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IMPACT OF *CALLISTEMON LANCEOLATUS* LEAF OIL VOLATILES ON REPRODUCTIVE BIOLOGY OF *CORCYRA CEPHALONICA* (STAINTON) (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

When freshly laid eggs of *Corcyra cephalonica* were exposed to 20, 40, 80 or 160 µl volume of *Callistemon lanceolatus* oil volatiles for 6 or 12 hour duration a significant reduction in percent egg hatchability was recorded only with 80 or 160 µl volume of the oil. When exposure period were increased up to 24, 48 or 72 hour a highly significant reduction in percent egg hatchability was observed at 40, 80 or 160 µl of the oil. The reproductive potential of *C. cephalonica* was affected variably when new born larvae of this pest were reared in a programmed manner with 20, 40, 80 or160 µl oil volatiles of *C. lanceolatus.* When freshly emerged male and female was exposed to *C. lanceolatus* oil volatiles emanating from 20, 40, 80 or 160 µl for 3 hours, there was increase, though not significant, in egg output and egg hatchability of breeding pairs. But when exposure period was increased up to 6 hour there was a significant decreased in reproductive potential of breeding pairs. A significant reduction in Glycogen level, Total lipids, Total protein and Total free amino acids level was recorded in the testes and ovaries of adult moths exposed to oil volatiles action of *Callistemon lanceolatus*, for 6 hours emanating from 20, 40, 80 or 160 µl volume of this oil.

INTRODUCTION

The Rice moth, *Corcyra cephalonica (*Stainton) (Lepidoptera: Pyralidae) is a major pest of stored grain commodities in the tropics (Piltz, 1977). Information is available pertaining to specified plant components effect (Pathak and Krishna, 1985, 1987, 1991, 1992; Mani *et al.*, 1993; Pathak *et al*., 1994; Ansari and Krishna, 1987) on the insect reproductive potential and egg hatchability. However, nothing is known about the changes that are likely to occur in the post embryonic development and reproduction in this insect, by the action of *Callistemon lanceolatus* leaf oil volatiles during rearing or breeding. *Callistemon lanceolatus* (Family: Myrtaceae) commonly known as red bottle brush, is frequently cultivated throughout India in gardens as ornamental plant. The essential oils from leaves are reported to possess antibacterial and antifungal activity (Oyedeji *et al.,* 2009). The antimicrobial, antistaphylococcal, antithrombin, anti inflammatory activity of the plant are well reported in traditional medicine (Chistokhodova *et al.,* 2002; Sudhakar *et al.,* 2004; Saxena and Gomber, 2006; Gomber and Saxena, 2007; Hee JK *et al.,* 2009).

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Therefore, it was thought desirable to ascertain the impact of the *Callistemon lanceolatus* oil volatiles on egg hatchability, embryonic development, reproductive potential and biochemical changes, if any, in ovary and testis of adults of this pest.

MATERIALS 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 (Mishra and Krishna, 1979). Newborn larvae thus obtained, were then allowed to develop singly inside muslin capped glass vials (20 mm diameter, 50 mm. height) unless otherwise stated, on similar dietary medium into moths for eventual use as experimental animal in the various tests included in this study.

Collection of plant material and extraction

The leaves of *Callistemon lanceolatus* were collected from the campus of D.D.U. Gorakhpur University Gorakhpur, Uttar Pradesh. Fresh and mature leaves of *C. lanceolatus* were collected, washed thoroughly with distilled water, hydro

distilled for 6 hours in Clevenger apparatus. A clear dark yellow colour oily layer was obtained on the top of the aqueous distillate, separated and collected in small 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 was kept in another petridish of same diameter, impregnated with 20, 40, 80 or 160 µl of *Callistemon lanceolatus* leaf oil volatiles. This experimental setup was kept in a glass chamber having 30 cm. diameter and 13 cm. height from inside. In first experiment after 6 hours, in second experiment after 12 hours, in third experiment after 24 hours, in fourth experiment after 48 hours, in fifth experiment after 72 hours, the impregnated paper discs were removed and eggs were shifted from vapours to normal environment, where in their hatchability was monitored daily (Pathak *et al.,* 2010; Pathak and Sangita Pandey 2011).

Larval exposure to the oils

Larval exposure was done by the way according to Pathak & Krishna (1991). Three types of exposure regimens were constituted. New born larvae of parents exposed to the leaf oil volatiles of *C. lanceolatus* **(a)** for first 15 days of their lives, **(b)** from 16th day for 15th days, and **(c)** continuous till 30th days and then emerged male and female paired with the opposite sex. These pairs were then monitored to determine reproductive potential (total numbers of egg laid / egg hatchability) (Pathak *et al.,* 2010; Sangita Pandey and Pathak 2008).

Adult exposure to the oils

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 volatiles of this oil were placed (Pathak *et al.,* 2010; Pathak and Sangita pandey 2011). All tests, performed at 27º C ± 2º C and 85 ± 5% r. h. 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 (Paterson, 1939).

BIOCHEMICAL STUDIES

Effect of 6 hour adult exposure of *C. lanceolatus* oil volatiles showed a highly significant reduction in eggs yield and their hatchability, at different volume of 20, 40, 80 or 160 µl. Testes and ovaries of adult moth were excised from laboratory reared unmated males and virgin female individuals, unexposed (control) and exposed to 20, 40, 80 or 160 µl volume of *C. lanceolatus* oil volatiles for 6 hour. The isolating organs were separated from flowed out haemolymph and other adhered visceral materials. These were quickly shifted to separate glass plates and their fresh weight was recorded. Subsequently glycogen levels were estimated according to Anthrone method of Van der Vies (1954). Method of Folch *et al.* (1957) was followed for the extraction of total lipids and its quantitative measurement was carried out by applying the simple charring method of Marsh and Weinstein (1966). Total protein was estimated According to the method of Lowery *et al.* (1951) and total Free amino acids (FAA) was measured according to the method of Spies (1957).

RESULTS AND DISCUSSION

When freshly laid eggs of *C. cephalonica* were exposed to different volumes of *C. lanceolatus* oil volatiles for 6 hour or 12 hour duration, a significant reduction (P<0.01<0.05) in percent egg hatchability was observed with 80 or 160 µl volume out of 20, 40, 80 or 160 µl of oil. While 24, 48 or 72 hour exposure period causes drastic decline (P<0.01) in percent egg hatchability was noticed with 40, 80 or 160 µl volume of oil. It was recorded that 20 µl oil volatiles exposure has no significant effect with any exposure period where as 40 µl *Callistemon* oil volatiles have no effect on percent hatchability with 6 and 12 hour exposure period (Table1).

Table 1. Estimates of Percent hatchability of eggs laid by *C. cephalonica* following their programmed exposure to different volumes of *Callistemon lanceolatus* oil volatile

Volume of oil in µl	Percent hatchability after exposure period				
	6 hour	12 hour	24 hour	48 hour	72 hour
Control (0)	85.6^{a}	85.6^{a}	85.6^{a}	85.6^{a}	85.6^{a}
20	85.0^{a}	84.2^{a}	83.6^{ab}	83.0^{a}	81.8^{a}
40	83.8^{ab}	83.4^{ab}	80.0^{b}	78.0^{b}	75.0^{b}
80	81.0^{b}	80.2^{b}	76.0^{c}	71.0^{c}	56.0^{c}
160	77.0^{c}	75.8^{c}	68.0^{d}	60.0^{d}	51.0^{c}
Mean	82.48	80.64	78.64	75.52	69.88
LSD 5%	3.69	4.13	3.94	4.31	5.61
1%	5.03	5.63	5.37	5.88	7.65

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

Similar type of reduction in percent egg hatchability was observed by Pathak *et al.*, 2010; Pathak and Sangita Pandey, (2011) where these eggs were exposed to different oil concentration for different time duration. G. Nattudurai *et al.* (2012) recorded that camphor and eucalyptus oil when treated individually affected the egg hatchability of *T. castaneum* beetles drastically at higher concentrations viz. 40, 80 µl. Presumably, the volatiles liberated from this oil 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, this oil through their volatiles 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 result presented in table 2 indicates that the higher concentration of oil volatiles and longer time duration, significantly reduces egg output and their hatchability in immature stage in this pest. When new born larvae of *C. cephalonica* exposed with 80 or 160 µl volume of *Callistemon* oil volatiles for first 15 days or from 16th day for 15th days or continuously till 30th days, a severe reduction (P<0.05 or < 0.01) both in egg output and egg hatchability in breeding pairs

Table 2. Estimates of mean eggs laid/eggs hatched by *C. cephalonica*, following their programmed exposure, during their immature stages, to different volumes of *Callistemon lanceolatus* oil volatile, during rearing

Experimental Regimen	Volumes of oil (in µl)							
	20		40		80		160	
	Eggs laid	Eggs hatched	Eggs laid	Eggs hatched	Eggs laid	Eggs hatched	Eggs laid	Eggs hatched
No exposure (Control)	302.4 [a]	293.2 [a]	302.4 [a]	293.2 [a]	302.4[a]	293.2 [a]	302.4[a]	293.2[a]
First 15 days Exposure	300.2 [a]	290.0 [a]	292.0[ab]	284.0[ab]	276.2[b]	265.8 [b]	272.0[b]	262.0[b]
From 16th days for 15 days after hatching	285.8 [a]	277.0[a]	275.6[b]	265.2 [b]	258.4 [b]	246.0 [b]	204.0[c]	195.0[c]
Continuous exposure till 30th day	260.2[b]	248.4 [b]	250.2 [c]	238.0 [c]	220.0 [c]	205.4[c]	185.0 [c]	176.0[c]
Mean	287.15	277.15	280.05	270.10	264.25	252.6	240.85	231.55
LSD 5%	23.74	21.96	22.32	22.47	24.65	23.42	23.59	23.57
1%	32.71	30.26	30.75	30.96	33.97	32.27	32.50	32.48

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

Table 3. Mean number of eggs laid and their hatchability in *C. cephalonica* following their programmed exposure to the different volumes of Callistemon lanceolatus oil volatile, during their adult stage for 3 hours or 6 hours, after emergence

Experimental regimen (oils in µl)	Time of adult Exposure			
	3 hours		6 hours	
	Mean Eggs laid (±SE)	Mean Eggs hatched (±SE)	Mean Eggs laid (±SE)	Mean Eggs hatched (±SE)
0 (control)	300.2 ±6.52[NS]	292.2 ± 6.52[NS]	300.2± 6.52 [a]	292.2± 6.52 [a]
20	292.0 ± 6.87[NS]	284.0 ± 7.64[NS]	290.0 ± 8.18[a]	280.4± 7.87[a]
40	287.0± 8.19[NS]	278.0 ± 8.22[NS]	265.4 ± 7.67 [b]	255.6± 8.22[b]
80	317.0± 6.98[NS]	309.4 ± 6.53[NS]	234.2 ± 8.10 [c]	223.0± 6.22[c]
160	318.0 ± 6.68[NS]	311 ± 7.10 [NS]	194.0 ± 6.38 [d]	180.0± 7.50[d]
Mean	303.44	294.92	256.76	246.24
LSD at 5%	21.54	21.27	21.79	21.50
LSD at 1%	29.38	29.01	29.73	29.33

Mean followed by different letters differs significantly with control at 5% or 1% by Least Significant Difference (LSD) test. SE = Standard Error

was recorded, but with 40 µl volume of this oil volatiles, when acted for first 15 days had no significant effect, while from 16th day for 15th days and continuously till 30th days there was a significant reduction (P<0.05 or P <0.01) both in eggs laid and eggs hatched. While with lower dose of 20 µl for first 15 days or from 16th day for 15th days there was no significant effect with control except continuous exposure for 30 days (P<0.01) which was dose and time depended. A similar results in moths reproductive potential was reported by Sangita Pandey and Pathak (2008), Pathak *et al.* (2010) in breeding pairs, with Neem and *Eucalyptus* oil or Garlic extract and Mint oil volatiles. When freshly emerged males and females were exposed to *C. lanceolatus* oil volatiles emanating from different doses for 3 hours there was increase, though not significant, in egg output and egg hatchability of breeding pairs but when the exposure period was increased for 6 hours, a significant reduction (P <0.01) in egg yield and their hatchability was observed at 40, 80 or 160 µl volume of the oil (Table 3). Pathak *et al.* (2010), Pathak and Sangita Pandey (2011) also recorded reduction in reproductive potential of *C. cephalonica* with different oils volatiles. The outcome of such fall in the reproductive potential is possibly due to some kind of spermicidal effect of the volatiles action of *Callistemon* 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 behavior of lepidopterans are still limited (Engelmann, 1970; Feeny et al., 1983; 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).

For 6 hour exposure of adults to *Callistemon* oil volatiles emanating from 40, 80 or 160 µl volumes of oil testes Glycogen level was reduced significantly (P<0.05 or < 0.01) up to 55.51%, 46.45%, and 41.33% of control and in ovaries of females glycogen level were reduced significantly (P<0.05 or < 0.01) up to 51.59%, 43.28%, and 37.53% of control. While with 20 µl volume of this oil volatiles decrease in Glycogen level both in testes and ovaries of adult moth were not significant with control (Table 4). The reduction in the level of glycogen in the testes may be due to inhibition of synthesis and / or storage of glycogen in the testicular cells which may create energy crisis, thereby adversely affecting the spermatogenesis. A sharp decline in the glycogen content of ovaries due to *Callistemon* oil volatiles, which presumably, adversely, affects the synthesis of glycogen in the oocytes, inactivating glycogen synthetase and / or by blocking the passage of raw materials for glycogen synthesis into the oocytes (Engle's and Drescher 1964 ; Bonhag, 1956; Ramamurty, 1968). Total lipids level was reduced significantly (P< 0.01) up to 75.21%, 60.56%, and 50.59% of control in testes and up to 65.41%, 38.22%, and 30.94% of control, in ovaries of adult female moths at the exposure of different volume viz., 40, 80 or 160 µl of *Callistemon* oil volatiles, while 20 µl volume of this oil volatiles total lipid level reduced both in testes and ovaries of adult moths but not significant with control (Table 4).

A pronounced decline in the lipid level of testes of *C. cephalonica* was noticed with the increasing volumes of *Callistemon* oil volatiles. Lipids has role in bioenergetics and as component of biological membrane. Lipids serves primarily for the storage and liberation of metabolic energy and other perform a structural role in biological membrane. Lipid oxidation may provide energy for metabolic maintenance

Table 4. Changes in Glycogen level, total Lipids, total Protein and total Free Amino Acids (FAA) level (in µg / mg) in the testis and ovaries of adult males and females of *C. cephalonica*, unexposed (control) /exposed (treated) to the action of *Callistemon lanceolatus* oil volatile for 6 hours

Quantity of oils (in µl)	Glycogen Level		Total Lipids		Total Protein		Total Amino Acids	
	Testis	Ovary	Testis	Ovary	Testis	Ovary	Testis	Ovary
0	2.54 ± 0.34	2.19 ± 0.20	44.1 ± 0.74	69.36 ± 0.80	36.06 ± 0.74	43.98 ± 1.21	19.16 ± 0.87	22.17± 0.96
(Control)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)
20	2.18 ± 0.46^{NS}	1.85± 0.42^{NS}	42.96 ± 0.62^{NS}	66.61 ± 0.91^{NS}	35.41 ± 1.0^{NS}	41.07 ± 1.0^{NS}	20.67 ± 0.66^{NS}	24.61 ± 0.75^{NS}
	(85.82)	(84.47)	(97.41)	(96.03)	(98.19)	(93.38)	(107.88)	(111.00)
40	1.41 ± 0.18*	1.13± 0.27*	33.17 ± 0.97**	45.37 ± 0.57**	30.51 ± 0.73**	37.35 ± 0.86**	24.61 ± 0.80**	28.94 ± 0.75 **
	(55.51)	(51.59)	(75.21)	(65.41)	(84.60)	(84.92)	(128.44)	(130.53)
80	1.18± 0.17**	0.948 ± 0.12**	26.71 ± 0.59**	26.51± 0.81**	25.87 ± 0.57**	24.41 ± 0.69**	31.01 ± 0.94**	37.59 ± 0.66**
	(46.45)	(43.28)	(60.56)	(38.22)	(71.74)	(55.50)	(161.84)	(169.55)
160	1.05± 0.08**	0.822 ± 0.13**	22.31 ± 0.65**	21.46 ± 0.78**	16.17± 0.81**	15.19± 0.86**	34.62± 0.68**	45.67 ± 0.87**
	(41.33)	(37.53)	(50.59)	(30.94)	(44.84)	(34.53)	(180.68)	(205.99)

- 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.

during stress condition. (Downer, R., 1972 ; Downer and Mathews,1976 ; Downer, R., 1978 ; 1981 a ; 1985 ; Gilbert and Chino ,1974 ; Beenakkers *et al.*, 1981). Pronounced loss in lipid content in ovaries of adult females held in *Callistemon* oil volatiles regimen can presumably be considered as a reflection of serious dislocation brought about by such treatment, in the physiological operations connected with the movement of lipids, which under normal circumstances, occur from fat body to ovary via haemolymph for the purpose of vitellogenesis in the reproductive life of a female insect (Dean *et al*., 1985; Keeley, 1985; Kunkel and Nordin, 1985; Mullins, 1985; Bownes, 1986; Rabbe , 1986; Bhola and Shrivastava , 1986 ; Shrivastava and Krishna, 1992; Das *et al*., 1993). Total protein level was reduced significantly (P< 0.01) up to 84.60%, 71.74%, and 44.84% of control, in testes of adult males and up to 84.92%, 55.50%, and 34.53% of control level in ovaries of adult females at the exposure of different volume 40, 80 or 160 µl of *Callistemon* oil volatiles, while 20 µl volume of this oil volatiles total protein level reduced both in testes and ovaries of adult moths but not significant with control (Table 4). The decrease in protein level observed in present investigation may be due to their degradation and possible utilization for metabolic purposes. Decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery. The quantity of protein is depends on the rate of protein synthesis or on the rate of its degradation. (Pathak *et al*, 2011).

After 6 hours adult exposure to the *Callistemon* oil volatiles, total free amino acids level was induced significantly (P< 0.01) up to128.44%, 161.84% and 180.68% of control in testes and up to 130.53%, 169.55% and 205.99% of control, in ovaries, emanating from 40, 80 or 160 µl volume of this oil, while 20 µl volume of this oil volatiles total free amino acid level induced both in testes and ovaries of adult moths but not significant with control (Table 4). It was interesting to notice that the total free amino acids (FAA) level in the testes and ovaries of adult moths, which were exposed in varying volume of *Callistemon* oil volatiles for 6 hours, was found to be increased significantly, unlike glycogen, lipids and proteins level with the increasing volume of this oil. The increased FAA level suggests tissue damage probably due to increased proteolytic activity under volatile stress. However, the elevated levels of total FAA can be utilized for energy production by feeding them into the TCA cycle through amino transferase reaction.

The increase in the level of FAA can also be attributed to the synthesis of Amino Acids in addition to their elevation by protein hydrolysis. A third possibility for increased FAA level might be due to transamination and animation of keto acids.

Conclusion

In this study, leaf oil volatile of *C. lanceolatus* was found to be effective on egg hatchability, postembryonic development and reproductive potential of *Corcyra 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. The activity of plant oils mainly depends upon the major volatile components they possess. We conclude that leaf oil volatile of *C. lanceolatus* is a good source of insecticidal compounds. Its future lies in isolation of bioactive molecules and their characterization by advanced analytical techniques.

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