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Studies for colour-selection of *Rhynchophorus ferrugineus* pheromone trap

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The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae/ Rhynchophoridae/Dryophthoridae), has been reported from Southern Asia as a pest of coconut for more than a century. Since mid-1980s it has expanded its geographical range westwards to reach United Arab Emirates and Saudi Arabia and afterwards spreading to rest of the Middle-East region (Murphy and Brisco, 1999; Abbas *et al*., 2006). It has caused heavy economic losses to date palm growers by mortally infesting the trees (Vidyasagar *et al*., 2000; Faleiro, 2006). In Europe, the RPW was reported as an alien pest in 1995 and continues to seriously threaten Canary Island palm (Ferry and Gomez, 2002; Haddad, 2009). More recently, *R. ferrugineus* was reported from Aruba, Curacao Islands (EPPO 2009) and subsequently from Laguna Beach, California, USA (CDFA, 2011). Among the effective control methods against RPW is the use of pheromone traps made of plastic buckets baited with food for attracting and killing the adults (Abozuhairah *et al*., 1996; Abraham *et al*., 1999). While much attention was paid to make the standard Saudi trap (Vidyasagar *et al*., 2000), a modification of plastic bucket, attractive to *R. ferrugineus* adults through improvement in design, height, food very little information is available on the role of trap colour in increasing the efficiency. But, can colours as visual cues help RPW adults orient themselves to their hosts or they are dependent on olfactory cues alone? True colour vision has only been shown for a limited number of insect species and was not shown in coleopterans (Menzel and Backhaus, 1991; Briscoe and Chittka, 2001). However, many studies suggest the role of visual cues for curculionids in locating the host plants in the field (Reddy and Raman, 2011; Tsuchimatsu *et al*., 2014; Gadi and Reddy, 2014). Recently, there is a renewed interest on the relative attraction of different colour traps to adult RPW, but there is no unanimity among the investigators. While most studies have suggested different colours, such as, green (Ajlan and Abdulsalam, 2000), brown-reddish (Sansano *et al*., 2008), red (Al-Saoud *et al*., 2010) and black (Abuagla and Al-Deeb, 2012) as the most effective trap colour in capturing RPW, Kalleshwaraswamy *et al.* (2006) reported no significant difference in colour preferences of RPW. Also, apart from ambiguity in colours, all the previously quoted work with RPW did not include any laboratory studies to ascertain attraction of RPW towards light and various colours of the visible spectrum. In the present study, an attempt was made to find out the colour/s showing maximum attraction of adult weevils. The darkness was compared with various colour-lights in choice tests, and orientation of adults to colours was tested in a multiple choice bioassay in laboratory. Six colours from across the span of visible spectrum and two neutral colours, white and black were chosen for the study. A field experiment was also conducted for all the eight colour pheromone traps

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baited with food to confirm the results of laboratory tests and ascertain the most effective trap colour/s to capture red palm weevils

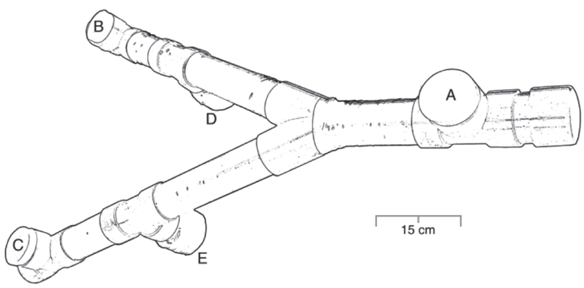

Fig. 1. The fabricated Y-tube apparatus for red palm weevil colour choice test

A Y-tube was fabricated (Fig. 1) for conducting the choice test with adult RPW. PVC pipe of 3 mm thickness and 7 cm diameter was used with joints of the same material. The test insects were introduced through the opening 'A' of the Y-tube and lid was closed. Each arm of the device was equipped with a holder for introduction of colour light source or a stopper to create darkness. On each arm a circular exit hole of 5 cm was made wherein the plastic pet bottles were screwed to collect the test insects from that arm. For every experiment, 20 healthy male and female adults (1:1 ratio) were placed inside the entry point of Y-tube. The experiment was randomized for the dark and colour arms of the Y-tube and the light intensity was kept constant in all the experiments with colour lights. The light bulbs, CFL DEMO 15W, 220V, 50/60Hz (Philips) of blue (475-495 nm), green (510-520 nm), yellow (570-580 nm), orange (590-600 nm), red (650-670 nm) and brown reddish (670-690 nm) colours were used besides white. For simulating black colour in the choice test, one arm of the Y-tube was covered to make it a dark chamber. The light intensity of all colour bulbs, was measured by the Testo 545 light meter (DM/043563), and adjusted at 300 Lux in order to expose the test insects to the same level of intensity of light of different colours. In addition, the light intensity was measured between the release point 'A' and the capture points D or E and was recorded as 19 and 300 Lux respectively. Measurement was also taken in the dark chamber of Y-tube and it showed zero Lux indicating total darkness or black. Eight replications were set up for each colour and dark, and the experiment was terminated when all insects walked in to D or E collection chambers provided in each arm of the Y-tube. Maximum time recorded for completion of the experiment was 3 hours. After the experiment the insects were taken back in to their feeding bottles in pairs and allowed to feed on sugarcane till the next experiment. At no time two experiments were conducted on the same day to eliminate any kind of bias or preference due to training.

For conducting the multiple choice test, a circular arena of 2.5 inch thick thermocol sheet was prepared with a 50 cm radius (Fig. 2). Eight equal-sized triangular colour-sheets of each of the eight tested colour were pasted on the arena to fill it completely. The six colours of the visible spectrum were arranged starting with white followed by increasing wavelength, proceeding anticlockwise,

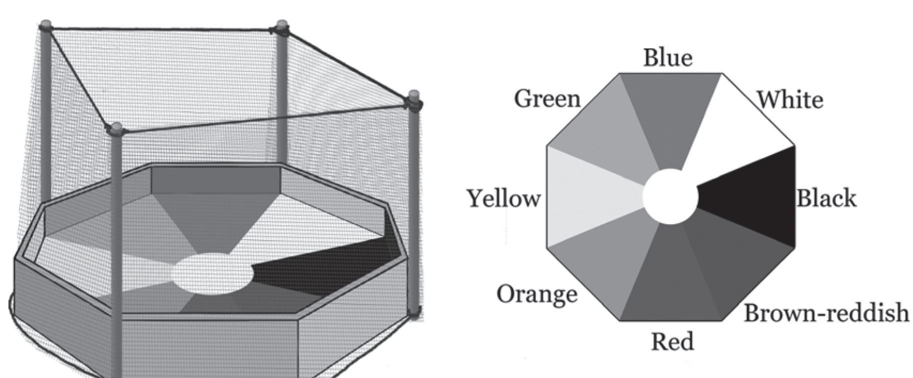

Fig. 2. An arena fabricated for multiple-choice test

and ending with black. A white paper disc of 8 cm diameter was glued in the center of this eight coloured arena. This central disc was used as the release point for adult test insects. The perimeter of the arena was encircled with a 5 inch plastic sheet and the entire arena was secured inside a wire mesh cage. Twenty male and female adult insects (1:1) were released on the white disc center and observations were taken after 30 min on the movement of adults. The number of adults clustering in each coloured area was recorded. The adult weevils which didn't move from the central disc for 30 min were assumed to prefer white colour. The experiment was replicated for eight times.

The field test was conducted in Al Mubarak date farm, located in Al Kharj, Central Province, Saudi Arabia (GPS coordinates: 24° 8' 54" North, 47° 18' 18" East). The palms in the farm were about 10 years in age with a moderate level of RPW infestation. The field design of the experiment was RBD having eight treatments of colour traps with six replicates and a total numbers of 48 traps. The traps in each replication were rotated randomly every week till the end of the experiment. The spacing between the two treatments was between 35-40 m and distance between two replications was about 80-90 m. All replications of the experiment were set up in the same orchard. The traps were prepared from the 6 L buckets of blue, green, yellow, orange, brown reddish, red, white and black colours, procured from the local market. The trap colours were measured using Chroma meter of Minolta Model CR-100 set on the L*, a*, b* system, where L* measures relative lightness, a* relative redness and b* relative yellowness (Table 1). The standard Saudi trap without wrapping was prepared by making four rectangular (2 x 4 cm) holes at about 2 inches below the top of each plastic bucket. The outer surface of the bucket was roughened to make it easy for the attracted adult weevils to crawl and enter the bucket. The Pheromone lures, *Ferrolure+* (a 9:1 mixture of 4-methyl-5-nonanol and 4-methyl-5-nonanone, with 98% purity), manufactured by Chem Tica International, Costa Rica, were used in this study. Each lure sachet with ~ 600 mg content was suspended inside the bucket from the lid. Besides lure, 40 mL plastic dispenser vial containing ethyl acetate (98%) was also suspended from inside the bucket lid. About 200 g ripe date fruits and 2 L water along with 2 g yeast and Carbaryl 10 WP @2g per trap were put in the bucket trap. The traps were tied with an iron wire to the clean trunk of date palm trees at a height of 1.3 m from the ground. Food baits, yeast, pesticide and water were changed every week throughout the experimental period of five weeks, after taking observations on the number of weevils captured and removing them from each trap.

Table 1. The surface colour values of the pheromone bucket traps as measured by chromameter

Trap colour	L*	a*	b*
Red	40.71	34.18	24.58
Green	59.66	-43.40	14.20
Blue	38.00	5.16	-37.13
White	79.28	-2.15	4.54
Orange	46.86	26.27	32.06
Black	22.13	0.11	-0.19
Brown reddish	42.28	36.54	25.52
Yellow	65.93	-5.14	62.78

L = relative lightness, a = relative redness and b* = relative yellowness, average value recorded

The data collected from the Y-tube choice test was analyzed for percentage catches in the selected source of colour with the null hypothesis that the weevil attracted equally likely to dark or colour trap, using the one-sample t-test procedure of SPSS 19.0 (IBM Corp., SPSS). Data from multiple choice and field tests were analyzed and mean values are presented. For comparing the proportion of catches in different colour traps, analysis of variance (ANOVA) was employed. Inference on paired comparisons was obtained through the Post-Hoc procedure Tukey's B Multiple Range Test.

The food baited plastic buckets with pheromone lure are used for trapping of red palm weevil for almost two decades in Saudi Arabia (Vidyasagar *et al*., 2000; Faleiro 2006). After several field experiments, a standard upright bucket trap with a wrap was developed for use in mass trapping program in Saudi Arabia (Abozuhairah *et al*., 1996). But regarding the colour of the trap, there are different opinions. While the evidence exists since very long that behavior of curculionid weevils is affected by colours, (Reddy and Raman, 2011; Gadi and Reddy, 2014; Tsuchimatsu *et al*., 2014), the most attractive colour of the trap for red palm weevil adults by providing, in addition to the olfactory, visual cues for their faster navigation to traps, is still debated. A comparison of many examples in the literature led us to believe that there could be generally two types of curculionid weevil species – the ones which show positive phototaxis and a preference for colours of lower wavelengths of visible spectrum such as violet, blue and green; (Smith and Hough-Goldstein, 2013; Chen *et al*., 2013; McQuate, 2014), and the ones which show negative phototaxis with preference for colours of higher wavelengths such as orange, brown reddish and red (Reddy *et al*., 2011; Otalora-Luna *et al*., 2013). However, this could well be over-simplification because examples of differences in colour preferences at the gender level within the species (Crook *et al*., 2009), and very recently, location (Gadi and Reddy, 2014), are known. The case of RPW behavior seems more akin to the category showing negative phototaxis and preference for dark/black (Abuagla and Al-Deeb, 2012) or colours of higher wavelengths, *viz*., brown-reddish (Sansano *et al*., 2008) and red (Al-Saoud *et al*., 2010), except one study (Ajlan and Abdulsalam, 2000) which suggests green, one of the two middle colours of the visible spectrum, for the food-baited pheromone traps. The results obtained in the current study also support the fact that the RPW adults prefer darkness and are attracted more towards colours of higher wavelengths in the right-half of the visible spectrum.

Table 2. Mean numbers of *R. ferrugineus* adults attracted by two neutral and six colours from the visible spectrum

Treatments	Mean ±SE	
	Choice Test	Multiple-Choice Test
Black*	7.88 ± 1.008 a	3.88 ± 0.295 a
Red	1.63 ± 0.324 b	2.75 ± 0.412 abc
Brown reddish	2.25 ± 0.366 b	2.63 ± 0.183 abc
Orange	2.00 ± 0.423 b	2.88 ± 0.398 ab
Yellow	1.13 ± 0.350 b	2.13 ± 0.350 bc
Green	2.00 ± 0.500 b	2.63 ± 0.324 abc
Blue	1.88 ± 0.350 b	2.38 ± 0.375 bc
White	1.25 ± 0.366 b	1.38 ± 0.183 c
F, P	18.874, < 0.0#	4.701, < 0.0#

*For Choice test, dark chamber without any light was used to simulate black colour; Means (± SE) within the columns not followed by the same letter are significantly different at the P = 0.05 level (df = 7, 56; Tukey's B[a]; and LSD test) #Significant till more than four decimal places.

The colour lights in the Y-tube laboratory choice test showed significantly less attraction by adult weevils as compared to the dark chamber in the other arm (Table 2) which clearly indicated that the *R. ferrugineus* adult weevils did not prefer the white or any other colour light sources over dark, thus showing negative phototaxis. In the multiple-choice test too, the highest orientation of *R. ferrugineus* adult weevils was observed towards black area (Table 2), and significantly more than blue, yellow and white colour areas. However, the mean numbers of adult weevils from orange, red, brown-reddish and green colour areas were not significantly different from black colour. Notably, the possibility that adult weevils may exhibit negative phototaxis due to escaping behavior for refuge was negated by the fact that the complete recovery of the test insects from each colour area / arm took as long as 3 hours, and also the results of the field test. In the field experiment (Fig. 3), results showed a trend similar to the findings of laboratory tests, as the black colour attracted highest mean number of adult weevils. Despite being the highest, the black trap-catch was not significantly different than the mean number of adult weevils caught in orange and red traps and the green, brown-reddish and yellow traps showed moderate attraction followed by least attractive blue and white coloured traps.

Therefore, the two top preferred colours after black by RPW adults include orange and red, as seen both in laboratory and field experiments. The

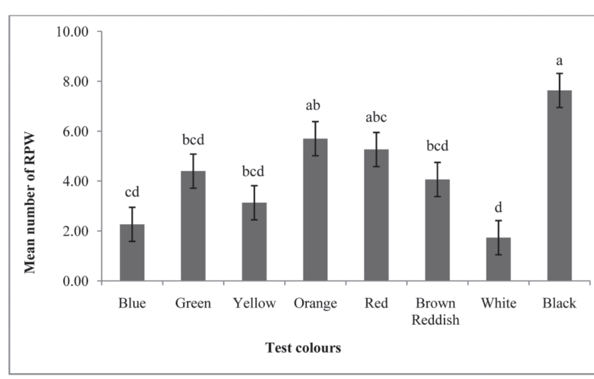

Fig. 3. Mean number of *R. ferrugineus* adults captured over the period of five weeks in different colour traps. Means (± SE) in a bar of the test colour not followed by the same letter are significantly different at the P = 0.05 level (df = 7, 232; Tukey B[a]; and LSD test).

orange colour, notably, was not highlighted by any of the previous studies. However, in conformity to the previous studies, the white, blue and yellow colours attracted least mean number of adult weevils across the laboratory and field tests. Amongst the tested colours, the black colour emerged as the ideal trap colour for maximizing the adult RPW catches in both laboratory and field tests This study brings forth the experimental evidence for higher preference of black colour by both female and male adult weevils. Therefore, though secondary to the olfactory cues, black trap-colour plays an important role in maximizing the catch. However, for implementing an effective management programme of red palm weevil, in addition to trapping, the other components of IPM including appropriate irrigation method, farm- and phyto-sanitation, chemical treatment of physically damaged parts of the tree, timely detection of infestation and curative measures are also essential.

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