Electrophysiological and Behavioural Responses of Coconut Black Beetle (*Oryctes rhinoceros* L.) (Coleoptera: Scarabaeidae) to Selected Plant Volatiles

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Abstract- *Rhinoceros beetle (RB) (Oryctes rhinoceros L.) is one of the devastating pests of coconut and other palm species. The main strategy for the management of RB is the use of highly toxic synthetic pesticides. However, banning of toxic pesticides in Sri Lanka urges finding alternative repellant materials. The use of semiochemicals especially behaviour-modifying plant volatiles is a recent trend in agriculture pest management. Therefore, this study was conducted to identify the repellent plant volatiles to formulate a semiochemical-based pest management strategy. Electroantennographic (EAG) technique employed to explore the antennal response of male and female RB to 12 plant volatiles using a commercially available EAG system. Results revealed that mean antennal responses were significantly higher (p<0.05) on both males and females to plant volatiles than control. The largest response evoked by aggregation pheromones (Male 0.7698 ± 0.130 mV, Female 0.9504 ± 0.232 mV) then evoked the higher responses by the male to Ethyl butyrate, Limonene (+), 1-Octane-3-ol, α-pinene, and Propyl butyrate. Whereas female evoked higher responses to 1-Octane-3-ol, Limonene (+), Citronella, and 3-Hexene-1-ol. The dose-response study indicated the male responses had decreasing trend with increasing doses from 1% to 10%, while the female was erratic. Moreover, dual choice olfactometer studies revealed that males and females were attracted to RB aggregation pheromone, Limonene (+), Ethyl propionate, and β-Myrcene where they were repelled by Citronellol, 2-hexane-ol, 1-Octane-3-ol, and α-Pinene. Therefore, Citronellol, 2-hexane-1-ol, α-Pinene and 1-Octane-3-ol, can be used as a potential plant volatile to formulate the repelling compounds to manage the O. rhinoceros.*

Keywords: Volatiles, EAG, Repellent, Rhinoceros beetle

I. INTRODUCTION

The Coconut rhinoceros beetle (RB), commonly known as Black beetle, *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) is one of the devastating pests of coconut palms, prevalent in all coconut growing areas in Sri Lanka. There were several species of rhinoceros beetles of the family Scarabaeidae that are distributed throughout the world, such as *Oryctes boas*, *Oryctes Monoceros*, *Oryctes elegans*, *Scapanes australis* and *Strategus aloeus* (Singh and Rethinam, 2005). The metamorphosis of *O. rhinoceros* is complete with four stages; egg, larvae, pupa, and adult. The total cycle from egg to emergence of the adult from the pupal cocoon lasts 4 - 9 months, the mean period being about 6 months (Lever, 1969). In Sri Lanka the average period from egg to adult is thus 154 days (range 128 to 178 days). The adult life is on average 89 days (range of total life span 193 to 292 days) (Goonewardena, 1958). Although *O. rhinoceros* is found in several regions of the world, its shape, size, and colour are generally consistent (Manjeri et al., 2013). Both males and females possess a similarly sized horn used for leverage when moving within tightly-packed leaves or the cavities, they create in the crown of palms, the horn length is longer on average for males (Doane 1913). The antennae of the rhinoceros beetle, *O. rhinoceros* (Coleoptera: Dynastidae), comprise 4 parts: the scape, the pedicel, a funicle, and a club of 3 lamellate segments (Renou et al., 1988).

Oryctes rhinoceros mainly damages the young palms and seedlings and occasional death of seedlings occurs. The damage is caused by the adult beetle which bores and enters into the soft bud region, continues feeding on the soft tissues, resulting in damage to the unopened leaves and their petioles (Bedford, 2014). When the leaves grow out, the damage appears as V-shaped geometric cuts or holes through the base of the fronds. If the damage to the petiole is extensive, breaking of the flag leaf could occur. The damage often causes choking of the developing leaves in seedlings, resulting in the formation of crooked and malformed leaves while the damage to the growing point leads to the death of seedlings (Dornberg, 2015). It may also provide an entry point for lethal secondary attacks by the red palm weevils or by pathogens. When the attack is on the unopened spathe, the inflorescence gets destroyed (Kumara, 2015).

The palms with 50 % frond damage corresponded to a reduction of 13 % leaf area caused a 23 % reduction in nut yield (Singh and Rethinam, 2005) as compared with undamaged palms. A recent survey conducted by the Coconut Research Institute revealed that the damage caused by black beetle is the most widespread pest damage in coconut. The survey further indicated that 72 % of growers claimed that the black beetle damage had existed in their lands, but only 52 % were aware that it is a serious problem in coconut cultivation (Peiris, *et al.*, 2006). For management of the *O. rhinoceros*, can be directed at either the larval or adult stage of the life cycle. Several control methods can be practiced such as cultural, chemical and biological but the integration of all these methods is the most effective way of managing the pest. Hence, it is urged to identify the new effective and environment-friendly compounds for repelling the beetle from the plantation. Mainly on the development of semiochemical based product used this pest management method. Semiochemicals are mainly emitted by plants or by an insect for inter and intraspecies communications. These are volatile compounds and are conspecific and behavioural modifying chemicals.

Mainly Electroantennogramme (EAG) technique and behavioural studies can be effectively used for identification of semiochemical responses. The responsive compounds act as attractants and some act as a repellent (Schneider, 1957; Schneider *et al*., 1967). Therefore, the objective of this study is identification of effective repellent plant volatiles through EAG and behavioural studies and thereby can be used effective environmentally friendly semiochemicals formulation as a green pest management product to management of *O. rhinoceros.*

II. MATERIALS AND METHODS

The following plant volatiles were used to investigate the EAG and Behavioural studies *i.e.* Limonene (+), α-pinene, Ethyl propionate, Nonanoic acid, 4-Phenyl-2-butanone, Propyl butyrate, 2-Hexene-1-ol, Citronella, 1-Octane-3-ol, β-Myrcene, 3-Hexene-1-ol, and Ethyl butyrate. While Black beetle pheromone lure (Recommended and commercially available), Hexane (Solvent) were used to compare the volatiles as controls.

A. Insect culture/ insect rearing

The late instar larvae of *O. rhinoceros* were collected from the field and reared in the insect rearing glass boxes by providing decaying organic manure especially coconut log pieces and coir dust. Subsequently, after the emergence of adults both male and female were separated and maintained separately for Electroantennogramme and behavioural studies.

B. Electroantennogramme (EAG) Studies

Electroantennogramme studies were conducted to determine the electrophysiological responses of both male and female antenna to selected plant volatiles. EAG studies were conducted using a commercially available SYNTEC EAG system at the electrophysiological study laboratory of the Coconut Research Institute, Lunuwila. Five microliters of each selected volatile solution were used for EAG and 1% solution of *O. rhinoceros* aggregation pheromone, fresh air and hexane were used as positive and negative control treatment respectively. The selected plant volatiles were diluted in Hexane to form a 1% solution. A filter paper (3 cm x 0.5 cm) was inserted into the Pasteur pipette. Then it was treated with 05 µl of the test solution which was either a volatile organic compound or a pheromone, and allow some time to evaporate the volatile. The beetle antenna was removed and the antenna was placed in between the two terminals of the forked type electrode which were fixed with electrically conductive gel (salt-free electrode gel) and adjustments were made under the medium power of a stereo microscope for further stabilization of the antenna (Kumara, 2015). The treated Pasteur pipette was inserted through the air-carrying duct in a way that the airflow could carry the volatile compound or the pheromone through the passage towards the antenna. An electric impulse was given just when the airflow was released towards the antenna. The electroantennogram for their relevant volatile cue or the pheromone was recorded by using Auto spike software. Each volatile was replicated five times and the triggered voltage values were analyzed using One Way ANOVA followed by Tukey post hoc test.

C. Behavioural Studies for selected plant volatiles

A dual choice olfactometer study was conducted to determine the behaviour of the beetle against selected electrophysiologically active plant volatiles. The study was conducted at the behaviour testing laboratory in the Coconut Research Institute, Lunuwila using a dual choice behavioural testing olfactometer system developed by Kumara (2015).

The experiment was conducted for both males and females separately and a mixture of beetles. Five male beetles and five female beetles were transferred to the center of the apparatus from the rearing box. Freshly cut coconut frond pieces were placed equally at both the arms of the apparatus. Fifty microliters of 1 % volatile solutions were used for the behavioural studies. The coconut frond pieces, placed at the one arm was treated with the hexane which was considered to be the control, and the coconut frond pieces, placed at the other arm was treated with the test sample which was a volatile organic compound. The setup was kept overnight and observation of searching behaviours was recorded at 4 hrs. intervals. The number of beetles that could be recovered from each end and the center were recorded in the morning. Each test was replicated with 30 individuals and the number of responded beetles for the choices were compared using the chi-square test assuming equal probabilities for both ends.

D. Dose-response EAG for selected repellent plant volatiles

EAG studies were conducted to determine the effective dose of selected electrophysiologically active volatile compounds. Series of dilution were prepared *i.e*. 1 %, 5 %, 10 %, 15 %, 20 %, and 25% and EAG studies were conducted for both male and female beetles. A dose-response curve was prepared and the effective dose of maximum responses was determined.

III. RESULTS AND DISCUSSION

A. Electrophysiological responses of O.rhinoceros against selected plant volatiles

The depolarization response results showed that there were significant antennal responses against selected volatiles on both male and female beetles ($p < 0.05$) (Table 1) compared to the control (Air and Solvent). Both male and Female *O. rhinoceros* (Male, 0.7698 ± 0.130 mV, Female, 0.1982 ± 0.015 mV) gave the highest response for aggregation pheromone from the tested volatiles. The aggregation pheromone being more effective with obvious results to attract both male and female beetles in the fields and it is already recommended for commercial use in most coconut growing countries including Sri Lanka for trapping the *O. rhinoceros*. However, the highest response of aggregation pheromone was not significantly different with certain plant volatiles giving the highest responses. The response of female beetle antenna for aggregation pheromone followed by the plant volatiles *viz*. 1-Octane 3-ol, Limonene (+), Citranellol, 3- hexene – 1-ol, Propyl butyrate, α- pinene, and β- Myrcene respectively (Table 1). Whereas the male antennal responses were higher in aggregation pheromone followed by plant volatiles were Ethyl butyrate, α-pinene, Limonene (+), 1- Octane 3-ol, and Propyl butyrate respectively (Table 1). Considering the male and female responses against the number of volatiles, the male has a broader range of volatiles than the female. However, these results indicating that both male and female responses were common to certain plant volatiles *viz* Limonene (+), Propyl butyrate, and 1-Octan-3-ol.right-justified.

Table 1: EAG Responses of *O. rhinoceros* against plant volatiles

	Female	Male
Volatile	**Mean (mV) ± SE**	**Mean (mV) ± SE**
Air	0.1792 ± 0.014	0.1162 ± 0.030
Limonen (+)	0.7974 ± 0.184	0.6868 ± 0.137
α- Pinene	0.3696 ±0.024	0.7342 ± 0.153
B-Myrcene	0.5346 ± 0.186	0.5776 ± 0.103
Ethyl propionate	0.3572 ± 0.033	0.4580 ± 0.064
Nonanoic acid	0.4850 ± 0.192	0.4460 ± 0.072
4-Phenyl-2-butanone	0.4400 ± 0.174	0.4390 ± 0.170
Propyl butyrate	0.5012 ± 0.194	0.6874 ± 0.406
Ethyl butyrate	0.4958 ± 0.186	0.7358 ± 0.148
2-Hexene-1-ol	0.3060 ± 0.043	0.3264 ± 0.007
Citronella	0.6858 ± 0.204	0.4460 ± 0.051
1-Octain-3-ol	0.9260 ± 0.240	0.6842 ± 0.158
3-Hexene-1-ol	0.7286 ± 0.269	0.7116 ± 0.143
B/B Pheromone	0.9504 ± 0.232	0.7698 ± 0.130
Hexane	0.1982 ± 0.015	0.5466 ± 0.036

F value(df)	1.875 (12, 58)	1.693 (12, 58)
P value	0.020	0.044

Mean antennal response (mV) of males and females of RB for plant volatiles, SE indicate the standard error of the mean

B. *Dose-response EAG studies of O. rhinoceros against selected plant volatiles.*

Dose-response EAG results revealed that there was a decreasing trend across the increasing doses of selected volatiles for male. The maximum response was recorded for the 1 % volatile dose (Fig. 1) among the tested doses. However, the declining trend was seen up to 15 % dosage and again it increased with the 20% dosage. The results further indicated that the male beetles were highly sensitive to the above responsive volatiles and to take optimum dosage further studies are needed with doses below 1%. The females dose-response EAG indicating that increasing trend towards the increasing dose of volatiles up to 15 % and then it was seen in decreasing trends (Fig 2). To obtain optimum doses for responsive volatiles further studies are needed with a broad range of doses with all responsive chemicals.

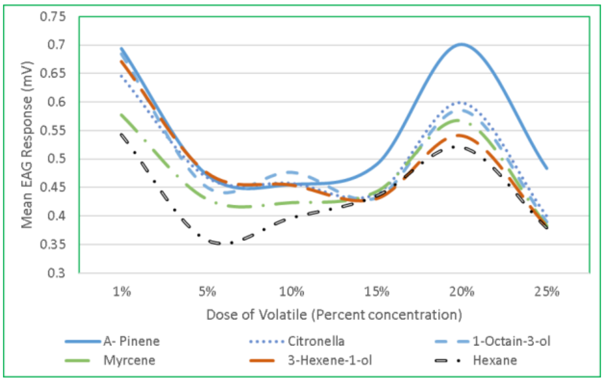

Figure 1: Antennal response of male *O. rhinoceros* for selected volatiles with different doses

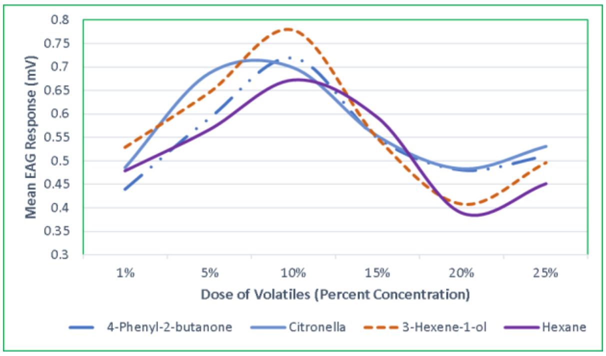

Figure 2: Antennal response of female *O. rhinoceros* for selected volatiles with different doses.

C. *Behavioural Studies*

Dual choice olfactometer studies were conducted to determine the behavioural response against the selected plant volatiles. Both male and female beetles responses were indicated two types of behaviours. Either the beetles were attracted towards the volatiles treated arm (Positive) called attraction behaviour of beetles were attracted towards the control arm (Negative) that indicating they were repelled from the volatile compounds. Male beetles showed the sensitive behaviour for selected all volatiles and maximum attraction behaviour reported towards the *O. rhinoceros* aggregation pheromone and followed by Limonene (+), Ethyl propionate, Myrcene, Propyl butyrate, and Ethyl butyrate. Whereas Citronellol, 2-Hexene-1-ol, 1-Octan-3-ol, and α – Pinene were repelled the male beetles (Fig. 3). Female beetles were more attracted towards the *O. rhinoceros* aggregation pheromone followed by β-Myrcene, Limonene (+) and Ethyl butyrate attracted the beetles. Whereas Citronellol, α-Pinene, 4-Phenyl-2-butanone and 2-hexane-ol were repelled the female beetles (Fig. 4).

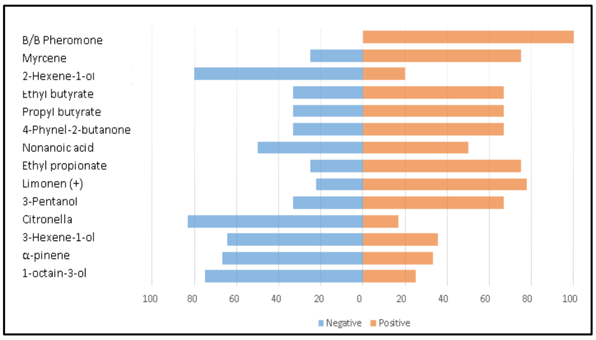

Figure 3: The behavioural response of male RB to the Plant volatiles. The bars indicate the percentage of beetle responses towards the tested volatiles, the negative indicates the beetle repel from the test volatile while positive indicates the attraction of beetles towards the volatile treated side of the Olfactometer.

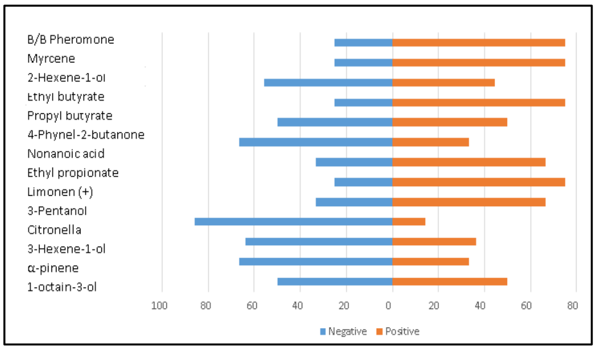

Figure 4: The Behavioural response of female RB to the Plant volatiles. The bars indicate the percentage of beetle responses towards the tested volatiles, the negative indicates the beetle repel from the test volatile while positive indicates the attraction of beetles towards the volatile treated arm of the Olfactometer.

For the management of the *O. rhinoceros* it is important to identify the repellence action of plant volatiles to formulate a semiochemical due to the availability of aggregation pheromone to attract and trap. Application of burnt engine oil to the leaf base, application of carbofuran granules to the leaf axis and application of naphthalene balls to the leaf axis are some recommended repellents used in the field for management of *O. rhinoceros*. However, banning toxic chemical pesticides, due to side effects of other recommended products, urged for finding out of alternative repellents. In this, study it was identified the repellent plant volatiles those were effective for both male and female such as Citranellol, 1-Octane-3-ol, α-Pinene, 4-Phenyl-2-butanone and 2-hexane-ol, which can be suggested as a potential plant volatile for the formulation of the repellence for management of *O. rhinoceros*. The dose-response studies revealed that there were no significant differences among the tested doses hence, further beetle can be responses of very low doses and it can be suggested to test low doses. Based on the tested doses, dose of 1% to 10 % can be suggested for formulating the green pesticides, as they will be environmentally and economically feasible for large-scale usage.

IV. CONCLUSION

Both male and female *O. rhinoceros* evoked the highest antennal response to their aggregation pheromone followed by electrophysiologically active plant volatiles *viz* 3-Hexen-1-ol, Limonene (+) and 1-Octan-3-ol. Whereas both male and female showed repellent behaviours against Citronella, α-pinene and 3-Hexene-1-ol plant volatiles. Based on the results of EAG, Olfactometer and dose-response studies, the repellent volatiles such as, Citronellol, 1-Octane-3-ol, 3-Hexen -1-ol and α-Pinene with the effective doses of 1% to 10 % concentrations can be used to formulate the repellent compounds as a green pest management strategies for the management of *O. rhinoceros* in coconut cultivation.

REFERENCES

Bedford, G.O. (2014). Advances in the control of rhinoceros beetle, *Oryctes rhinoceros* in oil palm, *Journal of Oil Palm Research*, vol. 26(3), pp. 183-194.”

Doane, R.W. (1913). How *Oryctes rhinoceros*, a dynastid beetle, uses its horn. *Science, New Series* ,vol. 38.990, pp. 883.

Dornberg, M. (2015). Introduction-Distribution-Description-Biology-Host Plants-Economic Importance-Management and Control-Selected References, Division of Plant Industry, *Florida Department of Agriculture and Consumer Services*. EENY-629.

Goonewardena, H. F. (1958). The rhinoceros beetle (*Oryctes rhinoceros* L.) in Ceylon part I. Introduction, distribution and life history, *Trop. Agric. Ceylon* 114: pp. 39-60.

Kumara, A. D. N. T., Chandrashekharaiah, M., Kandakoor S. B., Chakravarthy, A. K. (2015). Status and Management of Three Major Insect Pests of Coconut in the Tropics and Subtropics, A. K. Chakravarthy (ed.), New Horizons in Insect Science: Towards Sustainable Pest Management, © *Springer*, pp 359-381.DOI 10.1007/978-81-322-2089-3_32

Kumara, A.D.N.T. (2015). Electrophysiological and Behavioural responses of Coconut black headed caterpillar *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) to Plant semiochemicals, *Ph.D. Thesis*, University of Agricultural Sciences, Bangalore, pp 20-30.

Lever, R.J.A.W. (1969). Pests of coconut palm, *Food and Agriculture Organization of the United Nations*, Rome, ISBN 92-5-100857-4, pp. 125 – 133.

Manjeri, G., Muhamad R, Faridah, Q.Z., Tan, S.G. (2013). Morphometric analysis of Oryctes rhinoceros (L.) (Coleoptera: Scarabaeidae) from oil palm plantations, *The Coleopterists Bulletin* 67: pp. 194-200.

Peiris, T.S.G., Appuhamy, P.A.H.N., Nainanayake, N.P.A.D., Bandaranayake, C.K., Fernando, M.T.N. (2006). Coconut Research, Development and Dissemination of Technologies-Growers Perception, A Diagnostic Survey Report, *Coconut Research Institute, Lunuwila, Sri Lanka*. pp. 57 – 62.

Renou, M., Tauban, D., Morin, J.P. (1998). Structure and function of antennal pore plate sensilla of *Oryctes rhinoceros* (L.) (Coleoptera: Dynastidae), *International Journal of Insect Morphology and Embryology*, Volume 27, Issue 3, pp. 227-233.

Schneider, D. (1957). Elektrophysiologische Untersuchungen von Chemound Mechanoreceptoren de Antenne des Seidenspinners *Bombyx mori* L. Z. Vergl, *Physiol*, 40. pp. 8-41.

Schneider, D., Block, B.C., Boeckh, J., Priesner, E. (1967). The reaction of the male silk moth to Bombykol and its isomers: electro-antenna diagram and behavior, *Journal of Comparative Physiology*, *Olfaction and Taste: Proceedings of the Third International*. volume 54, pp 192 - 209.

Singh, S.P. and Rethinam, P. (2005). Rhinoceros beetles, *Asian Pacific Coconut Community. Jakarta, Indonesia*, pp. 21 – 22.