Red Palm Weevil in Coconut

Knack to Crack Trajectory

ICAR-Central Plantation Crops Research Institute
Kasaragod – 671 124, Kerala, India

Consortium Research Platform on Borers in Network Mode

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Preface

ICAR- Consortium Research Platform on Borers is a well conceived programme proposed by Dr. N.K. Krishnakumar, former DDG (Horticultural Sciences) during his brief stint as Director, ICAR-NBAIR, Bengaluru. Several rounds of discussion with all stakeholders were held to streamline the broad objectives and probable deliverables cutting across all crop-based institutes. The representations are even from Andaman and Nicobar Islands, Jammu and Kashmir, Kerala and Rajasthan in this national flagship programme. As borers are key pests in most of the crops, a systematic documentation was envisaged in all crops so that, it could be a policy forming framework. Emphasis was also given on different geo-morphs, morphological and molecular identification, understanding bionomics and missing links of borer pest complex, deciphering semiochemicals in all borer complex associated with different crops.

It was a great privilege ICAR-CPCRI was also part of this mega programme in which coconut red palm weevil was identified as a key pest to work with. The authors of this bulletin are extremely pleased to document the accomplishments of the programme undertaken at the Regional Station, Kayamkulam on diversified aspects such as chitin synthesis inhibitor, gut microbiome, synthetic diet and biology, pheromone field delivery, compatibility of botanical cakes and entomopathogenic nematodes, modification of pheromone trap design for the holistic and sustained management of *Rhynchophorus ferrugineus*.

Salient outcome of the project and the methodology are outlined in a lucid manner for the readers to comprehend and suggest meaningful ideas in the next phase of the programme. Authors sincerely thank the two senior research fellows Sri M. Rajumon and Ms. A.S. Anjali for their sincere contributions in making the project accomplish the target in the allotted time period. The services of two post-graduate students as part of their dissertation are also thankfully acknowledged.

The meticulous support extended by all team members in making the project so specific and achieve targets is praiseworthy. The authors are pleased to thank the National Project Coordinator, Dr. A. Krishnamoorthy, Principal Scientist, ICAR-IIHR, Bengaluru and later by Dr. P.V. Rami Reddy, Principal Scientist, ICAR-IIHR for the magnanimous support and timely sanction of funds to make the programme more realistic. The statistical analysis made by Dr. C.K. Nampoothiri, ACTO (Statistics) is profusely revered. Authors place on record the unstinting patronage provided by DDG (Horticultural Sciences) for the overall success of the programme.

Editors

Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier)

Introduction

Coconut, a versatile crop of multifaceted utilities, ensures livelihood security to more than 12 million farm families in our country. Coconut cultivation is spread out in 2.0 m ha with an annual production of 20.4 billion nuts in India. Kerala, the only State in our republic country named after the *kalpavriksha,* coconut delivers the natural bounty on God's own country through its soil binding roots along the coastal zones and hovering leaflets braving the sea breeze. Despite making significant strides, the productivity of coconut in Kerala on per ha basis has plummeted down considerably. Many a times farmers fail to understand the key production constraints and hidden villains associated with the crop which sometimes totally ruin the palms and affect its production significantly. Among the key biotic stresses associated with the crop, infestation by red palm weevil (RPW), *R.ferrugineus* has assumed threatening proportions mainly because the farmers fail to diagnose the pest attack in advance. The RPW damage symptoms are diagnosed by farmers mainly at an irrecoverable stage leading to complete loss of palm. By the time RPW damage is diagnosed, it becomes too late for any curative technique to save the palm. Proper awareness to detect the attack at the early stage of infestation is the most important aspect that needs to be focused.

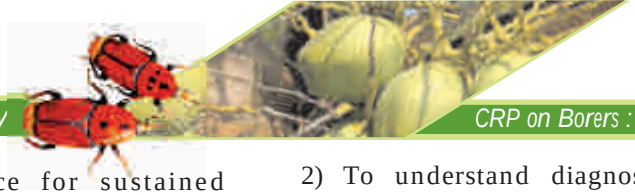
Orientation of gravid females is normally linked to coconut volatiles emanating from injuries imparted by bad agricultural practices or frictional contacts of leaflets during natural calamities. Injuries could also emerge due to improper nutrition leading to breakage of petioles or due to succulence attributed by genetic traits in Dwarf cultivars. Closer planting of palms is one of the major reasons responsible for increased release of volatiles in the surrounding leading to faster attraction of weevils for egg laying. Continuous feeding by a large number of grubs on the terminal crown portion will devastate the growing spear leaf and kills the palm. This will never happen all of a sudden and sustained feeding by the grubs for more than two months is a prerequisite for crown collapse. RPW is reported to attack 17 palm species worldwide. It is one of the key pests of coconut causing mortality of juvenile palms (aged 5-15 years) to the tune of 3-5% in different tracts of the country. In addition to coconut, RPW has become a devastating pest of date palm (*Phoenix dactylifera* Linn.) in Middle East countries. Currently, the pest is reported in 15% of the coconut-growing countries and in nearly 50% of the date palm-growing countries.

Symptom diagnosis

1) Feeding activity of grubs rots the nourishing zone which could be visualized through the health condition of the spear leaf. Choking of spindle, improper unfurling and rotting of spear leaf could be the visible explicit symptoms.
2) Yellowing of mid whorl leaves due to arrest of nutrient translocation.
3) Oozing out of brown foul-smelling fluid along the trunk from the feeding zone. Grubs feed by rotting the tissues with the help of microbes in the tubular gut.
4) Bore-holes on the trunk due to the feeding of grubs.
5) Gnawing sound of the grubs could be heard along the trunk similar to the crushing sugarcane during juice extraction.

Varietal preference

Among the Tall and Dwarf genotypes of coconut, Dwarf cultivars are relatively susceptible to attack by RPW may be due to the presence of specific volatile cues and



preferential substance for sustained feeding. Among the Dwarf genotypes, Chowghat Green Dwarf, Gangobondam *etc.*, are highly susceptible due to weak leaf axils which are unable to withstand the heavy nut load leading to fissures and injury. Though farmers prefer Dwarf genotypes and many are found resistant to root (wilt) disease, strict monitoring and good agricultural practices would be the key for success in Dwarf palms.

Missing links

Incidence of RPW in Southern Kerala, India is mainly due to the prevalence of root (wilt) disease which emanates characteristic odour attracting adult weevils for egg laying. Early diagnosis is one of the key factors for the timely management of the pest. Entire life stages (eggs, grubs, fibrous pupae & weevils) are confined within the palm and, therefore, the correct diagnosis and curative management will reduce the pest population significantly. Not less than nine larval instars are reported and this forms one of the weakest links of growth modulation that could be exploited through use of hormone mimics. Tremendous variation in size, shape, colour and stripes is prominent among the adult weevils collected from different locations. Feeding by grubs rotten the succulent palm crown (cabbage) with the help of gut microbiome is a researchable issue which inhibits the establishment of bioagents.Communication signaling through pheromones and pull-push strategy in taking weevils away from the main field was studied.

Keeping this in mind the following objectives were finalized in the project launching workshop.

Objectives

1) To identify potential bio-control agents (entomopathogenic nematodes/fungi/bacteria), botanicals and insect growth regulators for efficient delivery mechanism.

2) To understand diagnostic symptoms and characterize gut microbiomes of *R. ferrugineus*.

3) To collect, catalogue and characterize *R. ferrugineus* from different geographical locations *vis-à-vis* crop phenology and climate extremities.

4) To harmoniously integrate intra-specific and inter-specific info-chemicals for attraction and repellency strategy.

Methodology

I Identification of potential bioagent/insect growth regulator

a) Standardization of artificial diet for insect rearing

An artificial diet component was selected from the existing literature to initiate rearing of *R. ferrugineus* and deliver insects for experiments. Part I of the diet components are homogenized with water and admixed with part III. Part II components are added after boiling the ingredients with agar before solidification so that the protein, antibiotic and vitamins are not degraded. Before solidification diet slurry is transferred to required containers and activated at 5°C in refrigerator for 24 h before provided to grubs. A base model of diet used for optimum rearing standard of RPW grubs and its composition are given in Table 1.

Emerging neonates from RPW eggs were transferred into the diet in different containers. Early stages were confined on smaller containers which were later transferred into bigger containers when the larval stages advanced. After obtaining a critical weight of >1.0g, the grubs were transferred to natural diet of coconut petiole to reduce mortality and prepare for pupation which is normally a fibrous cocoon type. Head capsule width, body weight as well as body length *vis-à-vis* different instars of RPW were worked out and documented.

Table 1. Diet composition for rearing RPW grubs

Part I		Part II	
Corn flour	50 g	Ascorbic acid	4.5 g
Wheat germ	50 g	Chloramfenicol	0.5 g
Yeast	50 g	Coconut fibre	15.0 g
Methyl *p*-hydoxy benzoate	1.8 g	Vitamin and amino acid additive	1 capsule
Benzoic acid	1.8 g	Cholesterol	0.5 g
Milk powder	1.0 g	Protein	17.0g
Formaldehyde	1.0 ml	**Part III**	
Sucrose	2.0 g	Bacto agar	20 g
Distilled water	440 ml	Distilled water	440 ml

b) Studies on RPW biology in artificial diets

Male and female adult weevils were separated through morphological identification marks and maintained in the mating chamber of volume 100 cc along with sucrose soaked in cotton. Eggs laid by weevils on the cotton were collected and transferred to Petri dishes containing moist filter paper using fine brush. Emerging larvae were transferred to artificial diet for the study on biology and number of instars was determined using Dyar's principle. Male and female weevils were closely observed for any morphological distinct features besides the snout hairs and pygidial hairs for easy identity.

c) Effect of insect growth regulator

Larvae collected from the field were separated into different categories based on head capsule width and body weight. Grubs belonging to similar group ranging from 1.0-2.0 g were used for bioassay with the insect growth regulator, lufenuron (Cigna 5.4% w/w EC; Syngenta). Six different doses of lufenuron *viz.*, 0.00054% (0.1 ml per litre), 0.0011% (0.2 ml per litre), 0.0054% (1.0 ml per litre), 0.0065% (1.2 ml per litre) and 0.0081% (1.5 ml per litre) were exposed to uniform-sized grubs and the growth and development abnormalities, if any, was recorded at regular time intervals. Twenty grubs were used in the laboratory bioassay experiment and the number of grubs that transformed into malformed / dead pupae was calculated. The data was subjected to probit analysis to determine the median effective dose and the homogeneity of data was confirmed through chi-square test. Grubs after treatments were maintained in coconut petiole under standard laboratory conditions of temperature 25±2°C and relative humidity 80±5%. Compatibility of lufenuron with different concentrations on *Beauveria bassiana* was also conducted through poison food technique.

d)Delivery of entomopathogenic nematodes

Prophylactic leaf axil filling of *Heterorhabditis indica*-infected *Galleria mellonella* larvae in filter paper sachets along with botanical cake developed by ICAR-CPCRI was studied on twenty juvenile palms for the infestation of rhinoceros beetle as well as RPW.

II Gut microbiome

a) Isolation of gut microbes in RPW and biochemical characterization

Fully grown grubs collected from the infested palm were dissected out in ice-cold sterile water and streaked on nutrient agar and yeast extract peptone dextrose agar for the development of gut microbes. Individual colonies were picked out and studied for diversity. Microbial diversity in the gut streaked on nutrient agar and yeast extract peptone dextrose agar was ascertained. Well isolated single colonies from the dilution set

were further purified by quadrant streaking. The purified bacterial colonies from each sample were maintained individually as slant at 4°C on respective media with periodic sub culturing. The isolated cultures from gut of RPW was characterized morphologically and physiologically as per Bergey's Manual of Systematic Bacteriology which included Gram's staining, capsule staining, motility test and different biochemical tests such as IMViC (Indole, Methyl red, Voges-Proskauer, Citrate utilization) and sugar fermentation test. Fresh culture was used for all the tests.

b) Molecular characterization

(i) Isolation of genomic DNA using QIAampminikit

The 24 h old pure cultures from single cell colonies of the bacterial isolate RPB-1, 2, 4,5,6,7,8,9 &11 multiplied on LB broth were used for isolation of genomic DNA. The genomic DNA of the bacterial isolates under study was isolated using the QIA-ampminikit (Qiagen Inc., Chatsworth, CA, USA) with slight modifications in manufacturer's instruction.

(ii) Polymerase chain reaction (PCR)

PCR was performed as per the procedure detailed by Sambrook and Russel (2001) with a few modifications. Twenty five microlitres (25 µl) of reaction mixture containing 150-200 ng of DNA extracted from bacterial isolates under study, 2.5µl of 10X Taq polymerase buffer supplied along with the enzyme, 1.5 mM $MgCl_2$, 200 µM each of dNTPs, 100 ng each of forward and reverse primers and 2.0 units of Taq DNA polymerase (Merck Biosciences) were used to amplify the gene of interest. All the PCR amplifications were carried out in Techne Flexigene thermal cycler.

(iii) Primers

All the primers were synthesized from Eurofins Genomics India Pvt. Ltd in salt free status. The primers F27 - 5´ AGAGTTTGATCMTGGCTCAG-3´ and R1492- 5´-TACGGYTACCTTGTTAC GACTT-3´ (Wang and Wang, 1996) were used for the amplification of the16S rRNA region where M= A or C and Y=C or T.

(iv) Amplification of 16S rRNA region

PCR was carried out with an initial denaturation at 95ºC for 4 min followed by 35 cycles of denaturation at 94ºC for 30 seconds, annealing at 56ºC for 1 min and extension at 72°C for 1 min. Final extension at 72ºC for 10 min was given for end filling and the reaction products were analyzed in one per cent agarose gel.

(v) Agarose gel electrophoresis

Required amount of agarose (w/v) was weighed and melted in 1X Tris-borate EDTA (TBE) buffer until a clear transparent solution was obtained. The solution was cooled to about 50°C and ethidium bromide was added from the stock and mixed well. Comb of appropriate size was placed on one end of the gel tray. The agarose solution was poured into the gel tray to a thickness of 4-5 mm and allowed to solidify. The comb was removed after gel solidification and the gel was placed in electrophoresis chamber containing 1X TBE buffer. The DNA samples were mixed with 6X loading buffer at 5:1 and loaded into the well. Electrophoresis was carried out at 60 V (Sambrook and Russel, 2001) and the gel was documented using Biorad Molecular Imager Gel doc XR system, USA. The 1 Kb DNA ladder from NewEngland Biolabs Ltd was used as marker for comparing the size of the amplicon.

(vi) Purification of amplicons & sequencing

The amplicons obtained were purified from each reaction mixture after electro-phoresing in one per cent agarose gel. The bands at desired basepair level were excised and purified using QIAquick gel extraction

kit (Qiagen Inc., Chatsworth, CA, USA). This purification was performed to remove primer dimers and other residues from the PCR amplified product. The purified products were mailed for sequencing at Scigenom labs Pvt. Ltd., Kochi, Kerala.

(vii) Assignment of cloned sequences to established phylogenetic divisions

The nucleotide sequences of different bacterial isolates/strains used for comparison were from GenBank (Benson *et al.*,1999). The BLAST programme (Altschul *et al.*, 1990) was used to identify related sequences available in the GenBank database. Multiple sequence alignments were made using ClustalW. Phylogenetic tree was constructed with 16SrRNA sequences using Mega 6.0 software by Neighbour joining method with 1000 replications for bootstrap analysis.

III Morphometric analysis of geo-morphs

a) Collection of geo-morphs

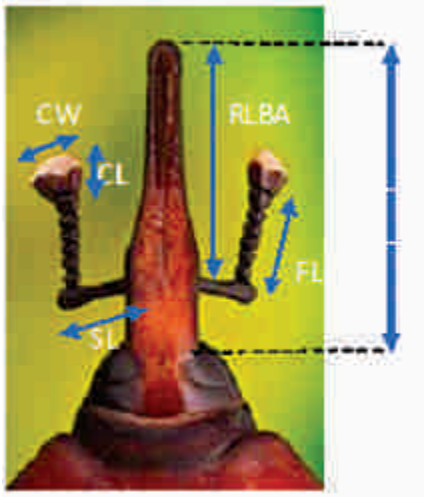
RPW infested palms were identified in different locations, *viz*., Kayamkulam and Kasaragod, Kerala as well as Pollachi and Tenkasi, Tamil Nadu based on the damage symptoms described earlier. Different life stages of the pest were collected from the infested palm and brought to the laboratory and reared to adult stage. Three male and female adults from each location was subjected to morphometric analysis of different body parts of adult weevils. Measurements were made on each specimen using a binocular stereo-microscope as outlined in Fig.1. Measurements include 13 characters such as body length (BL), body width (BW), elytron length (EL), elytron width (EW), pronotum length (PL), pronotum width (PW), rostrum length (RL), rostrum length before antennal scrobe (RLBA), scape length (SL), funicle length (FL), club length (CL) and club width (CW). Comparison on morphometric parameters between male and female and among localities were analysed by one-way ANOVA followed by Fischer's test.

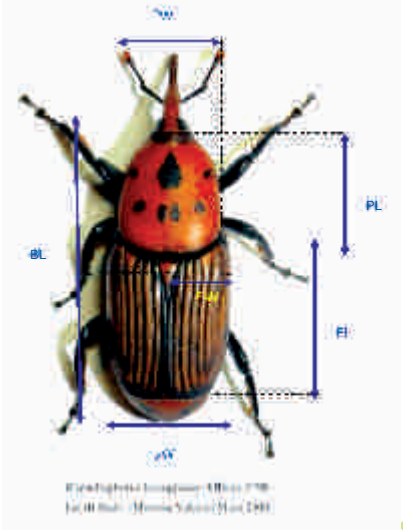
IV Delivery of semiochemicals through push-pull strategies

a) Essential oil versus repellency to RPW

Essential oils such as citriodora oil, black pepper, ajowan, garlic extract, nutmeg, methyl chavicol, betel leaf and cashew nut shell liquid @1% were evaluated in Y-tube choice assay and the reaction of weevils to the essential oil was measured through its orientation / disorientation from the source. The most repellent and efficacious molecule was further subjected to wind tunnel

Fig. 1 Morphometric measurements studied





bioassay to confirm the flight response of weevil as well as to ascertain the behavioural reaction of the weevil *vis-à-vis* essential oil.

b) Delivery matrix of essential oil and aggregation pheromone of RPW

The delivery of aggregation pheromone through capillary vials, polymer membrane sachets and nanoporous matrix have been standardized by ICAR-CPCRI and validated for field efficacy. A novel calcium alginate bead impregnated with essential oil or aggregation pheromone was developed for slow release of the molecule and is being field evaluated. Gel-based slow release matrix (calcium alginate) impregnated with essential oil (5 %) and placed on coconut leaf axil @ 5 g /palm in two sachets, and the pheromone trap was installed outside the garden to reinforce the push-pull strategy. Para pheromone (9:1 mixture of 4-methyl 6-nonanol and 4- methyl 5- nonanone) loaded in calcium alginate matrix was also evaluated at Chittarikkal, Kasaragod, Kerala.

c) Modification of trap design for durability and slow release of aggregation pheromone

The bucket trap design is slightly modified using PVC tube for enhancing the longevity of the trap in the field, slow release of aggregation pheromone as well as *in situ* umbrella effect to reduce the loss of the impregnated molecule. The efficacy of the newly designed trap is under evaluation.

Results

I a) Standardization of diet

The diet composition as outlined in Table 1 was found to be optimum with regard to the growth and development of RPW, however, the pre-pupating insect required the natural host of coconut petiole for the construction of fibrous cocoon. The emerging neonates transferred into the artificial diet were maintained in the diet only up to 23 days when the grubs weighed around 1.12 g.

Afterwards, the grubs were maintained in coconut petiole until pupation. Maintaining the grubs after 23 days in artificial diet enhanced mortality of grubs may be due to improper aeration in the rearing container which will be standardized in due course of time. From an initial weight of 0.0014g, the grubs attained a weight of 1.12 g in a period of 23 days (Fig.2). After transfer into coconut petiole there was slight drop in the body weight which was recouped in a couple of days. Grubs took an average of 21-23 days to attain a weight of 1.0 g in artificial diet and thereafter the growth was found faster when transferred into natural diet indicating further refinement required for the diet. Biology of RPW and damage symptoms on palms are presented in Fig. 3.

Ib) Determination of larval instars using Dyar's law.

As the number of larval instars was found to

Fig. 2 Growth of RPW grubs in artificial diet / natural host

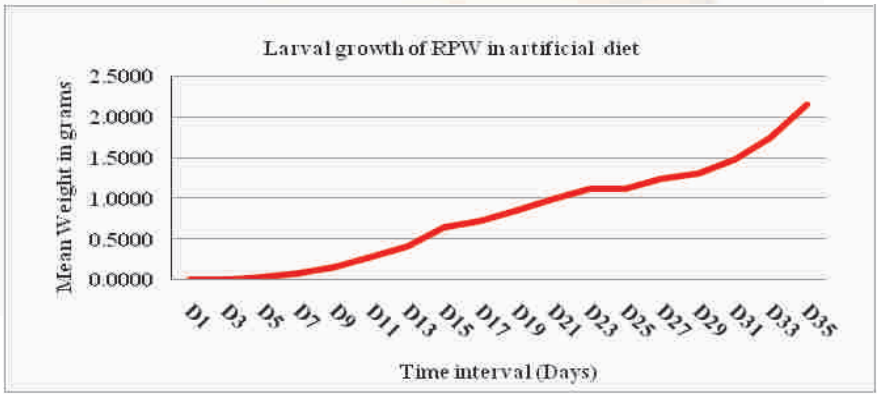


Fig. 3 Biology of RPW and damage symptoms

be high, a study was made to understand the number of larval instars by measuring the head capsule width and subjecting to Dyar's law. The head capsule width (mm) of grubs belonging to each instar was recorded and the measurements were plotted against number of instars. According to Dyar's law, a linear progression of head widths could be represented by a straight line, if the measurements (Y-axis) are plotted against the number of instars (X-axis). The Dyar's law was calculated using the equation

$$\frac{II/I + III/II + IV/III + \ldots\ldots n^{th} \text{ instar head capsule width} / (n-1)^{th} \text{ instar head capsule width}}{n}$$

Graphical representation of head capsule width in mm *vis-à-vis* different larval instars are presented in Fig. 4

The regression equation was found to be $Y=0.541x$ with R^2 value 0.9244. The ratio obtained subjecting to Dyar's law was found to be 1.16 which is within the permissible limit of 1.2 to 1.4 indicating that the head capsule width increases in linear dimension with one instar to the next in this ratio (Fig.4). A total of 10 larval instars could be demarcated in this study. Body weights (g) as well as length (mm) *vis-à-vis* different larval instars are presented in Fig. 5 & 6, respectively.

The weight gain of RPW grubs during the initial five larval instars was found to be slow attaining less than 100 mg whereas it increased significantly during VI-X instar constituting a J-shaped curve and attaining a

Fig. 4. Head capsule width *vis-à-vis* larval instars of RPW

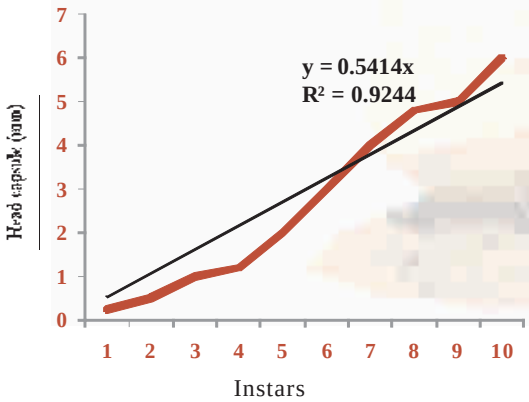


Fig. 5. Body weight *vis-à-vis* larval instars of RPW

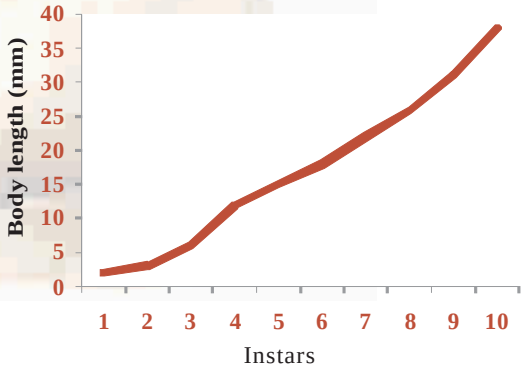
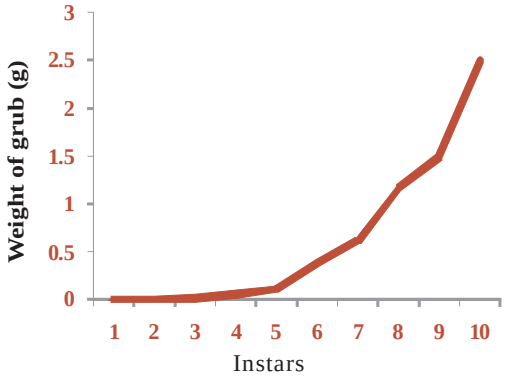


Fig. 6. Body length *vis-à-vis* larval instars of RPW



weight of 2.5 g (Fig. 5). Body length (mm) was found to have linear relationship with different instars with regard to RPW grubs (Fig.6).

Morphological features of adult male and female weevils were closely studied. In addition to the presence of hairs on the snout, males do have conspicuous and thick setae arranged on tibia as well as femur on forelegs. The inner hairs arranged on male tibia are quite long and these extraneous growths of hairs could be involved in copulation process for effective clasping of females during mating. The general feeling when the adult males are held in our fingers are extremely sensible and itchy due to the presence of these hairs and such an intense itchy feeling is not well pronounced in case of adult females as the hair growth is inconspicuous. Such a unique characteristic feature of hairs on foreleg of adult male weevil is observed and reported for the first time. The conspicuous growth of tibial hairs in adult male weevils and their inconspicuous nature in female is presented in Fig. 7 & 8, respectively.

Ic) Influence of lufenuron on RPW grubs

Influence of lufenuron on the formation of larval-pupal aberration is determined and presented in Table 2. At the lowest concentration (0.00054%) evaluated in the study, there was slight effect on the treated grub but the adults turned malformed. However, the growth rate was accelerated and the treated grubs entered the wandering phase of pupation quite faster. With the increase in concentration of lufenuron, there was visible malformation on the grubs. At the highest concentration (0.0081%) studied, 70% of the grubs were malformed. A dose-depended increase in malforamtion of grubs was observed with lufenuron (Table 2). Malformation percentage increased with increase in the concentration of lufenuron inferring its deleterious effect on the cuticle

Fig. 7 Foreleg of male weevil

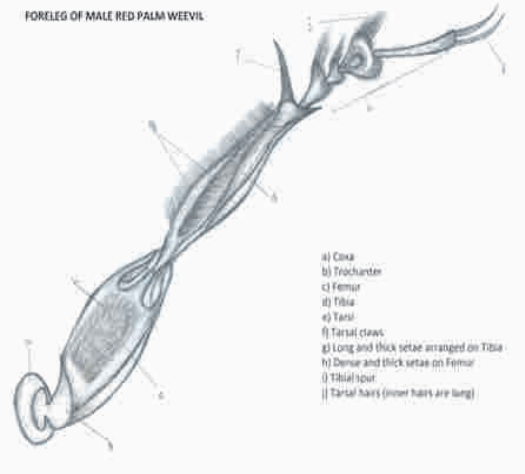


Fig.8 Foreleg of female weevil

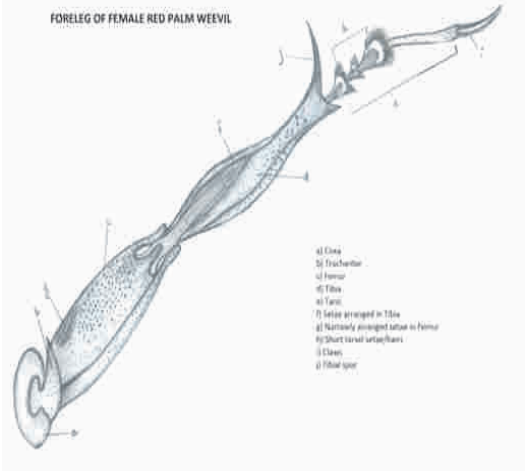


Table 2 Effect of lufenuron on RPW grubs

Lufenuron concentration (%)	Malformation (%) (n=20)
0	Nil
0.00054	25.0
0.0011	30.0
0.0054	35.0
0.0068	40.0
0.0081	70.0

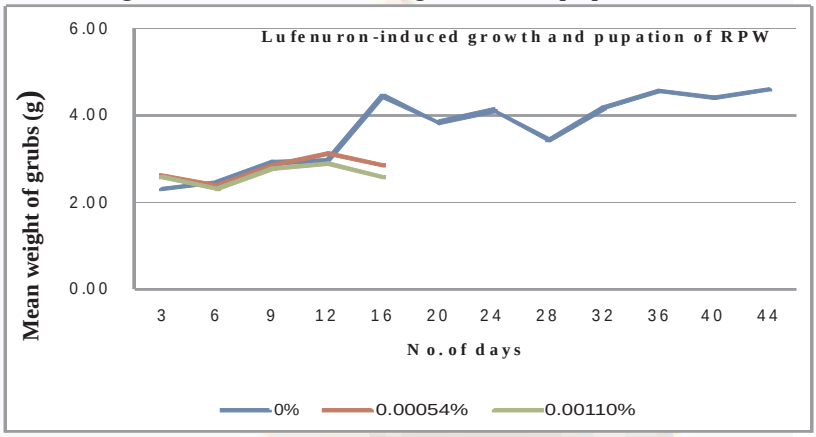
development during moulting. The cuticle became soft and fragile with boil-like pustules; lesion turn brown and sometimes eruption of fluid was observed. Pupating insects were busted at certain points and the fibrous cocoon formed was also not well-woven with low tensile strength. The experiment indicated that lufenuron interfered with larval-pupal transformation of RPW grubs and pupation was found abnormal at the highest concentration evaluated. This is the first report on the influence of insect growth regulator on the growth and development of RPW grubs. With the aberration on larval cuticle by lufenuron, entry of entomopathogens through the wound would be quite easier and a combination of lufenuron and entomopathogens was found promising. The data in Table 2 was subjected to probit analysis to arrive at the median effective dose of lufenuron against RPW grubs. Median effective dose of lufenuron is presented in Table 3.

Table 3. Median effective dose of lufenuron against RPW grubs

IGR	ED_{50}	LFL	UFL	Reg. Equ.	Chi-square Test
Lufenuron	0.00779%	0.0057%	0.0136%	Y=0.205x-1.026	6.45 (p=0.168)

Chi-square non-significant Table value 9.49 (P=0.05)

Fig . 9 Lufenuron-induced growth and pupation of RPW



From the Table 3, it was found that the median effective dose of lufenuron against RPW was found to be 0.00779% and at this concentration 50 per cent of the RPW grubs turn malformed. Based on the non-significance of the chi-square value the data was found to be homogenous to ascertain the ED_{50} value within the fiducial limit values.

Influence of lufenuron on the growth of RPW grubs and initiation of pupation is presented in Fig. 9. Pupation of RPW grubs treated with lufenuron was completed in a period of 16 days, whereas the healthy

insects pupated only after 44 days. Weight gain was reduced with increase in the dosage of lufenuron. Lufenuron-treated insects attained a maximum weight gain in 10 days and healthy insects reached that stage only after 17 days. This indicated accelerated feeding of RPW grubs after exposure to lufenuron leading to precocious pupation and abnormalities. Body weight gain in treated insect was less than the healthy counter parts before pupation (Fig. 9). Malformations of grubs, pupae and adult weevils are presented in Fig. 10,11&12.

Fig. 10 Lufenuron-induced malformed RPW grubs

Blackening of cuticle *Boil-like pustules on larval cuticle*

Fig.11 Lufenuron-induced malformed pupae

Improper melanization *Breaking of pupa*

Fig.12 Lufenuron-induced malformed weevils

Twisted antennae, fragile wings & retention of exuviae

Table 4. Interaction of lufenuron and *B. bassiana* on poison food technique

Lufenuron (%)	Mycelial growth (cm)
0	$2.81^{b} \pm 0.80$
0.001	$2.37^{cd} \pm 0.87$
0.004	$2.26^{d} \pm 0.78$
0.007	$2.70^{bc} \pm 0.44$
0.01	$3.11^{ab} \pm 0.61$
0.013	$3.26^{a} \pm 0.44$
CD (p=0.05)	0.428

The mycelial growth of *B.bassiana* was not affected even at the highest concentration of lufenuron @ 0.013% indicating its compatibility and the mycelial mat grew to the maximum (3.26 cm) when exposed to the highest concentration of lufenuron (0.013%).

Id) Field delivery of entomopathogenic nematodes (EPN)

Most of the coconut pests are subdued by biological means and therefore a keen look out for entomopathogens in the management of RPW has been in vogue since quite long. Entomopathogenic nematode belonging to *Heterorhabditis indica* was found effective in the bio-suppression of RPW grubs in synergy with imidacloprid. Placement of three filter paper sachets containing 12-15 *H.indica*-infected *G. mellonella* cadavers on the leaf axils after application of 0.002% imidacloprid could recover 60% of infested palms.

In our earlier experiments, *H.indica* was found superior in the suppression of RPW than *Steinernema* spp. and subsequently *H. indica* was alone attempted. In order to dispense with the chemical, imidacloprid, a botanical cake developed by ICAR-CPCRI was used in combination with the *H. indica*-infected *G. mellonella* cadavers through filter-paper delivery technique (Fig. 13). Prophylactic delivery of filter paper sachets containing ten *H.indica*-infected *G. mellonella* cadavers in combination with tablet-shape botanical cake on the leaf axils could reduce 43.1% rhinoceros beetle attack (Table 5) and was also observed to safeguard palms from RPW invasion during monsoon period. This field delivery of filter paper sachets containing botanical cake and *H. indica* infected *G. mellonella* cadavers will be further refined for sustainable pest management.

Table 5. Interaction of botanical cake and EPN suppressing rhinoceros beetle in juvenile palms

Leaf damage (%)			
Treatments (N=20)	**Pre-treatment**	**Post-treatment**	**Reduction**
Control	31.5	30.5	3.2
EPN plus cake sachets	32.5	18.5	43.1*

**Significant t<0.01%*

Fig.13. Field delivery of EPN plus botanical cake in filter-paper sachets

Botanical cake developed by ICAR-CPCRI

H. indica infected G. mellonella

Botanical cake plus EPN

IIa) Isolation of gut microbes in RPW

The dissected gut of RPW grub was found to be at least three-fold longer than the body size of the insect. When body length of the grubs was found to be 3.5 cm, the length of the gut was recorded as 10.5 cm. Though the grubs feed on the fibrous coconut tissue, the gut content was devoid of any fibrous tissues and comprised of mainly fluid materials. Nearly 5.12% of the total bacterial colonies constituted cellulose degrading type in the gut. Nine predominant and distinct bacterial colonies *viz.*, RPB-1, RPB-2, RPB-4, RPB-5, RPB-6, RPB-7, RPB-8, RPB-9 and RPB-11 were isolated from the gut of RPW. Important colony features of these bacterial isolates are presented in Table 6.

Table 6. Colony characteristics of bacterial isolates

Isolates	**RPB-1**	**RPB-2**	**RPB-4**	**RPB-5**	**RPB-6**	**RPB-7**	**RPB-8**	**RPB-9**	**RPB-11**
Form	Regular	Regular	Regular	Regular	Irregular	Regular	Regular	Regular	Regular
Elevation	Raised	Flat	Raised	Raised	Flat	Flat	Flat	Raised	Raised
Pigmentation	Red, shiny	Clear	Creamy mucoid	Creamy shiny	Whitish	Whitish to Creamy	Greyish white	Whitish	Whitish
Size	Small	Pin point	Medium	Medium	Medium	Medium	Small	Small	Small

As revealed from the Table 6, it was found that eight bacterial isolates were regular in form and RPB-6 was found be irregular. Regarding elevation, four bacterial isolates (RPB-2, RPB-6, RPB-7 & RPB-8) were found as flat and other five isolates were slightly elevated. In terms of pigmentation, RPB-1 was found as red and shiny, RPB-7 as greyish-white and other isolates were creamy clear and whitish. While RPB-2 was found as pin point in size, RPB-4, RPB-5 , RPB-6 and RPB-7 were medium in size and other isolates (RPB-1, RPB-8, RPB-9 & RPB-11) were categorized as small. Important biochemical tests such as Gram staining, capsule staining, endospore staining, oxidase, IMViC, carbohydrate fermentation and starch hydrolysis, were performed on the nine predominant isolates and are briefly presented in Table 7.

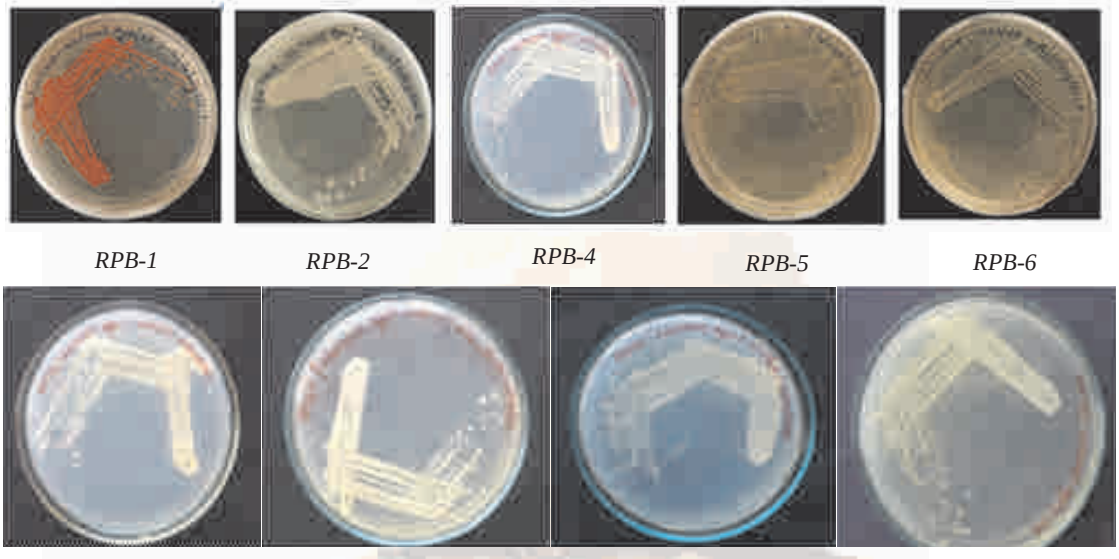
The biochemical tests presented in the Table 7 indicated that RPB-2, RPB-6, RPB-7 and RPB-8 are Gram Positive and other five isolates (RPB-1, RPB-4, RPB-5, RPB-9 & RPB-11) as Gram Negative. While the shape of RPB-2 was found as cocci others were all designated as bacilli. Five isolates *viz.*, RPB-4, RPB-6, RPB-7, RPB-9 and RPB-11 exhibited capsule around cell wall with a characteristic blue colour whereas others could not. RPB-9 is the only isolate which had the ability to produce cytochrome oxidase activity. Regarding IMViC test, all the isolates showed negative indole reaction, RPB-2 and RPB-11 alone produced positive reaction for methyl red test. RPB-1, RPB-6, RPB-7, RPB-9 and RPB-11 had the ability to ferment glucose and the bacterial issolates. RPB-1, RPB-4, RPB-5, RPB-9 and RPB-11 used citrate as source of carbon. Seven isolates *viz.*, RPB-2, RPB-4, RPB-6, RPB-7, RPB-8, RPB-9 and RPB-11 were found to be lactose fermenting types whereas all isolates except RPB-5 fermented mannitol to produce acid end products. In addition, all bacterial isolates could use sucrose as a carbon source and except for RPB-11 all isolates were found positive for nitrate reduction test.

The pure culture of the nine bacterial isolates in simple media is presented in Fig.14.

Table 7. Biochemical characteristics of bacterial isolates

	RPB-1	RPB-2	RPB-4	RPB-5	RPB-6	RPB-7	RPB-8	RPB-9	RPB-11
Gram's reaction	-	+	-	-	+	+	+	-	-
Shape	Bacilli	Cocci	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Arrangement	Single	Cluster	Single	Single	Chain	Chain	Single	Single	Single
Motility	+	-	-	+	+	+	+	-	+
Capsule staining	-	-	+	-	+	+	-	+	+
Oxidase	-	-	-	-	-	-	-	+	-
Indole	-	-	-	-	-	-	-	-	-
Methyl Red	-	+	-	-	-	-	-	-	+
Voges-Prausker test	+	-	-	-	+	+	+	+	-
Citrate Utilization test	+	-	+	+	-	-	-	+	+
Glucose	+	+	+	+	+	+	+	+	+
Lactose	-	+	+	-	+	+	+	+	+
Mannitol	+	+	+	-	+	+	+	+	+
Sucrose Nitrate	+	+	+	+	+	+	+	+	+
Reduction test	+	+	+	+	+	+	+	+	-

Fig.14 Pure bacterial cultures in simple media



IIb) Molecular characterization and identification of bacterial isolates

PCR amplification and sequencing of 16S rRNA genes provides the most comprehensive and flexible means of identification of bacterial isolates. DNA samples from RPB-1, 2, 4, 5, 6, 7, 8, 9 & 11 were found positive to the universal primers (F27-R1492) in PCR. Agarose gel analysis of the PCR products revealed the presence of

approximately 1500 bp amplicons in all the DNA samples from the isolates under study (Fig.15). PCR amplified fragments were sequenced for the identification of the bacterial isolate based on the 16S rRNA sequences.

Fig. 15. Agarose gel analysis of PCR amplified products of bacterial isolates

Lane 1-1kb DNA ladder; lane 2-10-RPB-1to 9

The DNA sequence was blasted with the existing bacterial 16S rRNA sequences in the NCBI gene bank (Table 8; Fig.16) and the identification was confirmed based on the genetic relatedness of the existing bacterial sequences (Fig.16). In closeness with the phylogenetic tree of 16S rRNA sequences of bacteria isolated from the gut of RPW, the following nine bacterial species were identified. In the phylogenetic tree, Gram positive and Gram negative bacterial isolates got clustered in two distinct branches.

RPB-1

The colony characteristics and results of the various biochemical tests indicated that RPB-1 may be an isolate of *Serratia marcescens*. The 16S rRNA gene sequences of RPB-1 showed 100% similarity with sequences of *Serratia marcescens* isolates (Accession No. JX872284.1, KJ721215.1) in Genbank. Based on the morphological, biochemical and molecular characterization the isolate RPB-1 was identified as *Serratia marcescens.*

RPB-2

The Gram positive, catalase negative cocci occurring in groups may be *Streptococcus* spp. or *Enterococcus* spp. The 16S rRNA gene sequences showed 98-99.7% similarity with *Enterococcus* spp. In the phylogenetic tree constructed using the CLUSTAL aligned sequences, the RPB-2 grouped with *E. casseliflavus* strain CCFM8319, CCFM83198 and TS4E1(Accession No. KJ803876.1, KJ803875.1, KJ571214.1). Based on the morphological, biochemical and molecular characterization the isolate RPB-2 was identified as *Enterococcus casseliflavus.*

RPB-4

The Gram negative, rod-shaped, oxidase negative and catalase positive RPB-4 isolate with capsule was identified as *Klebsiella* sp. based on biochemical tests. This was further confirmed by the molecular characterization of 16S rRNA gene sequences. The nucleotide sequence of RPB-4 showed 99.6 to 99.8% similarity with the sequence of *Klebsiella variicola* available in Genbank. In the phylogenetic tree constructed using the CLUSTAL aligned sequences, the test isolate grouped with various strains of *K. variicola* (Accession no. KC853306.1 and KC853294.1) and hence, the RPB-4 isolate was identified as *Klebsiella variicola* .

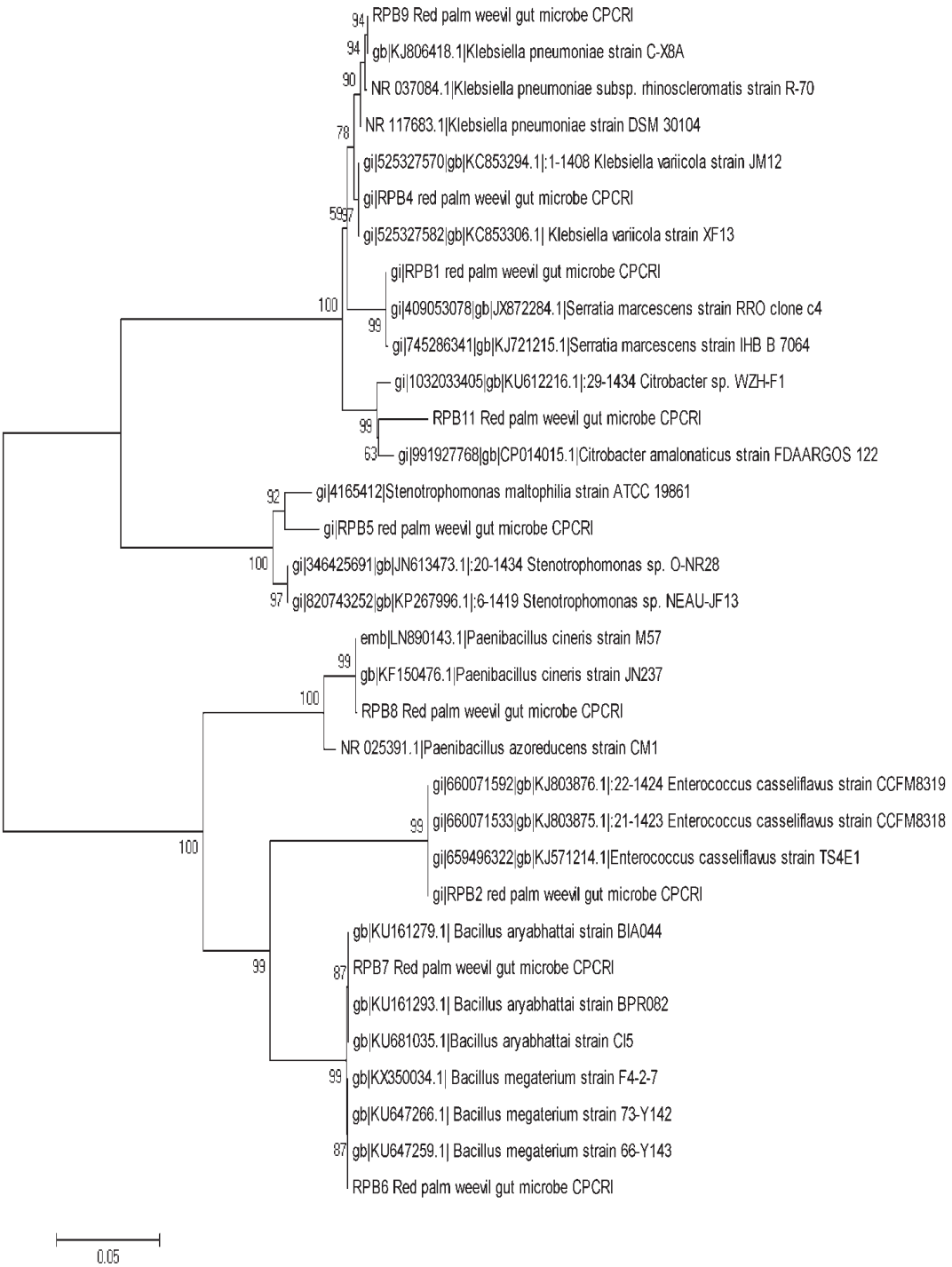
RPB-5

The Gram negative, rod-shaped, motile, catalase and oxidase negative RPB-5 isolate was found to be a member of the family Enterbacteriaceae as evident from the results of the biochemical tests. The 16S rRNA sequence of the isolate showed 96-97% similarity with two genera *viz.*, *Stenotrophomonas* spp. and *Xanthomonas* spp. In the phylogenetic tree constructed using neighbor joining method, RPB-5 clustered along with *Stenotrophomonas maltophilia* strain ATCC 19861 (Accession No. AB021406.1). Thus, the isolate RPB-5 was identified as with *Stenotrophomonas maltophilia.*

Table 8 Accession numbers of selected bacterial 16S rRNA sequences used for analysis of isolates of RPW gut microbes

No.	Accession No.	Description of organism	Gen ID (gi) No.
1.	JX872284.1	*Serratia marcescens* strain RRO clone c4	409053078
2.	KJ721215.1	*Serratia marcescens* strain IHB B7064	745286341
3.	KJ803876.1	*Enterococcus casseliflavus* strain CCFM8319	660071592
4.	KJ803875.1	*Enterococcus casseliflavus* strain CCFM8318	660071533
5.	KJ571214.1	*Enterococcus casseliflavus* strain TS4E1	659496322
6.	KC853306.1	*Klebsiella variicola* strain XF13	525327582
7.	KC853294.1	*Klebsiella variicola* strain JM12	525327570
8.	KP267996.1	*Stenotrophomonas* sp.NEAU-JF13	820743252
9.	JN613473.1	*Stenotrophomonas* sp. O-NR28	346425691
10.	AB021406.1	*Stenotrophomonas maltophila* strain ATCC19861	4165412
11.	KU681035.1	*Bacillus aryabhattai* strain CI5	1035288476
12.	KU161293.1	*Bacillus aryabhattai* strain BPR082	1024297416
13.	KU161279.1	*Bacillus aryabhattai* strain BIA044	1024297402
14.	KX350034.1	*Bacillus megaterium* strain F4-2-7	1038485818
15.	KU647266.1	*Bacillus megaterium* strain 73-Y142	987385956
16.	KU647259.1	*Bacillus megaterium* strain 66-Y143	987385949
17.	LN890143.1	*Paenibacillus cineris* strain M57	939460408
18.	KF150476.1	*Paenibacillus cineris* strain JN237	513798816
19.	NR025391.1	*Paenibacillus azoreducens* strain CM1	219878252
20.	KJ806418.1	*Klebsiella pneumoniae* strain C-X8A	639127992
21.	NR037084.1	*Klebsiella pneumoniae subsp. rhinoscleromatis* strain R-70	310975220
22.	NR117683.1	*Klebsiella pneumoniae* strain DSM 30104	645320489
23.	KU612216.1	*Citrobacter* sp. WZH-F1 16S	1032033405
24.	CP014015.1	*Citrobacter amalonaticus* strain FDAARGOS_122	991927768

Fig.16. Phylogenetic tree of 16S rRNA sequences of bacterial isolates from gut of RPW



RPB-6

The colony characteristics and results of the various biochemical tests indicated that RPB-6 may be an isolate of *Bacillus megaterium*. The 16S rRNA gene sequences of RPB-6 showed 99% similarity with the sequences of *Bacillus megaterium* isolates (Accession No:KX350034.1 and KU647266.1) in Genbank. Based on the morphological, biochemical and molecular characterization the isolate RPB-6 was identified as *Bacillus megaterium*.

RPB-7

The Gram positive, oxidase negative bacilli occurring in groups indicated as *Bacillus aryabhattai*. The 16S rRNA gene sequences showed 99% similarity with *Bacillus aryabhattai*. In the phylogenetic tree constructed using the CLUSTAL aligned sequences RPB-7 grouped with *Bacillus aryabhattai* (Accession No:KU681035.1, KU161293.1). Based on the morphological, biochemical and molecular characterization the isolate RPB-7 was identified as *Bacillus aryabhattai*.

RPB-8

The Gram positive, indole and nitrate reduction negative bacilli occurring as groups was suggested as *Paenibacillus* sp. When subjected to molecular characterization, the 16S rRNA genes showed 99.8% similarity with *Paenibacillus cineris* (Accession No: LN890143.1, KF150476.1) and clustered with these isolate in the phylogenetic tree. Based on the 16S rRNAgene sequencing the RPB-8 was identified as *Paenibacillus cineris*.

RPB-9

The Gram negative, rod-shaped, oxidase negative and non-motile RPB-9 isolate with capsule was identified as *Klebsiella pneumoniae* based on biochemical tests. This was further confirmed by the molecular characterization of 16S rRNA sequences. The nucleotide sequence of RPB-9 showed 100% similarity with the sequences of *Klebsiella pneumoniae* available in Genbank (Accession No: KJ806418.1). In the phylogenetic tree constructed using the CLUSTAL aligned sequences and the test isolate grouped with various strains / isolates of *Klebsiella pneumoniae* and hence, the RPB-9 isolate was identified as *Klebsiella pneumoniae*.

RPB-11

The Gram negative, rod-shaped, methyl red and citrate utilization positive isolate with capsule was identified as *Citrobacter* sp. based on colony characteristics and biochemical tests. The 16S rRNA gene sequences showed 98% similarity with *Citrobacter amalonaticus* strain FDAARGOS (Accession No. CP014015.1) in Genbank. Hence, the RPB-11 isolate was identified as *Citrobacter* sp.

Based on the biochemical and molecular characterization, the nine predominant bacterial isolates occurring inside the gut of *R. ferrugineus* was identified as

RPB-1 - *Serratia marcescens*

RPB-2 - *Enterococcus casseliflavus*

RPB-4 - *Klebsiella variicola*

RPB-5 - *Stenotrophomonas maltophilia*

RPB-6 - *Bacillus aryabhattai*

RPB-7 - *Bacillus megaterium*

RPB-8 - *Paenibacillus cineris*

RPB-9 - *Klebsiella pneumonia*

RPB-11- *Citrobacter* sp.

A holistic characterization of culturable and non-culturable bacterial colonies would be the key for understanding the microbiome dynamics to greater insights in *R. ferrugineus* so as to exploit for innovative pest management strategies.

The putative functional role of these microbes in *R. ferrugineus* is presumed as

Bacterial Isolates	Putative functions
Serratia marcescens	Cellulose degradation & host fitness against parasitoids
Enterococcus casseliflavus	Synergistic effect on entomopathogens
Klebsiella spp.	Survive on high C / N ratio medium
Stenotrophomonas maltophilia	Imparts resistance against fungal invasion
Bacillus aryabhattai	Ability to withstand harsh environment
Bacillus megaterium	Imparts immunity against fungi,bacteria and virus due to the presence of antifungal and antiviral principles
Paenibacillus cineris	Produce antimicrobial substances thus imparts immunity
Citrobacter sp.	Nitrogen fixation

III Documentation of geo-morphs

Extreme variation in size and body markings of the adult weevils particularly the black spots on pronotum are observed on weevils collected from different regions. Diversity in size morphs is a common feature and the morphometric parameters are presented in Table 9.

Body length was found to be significant ($F = 5.09$, $p < 0.05$) between locations and the longest being Tenkasi female weevil (33 mm) and the smallest being Kayamkulam female (24 mm). Elytra width was found as significant ($F = 4.25$, $p < 0.05$) between the locations, the broadest being Tenkasi male (11.8 mm) and the narrowest being Kayamkulam male (5.42 mm). Significance ($F = 5.84$, $p < 0.05$) between locations also existed for pronotum length, the longest being Pollachi male (13.5 mm) and the shortest being Kayamkulam female (8.06 mm). With regard to pronotum width, significance ($F = 5.18$, $p < 0.05$) existed between locations, the broadest being female Pollachi (11.7 mm) and the narrowest being female (8.12 mm) collected from Kayamkulam. The rostrum length was found significant for both locations ($F = 5.83$, $p < 0.05$ as well as sexes ($F = 12.49$, $p < 0.01$). Among the four locations studied, Tenkasi female had the longest rostrum (13.1 mm) and the smallest rostrum was observed in Kayamkulam male weevil (8.62 mm). Among the two sexes, the longest rostrum was observed in female and male weevils recorded the least rostrum length. Rostral length before the antennal scrobe was found to be significant for both locations ($F = 6.11$, $p < 0.05$) as well as sexes ($F = 14.87$, $p < 0.01$). Among the locations studied, rostrum length before scrobe was found to be the highest for Tenkasi female (8.87 mm) and the lowest for Kayamkulam male (5.24 mm). Among the two sexes, rostrum length before scrobe was found the highest for females and males recorded the least rostral length. Scape length was found to be significant ($F = 5.93$, $p < 0.05$) between the sexes. Pollachi female recorded the highest scape length (4.33 mm) and the lowest was observed in Kayamkulam male (3.15 mm). With respect to funicle length, significant difference ($F = 12.67$, $p < 0.01$) existed between locations. Tenkasi female recorded the longest funicle

Table 9. Morphometrical measurements of different body parts of RPW male and female collected from different locations

Locality	Sex	BL	BW	RL	RW	PL	PW	RL	RLBA	SL	FL	CL	CW
Pollachi	Female	30.66±0.66	13.33±0.66	14.66±0.33	5.66±0.33	11.00±0.36	11.66±0.88	10.83±0.30	6.73±0.11	4.33±0.33	2.59±0.10	1.72±0.06	1.63±0.36
	Male	30.33±1.20	13.26±0.32	14.33±0.66	7.23±0.57	13.50±0.28	10.23±0.50	9.64±0.15	6.54±0.06	3.57±0.10	2.48±0.13	1.33±0.06	1.41±0.12
	Total	30.50±0.61	13.29±0.33	14.50±0.54	6.45±0.46	12.25±0.62	10.95±0.55	10.23±0.29	6.63±0.07	3.95±0.24	2.53±0.07	1.53±0.09	1.52±0.13
Tenkasi	Female	33.00±0.57	14.00±0.57	14.66±0.33	7.96±3.54	9.33±1.16	10.56±0.34	13.10±0.23	8.87±0.27	4.33±0.33	2.83±0.22	2.19±0.08	1.55±0.12
	Male	30.33±1.20	11.83±0.45	13.56±0.30	11.81±0.55	10.32±0.67	11.29±0.45	10.83±0.05	6.20±0.08	3.51±0.18	2.61±0.08	1.75±0.04	1.33±0.05
	Total	31.66±0.84	12.92±0.58	14.21±0.31	9.88±1.80	10.07±0.61	10.93±0.31	11.74±0.51	7.54±0.61	3.92±0.21	2.72±0.11	1.97±0.10	1.44±0.07
Kanyakumari	Female	28.66±1.52	13.66±1.85	10.66±0.88	6.50±0.28	10.33±0.88	8.50±0.28	10.86±0.19	7.04±0.10	3.44±0.08	1.98±0.07	1.54±0.10	1.16±0.02
	Male	29.00±1.73	11.80±0.20	10.33±0.33	6.60±0.52	10.07±0.26	8.23±0.11	8.94±0.58	6.37±0.08	3.19±0.03	1.52±0.11	1.41±0.11	1.12±0.07
	Total	28.50±1.05	12.73±0.93	10.50±0.42	6.25±0.29	10.20±0.41	8.15±0.15	9.90±0.51	6.70±0.16	3.27±0.05	1.60±0.07	1.47±0.07	1.14±0.01
Kayyunkulam	Female	24.00±4.00	11.75±1.39	14.00±2.78	5.53±0.76	8.06±1.15	8.12±1.05	9.45±1.16	6.27±0.36	3.53±0.30	2.71±0.25	1.38±0.07	1.23±0.11
	Male	24.16±4.00	10.63±2.05	12.53±1.81	5.42±1.26	9.06±1.73	8.66±2.02	8.62±1.40	5.24±1.04	3.15±0.65	2.03±0.39	1.39±0.19	1.18±0.14
	Total	24.08±2.53	10.69±1.11	13.26±1.90	5.48±0.66	8.56±0.95	8.39±1.03	9.02±0.83	5.75±0.54	3.34±0.33	2.07±0.21	1.35±0.09	1.20±0.08

(2.83 mm) and the least by male Kasaragod (1.52 mm). Club length was found to be significant for both locations ($F=16.14$, $p<0.01$) and sexes ($F=10.50$, $p<0.01$). Among the four locations studied, club length was found to be the highest in Tenkasi female (2.19 mm) and lowest in Kayamkulam female (1.30 mm). In general, females had higher club length than males. Club width was also found to be significant ($F=3.43$, $p<0.05$). Club width was found to be the highest for female Pollachi (1.63 mm) and the least in male Kasaragod.

Despite small insect population examined for morphometric features, tremendous variation existed in most of the features studied cutting across the specimens indicating diversity and its adaptation according to the locations. In a very narrow population studied wide diversity existed indicating the success nature of the pest and the challenge in suppression. In general, Tamil Nadu specimens were robust in size and Kerala specimens were smaller in size. Diversity in size morphs as well as multi - numero spots on pronotum of adult weevils are presented in Fig. 17 & 18.

Fig. 17. Variation in size morphs of adult weevils collected from different zones

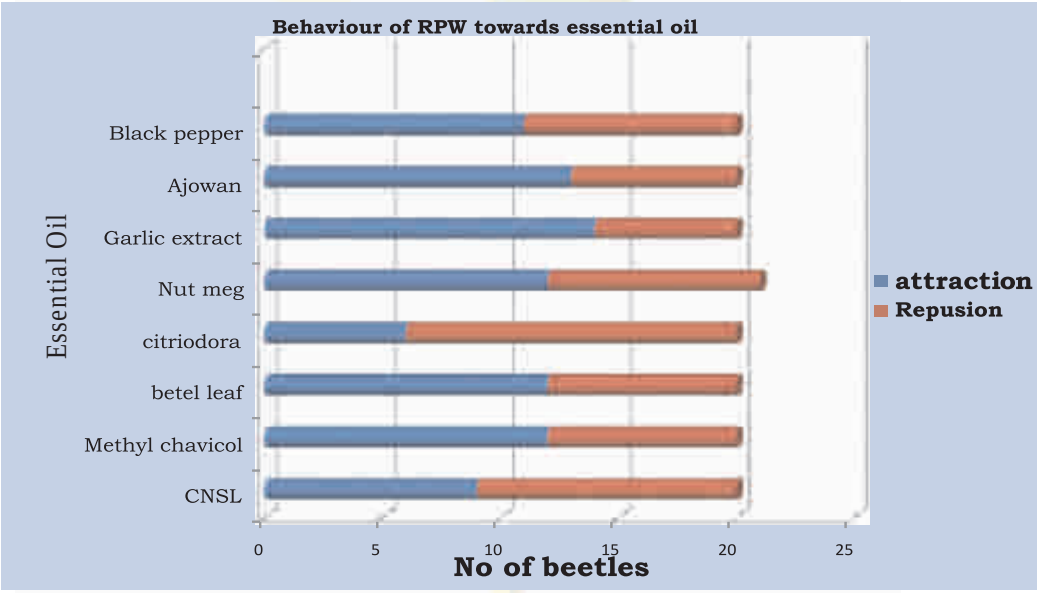
Fig.18. Multi-numero spots on pronotum of adult weevils

IVa) Essential oil and repellency of RPW

Botanical repellents (essential oils) were screened by Y tube choice assay against RPW (Fig.19). Citriodora oil (1%) exhibited the highest repellency, and induced 70% repulsion at 1000 ppm concentration closely followed by CNSL that caused 60% repulsion of adult weevils. Repellent property of citriodora oil was further confirmed by wind tunnel assay in which 13.33% beetles merely oriented towards wind flight

response, 23.33% beetles exhibited downwind flight response after moving up to mid point and 63.33% beetles remained at the point of release in the wind tunnel. Higher percentage of weevils remained at the point of release avoiding movement towards the source, as an attributable behavioral response confirming the repellency of the oil. This oil will be further evaluated against RPW in the field for slow release as well as for pushing the weevils away from the field.

Fig 19. Orientation and behavior response of RPW towards essential oil



IVb) Delivery of aggregation pheromone

ICAR-CPCRI has successfully validated the field efficacy of the aggregation pheromone through different delivery matrices and established the supremacy of the nanoporous matrix in effective dispensation of the semiochemical. The different dispenser strategies employed was presented in Fig. 20.

A novel gel based slow-release matrix for aggregation pheromone was developed and this delivery matrix was field evaluated for the trapping of RPW. Placement of two gel-matrix sachets on coconut leaf axil @ 5 g

Fig.20 Different modes of pheromone delivery strategies in RPW trapping

Capillary tube mechanism *Polymer membrane* *Nanoporous matrix* *Gel-based delivery matrix*

Fig.21. Umbrella studded redesigned PVC trap

per palm and pheromone trap outside the garden reduced RPW infestation on coconut from 5.19% to 1.3%. Dissipation of (5%) citriodora oil from sodium alginate beads was found to be 12%, 20%, 25% and 34%, respectively in a period of one to four weeks.

IVc) Re-designing pheromone trap

The plastic bucket trap used in the trapping of RPW is designed with an umbrella mode as well as PVC tube for enhanced durability and better catch of weevils. The design is also focused to reduce the slippages of weevils and orient them perfectly in to the trap. On account of its PVC make the durability of the trap is enhanced and simultaneously the umbrella could reduce the dissipation loss of the aggregation pheromone considerably (Fig.21).

IVd) Ecological engineering

A pest suppressive coconut-based agro-ecosystem was designed through ecological infrastructure within the cropping system such as attraction of defenders, volatile cue repulsion, refuge site, raising eco-feast crops, installation of bird perches and attraction of predatory birds *etc.*, (Fig.22). Such crop-habitat diversification approach could avoid pest entry into the system through stimulo-deterrent diversionary strategy. Growing intercrops such as nut meg, rambuttan, curry leaf, papaya, banana, turmeric, marigold *etc.*, distracted RPW from egg laying in diversified cropping system in coconut gardens due to volatile confusion in host location. Maintenance of such coconut-based diversified cropping system through ecological engineering could reduce pest attack in coconut than in mono-cropped gardens.

IPM strategies

- Close scrutiny and regular inspection of palms for early diagnosis.
- Planting coconut with correct spacing (Tall 8 x 8 m; Dwarfs 7x7 m) to reduce odour cues.
- Constant vigil and prophylactic treatment for rhinoceros beetle damage, leaf rot and bud rot diseases.
- Avoid physical injuries on palms by following palm and farm hygiene as well as good agricultural practices.
- Crop-habitat diversification through compatible intercropping and sequential cropping in coconut gardens reduced pest occurrence in palms through stimulo-deterrent diversionary strategy.
- Prophylactic delivery of filter paper sachets containing ten *Heterorhabditis indica*-infected *Galleria mellonella* cadavers in combination with botanical cake on the topmost three leaf axils.
- Curative treatment by spot application of imidacloprid 0.02% (1ml per litre) or indoxacarb 0.04% (2.5 ml per litre) recovered the pest attack.
- Installation of pheromone traps @ 1 trap / ha through slow release delivery in community mode.

Fig. 22 Ecological engineering through push-pull strategies

Table 10. Budget outlay and expenditure (4001-1331)

Year	Amount released from ICAR-IIHR (Rs)	Opening balance (Rs)	Expenditure (Rs)	Balance (Rs)
2014-2015	1,10,000	1,10,000	1,07,583	2417
2015-2016	3,27,583	3,30,000	3,29,255	745
2016-2017	2,50,000	2,50,745	2,50,745	0
Total	6,87,583		6,87,583	0

Epilogue

1) Lufenuron, an insect growth regulator, induced larval-pupal and pupal-adult aberrations and intermediates in RPW with a median effective dose (ED_{50}) of 0.00779%. RPW grubs accelerated pupation when exposed to lufenuron indicating modulation in hormone titre disfavouring morphogenetic moults.

2) Dyar's ratio (1.2) confirmed the presence of ten larval instars in RPW which could be one of the weakest links for exploitation in pest management through hormone mimics.

3) Based on biochemical and molecular characterization, nine cultural bacterial strains could be identified as *Serratia marcescens, Enterococcus casseliflavus, Klebsiella variicola,, Stenotrophomonas maltophilia, Bacillus aryabhattai, Bacillus megaterium, Paenibacillus cineris, Klebsiella pneumoniae* and *Citrobacter* sp. Putative functional role in the fitness behaviour of RPW involving protection against entomopathogens, parasitoids, sustaining in high C/N ratio medium and production of antibiotics conferring immunity could be linked.

4) Citriodora oil (1%) induced effective repulsion of RPW in wind tunnel bioassay and a novel slow release gel based delivery of aggregation pheromone as well as the trap design were refined for effective trapping of weevils in the field. Placement of two gel-matrix sachets on coconut leaf axil @ 5 g per palm and pheromone trap outside the garden reduced the RPW infestation from 5.19% to 1.3%.

5) Extreme modulation in size and colour morphs of *R. ferrugineus* could be observed based on morphometric analysis indicating robustness in Tamil Nadu RPW and smallness of Kerala specimens clearly adapting to palm health status.

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