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Changes in the Content of Certain Oxidative Defense Enzymes and Pigments in Leaves of Some Date Palm Cultivars Due to Infestation by the Red Palm Weevil in Egypt

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Abstract: The most dangerous insect pest of planted date palm trees in numerous countries is the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Oliv.) (Coleoptera: Curculionidae). Current work aimed to evaluate the effect of (RPW) infestation on some biochemical characteristics of infested date palm cultivars defensive oxidation enzymes, Peroxidase, Polyphenol oxidase enzyme activity and photosynthetic pigments content Chlorophyll a and b in infested date palm cultivars from three governorates, Giza, El Beheira and New Valley compared to healthy (non infested) date palm from the same cultivars. Obtained results indicated that the infestation of RPW to different date palm cultivars affected oxidative enzymes, peroxidase, polyphenol oxidase activities and pigments, chlorophyll a and chlorophyll b contents of the infested cultivars. Data showed significant increase in peroxidase and polyphenol oxidase activities of infested date palm cultivars (Zagloul, Siwi and Bartamoda) with red palm weevil compared to healthy date palm of the same cultivar. While, the obtained results of chlorophyll a and chlorophyll b recorded significant decrease for the tested cultivars (Zagloul, Siwi and Bartamoda) compared to the healthy date palm of each cultivar. Through these results, it can be concluded that the infestation with RPW to various date palm cultivars, whether the fresh, semi dry and dry cultivars represented by Zaghloul, Siwi and Bartamoda, respectively led to a significant increase in the activity of some defensive enzymes peroxidase and polyphenol oxidase activities in the infested date palm, cultivars at the same time, there is a decrease in the level of pigments, chlorophyll a and chlorophyll b of infested palms compared to healthy date palm from each cultivar separately.

Key words: *Rhynchophorus ferrugineus* · RPW · Biochemical characteristics · Peroxidase · Polyphenol oxidase · Chlorophyll a · Chlorophyll b defensive enzymes and pigments

INTRODUCTION

Phoenix dactylifera L., the date palm, is fairly successfully cultivated, particularly in tropical and semitropical areas. It is regarded as a significant fruit crop in Egypt and the Middle East economically after citrus, banana and mango. Date palm fruits are a vital food source with many therapeutic properties [1-5]. Many damaging and dangerous pests are drawn to palm trees, but the red palm weevil is the most significant of them all [6-10]. The primary borer attacking palm trees is the red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) [11-12]**.** According to Sallam *et al.* [13]**,** the majority of popular date palm cultivars are also appropriate for red palm weevil RPW infestations. Due to the widespread circulation of RPW, it was able to spread to countries in Asia, Africa, Europe, and America, severely harming palm production [14]. First noted RPW as an invasive pest in date palm plantations in the Egyptian governorates of Sharqia and Ismailia [15]. Since then, it has evolved into the primary pest of date palm [16]**.**

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After date palm infestation reached 1%, a lower economic threshold was needed for the implementation of effective control techniques due to the significant risk of RPW [17]. In order to safeguard palm trees, it is crucial to monitor the population density of *R. ferrugineus* [18]. This monitoring was done using pheromones and other semi-chemicals in traps to ascertain the best time to use the measures of control [16]. Due to the lack of evident indicators of infection, particularly in the early stages of infestation, it is challenging to identify infested palm trees in palm plantations. Physical symptoms become more obvious as the infestation gets worse [19]. In order to find infested palms early on, alternative methods of infestation detection were investigated. Examples include observing acoustic signals linked to RPW larva chewing and using trained sang dogs [20-21]. Each option, meanwhile, has drawbacks, such as low precision or a lack of viability for extensive plantations. As a result, the traditional method of identification continues to rely on identifying the visible signs of RPW infestation on palm trees. The physical signs of infestation in the cases of *C. nucifera*, *Phoenix dactylifera*, and *P. canariensis* have been well-documented. Based on the degree of the infestation shown in *P. canariensis,* and the phases of RPW infestation divided into five categories [22]. Growers' visual inspection is currently the most effective monitoring technique for spotting RPW infestation [23]. However, the procedure is frequently drawn out and doesn't become apparent until the RPW infestation has gotten out of hand. Recent research has looked into how RPW infestation affects the physiology of the host. According to Stephens and Westoby [24] and Golomb *et al*. [25], insect herbivory activity can cause physiological changes in terms of growth, temperature, photosynthetic activity, fruit output, and reproductive capacity.

This study was established to study the effect of infestation of some date palm cultivars by red palm weevil on some biochemical aspects of infested palm trees such as oxidative stress and pigments changes to find out the possibility of using this effect in determining whether or not infested trees.

MATERIAL AND METHODS

These experiments were carried out during 2022 in certain date palm plantations, which infested by the Red Palm weevil (RPW). The chosen areas were farms in Giza, Beheira and New Valley governorates, Egypt.

Sampling of Date Palm Leaves: Date palm leaf samples were collected from the upper third of the leaves located around the top of the developing palm with no more than 50 cm from the top. Samples were taken from the cultivars Zagloul, Siwi and Bartamoda which representing the three groups of date palm cultivarsfresh, semi dry and dry respectively. Samples were taken from palm trees of similar ages, from 10 to 12 years, in November 2022, with the same degree of infestation with the red palm weevil (medium degree).Three replicates were taken from each infested cultivar by RPW. All samples were compared to three replicates of the healthy (non infested palm tree) of the same cultivar and the same age and size of the infested cultivar.

Biochemical Assay: Bioanalyses of the leaves were carried out in the Virus Research Department at the Institute of Plant Diseases and the Soils, Water, and Environment Unit of the Soils, Water, and Environment Research Institute) at the Agricultural Research Center in Egypt.

The supernatant of different collected samples from the leaves of date palm trees cultivars infested with the red palm weevil in addition samples of healthy (non infested) date palm trees as control, were subjected determinations phytochemicals assay.

Determination of Peroxidase (POD):

A-Peroxidase Activity: In order to measure the activity of peroxidase, oxidation in the presence of the enzyme and hydrogen peroxide is measured using a UV- 2401 PC UV-Vis recording spectrophotometer in a 3 ml light path cuvette at intervals of 30 seconds. Unless otherwise noted, the reaction mixture comprised 8 moles of hydrogen peroxidase, 60 moles of guaiacol, and 60 moles of sodium acetate buffer in a volume of 3 ml. PH 5.6 and peroxidase doses that produced a linear response over 3 minutes. A unit of peroxidase activity is defined as the amount of enzyme that causes one optical density (OD) change per minute [26]. The enzyme is introduced and mixed to start the reaction.

Calculations: The activity is estimated using the tetraguaiacol extinction coefficient (26.6 $mM^{-1}.Cm^{-1}$). The amount of enzyme required to convert one micromole of guaiacol into tetraguaiacol per minute is known as the unit.

Determination of polyphenol oxidase (PPO):

A. Polyphenol oxidase activity: Using a 24-01 PC UV- Vis recording spectrophotometer, the initial rate of quinine formation–indicated by an increase in absorbance at 420 nm–was measured in order to determine the activity of

polyphenol oxidase [27]. PPO activity was assessed in triplicate measurements, with one unit of enzyme activity being defined as the amount of enzyme that generated a change in absorbance of 0.001/min. 2.95 ml of a 20 nm catechol solution in 0.1 M phosphate buffer made up the sample cuvette. 0.05 ml of the enzyme solution at pH 6.0. The amount of substrate solution in the blank sample was just 3 ml.

Determination of Photosynthetic Pigments: As per Wettstein [28], chlorophyll a and b were extracted and estimated. As the subsequent process: To neutralize organic acids in the fresh leaf homogenate, fresh leaf samples (0.5 g) were homogenised in a mortar with 85% acetone, washed dried sand and a little amount of $CaCO_3$ (0.1 g). After that, a sintered glass funnel was used to filter the homogenate. Acetone was used to wash the residue numerous times until the filtrate was colourless. A spectrophotometer was used to measure the optical density of this extract at 660 and 640 nm for Chl. a and b, respectively.

Calculation:

Chlorophyll a = 9.784 × E 660 - 0.99 × E 640 mg/L.
Chlorophyll b = 21.426 × E 660 – 4.65 × E 640 mg/L.

The results were calculated as mg g^{-1} fresh weight.

Statistical Analysis: Data of analysis of date palms cultivars leaves quantity of Peroxidase, Polyphenol oxidase Enzyme activity and photosynthetic pigments content (Chlorophyll a and b) in infested date palm cultivars compared to healthy (control) date palm from the same cultivars were examined with SAS's Proc., ANOVA [29].

RESULTS AND DISCUSSION

Oxidative Enzymes Activity Changes on Infested Date Palm Trees Cultivars with the Red Palm Weevil Compared to Healthy Date Palm: Data in Table 1 showed significant increasing in peroxidase activity in the leaves of the infested date palm with RPW from cultivars, Zagloul, Siwi and Bartamoda which recorded (6.81, 5.20 and 6.35 Unit) respectively compared to the healthy date palm (control) from each tested cultivar which were (1.60, 3.66, and 3.64 Unit) respectively. The same trend were recorded for polyphenol oxidase activates which indicated a clear significant increasing in the infested date palm tested cultivars, Zagloul, Siwi and Bartamoda (4.52, 3.52, and 3.23 Unit) compared to the healthy date palm (control) from each tested cultivar which were (1.10, 2.29, and 2.46 unit) respectively.

Table 1: Effect of date palm trees cultivars infestation with red palm weevil insects on oxidative enzymes activity.

Date palm group	Date palm Cultivars	Peroxidase (POD) activity Unit	Polyphenol oxidase (PPO) activity Unit
Fresh date cultivars	Healthy Zagloul Cultivar	1.60 b	1.10 b
	Infested Zagloul Cultivar	6.81 a	4.52 a
	Pr	<0.0001	<0.0001
	L.S.D.	0.493	0.247
Semi-Dry date cultivars	Healthy Siwi Cultivar	3.66 b	2.29 b
	Infested Siwi Cultivar	5.20 a	3.52 a
	Pr	0.001	0.015
	L.S.D.	0.538	0.831
Dry date cultivars	Healthy Bartamoda Cultivar	3.64 b	2.46 b
	Infested Bartamoda Cultivar	6.35 a	3.23 a
	Pr	0.001	0.006
	L.S.D.	0.829	0.401

Unit Peroxidase = (Chang in absorbance at 420/20sec. g^{-1} fresh leaves).
Unit Polyphenol Oxidase = (Chang in absorbance at 420 hr^{-1} g^{-1} fresh leaves).

The obtained results indicate that the infested date palm trees with the red palm weevil have shown a reaction in the bio-chemicals related to the resistance such as peroxidase and polyphenol oxidase enzymes to resist the infestation. Since the tested palm trees were not infested with any other insects or diseases other than the red palm weevil or lack of elements, the increase in the activity of these enzymes is clearly due to the infestation of these date palm cultivars by RPW. This observation is in accordance with El-Deeb *et al.* [30] who observed that there was highly significant variance recorded on two defense related enzymes, namely peroxidases (POD) and polyphenol oxidases (PPO) in different date palm varieties, the means of POX and PPO were (5.76 & 2.012 mg) respectively, for healthy date palm while, they were (7.16 & 2.742) mg for infested date palm with RPW. The obtained values for peroxidase and polyphenol oxidase were higher in infested date palm trees with red palm weevil compared to healthy date palm trees. Also, Manzoor *et al.* [31] mentioned that there was enhanced peroxidase activities after RPW infestation of date palm cultivars in Pakistan.

Phytochemicals Changes on Infested Date Palm Trees Cultivars with the Red Palm Weevil: Changes in some

Table 2: Effect of date palm trees cultivars infestation with red palm weevil insects on photosynthetic pigments content Chlorophyll a and b.

Date palm group	Date palm Cultivars	Chlorophyll a mg g^{-1}	Chlorophyll b mg g^{-1}
Fresh date cultivars	Healthy Zagloul Cultivar	5.46 a	7.72 a
	Infested Zagloul Cultivar	2.47 b	1.83 b
	Pr	0.0405	<.0001
	L.S.D.	2.780	1.057
Semi-Dry date cultivars	Healthy Siwi Cultivar	4.41 a	2.07a
	Infested Siwi Cultivar	2.50 b	0.91 b
	Pr	0.0201	0.0002
	L.S.D.	1.414	0.314
Dry date cultivars	Healthy Bartamoda Cultivar	4.49 a	1.16 a
	Infested Bartamoda Cultivar	1.95 b	0.92 b
	Pr	0.001	0.033
	L.S.D.	0.873	0.202

leaves biochemical components associated with infested date palm trees with RPW were conducted in healthy and infested date palm trees including Chlorophyll a and b. Obtained data in Table 2 indicated that chlorophyll a recorded a significant decrease in the three tested cultivars infested with RPW (Zagloul, Siwi and Bartamoda) which record (2.47, 2.50, and 1.95 mg g^{-1}), respectively, compared to the healthy for the same cultivars, which recorded (5.46, 4.41, and 4.49 mg g^{-1}) respectively. The same trend was recorded for chlorophyll b, where a significant decrease was also recorded in the three infested tested cultivars, which were (1.83, 0.91, and 0.92 mg g^{-1}) for Zagloul, Siwi and Bartamoda, respectively, compared to the control healthy for the same cultivars, which recorded (7.72, 2.07, and 1.16 mg g^{-1}) respectively. Our findings in the present work were similar to Bannari et *al.* [32] according to their findings; The Green Normalised Difference Vegetation Index (gNDVI) and the Structure Insensitive Pigment Index (SIPI) were the two metrics that were most sensitive to the bio-physiological agitation that RPW attacks caused in palm trees. They successfully distinguished between the classes under consideration, with excellent r 2 values of 93% and 98% for SIPI and gNDVI, respectively. These first findings led to the conclusion that remote sensing research has potential substitute for RPW detection. Kang *et al.* [33] indicated that chlorophyll is one of the spectral indicators that helps in discovering the vital physiological differences within plants. All the results that were collected from the crown of palm trees were found to be very correlated with the values of chlorophyll, through which it is possible to determine whether the palm is infested with the red palm weevil or not, as well as knowing the degree of infestation with the red palm weevil.

CONCLUSION

These results could be concluded that the infestation of date palm trees with the red palm weevil insect affected the activity level of some oxidation enzymes such as, peroxidase and polyphenol oxidase, and led to a significant increase in their activity in the three infested tested date palm cultivars, which represent the three main groups of date palm cultivars in Egypt, compared to the healthy date palm of the same cultivars. On the contrary, it was found that the infestation with the red palm weevil led to a significant decrease in the level of chlorophyll a and b dyes in the leaves of the three tested date palm cultivars compared with the healthy (control) palm of the same cultivars. Accordingly, it is possible to determine whether or not the palm tree is infested with the red palm weevil through conducting this analysis, provided that the tested palm tree is not infested with any other insect, disease or lack of elements, so the change in this case is attributed to the infestation of the red palm weevil. This difference in the activity of these resistance enzymes and pigments, chlorophyll a and chlorophyll b contents is considered a reaction of the infested date palm to the infestation with the red palm weevil, and this point needs further study and evaluation.

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