



## Efficacy of aggregation pheromone in trapping red palm weevil (*Rhynchophorus ferrugineus* Olivier) and rhinoceros beetle (*Oryctes rhinoceros* Linn.) from infested coconut palms

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### Abstract

Red palm weevil and Rhinoceros beetle are the major pests inflicting severe damage to coconut palms. Due to ineffectiveness of the current management practices to control the two important pests on coconut, a study was conducted to know the attractiveness of red palm weevil and rhinoceros beetle to aggregation pheromone. Olfactometer studies indicated that the aggregation pheromone of red palm weevil and rhinoceros beetle attracted significantly more number of weevils (13.4 females and 7.6 male weevils) and beetles (6.5 male and 12.3 female beetles), respectively than control. Similarly, field studies found that both 750 and 1000 mg pheromone dosage lures of red palm weevil and rhinoceros beetle trapped significantly higher numbers of weevils (695.80 and 789 weevils, respectively) and beetles (98 and 108 beetles, respectively) in traps ( $P < 0.05$ ), respectively. On an average ( $n=6$  field trials) 80-85% red palm weevil and 72-78% rhinoceros beetle population got trapped. Observations indicated activity of red palm weevil throughout the year and of rhinoceros beetle from September to March around Bangalore, South India. Pheromone traps for red palm weevil can be placed in fields from June to August and October to December and September to February for rhinoceros beetle. Population reductions of the two coleopteran pests by pheromone traps are compatible with mechanical and cultural management tools with cumulative effects.

### Key words

Aggregation pheromone, Olfactometer, Red palm weevil, Rhinoceros beetle

### Introduction

Coconut (*Cocos nucifera* Linn.) is a major, commercially grown plantation crop in 93 countries of the world. India and Sri Lanka together account for 78% of total coconut world production. India contributes 15.53 % area and 22.34 % of the global coconut production. It contributes more than US \$ 1400 million to the country's gross domestic product apart from an export earning of US \$ 80 million. It also provides livelihood securities to more than 10 million people (Rethinam and Singh, 2007). The coconut palm is susceptible to attack by a large number of pests, of which red palm weevil (Faleiro, 2005) and rhinoceros beetle (Valentine *et al.*, 2007) are serious and cause enormous damage to coconut palms.

Red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) is considered as the most devastating insect pest of palms in South and Southeast Asia (Abraham *et al.*, 2002; Faleiro *et al.*, 2003). According to Faleiro (2005), *R. ferrugineus* damages 12 % of young coconut palms (5 to 20 years) in India. Detection of pest infestation is difficult because the grub starts feeding from inside the palm and never comes outside till adult emergence. The damage symptoms include occurrence of small holes on crown and stem, drying up of the young leaves and splitting of the petioles near the area of attack. Often the attack of weevils is noticed only when the trees have been severely infested or beyond repair. Initially, red palm weevil infestation is identified by presence of holes on the stem along with protruding chewed fibrous material. Other damage symptoms include

presence of fermented odor from the trunk or topping of the crown (Kaahkeh *et al.*, 2001).

The rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Dynastidae), is one of the most damaging insect to coconut palm. The adult beetles feed on the growing portion of the palm leading to ragged appearance. A severely attacked palm will die or gets exposed to damage by secondary pests (Molet, 2013). The life cycle of this pest lasts from 4 to 9 months allowing more than one generation (Giblin-Davis, 2001) in a year. The beetle breeds in dead standing coconut palms killed by pest, disease, lightning, decaying organic materials like compost and sawdust heaps. Floating logs containing larvae in tunnels might spread the pest to new areas (Howard *et al.*, 2001). The rhinoceros beetle bores into the base of cluster of spears, causing wedge shaped cuts in the unfolded fronds. In younger palms the effect of damage can be more severe. Attack by adults may reduce yield and kill seedlings. They may provide entry points for lethal secondary attacks by the red palm weevil or pathogens (Molet, 2013).

When an individual of the same species comes in contact with pheromone, it elicits a response, depending on the type of pheromone. In this way, specific information is conveyed. Pheromones that cause clumping or clustering behavior in a species, which bring individuals into a closer proximity, are referred to as aggregation pheromones (Figueiras and Lazzari, 1998). In general, male-produced sex attractants have been called aggregation pheromones, because they usually result in the arrival of both sexes at a calling site, and increase the density of conspecifics surrounding the pheromone source (Karlson and Luscher, 1959). Aggregation pheromone functions in defense against predators, mate selection, finding shelter and to overcoming host resistance by mass attack (Figueiras and Lazzari, 1998). Cockroaches produce a specific pheromone with their excrement when they find safe shelter, which attracts other members of their species. As a result, aggregation pheromones function such that individuals aggregate around a "good position" with positive feedback. Pheromones evaporate with some rate. This prevents other individuals to aggregate to a local position (Tsutsui, 2004).

Generally, insecticide application are executed via trunk injection or root feeding for red palm weevil (Faleiro, 2006), but these are highly combursum and farmers are reluctant to apply insecticide using these methods. These methods lead to other side effects. More importantly insecticide applications leave toxic residues in coconut water posing health hazards (Ranasinghe *et al.*, 2003). This happens particularly against rhinoceros beetle when insecticide in granular form are incorporated in to the holes (Faleiro, 2006). Application of insecticides on palms is a major constraint because of greater height (usually 10-20 m) to which palms grow. Insecticides are also undesirable because of inimical effects on beneficial (Faleiro, 2006). The structure and architecture of palms make difficult the implementation of

mechanical/physical tools of pest management. Non availability of skilled laborers to execute pest management practices in coconut cultivated tract is another constraint.

In view of the above impediments, the effect of pheromone quantity on captures and activity of red palm weevil and rhinoceros beetle, feasibility of implementing the pheromone management technology in conjunction with other IPM tools at field level was researched.

## Materials and Methods

**Red palm weevil and rhinoceros beetle culture :** The field collected red palm weevil adults caught in traps baited with fermented coconut palm trunk pieces and synthetic pheromone, respectively were brought to the laboratory. Males and females were kept separately under laboratory conditions ( $25\pm 2^\circ\text{C}$ ; R.H.: 75-90%; L13:D11) for 5 days and provided with pineapple (*Ananas comosus* Linn.) slices as a conditioning to the new environment. The day before experimentation, weevils and beetles were isolated in plastic boxes (0.1×0.1 m) without food. The boxes containing weevils were transferred to a bioassay room and allowed to acclimate for at least 30 min before testing.

**Olfactometer study :** The synthetic aggregation pheromones were imported from USA, were mixed and lures were formulated and prepared in Bangalore ( $12^\circ 58' \text{N}$ ,  $77^\circ 34' \text{E}$ , 982 m amsl), India. The aggregation pheromone, 4-methyl-5-nonanol and 2,4-methyl-5-nonanone in 9:1 ratio formulated as, RPW - CATCH against weevil and Ethyl 4-methyloctanoate as RHINO - CATCH against beetle was used in the present study. Synthetic aggregation pheromone with more than 99 % and 98 % for red palm weevil and rhinoceros beetle were used, respectively. The pheromones were stored at  $-50^\circ\text{C}$  in a freezer. Before use, the pheromones were maintained at room temperature ( $25\pm 10^\circ\text{C}$ ) for an hour. The pheromone component was impregnated with filter paper and left it for one min for the olfactometer studies.

The responses of red palm weevils and rhinoceros beetle males and females ( $n = 30$ ) to aggregation pheromone was tested in a Y-tube glass olfactometer (7 cm diameter, 60 cm main tube, 25 cm each arm), operated at airflow of  $4 \text{ l min}^{-1}$  and with  $25\pm 1^\circ\text{C}$  and  $75\pm 5\%$  RH. Each male and female was introduced into the olfactometer and behavior was observed in 10 min periods. The observation periods were repeated 12 times with freshly collected weevils and beetles. It was recorded as 'no-choice' if the weevils and beetles remained in the main tube and as 'choice' if they entered in one of the arms. Individuals that did not choose a particular arm were excluded from the statistical analysis. Each individual was tested only once. Chi-square test was used to find significant differences in responses of red palm weevils and rhinoceros beetle to the pheromone source.

**Field study :** Field trials were conducted at Ukkada, Maralvadi and Honnaganahalli, Satnur, Kanakpura, Ramanagara district,

Karnataka, India to evaluate the efficacy of pheromone lures of red palm weevil (RPW – CATCH) and rhinoceros beetle (RHINO – CATCH) infesting coconut palms.

To know the effective dosage for attracting maximum weevils and beetles, four different dosages viz. 250, 500, 750 and 1000 mg pheromone lures were impregnated in wooden blocks (0.025×0.025 m) for one min and packed in plastic cover (0.075×0.075 m) and sealed airtight in trilaminated pouches (0.075×0.075 m). These bucket traps (2 liter. vol.) wrapped with gunny cloth having four holes of diameter 4.5 cm were used for trapping the weevils and beetles. Food bait consisting ripened pineapple fruit pieces with one liter water and carbofuron 3g @ 5g and old coconut frond pieces was used in each trap for weevils and beetles, respectively. The pheromone lure was tied to the inner surface of the bucket trap lid. The trap was tied to the coconut palms at 0.6 m height. A total of 20 traps were installed at each location from 1/3/2012 to 7/3/2012 so that trapping coincided with the activity of weevils and beetles. The traps erected in the field and water, food baits were changed once a month. Data on trap catches were recorded at two weeks intervals. The trap layout followed randomized complete block design with four treatments and five replications each. Observations on weevils and beetle catches were recorded weekly once for a year. The data were subjected to ANOVA test.

### Results and Discussion

**Olfactometer study :** Synthetic aggregation pheromone attracted significantly more number of red palm weevil than control (Chi-square = 24.47,  $P \leq 0.001$ ,  $n=30$ ). A mean of 21 weevils ( $n=30$ ) responded to pheromone of which 13.4 were females and 7.6 males. Few weevils (mean of 0.9) moved towards control where only filter paper without pheromone was placed. Weevils remained quiet for 2-3 min immediately after release, then started moving towards source, intermittently making short flights (Table 1). The results are in confirmation with the study of Poorjavad (2009), female weevils responded to the pheromone more than males. Differential responses to aggregation pheromones by sex have been reported in a number of other beetles (Graaf *et al.*, 2005). Present study suggests that females may have a higher sensitivity to the aggregation pheromone than males. This is due to the fact that red palm weevil females have more basiconic sensillae on antenna than males (Avand-Faghieh, 2004). The basiconic sensillae in the weevil, *R.*

*palmarum*, are known to be sensitive to the aggregation pheromone (Said *et al.*, 2003).

Similarly in case of rhinoceros beetle synthetic aggregation pheromone attracted significantly more number than control (Chi-square = 24.14,  $P \leq 0.001$ ,  $n=30$ ). It was observed that 6.5 male and 12.3 female rhinoceros beetles were attracted to filter paper containing synthetic pheromone, while only 1.33 beetles were found in control. Compared to ethyl 4-methyloctanoate alone, combinations of three male-produced compounds did not increase attraction, where as addition of freshly rotting oil palm fruit bunches to pheromone-baited traps significantly enhanced attraction. With increasing dose, captures of *O. rhinoceros* increased, but doses of 6, 9, and 18 mg day<sup>-1</sup> were on par with 30 mg day<sup>-1</sup> lures. Newly designed vane traps were more effective in capturing beetles than barrier or pitfall traps. Results of this study indicated that there is potential for using aggregation pheromone in operational programs to suppress *O. rhinoceros* in oil palm plantations (Table 1). Similar study was done by Rochat *et al.* (2004) in electroantennography and field trapping experiments that compound 4-methyloctanoic acid is an essential component of male aggregation pheromone of *Oryctes elegans*. Rochat *et al.* (2002) reported that rhinoceros beetle, *Scapanes australis* were captured in traps baited with synthetic racemic 2-butanol plus 3-hydroxy-2-butanone (2.5- to 2500 mg day<sup>-1</sup> doses).

Earlier, workers tested the pheromone lures is select concentrations. Present study tested a wide range of pheromones concentration to determine, if lower concentration would effective in trapping the targeted insects. Workers in the past dispersed pheromone lures in plastic vials. This did not persist for longer period in the present study. In the present study, however lures were dispersed in porous wooden blocks and the blocks were covered with polyethylene films, which made the pheromone chemicals to persist for longer period (60-70 days more). This was validated in laboratory experiment.

**Activity of red palm weevils and rhinoceros beetle :** The average number of weevils per trap per week differed in all the weeks both in Ukkada and Honnaganahalli. The red palm weevil catches were recorded 9 weeks after installation of traps, the rate of capture varied from 0.25 to 13.25 beetles per trap per week in Honnaganahalli during 9<sup>th</sup> to 53<sup>rd</sup> week after installation of traps. Where as in Ukkada, the beetle activity varied on an average from

**Table 1 :** Response of adult red palm weevil and rhinoceros beetle to synthetic pheromone in wind tunnel

Treatment	Number of weevils/beetles positively responded							
	Rhinoceros beetle*				Red palm weevil*			
	Male	Female	Total	Control	Male	Female	Total	Control
Pheromone	6.50±1.95	12.30±2.75	18.80±3.85	1.30±0.48	7.60±2.01	13.40±2.67	21.00±3.92	0.90±0.73

\* For 10 min period; Values are mean of five replicates ±SD

0.25 to 13.5 beetles per trap per week during 10<sup>th</sup> to 53<sup>rd</sup> week after installation of traps (Fig. 1a,b). Present finding is in accordance with Al-Saoud (2010) who found weevil activity throughout the study period in the date palm cultivated area in Al-Rahba. The highest catch was found during March and April. The lowest red palm weevil numbers was recorded in December and January. Faleiro (2005) studied the weevil seasonal activity using pheromone traps set throughout Goa, during 1999. Month-wise weevil captures between August 1999 and December 2001 showed that weevil activity was high after South West monsoon between October and November, while low activity of the pest was noticed during monsoon between June and July. The weevil captured were female dominated and for every male weevil trapped, two female weevils were captured.

The peak activity of beetles was found during 29<sup>th</sup> to 53<sup>rd</sup> week i.e., from September to March. The beetles caught in the traps ranged from 0.0 to 4.5 beetles per trap per week recorded

from 29<sup>th</sup> to 53<sup>rd</sup> week in Honnaganahalli and Ukkada the beetles caught in the trap varied from 0.25 to 1.75 beetles per trap per week recorded from 29<sup>th</sup> to 53<sup>rd</sup> week. Rhinolure is an aggregation pheromone effective in mass trapping both males and females of rhinoceros beetle. The active compound, 4-methyl octanoate serving as an attractant, is supplied as a bubble formulation in sachets and the chemical is suspended in septa. The trap should be installed at about 0.8 m from the ground level and is effective @ 1/ha. The septa can be suspended in the upper lid of the bucket, taking care to avoid direct sunlight as it would be affecting the attractiveness of the lure to the beetles. The bucket should contain holes and rough corrugations on the lateral sides just below the upper lid so that the beetles that are attracted, alight on this rough surface before entering the holes (Oehlschlager, 2007). Abuagla and Ali (2012) reported that the black pheromone trap containing 100 g of date palm significantly enhanced *R. ferrugineus* attractiveness and helped considerably in reducing the red palm weevils' deleterious impact on coconut palms.

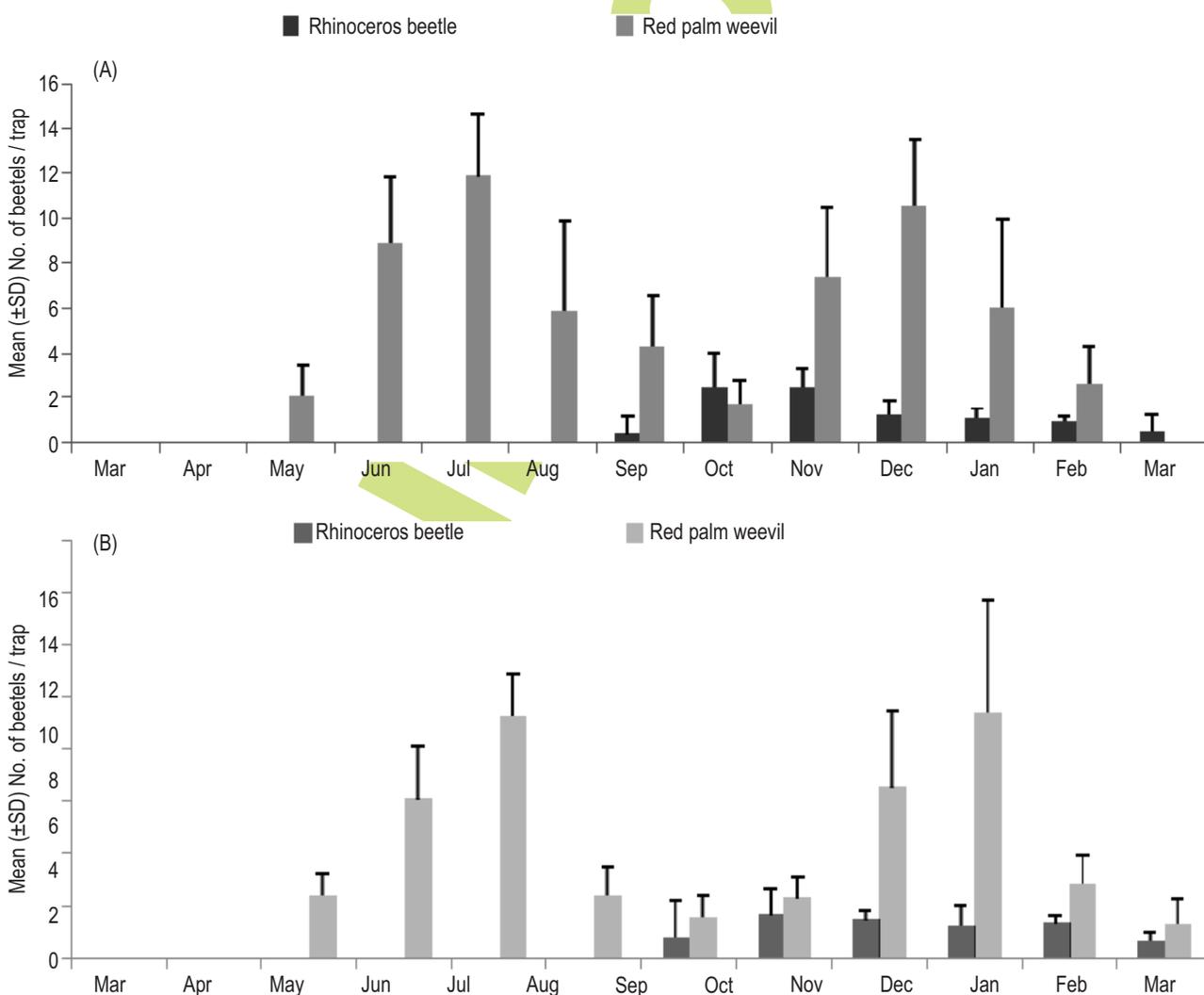


Fig. 1 : Number of rhinoceros beetle and red palm weevil recorded in pheromone traps at (a) Honnaganahalli (b) Ukkada

**Table 2 :** Numbers of rhinoceros beetle and red palm weevil trapped in traps at four different pheromone concentrations

Treatments (mg)	Mean no. of beetle and weevil/trap	
	Rhinoceros beetle	Red palm weevil
250	33.00 (5.78) <sup>c</sup>	78.00 (8.85) <sup>c</sup>
500	42.00 (6.51) <sup>b</sup>	264.00 (16.26) <sup>b</sup>
750	98.00 (9.91) <sup>a</sup>	695.80 (26.38) <sup>a</sup>
1000	108.00 (10.41) <sup>a</sup>	789.00 (28.10) <sup>a</sup>
CD	0.34	0.27
SEm	0.11	0.09

\*Mean of 10 traps per concentration, values with same letter as superscript are non significant at 5% (P < 0.05)

**Field study :** Total number of red palm weevil trapped in the pheromone traps varied from place to place. The statistical analysis showed that 750 and 1000 mg pheromone lures were found attractive and trapped significantly higher numbers of weevils. A total of 695.80 and 789 red palm weevils were trapped in 750 and 1000 mg concentrations of lure, respectively. There were non significant differences in the number of weevils trapped in 250 mg (78 weevils) and 500 mg (264 weevils) (P < 0.05 at df=15) traps (Table 2). The results are in confirmation with the study of Faleiro (2005) who found that Pherobank red palm weevil lure (Holland) at 400 mg and Ferrolure+ (Costa Rica) at 800 mg caught significantly higher number of weevils than Pherobank red palm weevil lure 700 mg, Pherobank red palm weevil lure mg, ISCA Technology (USA) 900 mg, CPCRI lure (India) 157 mg, Ferrolure+ only (no food) 800 mg and food only (coconut petiole).

Studies with synthetic pheromone (4S)-ethyl 4-methyloctanoate and the racemic mixture were equally attractive and 10 times more effective in attracting rhinoceros beetle. Ethyl 4-methylheptanoate was as attractive as ethyl chrysanthemumate and more attractive than 4-methyloctanoic acid, but further studies are required before it can be classed as an aggregation pheromone. Rhinoceros beetles were caught in the pheromone traps varied with concentrations, 98 and 108 rhinoceros beetles were caught in pheromone traps baited with pheromone lure 750 and 1000 mg concentrations, respectively, and were found significantly superior than other treatments. 250 and 500 mg lure traps proved inferior with only 33 and 42 beetles in the pheromone traps (Table 2). Compared to ethyl 4-methyloctanoate alone, combination of three male-produced compounds did not increase attraction, where as addition of freshly rotting oil palm fruit bunches to pheromone-baited traps significantly enhanced attraction. With increasing dose, captures of *O. rhinoceros* increased, but doses of 6, 9, and 18 mg day<sup>-1</sup> were on par with 30 mg day<sup>-1</sup> lures. Newly designed vane traps were more effective in capturing beetles than barrier or pitfall traps. Results of this study indicated that there is potential for using ethyl 4-methyloctanoate in operational programs to suppress *O. rhinoceros* in oil palm plantations. The results are in

confirmation with Rochat *et al.* (2004). Also similar results were observed electroantennography and field trapping experiments demonstrated that compound, 4-methyloctanoic acid is an essential component of male aggregation pheromone of *Oryctes elegans* L. Rochat *et al.* (2002) reported that extensive field trapping trials with various synthetic pheromone mixtures and doses showed that 2-butanol and 3-hydroxy-2-butanone1 (formulated in polyethylene sachets in 90:5 v/v ratio) were necessary and sufficient for optimum long-range attraction. Beetles were captured in traps baited with racemic 2-butanol plus 3-hydroxy-2-butanone, with or without a stereoisomer mixture of 2,3-butanediol (2.5- to 2500-mg/day doses). Plant pieces, either sugarcane or coconut, enhanced captures by the synthetic pheromone, which was active alone in rhinoceros beetle, *Scapanes australis*.

A nanomatrix and polymer composite was developed to load the rhinoceros beetle pheromone, ethyl 4 methyl octonate. The pheromone loaded to nanomatrix showed extended duration of release when subjected to thermal gravity analysis. Field evaluation of traps revealed that nanomatrix loaded pheromone (240 mg) trapped 18.0 beetles/trap/month, followed by nanomatrix polymer composite that trapped 15.8 beetles/trap/month. The commercial lure (containing 800 mg pheromone) trapped 12 beetles/trap/month. Studies on longevity of pheromone lures indicated that the commercial lure was exhausted in three months where as in the nanomatrix, it remained active up to 8 months (CPCRI, 2012).

Through this study, percent trapping of two major insect pests of coconut viz., red palm weevil and rhinoceros beetle were accomplished. Persistence of pheromone components for longer periods was achieved with wooden blocks covered with polyethylene film. These findings have facilitated effective and efficient trapping of red palm weevil and rhinoceros beetle in coconut cultivated ecosystems under varied habitat conditions. The study has also lead to better understanding of ecology and behaviour of red palm weevil and rhinoceros beetle in coconut cultivated ecosystems.

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