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Ecofriendly management of Coconut Rhinoceros Beetle Grubs, *Oryctes* rhinoceros Linnaeus (Scarabaeidae: Coleoptera) using Botanical Extracts under Laboratory Condition

K. Manivasagam[1]*, M. Muthuswami[1], T. Srinivasan[1] and K.K. Kumar[2]

[1]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[2]Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

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ABSTRACT

Coconut rhinoceros beetle, *Oryctes rhinoceros* L. (Scarabaeidae: Coleoptera) is a serious pest which causes greatest impediment in the early establishment of juvenile palms. The experiment was conducted to test the insecticidal property of different botanical extracts (Aqueous extract) *viz.*, Neem based products (Neem cake, Neem leaf, Neem oil), Vitex (*Vitex negundo)* leaf, veldt grape (*Cissus quadrangularis*) leaf, Holy basil (*Ocimum sanctum*)leaf and Sweet flag (*Acorus calamus*) Rhizomeagainst the 2nd and 3rd instar grubs of Coconut Rhinoceros Beetle, *O. rhinoceros*. It was founded that Neem cake 30% was highly effective against Rhinoceros Beetle grubsthat elicits 54.00% and 50.00% mortality in case of 2nd and3rd instar grubs respectively. Followed by Neem cake, Neem oil shows good response of 49.33% and 40.67% mortality over 2ndand 3rd instar grubs. The mortality data fits good with the probit data in regard with chi-square($\div^2$) value. The lowest LC_{50} value (22.31%,26.19%) was obtained in Neem cake treatment in both 2nd and 3rd instar respectively. Followed by Neem cake, Neem oil, *Acorus calamus*Rhizome extract,Neem leaf extract, *Vitex negundo* leaf extract, *Cissus quadrangularis* leaf extract and *Ocimum sanctum* leaf extract having median lethal concentrations of about 30.07, 52.86, 63.11, 74.37, 85.42 and 85.53%. In case 3rd instar followed by Neem cake, *Acorus calamus* leaf extract,Neem oil, Neem leaf extract,*Vitex negundo* leaf extract, *Cissus quadrangularis* leaf extract and *Ocimum sanctum* leaf extract having median lethal concentrations of 38.00, 54.71,72.79, 95.35,95.71and 99.60%. Thus, the mortality data and LC_{50} value shows among different treatment Neem Cake shows high efficacy then compared to the others. Hence, it can be utilized for the effective management of Rhinoceros beetle grubs in case of immature larval stages by using drenching method.

Key words*: Oryctes rhinocerosL., Botanicals, Larval mortality, Median lethal concentration*

Introduction

Coconut, *Cocus nucifera* L.an important plantation crop belongs to Arecacaefamily, is the only living species in the genus of *Cocus*.Coconut is grown in 93 countries around the world. Among them, India ranks third in the global producer of coconut followed by Indonesia and Philippines. Kerala, Karnataka, Tamil Nadu, and Andhra Pradesh are

***Corresponding author's emai:** manivasagam453@gmail.com

the major coconut growing states of India. In India, Tamil Nadu is the second largest coconut producing state having annual production of about 3701.01 thousand metric tonnes (indiastat, 2019-2020). The coconut palms are popularly called as "Kalpa Viruksha" which means tree of life because of its valuable importance to humans (Nair *et al*.,2016).

The coconut palm is attacked by a number of insect pests throughout the year (Thampan, 1975). Among them, coconut rhinoceros beetle, *Oryctes rhinoceros* L. (Scarabaeidae: Coleoptera) is a serious pest which causes the greatest impediment in the early establishment of juvenile palms causing more than 20 percent damage (Catley, 1969).Even though *Oryctes sp*. have a wide host range such as banana, sugarcane, papaya and pineapple, they mostly prefer to feed on coconut palms.Grub of *O. rhinoceros* mainly finds its niche in manure pits, compost yards and in logged trees.Grubs of *Oryctes* don't damage the crop, only the adults cause severe economic damage to the coconut plantation. Beetles bore into the collar region of the young palms resulting in dead heart, twisted spindle with elephant-tusk like symptoms and perverted leaflets. In adult palms, the beetle bores into the unopened fronds/spindle region/spear leaf/spathes/nuts. The affected frond shows the characteristic diamond shaped cuts (V-shaped) upon unfurling as well as exposing chewed up fibers from the feeding site. Severely damaged palm will be prone to attack by red palm weevil and may also invites other disease-causing pathogens (Thampan, 1975).

Botanicals are the best alternate for ensuring the environmental safety when compared to chemical management. The compounds of plant origin are responsible for inducing some allergic reactions and metabolic changes in insects that could be used as an effective pest management option. The key strength of botanicals is their specificity, as they proven to be highly safe to animals as well as human beings (Miresmailli *et al.,* 2014 and Stevenson *et al.,* 2017).

Most of the management practices were focused to control the adults but still it is not effective due to inapplicability, in case of both established plantations and large coconut farms. Targeting the immature stages of *O. rhinoceros* will enhance an effective management of this pest before causing economic damage. And also, grub management is quite easier and effective by applying the management options in breeding sites such as manure pits or other compost yards. Hence, the present experiment was focused to evaluate the mortality percentage and median lethal concentration of different locally available botanicals against 2nd and 3rd instar grubs of *O. rhinoceros*.

Materials and Methods

Site of study

The present study was conductedat the Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore (Latitude, 11^{0}0′ 50" Nand Longitude, 76^{0}55′ 51" E)during August 2021. The test insects were collected from the Central farm, Tamil Nadu Agricultural University.

Botanicals

Neem based products (Neem cake, Neem leaf, Neem oil) which contains bioactive compound Azadirachtin (Schmutterer, 1985), Vitex (*Vitex negundo)* leaf which contains 22,23-dihydro-a-spinasterol-b-D-glucoside and salicylic acid (Chowdhury *et al.,* 2011), Veldt grape (*Cissus quadrangularis*) leaf which contains numerous bioactive alkaloids and flavonoids (Kumar *et al.,* 2012), Holy basil (*Ocimum sanctum*) leaf which contains camphor, limonene and â-caryophyllene (Singh *et al.,* 2014) and Sweet flag (*Acorus calamus*) Rhizome which contains á-asarone and â-asarone (Paneru *et al.,* 1997 and Kafle*et al.,* 2017) were obtained from Khadi Gramodyog Bhavan, Vadavalli and used in this experiment.

Insect culture

The pupae of *O. rhinoceros* were initially collected from the breeding sites such as manure pits and logged trees. The collected pupae are then stored in the plastic container (20×30 cm) filled with 5:1 ratio of cow dung and coir pith dust. The mouth of the container was covered with large khada cloth which is tightened with the help of elastic rubber bands. The culture setup was maintained in laboratory conditions 26±2^{0}C, 70±5%RH for 34 days. After adult emergence, the male and female beetleswere segregated based on the abdominal characters (Female's presence of fuzzy hairs at the tip of the abdomen whereas in males, the abdomen is smooth and glossy).Two pairs of such adults were released in the ratio of 1:1 (male: female) into the plastic container (20×30 cm) which contains a layer of fine sand

(3 cm thick) at bottom followed by a layer of coir dust (5 cm thick). After 10days, 2 cups of farmyard manure (100g each) were placed in the container as a substrate for oviposition. Container were examined once in a week and eggs were collected and incubated for hatching. Minimum hatching period is 10days. Newly hatched grubs were stored in pot culture condition. The plastic container of 20×30 cm (Depth×Height) filled with 3/4th with farm yard manure were used to rear grubs. The mouth of the container was sealed with the help of shade net. The pots were watered daily to maintain the average humidity. The grubs are reared until the pupation and next cycle of culture is followed as discussed above. (Suhasini *et al.,* 2017).

Preparation of plant extracts

For preparingbotanical extracts, 50 grams of each plant powders *viz.,*Neem leaf, *Vitex negundo* leaf, *Cissus quadrangularis* leaf, *Ocimum sanctum* leaf, *Acorus calamus* Rhizome were taken in 500ml beakers and added with 100 ml of distilled water. The botanical powder along with water was agitated at 6000 rpm for 30 minutes with a magnetic stirrer kept undisturbed for 24 hours (Shah *et al.,* 2008). After incubation, the extract was filtered using Whatman's No.2 filter paper and the filtrate was collected in a separate beaker.Then, the extract stored at 4 ^{0}C for further analysis.

Six different concentrations of botanical extracts (10, 15, 20, 25, 30, 35 %) were prepared by diluting condensed extracts of different botanicals with the help of distilled water. (Mansoor-ul-Hasan *et al.,* 2014 and Loko *et al.,* 2017). In case of Neem cake and Neem oil, the serial dilution is carried out directly to preparethe different concentrations.

Bioassay (*In vitro* condition)

Larval dipping/immersion method is used for the laboratory bioassay to test the efficacy of different botanicals (Paramasivam and Selvi, 2017). Two sets of bioassays were carried out separately by using the 2nd and 3rd instar grubs. The grubs were dipped in the aqueous extracts of different botanical concentrations for a period of 3 secs. For control, the grubs were dipped in distill water for 3 secs with help of forceps (Ricaño *et al.,* 2013). The treated grubs were released into the ventilated plastic containers for the observation of mortality. For each concentration, 30 grubs were released. Each concentration of botanicals was replicated thrice with 10 grubs in each replicate. Observation on percent mortality was recorded at 2 days' time interval for the period of 10 days (Sutanto *et al.,* 2021). Among the different concentration of botanicals, the best concentration is selected based on results of bioassay and used for further analysis. The LC_{50}value for each botanical were determined using the technique outlined above.

Statistical analysis

LC_{50} for different botanicals was calculated using Probit analysis (Finney, 1971). The percent mortality was analyzed using one way ANOVA through completely randomized block design, and the means were compared using Duncan's multiple range test (DMRT) with a P value of 0.05. (Gomez and Gomez, 1984). Before the analysis, the necessary data transformations were performed and the calculations were done by using the computer-based IBM SPSS (Statistical Package for the Social Sciences) version 23 software.

Results

Effect of botanicals on 2nd instar grubs

In terms of percent larval mortality, there were substantial variations across the seven treatments (Table 1). Among the treatments, highest larval mortality was recorded from Neem cakewhich was 54.00%followed by Neem oil extract (49.33%), *Acorus calamus* rhizome extract (41.33%), Neem leaf extract (37.33%), *Vitex negundo* leaf extract (33.33%), *Cissus quadrangularis* leaf extract (30.00%) and *Ocimum sanctum* leaf extract (26.67%) on comparison with control. Highest percent reduction over control was recorded in Neem cake extract (51.06%) followed by Neem oil extract (46.10%). The least per cent reduction was recorded with *Ocimum sanctum* leaf extract(21.99%).

Effect of botanicals on 3rd instar grubs

In case of 3rd instar (Table 3), the Neem cake (50.00%) was found to be effective followed by Neem oil (40.67%), *Acorus calamus* rhizome extract (34.67%), Neem leaf extract (29.33%), *Vitex negundo* leaf extract (25.33%), *Cissus quadrangularis* leaf extract (20.67%) and *Ocimum sanctum* leaf extract (14.67%) to manage the3rd instar grubs of Rhinoceros beetle, *O. rhinoceros*. Highest percent reduction over control was recorded in Neem cake extract (47.18%)

Table 1. Efficacy of botanical extracts on 2nd instar grubs of Rhinoceros beetle, *Oryctes rhinoceros.*

Sl. No.	Treatment		% Larval mortality of 2nd instar grubs at different time period*±SE					Overall Mean (%)	Percent reduction over control (%)
			2 DAT	4 DAT	6 DAT	8 DAT	10 DAT		
1.	Neem cake (30%)		46.67±0.33 (43.09)^{a}	50.00±0.00 (45.00)^{a}	53.33±0.33 (46.91)^{a}	56.67±0.33 (48.83)^{a}	63.33±0.33 (52.73)^{a}	54.00	51.06
2.	Neem leaf extract (30%)		30.00±0.00 (33.21)^{cd}	33.33±0.33 (35.26)^{bc}	36.67±0.33 (37.27)^{cd}	40.00±0.00 (39.23)^{cd}	46.67±0.33 (43.09)^{cd}	37.33	33.33
3.	Neem oil (30%)		40.00±0.33 (39.23)^{ab}	46.67±0.33 (43.09)^{a}	50.00±0.00 (45.00)^{ab}	53.33±0.33 (46.91)^{ab}	56.67±0.33 (48.83)^{ab}	49.33	46.10
4.	*Vitex negundo* leaf extract (30%)		26.67±0.33 (31.09)^{cde}	30.00±0.00 (33.21)^{bc}	33.33±0.33 (35.26)^{cd}	36.67±0.33 (37.27)^{de}	40.00±0.00 (39.23)^{de}	33.33	29.07
5.	*Cissus quadrangularis* leaf extract (30%)		23.33±0.00 (28.88)^{de}	26.67±0.33 (31.09)^{bc}	30.00±0.00 (33.21)^{cd}	33.33±0.33 (35.26)^{de}	36.67±0.33 (37.27)^{e}	30.00	25.53
6.	*Ocimumsanctum* leaf extract (30%)		20.00±0.00 (26.57)^{e}	23.33±0.33 (28.88)^{c}	26.67±0.33 (31.09)^{d}	30.00±0.00 (33.21)^{e}	33.33±0.33 (35.26)^{e}	26.67	21.99
7.	*Acorus calamus* rhizome extract (30%)		33.33±0.33 (35.26)^{bc}	36.67±0.33 (37.27)^{ab}	40.00±0.00 (39.23)^{bc}	46.67±0.33 (43.09)^{bc}	50.00±0.00 (45.00)^{bc}	41.33	37.59
8.	Control (Water)		0.00±0.00 (0.00)^{f}	3.33±0.33 (10.52)^{d}	6.67±0.33 (14.96)^{e}	10.00±0.00 (18.43)^{f}	10.00±0.00 (18.43)^{f}	6.00	-
9.	SEm		0.73	1.17	1.10	0.72	0.71	-	-
10.	C.D (P= 0.05)	2.19	3.52	3.29	2.15	2.13	-	-	

Note: *Mean of three replications for 2nd instar grubs.Value in parentheses is arc sine transformed values; Means with the same letter are not significantly different at $p < 0.05$; S.E: Standard Error; DAT: Days After Treatment; SEm: Standard error of mean; C.D: Critical difference.

followed by Neem oil (47.06%). As like 2nd instar grubs, least per cent reduction was recorded with *Ocimum sanctum* leaf extract (37.33%). In both instars the neem cake extracts show highest percent mortality then compared to the other botanical extracts.

Median lethal concentrations (LC_{50}) for 2nd instar grubs

The median lethal concentrations (LC_{50}) of several botanical extracts against 2nd instar grubsare presented in Table 2. The experimental results showed that Neem cake extract was highly effective having lowest LC_{50} value (22.31%) followed by Neem oil extract, *Acorus calamus* rhizome extract,Neem leaf extract,*Vitex negundo* leaf extract, *Cissus quadrangularis* leaf extract and *Ocimum sanctum* leaf extract having median lethal concentrations of 30.07,52.86, 63.11, 74.37, 85.42 and 85.53%.

Median lethal concentrations (LC_{50}) for 3rd instar grubs

The median lethal concentrations (LC_{50}) of different botanical extracts against 3rd instar grubs are presented in Table 4. The experimental results showed that Neem cake extract was highly effective having lowest LC_{50} value (26.19%) followed by Neem oil, *Acorus calamus* rhizome extract, *Vitex negundo* leaf extract, Neem leaf extract, *Cissus quadrangularis* leaf extract and *Ocimum sanctum* leaf extract having median lethal concentrations of 38.00, 54.71, 72.79, 95.35, 95.71 and 99.60%.

Discussion

Because of its insecticidal and anti-feedent capabilities against insects, neem-based products had the highest percentage of larval death. Ranger et al (2009) reported the lowest medial lethal concentration of 1.13ml/l for Neem based product Azatin against white grub, Popillia japonica shows similar result that Neem based products shows lower LC_{50} value than compared to others and this finding greatly supports our result.Another finding by Mohammad et al (2010) studied the pathogenicity of Neem against red palm weevil, Rhynchophorus ferrugineus and founded that highest mortality rates attained in prepupae at higher concentration of neem (500 ppm) respectively. This result is upholding our research finding and suggest neem-based formulations is highly effective against coleopteran pest such as O. rhinoceros.

Conclusion

When compared to control, Botanical extracts had the good response to the 2ndand 3rd instar grubs of the Rhinoceros beetle, *O. rhinoceros*. According to the results, Neem cake extract 30% was most effective against Rhinoceros beetle grubs, *O. rhinoceros* followed by Neem oil extract 30% in case of 2ndand 3rdinstar grubs within 2 days because of having the potential insecticidal properties. Mortality percentage increase over time and the cumulative mortality percentage shows that Neem cake treatment shows better toxicity against the grubs then compared to other treatments. Regarding the Medial Lethal concentration (LC_{50}), Neem cake shows less LC_{50} value then compared to the other treatment in both the instars. These natural plant-based compounds pose no health risks to environment andthey are also inexpensive. Thereby these findings suggest that botanically based biopesticides might be used as drench treatments to reduce rhinoceros beetle grubs, *O. rhinoceros* that found in manure dumps and logged tree trunks. The vast variety of plant compo-

Table 2. LC_{50} of different botanical extract for 2nd instar grubs of Rhinoceros beetle, *Oryctes rhinoceros.*

Botanicals	LC_{50}(%)	Regression Equation	Fiducial Limit	χ^2	
				Calculated	Table
Neem cake	22.31	y = 0.8616x + 3.8381	13.58-36.65	0.2598**	9.4877
Neem leaf extract	63.11	y = 0.9624x + 3.2643	17.50-227.64	0.2537**	9.4877
Neem oil	30.07	y = 0.7726x + 3.8562	14.65-68.69	0.0766**	9.4877
Vitex negundo leaf extract	74.37	y = 1.0135x + 3.0993	18.18-304.16	0.2379**	9.4877
Cissus quadrangularis leaf extract	85.42	y = 1.0829x + 2.904	18.96-384.79	0.2329**	9.4877
Ocimum sanctum leaf extract	85.53	y = 1.4004x + 2.2656	25.40-287.99	0.2251**	9.4877
Acorus calamus rhizome extract	52.86	y = 0.9246x + 3.4072	16.86-165.74	0.2437**	9.4877

Note: χ^2 Chi-square test
** χ^2 Significant at p=0.05

Table 3. Efficacy of botanical extracts on 3rd instar grubs of Rhinoceros beetle, *Oryctes rhinoceros*.

Sl. No.	Treatment	% Larval mortality of 2nd instar grubs at different time period*±SE					Overall Mean (%)	Percent reduction over control (%)
		2 DAT	4 DAT	6 DAT	8 DAT	10 DAT		
1.	Neem cake (30%)	36.67±0.33 (37.27)^{a}	46.67±0.33 (43.09)^{a}	50.00±0.58 (45.00)^{a}	56.67±0.33 (48.83)^{a}	60.00±0.58 (50.77)^{a}	50.00	47.18
2.	Neem leaf extract (30%)	13.33±0.33 (21.42)^{bcd}	26.67±0.33 (31.09)^{bc}	30.00±0.00 (33.21)^{bcd}	36.67±0.33 (37.27)^{bcd}	40.00±0.00 (39.23)^{cd}	29.33	25.35
3.	Neem oil (30%)	23.33±0.33 (28.88)^{b}	36.67±0.33 (37.27)^{ab}	43.33±0.33 (41.17)^{ab}	46.67±0.33 (43.09)^{ab}	53.33±0.33 (46.91)^{ab}	40.67	37.33
4.	*Vitex negundo* leaf extract (30%)	16.67±0.33 (24.09)^{bc}	20.00±0.00 (26.57)^{cd}	23.33±0.33 (28.88)^{cd}	30.00±0.00 (33.21)^{cde}	36.67±0.33 (37.27)^{de}	25.33	21.13
5.	*Cissus quadrangularis* leaf extract (30%)	10.00±0.00 (18.43)^{cd}	16.67±0.33 (24.09)^{de}	20.00±0.00 (26.57)^{de}	26.67±0.33 (31.09)^{de}	30.00±0.00 (33.21)^{ef}	20.67	16.20
6.	*Ocimumsanctum* leaf extract (30%)	6.67±0.33 (14.96)^{d}	10.00±0.00 (18.43)^{e}	13.33±0.33 (21.42)^{e}	20.00±0.00 (26.57)^{e}	23.33±0.33 (28.88)^{f}	14.67	9.87
7.	*Acorus calamus* rhizome extract (30%)	20.00±0.00 (26.57)^{b}	30.00±0.00 (33.21)^{bc}	36.67±0.33 (37.27)^{bc}	40.00±0.00 (39.23)^{bc}	46.67±0.33 (43.09)^{bc}	34.67	30.99
8.	Control (Water)	0.00±0.00 (0.00)^{e}	3.33±0.33 (10.52)^{f}	6.67±0.33 (14.96)^{f}	6.67±0.33 (14.96)^{f}	10.00±0.00 (18.43)^{g}	5.33	-
9.	SEm	1.23	1.16	1.23	1.08	0.83	-	-
10.	C.D (P= 0.05)	3.67	3.48	3.70	3.26	2.49	-	-

Note: * Mean of three replications for 3rd instar grubs.Value in parentheses is arc sine transformed values;Means with the same letter are not significantly different at $p < 0.05$.S.E: Standard Error; DAT: Days After Treatment; SEm: Standard error of mean; C.D: Critical difference.

Table 4. LC_{50} of different botanical extract for 3rd instar grubs of Rhinoceros beetle, *Oryctes rhinoceros.*

Botanicals	LC_{50}(%)	Regression Equation	Fiducial Limit	χ^2	
				Calculated	Table
Neem cake	26.19	y = 0.962x + 3.6353	15.90-42.13	0.5965**	9.4877
Neem leaf extract	72.79	y = 1.4898x + 1.71	21.69-244.20	0.5653**	9.4877
Neem oil	38.00	y = 1.2761x + 2.9849	21.05-68.59	0.0830**	9.4877
Vitex negundo leaf extract	95.35	y = 1.3176x + 2.357	23.63-384.68	0.2309**	9.4877
Cissus quadrangularis leaf extract	95.71	y = 1.5346x + 1.9261	26.25-348.92	0.1964**	9.4877
Ocimumsanctum leaf extract	99.60	y = 1.8743x + 1.1329	29.89-331.81	0.5496**	9.4877
Acorus calamus rhizome extract	54.71	y = 1.2174x + 2.8759	22.07-135.59	0.3150**	9.4877

Note: χ^2 Chi-square test
** χ^2 Significant at p=0.05

nents and their insecticidal toxicity was evaluated in this study which helps to choose better plant compound in orderto manage the grubs at their non infective immature stage.

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Conflict of interest

The authors have no conflict of interest to declare.

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