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Reproductive impairment and lethal effects of selected combinations of some essential oils against the rice moth, *Corcyra cecphalonica*

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ABSTRACT

Laboratory experiments were conducted to study the lethal and ovicidal effects of fifteen different combinations of six essential oils i.e. Cedarwood (Cedrus deodara), Camphor (Cinnamomum camphora), Eucalyptus (Eucalyptus globulus), Lemongrass (Cymbopogon flexuosus), Peppermint (Mentha piperita), and Bitter orange (Citrus aurantium) on the 4th instar larvae of Corcyra cephalonica via contact application. Lowest fecundity was recorded by the application of 1:1 combination of Cedarwood+Eucalyptus which was followed by combinations of Cedarwood+Peppermint, Cedarwood+Camphor oil, Cedarwood+ Lemongrass and Cedarwood+ Bitter orange oil. Drop in fecundity of treated females was caused by interference of these essential oils with the normal development of the eggs. All the tested combinations of essential oils exhibited mortality effect on the larvae of C. cephalonica, but the highest effect was shown by Cedarwood+Camphor having Lc_{50} 1.05%. This study highlights the importance the essential oils could have in the framework of an integrated pest management program for the control of rice moth, providing an alternative strategy to the current management practices.

Keywords: *Corcyra cephalonica,* Reproduction, Essential oils, Fecundity.

INTRODUCTION

Corcyra cephalonica is a serious lepidopteran pest of stored cereals such as wheat, rice, sorghum, maize, millet etc. in tropical and subtropical regions of the world [1]. The larvae alone damage the grains by feeding under silken webs. The insects cause substantial damages in stored food grains such as dry weight losses, impaired nutritional qualities, seed viability reduction and low market value [2-3]. Simultaneously, the continued boom in human population has posed a great problem of food scarcity. In such a scenario, protection of stored grains and agricultural products from insect infestation is an urgent need. The widespread and intensive use of synthetic insecticides for the control of stored grain insects has led to serious problems including insecticide resistance, poisoning of workers, rising cost of production and lethal effects on non-target organisms [4-7]. Thus it appears necessary to develop new alternatives that must be ecologically safe without any residual activity. In this direction, many plant products have been evaluated for their toxic properties against different stored grain pests. Plant essential oils are the odorous components and secondary metabolites that can be separated from various plant tissues through steam distillation. Most of these essential oils are quite complex mixtures of mono and sesquiterpenes (eg., α – terpineol and pulgeone) and biogenically related phenolics or monophenols (eg., thymol, carvacrol and eugenol). They are often quite volatile and are commonly used as fragrances and as flavouring agents or food additives. More recently they have become the focus of interest in developing ecologically sensitive pesticides [8]. Major constituents from aromatic plants, mainly monoterpenes, are of special interest to industrial markets because of other potent biological activities in addition to their toxicity to insects [9]. Essential oils being volatile can act like fumigants [10], repellents [11], contact insecticides [12], and also reproduction inhibitors [13]. Keeping these facts in mind, in the present investigation several combinations of six commonly available essential oils were tested against *C. cephalonica.*

MATERIALS AND METHODS

The six essential oils used in present study viz. Cedarwood (*Cedrus deodara*), Camphor (*Cinnamomum camphora*), Eucalyptus (*Eucalyptus globulus*), Lemongrass (*Cymbopogon flexuosus*), Peppermint (*Mentha piperita*), and Bitter orange (*Citrus aurantium*) were of commercial grade and obtained from SNN Natural Products, Delhi. The eggs of *C. cephalonica* were obtained from Tropical Forest Research Institute, Jabalpur. The culture of *C. cephalonica* was maintained in laboratory on a dietary medium composed of coarsely ground maize, streptomycin, 5% (w/w) powdered yeast in large glass containers at 26 ± 1°C and 75 ± 5 % RH.

To examine the contact toxicity of the essential oils, 3rd instar larvae were sorted out and maintained in separate jars. They moulted to 4th instar larvae within 7 to 8 days. The newly moulted 4th instar larvae were then treated with different concentrations of essential oils. Fifteen different combinations of essential oils i.e cedarwood+ eucalyptus, cedarwood+ camphor, cedarwood+ peppermint, cedarwood+ lemongrass, cedarwood+ bitterorange, eucalyptus+ camphor, eucalyptus+ peppermint, eucalyptus+ lemongrass, eucalyptus+ bitterorange, camphor+ peppermint, camphor+ lemongrass, camphor+ bitterorange, peppermint+ lemongrass, peppermint+ bitterorange, lemongrass+ bitterorange were prepared. Essential oils were mixed in equal proportion and required concentrations i.e 1%, 1.25%, 1.5%, 1.75%, 2% and 2.25% were prepared by serial dilution in acetone. Aliquots of 1ml of various combinations were applied into the petridish (5cm dia) and allowed to evaporate for 5 min. For each concentration ten 4th instar larvae were treated. Larval mortality at 24 and 48 hrs was recorded. Parallel controls, using solvent only, were also maintained for comparison. The petridishes containing different essential oil combinations and insect larvae were kept at 26±1°C until the emergence of adults. Adults were then transferred to separate jars in pairs. Daily fecundity of each treated and control adults was recorded. Females were also dissected out within 24 hrs of emergence to observe their reproductive system.

The data is expressed as Mean ± SE based on 5 replicates. The data so obtained at 48 hrs was subjected to probit analysis as described by Finney, 1971. The t-test was done by statistical program SPSS 12.0.

RESULTS

3.1 Insecticidal activity of Essential oils against *Corcyra cephalonica*

The effects of all these essential oils at different concentrations were tested against the 4th instar larvae of *Corcyra cephalonica*. Mortality and the LC_{50} values of different combinations of various concentrations of essential oils after 48 hrs of treatment are listed in Table 1.

3.2 Reproductive effect of essential oils on *Corcyra cephalonica*

The female reproductive system of *C. cephalonica* consists of a pair of ovaries. Each ovary is composed of four ovarioles. Distal end of each ovariole is called germarium which is followed by the vitellarium. Vitellarium includes major portion of the ovariole in which a series of immature eggs become progressively larger [Fig.2 (a)]. There were on an average 240-275 egg in various stages of development in the ovaries of control female adults of *C. cephalonica*. The effect of different combination of essential oils (cedarwood + eucalyptus, cedarwood + peppermint, cedarwood + camphor, cedarwood + lemongrass and cedarwood + bitterorange) on fecundity expressed as the number of eggs per female is summarized in Table 2. cedarwood oil in combination with eucalyptus, peppermint and camphor oil was a potent fecundity inhibitor as evident by appearance of numerous empty spaces within the ovarioles. The distribution and arrangement of oocytes/ova was also found disturbed and disorderly with two or more ova coalescing and fusing to form a lumpy mass within the ovarioles. In contrast, in the control set there was no apparent deformity in the ovarioles, the developing oocytes were evenly distributed without fusing to one another, becoming progressively larger towards the lateral oviducts with hardly any empty space in between.

The gaps which appeared within the ovarioles of treated sets were presumably due to disintegration and resorption of oocytes therby significantly reducing the number of eggs laid by affected females. In such females the overall ovariole length was also visibly reduced. However, cedarwood oil in combination with lemongrass and bitterorange was not as potent reproductive inhibitor and had relatively moderate effect. Other than cedarwood, the remaining essential oils used in the present study did not produce any adverse effect on the reproductive potential of surviving adults when applied on last instar larvae of *C. Cephalonica.*

Table 1. Relative mortality of *C. cephalonica* against various concentrations of different essential oil combinations

Essential oil	Conc. (%)	% Mortality	% Corrected Mortality	LC_{50} (%)	t(df)*
Cedarwood+ Eucalyptus	Control	2			
	1.00	38	36.73		07.060(4)
	1.25	68	67.35		26.944(4)
	1.50	88	87.76		21.500(4)
	1.75	100	100		49.000(4)
	2.00	100	100		49.000(4)
	2.25	100	100		49.000(4)
Cedarwood+ Camphor	Control	2			
	1.00	42	40.82		06.325(4)
	1.25	52	51.02		15.811(4)
	1.50	62	61.22		18.974(4)
	1.75	88	87.76		35.109(4)
	2.00	100	100		49.000(4)
	2.25	100	100		49.000(4)
Cedarwood+ Peppermint	Control	2			
	1.00	32	30.61		09.487(4)
	1.25	42	40.82		12.464(4)
	1.50	62	61.22		18.974(4)
	1.75	100	100		49.000(4)
	2.00	100	100		49.000(4)
	2.25	100	100		49.000(4)
Cedarwood+ Lemongrass	Control	4		1.27	
	1.00	34	31.25		06.708(4)
	1.25	40	37.50		07.060(4)
	1.50	60	58.33		10.983(4)
	1.75	100	100.0		39.192(4)
	2.00	100	100.0		39.192(4)
	2.25	100	100.0		39.192(4)
Cedarwood+ Bitterorange	Control	2		1.27	
	1.00	38	36.73		09.487(4)
	1.25	48	46.94		08.944(4)
	1.50	62	61		22.862(4)
	1.75	100	100		18.500(4)
	2.00	100	100		49.000(4)
	2.25	100	100		49.000(4)
Eucalyptus+ Camphor	Control	2		1.29	
	1.00	42	40.82		12.649(4)
	1.25	50	48.98		24.000(4)
	1.50	62	61.22		18.974(4)
	1.75	68	67.35		26.944(4)
	2.00	82	81.63		25.298(4)
	2.25	100	100		49.000(4)
Eucaylptus+ Peppermint	Control	2		1.32	
	1.00	32	30.61		09.487(4)
	1.25	42	40.82		08.944(4)
	1.50	58	57.14		22.862(4)
	1.75	76	75.51		18.500(4)
	2.00	100	100		49.000(4)
	2.25	100	100		49.000(4)
Eucalyptus+ Lemongrass	Control	2		1.38	06.325(4)
	1.00	22	20.41		
	1.25	38	36.73		14.697(4)
	1.50	52	51.02		15.811(4)
	1.75	76	75.51		18.500(4)
	2.00	100	100		49.000(4)
	2.25	100	100		49.000(4)
Eucalyptus+ Bitterorange	Control	2		1.41	
	1.00	24	22.45		11.000(4)
	1.25	38	36.73		14.697(4)
	1.50	52	51.02		15.811(4)
	1.75	66	65.31		26.128(4)
	2.00	84	83.67		21.915(4)
	2.25	100	100		49.000(4)
Camphor+ Peppermint	Control	4		1.54	
	1.00	20	16.67		06.532(4)
	1.25	26	22.92		11.000(4)
	1.50	38	35.42		06.668(4)
	1.75	56	54.17		26.000(4)

	2.00	82	81.25		15.922(4)
	2.25	100	100		39.192
	Control	4			
	1.00	18	14.58		05.715(4)
	1.25	22	18.75		09.000(4)
Camphor+ Lemongrass	1.50	32	29.17	1.56	14.000(4)
	1.75	68	66.67		16.000(4)
	2.00	74	72.92		29.000(4)
	2.25	100	100		39.192(4)
	Control	2			
	1.00	22	20.41		06.325(4)
	1.25	30	28.57		14.000(4)
Camphor+ Bitterorange	1.50	38	36.73	1.57	14.697(4)
	1.75	60	59.18		29.000(4)
	2.00	72	71.43		22.136(4)
	2.25	100	100		49.000(4)
	Control	4			
	1.00	22	18.75		04.811(4)
	1.25	28	25.00		09.798(4)
Peppermint+ Lemongrass	1.50	42	39.58	1.58	10.156(4)
	1.75	62	60.42		15.501(4)
	2.00	100	100		39.192(4)
	2.25	100	100		39.192(4)
	Control	2			
	1.00	24	22.45		11.000(4)
Peppermint+ Bitterorange	1.25	32	30.61		06.708(4)
	1.50	38	36.73		09.000(4)
	1.75	46	44.90	1.77	11.000(4)
	2.00	68	67.35		26.944(4)
	2.25	100	100		49.000(4)
	Control	4			
	1.00	16	12.50		03.207(4)
	1.25	28	25.00		09.798(4)
Lemongrass+ Bitterorange	1.50	36	33.33	1.80	16.000(4)
	1.75	46	43.75		11.225(4)
	2.00	66	64.58		10.633(4)
	2.25	82	81.25		20.846(4)

*Lc_{50} = lethal concentration that kill 50% of the exposed larvae; 50 larvae (5 replicates of 10 each) were treated at each dose; *Significant at $P<0.05$.*

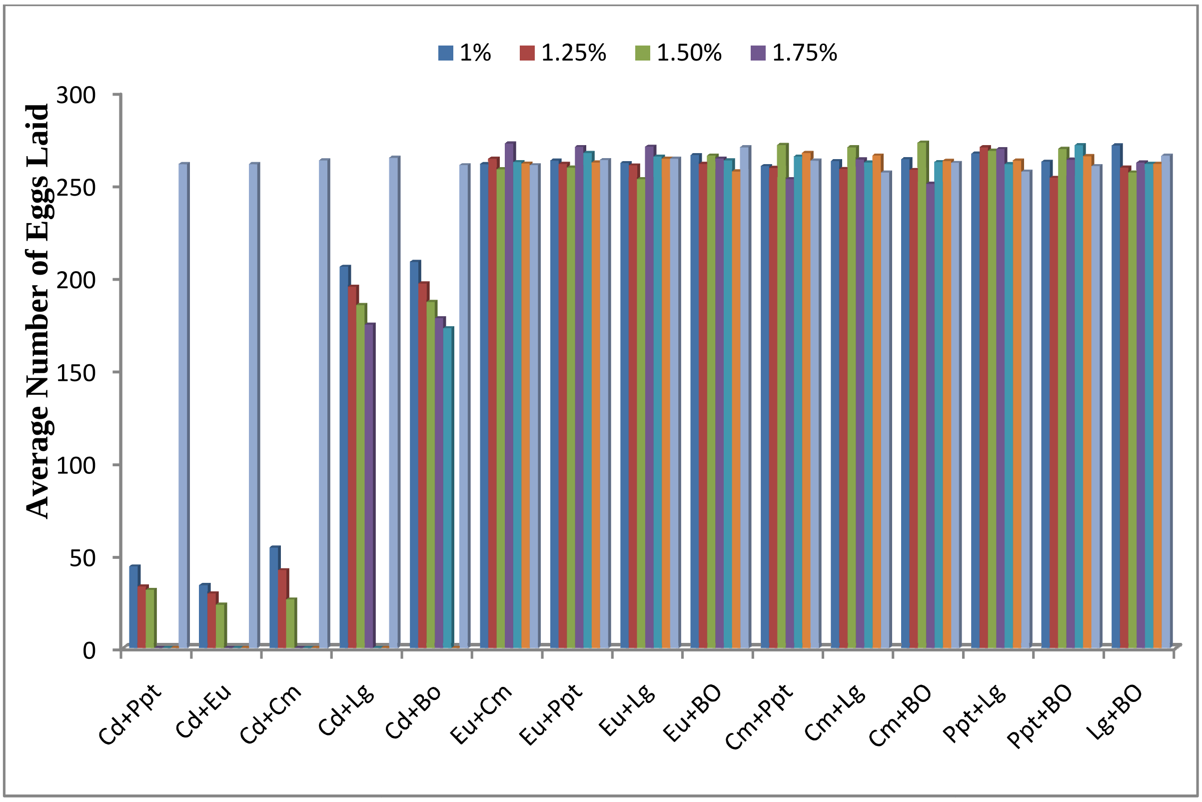
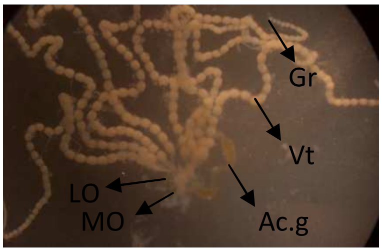
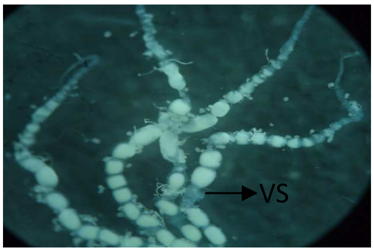
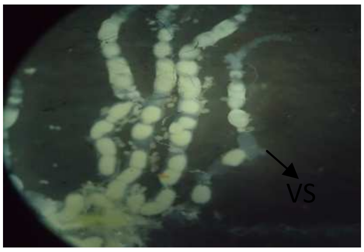


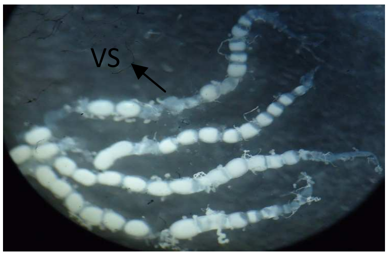
Figure 1. **Average number of eggs laid by *C. cephalonica* via contact application of different oils at varying concentrations**

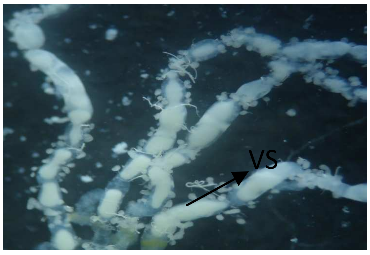


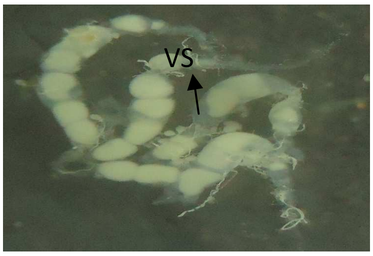




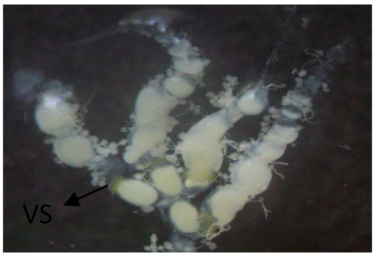
(a). Control (b). 1% (Cedarwood+ Eucalyptus) (c). 1.25% (Cedarwood+ Eucalyptus)







(d). 1.5% (Cedarwood+ Eucalyptus) (e). 1% (Cedarwood+Peppermint) (f). 1.25% (Cedarwood+ Peppermint)



(g). 1.5% (Cedarwood+ Peppermint) (h). 1% (Cedarwood+Camphor

Figure 2. Ovarioles of the females emerged from control (a) and treated with different combinations of essential oils (b-h). Gr (Germarium), Vt (Vitellarium), VS (Vacant Space), LO (Lateral Oviduct).

Table 2. Fecundity of treated *C. cephalonica* against various concentrations of different essential oil combinations

Essential oil	Conc. (%)	Mean ± SE (No of eggs laid)	% ODI
Cedarwood+Eucalyptus	Control	261.2 ± 5.258	
	1.00	34.0 ± 0.707	76.96
	1.25	29.4 ± 1.248	79.76
	1.50	23.4 ± 0.927	83.55
	1.75	Nil	Nil
	2.00	Nil	Nil
	2.25	Nil	Nil
Cedarwood+Peppermint	Control	260.6 ± 4.936	
	1.00	44.0 ± 0.707	71.10
	1.25	33.2 ± 0.969	77.39
	1.50	31.4 ± 0.509	78.49
	1.75	Nil	Nil
	2.00	Nil	Nil
	2.25	Nil	Nil
Cedarwood+Camphor	Control	263.2 ± 3.291	
	1.00	54.2 ± 0.860	65.84
	1.25	42.0 ± 0.707	72.47
	1.50	26.2 ± 1.067	81.89
	1.75	Nil	Nil
	2.00	Nil	Nil
	2.25	Nil	Nil
Cedarwood+Lemongrass	Control	264.6 ± 2.539	
	1.00	205.8 ± 1.593	12.50
	1.25	195.0 ± 1.139	15.14
	1.50	185.2 ± 0.860	17.65
	1.75	Nil	Nil
	2.00	Nil	Nil
	2.25	Nil	Nil
Cedarwood+Bitterorange	Control	260.6 ± 3.904	
	1.00	208.4 ± 1.938	11.13
	1.25	196.8 ± 1.157	13.94
	1.50	186.8 ± 1.496	16.49
	1.75	Nil	Nil
	2.00	Nil	Nil
	2.25	Nil	Nil

DISCUSSION

The use of natural products can be considered as an important alternative for the control of pests of stored products. Many essential oils and their constituents have been found to possess potential as alternative compounds to be used as insect control agents [14-18]. Apart from their insecticidal properties, these essential oils have pleasant odour which makes them ideal agents to be used in various additives and perfume formulations. Furthermore, when applied to food grains stored in granaries and godowns, these oils impart fragrance contrary to other conventional alternatives. Among the fifteen different combinations of essential oils that were assayed in our laboratory, only the combinations of cedarwood oil with other essential oils had ostensible effect on the female reproductive system so the results of only these combinations have been presented in our study. Furthermore, (cedarwood + eucalyptus oil), (cedarwood + peppermint oil) and (cedarwood+ camphor) exhibited highest reproductive inhibition while the other tested combinations of cedarwood oil were found not to alter the reproductive parameters.

Among the fifteen tested combinations of essential oils, cedarwood+ eucalyptus showed the highest insecticidal activity followed by other combinations of cedarwood. If we compare the results of present study with our earlier such study [19], wherein we demonstrated the effects of these essential oils against the same pest i.e. *Corcyra cephalonica*, it is evident that when used in combination these essential oils have enhanced efficacy rather than when used individually e.g. at a concentration of 1.75% the observed mortality of eucalyptus oil and cedarwood oil was 68% and 74% respectively but the mortality as high as 100% is achieved when these two essential oils are used in combination at the same concentration.

These results coincide with the reports of other authors who have investigated the biological activity of various essential oils towards stored grain pests [20]. Combined action of volatile oils of *Azadirachta indica* and *Eucalyptus sp.* against *C. cephalonica* has been shown to markedly decrease the egg output and egg hatchability. In earlier studies the insecticidal and reproductive inhibitory effects of these essential oils have been determined in *Corcyra cephalonica*. The 24 h LC_{50} values for combination of *Trachyspermum ammi* and *Anethum graveolens*, *A. graveolens* and *Nigella sativa*, and *N. sativa* and *T. ammi* essential oils were 11.6, 8.7 and 11.2 µl respectively against *Callosobruchus chinensis.* All these combinations also significantly reduced the oviposition potential of this pest [21]. Significant effect on the number of oocytes per ovariole induced by several insect growth regulators has also been reported by some workers [22, 23].

The essential oil content of aromatic plants is about 1–3% [24]. Therefore large quantities of plant material have to be processed in order to obtain the essential oils in quantities sufficient for commercial-scale tests [25]. It would be useful to breed the plants containing desired essential oils in elevated quantity. The high activity of these compounds could make these potential substitutes for various conventional pesticides used in stored-product control programs and can be used in coordination with microbial insecticides, attractants and traps, and natural enemies of pests as a component of the integrated pest management.

CONCLUSION

From these findings we can conclude that all the six essential oils tested are toxic to *Corcyra cephalonica*. A good level of control of the test insect (especially anti-reproductive effect) in this study was achieved by applying the cedar wood oil alone and along with different combinations as well. Application of essential oils to stored grains is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptance of this technology by farmers.

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