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ORIGINAL RESEARCH

Evaluation of *Cinnamomum tamala* Leaf and Bark Essential Oil as an Insecticidal Agent against Rice Moth, *Corcyra cephalonica*

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ABSTRACT

Corcyra cephalonica (Lepidoptera: Pyralidae), the rice moth, isa notorious insect damaging grain and processed food products under storage. The larval stage of this moth feeds externally on the grains andits products, damaging both quantitative and qualitative. For the management of this insect pest and to reduce its outcomes in terms of losses in food products, several insecticides have been formulated, synthesized and used in various forms. Since these synthetic insecticides affect human environment, health and non-target organisms as vital life-sustaining parameters of air, soil and water, scientific communities focused on developing green eco-friendly alternatives. In the present study, essential oil from bay plant cinnamon, *Cinnamomum tamala* (Family: Lauraceae) leaf and bark was extracted by hydrodistillation in Clevenger apparatus and investigated for their potential as an insecticidal agent in *C. cephalonica*. These two essential oils repel the adults in the repellency assay and cause lethality in larvae and adults both in fumigant toxicity assays. These two essential oils reduce oviposition potential in insects and interfere with the metamorphic transformation of larva to pupa to adult besides inhibiting the hatching of eggs when fumigated. *C. tamala* leaf and bark essential oils also cause deterrence in the feeding habit of *C. cephalonica* larvae. In conclusion, the essential oils isolated from *C. tamala* leaf and bark can be used as an important component in developing eco-friendly insecticide formulations.

KEY WORDS: Antifeedant activity, Cinnamon, Essential oils, Rice moth

INTRODUCTION

Infestation and damage of grain by insects under storage started with the beginning of storage practices resulting in both quantitative and qualitative losses. In developing countries using traditional technologies for protecting and storing grains, such losses at the level of the farm vary between 10-40% and are estimated to be 10% of the total yield (Lal, 1988; Shaaya *et al.,* 1997). In India, this loss has been estimated at 5-10% of stored grains (Champ & Dyte, 1977; Tooba *et al.,* 2005). These damages cause spoilage of food grains and processed products as well as raise concerns of security issues especially in developing countries. The rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) is a serious insect pest of grains under storage. It is commonly found in India and other parts of the world like Asia, Africa, Brazil, Europe, Indonesia, Myanmar, North America and Thailand (Osman, 1984; Allotey & Azalekor, 2000; Roopa *et al.,* 2021). Its larval stage is an external feeder damaging grains like corn, cotton seeds,jowar, groundnuts, pulses, rice and sorghum. The feeding larval stage makes silken webs in grains in serious infestation making the grain unpalatable for human consumption (Alam, 1972; Frenemore & Prakash, 1992; Atwal & Dhaliwal, 2008; Samanta & Yadav, 2021).

To manage such losses by insect pests, several chemicals of synthetic origin viz., organophosphates, organochlorines and carbamates were developed and used in different forms indiscriminately, but, besides paying success in reducing insects population and its infestation outputs, these insecticides have cause resistance development in most of the insects due to its high

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persistence in the environment (Elzen & Hardee, 2003; Benhalima *et al*., 2004; Islam & Talukdar, 2005). These synthetic insecticides are responsible for the origin of different chromosomal aberrations and in forming DNA adducts (Le Goff *et al*., 2005; Muniz *et al*., 2008; Simoniello *et al*., 2008). These synthetic pesticides are also responsible for various environmental issues like thinning of ozone layer, poor consumable quality of soil, air and water, toxic activity in others animals like beneficial insects and cause variety of health problems like neurotoxicity, carcinogenicity and teratogenicity (WMO, 1991; Lu, 1995; UNEP, 2000; Beckel *et al*., 2007; Regnault-Roger *et al*., 2004).

These serious outcomes forced the reduction of the use of such synthetic insecticides and exploration of new ecologically safe plant based alternative methods. Essential oils are nowadays considered as alternatives to these synthetic insecticides to protect stored grains from insect infestation.

Cinnamomum tamala (Family: Lauraceae) commonly called Bay plant, is a native tree of Bangladesh, Bhutan, China, India and Nepal. Its leaves known as tejpatta, possess aromatic properties and, thus, are used as spice and in the preparation of several medicines. Being one of the oldest spices and is mainly used as a flavouring agent and in the preparation of antidiabetic, stimulating, carminative agents (Hussain *et al*., 1980). In Ancient literature, the leaf and bark of the bay plant were used in the treatment of anemia and fever and in reducing body's odour. The powder of the seeds of this plant was recommended with honey or sugar for the treatment of dysentery and cough in children (Edwards, 1993). Its leaves are placed with clothes and often chewed to remove the mouth's foul odour due to their aromatic nature. *C. tamala* leaf is used in the treatment of fungal disease of skin, fractures and eye diseases, oral diseases and in curing disorders of breast milk. Leaves of *C. tamala* produce colourless volatile oil on hydrodistillation. The major chemical constituents present in the leaf oil are eugenol, α-phellandrene and β-phellandrene. The other minor components of the oil includecis-caryophyllene, elixene, α-pinene, limonene and myrcene (Vishwam, 2015). *C. tamala* leaf possesses antibacterial effects and isused in soap as a fragrance component (Minakshi *et al*., 1999). The aqueous solution of *C. tamala* bark shows antidiabetic effects (Roux *et al*., 1998). Several barks extracts of this plant show antimicrobial activity against a variety of microbes (Palmer *et al*., 1998; Prabussenivasan *et al*., 2006). Both the dried leaf and bark have been given along with honey for curing fever and anemia (Shah & Panchal, 2010). *C. tamala* bark essential oil includes cinnamaldehyde, δ-cadinine, γ-gurjunine, α-muurolol, γ-cadinine and α-muuroleneas major constituents (Mohanty *et al*., 2024). Bark essential oil shows antimicrobial activity against a variety of bacteria and fungi (Taha & Eldahshan, 2017). In the present study, essential oil from *Cinnamomum tamala* (Family: Lauraceae) leaf and bark was extracted and evaluated for its insecticidal effects on *C. cephalonica*.

MATERIAL AND METHODS

Plant Collection

Leaf and bark of cinnamon, *C.tamala* were collected locally from the market. The collected plant material, leaves and bark were dried at room temperature (20-25°C) in air for one week.

Essential oil extraction

Dried leaf and bark were grounded in mixer and subjected to hydrodistillation in 1L of distilled water in a Clevenger apparatus for 4-hours. The essential oil was collected and subjected to sodium sulphateto make it anhydrous. Finally, essential oils were kept in eppendorff tubes, sealed and stored at 4-5°C.

Insects

The laboratory culture of rice moth, *C. cephalonica* was done in a glass jar (30 cm height, 20 cm diameter). Insects were fed crushed rice grain mixed with 5%(w/w) and kept in the rearing room at 28±1ºC of temperature, 65±5% RH and (12:12 h) h light, dark photoperiod. Young adults (2-3 days old) were utilized for bioassays.

Repellent Activity

Repellency assay was carried out in a Y-shaped glass tube (each arm with a diameter of 2 cm and 10 cm length). Five grams of crushed rice mixed with different concentrations of *C. tamala* leaf/bark oils was taken in one of the paired arms (experimental arm),while the other arm was provided only crushed rice (control arm). Twenty young individuals of *C. cephalonica* were released into the third arm (median arm) of the Y-tube. All the terminal ends of theY-shaped glass tube were closed using cotton wool. The experimental set up was left for three hours, and then, number of insects was counted in all the three arms (experimental, control and median arms). Each concentration was replicated six times. The repellent assay was calculated using formula:

$$\mathbf{PR = [(N_{UT} - N_T / N_{UT} + N_T]100}$$

Where, N_{UT}=Total insect in control area and N_T=Total insects in experimental area.

Fumigant toxicity

Experimental solutions of essential oils prepared in acetone were used to evaluate the fumigant toxicity of *C. tamala* leaf and bark oils. Ten adults/3rd instar larva of *C. cephalonica* were removed from laboratory culture and transferred them into a glass jar (15 cm height 10cm diameter) floored by Whatman filter paper No. 1. A paper strip of 2 cm diameter treated with different concentrations of essential oils was pasted on the inner surface of the glass cover of glass jar. To avoid contact with the treated filter, the test insect was closed with a wire mesh. Finally, the glass jar was covered with a glass cover and sealed with parafilm. All the closed glass jars were kept in the dark in conditions maintained for insect culture. Six replicates were used for each concentration of oil. After completion of 24 hours of the fumigation period, dead adults and larvae were counted.

Oviposition inhibition

Effect of *C. tamala* leaf and bark oils on the oviposition potential was tested against *C. cephalonica* by fumigation method. Ten pairs of 1-2 old adults were placed in a glass jar (15 cm height 10cm diameter) floored by Whatman filter paper No. 1. A paper strip (2 cm diameter) treated with two different sub-lethal concentrations of essential oils,viz. 40 and 80% of 24 hours LC_{50} was pasted on the inner surface of the glass cover of the glass jar. Finally, the glass jar was closed with a glass cover and sealed. All the glass jars were kept in the rearing room in conditions used for insect culture. For each concentration of oil as well as control group, six replicates were set. After 24hours of fumigation, the treated adults were transferred to fresh glass jar of similar measurement. After four days of treatment, eggs laid were counted in treated as well as control groups. Effect on oviposition was estimated by calculating

Percent Oviposition Deterrence Index (%ODI)

$$\%ODI = [(C-T)/(C+T)]100$$

where, C = Number of eggs in control, T = Number of eggs in test.

Table 1: Effect of *C. tamala* leaf and bark oils as repellent on *C. cephalonica*

Concentration (vol:vol)	Leaf oils Percent repellency (PR)	Bark oils Mean±SE
2	26.51±1.13	36.65±0.85
4	51.56±2.06	60.00±2.12
8	95.00±1.02	93.35±1.34
16	100±0.00	100±0.00

Percent repellency (PR) was calculated using formula: PR = $[(N_{UT} - N_T / N_{UT} + N_T] 100$, where N_{UT}=numberof insect in control arm and N_T= numberof insects in experimental arm

Ovicidal assay

In ovicidal assay, twenty five freshly laid eggs were placed on Whatman filter paper No. 1 and then placed at the bottom of glass Petridish (10cm in diameter and 1.0 cm in height). These eggs were fumigated with test solutions prepared by diluting *C. tamala* leaf and bark essential oils with acetone. A 100 µl aliquot of test solution was applied on a filter paper strip (2.5 cm diameter), and the solvent was allowed to evaporate for 5 minutes. The filter paper was pasted to the inner surface of glass Petridish and incubated for 72 hours in conditions maintained for insect culture. After completion of fumigation, the number of eggs hatched was recorded. For each type of essential oil, three different concentrations were used, and for each concentration of oil as well as control group six replicates were set. Effect on egg hating was estimated by calculating Percent Hatching Inhibition Rate (%HIR).

$$\%HIR = [(C-T)/C] 100$$

where, C = Number of adults in control, T = Number of adults in test.

Developmental inhibition

Developmental inhibitory activity of *C. tamala* leaf and bark oil was tested against newly emerged 3th instar larvae of *C. cephalonica*. Twenty larvae were fumigated with two sub-lethal concentrations, viz. 40 and 80% of 48h-LC_{50} of volatile oil in Petri dish for 48-hours as was done in larvicidal assay and then the treated larvae were transferred to fresh glass jars and supplied with crushed rice with 5% (w/w) yeast powder. The number of larva transformed into pupa and the number of adults emerged from pupa was recorded. Six replicates were set for each concentration of oil as well as control.

Antifeedant activity

Antifeedant activity of *C. tamala* leaf and bark oils was tested in *C. cephalonica* larvae. For this assay, food was prepared by crushing 10gm of rice grains and mixing 5% (w/w) yeast powder in it. Now, food was mixed thoroughly with 0.3µl/gm, 0.6µl/gm and 1.2µl/gm *C. tamala* leaf and bark essential oils. Now ten 3rd instar *C. cephalonica* larvae were placed on Whatman filter paper No. 1 and then placed at the bottom of glass Petridish (10cm in diameter and 1.0 cm in height). Now, 10gm of food prepared was spread around the larvae, covered the petri dish and it was kept in laboratory conditions applied for insect rearing. After the end of the larval life, antifeedant activity was calculated using

$$AFA = [C - T/C] \times 100$$

where, C = consumption of food in control group, and T = consumption of food in treated group.

Table 2: Summary of fumigant toxicity assay of *C. tamala* leaf and bark oils on *C. cephalonica*

Oil	Parameter	Exposure Period	LC_{50} (µl/L)	g-value	t-ratio	Hetero-geneity	Regression Equation	Correlation Coefficient
C. tamala leaf oil	Adult mortality	24h	112.67	0.28	3.84	0.36	Y = -3.78+1.15X	0.98
		48h	73.95	0.29	3.64	0.33	Y = -6.31+3.96X	0.99
	Larval mortality	24h	77.36	0.29	4.12	0.37	Y = -4.21+4.62X	0.98
		48h	48.59	0.27	3.36	0.32	Y = 2.39+4.21X	0.98
C. tamala bark oils	Adult mortality	24h	131.87	0.28	3.85	0.39	Y = -3.39+3.64X	0.99
		48h	78.52	0.25	3.12	0.32	Y = -2.21+7.36X	0.99
	Larval mortality	24h	81.75	0.28	4.63	0.37	Y = -4.97+3.64X	0.98
		48h	52.64	0.29	3.45	0.34	Y = 3.28+4.39X	0.99

DATA ANALYSIS

Median lethal concentrations (LC_{50}) of the essential oils against adults and larvae calculated using POLO programme (Russel *et al.,* 1977). The concentration and response relationship was accessed by correlation and linear regression analysis (Sokal & Rohlf, 1973). To test the equality of the regression coefficient and to test the significance of data, analysis of variance was applied (Sokal & Rohlf, 1973).

RESULTS

Repellency

Results of the repellency assay indicated that essential oils of *C. tamala* leaf and bark were repellent to C. *cephalonica* adults. The percent repellency (PR) was recorded 26.51, 51.56, 95 and 100%, and 36.65, 60.00, 93.35 and 100% at 2, 4, 8 and 16µl of *C. tamala* leaf and bark essential oil, respectively (Table 1).

Fumigant toxicity

Median lethal concentrations (LC_{50}) were recorded 112.67 and 73.95µl/L, and 131.78 and 78.52µl/L air against *C. cephalonica* adults for *C. tamala* leaf and bark oils after 24h and 48h exposure periods,respectively (Table 2). On the other hand, median lethal concentrations were 77.36 and 48.59µl/L, and 81.75 and 52.64µl/L air against *C. cephalonica* larvae for *C. tamala* leaf and bark oils after 24h and 48h exposure period, respectively (Table 2). The g-value represented the index of the significance of

Table 3: Effect of *C. tamala* leaf and bark oils on oviposition behaviour of *C. cephalonica*

Oil	Concentration	Number of larvae emerged/adult (Mean±SE)	% ODI	F-value* (df=2,15)
Control	-	73.83±3.85 (100)	-	-
C. tamala leaf oil	40% 48h-LC_{50}	43.33±2.20(58.68)	38.41	35.18
	80% 48h-LC_{50}	25.83±1.43 (34.98)	48.16	
C. tamala bark oil	40% 48h-LC_{50}	44.83±2.02 (60.72)	25.00	37.95
	80% 48h-LC_{50}	23.83±1.06 (32.37)	51.19	

Values in parentheses indicate per cent change with respect to control group
*Significant at P<0.01

Table 4: Effect of *C. tamala* leaf and bark oils on the hatchibility of *C. cephalonica* eggs.

Oil	Concentration (µl/L)	Number of larvae emerged (Mean±SE)	%HIR	F-value*
Control	-	23.50±0.48(100)	-	-
C. tamala leaf oil	25	19.50±0.41(82.97)	17.02	122.95
	50	14.33±0.53(60.97)	39.02	
	75	10.50±0.61(44.68)	55.31	
C. tamala bark oil	25	18.17±0.53(77.32)	22.77	179.14
	50	13.50±0.42(57.44)	42.56	
	75	10.67±0.37(45.40)	54.60	

Values in parentheses indicate per cent change with respect to control group.
*Significant at P<0.01

potency. The g-value was less than less than 0.5, indicating that the mean value was within the limits of all probability levels (P<0.1, 0.5 and 0.01). T-ratios were greater than 1.6, indicating that regression was significant. Heterogeneity factor was less than 1.0, representing the model fits the data adequately. Regression analysis of the data reflects alinear relation between concentration and mortality in *C. cephalonica* adults and larvae (Table 2).

Oviposition inhibition

The oviposition potential of *C. cephalonica* adult was decreased significantly when fumigated with the *C. tamala* leaf and bark oils. The oviposition was reduced to 58.68 and 34.98%; and 60.72 and 32.37% when *C. cephalonica* adults were fumigated with 40 and 80% of 48h-LC_{50} of *C. tamala* leaf and bark oils respectively (For *C. tamala* leaf oil F = 35.18; and for *C. tamala* bark oil F = 37.95; P<0.01; Table 3).

Ovicidal activity

The percentage of egg hatching was decreased significantly with an increase in concentration of *C. tamala* leaf and bark oils. Egg hatching was reduced to 82.97, 60.97 and 44.68%; and 77.32, 57.44 and 45.40% of the control when eggs were fumigated with 25, 50 and 75 µl/L of *C. tamala* leaf and bark oils respectively (For *C. tamala* leaf oil F = 122.95; and for *C. tamala* bark oil F = 179.14; P<0.01; Table 4).

Developmental inhibition

The larval transformation into the pupa and adult emergence from pupa was decreased significantly with in *C. cephalonica* by the vapours of *C. tamala* leaf and bark oils. The transformation of larva into pupa was reduced to 72.42 and 46.56%; and 66.37 and 39.67% of the control when larvae were fumigated with 40 and 80% of 48h-LC_{50} of *C. tamala* leaf and bark oils respectively (For *C. tamala* leaf oil F = 112.64; and for *C. tamala* bark oil F = 323.77; P<0.01; Table 5). Adult emergence from the pupa was reduced to 60.00 and 33.31%; and 48.42 and 29.54% of the control when larvae were fumigated with 40 and 80% of 48h-LC_{50} of *C. tamala* leaf and bark oils respectively (For *C. tamala* leaf oil F = 262.25; and for *C. tamala* bark oil F = 279.93; P<0.01; Table 5).

Antifeedant assay

C. tamala leaf and bark oils reduced the consumption of food by *C. cephalonica* larva as antifeedant activity was found to increase with increase in oil concentration. Consumption of food by 3rd instar larva was significantly reduced to 82.15, 65.37 and 38.20%, and 83.05, 65.49 and 37.55% of the control at 0.3, 0.6 and 1.2 µl/gm of *C. tamala* leaf and bark oil respectively (For *C. tamala* leaf oil F = 166.93; and for *C. tamala* bark oil F = 181.18; P<0.01; Table 6).

Table 5: Effect of *C. tamala* leaf and bark oils on the development larva and pupa of *C. cephalonica*

Oil	Concentration	Number of pupa transformed (Mean±SE)	F-value*	Number of adult emerged (Mean±SE)	F-value* (df=2,15)
Control	-	19.33±0.22(100)		17.50±0.23(100)	-
C. tamala leaf oil	40% 48h-LC_{50}	14.00±0.32(72.42)	112.65	10.50±0.21(60.00)	262.25
	80% 48h-LC_{50}	9.00±0.26(46.56)		5.83±0.19(33.31)	
C. tamala bark oil	40% 48h-LC_{50}	12.83±0.39(66.37)	323.77	7.67±0.28(43.82)	279.93
	80% 48h-LC_{50}	7.67±0.31(39.67)		5.17±0.31(29.54)	

Values in parentheses indicate per cent change with respect to control group.
*Significant at P<0.01

Table 6: Effect of *C. tamala* leaf and bark oils on food consumption by of *C. cephalonica*

Oil	Concentration (µl/gm)	Food consumed (gm) (Mean±SE)	AFA	F-value*(df=3,20)
Control	-	8.52±0.36 (100)	-	-
C. tamala leaf oil	0.3	7.00±0.41 (82.15)	17.85	166.93
	0.6	5.57±0.39 (65.37)	34.63	
	1.2	3.37±0.23 (38.20)	61.80	
C. tamala bark oil	0.3	7.06±0.54 (83.05)	16.85	181.18
	0.6	5.58±0.32 (65.49)	34.51	
	1.2	3.20±0.28 (37.55)	62.45	

Values in parentheses represent per cent change with respect to control group.
*Significant at P<0.01

DISCUSSION

Several volatile products of botanical origin have been reported for their repellent, toxic, oviposition inhibitory, developmental inhibitory and antifeedant activities against a variety of insect pests of stored grains and products. The present study reported that *C. tamala* leaf and bark oils repelled adults and caused acute toxicity in adults as well as larvae of *C. cephalonica*. The repellent and rapid toxicity of the essential oils is caused due to volatile nature showing their low persistence in the environment and probable neurotoxicity. Several essential oils have been known for their neurotoxicity in insect pests (Chaubey, 2012; Rani, 2012; Kumar & Tiwari, 2018; Ebadollahi *et al*., 2022). These oils interfere with neuromodulator octopamine or GABA-gated chloride channels and cause disruption in the nervous system in insects (Hollingworth *et al.*, 1984; Enan, 2005; Tong & Coats, 2012).

C. tamala leaf and bark oils inhibit oviposition and egg hatching in *C. cephalonica* by vapour action Several essential oils like *Trachysper mummammi*, *Piperbetle*, *Eucalyptus citriodora*, *Cymbopogann ardus*, *Pelargonium graveolens*, *Rosmarinus officinalis* and *Ocimum basilicum* essential oils produce similar effects on the viability of eggs (Sowmya *et al*., 2023). Different combinations of *Cedrus deodara*, *Cinnamomum camphora*, *Eucalyptus globulus*, *Cymbopogon flexuosus*, *Menthapi perita* and *Citrus aurantium* essential oils have been reported for ovicidal and developmental inhibitory effects via contact application (Jacob & Qamar 2013).The mode of action of these essential oils has not yet been established, but it seems that reduction in oviposition and development is the result of suffocation or interference with biosynthetic pathways in insects (Don-Perdo, 1989). This speculation has been supported by some essential oils, which have been known to reduce protein, glycogen and lipid contents. These oils reduced amylase and protease enzyme activities along with the reduction in consumption index, relative consumption rate and relative growth rate (Ebadollahi *et al*., 2022). *C. tamala* leaf and bark oils reduce food consumption by larvae of *C. cephalonica.* This reflects that essential oils reduce damaged grains by feeding action, thus reducing quantitative and qualitative losses. The damage to grains causes deterioration and contamination due to microbial action, as well as the reduction in protein and carbohydrate levels.

In conclusion, *C. tamala* leaf and bark essential oils show repellent, toxic, oviposition inhibitory, developmental inhibitory and antifeedant activities on *C. cephalonica*. Since these two volatile oils are of botanical origin and are parts of the human diet, they are safe for humans when used in insecticide formulations. Their high fumigant actions show low persistence in the environment, thus eliminating residual properties. Thus, these *C. tamala* leaf and bark essential oils can be used in developing eco-friendly insecticide formulations based on volatile organic chemicals.

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