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Efficacy of SPLAT in entrapping the invasive insect pest Red Palm Weevil in Toddy farming areas of Nizamabad, Telangana, India

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Abstract
The red palm weevil (RPW, *Rhynchophorus ferrugineus*) (Oliver) an invasive insect pest of coconut, oil palm, date palm and sago across the globe and continued to spread to different parts of India damaging the palm trees within months following infestation. However, very little research is focused on RPW a serious insect pest which is threatening the very existence of palm trees particularly toddy plants. Toddy cultivation is an important food source for several communities across the Telangana, India. Therefore, we have developed Specialized Pheromone and Lure Application Technology (SPLAT) technology for effective trap of RPW. For trapping RPW, SPLAT-1 and SPLAT-2 in combinations with food bait (sugarcane, apple and banana) and insecticide has attracted a greater number of insects when compared to individual application within a period of one month. Interestingly, both male and female populations (more females) were captured due to attractive nature of ferrugineol. From the current studies, it is strongly recommended to combine bait and insecticides along with the SPLAT for efficient capture of RPW to control its widespread across the globe.

Keywords: Red Palm Weevil, RPW, SPLAT, Pheromones, Invasive Insects

Introduction:

Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is one of the most devastating invasive insect pests of palms prevalent in India, Middle East, and invaded at global level (Asia, Africa, Europe, Middle East, Oceania, and United States of America) threatening almost all palm species (coconut, date palm, oil, sago etc.) (Soroker et al. 2005). Particularly in South India, Telangana and the Andhra Pradesh States are very much familiar for growing Toddy Palms (*Phoenix sylvestris* and *Borassus flabellifer*) wherein RPW infestation is seen at all geographical locations of Telangana. In the State of Telangana, toddy is a popular drink in rural parts that is frequently consumed by the labor at the end of the day. Toddy tappers will tap the apex trunk region of the toddy palm for extracting the toddy which in fact help RPW to locate and infest the host plant. Since last two decades, the majorities of the toddy palms was threatened for its survival and were destroyed due to a huge infestation of RPW.

The adult female RPW (post mating) oviposit in wounds, cracks, and crevices of the palm tissues and upon hatching the larvae (grubs) chew the surrounding plant tissue and penetrate into the interior of palm trunks, leaving behind frass (plant fibers). In due course of time, the grubs reach the bud region causing fatal damage which ultimately leads to the death of the palm (Hussain et al., 2013b). Till date, several integrated pest management (IPM) strategies were attempted in order to control the infestation of RPW such as cultural control (El-Ezaby 1997, Abo-El-Saad et al. 2001), chemical control, mass pheromone trapping (Faleiro and Chellapan 1999, Hallett et al. 1999), biological control (Gindin et al. 2006, Mazza et al. 2014), Sterile Insect Technique (SIT) approach (Paoli et al. 2014), mechanical control, quarantine control etc. Among these, RPW control was mostly attempted with the application of insecticides causing long-term damage to the environment and human health. Due to several disadvantages associated with chemical control, there is a strong emphasis on the development of IPM which integrates pheromone traps and biological control rather than insecticides application alone (Faleiro, 2006). Though pheromone traps had been incorporated into RPW-IPM programs, very few reports exist on the successful trap of RPW with respect to the type of trap used against time period (El-Shafie et al., 2011; Haris et al., 2014).

Insect pheromones which are volatile organic molecules with low molecular weight, enhance behavioral response and communication from insects between the same or the opposite sex (Phillips, 1997). Pheromone trapping system using Ferrolure (Ferrugineol, a mixture of 4-methyl-5-nonanol and 4-methyl-5-nonanone is the specific pheromone of red palm weevil) in food baited bucket traps containing (or not) insecticide(s), has become a vital component of the IPM strategy (Hallett et al., 1993). RPW pheromone traps not only serve as monitors, indicating the presence of the pest in and around the plantation, but also play a vital role in reducing the RPW population levels in the field (Kaakeh et al., 2001). In the recent past, Specialized Pheromone and Lure

Application Technology (SPLAT) has been effective in controlling several insect pests (Vidyasagar et al., 2000a, b). As per El-Shafie (2011), the bait free attract and kill technology (Hook™ RPW) has reported in the successful trap of RPW (2.47 weevils/ha/wk). Therefore, we attempted a novel SPLAT method (Sentomol containing 4-methylnonan-5-ol and 4-methylnonan-5-one) to enhance the trap rate of RPW.

Materials and methods:

Field Survey of Toddy Palms and intensity of damage caused by RPW:

During December 2014 and January 2015 we have selected three villages (Yanampally 18° 37' 52'' N latitude and 78° 15' 96'' E longitude, Sampally 18° 63' 92'' N latitude and 78° 21' 70'' E longitude, and Mentrajpally 18°65'53'' N latitude and 78°20'63'' E longitude) of Nizamabad District (TS) India, which were hugely infested with RPW. All the above three mentioned sites are being maintained by toddy farmers with whom we have made MoA to reduce the RPW infestation. Approximately 500 *P.sylvestris* exists in each identified site, wherein majority (~50%) of the *P.sylvestris* were already got infested with RPW. It is intriguing to mention that few of the *P.sylvestris* which is not prepared for toddy extraction was not infested with RPW and remained healthy.

Preparation of Trap:

Following survey, we have initiated the RPW trap using novel pheromone lure, wherein SPLATs were examined for efficacy of the traps. Until unless stated, we have carried out all the experiments using black colored buckets as traps as per Abuagla et al., (2012). The traps were optimized in such a way that the sides of the traps were covered by jute bag (rough surface) to allow weevils to crawl inside, four entry holes were made on the sides (1.5 x 5 cm^2) of the trap and three holes on the top for easy entry of the RPW.

Traps Installation using SPLAT:

Black coloured bucket traps containing SPLAT, food bait and insecticide were placed in the infested sites to record the peak weevil activity. As suggested by Oehlschlager 1994; Soroker et al., 2005; Faleiro et al., 2011 and Giblin-Davis et al., 2013, 1-10 traps were placed per hectare for small holdings and in the field of uneven topography, one trap for every two hectares of large-scale fields of homogenous lands. Traps were placed under shade to retain for long time of SPLAT, food bait and insecticide. To increase the capture rates of SPLAT traps the food bait was included.

Type of Food Bait and Insecticide concentration:

The type of food bait also influences the ability of SPLAT, so in our study we used various combinations of food as bait such as fresh sugarcane slices, apples, and banana (each pprox. 200 gms) which were then mixed with 5 liters of water containing 5 ml of non-repellant insecticide. The food bait and pheromone lure in combination will attract and trap RPW adults, while the insecticide immobilizes and kills the trapped weevils. Food bait, insecticide, and trapped weevils were replaced with fresh ones at least once in 10 days.

Statistical Analysis:

In all the tests performed with different treatments, data pertaining to a number of trapped RPW with different treatments were recorded every 3 days. The data were analyzed statistically according to One Way ANOVA (Dunnetts Test) ($p<0.05$) by using statistical analysis software SigmaPlot14.

Results:

Trapping efficiency using SPLAT-1 and SPLAT-2:

For trapping the RPW using SPLAT-1 and SPLAT-2 in different biological replicates, the highest number (#11.6) with SPLAT-1 (Table-1) and (#9) with SPLAT-2 (Table-2) in presence of food bait, and insecticide whereas very less number of RPW trapped with SPLAT-1 (#1) and SPLAT-2 (#4.6), without bait and insecticide. The sex ratio of captured RPW was the highest female biased [(n=18 female RPW (51%); n=17 male RPW (49%)] with SPLAT-1 and [(n=18 females (66%); n=9 males (34%)] with SPLAT-2. It was also observed that RPW flight activity was crepuscular or nocturnal. Female RPW were 51% (n=18) and 66% (n=18) and male RPW were 49% (n=17) and 34% (n=9) with SPLAT-1 and SPLAT-2. All the treatments were examined and captured during the day time.

Table-1: The list of total number of RPW trapped SPLAT-1 various combinations of treatments with three different biological replicates.

S. No.	Treatment*	Test-1	Test-2	Test-3
1.	B + S + I	13 (5♂+8♀)	12 (5♂+7♀)	10 (7♂+3♀)
2.	B + S + W	13 (6♂+7♀)	10 (1♂+9♀)	9 (4♂+5♀)
3.	S + I	9 (3♂+6♀)	8 (3♂+5♀)	3 (♀)
4.	S + W	3 (1♂+2♀)	3 (2♂+1♀)	1 (♀)
5.	B + I	0	0	0
6.	B + W	1 (♀)	2 (♂)	0
7	I	0	0	0
8	W	0	0	0
9	EBB	0	0	0

*B- Bait (Mixture of Sugarcane, Apple & Banana); S- SPLAT-1 Lure; I- Insecticide; W- Water; EBB- Empty black bucket; ♂- Male; ♀- Female.

Table-2: The list of total number of RPW trapped SPLAT-2 various combinations of treatments with three different biological replicates.

S.No.	Treatment*	Test-1	Test-2	Test-3
1.	B + S + I	10 (3♂+7♀)	10 (4♂+6♀)	7 (2♂+5♀)
2.	B + S + W	9 (4♂+5♀)	8 (3♂+5♀)	5 (1♂+4♀)
3.	S + I	6 (2♂+4♀)	6 (3♂+3♀)	6 (2♂+4♀)
4.	S + W	5 (2♂+3♀)	6 (2♂+4♀)	3 (1♂+2♀)
5.	B + I	0	2 (♂)	1 (♀)
6.	B + W	0	1 (♀)	1 (♂)
7	I	0	0	0
8	W	0	0	0
9	EWB	0	0	0

*B- Bait (Mixture of Sugarcane, Apple & Banana); S- SPLAT-2 Lure; I- Insecticide; W- Water; EWB- Empty white bucket; ♂- Male; ♀- Female.

Figure 1: RPW Pheromone traps containing bait and Insecticide in the infested fields: A) Preparation of SPLAT mixture; B). Exposure of SPLAT in open areas of toddy fields; and C). RPW trap in SPLAT mixture after 4 weeks in the toddy fields.

Influence of food bait on SPLAT -1 & SPLAT-2 for increased efficiency of RPW trapping:

In the given study, we have initially conducted experiments with various combinations of treatments such as Bait + SPLAT + Insecticide, BSI; Bait + SPLAT + Water, BSW; SPLAT + Insecticide, SI; SPLAT + Water, SW; Bait + Insecticide, BI; Bait + Water, BW. Among all the treatments tested, we observed maximum number of RPW trapped in SPLAT-1 (# 13) and SPLAT-2 (# 10) which is statistically significant in both BSI and BSW combination when compared with others (Fig.2 & Fig. 3). Similar to our study, several other researchers have also performed experiments using SPLAT food bait studies. Our current findings suggest that presence of pheromone lure (SPLAT) along with food bait led to the entrapment of a greater number of RPW, while there were very less RPW trapped (# 2) and (# 1) in presence of only food bait. This signifies that pheromone is definitely increasing the

entrapment of RPW, but an additional supplementation of food bait along with pheromone seems to exhibit an enhancement in trapping RPW irrespective of sex.

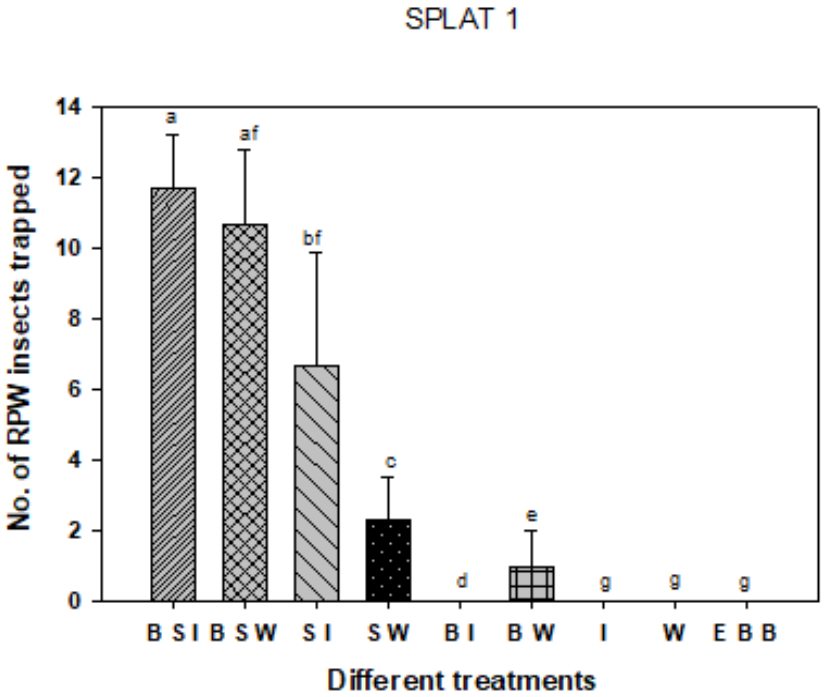

Figure-2: Mean number of adult weevils (Red Palm Weevil) captured per trap tested in three different biological replicates.

The treatment B+S+I indicate supplementation of SPLAT-1 along Bait and Insecticide. The treatment B+S+W indicate supplementation of SPLAT-1 along Bait and Water. The data presented are the mean values of triplicates performed at different locations. Bars labeled with different letters are significantly different at $P<0.050$ according to One Way ANOVA (Dunnett's Test).

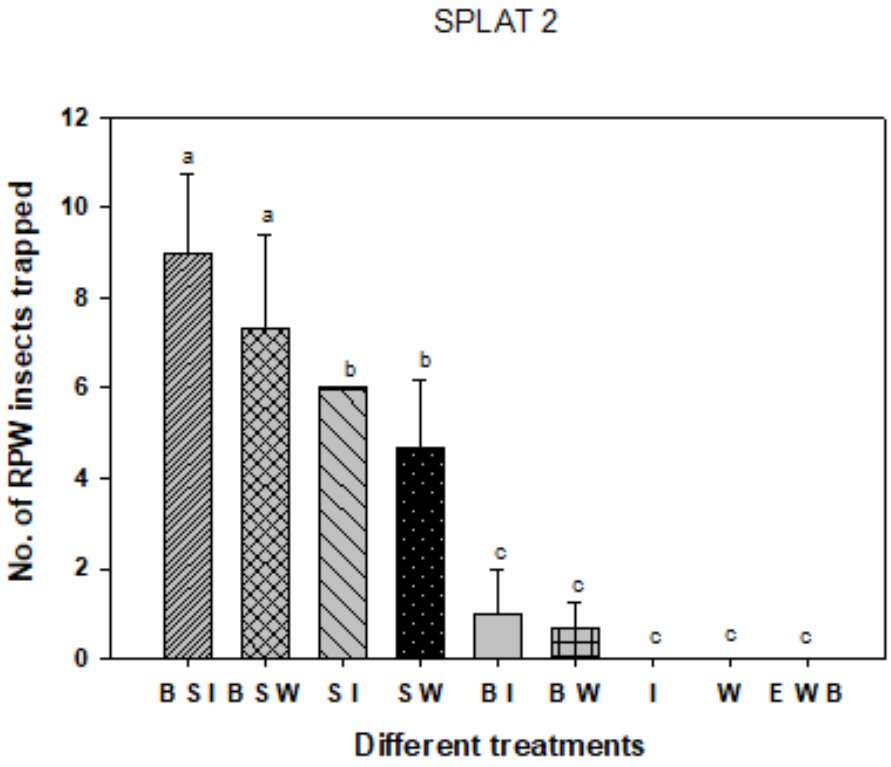

Figure-3: Mean number of adult weevils (Red Palm Weevil) captured in three different biological replicates.

The treatment B+S+I indicate supplementation of SPLAT-2 along Bait and Insecticide. The treatment B+S+W indicate supplementation of SPLAT-2 along Bait and Water. The data presented are the mean values of triplicates performed at different locations. Bars labeled with different letters are significantly different at P<0.050 according to One Way ANOVA (Dunnett's Test).

Gender specificity of SPLAT-1 and SPLAT-2 in entrapment of RPW:

In the given study, among all the treatments tested, we observed maximum number of female RPW entrapped in SPLAT-1 (# 9) and SPLAT-2 (# 7) (Fig. 4 & Fig. 5). In detail, the B+S+W combination with SPLAT-1 trapped 9 female RPW whereas the B+S+I combination with SPLAT-2 trapped 7. This signifies that both SPLAT-1 and SPLAT-2 have been predominantly entrapping females RPW rather than males by virtue of females aggregating nature.

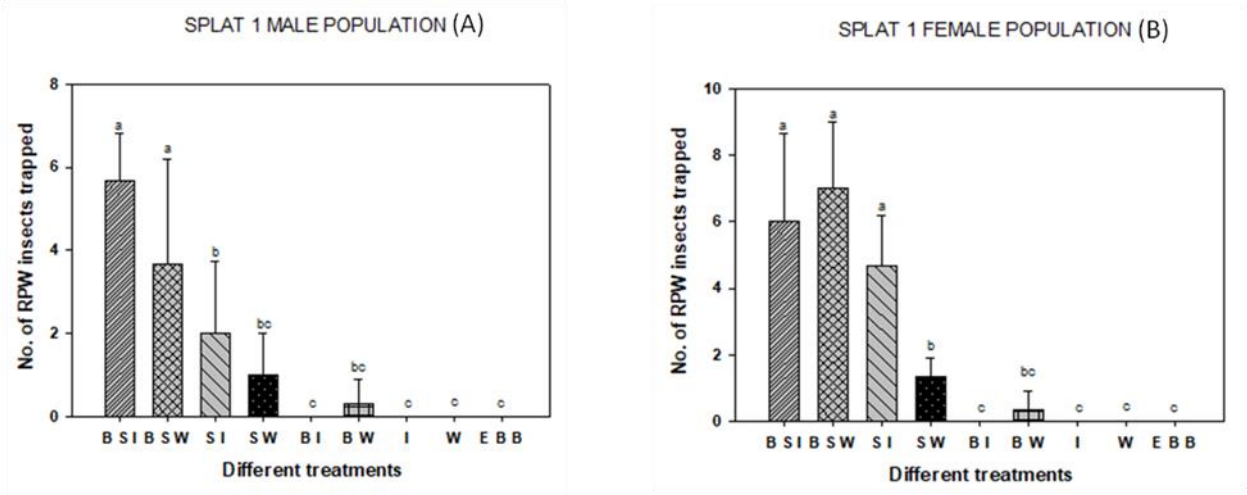

Figure-4: Mean number of adult male(A) and female(B) RPW captured in three different biological replicates.

Bars labeled with different letters indicate statistical significance (P<0.05) as per One Way ANOVA (Dunnett's Test).

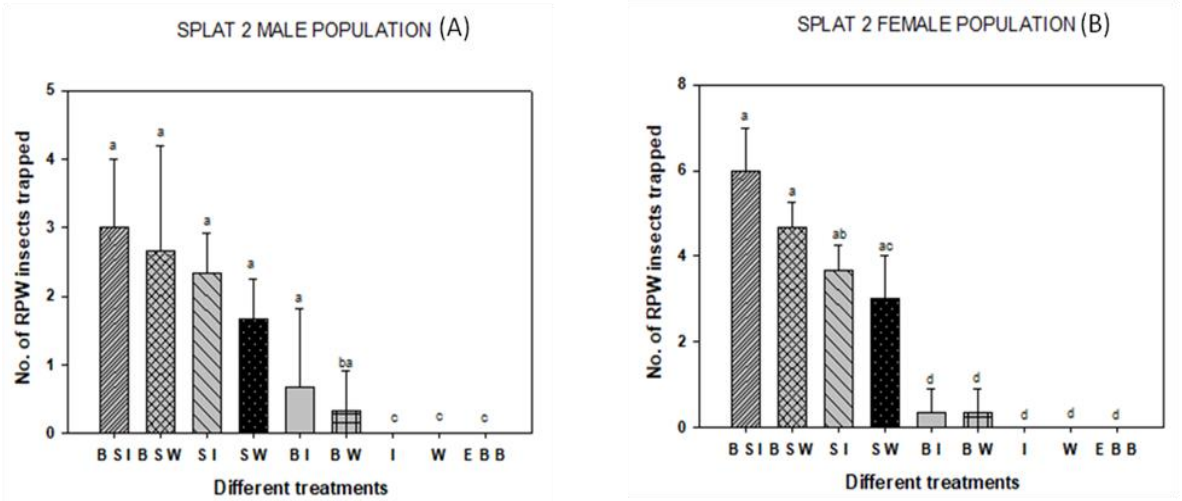

Figure-5: Mean number of adult male(A) and female(B) RPW captured in three different biological replicates.

Bars labeled with different letters indicate statistical significance (P<0.05) as per One Way ANOVA (Dunnett's Test).

For a decade, the deployment of SPLAT (Specialized Pheromone Lure Application Technology) in trapping several insects has become more pronounced. Even in our study, we have used SPLAT for trapping RPW. As per previous reports on trapping RPW, we observed a good number of insects trapped in presence of pheromones with other host plants. In our study, we have attempted to trap RPW in the geographical area where there is an existence of *Phoenix sylvestris* as predominant. Apart from this, we have attempted to improvise trapping by additional supplementation of food bait along with pheromone lure. This led to the enhanced trapping of RPW

compared to SPLAT/Food bait alone in a short time. Our results were concurrent with the previous reports on optimizing components of pheromone-baited traps for the management of red palm weevil in date palm agro-ecosystem by Faleiro, 2006.

In the given study, we have used multiple combinations of treatments involving BSI, BSW, SI, SW, BI, and BW with both SPLAT-1 and SPLAT-2. Among all tested treatments, we found SPLAT-1 with BSI exhibited significantly high efficiency (35 insects/ trap) within a period of 31 days. While the tested samples with SPLAT-2 with BSI combination resulted in entrapment of 27 insects/ trap. Similar studies were carried out by several other researchers who explained that in order to attain high weevil captures in RPW pheromone traps it is essential to adopt the best trapping protocols (Hallett *et al*., 1999; Faleiro, 2006) with respect to the trap design, trap components (lure, bait and water), trap servicing, trap density etc.

Apart from the above study, we segregated the ratio of females/males trapped from the obtained data with above respective treatments in both SPLAT-1 and SPLAT-2. It is well known that the pheromone is attractive to both sexes, several studies on traps baited with ferrugineol captured more females than males, with the average reported ratio of captured RPW being 2:1, female to male (Oehlschlager et al., 1994; Hallett et al., 1999; Abraham et al., 1999; Vidyasagar et al., 2000a, b; Faleiro et al., 2003; Soroker et al., 2005). Our findings are in line with the previous reports with a 2:1 ratio of female to male.

We further performed comparison studies between SPLAT-1 and SPLAT-2 in their efficacy to capture RPW. From our results it was quite evident that the SPLAT-1, entrapped high number of insects with more specificity compared to SPLAT-2 entrapped less number of insects with other nonspecific insects. Finally, we come to a conclusion that SPLAT-1 is more effective in presence of food bait with more specificity towards RPW compared to SPLAT-2.

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