

Olfactometer Response of Red Palm Weevil (RPW) (*Rhynchophorus ferrugineus*) Coleoptera: Curculionidae and its Biology for the Evaluation of Resistance in the Commercially Grown Date Palm (*Phoenix dactylifera* L.) Cultivars in Pakistan

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Abstract:

Date palms (*Phoenix dactylifera* L.) are prominent in Kingdom of Saudi Arabia (KSA), Iran, UAE, Iraq as well as Algeria, Egypt and Pakistan. The commercially grown cultivars in Pakistan are Aseel, Dhaki, Falsi, Karbalai, Mozawati, Jan Swore, Kohra, Hillawi, Kohra and Begum Jhangi. *Rhynchophorus ferrugineus* considered as hazardous insect pest responsible for damage in palm orchards. This work describes the infestation of RPW in eleven different palm cultivars (Hillawi, Mozawati, Kechanr, Aseel, Shamrani, Khudravi, Dhaki, Zeri, Kobra, Zaidi, Denda) which frequently grow in different regions of Pakistan associated through impulsive expulsion from palm leaves using Y-shaped olfactometer for research. In addition, level of both antixenosis and antibiosis was noticed by examining the various parameters such as percentage lure of weevils of mature female, general count of eggs laid in addition to their activeness. Furthermore, obtained results depicted that a positive contact was establish with minimum antixenotic effect in place of consequence revealed by a cultivar, "Hillawi" among most hold of RPW adults (22.32%), number of eggs laid (16.66%) and egg hatching (84.66%). While other cultivars including Mozawati, Aseel, Kechanr, Shamrani, Khudravi, Dhaki, Zeri and Zaidi exhibited level of antixenosis with percent weevils attracted (18.13, 17.25, 14.67, 6.17, 5.88, 5.59, 3.5 and 1.5%), no. of eggs laid (15, 14, 12.66, 10, 5.66, 4.33, 2.33 and 1.33) along with egg hatching percentages (82, 72.66, 68.33, 53.66, 37.66, 31, 13.33 and 5.66%) respectively. Moreover, "Hillawi" documented the maximum oviposition rate similar with strains like Kechanr, Mozawati and Aseel. While Kobra and Denda showed minimum attraction by RPW. Maximum RPW (6) were attracted in an olfactometer assay of sugarcane varieties.

Keywords: *Phoenix dactylifera*, *Rhynchophorus ferrugineus*, Olfactometer, Antixenosis

Introduction

Date palms are significant crops of present and past possessing high dietetic and cultural values (Lee, 1963; Abouziena *et al.*, 2010). Maximum commercial production and export of palm variety aseel was reported in Sindh Province of Pakistan (FAO, 2021; Soomro, M.H. *et al.* 2022). Around 300 distinct cultivars of date palms which are cultivated on commercial scales are: Falsi, Aseel, Begum jhangi, Karbalain, Mozawati, Dhaki, Jan swore, Hillawi, Dashtiari, Kohra and Rabai. In Saudi Arabia approximately 400 distinct date palm cultivars are grown on commercially, from these there are two main cultivars (Sheshi and Reziz) which are mainly superior (Arabia, 2006; Ata, 2011; Ashraf & Hamidi-Esfahani, 2011; Al-Abdoulhadi *et al.*, 2011). Furthermore, in Tunisia, farming of such date palms is generally carried out in the southern states where the climatic environments are highly appropriate for the production of date varieties (dessert inhibited) like Deglet Nour which encompass 60% of overall production of palm in the nation state as documented by Triki *et al.* (2003). In

the Middle East, Red Palm Weevil (RPW) is supposed to be "Pakistani weevil" on the base of its desired origin from Pakistan. However, controversy in the RPW status of origin still exists that either its native or invasive to Pakistan (Yasin *et al.*, 2016; Muhammad *et al.*, 2020; Manzoor *et al.*, 2020; 2022). In case of resistance, three substantial phenomenon in insect pests are presented which include antixenosis, antibiosis as well as tolerance (Smith, 2005). These joint strengthening resistant modalities may encompass antixenosis plus antibiosis hence; resistance mechanism of antibiosis insolence arthropodes biotypes might be influenced in contradiction of antixenosis after plant colonization (Hesler & Dashiell 2011). Whereas, in case of United State of America, episodic manifestations of soybean linked with aphids via solicitation of insecticides which lengthen the resistance progression in case of host plant relations which have a key role in pivot of defensible pest management system (Rutledge *et al.*, 2004; Wiseman, 1999; Panda & Khush 1995). On another

hand, tolerance is a plants protective response to safe itself from injury by arthropods or due to invasive body. However, antibiosis badly disturbs the persistence, reproduction as well as arthropods development to avoid them from settling host plant. The studies of Barranco *et al.* (2000) and Dembilio *et al.* (2010) examined the feedback for process of antixenosis along with antibiosis similar to *Chamaerops humilis L.* and *Washingtonia filifera* in contradiction to RPW. Various factors were discussed in this research in relation to imitation as well as development of weevil responsible for destruction of palm in several palms cultivars exposing that both *W. filifera* and *P. canariensis* are highly parishioner vegetation (Ju *et al.*, 2011). Research was also performed to analyze the resistance of distinct palm cultivars in relation to palm weevil and it was applied to various significant varieties of palm in the Qatar such as Shahal, Khalas, Sheshi, Reziz, and Khasab (Al-Dous *et al.*, 2011). Currently, the key sources for development of palms cultivars are seedlings, as there is possibility of being resilient or susceptible established from two contrast progenies getting aid from multiple resistance gene (Johnson *et al.*, 2013). The palm cultivars which possess high sugar level are most preferential in case of egg placement and oviposition by *R. ferrugineus* on the other hand calcium delayed the development and growth of RPW (Farazmand, 2002). Faleiro *et al.* (2006) and Abraham *et al.* (1998) defined those tissues of palm plantations are important components which perform a key role for appealing mature red palm weevils through the discharge of volatiles and eggs preferably laid on damaged areas of palm plant which help in providing better nourishment for initial premature stages, resulting severe deterioration in palms trees. Several evidences suggest that date palms are attacked by RPW and no survey previously has been conducted to investigate RPW infestation on commercially grown date palm cultivars in Pakistan. The subsequent research effort was carried out for the assessment linked with resistance mechanism in eleven cultivars of palm which are being cultivated commercially in Pakistan. It is expected that this research work will enhance our understanding about the olfactometer based behavioral response of RPW towards commercially grown date palms in Pakistan.

Materials and Methods

Insect samples used for analysis: Food baited pheromones traps were used to collect the adult beetles along with hand picking. The pheromones of Ferrulure™ trade label was used. Then, adult plus larvae were nurtured on synthetic food in IGDCB laboratory, PARS campus, UAF. Fresh sugarcane slices were provided them as a food while plastic cages used for reconditioning with relative humidity ($76 \pm 4\%$) and temperature (28 ± 2 °C). Rearing was done in the Integrated Genomics Developmental Cellular and

Biotechnology Laboratory (IGDCB), Postgraduate Agricultural Research Station (PARS), University of Agriculture, Faisalabad (UAF). Two instars of *R. ferrugineus* were selected (2nd and 3rd) having weight of 0.1 g and they were used for the Laboratory trials.

Study based on Olfactometer (Y-shaped): A glass made olfactometer having arm of 14 cm length, stem length and internal diameter was 16 cm and 2 cm respectively was used. A black cage fitted on a tripod in a steel frame with dimensions (676 cm x 60cm x 660 cm) where it was fasten. Black card board paper along with 2 florescent bulbs (Luminux; 70W) was used to cover it in an inclined position approximately 25 cm above the intersection. Two electric air pumps were employed which maintained the air flow to remove chemical contamination adjusted lean with the olfactometer. Chemical contaminants were removed by the using charcoal. Two flowmeters were adjusted in such a way to keep the flow rate of air same in both sides. On the way to olfactometer, filtration in the bottles was done by employing filter paper where both treatment as well as control samples were used. RPW were placed in aspirators with 2 hours fasting to get rid of distiction in the rearing cages for each test. Ten test insects were used for each replication and all experiments were further replicated ten times. In order to remove the position effect entire apparatus was set to 180° rotation. Ten minutes stay was allowed for each test insect to decide relative movement either right or left. Entire bioassay studies were done in the dark environment which had extraction fan with relative humidity 40 % as well as 2261 µC. For present bioassays date palm cultivars were collected from commercially cultivated gardens with owner's permission. The varieties under studies were Khudravi, Mozawati, Hillawi, Shamrani, Kechanr, Dhaki, Aseel, Zeri, Kobra, Denda and Zaidi. From date palm orchards exposed seedlings were collected and small pieces were obtained after cutting which provided a large area for volatiles emissions. Newly growing fronds and leaves were taken together at similar time and at the same point from these cultivars ie. 0 to 3 hours, 3 to 7 hours, 7 to 20 hours, 20 to 30 hours, 30 to 45 hours, 45 to 55 hours, 55 to 70 hours of outbreak of these palm weevils.

Attraction to date Palm Tissue cultivars: Above mentioned varieties were used for olfactometer based assays of emitted volatiles in the fresh palms tissues. In each side of the olfactometer, freshly cut palm pieces were kept for behavioral study of the red palm weevils while in case of cultivar comparisons made, two different cultivars pieces (1 x 1 x 5 cm) were placed in both sides in each Inlet Odor Source (IOS). Two experiments consisting of 11 cultivars were made for the analysis of freshly emitted volatiles from 'Hillawi'- 'Kechanr'- 'Mozawati'- 'Zeri'- 'Aseel'- 'Khudravi'- 'Shamrani'- 'Kobra'- 'Dhaki'- 'Zaidi' as well as 'Denda' whereas the

later both cultivars (Kobra and Denda) were utilized as control due to zero attraction and infestation level made. Fifteen days old gravid female adults were collected from previously maintained population in Integrated Genomics Cellular Developmental and Biotechnology Laboratory, University of Agriculture, Faisalabad and they were. In the insect release device of the Olfactometer (Fig. 1), only 5 insects were kept for analysis. After short period of time (5 minutes), insects were collected from insects isolation trap inclined to the opposite side of the olfactometer and counted. Time

elapse between putting the cultivar samples and 5 weevils was only 1 minute. Time between two replications was 10 minute and 8 replications were done for the whole experiment. Both samples (cultivars and insects) were discarded after each replication and new palm tissues and insects were used for the next replication assays. Inlet odor source was removed sequentially for each replication creating the possibility for each treatment on the same arm in each trial for minimizing the biasness created by the instrument or even by the environment.

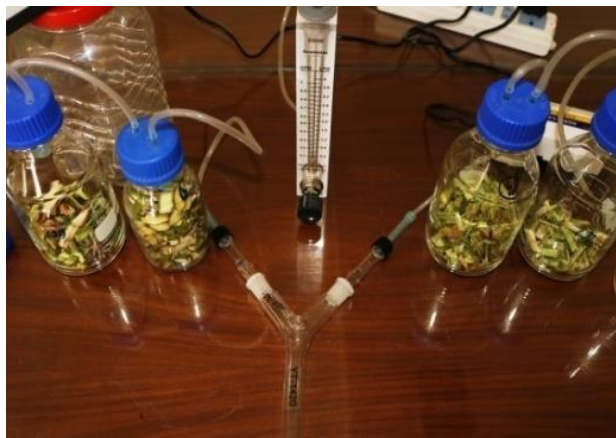


Fig.1. Red palm weevil preview of nurturing at the IGCD laboratory for inspecting their developmental phases

Egg Laying and Hatching Capability of RPW: In a firm cylinder (2cm x 5cm) new green colored fronds were set. Gravid fertile females from reared pool were released on these fronds for egg laying purpose. In addition two male weevils along with other female weevils were added in the same insect boxes with dimensions (35cm x 40cm x 60cm) and check it after a 1 week. Egg laying as well as hatched larvae were recorded as depicted in Fig.2. With the help of fine camel hair brush eggs were taken out from the petiole fiber and placed on the Whatman paper slant to plastic dish with dimension (6 x 1.5 cm). To ensure egg hatching these plates were placed under control conditions. For the oviposition trials six replications were done and for egg hatching 5 replications were recorded.

Several attractants employed in olfactometer to inspect behavioral feedback of RPW

RPW attraction towards pheromones, sugarcane varieties and ethyl acetate: Two distinct pheromones namely ferrugineone and ferrugineol were used alone as well as in amalgamation with other chemical compounds i.e., ethyl acetate, dates and sugarcane (*S. officinarum*) in this behavioral investigation by olfactometer. Bottles of olfactometer on both sides were filled with fresh sliced leaves (100 g) of palms. In combination treatment 50 g of ragged sugarcane was mixed approximately. Hillawi

plus Mozawati varieties were used in this investigation. Each treatment used separately in which a single female weevil permitted to move in Y shaped olfactometer and time was recorded. Before performing experiment female weevils were set for fasting for 2 hours in order to reduce differences. For aeration charcoal was placed on both arms of olfactometer to keep the sides aerated.

Statistical analysis: Standard dispersal was confirmed by analysis of data by t-test obtained from olfactometer. Log altered the whole data which was obtained from seedlings and volatile reactions of palm cultivars. Split pot design used for comparing the key effect of treatments, their groupings and time plus relation between couple of factors. Means were separated using Tucky's and LSD test at $p = 0.05$ with 95% confidence interval. Information obtained from collective effects of cultivars while examination of control treatments was studied through paired t-test. Statistical investigations were undertaken through GenStat statistical system (VSN-10th edition Int. limited, Hemel, Hemp. UK).

Results

The consequences attained by means of olfactometer checkup have been exhibited in Fig.2. on percentage incursion of palm weevil in contrast to various palm cultivars which are commercially cultivated in Pakistan and representing that mature feminine weevils were noticeably appealed largely toward Hillawi ("P =

0.017"; "F = 4.38") with %age RPW appealing of 22.33%. In other case, Zeri cultivar has reduced %age attraction of RPW (1.5%) but treatments employed in term of control had weevil's absence revealing high grade antixenosis. However consequences having connection with the egg laying displays Kechanr (14), Mozawati (15) and Hillawi (16.66) with maximum capacity of egg laying, but two cultivars where no egg laying were observed are Kobra and Denda. Moreover, there was a less number of egg laying noted on another

palm cultivar Zeri (1.33). In case of Kechanr, Hillawi, Mozawati, least grade of antixenosis was exhibited but in another case maximum antixenosis was reported in both of the cultivars including Kobra and Denda. Out of these aforesaid cultivars, Hillawi was the highly favored variety in bio-assays that were performed via olfactometer for tissue volatiles as well as this mentioned cultivar was recorded as extremely susceptible variety.

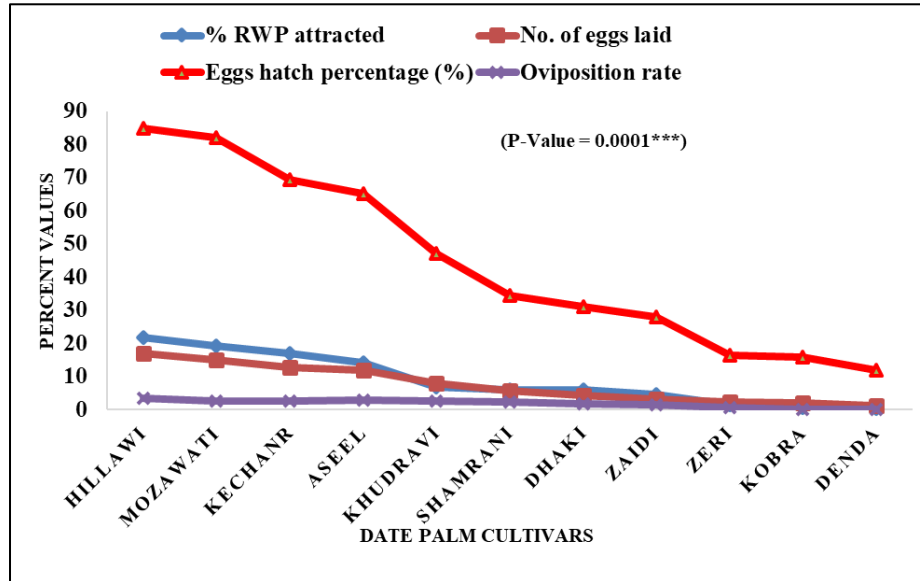


Fig.2: *Rhynchophorus ferrugineus* (RPW) attraction, Number of eggs laid, eggs hatch percentage and ovipositional rate of female RPW toward various commercially cultivated cultivars of date palms (*P. dactylifera* L.).

The emergence of immature weevil from eggs was also founded on resilient as well as vulnerable palm varieties. Maximum egg setting was accomplished via RPW on three cultivars including Kechanr, Hillawi plus Mozawati with percentage of 72.6, 84.6 and 82% correspondingly via statistical means were same (F value=2.40, P value<0.0001) as exhibited in the Fig.3. Minimum oviposition rate and hatching of eggs was noted on two cultivars Kobra and Denda which were retained as control. Highest oviposition rate was found on Hillawi followed by Mozawati cultivar of date palm. Our results indicate that significant function included in appealing of this weevil against several palm cultivars perform its key role in the first desirability of RPW in case of palms varieties is persuaded to mechanism of antixenosis with the magnitude of oviposition via egg hatchability. The

volatiles associated with tissues of various cultivars such as Kechanr, Hillawi and Mozawati resulted lot of weevil attraction, consequent range of egg laying as well as egg hatchability. Nonexistence of antibiosis in aforementioned cultivars of date palms including Kechanr, Hillawi and Mozawati lessened their capability to provide protection counter to damage as well as infection that take place because of RPW later the oviposition. Means of the difference was calculated via graphpad prism (Version 9.4.1) as shown in the fig. 4. A significant correlation was found in all treatments with 95% confidence interval. A significant pearson correlation was also calculated between No. of weevils attracted and Average time/seconds for RPW attraction as shown in fig. 5.

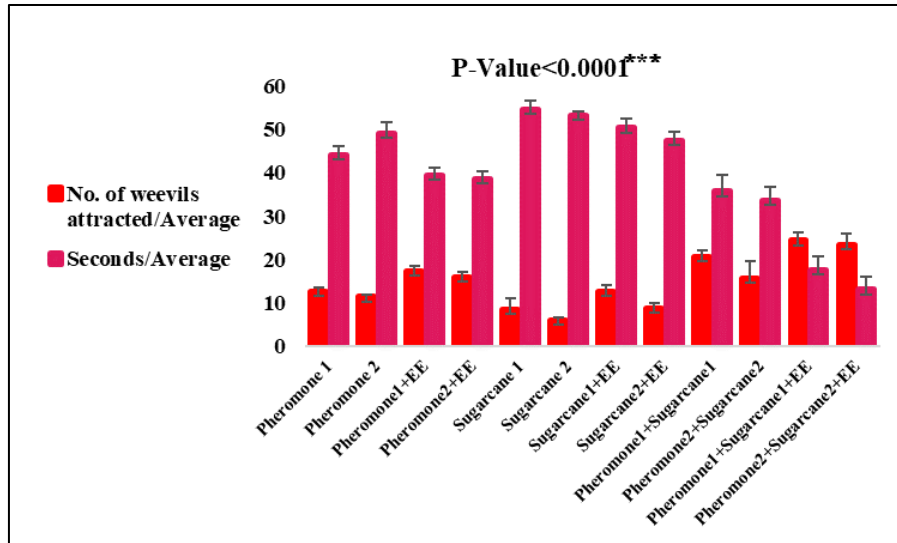


Fig.3: Several attractants employed in olfactometer to inspect behavioral feedback of RPW. Bars exhibits standard error. (Dates 1= “Hillawi”-, Dates 2= “Mozawati”-, Pheromones 1= “Ferrugineol”-, Pheromone 2= “Ferrugeneone”-, EE = “Ethyle acetate”)

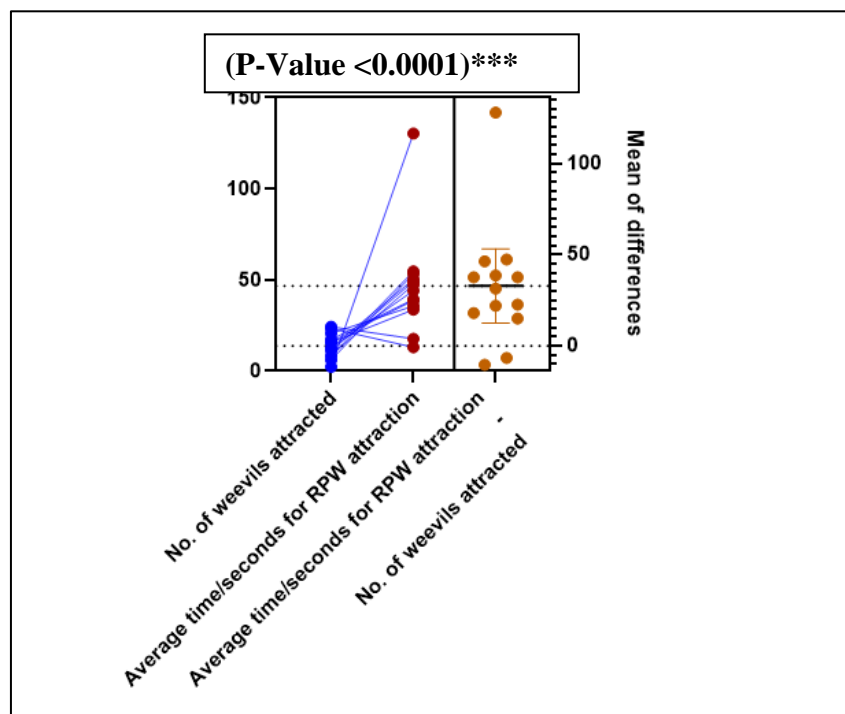


Fig.4. Estimation plot: paired t-test of correlations between Number. of weevils attracted and Average time/seconds for RPW attraction

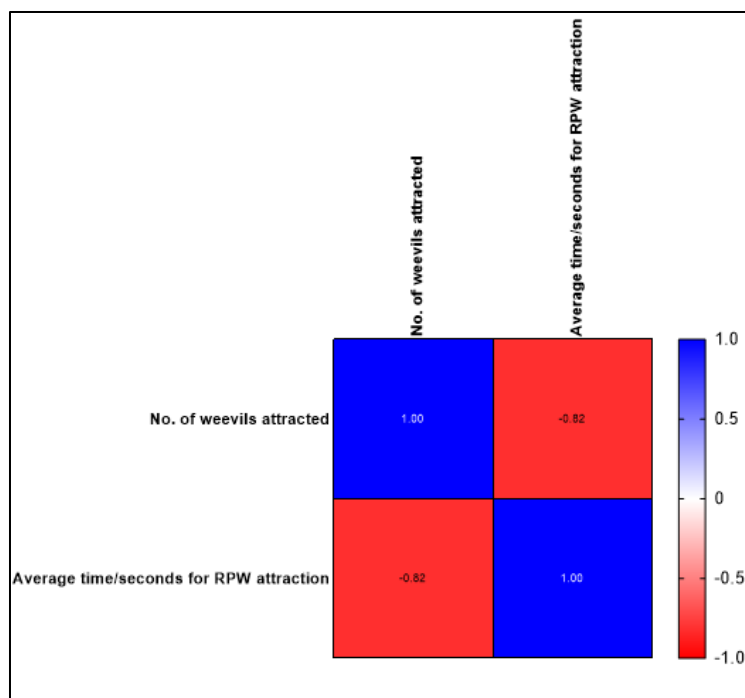


Fig.5. Pearson correlation (r) between Numbers of weevils attracted and average time/seconds for weevils attraction

RPW feedback in distinction with numerous combinations of sugarcane-, dates-, pheromones, and additionally blends of ethyle acetate were employed for behavioral scrutiny to get knowledge about the mechanisms responsible for resistance in cultivars concerning time factor. Extended stay duration (8 minutes) of this weevil was observed when dates palms combination of both Hillawi and Mozawati was used with sugarcane, pheromones as well as EE (Ethyle acetate) while this dates combination with pheromones plus sugarcane excluding the ethyle acetate presented the weevil stay duration of 4.5 minutes. Cultivars date 1 and 2 were Hillawi and Mozawati while Pheromone 1 and 2 were ferrugineol and ferrugineone respectively. Moreover, minimum time length (less than 1 minute) for the weevil stay was noted while pheromones trials were performed separately.

Discussion

Usually, the tissue attractants linked to various varieties of date palm such as Hillawi, Mozawati as well as Kechanr were extremely appealing to red palm weevil. Hillawi and Mozawati combination along with pheromones like ferrugineol, ferrugineone, sugarcane and ethyle acetate depicted strong to palm weevil allowed them to stay here for long on the olfactometer. Our research findings are closely related to the previously work done by Riolo *et al.* (2017) in which he analyzed the behavior of *Hyalesthes obsoletus* by using Y-shaped olfactometer and spotted that the male insects

preferably moved to methyl silicate, (E)-beta-farnesene, (E)-beta-caryophyllene, 1, 8-cineole signals whereas maximum female insects moved towards the mixture when it possess methyl benzoate, similarly, more weevils were attracted when we used mixture of dates, pheromones, sugars and ethyle acetate. A research work on saproxylic beetles was just similar to present studies in which volatile organic compounds (VOCs) of oak species (*Quercus pyrenaica*) were found to attract them was also discussed by Ramilo *et al.* (2017). Present research results support the previous research work done by Muhammad, N. & S.A. Khan (2022) in which the genotype KS-75 showed antixenotic effect against aphids (*L. erysimi*). Contrarily, oviposition behavior of larvae of Diamond back moth (*Plutella xylostella*) was not affected by the volatiles such as 0.01% in a triethyl citrate and *Brassica rapa* var. *perviridis* (Uefune *et al.*, 2017) while in our results show maximum weevils attracted to Hillawi (22.32%), number of eggs laid (16.66%) and eggs hatched (84.66%). High grade of egg setting was remarked for “Shahal” out of 25 cultivar where maximum egg laying potential found along least potential of antixenosis (Al-Shawaf *et al.*, 2013). A work similar to resistance mechanism in RPW that was observed in sorghum varieties against sorghum stem borer (*Chilo partellus*) was performed using similar protocols (Sharma *et al.*, 1992). A number of researches have been done in this field depicting the resistance against insects pests presented by different scientists in various varieties

of turf grass in USA while there is no clear evidence associated to resistance in the turf grasses, entire process of tolerance, antixenosis and antibiosis. Along with this a collective information enable us to reform the basics for sustainable resistant management (Baker *et al.*, 1981; Reinert, 1982; Ahmad *et al.*, 1986; Johnson-Cicalese *et al.*, 1989; Braman *et al.*, 1994; Reinert & Busey, 2001; Heng-Moss *et al.*, 2002; Gulsen *et al.* 2004; Rangasamy *et al.*, 2006; Eickhoff *et al.*, 2007). Research work has been conducted in Spain to treasure various mechanisms related to resistance in case of red palm weevil in several species of palm plantations and resulted that both *C. humilis* and *Washingtonia filifera* possess potential of antixenotic and antibiotic resistance while considered the *Phoenix canariensis* as best host for weevil *Rhynchophorus ferrugineus* (Dembilio *et al.*, 2009). Red palm weevil Rejoinder was reflected in China where *P. sylvestris* regarded as the most susceptible palm cultivar to red palm weevil depicting high potential to RPW as compare to *W. Filifera* and *P. canariensis*, presented less count of larvae resulting in maximum number of adult structures (Ju *et al.*, 2011). On the attack of RPW date palm plant tissues become harden and coagulate that usually get infection in age of about 20 year. As a result, such date palm cultivars inhibits spread of tarnished palm weevil (Abraham *et al.*, 1998; Faleiro, 2006). These weevils species causing infection in date plants are responsible for infection in various date palm cultivars in Saudi Arabia considered RPW life that supposed to be very high as in variety “Khalas” whereas distinctive conditions of Sukary as well as in Sillaj the life duration found to be least (Al-Ayedh, 2008) and these results from different studies are in concurrence with our findings. Experimentations were carried out on several cultivars of coconut for the purpose of investigating the mechanisms of preference plus non-preference in case of coconut cultivar “Chowghat dwarf green” as well as “Malayan dwarf” with high degree of egg laying on the cultivar designated as Chowghat dwarf green (Faleiro & Uanekar 2007). Date plants due to high sugar values are the preferred host of RPW because they lay eggs in large number in abovementioned varieties and consume these sugars in their growth. On another hand, calcium obstructs the RPW development (Farazmand, 2002). Both the practices including pruning and thinning experienced in the orchards of palms plantations result injuries in the date’s trees which are the major sites for RPW infection (Abraham *et al.*, 1998). To highlight the genes responsible for defence and protection mechanism in date plants because of its perennial and heterozygote nature. While research is continuous to colon entire genome of cultivar “Khalas” (Al-Dous *et al.*, 2011; Ma *et al.*, 2016). Genetic engineering can help to produce and analyze traits resistance, yield and quality (Faleiro *et al.*, 2014). RPW outbreak is associated with sugar content of date palm varieties which is linked with sugar

metabolic genes that are frequently supplemented in the chromosomal regions where minimum of nucleotide polymorphism occurs (Al-Mssallem *et al.*, 2013). Furthermore, in the Al-Ahsa zone of Saudi Arabia frequently grown cultivar is “Khalas”, much vulnerable RPW (El-Sabea *et al.*, 2009; Sallam *et al.*, 2012). The possible solution to mitigate the losses caused by the notorious pest will be the deployment and cultivation of commercially available resistant cultivars.

Conclusion:

Feeding preference and biology of field collected Red Palm Weevils (*R. ferrugineus*) was evaluated against different palm cultivars which indicates that Hillawi, Mozawati and Kechanr showed maximum attractions of red palm weevils while Denda, Kobra, Zeri and Zahidi varieties showed minimum attraction of weevils. In an olfactometer, maximum attraction of weevils was observed in sugarcane varieties in minimum attraction time followed by pheromones (ferrugineoneTM and ferrugineolTM) based attraction. It is concluded that resistant date palm varieties with less stress metabolites should be recommended in highly RPW infested areas. However, a detailed profiling is necessary for all commercially available date palm cultivars in Pakistan for the detection and evaluation of insect repellents.

Authors’ Contribution

All the authors have equal contribution in this manuscript

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Conflict of Interest

The authors have no potential conflict of interest.

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