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Chemical and Mechanical Properties of Palm Hearts from three Iranian Date Palm Cultivars Affect Physiological Responses of Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier, 1790) (Coleoptera: Dryophthoridae)

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ABSTRACT

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Dryophthoridae) is a serious insect pest of date palm trees worldwide. In this study, we aimed to assess the energy reserves and antioxidative defense response of *R. ferrugineus* larvae on palm hearts of three date palm cultivars (Piarom, Mazafati, and Kalute). Furthermore, the specific biochemical and mechanical properties of the palm hearts were evaluated. The insect was reared in a growth chamber at 29 ± 2°C, 80 ± 5% RH, and a photoperiod of 12:12 (L:D) and the experiments were conducted using the seventh instar larvae. The results revealed that the highest contents of sugar and lipid in *R. ferrugineus* larvae were on Piarom and Mazafati palm hearts, respectively. The antioxidant enzymes activities in *R. ferrugineus* larvae were significantly different on the tested palm hearts. According to the mechanical analysis, the Mazafati palm heart had the lowest tissue hardness. Biochemical analysis revealed that the total sugar content was high in Mazafati and Kalute palm hearts, and low in Piarom palm heart. The concentrations of phenol and flavonoid were also different in the tested palm hearts. Moreover, gas chromatography-mass spectrometry (GC-MS) profiling showed that alkaloids, terpenes, acids, and amides were the major volatile components of the tested palm hearts. Based on the results, Piarom showed traits associated with reduced larval performance, suggesting its potential use in integrated pest management (IPM) programs.

Keywords: Antioxidant enzymes; mechanical traits; phytochemicals; red palm weevil; energy reserves.

INTRODUCTION

Palm heart, often called heart-of-palm or palmito, is the palm tree stem cells, located near the apical meristem of tree (Mostafa, 2024). It is conventionally harvested from the inner core of date

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palm trees, *Phoenix dactylifera* L. (Arecales: Arecaceae), or gathered from a cluster of shoots developing from the main branch. This edible white cylindrical substance can be eaten raw or cooked in salads and various recipes (Quast and Bernhardt, 1978; Bovi *et al*., 2001). Palm heart with its tender texture and delicate flavor is a good source of carbohydrates, lipid, protein, vitamins, minerals, and dietary fiber not only for humans, but also for pests (Quast and Bernhardt, 1978). Various species of palm trees, particularly the date palm, are globally under severe threats by the attack of *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Dryophthoridae) or commonly known red palm weevil (RPW), which is considered as the most destructive pest of palm trees (Kubar *et al*., 2017; Wang *et al*., 2021).

The pest usually attacks young palms (generally less than 20 years old) because of their soft and succulent tissues. Although adults may partially feed on stem, leaves, and flowers, but the most damaging life stage of the pest is larvae. The female of RPW uses its rostrum to drill a hole and then, lay eggs in it. While feeding on the internal tissues, ideally the palm heart, the larvae burrow into the trunks of palm trees and create sizable tunnels within it (Kubar *et al*., 2017; Manzoor *et al*., 2022), leading to the destruction of the palm trunk's vascular system.

A part of sustainable integrated management (IPM) programs to control *R. ferrugineus* outbreak is using resistant palm tree cultivars, an eco-friendly and long-lasting tool, which are extensively used in numerous countries of the world (Faleiro *et al*., 2014; Kubar *et al*., 2017). More than 3000 cultivars of date palm are known to exist all around the globe (Alrashidi *et al*., 2023). These cultivars have various morphological, chemical, and nutritional properties (Trabzuni *et al*., 2014), which their susceptibility or resistance to insect pests, particularly RPW is often linked to these traits (Manzoor *et al*., 2022). Faleiro *et al.* (2014) reported that among seven different date palm cultivars, Gaar, Khasab, and Shahal cultivars were poorly prone to attack by *R. ferrugineus*. According to Manzoor *et al.* (2022) assessment of *R. ferrugineus's* feeding preferences across 11 different date palm cultivars, the pest heavily infested the Hillawi cultivar. Insect larvae may choose the palm heart as a feeding location due to its soft and delicate tissues and nutritional value (Trabzuni *et al.*, 2014). Nearly, all plants usually use complicated strategies to combat pests (Al-Khayri *et al*., 2023). A phytochemicals variety, i.e. secondary metabolites are involved in their defense against insect pests. These phytochemicals may have repellent or deterrent effects on phytophagous insects, interfering with their physiological and nutritional performances (Bennett and Wallsgrove, 1994). During these interactions, the ingestion of the toxic phytochemicals by insects may induce toxic reactive oxygen species (ROS), which the associated oxidative stress in insect's cells targets biomolecules, leading to the damage of lipids, proteins, and DNA (Mythri *et al*., 2013). Investigating the biochemical profile of three Saudi date palm cultivars, Trabzuni *et al*. (2014) reported that their palm hearts were rich in antioxidants. Sahito *et al.* (2017) noted that the vulnerability of Aseel variety to *R. ferrugineus* was due to its soft trunk fiber and high nutritional value. Other researchers showed that plant defense metabolites increased oxidative stress in plant feeding insects (Kaur *et al*., 2017).

Considering the significance of adopting resistant cultivars for IPM programs, the current research was carried out to examine how the nutritional profile and antioxidant enzyme activities of *R. ferrugineus* larvae may alter while feeding on the palm hearts of various date palm cultivars. Furthermore, we wanted to know whether the mechanical and biochemical properties of palm hearts were different in the examined date palm cultivars. The obtained results may aid to identify the physiological response of *R. ferrugineus* larvae to cope with palm heart phytochemicals, which could be used in the development of sustainable control approaches against the pest in date palm orchards.

MATERIALS AND METHODS

Insect rearing

Rhynchophorus ferrugineus was reared in a growth chamber at 29 ± 2°C, 80 ± 5% RH, and a 12:12 (L:D) photoperiod at the Agricultural Research Center of Jiroft, Kerman province, Iran. Adults were collected from date palm orchards in Jiroft County (570637˚N, 3152288˚E) and placed in plastic boxes containing a cotton ball saturated with 10 % (w/v) bee pollen solution. Eggs were collected and incubated in Petri dishes (8 cm diameter, 2.5 cm height), lined with moist filter papers. Then newly hatched larvae (30 larvae per each cultivar) were maintained in plastic container (10 cm diameter, 20 cm height) containing fresh grated-palm heart (100 g) of each date palm cultivar as substrate. The food was provided daily. The palm hearts were grated to facilitate the first instar larvae feeding. After reaching to the second instar, the larvae were individually transferred on the newly cut young shoots (one larva per one shoot) (each having a palm heart surrounded with young leaves) from each date palm cultivar. At the seventh instar, as the most damaging larval instar, they were used in experiments for evaluating their antioxidant enzymes activities and energy resources.

Preparation of palm heart samples

Palm hearts of three commercially important date palm tree cultivars in Iran, including Piarom, Mazafati, and Kalute were randomly obtained from date palm orchards in Karimabad-e Sofla (28˚ 29′ 07″ N, 57˚ 42′ 58″ E) (Jiroft County, Kerman Province, Iran). The selected cultivars are among the most widely grown date palm cultivars in date-producing areas in Iran (Yektankhodaee et al., 2010) as well as the most attacked ones by RPW based on our observation in Kerman Province. They were harvested from the same-aged date palm trees (each cultivar) then, carefully peeled and freed from other plant materials. The fresh palm hearts of each studied cultivar were used for further mechanical analysis. For biochemical analysis of fresh palm hearts; they were chopped into small pieces (100-200 g) and dried in shade, in a ventilated room at room temperature. The dried samples were completely powdered using an electric grinder and stored (4˚C) in sealed containers for further use.

Determination of energy reserves of *R. ferrugineus* larvae

Sugar content

The samples were prepared as described by Foray *et al*. (2012). The larvae (3 larvae) were homogenized using a glass homogenizer. Homogeneity was centrifuged at 5000 g for 15 minutes at 2°C in a refrigerated centrifuge. After centrifugation, the precipitate (containing sugars) was collected and oven-dried at 90-110˚C. The obtained condensed sample (precipitate) was reacted with anthrone reagent. The reagent was prepared by dissolving 75 mg anthrone in 150 mL distilled water and then, mixed with 380 mL H_2SO_4. After increasing the volume of the mixture to 5 mL, it was added to the condensed samples. The mixture was incubated for 17 min at 90-110˚C. The amount of sugar was recorded at 625 nm by spectrophotometer (S2100SUV, UNICO, USA). Estimation of sugar content was carried out in three replicates.

Lipid content

The lipid content of *R. ferrugineus* larvae was quantified following the method described by Foray *et al*. (2012). First, the supernatant obtained by centrifugation of samples (3 larvae); was collected and diluted with distilled water (2 mL). After that, the mixture was re-centrifuged at 3000 *g* for 1 min. The precipitate thus obtained was oven-dried at 90-110ºC. Then, H_2SO_4 (98 %, 0.2 mL) was added to the lipid sample and incubated at 90-110ºC for 10 min. Finally, vanillin reagent

(60 mg of vanillin solution dissolved in 10 mL hot water and kept in dark) was added to the mixture. The absorbance was read at 530 nm (S2100SUV, UNICO, USA). Measurements were technically done in three replicates.

Protein content

Protein concentration of RPW larvae was assayed in three replicates, according to Greenfield (2018) method. After homogenization of samples (3 larvae) with a glass homogenizer and following their centrifugation, the Bradford reagent (190 µL) was added to the supernatant (10 µL). The absorbance was recorded at 630 nm (S2100SUV, UNICO, USA).

Determination of antioxidant activity of *R. ferrugineus* larvae

Extraction of enzymes

For preparation of enzymes extract from each cultivar, 10 larvae previously fed on the related date palm cultivars were randomly collected. Then, the larvae were homogenized in phosphate buffer (10 mM, pH 7.5, 200 µL) using a homogenizer. The homogenates obtained for each treatment were centrifuged at 13000 *g* at the temperature of 4˚C for 15 min. For enzymatic evaluates, the supernatants were collected in micro tubes and stored at −20°C. All enzyme assays were done in three replications.

Catalase activity

Catalase activity of *R. ferrugineus* larvae was measured following the methodology of Aebi (1984). For this purpose, 50 µL of crude enzyme extract, 225 µL H_2O_2 225mM, and 70 mM phosphate buffer 225 µL (pH 7.5) were mixed and the absorbance was read at 240 nm by spectrophotometer.

Esterase activity

The activity of this enzyme was calculated according to van Asperen (1983). Briefly, enzyme extract (15 µL), α-naphthyl acetate (10 mM in acetone, 10 µL), and phosphate buffer (70 mM, pH 7.4, 40 mL) were incubated for 10 min at 30°C. Next, 50 µL Fast Blue RR salt (50 mg in sodium phosphate buffer (40 mM, pH 7, 50 mL)) was added to the reaction mixture. The absorbance was recorded at 450 nm using a spectrophotometer.

Peroxidase activity

This reaction was performed by Bergmeyer methodology (1974). For this reason, enzyme extract (50 μL), H_2O_2 (225 mM, 225 μL), guaiacol buffer (45 mM, pH 7, 225 μL), and potassium phosphate buffer (50 mM, pH 7, 50 μL) were used as the assay mixture. Change in absorbance was measured at 470 nm by spectrophotometer.

Determination of palm hearts mechanical properties

The mechanical features (force, breaking deformation, stress, modulus of elasticity, and energy) of fresh palm hearts were determined using a Universal testing machine (model MRT-5, Santam, Iran) (Fig. 1). First, 500 g fresh palm hearts were placed upon a flat plate and the hardness was measured with a 4.8-mm-diameter stainless steel puncture. Three palm hearts were experimented with 10 mm penetration depth for each cultivar. The maximum force required to penetrate each palm heart was calculated according to Ghanbarzadeh (2019) and Najafi *et al*. (2022). The highest value recorded by the puncture while passing through each tested palm heart, in Newton (N), was considered as hardness of the palm heart. The elasticity was calculated according to the force-time curve, using the same probe. The force-deformation curves were used to determine the mechanical features.

Determination of palm hearts total sugar

To measure the total sugar content, 40 mg the powder of each palm heart cultivar (taken from a fresh sample as described previously) was homogenized in ethanol (80 %, 5 mL) to remove sugars. Residue was precipitated by placing the mixture in a water-bath set at 70˚C for 10 min. The residue was washed repeatedly with ethanol (80 %) until all the soluble sugars were completely extracted. The sample extract was mixed with 3 mL anthrone reagent (150 mg anthrone dissolved in H_2SO_4 (13 M, 100 mL). Afterwards, the mixture was put in a water-bath set at 100 °C (20 min) then, cooled to room temperature. Absorbance was measured in triplicate for each treatment at 630 nm (McCready *et al*., 1950).

Determination of palm hearts phytochemicals

Preparation of extracts

Each palm heart powder (1 g) was extracted with methanol (20 mL) for a period of 24 h in dark situation. Then, each sample was centrifuged at 1000 *g* for 5 min.

Total phenol content

The amount of total phenolic content of the examined cultivars of palm hearts was determined using the Folin-Ciocalteu reagent, based on the method defined by Soland and Lima (1999). Briefly, each palm heart extract (0.2 mL) was mixed with Folin-Ciocalteu reagent (50 %, 0.4 mL) and sodium carbonate (Na_2CO_3) solution (5 %, 0.8 mL). The reaction mixture was allowed to develop for 2 h in dark and the absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as standard. The assays were carried out in three replicates for each palm cultivar.

Total flavonoid content

The total flavonoid content in the palm hearts was determined according to the procedure of Jia *et al*. (2015). First, each palm heart extract (500 µL) was mixed with bicarbonate (5%, 75 µL). After 5 min, $AlCl_3$ (10 %, 150 mL) was added to the mixture and let to stand for another 5 min at room temperature. Then, NaOH (1.0 M, 500 ml) and distilled water (275 µL) were added. The absorbance was read at the wavelength of 510 nm. Each tested extract included three replicates. The total flavonoid of the treatments was expressed as µg of quercetin equivalent per gram dry weight (DW).

Gas chromatography-mass spectrometry (GC-MS) analysis

The palm heart's powder of each date palm cultivar (20 g) was suspended in aqueous methanolic solution (1: 2/*v*:*v*) (200 mL) in a container and then, placed on a rotary shaker for 48 h. After that, the mixture filtered and poured into falcon tubes, then centrifuged at 6000 *g* for 3-5 min. The resulting extracts were subjected to GC-MS (Agilent Technologies 7890 A, Germany) to evaluate the phytochemical profile of the treatments. The analysis was performed on a HP-5-MS capillary column (30 m × 250 µm i.d., film thickness 0.25 µm, temperature program of 50-280˚C). The injector temperature was 50˚C raised to 260˚C with normal injection mode and the flow rate of carrier gas (Helium) was 1.0 mL/min. All MS was operated at EI mode (70 eV ionization energy, 250˚C EI ion source temperature). The results were compared by using WILEY, Demo and NIST Spectral libraries search programs.

Data analysis

All data were analyzed by one-way analysis of variance (ANOVA) appropriate to completely randomized design using the statistical software of SPSS ver. 26.0 (Stata Corp., College Station, TX, USA). The means separation was performed by a Tukey's test at P < 0.05. The data were examined for normality using Kolmogorov–Smirnov test before analysis. The graphs were created in SigmaPlot.

RESULTS

Energy reserves of *R. ferrugineus* larvae

The concentrations of storage macromolecules in *R. ferrugineus* larvae fed on palm hearts from different date palm cultivars are shown in Fig. 2. Larvae fed on Piarom palm heart had the highest sugar content (0.484 mg/g) ($F_{2,9}$ = 11.96; P = 0.003). Furthermore, lipid content was highest when larvae fed on Mazafati palm heart (16.2 mg/g), while it was lowest when the larvae reared on Piarom palm heart (8.8 mg/g) ($F_{2,9}$ = 553.62; P < 0.0001).

Antioxidant activity of *R. ferrugineus* larvae

The antioxidant activities of *R. ferrugineus* larvae were significantly different on the tested cultivar, except that of esterase ($F_{2,9}$ = 2.51; P = 0.136) (Fig. 3 B). The catalase activity of larvae was highest on Kalute palm heart (0.290 U/mg) and lowest on Mazafati palm heart (0.202 U/mg) ($F_{2,9}$ = 5.71; P = 0.025) (Fig. 3 A). The highest and lowest peroxidase activities were observed on Mazafati (0.154 U/mg) and Piarom (0.128 U/mg) palm hearts, respectively ($F_{2,9}$ = 6.17; P = 0.021) (Fig. 3 C).

Palm hearts mechanical properties

There were significant differences among the cultivars regarding the mechanical properties of their palm hearts. The maximum force required to puncture the palm hearts was recorded in Kalute palm heart, while the minimum force was obtained in Mazafati palm heart ($F_{2,6}$ =62.37; P < 0.0001) (Table 1). The highest value of breaking deformation was in Mazafati palm heart ($F_{2,6}$ = 14.41; P < 0.0001) (Table 1). The stress was highest in Kalute palm heart, followed by Piarom and Mazafati palm hearts ($F_{2,6}$ = 11.74; P < 0.0001) (Table 1). The highest and lowest modulus of elasticity were in Kalute and Mazafati palm hearts, respectively ($F_{2,6}$ = 3448.34; P < 0.0001) (Table

1). The highest energy was consumed for puncturing Kalute palm heart. However, the lowest energy was used up in Piarom palm heart ($F_{2,6} = 1936.56$; $P < 0.0001$) (Table 1).

Palm hearts total sugar content

According to the results, there was a significant variation in total sugar contents of palm hearts ($F_{2,9} = 50.35$; $P < 0.0001$). The total sugar content was higher in Mazafati (0.679 mg/g DW) and Kalute (0.588 mg/g DW) palm hearts than in Piarom (0.345 mg/g DW) (Fig. 4).

Palm hearts phytochemicals

The results indicated that the total phenol and flavonoid contents of palm hearts were significantly different ($F_{2,12} = 58.86$; $P < 0.0001$, $F_{2,12} = 27.35$; $P < 0.0001$, respectively). The higher amounts of phenol were detected in Piarom (34.6 µg/g DW) and Mazafati (34.3 µg/g DW) palm hearts. However, its lowest amount was found in Kalute palm heart (30.1 µg/g DW) (Fig. 5). The flavonoid content was highest in Mazafati palm heart (50.2 µg/g DW), whereas it was lower in Piarom (31.2 µg/g DW) and Kalute (28.2 µg/g DW) palm hearts (Fig. 5).

According to GC-MS analysis, different compounds with various antibiotic activities (i.e., antimicrobial, insecticidal, larvicidal, and fungicide activities) were identified in the extracts of palm hearts of Piarom, Mazafati, and Kalute, representing 57.70 %, 89.83 %, and 49.48 % of the total sample, respectively. Two alkaloids including Dimpylate and Bis(2-ethylhexyl) phthalate were the main constituents in Piarom palm heart (Table 2). The predominant constituents in Mazafati palm heart were classified as terpenes (9-octyl-heptadecane and Eicosane) and alkaloids (Bis(2-ethylhexyl) phthalate) (Table 2). Furthermore, the major compounds in Kalute palm heart were acids (Acetic acid, chloro-, 2-butoxyethyl ester and Butanoic acid, 2-oxo-) and amids (N-methoxymethyl-N-methylacetamide) (Table 2).

DISCUSSION

The consumption of *R. ferrugineus* larvae on palm hearts of various date palm cultivars greatly affected the energy reserves of the larvae. The larvae fed on Piarom palm heart had high level of sugar. This value was about 1.3-fold higher than those fed on Mazafati and Kalute palm hearts. Interestingly, the total sugar content of palm hearts either in Mazafati or in Kalute was higher than that in Piarom, emphasizing that the Mazafati and Kalute palm hearts were high-nutrient foods for the pest. Similarly, Farazmand (2002) reported that Mazafati was nutritionally suitable food for *R.*

ferrugineus larvae. For long-term use, the majority of ingested carbohydrates are usually stored as glycogen or triglycerides in fat bodies to meet the insects' energy requirements for reproduction and metamorphosis (Enriquez and Visser, 2023) as well as to assist them withstand harsh environmental conditions (Sinclair, 2015). It seems that feeding of *R. ferrugineus* larvae on sugar-rich diets, i.e. Mazafati and Kalute, can impose low metabolic and fitness costs to the pest, thus it readily converts the received dietary sugars to lipids. However, the high sugar level in larvae body on Piarom palm heart indicates that it was used mainly for instant energy needs, such as energy production, growth and development, immune or stress response, etc., but not or less for future use (in the forms of glycogen or lipids). Based on the results, the lipid level of RPW larvae was highest when fed on Mazafati palm heart, while it was lowest feeding on Piarom palm heart, being almost 1.8-fold less than the related value obtained on Mazafati palm heart. As a result, the significant quantity of lipid discovered in larvae feeding on palm heart of Mazafati may support the concept that the insect transforms dietary carbohydrates to lipids to endure adverse circumstances in later stages of development.

The Mazafati suitability for *R. ferrugineus* larvae was supported by the of mechanical properties effects of its palm heart. According to the results, the lowest amounts of force, mechanical stress, and modulus of elasticity were recorded for Mazafati palm heart, emphasizing that it had low tissue hardness, which can be favored by *R. ferrugineus* larvae. However, the palm heart of Kalute had a high puncture resistance, as shown by its increased force, modulus of elasticity, mechanical stress, and puncturing energy. This may decrease the feeding of RPW larvae by supplying comparatively hard tissues. Even though, the sugar content of Kalute was suitable for *R. ferrugineus* larvae, but the stiffness of its palm heart tissue may impose stress to the pest, probably leading to increased catalase activity of larvae. High sugar intake may increase the insect's metabolism and catalase activity due to production of ROS (Kauldhar and Sooch, 2016). Therefore, reduced fitness and host acceptance, as well as low feeding performance in insects may be associated with the mechanical properties of plant tissue (Hochuli, 1996, Stevanato *et al*., 2020; Nalam *et al*., 2021).

The increased activities of catalase and even esterase in *R. ferrugineus* larvae feeding on Kalute palm hearts and peroxidase in larvae feeding on Mazafati and Kalute palm hearts indicate the occurrence of oxidative stress in RPW larvae body. Mohamed *et al*. (2022) reported that the up-regulation of antioxidant enzymes provided protection to *R. ferrugineus* larvae from the cytotoxic effects of ROS, which is consistent with our findings. Antioxidant enzymes generally reduce the level of ROS (Dmochowska-Ślęzak *et al*., 2015 Wei *et al*., 2020). The ingestion of plant secondary substances is reported to generate ROS in plant-feeding insects and gives rise to oxidative radicals (Umar *et al*., 2022, Krishnan and Kodrik, 2006). Piarom had the highest amount of phenol and Mazafati had the highest amounts of phenol and flavonoid. High phenolic content in a plant (Piarom in our study) is usually associated with its resistance, as it may interfere with the digestion or physiology of the pest (Sattari Nasab *et al*., 2018). However, it is worth to note that just because the levels of phenol and flavonoid were high in Mazafati palm heart does not mean that this cultivar was resistant to the pest. Since, the strong antioxidant activity in larvae, especially peroxidase might have contributed in detoxification of these compounds. The phenol concentration levels in this investigation are lower than, those reported by Trabzuni *et al*. (2014) for date palm heart extracts from the Solleg and Naboat Saif cultivars. The amount of defensive metabolites in various date palm cultivars may be influenced by genetic and age variability, farming management variations, and environmental factors (Umar *et al*., 2022, Al-Farsi *et al*., 2005). According to the results, the antioxidant enzymes activity of RPW larvae was also high in Kalute palm heart. Surprisingly, the amount of either phenol or flavonoid was lowest in palm heart of this cultivar, emphasizing that phytochemicals other than phenol and flavonoid might have increased oxidative radicals in larvae body. GC-MS analysis indicates that the main component in Kalute palm heart was acidic molecules with insecticidal action, such as Butanoic acid, 2-oxo-, which may disrupt insect pests' normal physiological processes, causing paralysis and death (Robert *et al*., 2015). Based on the results, alkaloids (Bis (2-ethylhexyl) phthalate) and terpenes (9-octyl-heptadecane, and Eicosane) were the predominant compounds in Mazafati palm heart . Waris *et al*. (2018) reported that Eicosane in volatiles of rice plants elicited behavioral responses in *Nilaparvata lugens* Stål by simply attracting the pest to the plants. However, exposure to compounds like Di (2-ethylhexyl) phthalate can induce antioxidative stress in insect pests (Rivas *et al*., 2023), emphasizing that alkaloids can negatively affect insect pests. The amounts of phenol and alkaloids (Dimpylate and Bis (2-ethylhexyl) phthalate) were high in Piarom palm heart, but the larvae on this cultivar had the lowest activity of peroxidase. It is possible that, some allelochemicals will inhibit the activities of antioxidant enzymes and increase the level of free radicals as well as oxidative stress in some species (Krishnan and Kodrik, 2006, Mythri *et al*., 2013, Yang *et al*., 2019). Moreover, the larvae may use antioxidant enzymes other than peroxidase to cope with stress caused by secondary metabolites (Wei *et al*., 2020). The number of experiments to address the ambiguities regarding the behavior of RPW is very low, and more experiments are needed to address the dark spots in the behavior of this pest.

In conclusion, RPW larvae were able to feed on palm hearts of the studied date palm cultivars. However, not all date palm cultivars appear to be equally appropriate as hosts for this pest. The Piarom palm heart was relatively unsuitable host for RPW larvae in terms of low sugar content and high phytochemicals contents (especially phenol). Some mechanical traits and phytochemicals of this palm heart increased stress in larvae. However, the antioxidant enzymes of larvae via adaptive responses may moderate the suppressive effect of date palm trees' defenses against *R. ferrugineus*. Using the date palm-derived resistance traits, i.e., phytochemicals and mechanical defense, offers an effective approach for strengthening IPM strategies against RPW, particularly in combination with other control measures. To control *R. ferrugineus*, collecting further applicable data is suggested to assess the demographic parameters and other physiological responses of this pest.

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Table 1. Mechanical properties (mean ± SE) of palm hearts of three date palm cultivars.

Cultivar	Force (N)	Breaking deformation (mm)	Stress (MPa)	Modulus of elasticity (MPa)	Energy (mJ)
Piarom	47.16± 0.31ab	3.24± 0.98b	4.38±0.97ab	81.99± 1.12b	90.97± 2.22b
Mazafati	35.13± 1.01b	9.87± 0.59a	2.88± 0.57b	44.62±0.55c	158.51± 1.39ab
Kalute	55.66± 1.99a	3.50± 1.18b	5.5± 0.29a	179.86±1.62a	226.21± 0.57a

Means followed by different letters in each column are significantly different (Tukey test, P<0.01).

Table 2. Phytochemicals identified in palm hearts of three date palm cultivars using gas chromatography-mass spectrometry.

Cultivars	IUPAC Name (Synonym)	Retention time (min)	Activity	Area (%)	Category
Piarom	Dimpylate*	23.82	Antimicrobial activity	30.21	Alkaloid
	Bis(2-ethylhexyl) phthalate	35.21	Antimicrobial activity Larvicidal activity	27.49	Alkaloid
Mazafati	9-octyl-heptadecane	30.15	Plant growth promotion activity Insecticidal activity	21.12	Terpene
	Bis(2-ethylhexyl) phthalate	32.89	Antimicrobial activity Larvicidal activity	50.89	Alkaloid
	Eicosane	39.89	Plant growth promotion activity Insecticidal activity	17.82	Terpene
Kalute	Acetic acid, chloro-, 2-butoxyethyl ester	13.98	Insecticidal activity	19.46	Acid
	Butanoic acid, 2-oxo-	13.16	Insecticidal activity Antimicrobial activity	22.79	Acid
	N-Methoxymethyl-N-methylacetamide	15.20	Fungicide activity Insecticidal activity Herbicidal activity Antimicrobial activity	7.23	Amid

Only major compounds were listed.

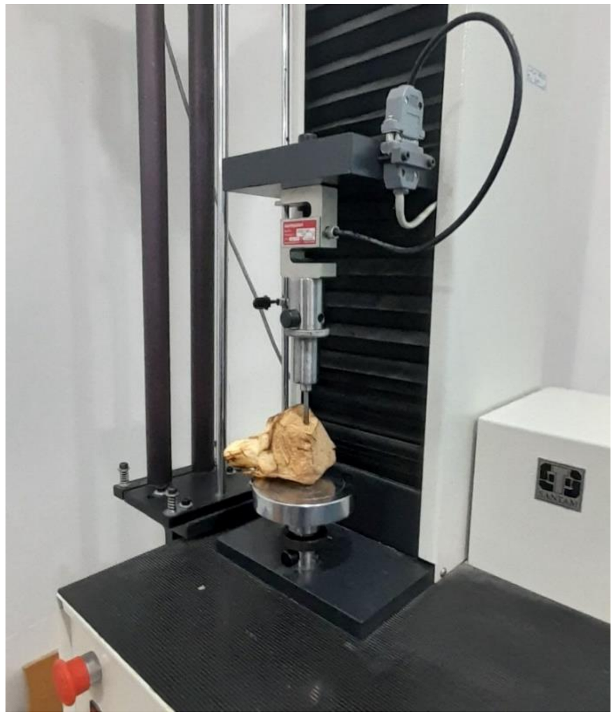

Figure 1. The Universal testing machine for determining the mechanical properties of palm hearts.

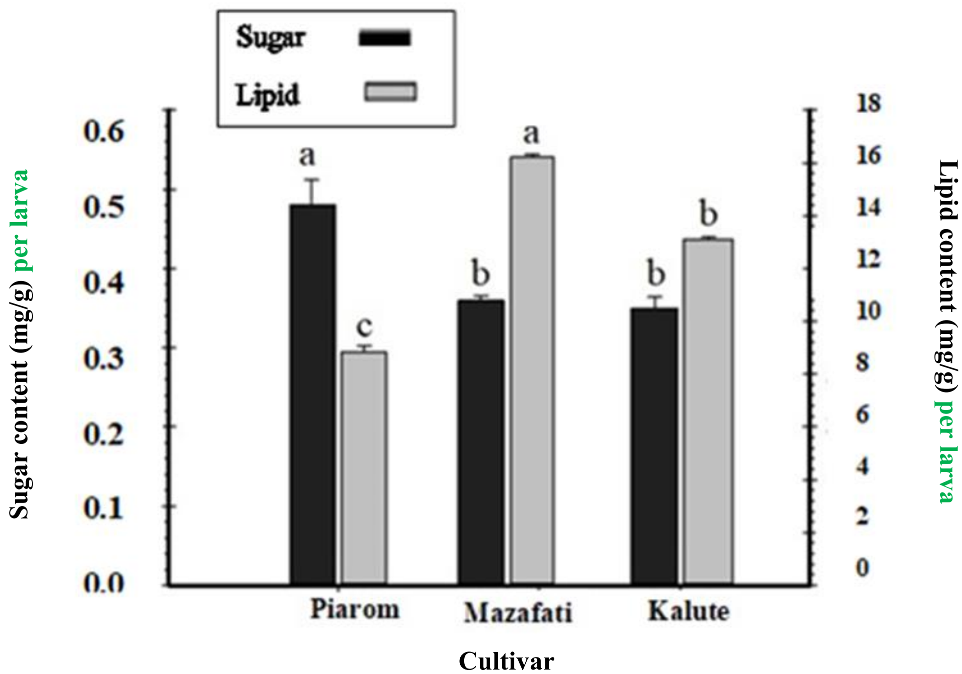

Figure 2. Energy reserves (mean ± SE) in *Rhynchophorus ferrugineus* larvae feeding on palm hearts of three date palm cultivars. Columns with different letters represent significant differences among cultivars (Tukey test, P<0.01).

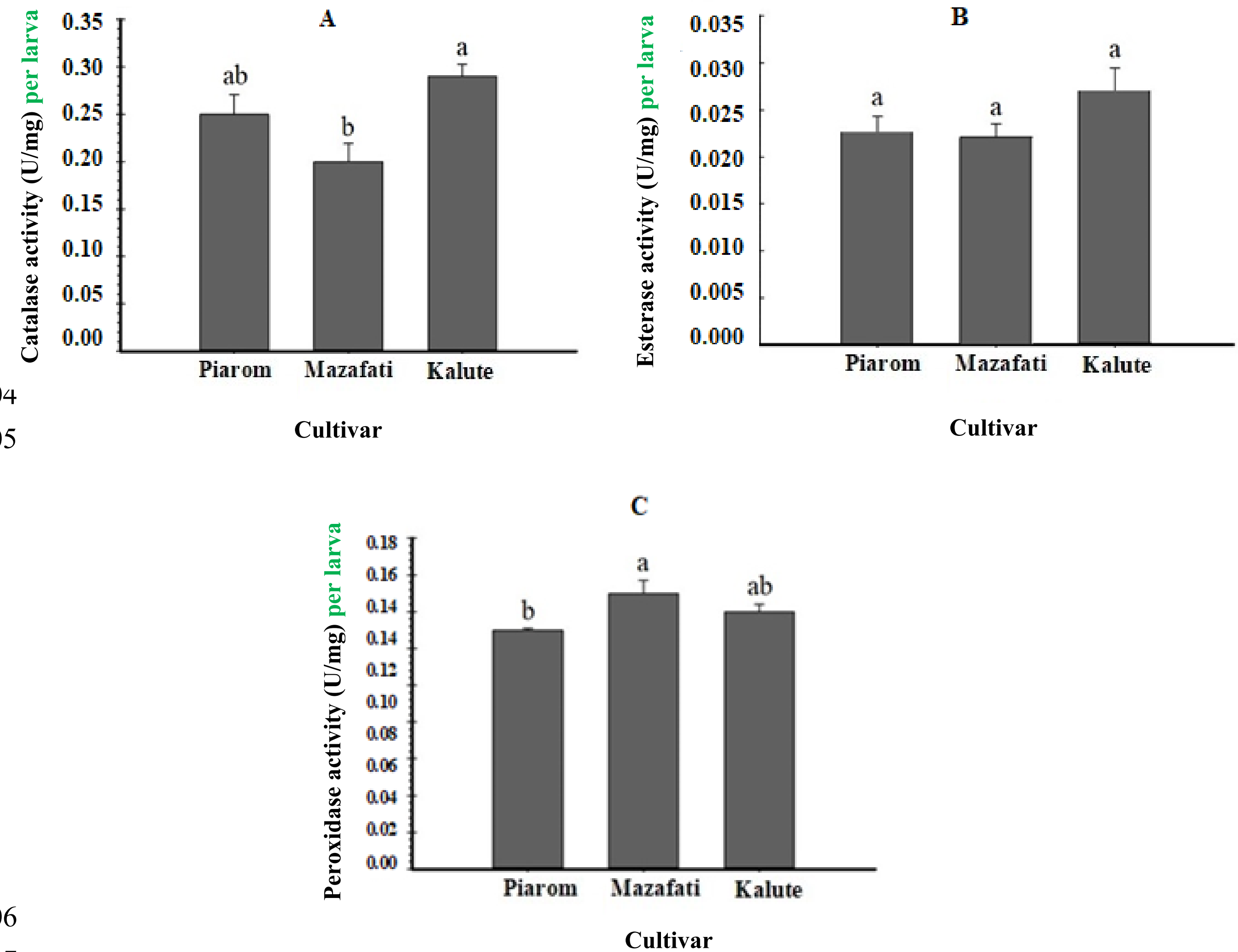

Figure 3. A, B, C: Antioxidant enzymes activities (mean ± SE) in *Rhynchophorus ferrugineus* larvae feeding on palm hearts of three date palm cultivars. Columns with different letters represent significant differences among cultivars (Tukey test, P<0.01).

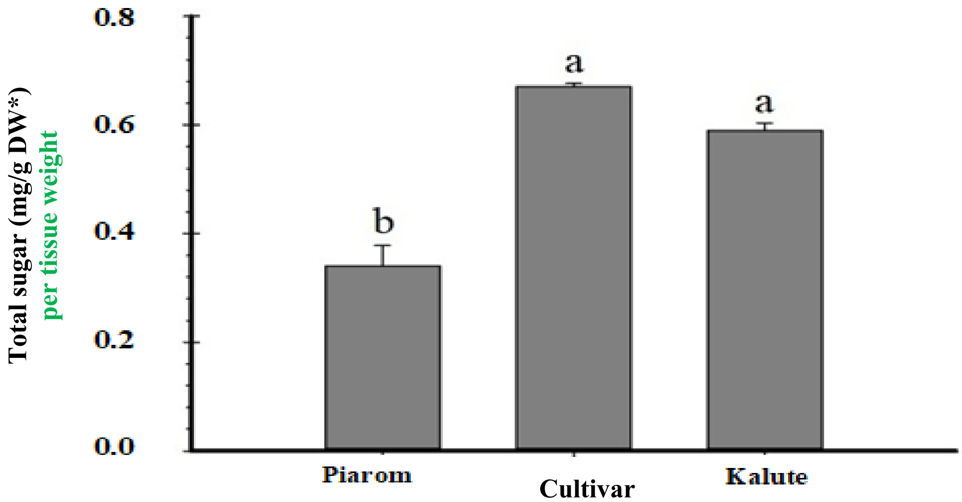

Figure 4. Total sugar content (mean ± SE) in palm hearts tissues of three date palm cultivars. Columns with different letters represent significant differences among cultivars (Tukey test, P<0.01). *DW: Dry Weight.

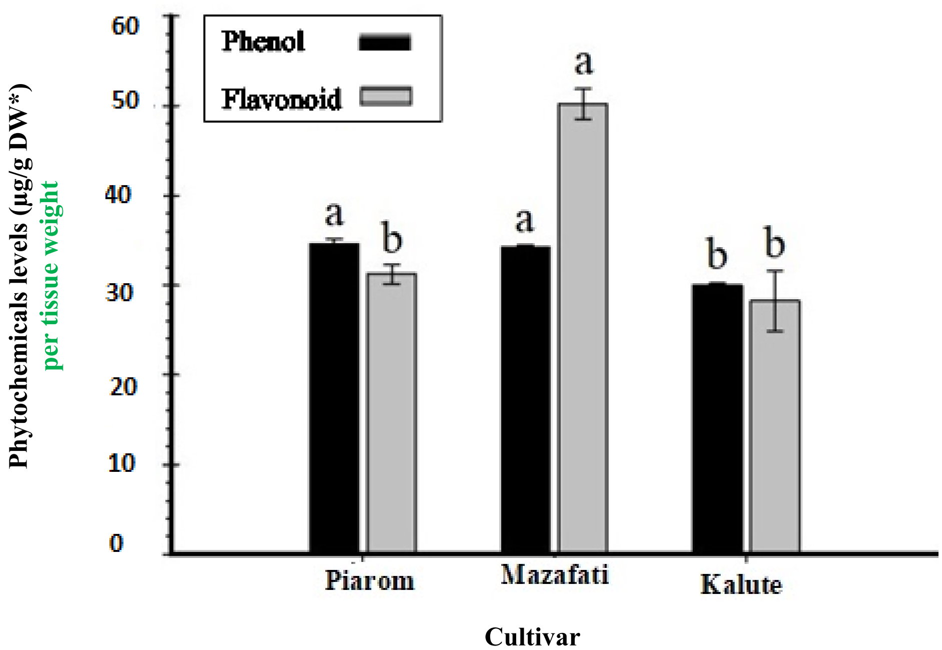

Figure 5. Mean (± SE) amounts of phytochemicals (phenol and flavonoid) in palm hearts tissues of three date palm cultivars. Columns with different letters represent significant differences among cultivars (Tukey test, P<0.01). *DW: Dry Weight.

بررسی ویژگیهای شیمیایی و مکانیکی پنیر سه رقم خرمای ایرانی و تأثیر آن بر پاسخ‌های فیزیولوژیکی سرخرطومی حنایی خرما، (*Rhynchophorus ferrugineus* Olivier, 1790) (Coleoptera: Dryophthoridae)

فاطمه خدادادی اسفیچار، مریم پهلوان یلی، و پیمان نامور

چکیده

سرخرطومی حنایی خرما، *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Dryophthoridae) آفت جدی درختان خرما خرما در سراسر جهان است. هدف از این مطالعه، ارزیابی ذخایر انرژی و واکنش آنتی‌اکسیدانی لاروهای *R. ferrugineus* روی پنیر خرمای سه رقم خرما (پیارم، مضافتی و کلوته) بود. علاوه براین ویژگی‌های بیوشیمیایی و مکانیکی پنیر خرما هم مورد بررسی قرار گرفت. پرورش حشره در شرایط آزمایشگاهی با دمای 2 ± 29 درجه سانتی‌گراد و رطوبت نسبی 5 ±80 درصد و دوره نوری 12:12 (روشنایی: تاریکی) روی لاروهای سن هفتم انجام شد. نتایج نشان داد که بیشترین ذخایر انرژی (قند و چربی) لارو به‌ترتیب روی پنیرهای خرمای پیارم ومضافتی بود. فعالیت آنزیم‌های آنتی‌اکسیدانی در لاروهای *R. ferrugineus* روی پنیرهای خرمای آزمایش شده به طور قابل توجهی متفاوت بود. طبق آنالیز مکانیکی، پنیرهای خرمای رقم مضافتی کمترین سختی بافت را داشت.آنالیزهای بیوشیمیایی پنیرهای خرمای مورد مطالعه نشان داد که محتوی قند کل در مضافتی و کلوته بالا و در پیارم پایین بود. همچنین، سطح فنل کل به طور معنی‌داری در پنیرهای خرمای آزمایشی متفاوت بود. نتایج کروماتوگرافی گازی-طیف سنج جرمی (GC-MS) نشان داد که آلکالوئیدها، ترپن‌ها، اسیدها و آمیدها مهم‌ترین ترکیبات فرار پنیرهای خرمای مورد بررسی بودند. بر اساس نتایج، پیارم ویژگی‌هایی مرتبط با کاهش عملکرد لاروی را نشان داد که نشان‌دهنده کاربرد بالقوه آن در برنامه‌های مدیریت تلفیقی آفات (IPM) است.