol*0.42 %; Hexadecane*0.46 %; Durohydroquinone*0.65 %; Caryophyllene oxide*0.87 %; Gibberelic acid*0.04 %; Levomepromazine*0.03 %.

CONCLUSION

Eighteen Volatile compounds were identified after the GC-MS and the major constituents in the leaf was 1,8 Cineole percentage than Alpha-terpineol. The oil showed activities against some insect pest, bacteria and fungi. Trace analysis of pesticides in *Callistemon lanceolatus* oils presents a significant analytical challenge due to the heavy matrix effects experienced. Highly selective and sensitive GC-MS/MS systems permit a dilution approach to be taken when analyzing *Callistemon lanceolatus* oils which brings a fast result to the laboratory. This yield of the plants essential oil that has been studied is important for the management of *Corcyra cephalonica* insect pest globally.

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REFERENCES

1. Amzad M Hossain and Syed M Salehuddin. Analytical determination of nicotine in tobacco leaves by gas chromatography–mass spectrometry Arabian Journal of Chemistry 2013; 6: 275–278.
2. Beckett AH, Triggs EJ. Gas chromatographic-mass spectrometric analysis Nature 1996; 211: 1415–1418. http://dx.doi.org/10.1038 /2111415a0
3. Berger BM, O Yegen, C Cakir. J. Agric. Food Chem. 1997; 45: 4821. http://dx.doi.org/10.1021/jf970110y
4. Burrows IE, PJ Corp, GC Jackson. Page, R.E.I. Analyst 1971; 96: 81–84. http://dx.doi.org/10.1039/an9719600081
5. Chapman RF. The Insects: Structure and Function - Hong Kong, ELBS and Hodder and Stoughton; 1982. p. 919.
6. Cleber J *et.al*. Chemical composition and antibacterial activities from the essential oil of Myrtaceae species planted in Brazil; 2010. Vol. 33(1).
7. Derwich E, Z Benziane, R Chabir, R Taouil. *In Vitro* Antibacterial activity and GC/MS analysis of the essential oil extract of leaves of *Rosmarinus officinalis* grown in Morocco, Int J Pharm Pharm Sci 2011; 3: 8995.
8. Derwich E, Z Benziane, R Taouil, O Senhaji and M Touzani. Aromatic Plants of Morocco: GC/MS analysis of the essential Oils of leaves of *Mentha piperita.* Advances in Environmental Biology 2010; 4(1): 80-85.
9. Dow I, K Hall J. Chromatogr 1978; 153: 521–525. http://dx.doi.org/ 10.1016/S0021-9673(00)95513-4
10. Dr Hayssam M Ali, Mohamed ZM Salem *et al*. Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: Antibacterial and antioxidant activities, total phenolic and flavonoid contents. Asian pacific Journal of tropical medicine 2013; 785-787.
11. Feyerabend C, T Levitt, MAH Russell. J. Pharmacol 1995; 27: 434–440. http://dx.doi.org/10.1111/j.2042-7158.1975.tb09474.x
12. Franzios G, M Mirotsou, E Hatziapostolou. J. Kral, ZG Scouras, P Mavragani Tsipidou. J. Agric. Food Chem 1997; 45: 2690. http://dx.doi.org/10.1021/jf960685f
13. Garg S, GP Talwar, SN Upadhyay. Immuno contraceptive activity guided fractionation and characterization of active constituents plants extracts. Journal of Ethnopharmacology 1998; 60(3): 235-246. http://dx.doi.org/10.1016/S0378-8741(97)00157-8
14. Gue Don, DJ BP Pasquier. J. Agric. Food Chem 1994; 42: 679.
15. Isaac PE, MJ Rand. Nature 1972; 236: 307–311. http://dx.doi.org 10.1038/236308a0
16. Masada Y. Analysis of essential oils by Gas Chromatography and Mass Spectrometry, Wiley, New York; 1976. p. 13–22.
17. Mill PJ. Structure and Physiology of the respiratory system. In: Comprehensive Insect Physiology Biochemistry and Pharmacology. Pergamon Press, Oxford 1985; 3: 517-593.
18. Miyazawa M, H Watanabe, H Kameoka. J. Agric. Food Chem 1997; 45: 677. http://dx.doi.org/10.1021/jf960398b
19. Moore MJ *et al*. J. Chromatogr 1993; 645: 273–278. http://dx.doi.org/ 10.1016/0021-9673(93)83387-8
20. Mousa S, V Loon, Houdi Crooks. J. Chromatogr 1985; 347: 405–410. http://dx.doi.org/10.1016/S0021-9673(01)95514-1
21. Munteanu LS. In: Dacia (Ed.) Plante Medicinale Si Aromatice Cultivate I˜N Romania, Cluj-Napoca; 1990. p. 154– 166.
22. Opeoluwa O *et al*. Chemical composition and antibacterial activity of the essential oil of *Callistemon citrinus* and *Callistemon viminalis* from South Africa; molecules 2009; 14: 1990-1998.
23. Paterson DD. Statistical Technique in Agriculture Research MC Graw Hill New York; 1939.
24. Pathak PH, A Pandey, S Pandey. Efficacy of combined action of garlic extracts and mint oil volatiles against rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae). Trends in Biosciences 2010; 3(2): 176-180.
25. Pathak PH, S Pandey. Impact of combined action of Neem and Eucalyptus oil volatiles on different stages of *C. cephalonica* (Lepidoptera: Pyralidae). Journal of Applied and Natural Science 2011; 3(2): 247-252.
26. Pathak PH, SS Krishna, HC Mani. Effect of certain volatile chemicals and other factor on the reproductive Biology *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae). Annals of Entomology 1993; 11(2): 59-65.
27. Pathak PH, G Gurusubramanian, SS Krishna. Changes in post embryonic development and reproduction in *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) as a function of Eucalyptus and Neem oil vapour action during or on adult. Z. Ang. Zool 1994; 80: 345-352.
28. Pathak PH, Kumari Kiran. Effect of citrus oil volatiles on the reproductive biology of the rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera pyralidae). J. Pharm. Sci Innov 2013; 2(6): 2277-4572.
29. Pathak PH, SS Krishna. Effect of odors of certain plant oils or leaves on the egg hatchability in *C. cephalonica* (Stainton) (Lepidoptera: Pyralidae) Mitt. Zool. Mas. Berl 1992; 68(1): 139-141. http://dx.doi.org/ 10.1002/mmnz.19920680108
30. Pathak PH, SS Krishna. Neem seed oil a capable ingredient to check rice moth reproduction. Z. Ang ent 1985; 100: 33 - 35.
31. Pathak PH, SS Krishna. Reproductive eficiency in *Earias vitella*. (F.) (Lepidoptera: Noctuidae) affected by oil vapour. Appl. Ent. Zool 1986; 21(2): 347-348.
32. Pathak PH, SS Krishna. The influence of odors of certain botanical components or an organic solvent dichloromethane during breeding on the reproductive efficiency of *Earias vitella* (F.) (Lepidoptera: Noctuidae). Uttar Pradesh J. Zool 1987; 7(2): 175-179.
33. Pathak PH, SS Krishna. Postembryonic development and reproduction in *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) on exposure to eucalyptus and neem oil volatiles. J. Chemical Ecology 1991; 17(12): 2553-2558. http://dx.doi.org/10.1007/BF00994602
34. Perkins KA. Pharmacol. Biochem. Behav 1999; 64: 295–299. http://dx.doi.org/10.1016/S0091-3057(99)00085-4
35. Pierre MD Jazet *et al*. Correlation between chemical composition and antifungal properties of essential oils of *Callistemon rigidus* and *Callistemon citrinus* of Cameroon against *Phaeoramularia angolensis*. J. of med. Plant research vil 2009; 3(1): 009-015.
36. Piltz H. *Corcyra cephalonica* (Stainton): In: J Kranz, H Schmutterer and W Koch (eds.) Diseases, pests and weeds in tropical crops. Verlag Paul Preey, Berlin and Hamburg; 1977. p. 439 – 440.
37. Refsgaard HHF, BR Nielsen, LH Skibsted. J. Agric. Food Chem 1995; 43: 1177. http://dx.doi.org/10.1021/jf00053a011
38. Satish K, A Sahoo. Extraction of essential oil using steam distillation. A thesis submitted in partial fulfillment of the requirements for the degree of bachelor of technology in chemical engineering, Department of Chemical Engineering National Institute Of Technology Rourkela; 2010. p. 1-30.
39. Sehnul F. Growth and life cycles in: Comprehensive Insect Physiology. Biochemistry and Pharmacology. (Ed. GA Kerkut and LT Gilbert) oxford, Pergamon Press 1985; 2: 1-86.
40. Unnithan *et al*. GCMS Analysis and antibacterial activity of essential oil of *Callistemon pallidus* of mekell, Ethiopia, Undergraduate Journal of Psychology at Berkeley 2013; 01(03): 42-44.
41. Wilfried A and H Detlev, Hochmuth. Enantio selective gas chromatography in flavor and fragrance analysis: strategies for the identification of known and unknown plant volatiles. Journal of Chromatographic Science 2004; 42.

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