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Leaf Toughness and Anatomical Characteristics of Balady Mango Seedling Leaves as Influenced by *Parlatoria oleae* (Colvee) Infestation Rate

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Abstract – **The main objective of this work is to evaluate the leaf toughness and anatomical characteristics of Balady mango seedling leaves as affected by *Parlatoria oleae* (Colvee) infestation rate at Esna district, Luxor Governorate during two successive experimental seasons through duration from early September 2016 till mid August 2018 years. The results revealed that the leaf toughness together with thickness both of palisade tissue dimensions (upper& lower), spongy tissue, collenchyma layers below the upper epidermis at midrib, number of xylem rows in the vascular bundle and length of midrib vascular bundle, increased significantly in the leaves of infested trees (light and heavy) as compared to the uninfested ones. While, in contrast, the thickness both of [epidermis layer dimensions (upper & lower), leaf midrib, widest xylem vessel in the vascular bundle and width of midrib vascular bundle] in leaves of uninfested mango trees was the thickest than those of the infested mango leaves (light and heavy). Based on *P. oleae* feeding mechanism and damage, whenever less thickness for both of (epidermis layer dimensions (upper& lower), leaf midrib, widest xylem vessel in the vascular bundle and width of midrib vascular bundle), would be helpful and facilitation for *P. oleae* feeding and achieving damage to mango leaves. That is, the infestation by pest increases as the thickness of these layers decreases (negative relationship). It was clear that the reflection of the insect infestation levels on the anatomical properties was positive, that refers to the proportional increase in the percentages of loss in the measured parameter i.e., thickness for epidermis layer dimensions (upper & lower), leaf midrib, widest xylem vessel in the vascular bundle and width of midrib vascular bundle as infestation rate was increased. Meaning, whenever increase infestation by pest, would increase the percentages of reduction in the thickness of these layers (positive relationship).**

***Keywords* – *Parlatoria oleae*, Mango, Leaf Toughness and Anatomy.**

I. INTRODUCTION

In Egypt, mango (*Mangifera indica* L.) occupies the third place in acreage after citrus and grapes. Mango fruits are desirable and popular fruits for the Egyptian consumers due to its good flavour, delicious taste, nutritive value and other fruit attractive features **(El-Said, 2006)**. Among several pests of mango trees, *Parlatoria oleae* is considered one of the most main destructive ones **(Bakr *et al*., 2009)**. This pest injures the shoots, twigs, main branches, leaves and fruits by sucking the plant sap with the mouth parts, causing thereafter deformations, defoliation, dryness of young shoots twigs, dieback, poor blossoming, death of twig by the action of the toxic saliva and subsequently affecting the commercial value of fruits. Infestation causes conspicuous pink blemishes around the feeding sites of the scales **(El-Amir, 2002).** A characteristic symptom of infestation by this pest is the appearance and accumulation of its scales on attacked mango parts **(Hassan *et al*., 2009)**.

The interaction between plants and insect pests suggest that the leaf quality in relation to insect preference could be related to leaf toughness and anatomical characteristics. Differences in variability to infestation by insect pests of a given plant species could be due to many factors or most likely due to interaction of these factors **(Chen *et al*., 2002).** Mango trees exhibit variable reactions to the insect infestation depending on many factors *i.e*., plant physical properties or chemical components of plant leaves and anatomical characteristics. For example, several factors can affect population density of the mealybug, *Icerya seychellarum* (Hemiptera: Monophlebidae) on mango such as leaf quality *i.e*., leaf secondary metabolites **(Abd-El-Rahman *et al*., 2006)**, leaf nutrients and its inhibitors **(Salem *et al*., 2006)** and leaf toughness and its anatomical characteristics **(Salem *et al*., 2007).**

Thus, the aggregation of insect species or/and natural enemy species in a plant host could be an indication or a reason of host preference by such species due to plant's physiological and behavioural attributes and may be an genetic factors, or phenotypic due to differences in environmental conditions **(Dale, 1988).**

The plant leaf characteristics are important factors to manage scale insect population in mango crop. Plant leaf features may impact the preference and performance of herbivores **(Gianoli and Hannunen, 2000).**

Having information about density and changes in population of *P. oleae* throughout the season and determination of their periods of activity will help in management of this pest. Because of the lack of the adequate informations concerning the relationship between the infestation rates by *P. oleae* and the leaf toughness together with the anatomical characteristics of mango leaf. So, it was too necessary to study this point, where there is no reports about the estimated relationship between the population density of *P. oleae* and some anatomical characteristics of mango leaves. Accordingly, the objective of this study was aimed to find out the relationships between the different population densities of *P. oleae* and leaf toughness and thickness of some layers and tissues of mango leaves, as it is necessity to foresee with the pest status and are essential to determine the most suitable in order to present an investigated management program for control studies.

II. MATERIALS AND METHODS

This study was carried out on twelve years old Balady mango seedlings trees grown in clay loamy soil under surface irrigation system in a private orchard located at Esna district, Luxor Governorate, Upper Egypt during two successive seasons throughout duration from early September 2016 till mid August 2018 year. It was aimed to investigate the relationship between *P. oleae* infestation rate and leaf toughness and thickness of some layers and tissues of leaves of mango trees. Two *P. oleae* infestation levels (light and severe), besides the free / uninfested trees were studied.

Two limits of infestation rates were decided according to the total number of alive scale exited on both lower and upper leaf surfaces after **(Salem et al., 2015).** Hence, at low infestation rate number of scales per leaf (upper and lower surfaces) ranged from 10 to 70 individuals, while with the severe infestation rate the number was above 70 individuals.

Thirty seedy Balady mango trees were randomly, selected for sampling purpose from different parts of the orchard were distributed in completely randomized blocks design. Each level of infestation consisted of 10 trees and similar to great extent in their vigour. Taking into consideration that all chosed trees were subjected to the same horticultural practices and NPK fertilization adopted in the region.

In this experiment three fields of study were included i.e., a- Population size and the monthly changes of the pest as numbers of total alive *P. oleae* individuals per leaf (upper and lower surfaces), b- the correlation between *P.oleae* infestation rates and (leaf toughness & some anatomical characteristics measurements) and c- the relationship between the changes in numbers of alive *P. oleae* individuals (X) and the percentages of reduction or increasing % in leaf toughness and some anatomical characteristics measurements (Y) as follows:

1. *Population Size of P. oleae and Changes in Total Numbers of its Alive Individuals*

Regular fortnightly samples each consisted of 20 leaves were randomly picked from four geographical directions and heights of every individual tree along the year around. Collected leaves samples were placed in polyethylene bags and immediately transferred to the laboratory, then leaves of each sample was individually inspected using a binocular microscope whereas the total number of alive *P. oleae* individuals on both upper and lower surfaces were counted and recorded. Thereafter, the monthly mean number of total population of *P. oleae* per every leaf for each tree was concerned in this study to express the pest population size.

2. *Leaf Toughness and Some Anatomical Characteristics of Mango Leaves*

2.1. *Leaf Toughness (Expressed as Threshold Weight Needed for Piercing Leaf)*

Specific leaf weight is an indicator of leaf toughness. So, the Specific leaf weight in this work expressed as the leaf toughness of mango.

- Measurements of leaf: Ten mature leaves per shoot (below panicles) in the mid of August of each per season of study were taken for calculating their length, width (cm) and leaf area.
- Leaf area (cm^2) was measured using the following equation as reported by **Ahmed and Morsy (1999): Leaf area (cm^2) = 0.70 (L x W) - 1.06.**

Where,

L: Maximum length of leaf, W: Maximum width of leaf.

- In addition, the fresh samples of uninfested and infested leaves were picked and washed with tap water and then by distilled water to remove the dust and any other residues. Afterwards, these samples were dried in an electric oven at 70ºC for 48 hours. The dry weight for mango leaves was recorded in all different rates of infestation.
- **Specific leaf weight (g/cm^2) = Leaf dry weight / Leaf area.**

2.2. *Some Anatomical Characteristics Measurements*

Thickness of epidermis layer [upper and lower (µm)], thickness of palisade tissue [upper and lower (µm)], thickness of spongy tissue (µm), thickness of collenchyma layers below the upper epidermis at midrib (µm), thickness of leaf midrib (µm), number of xylem rows in the vascular bundle, thickness of widest xylem vessel in the vascular bundle (µm) and midrib vascular bundle [length and its width (µm)] of mango leaves were measured. All samples of leaves transferred to the Laboratory of Faculty of Science, Ain Shams University, Cairo, Egypt.

The all studied measurements of mango leaves were recorded in order to determine the correlations between the measured parameters (X) in relation to the infestation rates by *P. oleae* (Y).

To determine the relationship between the changes in numbers of alive *P. oleae* individuals (X) and the percentages of reduction or increasing % in leaf toughness and some anatomical characteristics measurements (Y).

The amount of losses in any measured parameter was calculated according to the following equation: Loss % $= \frac{A-B}{A}$ X 100.

Where, A = mean of a given measurement of the uninfested trees, while B = mean of the same parameter of the infested trees.

Meanwhile, the relationships between the percentage of changes (±) in measured parameters of mango leaves represented as a dependent variable (Y) and the different infestation rates with *P. oleae* representative of the independent factor (X) were determined during two successive (2016/2017 and 2017/2018) experimental seasons. Herein, the simple regression was used to show the variability in percentages of reduction or increasing in the measured parameters that could be caused by the pest at various infestation levels. The equation of linear regression was calculated according to the following formula of **Fisher (1950)** and **Hosny *et al*. (1972): $Y = a \pm bx$.**

Where:

Y = Prediction value (Dependent variable), **a =** Constant (y - intercept),

b = Regression coefficient, **x =** Independent variable.

This method was helpful for demonstrating basic information about the amount of variability in the difference (±) % in the desirable studied parameters, and also to find out the explained variance (E.V. %).

Obtained data were subjected to statistical analysis of variance as complete randomized block design and means were compared according to the LSD test at 0.05 level using the letters for distinguishing between rates of infestation. All statistical analysis of the obtained data were carried out by computer **(MSTATC Program software, 1980).** The averages of total alive insect population and the measurements of either leaf toughness and some anatomical features of Balady mango trees were subjected to calculations and were depicted graphically by Microsoft Excel 2010.

III. Result and Discussion

1. *Population Size of P. oleae and Changes in Total Numbers of its Alive Individuals*

Data obtained during both (2016/2017) and (2017/2018) experimental seasons regarding the monthly changes in means number counted of alive *P. oleae* individuals per each single Balady mango leaf are presented in Table (1).

It is obvious that the general average of alive *P. oleae* individuals per leaf of infested Balady mango trees at two rates was considerably varied as both compared each other. Herein, the infested trees showed general average of (30.67 ± 0.80 and 36.15 ± 0.96) and (105.07 ± 2.54 and 119.78 ± 2.81) alive *P. oleae* individuals for the light and heavy infested trees during the first and second seasons, respectively. Table (1) reveals also that the increase in *P. oleae* population density in leaves of the severe infested mango trees over the analogus one of the low infestation rate reached approximately 3.31 to 3.43 times during (2016/2017) and (2017/2018) experimental seasons, respectively.

2. *Leaf Toughness and Some Anatomical Characteristics of Mango Leaves*

2.1. *Leaf Toughness*

Mean toughness in the uninfested leaves (free) was 0.0026 and 0.0027 g/cm^2 compared to 0.0030 and 0.0031 g/cm^2 for the light infested leaves and 0.0033 and 0.0033 g/cm^2 for the heavy infested leaves by pest during the first and second seasons, respectively (Table 2). The increasing percentages in leaf toughness than healthy leaves (control) reached 15.12 and 14.83% for light infestation, and 24.08 and 22.71% for heavy infestation rates, respectively. The difference in leaf toughness among free, light and heavily infestation leaves was highly significant (L.S.D. value was 0.003) for each of the two seasons. Moreover, the simple correlation coefficient (r) between the different infestation rates by pest and the leaf toughness were positive and highly significant (r values were +0.75 and +0.81) for the two seasons, respectively (Table 2).

2.2. *Anatomical Structure of Mango Leaves*

A. *Upper Thickness of Epidermis Layer*

Data tabulated in Table (2) revealed that the heavily infested leaves had thinner in the upper epidermis thickness (averages of 12.89 and 13.15 μm) for the two seasons, respectively compared to 14.91 and 15.21 μm for the light infested leaves and 16.93 and 17.10 μm for the uninfested leaves, respectively. Thus showing reductions in the upper epidermis thickness in the heavy infested leaves by 23.88 and 23.12%, respectively than those of the uninfested ones. These reductions were about 11.93 and 11.06% in case of the light infested leaves. The differences among different infestation rates in the upper epidermis thickness on mango leaves were highly significant (L.S.D. value were 0.79 and 0.80) (Table 2). As well as, the simple correlation coefficient (r) between the different infestation rates by pest and the upper epidermis thickness were negative and significant (r values were -0.46 and -0.45) for the two seasons, respectively (Table 2).

Table 1. Monthly mean numbers of total alive *P. oleae* individuals per mango leaf at Esna district, Luxor Governorate during 2016/2017 and 2017/2018.

Season	**Date of Inspection**	**Mean Number of Individuals Per Leaf ± S.E.**			
		Light Infestation		**Heavy Infestation**	
		First Season	**Second Season**	**First Season**	**Second Season**
Autumn	Sept.	33.50 ± 0.92	33.16 ± 0.78	120.60 ± 2.63	125.60 ± 2.52
	Oct.	43.92 ± 0.70	44.83 ± 0.49	151.39 ± 2.15	146.07 ± 1.92
	Nov.	30.52 ± 0.59	48.94 ± 0.90	133.12 ± 2.55	154.73 ± 2.83
	Average	35.98 ± 1.14	42.31 ± 1.31	135.03 ± 2.72	142.13 ± 2.65
Winter	Dec.	19.99 ± 0.27	33.33 ± 0.46	95.43 ± 1.30	147.45 ± 2.04
	Jan.	18.03 ± 0.23	18.13 ± 0.14	69.05 ± 0.94	69.52 ± 0.47
	Feb.	14.79 ± 0.19	16.82 ± 0.39	61.14 ± 0.78	60.48 ± 1.50
	Average	17.60 ± 0.42	22.76 ± 1.41	75.21 ± 2.78	92.49 ± 7.30
Spring	Mar.	30.65 ± 0.70	29.13 ± 0.69	71.60 ± 1.65	112.52 ± 2.65
	April	33.19 ± 0.71	39.38 ± 0.86	106.46 ± 2.28	131.46 ± 2.88
	May	30.22 ± 0.58	35.61 ± 0.51	96.50 ± 1.79	103.89 ± 1.49
	Average	31.35 ± 0.45	34.71 ± 0.88	91.52 ± 2.93	115.96 ± 2.53

Season	Date of Inspection	Mean Number of Individuals Per Leaf ± S.E.			
		Light Infestation		Heavy Infestation	
		First Season	Second Season	First Season	Second Season
Summer	June	36.94 ± 0.34	43.94 ± 0.26	117.80 ± 0.69	139.80 ± 0.87
	July	41.61 ± 0.25	48.26 ± 1.13	136.59 ± 1.25	147.19 ± 2.11
	Aug.	34.71 ± 0.49	42.30 ± 0.62	101.20 ± 1.39	98.64 ± 2.31
	Average	37.76 ± 0.65	44.83 ± 0.63	118.53 ± 2.87	128.54 ± 4.10
General Average		30.67 ± 0.80	36.15 ± 0.96	105.07 ± 2.54	119.78 ± 2.81

B. *Lower Thickness of Epidermis Layer*

The uninfested leaves of mango had thicker in lower epidermis thickness (17.83 and 18.81 µm) than the heavy infested leaves (two season means; 12.57 and 12.50 µm), the light infested leaves was (15.50 and 16.43 µm) for the two seasons, respectively (Table 2). There were highly significant differences in the lower epidermis layer thickness between the different infestation levels (L.S.D. value was 1.12 and 1.20) for both the two seasons, respectively. Also, the lower epidermis layer thickness of the heavy and light infested leaves was decreased by 29.54 and 33.55% and about 13.10 and 12.63% as compared to the uninfested ones (Table 2). Moreover, the simple correlation coefficient (r) between the different infestation rates by pest and the lower epidermis thickness were negative and highly significant (r values were -0.72 and -0.78) for the two seasons, respectively (Table 2).

C. *Upper Layer Thickness of Palisade Tissue*

As reported in Table (2) revealed that the heavy infested leaves had thicker in the upper layer of palisade tissue with an averages; 44.23 and 46.32 µm compared to 38.09 and 37.28 µm for the uninfested leaves and 43.16 and 45.55 µm for the light infested leaves during the first and second seasons, respectively. These data indicated that the upper layer thickness of palisade tissue of the *P. oleae* heavily infested leaves was increased by 16.11 and 24.26% than the uninfested ones, while the light infested leaves increased about 13.31 and 22.20% during the first and second seasons, respectively. These differences between the different infestation rates were highly significant (L.S.D. values; 2.29 and 3.25) (Table, 2). Data shows also, that the simple correlation coefficient (r) between the different infestation rates by pest and the upper layer thickness of palisade tissue were positive and significant (r values were +0.45) for the first season and highly significant (+0.47) during the second season (Table 2).

D. *Lower Layer Thickness of Palisade Tissue*

Results presented in Table (2) showed that the uninfested mango leaves had slender in the lower layer of palisade tissue with an average were (21.96 and 21.72) than the heavy (27.25 and 29.43) and the light (24.27 and 25.24) infested ones during the two seasons, respectively. The increasing percentages of lower layer thickness of palisade tissue in proportion to uninfested leaves were 10.51, 16.21% for light infestation, 24.07 and 35.48% for heavy infested leaves during the both seasons, respectively. Highly significant differences were found among different infestation levels in lower layer thickness of palisade tissue (L.S.D. values; 0.68 and 0.73) (Table, 2). As well, the simple correlation coefficient (r) between the different infestation rates by pest and the lower layer thickness of palisade tissue were positive and highly significant (r values were +0.79 and +0.87) for the two seasons, respectively (Table 2).

E. *Thickness of Spongy Tissue*

The uninfested leaves of mango had thinner in spongy tissue thickness (71.32 and 70.61 µm) than the heavy infested leaves (two season means; 84.13 and 89.18 µm), the light infested leaves was (77.60 and 79.93 µm) for the two seasons, respectively (Table 2). There were highly significant differences in the spongy tissue thickness between the different infestation levels (L.S.D. value was 3.21 and 3.45) for both the two seasons, respectively. Also, the spongy tissue thickness of the heavy and light infested leaves was increased by 17.96 and 26.30% and about 8.80 and 13.20% as compared to the uninfested ones, respectively (Table 2). Moreover, the simple correlation coefficient (r) between the different infestation rates by pest and the spongy tissue thickness were positive and highly significant (r values were +0.56 and +0.68) for the two seasons, respectively (Table 2).

F. *Thickness of Collenchyma Layers Below the Upper Epidermis*

The uninfested leaves of mango had thinner in thickness of collenchyma layers below the upper epidermis with an average (60.20 and 59.62 µm) than the heavy infested leaves (71.77 and 71.91 µm) and the light infested leaves was (64.74 and 66.58 µm) during the two seasons, respectively (Table 3). The increasing percentages of thickness of collenchyma layers below the upper epidermis in proportion to uninfested leaves were 7.54, 11.68% for light infestation, 19.21 and 20.62% for heavy infested leaves during the both seasons, respectively. There were highly significant differences in the thickness of collenchyma layers below the upper epidermis between the different infestation levels (L.S.D. values were 2.03 and 2.00) for the two seasons, respectively (Table 3). Also, the simple correlation coefficient (r) between the different infestation rates by pest and the thickness of collenchyma layers below the upper epidermis were positive and highly significant (r value was +0.55) for each of the season of study (Table 3).

G. *Thickness of Leaf Midrib*

Data in Table (3) revealed that uninfested leaves of mango had thicker in leaf midrib thickness (1419.27 and 1417.57µm), followed by the light infested leaves (1413.48 and 1410.65 µm), while heavy infested leaves showed the least thickness (1410.48 and 1394.68 µm) as averages during the two seasons of study, respectively. Statistical analysis revealed highly significant differences among different infestation levels; L.S.D. values were 2.88 and 2.84, respectively. Under heavy infestation by pest, the reduction percentages in thickness of leaf midrib reached about 0.62 and 1.61%, while these reductions were about 0.41 and 0.49% for the light infested leaves comparing with healthy ones for the two seasons, respectively. Moreover, the simple correlation coefficient (r) between the different infestation rates by pest and the thickness of leaf midrib were negative and significant (r-value was -0.45) for the first season and highly significant (-0.84) during the second season (Table 3).

H. *Number of Xylem Rows in the Vascular Bundle*

Results revealed more numbers of xylem rows in the heavy infested leaves with an averages of (4.40 and 4.50 rows), respectively than those of the uninfested ones (4.00 and 4.00 rows) and 4.20 and 4.20 rows in the light infested leaves, respectively. These values confirmed increasing in the number of xylem rows in the heavy infested trees by 10.00 and 12.50% than the uninfested ones during the two seasons, respectively, while the light infested trees increased about 5.00% for each of the season of study. Statistical analysis of data showed highly significant differences in the number of xylem rows among the different infestation levels (L.S.D. values were 0.55 and 0.32) (Table, 3). As well, the simple correlation coefficient (r) between the different infestation rates by pest and the number of xylem rows in the vascular bundle were feeble positively and insignificant (r value was +0.12 and +0.15) for the two season of study, respectively (Table 3).

I. *Thickness of Widest Xylem Vessel in the Vascular Bundle*

The uninfested leaves of mango had thicker in xylem vessel widest thickness in the vascular bundle as averages of (53.25 and 56.44 µm) than the heavy infested leaves (42.99 and 45.91 µm), the light infested leaves was (50.03 and 50.53 µm) during the two seasons, respectively (Table 3). There were highly significant differences in the xylem vessel widest thickness in the vascular bundle between the different infestation levels (L.S.D. value was 1.16 and 1.13) for the two seasons, respectively. Also, the thickness of widest xylem vessel in the vascular bundle of the heavy and light infested leaves was decreased by 19.26 and 18.65% and about 6.04 and 10.48% as compared to the uninfested ones during the two seasons, respectively (Table 3). Moreover, the simple correlation coefficient (r) between the different infestation rates by pest and the xylem vessel widest thickness in the vascular bundle, were negative and highly significant (r values were -0.74 and -0.69) for the two seasons, respectively (Table 3).

J. *Length of Midrib Vascular Bundle*

Data represented in Table (3) cleared that the uninfested leaves had smaller in the length of midrib vascular bundle with an averages of (243.63 and 253.37 µm) for the two seasons, respectively compared to 278.18 and 294.96 µm for the heavily infested leaves and 260.08 and 265.54 µm, respectively for the light infested leaves. So, the increasing in the length of midrib vascular bundle in the heavy infested leaves by 14.18 and 16.42%, respectively than those of the uninfested ones. As well, about 6.75 and 4.80% in case of the light infested leaves. The differences among different infestation rates in the length of midrib vascular bundle on mango leaves were highly significant (L.S.D. value were 4.76 and 4.68) (Table 3). As well as, the simple correlation coefficient (r) between the different infestation rates by pest and the length of midrib vascular bundle were positive and significant (r values were +0.36 and +0.43) for the two seasons, respectively (Table 3).

K. *Width of Midrib Vascular Bundle*

As reported in Table (3), the uninfested leaves of mango had thicker in width of midrib vascular bundle as averages of (189.24 and 235.70 µm) than the heavy infested leaves (two season means; 156.78 and 189.87 µm), the light infested leaves was (180.82 and 196.71 µm) for the two seasons, respectively (Table 3). There were highly significant differences in the width of midrib vascular bundle between the different infestation levels (L.S.D. values were 3.65 and 4.94) for both the two seasons, respectively. Also, the width of midrib vascular bundle of the heavy and light infested leaves was decreased by 17.15 and 19.44% and about 4.45 and 16.54% as compared to the uninfested ones (Table 3). Moreover, the simple correlation coefficient (r) between the different infestation rates by pest and the width of midrib vascular bundle were negative and significant (r values were -0.46 and -0.43) for the two seasons, respectively (Table 3).

3. *The Relationship between the Changes in Numbers of alive P. oleae Individuals and the percentages of Reduction or Increasing % in Leaf Toughness and some Anatomical Characteristics*

Measurements

Concerning, the relationship between the percentages of changes (±) % in the measured parameters for mango trees represented as dependent variable (Y) and the different infestation rates by *P. oleae* represented as independent factor (X) data obtained during two successive seasons of (2016/2017 and 2017/2018) are tabulated in Table (4).

Results revealed a strong highly significantly positive correlations between the rates of infestation with *P. oleae* and percentages of reduction in thickness of upper and lower epidermis layers, thickness of widest xylem vessel in the vascular bundle and width midrib vascular bundle (r values; +0.68, +0.69, +0.91 and +0.95) for the first season and (+0.68, +0.77, +0.81 and +0.59) during the second season was detected, respectively. As well, the calculated regression coefficient (b) indicated that an increase of one insect per mango leaf, would increase the percentages of reduction in such thickness of upper epidermis layer by (0.16 and 0.14%), and its lower layer about (0.21 and 0.24%), thickness of widest xylem vessel in the vascular bundle by (0.17 and 0.09%) and thickness of width midrib vascular bundle about (0.17 and 0.04%) during the both two seasons, respectively (Table 4).

However, the effect rates of infestation with *P. oleae* on the percentages of reduction in thickness of leaf midrib were insignificantly positive correlation (r-value was +0.38) during the first season and highly significantly positive relation (+0.91) through the second season. In the same time, the simple regression coefficient indicated that an increase of one insect per mango leaf, would increase the percentages of reduction in thickness of leaf midrib by 0.003 and 0.01% during the two seasons, respectively.

On the contrary, correlation between the effect of different infestation rates by pest and the percentages of increasing in the leaf toughness, lower layer thickness of palisade tissue, thickness of spongy tissue, thickness of collenchyma layers below the upper epidermis at midrib and thickness of length midrib vascular bundle as shown in Table (4) which reveals highly significant negative values (r were -0.86, -0.85, -0.61, -0.74 and -0.78) during the first season and (-0.94, -0.90, -0.73, -0.73 and -0.87) through second season, respectively. As well, the calculated regression coefficient (b) indicated that an increase of one insect per mango leaf, would increase the percentage of raising by (0.12, 0.18, 0.12, 0.16 and 0.10%) for the first season and (0.09, 0.23, 0.15, 0.11 and 0.14%) during the second season data, respectively (Table 4).

Furthermore, the statistical analysis of simple correlation (Table 4) showed that feeble negatively relations and insignificant between the different infestation rates by pest and the percentages of increasing in the upper layer thickness of palisade tissue (r values; -0.20 and -0.12), as well number of xylem rows in the vascular bundle (r values; -0.14 and -0.25) during both seasons of study, respectively. As well, the simple regression coefficient (b) indicated that an increase by one insect per mango leaf, would increase the raising percentages in the upper layer thickness of palisade tissue by (0.04 and 0.04%) and about (0.06 and 0.11%) increase in number of xylem rows in the vascular bundle for the two seasons, respectively.

In similar studies, **Shalaby (1998)** in Egypt, found that common bean, Giza variety which characterized by higher palisade and spongy tissue thickness in micron proved to be less susceptible to infestation with *B. tabaci* and *Aphis spp*., while, the infestation of these insects increased on Bronco variety which possessing the lower thickness of palisade and spongy tissues. **Koschier *et al.* (2002)** reported that nymphs and adults of *Thrips* *tabaci* feed on green leaf tissue, causing direct damage by destroying epidermal cells on onion plants. **Legrand and Barbosa (2003)** reported that increased plant morphological complexity decreased the efficiency of *Coccinella syptumpunctata* adults with aphids *Acyrthosiphon pisum* on pea plants. **Tantawy, Maha (2006)** indicated that the population density of aphids and whitefly were positively correlated with lower & upper epidermis and negatively correlated with palisade and spongy tissues. On the other hand, *T. tabaci* population was positively associated with palisade and spongy layers and negatively with upper and lower epidermis. **Abou-Zaid (2013)** stated that the population density of *T. urticae* infesting cucumber plants was negatively correlated with upper & lower epidermis and palisade layers and positive with spongy tissue layer. **Hanafy *et al.* (2014)** recorded that the population densities of (thrips, *Thrips tabaci*, aphids, whitefly, *Bemisia. tabaci* and the spider mite, *Tetranychus urticae*) pests on cucumber and kidney bean had positive relationships and significant with palisade and spongy layers. While, this relation was significantly negative with upper and lower epidermis. *i.e.*, infestation rates of all studied pests increased by increasing the thickest layers of palisade and spongy tissues and decreased by increasing the thickest of upper and lower epidermis. **Salem *et al.* (2007)** recorded that the Ewaisi and Alphonoso varieties of mango had the smallest population densities by *Icerya seychellarum* (Hemiptera: Monophlebidae) than the other Egyptian mango varieties and that their contains the highest toughness of leaves and resistance of pest.

Based on the data summarized in Tables (2–3), it could be concluded that the percentages of abnormality (% increase or % decrease) in relation to the standard measurements for the infested leaves (light and heavy) must be compared between uninfested leaves. General averages of population overall the every season were compared among the different infestation rates by *P. oleae* and were also linked with the quantitative changes of various anatomical measurements (Table, 4).

The results revealed that the thickness both of [epidermis layer dimensions (upper & lower), leaf midrib, widest xylem vessel in the vascular bundle and width of midrib vascular bundle] in leaves of uninfested mango trees was the thickest than those of the infested ones (light and heavy), would be helpful to prevention for *P. oleae* infestation on mango leaves. That is, the infestation by pest decreases as the thickness of these layers increases (negative relationship).

While, in contrast, the leaf toughness together with thickness both of palisade tissue dimensions, spongy tissue, collenchyma layers below the upper epidermis at midrib, number of xylem rows in the vascular bundle and length of midrib vascular bundle), increased significantly in the leaves of infested trees (light and heavy) as compared to the uninfested ones (Tables 2 and 3).

Based on *P. oleae* feeding mechanism and damage, whenever less thickness for both of (epidermis layer dimensions (upper& lower), leaf midrib, widest xylem vessel in the vascular bundle and width of midrib vascular bundle), would be helpful and facilitation for *P. oleae* feeding and achieving damage to mango leaves. That is, the infestation by pest increases as the thickness of these layers decreases (negative relationship).

A positive correlations between different population densities of *P. oleae* and leaf toughness together with thickness in each of palisade tissue dimensions, spongy tissue, collenchyma layers below the upper epidermis at midrib, number of xylem rows in the vascular bundle and length of midrib vascular bundle were measured and recorded (Tables, 2 and 3). It was clear that the reflection of the insect infestation levels on the anatomical properties was positive that refers to the proportional increase in the percentages of loss in the measured parameter *i.e.*, thickness for epidermis layer dimensions (upper& lower), leaf midrib, widest xylem vessel in the vascular bundle and width of midrib vascular bundle as infestation rate was increased (Table 4). Meaning, whenever increase infestation by pest, would increase the percentages of reduction in the thickness of these layers (positive relationship).

The harmful / destructive effects of the *P. oleae* infestation on the differential investigated the leaf toughness together with anatomical characteristics of Balady mango seedlings trees it could be logically explained / discussed depending upon the depletion / exhausting of the photosynthetics substances and other nutritive materials exhibited in the infested plants through sucking the cell sap with the pest mouth, either indirectly as stopping of plant tissue growth, which in turn manifests as leaf loss and may cause further leaf drop. In addition, toxic saliva secreted by the pest may result in malformed leaves and poor shoot growth. Consequently, a considerable shortage of such important nutritive components in tissues of various infested plants organs has been taken place and induced some interruption in different bio-physical processes to take place normally. So, *P. oleae* infestation was reflected negatively on some the desirable mango measurements particularly anatomical characteristics aspects.

From the explained results, it could be concluded that the P. oleae heavily infested leaves exhibited the highest reduction in studied measurements i.e. [thickness for epidermis layers (upper & lower), leaf midrib, widest xylem vessel in the vascular bundle and width of midrib vascular bundle], except those of (the leaf toughness together with thickness in each of palisade tissue dimensions, spongy tissue, collenchyma layers below the upper epidermis at midrib, number of xylem rows in the vascular bundle and length of midrib vascular bundle) as compared with the free and light infested leaves. Generally, it seems that the differences (increase or decrease) in the measured parameters was a summation of many factors including not only the investigated one (rate of infestation) but also others such as irrigation, nutritional programs adopted in the orchard and climatic condition prevailing in the region. Obtained results of our present research paper threw a green light towards the necessity for planning an applicable environmentally safe approaches to control such harmfull scale insects and like other pests through an integrate program, as it is necessity to foresee with the pest status.

Table 2. Leaf toughness and thickness of some (epidermis, palisade & spongy) measurements of Balady mango seedling leaves as affected by *P. oleae* infestation rates, as well, the relation of them during two consecutive seasons (2016/2017 and 2017/2018).

Measurements / *P. oleae* Infestation Rate	**General average of *P. oleae* Individuals per leaf**	**Leaf Toughness**		**Thickness of Epidermis Layer**				**Thickness of Palisade Tissue**				**Thickness of Spongy Tissue**	
		g/cm^2	**± (%)**	**Upper (µm)**	**± (%)**	**Lower (µm)**	**± (%)**	**Upper (µm)**	**± (%)**	**Lower (µm)**	**± (%)**	**(µm)**	**± (%)**
		2016/2017 Season											
Free	Zero	0.0026 c	…….	16.93 a	…….	17.83 a	…….	38.09 c	…….	21.96 c	…….	71.32 c	…….
Low	30.67	0.0030 b	(+) 15.12	14.91 b	(-) 11.93	15.50 b	(-) 13.10	43.16 b	(+) 13.31	24.27 b	(+) 10.51	77.60 b	(+) 8.80
Severe	105.07	0.0033 a	(+) 24.08	12.89 c	(-) 23.88	12.57 c	(-) 29.54	44.23 a	(+) 16.11	27.25 a	(+) 24.07	84.13 a	(+) 17.96
L.S.D. at 5%	1.62**	0.003**	……	0.79**	…….	1.12**	…….	2.29**	……	0.68**	……	3.21**	……

Measurements / *P. oleae* Infestation Rate	**General average of *P. oleae* Individuals per leaf**	**Leaf Toughness**		**Thickness of Epidermis Layer**				**Thickness of Palisade Tissue**				**Thickness of Spongy Tissue**	
		g/cm^2	**± (%)**	**Upper (µm)**	**± (%)**	**Lower (µm)**	**± (%)**	**Upper (µm)**	**± (%)**	**Lower (µm)**	**± (%)**	**(µm)**	**± (%)**
r-value		0.75**	…..	-0.46*	…..	-0.72**	…..	0.45*	……	0.79**	…..	0.56**	……
2017/2018 Season													
Free	Zero	0.0027 c	…..	17.10 a	…..	18.81 a	……	37.28 c	……	21.72 c	……	70.61 c	……
Low	36.15	0.0031 b	(+) 14.83	15.21 b	(-) 11.6	16.43 b	(-) 12.63	45.55 b	(+) 22.20	25.24 b	(+) 16.21	79.93 b	(+) 13.20
Severe	119.78	0.0033 a	(+) 22.71	13.15 c	(-) 23.12	12.50 c	(-) 33.55	46.32 a	(+) 24.26	29.43 a	(+) 35.48	89.18 a	(+) 26.30
L.S.D. at 5%	1.75**	0.003**	….	0.80**	…..	1.20*	…..	3.25**	…..	0.73*	…..	3.45**	……
r-value		0.81**		-0.45*		-0.78**		0.47**		0.87**		0.68**	……

± (%) refers to the percentage of increase (+) or reduction (-) in studied measurements exhibited by infestation two rates as compared to control (infestation free).

Means followed by the same letter/s within each column did not significantly differ at 5% level. r-value refers to the simple correlation *i.e.*, the relationship between the mean population density of *P. oleae* and anatomical measurements of Balady mango seedling leaves.

Table 3. Thickness of (collenchyma layers, leaf midrib, widest xylem, midrib length and its width) measurements, as well, no. of xylem rows in the vascular bundle of Balady mango seedling leaves as affected by *P. oleae* infestation rates, as well, their relationship to each other during two consecutive seasons (2016/2017 and 2017/2018).

Measurements / *P. oleae* Infestation Rate	**General average of *P. oleae* Individuals per leaf**	**Thickness of Collenchyma layers below the upper epidermis**		**Thickness of Leaf Midrib**		**Number of Xylem Rows in the Vascular Bundle**		**Thickness of Widest Xylem Vessel in the Vascular Bundle**		**Length of Midrib Vascular Bundle**		**Width of Midrib Vascular Bundle**	
		(µm)	**± (%)**	**(µm)**	**± (%)**	**No.**	**± (%)**	**(µm)**	**± (%)**	**(µm)**	**± (%)**	**(µm)**	**± (%)**
2016/2017 Season													
Free	Zero	60.20 c	....	1419.27 a	…….	4.00 c	…….	53.25 a	…….	243.63 c	…….	189.24 a	… …
Low	30.67	64.74 b	(+) 7.54	1413.48 b	(-) 0.41	4.20 b	(+) 5.00	50.03 b	(-) 6.04	260.08 b	(+) 6.75	180.82 b	(-) 4.45
Severe	105.07	71.77 a	(+) 19.21	1410.48 c	(-) 0.62	4.40 a	(+) 10.00	42.99 c	(-) 19.26	278.18 a	(+) 14.18	156.78 c	(-) 17.15
L.S.D. at 5%	1.62**	2.03**	……	2.88**	…….	0.55*	…….	1.16**	……	4.76**	……	3.65**	…...
r-value		0.55**	…..	-0.45*	…..	0.12**	…..	-0.74*	……	0.36**	…..	-0.46**	…...

Measurements / ***P. oleae* Infestation Rate**	**General average of *P. oleae* Individuals per leaf**	**Thickness of Collenchyma layers below the upper epidermis**		**Thickness of Leaf Midrib**		**Number of Xylem Rows in the Vascular Bundle**		**Thickness of Widest Xylem Vessel in the Vascular Bundle**		**Length of Midrib Vascular Bundle**		**Width of Midrib Vascular Bundle**	
		(µm)	**± (%)**	**(µm)**	**± (%)**	**No.**	**± (%)**	**(µm)**	**± (%)**	**(µm)**	**± (%)**	**(µm)**	**± (%)**
2017/2018 Season													
Free	Zero	59.62 c	…..	1417.57 a	…..	4.00 c	……	56.44 a	……	253.37 c	……	235.70 a	……
Low	36.15	66.58 b	(+) 11.68	1410.65 b	(-) 0.49	4.20 b	(+) 5.00	50.53 b	(-) 10.48	265.54 b	(+) 4.80	196.71 b	(-) 16.54
Severe	119.78	71.91 a	(+) 20.62	1394.68 c	(-) 1.61	4.50 a	(+) 12.50	45.91 c	(-) 18.65	294.96 a	(+) 16.42	189.87 c	(-) 19.44
L.S.D. at 5%	1.75**	2.00**	….	2.84**	…..	0.32*	…..	1.13**	…..	4.68*	…..	4.94**	……
r-value		0.55**	…..	-0.84*	……	0.15	…..	-0.69**	……	0.43**	…..	-0.43*	……

± (%) refers to the percentage of increase (+) or reduction (-) in studied measurements exhibited by infestation two rates as compared to control (infestation free).

Means followed by the same letter/s within each column did not significantly differ at 5% level. r-value refers to the simple correlation *i.e.*, the relationship between the mean population density of *P. oleae* and anatomical measurements of Balady mango seedling leaves.

Table 4. Simple correlation, regression values and linear regression equation when the counts of the mean population density of *P. oleae* were plotted versus the percentages of reduction in each of the anatomical measurements of the Balady mango seedling leaves through the two successive seasons (2016/2017 and 2017/2018).

Season / **Treatments**	**First Season (2016/2017)**						**Second Season (2017/2018)**					
	r	**b**	**S.E.**	**T-test Value**	**Y = a ± bx**	**E.V. %**	**r**	**b**	**S.E.**	**T-test Value**	**Y = a ± bx**	**E.V. %**
Leaf Toughness	-0.86	-0.12	0.017	7.12**	-11.63-0.12 x	73.83	-0.94	-0.09	0.008	11.57**	-1143-0.09x	88.17
Upper Epidermis Layer	0.68	0.16	0.041	3.90**	7.34+0.16 x	45.82	0.68	0.14	0.036	3.92**	6.15 + 1.04 x	46.10
Lower Epidermis Layer	0.69	0.21	0.053	4.03**	7.12+0.21 x	47.45	0.77	0.24	0.047	5.16**	4.36+0.24 x	59.69
Upper Layer of Palisade Tissue	-0.20	-0.04	0.047	0.88	-11.61-0.04 x	4.14	-0.12	-0.04	0.067	0.53	-19.88-0.04 x	1.52
Lower Layer of Palisade Tissue	-0.85	-0.18	0.027	6.81**	-4.93-0.18 x	72.07	-0.90	-0.23	0.026	9.02**	-7.89-0.23 x	81.89
Spongy Tissue	-0.61	-0.12	0.035	3.30**	-5.60-0.12 x	37.70	-0.73	-0.15	0.033	4.50**	-8.15-0.15 x	52.99
Collenchyma Layers	-0.74	-0.16	0.034	4.70**	-2.73-0.16 x	55.07	-0.73	-0.11	0.024	4.55**	-7.42-0.11 x	53.54
Leaf Midrib	0.38	0.003	0.001	1.73	0.34 + 0.003 x	14.33	0.91	0.01	0.001	9.26**	0.02+0.01 x	82.65

Season / Treatments	First Season (2016/2017)						Second Season (2017/2018)					
	r	**b**	**S.E.**	**T-test Value**	**Y = a ± bx**	**E.V. %**	**r**	**b**	**S.E.**	**T-test Value**	**Y = a ± bx**	**E.V. %**
No. of Xylem Rows	-0.14	-0.06	0.099	0.60	-4.88-0.06 x	1.97	-0.25	-0.11	0.098	1.10	-3.05-0.11 x	6.24
Widest Xylem Vessel in the Vascular Bundle	0.91	0.17	0.019	9.11**	0.95-0.17 x	82.17	0.81	0.09	0.016	5.93**	7.26-0.09 x	66.15
Length of Midrib Vascular Bundle	-0.78	-0.10	0.018	5.30**	-4.07-0.10 x	60.93	-0.87	-0.14	0.018	7.62**	-0.16-0.14 x	76.33
Width of Midrib Vascular Bundle	0.95	0.17	0.013	12.92**	-0.72 + 0.17 x	90.28	0.59	0.04	0.012	3.09**	15.26 + 0.04 x	34.68

r = Simple correlation, S.E = Standard error, Y = a ± bx (Regression linear equation), b = Simple regression, T-test = T-test calculated, E.V. % = Explained variance.

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