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EFFECTS OF CITRUS OIL VOLATILES ON THE REPRODUCTIVE BIOLOGY OF THE RICE MOTH, *CORCYRA CEPHALONICA* (STAINTON) (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT
Rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) is an important pest of stored food such as cereals, cereal products, oil seeds pulses dried fruits nuts and spices. A sharp reduction in percent egg hatchability was noticed at 72 hours exposure of the volatiles in comparison to 6, 12, 24 or 48 hours exposure period to the vapor action of 20, 40, 80, or 160 µl volume of Citrus oil. There was a marked decline in egg output and egg hatchability in reproductive pairs, whose larval development for the first 15 days/from 16th day till 30 days/continuous till 30 days took place in an environment emanating from 20, 40, 80 or 160 µl volume of this oil. Appreciable reduction in egg laying capacity and egg viability was noticed at 6 hour exposure of the volatiles in comparison to 3 hour exposure period to the action of different volumes of this oil. There was significant reduction in Glycogen level, Total lipids, Total protein and Total free amino acids level in testes and ovaries of adults following their exposure to the action of selected volatiles for 6 hours, emanating from 20, 40, 80 or 160 µl volume of Citrus oil.
Keywords: Reproductive potential, *Corcyra cephalonica*, Egg hatchability, Post embryonic development, *Citrus* sp.

INTRODUCTION

The Rice moth, *Corcyra cephalonica (*Stainton) (Lepidoptera: Pyralidae) is a major pest of stored grain commodities in the tropics[1]. Citrus fruits are mainly used by juice processing industries, while the peels are generally wasted. The peel of *Citrus* fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants[2]. It has been used as an anti-diabetic[3], antimicrobial[4], antifungal[5], Hypotensive agent[6], antioxidant[7-8], carminative, insect repellent, antibacterial, larvicidal, antiviral, uricosuric, anti yeast, anti hepatotoxic and anti mutagenic agent[9]. Information is available pertaining to specified plant components effect[10-16] 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 Citrus oil volatiles during rearing or breeding. Therefore, it was thought desirable to ascertain the impact of oil volatiles from the peels of citrus (*Citrus* sp.) on embryonic development, egg hatchability and reproductive potential of this pest.

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[17]. 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.

Plant material collection and extraction

The peels of *Citrus* sp. were collected from the fruit juice shops near the campus of D.D.U. Gorakhpur University Gorakhpur, Uttar Pradesh, India. The volatiles oil was extracted from peel of fruits. After cleaning with water peels were subjected to water-distillation through Clevenger apparatus. A clear light yellow color 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 were kept in another petridish of same diameter, impregnated with 20, 40, 80 or 160 µl of *Citrus* oil. 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 odorous to normal environment, where in their hatchability was monitored daily till 7 days [18-19].

Larval exposure to the oils

The general layout of the experiments, the methodology adopted to treat the larvae with vapor action of this oil and the parameters chosen to assess their impact on postembryonic development, adult emergence and /or reproductive potential of this pest was similar to those as outlined by Pathak and Krishna[12]. Three types of exposure regimens were constituted larvae of parents were exposed to the vapor of *Citrus* sp. oil (a) for first 15 days of their lives, (b) from 16th day till 30th day, and (c) continuous till 30th day, in a programmed manner and after emerged adults then paired with the opposite sex. These pairs were then monitored to determine reproductive potential (total numbers of egg laid / egg hatchability).

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 / cm^2) and tightly covered at the top with a muslin cloth fastened by elastic bands, served as a specially designed experimental chamber, in which the odors of this oil were placed[18-19]. All tests, performed at 27 ºC ± 2ºC 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[20].

Biochemical Studies

Testes and ovaries were taken out from laboratory - reared unmated males and virgin female individuals, unexposed (control) / exposed to 20, 40, 80 or 160 µl volumes of Citrus oil volatiles for 6 hours. The tissues of gonads, after isolating them from flowed out haemolymph and other adhered visceral materials, were subsequently, quickly shifted to separate glass plates for obtaining their fresh weight. Glycogen was measured, according to Anthrone method of Van Der Vies[21]. Method of Folch *et al*[22] was applied for the extraction of total lipid, and its quantitative measurement was carried out by applying the simple charring method of Marsh and Weinstein[23]. Total protein was estimated according to method of Lowery *et al.*[24] and total Free amino acids (FAA) was measured according to the method of Spies[25].

RESULTS AND DISCUSSION

When freshly laid eggs of *C. cephalonica* were exposed to the action of Citrus oil volatiles, for 6 hour duration, a significant reduction in percent egg hatchability was observed at 160 µl volume only ($P < 0.05$) out of 20, 40, 80 or 160 µl volume of oil. However, if the exposure period was increased for 12 hours duration a significant reduction in percent egg hatchability was observed at 80 or 160 µl volume ($P < 0.05$ or < 0.01). When exposure period was increased 24 hours a significant decline were noticed at 40, 80 or 160 µl volume ($P < 0.05$ or < 0.01) while, severe reduction in percent hatchability was noticed at 40, 80 or 160 µl volume of above mentioned volatiles, for 48 hours or 72 hours exposure ($P < 0.05$ or < 0.01) (Table 1). Reduction in hatchability was observed by Pathak *et al*[18] and Pathak and Sangita Pandey[19], when eggs of *C. cephalonica* was exposed to the combined action of Garlic extract and Mint or Neem and Eucalyptus oil volatiles for 3, 6, 12 or 24 hours exposure at 40, 80 or 160 µl volume of oils. Presumably, the volatiles liberated from these oils diffused into the eggs, like air[26], through the shell or they entered into them via aeropyles - tiny holes in the chorion connected with respiration of embryos[27-28]. 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 results were obtained by Pathak and Krishna[29], when eggs of *Earias vitella* – another, though taxonomically unrelated, insect were like- wise interacted with volatile of eucalyptus oil. They also reported total inhibition of hatchability, if eggs of *Earias vitella* were exposed to eucalyptus oil vapor for 4 days. When new born larvae were exposed for first 15 days /from 16th day for 15 days / continuous till 30th day, at 20 µl quantity of this oil, there were no marked reduction in egg yield and egg hatchability of breeding pairs metamorphosing from such exposed larvae, while on its continuous exposure for 30 days, a significant decline in eggs yield / egg hatchability were observed ($P < 0.05$) in emerging adults. However, in 40 µl oil exposure for first 15 days /from 16th day for 15 days / continuous till 30th day, reproductive pairs formed from adults metamorphosing from larvae showed a severe reduction in egg output and egg hatchability ($P < 0.05 < 0.01$) from those recorded for untreated controls. A marked decline both in egg output and egg hatchability occurred in this insect, when the larvae were exposed for first 15 days /from 16th day for 15 days / continuous till 30th day, in vapors emanating from 80 or 160 µl quantity of this oils ($P < 0.05 < 0.01$) (Table 2). A similar result in moth's reproductive potential was reported by Sangita Pandey and Pathak[30] and Pathak *et al*[18] in breeding pairs, with Neem and Eucalyptus or Garlic and Mint oil environment. Out of three types of exposure, 30 days continuous exposure is more effective. When newly emerged (< 24 hours old) adults of both sexes were exposed to the Citrus oil volatiles, for 3 hours, a significant reduction in eggs laid / egg hatchability were observed at 80 or 160 µl volume of oil ($P < 0.05$ or < 0.01), while 20 or 40 µl volumes of oil had no significant effect. However, if the exposure period is increased for 6 hours, a significant reduction in egg yield / egg hatchability ($P < 0.05$ or < 0.01) were observed at 40, 80 or 160 µl volume of Citrus oil volatile, while 20 µl volume had no significant effect (Table 3). A similar type of result was observed by Pathak *et al*[18] and Pathak and Sangita Pandey[19] who reported detrimental action of garlic extract and Mint oil or Neem and Eucalyptus oil volatiles for 6 hours exposure to the adult moths. Pathak and Krishna[10] reported effects of neem oil and certain volatile chemicals on the reproductive biology of *C. cephalonica* (Stainton). Thorayia *et al*[31] observed the fumigation of peppermint oil on adult *Callosobruchus maculatus* causes a significant reduction in the number of eggs laid. The outcome of such fall in the reproductive potential is possibly due to some kind of spermicidal effect of the vapor action of Citrus oil, leading to less number of eggs getting fertilized in the females[32]. Knowledge emphasizing the significance of odors from plant products, in regulating ovipositional behavior of lepidopterans is still limited[15-16, 33-38]. The *modus operandi* of such control linked with olfaction, needs deeper understanding according to Feeny *et al*[34]. 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*[39], on the basis of their preliminary physiological recording experiments, in *Menduca sexta* (tobacco hornworm) – a plant feeding lepidopteran. Glycogen level was reduced significantly up to 56.29 %, 45.66 % and 40.94 % of control, in testes of adult males following their exposure to the action of citrus oil volatile, emanating from 40, 80 and 160 µl of volume for 6 hours, while 20 µl had no significant effect. The glycogen level in ovaries of females were reduced significantly up to 60.73 %, 46.57 %, and 42.0 % of control, at 40, 80 and 160 µl volume, respectively (Table 4). The glycogen content reduced in testes and ovaries significantly after the treatment with vapors emanating from the action of citrus oil volatiles. 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[19].

Table 1: **Estimates of Percent hatchability of eggs laid by *C. cephalonica* following their Programmed exposure to different volumes of *Citrus* sp. oil volatile**

Volume of oil in µl	Percentage 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	89.4[a]	84.0[ab]	83.6[a]	83.2[a]	82.0[a]
40	87.0[ab]	81.0[b]	79.0[b]	78.0[b]	76.0[b]
80	82.0 [b]	77.0[c]	77.2[b]	70.0[c]	55.0[c]
160	75.0[c]	70.0[d]	67.0[c]	58.0[d]	47.0[d]
Mean	83.8	79.52	78.48	74.96	69.12
LSD 5%	5.65	3.90	4.27	5.04	5.15
1%	7.71	5.32	5.83	6.88	7.02

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

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

Experimental Regimen	Volumes of Oil / Extract (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)	284.6 [a]	278.6 [a]	284.6 [a]	278.6 [a]	284.6[a]	278.6 [a]	284.6[a]	278.6[a]
First 15 days Exposure	280.0 [a]	272.0 [a]	260.0 [b]	256.8 [b]	258.0[b]	252.0 [b]	242.0[b]	234.0[b]
From 16th days for 15 days after hatching	272.2 [a]	263.8[a]	251.0 [b]	245.2 [b]	242.0 [b]	235.2 [b]	209.0 [c]	200.2[c]
Continuous exposure till 30th day	250.0[b]	240.2 [b]	225.0 [c]	216.8 [c]	205.0 [c]	195.0[c]	180.0 [d]	170.0[d]
Mean	271.7	263.65	255.15	249.35	247.4	240.2	228.9	220.7
LSD 5 %	17.74	17.76	19.29	18.40	20.43	20.20	17.02	17.49
1 %	24.44	24.47	26.58	25.35	28.15	27.83	23.45	24.09

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 the action of *Citrus* sp. oil volatile, during their adult stage for 3 hours or 6 hours, after emergence

Experimental regimen (extract / 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)	299.2 ± 6.19 [a]	290.0 ± 6.79 [a]	299.2 ± 6.19 [a]	290.0 ± 6.79 [a]
20	304.0 ± 5.33 [a]	296.4 ± 5.60 [a]	288.0 ± 6.89[a]	278.2± 6.73[a]
40	290.0 ± 7.39 [a]	281.0 ± 6.74 [a]	267.8 ± 6.18 [b]	258.4 ± 5.29 [b]
80	270.2 ± 6.64 [b]	258.4 ± 6.24 [b]	225.0 ± 5.22 [c]	215.2 ± 4.92 [c]
160	220.0 ± 5.61 [c]	207.2 ± 4.81[c]	190.0 ± 4.64 [d]	175.0 ± 4.64[d]
Mean	276.68	266.6	254.0	243.36
LSD at 5 %	18.27	17.91	17.31	16.91
LSD at 1 %	24.92	24.43	23.61	23.07

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

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 *Citrus* sp. 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 (Control)	2.54 ± 0.34 (100.00)	2.19 ± 0.20 (100.00)	44.01 ± 0.74 (100.00)	69.36 ± 0.80 (100.00)	36.06 ± 0.74 (100.00)	43.98 ± 1.21 (100.00)	19.16 ± 0.87 (100.00)	22.17± 0.96 (100.00)
20	2.36 ± 0.46[NS] (92.91)	2.09 ± 0.25[NS] (95.43)	42.55 ± 0.83[NS] (96.48)	67.42 ± 0.73[NS] (97.20)	35.09 ± 0.86[NS] (97.31)	40.91 ± 0.87[NS] (93.02)	18.03 ± 0.80[NS] (94.10)	20.67 ± 0.66[NS] (93.23)
40	1.43 ± 0.17* (56.29)	1.33 ± 0.22* (60.73)	32.50 ± 0.77** (73.69)	43.41 ± 0.77** (62.58)	30.40 ± 0.83** (84.30)	36.22 ± 0.86** (82.35)	23.49 ± 0.66** (122.59)	26.69 ± 0.87 ** (120.38)
80	1.16 ± 0.15** (45.66)	1.02 ± 0.14** (46.57)	26.53 ± 0.69** (60.15)	28.60 ± 0.57** (41.23)	27.33 ± 1.03** (75.79)	23.44 ± 0.57** (53.29)	28.56 ± 0.87** (149.06)	36.83 ± 1.0** (166.12)
160	1.04 ± 0.06** (40.94)	0.92 ± 0.11** 42.00)	22.42 ± 0.64** (50.83)	20.61 ± 0.70** (29.71)	15.52 ± 1.12** (43.03)	14.06 ± 0.74** (31.96)	35.59 ± 0.70** (185.75)	46.05 ± 1.07** (207.71)

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

A sharp decline in the glycogen content of ovaries due to Citrus oil volatile, 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[40- 42]. Total lipids level was reduced significantly up to 73.69 %, 60.15 %, and 50.83 % of control in testes and up to 62.58 %, 41.23 %, and 29.71 % of control, in adult female moths at the exposure of different volume viz., 40, 80 or 160 µl of Citrus oil volatile, while 20 µl had no significant effect, respectively (Table 4). A pronounced decline in the lipid level of testes of *C. cephalonica* was noticed and the rate of reduction was observed to be significant with the increasing volumes of Citrus oil volatiles. Lipids has role in bioenergetics and as component of biological membrane. Lipid oxidation may provide energy for metabolic maintenance during stress condition[43-49]. Pronounced loss in lipid content in ovaries of adult females held in citrus oil volatile regimen can presumably be considered as a reflection of serious dislocation brought about by such treatment, occur from fat body to ovary via haemolymph for the purpose of vitellogenesis in the reproductive life of a female insect[50- 58]. Total protein level was reduced significantly up to 84.30 %, 75.79 %, and 43.03 % of control, in testes of adult males and up to 82.35 %, 53.29 %, and 31.96 % of control level in ovaries of adult females at the exposure of 40, 80 or 160 µl volume of Citrus oil volatile, 20 µl had no effect (Table 4). The protein level of testes and ovaries of *C. cephalonica* was found to be reduced significantly with the increasing volume of citrus oil. The decrease in protein level 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 may also be affected due to impaired incorporation of amino acids into polypeptide chains[19]. Total free amino acids level was induced significantly up to122.59 %, 149.06 % and 185.75 % of control in testes and up to 120.38 %, 166.12 % and 207.71 % of control, in ovaries of *C. cephalonica* after 6 hours exposure to the action of 40, 80 or 160 µl volume of citrus oil volatile (Table 4). The total free amino acids (FAA) level in the testes and ovaries of adult moths, which were exposed in varying volume of citrus oil volatile for 6 hours, was found to be increased significantly, unlike glycogen, lipids and proteins level with the increasing volume of oils, while 20 µl volume had no significant effect. 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

This study shows that the *Citrus* sp. volatile oil effects egg hatchability, postembryonic development and Reproductive potential of *C. cephalonica*. It may be toxic by penetrating the egg shell or insect body via the respiratory system. 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.

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