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ESSENTIAL OIL IN MANAGEMENT OF COCONUT RHINOCEROS BEETLE *ORYCTES RHINOCEROS* L.

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ABSTRACT

Plant derived product essential oil is used as a component in management of coconut rhinoceros beetle *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae). A study was undertaken to evaluate the bio efficacy of essential oil from *Ocimumbasilicum*, *Eucalyptus citriodora*, *Trachyspermumammi* and its major constituent thymolin management of *Oryctes rhinoceros*. These essential oilsderived from plant parts were characterized and itsphysiological response and biocidal activity was determined. Basil, citriodora, ajowan oil and thymol caused electrophysiological response in the antennae of *O. rhinoceros* adults.Behavioral response of beetles was tested in'Y' tube olfactometer having a choice between odour arm containing essential oil and control arm having zero air. Orientation of beetles to essential oil laden arm ranged from 15-30 %. Over 70-85% of the beetles moved towards the control arm. Citriodora and basil oil inhibited hatching of 70% of eggs and also caused larval mortality. The mean percent larval mortality ranged from 61-66%. The repellence, ovicidal and growth regulating effect of essential oil offers an ecofriendly option in management of coconut rhinoceros beetle.

Keywords: Behavioural response, essential oil, insect behaviour, rhinoceros beetle

The rhinoceros beetle, *Oryctes rhinoceros* L. (Scarabaeidae: Dynastinae) is a major pest of coconut in coconut growing tracts across the world (Singh and Rethinam, 2005). The black colored beetle bores holes and feeds on the unopened spear leaf and spathe. Upon unfurling, the damaged leaves show geometric cuts (V shaped) on leaflets. The beetles cause damage to seedling, young and adult palms. Damage when done to the leaves reduces the photosynthetic area and renders them unsuitable for thatching purpose, but when damage is done to spathe it causes direct crop loss. Apart from feeding damage, they serve as pre-disposers for red palm weevil attack, budrot and leaf rot infestation. Repeated damage done to the meristem especially on juvenile palm is lethal (Rajan et al*.,* 2009, Joseph Rajkumar et al*.,* 2015). Among the measures adoptedin rhinoceros beetle management, use of botano chemicals is widely accepted by the farmers especially in Kerala where homestead farming is practiced. Recommended botano chemicals for adult management include prophylactic treatment with neem / marotti / pongamia cake mixed with sand (250 g) or placement of botanical cakes developed by ICAR-CPCRI @ 3/palm on top most three leaf axils (Joseph Rajkumaret al., 2015).

Though botano chemicals are being used, it is imperative to search for plant derived parts with enhanced bioaction on beetles. On thisline, essential oils were screened for rhinoceros beetle management asthey possess broad spectrum of bioactivities *viz.*, insecticidal, repellence and growth regulating effect on insects (Attend and Mary Eapen,1980;Bakkaliet al*.,* 2008) that are exploited for pest control **(**Regnault Roger,1997). The chemical compounds in the essential oils acts as ligands for the odorant binding protein (OBPs) and are detected by the insects in small concentrations (Peng and Leal, 2001; Robert Anholt and Trudy, 2009). Chemical constituents of these oils interfere with the octopaminergic nervous system in insects (Kosyukovsky et al*.,* 2002). As the target site is not shared with mammals, most essential oil chemicals are relatively non-toxic to mammals and fish hence fulfilling the criteria for "reduced risk" pesticides (Murray Isman, 2000). The present study attempts to assess the efficacy of essential oil on coconut rhinoceros beetle *O. rhinoceros* management*.*

MATERIALS AND METHODS

Adult rhinoceros beetles were collected fromICAR-Central Plantation Crops Research Institute experimental farm and neighbouring field at Kasaragod (12^{o} 31'

40.06'' and 74^{o} 58' 06.99'') Kerala, during the period from December 2014 to June 2015.Adults were reared on coconut petioles (20cm length and 3-4 cm width) placed in a poly propylene plastic jars (1000ml). Ten pairs of adult males and females were kept in jars and maintained at 27±0.5°C. The fresh petioles as feed were replaced once in 4 days.

Essential oils (basil, citriodora, ajowan oil and thymol) obtained from Southern Spice products (India) Ltd., Madurai, Tamil Nadu were used for the study. Solvents used for diluting the essential oils were obtained from Merck (India) Ltd. and used without further purification. Dilutions of essential oilswere made in dichloromethane for behavioural response assay. The oils were diluted in paraffin oil for EAG assay.The constituents inbasil oil, citridora oil, ajowan oil and thymol were characterized using Gaschromatograph-Mass spectrometer (GC-MS Agilent 5975).1µl of essential oil diluted in dichloromethane was injected into GC-MS having HP 5 MS Phenyl methyl silox capillary column. During the run, oven temperature was raised from 40°C at the rate of 20° C/ minute to 280°Cand held at 300° C for 1 min. The injector and column temperature were maintained at 250° C. The total run was for 23 min. duration. The volatile constituents in the oils were identified using the NIST library. Major constituents in the oil were verified by co injecting the compounds.

Electroantennogram (EAG) responses of *Oryctesrhinoceros* adults (Male and female) were made using a commercially available electroantennographic system (Syntech, Hilversum, The Netherlands) consisting of a dual electrode probe for antenna fixation, CS-05 stimulus controller and an IDAC- 2 for data acquisition. The whole antennae excised from the head along with the scape was mounted on the ground electrode and the club was attached to the recording electrode using a saline gel. The antennae were exposed to 100ng essential oil and physiological response in the antennaewas recorded (Male and female adult beetle). Five replications were made per dose. Coconut rhinoceros beetle aggregation pheromone (Ethy l4 - methyl octanoate) was used as reference stimuli. Reponses were expressed in mV based on shape and amplitude of the response.

Y-tube olfactometer was used to study the orientation of the adult beetle to essential oils. The Y tube olfactometer made of glass had a stem length of 18 cm and 4 cm diameter. The arms were 8 cm long and 4 cm in diameter. It was separated at an angle of 60°. The opening of the stem was covered with Teflon septum with a hole in the center through which the air was drawn. Beetles were introduced in the base of the stem. The odour source (pieces of coconut petiole treated with essential oil (1000ng)) was placed in one arm and control (coconut petiole alone) in the other. Null test was carried out to check if there was any bias in orientation. Red colored transparent sheet was used to cover the Y-tube olfactometerso as to avoid disturbance if any due to visual stimuli. Response of the beetle to odour or control was recorded if they walked 3cm up on the side arms from the stem. If an insect did not respond for 3 minutes, they were considered as non-responders. Ten beetles (Male and female) were randomly chosen from the culture for the behavioural assay. After every five insectswere tested, the orientation of the olfactometer was reversed to check for position effects and after every batch of analysis, the olfactometer was cleaned in luke warm water and dried completely. Odour source were switched between left and right-side arms for each subsequent trial to minimize spatial effect on choices. Y-tube bioassay was conducted at 30±0.5°C.

Oryctesrhinoceros eggs (2-3 days old) (n=20) were exposed to essential oils (basil oil, citriodora oil, ajowan oil and thymol) at 0.2%, 0.6% and 1.0%. Eggs exposed to 0.3 % neem oilwere treated as positive control. Eggs exposed to solvent alone were treated as negative control. The treatments were replicated four times and mean per cent hatchability of eggs was tabulated. Freshly emerged healthy first instar grubs (one to two days old) were introduced into a jar containing sterilized vermicompost (50 gm) treated with various dilutions of essential oils, *viz.* 0.2%, 0.6% and 1.0% along with control in four replicated trials.Neem oil (0.3%) was kept as positive check. The per cent mortality of the grubs after 48 hours of exposure to essential oils were studied.

Varied dilutions of essential oil ranging from 1, 3 and 6 % were prepared (Ajowan oil, basil oil, citriodoraoil and thymol) in dichloromethane. One ml of the solution was taken in rounded glass jar (50 ml). It was then rolled on a flat surface at uniform speed so as to provide uniform coating on the internal surface of the container. Small pieces of petioles (size 2.0×2.5 cm) from west coast tall palms were placed in the container as food. Ten beetles were introduced into separate containers. Mortality of the beetles was recorded after 48 hours. Neem oil (0.3%) was maintained as positive check. Four replications were maintained per treatment.The electrophysiological response, ovicidal, larval and adult toxicity were subjected to one-way analysis of variance with Post hoc test. Behaviouralresponse of beetles to oils were subjected to Student's t-test.

RESULTS AND DISCUSSION

Chemical composition

The chemical compositions of basil oil, citriodora oil, ajowan oil and thymol are shown in Table 1. The main constituent of *basil oil was methyl chavicol (80.3%) and that of citriodora was citronellal (79.7 %)*. Ajowan oil consists of43.2 %γ–terpinene and 32.4% of thymol while Thyme oil contains 98.95% of thymol. In basil oil, among the terpenes, methyl chavicol was the major constituent (Ahmet et al., 2018). Thymol, γ-terpinene, and p-cymene were reported as a major constituent in ajowan oil (Mehdi et al., 2018). *Eucalyptuscitriodora* were reported to contain about 70% citronellal, which is a monoterpene product from the plant secondary metabolism (Tolba et al., 2017)

Antennal responsein adults

The electrophysiological response of antennae of adult rhinoceros beetle (male and female) was assayed byelectroantennogram. The aggregation pheromone ethyl, 4- methyl octanoatewas used as a reference to check the antennal response and it caused maximum response of 2.8mV. Basil oil, citriodora oil and thymolexposed @ 100ng to the adult antennae caused mean antennal responses of 0.94, 0.99 and 0.94 mV, respectivelywhichwere at par. The physiological response of the antennae to exposed oils confirms the presence of the receptors in them (Fig. 1).Male and female of *Ectropisgrisescens* adults showed EAG response to the essential oils from *Chenopodium ambrosioides*, *Mentha spicata* and *Artemesiaannua* in a dose-dependent manner, with the antennal response

Table 1. Chemical composition of essential oils

Components	Retention Time (Min.)	% compound			
		Citriodora oil	Basil oil	Ajowan oil	Thymol
γ -terpinene	7.15	_	_	43.2	_
Thymol	7.63	_	_	32.4	98.952
Linalool	7.853	_	10.769	_	_
Methyl chavicol	8.830	_	80.3	_	_
Anisaldehyde	9.358	_	2.254	_	_
p-cymene	9.83	_	_	20.7	_
α-pinene	11.907	0.231	_	_	_
β-pinene	13.449	0.595	_	_	_
Cymene	15.136	0.079	_	_	_
Cineol	15.393	1.010	_	_	_
Terpinolene	17.341	0.076	_	_	_
Citronellal	20.056	79.669	_	_	_
Menthone	20.259	0.268	_	_	_
Isopulegol	20.354	0.439	_	_	_
Dimethyl heptan -2-ol	21.474	0.583	_	_	_
β-citronellol	21.991	7.422	_	_	_
Citronellylformate	23.280	0.125	_	_	_
Citronellic acid	24.770	0.598	_	_	_
Citronellol acetate	25.563	1.667	_	_	_
Caryophyllene	27.762	1.243	_	_	_
Bromo methylcyclo-hexanol	40.027	0.545	_	_	_

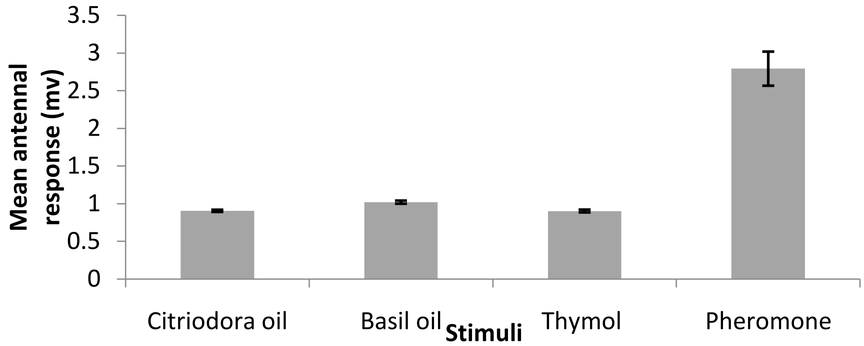

Fig.1. Electrophysiological response of adult O. rhinocerosto essential oils

strengthening first and then decreasing with the concentration of essential oil (Zhang et al., 2018).

Behavioral response ofadults

Essential oils that cause physiological response in insect's antennae need not be behaviourally active. Hence, the behavioural response of adultbeetles to essential oil was assessed by choice assay using Y-tube olfactometer. The adult beetles were provided with a choice between the odour arm containing food with essential oil and the control arm having food with clean air. Among the oils testedover 70 % beetles oriented to arm having clean air avoiding the arm having citriodora and basil oil. This was followed by ajowan oil, where over 63 % of the beetles moved towards the control arm in which the pure air was passed. In case ofthymol and neem oil, 58% beetles moved towards clean air arm respectively (Fig.2). On statistical significance of difference of proportion of 0.5 revealed that there was no effect but the t-test revealed that there exists a significant difference between the proportion of insects moving away from the essential oil (0.63) from 0.5(t-value of 9.07 which is significant at 1%). Citriodora oil derived from *E. citriodora* had citronellal (40%) that possess repellency to *Triboliumcastaneum* (Jesus et al., 2010). This clearly suggests that beetles showing the physiological response also had behavioral response by moving away from the arm having essential oils. The Y-tube olfactometer bioassay has also been used for testing repellency/ attractancy against coleopterans (Ndungu, et al., 1999).

Effect on eggs and grubs

The effect of essential oils on hatchability of *O.rhinoceros* eggs was studied. 2- 3 days old eggs

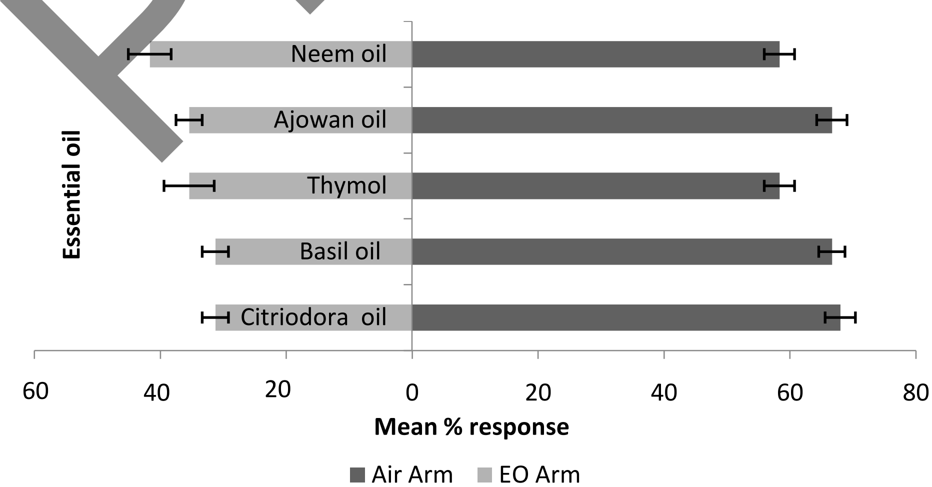

Fig.2. Behavioural response of adult O. rhinoceros to essential oils (0.1%).

were placed on sterilized oviposition substrate treated with various concentrations of essential oils. Among the essential oils tested, basil and citriodora oils at 1.0 % caused less than 31% and 37% hatchability respectively, whereas ajowan and thymol (1%) inhibited 50 per cent egg hatching. Across the essential oils, higher concentration (1%) of the oil was effective over the lower concentrations. Neem oil tested at all the doses did not have any impact on egg hatchability. It was on par with the control as evident from Table2. Oil derived from *E. citridora* inhibited the hatching of *Lutzomiyalongipalpis* at 40 mg/ ml. The higher dose of oil required to cause ovicidal effect was attributed to oils having neurotoxic effect as the compounds begin to act on nervous system after the growth of the embryo (Maciel et al., 2009). Essential oil of *T. ammi* exhibited larvicidal and pupicidalacivity against *M. domestica*. It also had strong oviposition deterrent activity against *M. domestica* (Aksorn and Mayura, 2018).

Toxicity of essential oils to first instar grubs of *O. rhinoceros* was assessed. Across the treatments, essential oil with higher dose (1%) was effective as compared to 0.2 and 0.6 %. Among the essential oils tested, basil oil at 1 % caused highest mortality followed by citriodora, ajowan oil and thymol which were at par. All the doses of neem oil tested caused lowest larval mortality (Table 2).

Contact toxicity to adult

Higher dose of essential oil *viz*., citriodora, basil, ajowan and thymol at 6 % caused adult mortality over 90 % as compared to lower doses of 3 and 1 %. Citriodora, basil and ajowan oil at 6 % caused over 95 % mortality. Neem oil at 6% caused over 60 % mortality of the beetles (Fig. 3). The insecticidal activity of essential oils to larvae and adult of *O. rhinoceros* may be due to compounds that have low molecular weight, high vapour pressure and hydrophobic nature. Low volatility

Table 2. Effect of essential oils on *O. rhinoceros* eggs and grubs

Essential oils	Conc. (%)	Egg hatchability (%)	Larval mortality (%)
Ajowan oil	0.2	83.75[bc]	8.75[ef]
	0.6	81.25[c]	25.00[cd]
	1.0	51.25[e]	46.25[b]
Thymol	0.2	83.75[bc]	15.00[cdef]
	0.6	81.25[c]	20.00[cde]
	1.0	52.50[e]	45.00[b]
Citriodora oil	0.2	82.50[bc]	11.25[def]
	0.6	75.00[cd]	27.50[c]
	1.0	37.50[f]	57.50[ab]
Basil oil	0.2	82.50[bc]	13.75[cdef]
	0.6	67.50[d]	27.50[c]
	1.0	31.25[f]	66.25[a]
Neem oil	0.2	98.75[a]	12.50[def]
	0.6	95.00[a]	11.25[def]
	1.0	93.75[a]	12.50[def]
Control		90.63[ab]	3.50[f]

Mean followed by same alphabet do not differ significantly by p=0.05 DMRT

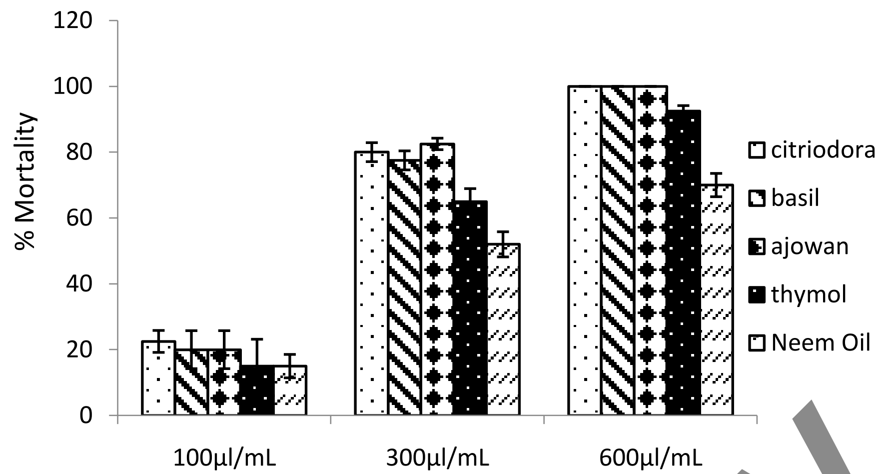

Fig.3. Contact toxicity of essential oils to adult O. rhinoceros

coupled with high vapour pressure helps in better activity of the compounds on the bio stages of the pest. The hydrophobic nature of the compounds in essential oils help to improve the binding on the cuticle of pest having wax layer and facilitate better penetration. Badawy et al., (2010) reported monoterpenes 1, 8-cineole, limonene and carvone having a strong fumigant activity against *Tetranychusurticae.* Volatile oil could penetrate organism via the respiratory system (Choi et al., 2004). Trans anethole, methyl chavicol and linalool the major constituents of sweet basil oil (10%) caused 90 % mortality of *Ceratatiscapitata, Bactroceracucurbitae* and *B. dorsalis* (Chang et al., 2009). Linalool caused fumigant toxicity to *O. surainamensis* (Eli Shaaya et al., 1991)

The essential oils, viz., basil oil, citriodora, ajowan oil and thymol caused physiological response in antennae of the adult beetle. Antennal neurons responding to the essential oils coupled with behavioural response to the beetles in causing repellence to beetles makes the essential oils as candidate to be integrated in management measures that are being used for rhinoceros beetle management. This repellence attribute can be exploited for the management of the pest by loading them in a suitable matrix. The essential oils in addition to causing physiological and behavioural response at lower dose cause insecticidal and growth regulatory effects as well. These phytoncides can be blended with the IPM practices of rhinoceros beetle management

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