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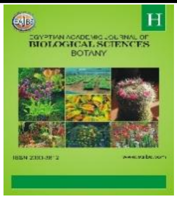

Multi-trait Analysis and Anatomical Characterization of Mango Cultivars: Insights into Mealybug Resistance and Horticultural Performance

Moustafa M.S. Bakry[1]; Lamiaa H.Y. Mohamed[1]; Rania M. Taha[*2]and Eman F.M. Tolba[3]

[1]Agricultural Research Center, Plant Protection Research Institute, Scale Insects and Mealybugs Research Department, 7, Nady El-Sayied Street, Dokki, 12619 Giza, Egypt.

[2]Botany and Microbiology Dept., Faculty of science, Minia University, El-Minia61519, Egypt.

[3]Plant Protection Dept., Faculty of Agric, New valley University, New valley, Egypt.

*E. Mail: md.md_sabry@yahoo.com ; ranya.taha@mu.edu.eg

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ABSTRACT

The mealybug *(Ferrisia virgate)* is a major agricultural pest, affecting various crops and potentially damaging mango trees. **Purpose:** Evaluate the relationships between the anatomical characteristics of the tested mango cultivar leaves and the mealybug infestation. **Results:** Zebda mango cultivar had the maximum number of *F. virgata,* possessed the thinnest upper and lower epidermal layers both on the main leaf and at the "wing". Its leaf midrib is thinner and narrower compared to other mango cultivars. Moreover, this cultivar exhibits smaller dimensions in several vascular tissues, including phloem resin canal, central vascular cylinder, and xylem vessel in the vascular bundle. These anatomical characteristics might contribute to higher pest susceptibility. In contrast, Taimour mango cultivar had the lowest *F. virgata* population density and characterized by thicker upper and lower epidermal layers, which may be a defensive response to the pest. This implies that certain anatomical characteristics may play a role in the resistance of mango trees to pests.

INTRODUCTION

Mango trees, or *Mangifera indica* L., are tropical fruit trees that produce juicy and delicious stone fruit (Elhalawany *et al.,* 2023). The most damaging pest for many crops is mealybugs, but they are particularly damaging for mango trees (Bakry, 2009). The striped mealybug, *Ferrisia virgata* (Cockerell) (*Hemiptera: Pseudococcidae*), is an invasive insect and one of the most common agricultural pests, found on a variety of commodity crops (Nabil *et al.,* 2020). It reported that mealybugs are some of the biggest global agricultural pests and can be harmful to mango trees. They are tiny, soft-bodied insects that surround themselves in large cottony masses of white, waxy material, found on leaf axils, veins, stems, branches, shoots, flowers, and fruit. Similar to aphids, mealybugs feed on the sap from the plant, which weakens the plant itself, causing a lot of stress on the plant and potentially killing the plant (Balboul, 2003; Garcia *et al.,* 2015; Ata *et al.,* 2019).

Mealybug infestations can cause leaf discoloration, stunted growth, and decreased quality of fruit. They can also cause fruit drop, probably because of the stress on the tree, and can leave branches weak, unhealthy, or dead. Mealybugs produce a sticky, fatty

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substance and excrete a waste called "honeydew." This substance can accumulate, so it seen on the leaves and stems of the plant or the fruit. Honeydew accumulates, and while everyone knows of it, the accumulation of honeydew may indicate a mealybug infestation. Honeydew will attract ants and sooty mold, which is a black fungus that can cover the leaves (Balikai *et al.,* 2011). In most cases, observing increased types of ants on a mango tree is a good indicator of mealybug infestations (Bakry and Fathipour, 2023). Mango trees experience direct and indirect damage from mealybug infestations, which shown to cause reduced yields in inhibitory fruit quality and quantity.

A plant may directly incur injury by the loss of sap, which can disrupt water flow (Bakry *et al.,* 2023) and the flow of nutrients, which can create nutrient deficiencies, stunted growth, and yellowing of leaves that help to weaken the plants (Mittler & Douglas, 2003). As Bakry *et al.* (2024) stated, farmers have often stated they have difficulty controlling mealybug problems once they have taken hold.

The interaction between insect pests and plants would indicate what is happening with leaf quality regarding the insect's preference for the toughness and anatomical features of leaves. Studies by Chen *et al.* (2002) suggest that the differences in vulnerability to insect pests in a given plant species may be caused by several factors or a combination of the factors. Mango trees have been variable in their response to the insect pests, which could be due to several factors, including the physical properties of the plants and chemical components of the leaves and anatomical traits. The population density of *Icerya seychellarum* (*Hemiptera: Monophlebidae*), which is a mealybug pest on mango, can be affected by several factors, including leaf quality, which includes the secondary metabolites of leaves (Abd-El-Rahman *et al.,* 2006), leaf nutrients and their inhibitors (Salem *et al.,* 2006), and leaf toughness and its anatomical traits (Salem *et al.,* 2007).

Because of the physiological and behavioral traits of the plant, the accumulation of insect species and/or population species in a plant host may also indicate or affect the suitability of host preference by those insect species. Additionally, for insect species, there may be genetic or phenotypic problems related to the variety of external climatic conditions (Dale, 1988). The traits of the plant leaves are crucial for controlling the scale of insects in the mango crop. Herbivore preferences and performance may be influenced by plant leaf characteristics (Gianoli and Hannunen, 2000).

Insects present biotic stress to the plants with which they are associated. There are some mango cultivars preferred by mealybugs (as well as other insect pests), and population levels may vary by cultivar (Karar *et al.,* 2009). Furthermore, the mango trees will respond as differently to insect outbreaks depending upon the morphology of the leaves. While physiological (or morphological) traits will affect how a pest feeds or how it engages in respective behaviors when additives utilized in feeding or ingested (Karar *et al.,* 2015). The phrase "plant resistance to insect pests" defined as the capacity of a plant to produce a characteristic that possesses resistance attributes in interaction with one or more elements of the association between an insect pest and a host plant (Stout, 2014). The form of genetic diversity and the soil nutrient- and mineral-rich environment of space cultivars of plants that are resistant to insects are vital for integrated pest management, which lessens pest damage (Bakry and Abdel-Baky, 2020). It is crucial to have some knowledge of the insect's related traits to choose cultivars that show minimal susceptibility (Salem *et al*., 2006). The leaves of mango cultivars with thicker cuticles may be impervious to insect penetration, according to Salem *et al.* (2007).

The authors are aware of very few studies that assessed how insect infestation affected the anatomical characteristics of several mango cultivars. Since Aswan, Egypt has the ideal climate for mango cultivation, the majority of the accession species grown there, particularly in Kom Ombo. The primary goal of this study is to evaluate the relationship between the striped mealybug populations and the anatomical responses of six different mango cultivars. These alterations show how mango trees react to pest attacks both structurally and physiologically, frequently as a defense mechanism or because of tissue damage.

MATERIALS AND METHODS

1- Population Estimates of Mealybug *F. virgata* of Some Mango Cultivars:
1.1 - Study Location of Mango Cultivars:

The field location for the assessments of mango cultivars in the 2022–2023 and 2023–2024 growing seasons was an exclusive mango orchard in Kom Ombo, Aswan Governorate, southern Egypt (24°30'55" N, 32°57'15"). The plantation is 240,000 m², strategically chosen along the Nile River, and has six mango cultivars of economic importance: Zebda, Ewaise, Taimour, Goleck, Hindi Bisinnara, and Sediek. These cultivars are mature and chosen because of their high commercial importance in the region. Cultivars selected at random, and no pest control measures applied to the randomly chosen trees.

1.2- Experimental Design and Sampling:

Six ten-year-old trees of each cultivar randomly selected, and all of the trees were cultivated using conventional horticultural methods. Leaf sample collections were made biweekly from the trees, with a sample of 40 leaves per tree (240 leaves per cultivar on each sampling date over the two-season program). During the two-year study; 69,120 leaves harvested and examined for the two-year study (i.e., 34,560 leaves per year). The sample collection procedure consisted of randomly selecting leaves from different strata and sections of the trees, putting the leaves in paper bags, and transporting them to a laboratory for examination with a stereomicroscope.

1.3 - Data Collection and Analysis:

The individual number of *F. virgata* on the mango leaf surfaces thoroughly counted and recorded. The data were prepared and organized in Microsoft Excel and subjected to statistical analysis in SPSS (1999). The means compared using Tukey's HSD test ($p \leq 0.05$) and an analysis of variance (ANOVA).

2- Measurements of Anatomical Traits of Mango Cultivars:

Fresh leaf samples from mango cultivars were collected, after which they were washed in tap water followed by distilled water to remove dust and other residues and were then preserved in formalin acetic acid alcohol solution (F.A.A.) embedded in paraffin wax for anatomical preparation and then serially sectioned at 10-15 µ according to the standard method (Johansen, 1940). Sections stained with a combination of crystal violet-erythrosine (saturated in clove oil). Micrometric leaf characteristics were examined and recorded using an Olympus© CHS Binocular Microscope. Photomicrography performed with a Sony® digital camera connected to a microscope. The triggers were performed automatically (scale 200 µm) by ImageJ® Tool software (Rasband, 2011). Description of each slide and reached mango cultivars leaves in the Central Laboratory for Microanalysis and Nanotechnology, Minia University, Egypt. This research may assist in determining the cause of infestation by pests and the triggering of pest attacks that might lead to widespread infestations.

Anatomical characteristics measured based on microscopic analysis, and the following measurements made:

Upper & lower epidermis layer thickness (µm), upper & lower epidermis at wing (µm), thickness of palisade tissue layer (µm), thickness of spongy tissue layer (µm), thickness of leaf midrib (µm), width of leaf midrib (µm), No. of phloem resin canals, No. of pith resin canals, width of largest phloem resin canal (µm), length & width of central vascular cylinder (µm), thickness of collenchyma layers below the upper and lower epidermis at midrib (µm), No. of xylem rows in the vascular bundle, and thickness of widest xylem vessel in the vascular bundle (µm) of mango leaves.

3- The relationships between counts of *F. virgata* and anatomical traits on six mango cultivars.

The Pearson's correlation coefficient utilized to investigate the association between the mean count of *F. virgata* individuals per leaf/season of each mango cultivar and anatomical traits on six mango cultivars, analysed using SPSS (1999). All the information collected was calculated, portrayed, and illustrated using Microsoft Excel 2010. Pearson's simple correlation coefficients between the several attributes computed in R (R Core Team, 2023).

4- Heat maps and hierarchical clustering for anatomical trait-related parameters on six mango cultivars as a result of infestation by *F. virgata* over the average of two seasons studied.

As the parameters being studied, the hierarchical clustering analysis (HCA) using Euclidean distance using the un-weighted paired group method with arithmetic average (UPGMA). A dendrogram of six mango cultivars based on a similarity coefficient created using two-season means of anatomical traits, analysed in R software (R Core Team, 2023).

RESULTS AND DISCUSSION

1- Population Ecology of *F. virgata* Infesting Different Mango Cultivars:

The presence of *F. virgata* was observed on all mango cultivars throughout the two-year period. The Zebda cultivar had the greatest mean number of *F. virgata* numbers per leaf (9.73 ± 1.32 individuals per leaf) over the course of the two-year average. But the cultivar that acquired the smallest number of *F. virgata* was Taimour, which averaged 2.52 ± 0.32 insects per leaf. However, as the findings in Figure 1 demonstrate, the date palm cultivars Goleck, Hindi Bisinnara, Sediek, and Ewaise exhibited moderate infestation. The average number of individuals per leaf was 6.56 ± 0.67, 6.25 ± 0.72, 4.48 ± 0.49, and 3.88 ± 0.37, respectively, as shown in Figure 1.

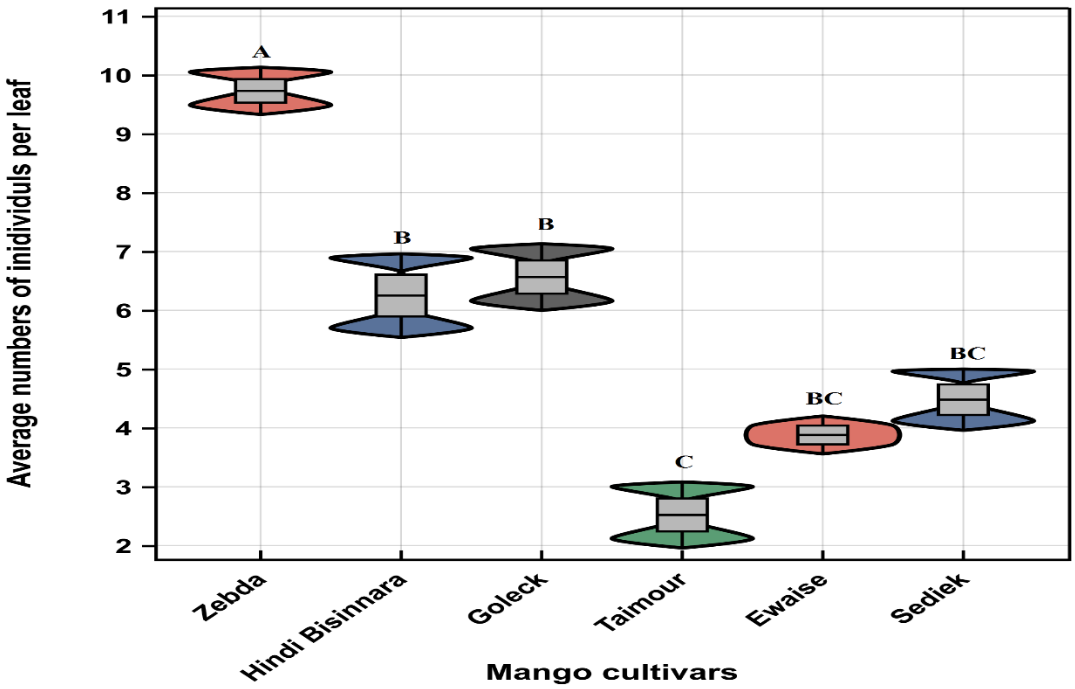

Fig. 1. Violin plot displaying the average numbers of *F. virgata* counts per leaf on tested mango cultivars through the two-year average. Different letters indicate significant difference among the evaluated mango cultivars (ANOVA, Tukey's HSD test, $p \leq 0.05$).

The present findings are largely consistent with currently available findings, albeit with the possible differences in mango varieties, infestation sites, and insect pests. Selim (2002) investigated how susceptible five mango varieties Hindy, Mabrouka, Dabsha, Kobania, and Taimour were to infestation by the two scale insects *Insulaspis pallidula* (Green) and *Aonidiella aurantii* (Mask.). Similarly, in our research on mealybugs, he found Taimour to exhibit resistance to infestation from both scale insect pests, while Hindi Bisinnara found to be the most susceptible. Bakry (2009) similarly measured four mango varieties in Egypt to describe differences in susceptibility to *I. pallidula* and *A. aurantii*, and then reported/expressed both Hindi Bisinnara and Goleck as having moderate infestations. Bakry and Dahi (2020) similarly reported that, for the total population size of the scale insect pest *Parlatoria oleae* (Colvée), the Balady mango cultivar had the highest number of individuals and classified that mango variety as very sensitive. The Zebda variety reported as comparatively resistant, while the Ewaise and Goleck mango varieties classified as sensitive. Conversely, the Sediek variety reported with the lowest population estimates and a moderate level of pest resistance over the year. Mokhtar (2022) reported that the Fagri Klan mango cultivar had the greatest average total population of *Icerya seychellarum* (Westwood) in Egypt, but the Skare and Ewaise varieties exhibited a high level of resistance, in concurrence with our mealybug research, and displayed no signs of infestation from a scale insect pest.

2- Evaluation of the Anatomical Characteristics of the Leaves of the Tested Mango Cultivars:

Figure 2 showed the anatomical characteristics of the tested mango cultivar leaves. The results exhibited that Zebda mango cultivar had the maximum number of *F. virgata* and possessed the thinnest upper and lower epidermis layers, both on the main leaf and at the "wing." Additionally, its leaf midrib is thinner and narrower compared to other mango cultivars. Moreover, this cultivar exhibits smaller dimensions in several vascular tissues, including the narrowest largest phloem resin canal, central vascular cylinder, and widest xylem vessel in the vascular bundle. Conversely, it had thicker palisade and spongy tissue layers, a greater length of the central vascular cylinder, and thicker collenchyma layers beneath both the upper and lower epidermis at the midrib. Finally, it is characterized by a higher count of both phloem and pith resin canals, and these canals are generally thicker than those found in other mango cultivars, as shown in Table 1.

In this context, the Taimour mango cultivar leaves show distinct anatomical characteristics when compared to other mango cultivars. They exhibit the lowest number of *F. virgata.* Furthermore, Taimour leaves have the thickest upper and lower epidermis layers, which may be a defensive response against the pest. Their largest phloem resin canal, central vascular cylinder, and widest xylem vessel in the vascular bundle are also the thickest. Conversely, they possess the thinnest central vascular cylinder length and the thinnest collenchyma layers under the upper epidermis at the midrib (Table 1). These changes may reflect defense mechanisms and contribute to cultivar resistance.

All the anatomical characteristics examined on the measured mango cultivars showed highly statistically significant variations, as shown in Table 1.

When insect infestation occurs, vascular tissues alter, mesophyll or xylem cell layers shrink because of nutritional degradation, and leaf tissues enlarge, affecting transpiration rates and water balance. Transport of nutrients and water hampered by tearing and blockage of phloem and xylem tissues. To reduce water loss caused by insect infestation, stomata density or closure patterns also change. Furthermore, the plant's general growth and photosynthesis performance could be hampered, which would make recovery and resistance to infestations more difficult. (Bakry *and* Abdel-Baky, 2020).

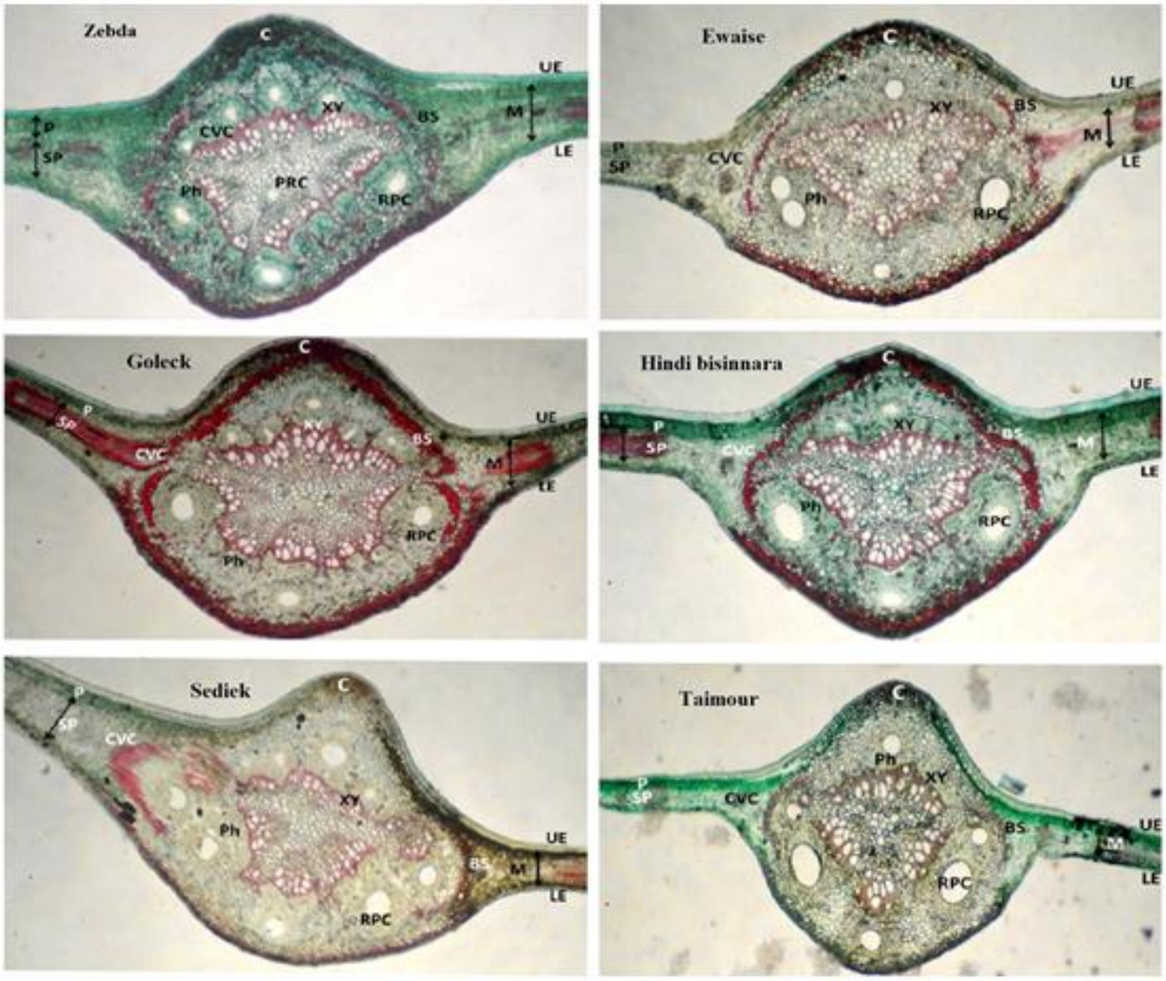

Fig. 2: The anatomical characteristics of the tested mango cultivar leaves.

Table 1. Impact of infestation by *F. virgata* on anatomical characteristics of the leaves of the tested mango cultivars, over the two-year average.

Cultivars / Measurements	Mean of parameters						P-value
	Zebda	Hindi Bisinnara	Goleck	Taimour	Ewaise	Sediek	
Upper epidermal thickness	10.25 f	18.55 a	17.45 b	12.43 e	14.62 d	16.36 c	0.000
Upper epidermis at Wing	13.26 e	22.43 a	22.43 a	16.50 d	18.65 c	19.25 b	0.000
Lower epidermal thickness	5.35 f	9.45 a	8.94 b	6.38 e	7.458 d	8.36 c	0.000
Lower epidermis at Wing	7.40 f	11.32 a	10.75 b	8.28 e	8.90 d	10.23 c	0.000
Thickness of palisade tissue layer	47.40 a	31.40 c	27.25 d	46.5b a	39.84 b	32.35 c	0.000
Thickness of spongy tissue layer	184.87 a	101.11 e	81.75 f	173.25 b	151.38 c	110.64 d	0.000
Thickness of leaf mid rib	666.25 e	1261.57 a	1256.40 a	844.90 d	880.83 c	1112.48 b	0.000
Width of leaf mid rib	726.21 e	1450.81 a	1444.86 a	946.29 d	1004.14 c	1268.23 b	0.000
Number of phloem Resin Canals	7.25 a	5.50 c	5.00 c	7.00 ab	6.50 ab	6.00 bc	0.000
No. of pith resin canal	1.00 a	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.000
Width of largest phloem resin canal	66.75 d	140.00 ab	144.25 a	123.50 c	126.50 c	134.00 b	0.000
Length of central vascular cylinder	853.25 a	565.20 c	490.50 d	815.29 a	697.25 b	582.30 c	0.000
Width of central vascular cylinder	733.47 e	1523.35 a	1531.55 a	955.75 d	1024.22 c	1318.96 b	0.000
Thickness of collenchyma layers below the upper epidermis at midrib	73.52 a	36.41 e	29.98 f	67.56 b	54.78 c	41.28 d	0.000
Thickness of collenchyma layers below the lower epidermis at midrib	67.64 a	23.81 e	24.13 e	60.80 b	48.77 c	35.90 d	0.000
Number of xylem rows in the vascular bundle	4.00 a	3.00 b	3.00 b	3.25 b	3.00 b	3.00 b	0.000
Thickness of widest xylem vessel in the vascular bundle	33.00 f	49.00 b	55.00 a	34.75 e	37.00 d	44.00 c	0.000

According to Tukey's HSD test, means that are followed by similar letters in each row have no substantial differences at the 0.05 level of probability. This suggests that the data patterns can be better understood by grouping the means according to their similarities.

3-The Relationships between *F. virgata* Estimates and the Tested Anatomical Characteristics Of Certain Mango Cultivar Leaves.

The triangular heatmap in Figure 3 summarized a correlation matrix that delineates the relationships among the various variables and demonstrates correlations among different variables associated with anatomical traits and mealybug infestation. Mealybug population estimates exhibited a strong negative correlation (dark blue) with almost all other variables, including the upper & lower epidermis layer thickness (UET & LET), upper & lower epidermis at wing (UEW & LEW), thickness of leaf midrib (TLMR), width of leaf midrib (WLMR), width of largest phloem resin canal (WLPRC), width of central vascular cylinder (WCVC), and number of xylem rows in the vascular bundle (NXR). In general, as the measurements of the anatomical traits studied increased, the number of mealybugs decreased. Strikingly, these parameters are indicators of plant health and growth, and they negatively affect pest infestation.

The results indicated that estimates of *F. virgata* individuals had a strong positive correlation with anatomical parameters (dark red), including thickness of palisade tissue layer (TPTL), thickness of spongy tissue layer (TSTL), number of phloem resin canals (NPhRC), number of pith resin canals (NPiRC), length of central vascular cylinder (LCVC), thickness of collenchyma layers below the upper and lower epidermis at midrib (TCLBUEM & TCLBLEM), and thickness of widest xylem vessel in the vascular bundle (TWXV). This indicates that as measurements of the studied anatomical characteristics increase, the number of mealybugs tends to increase. This is a very important result. These measurements indirectly contribute to increased pest infestation (**Figure 3**).

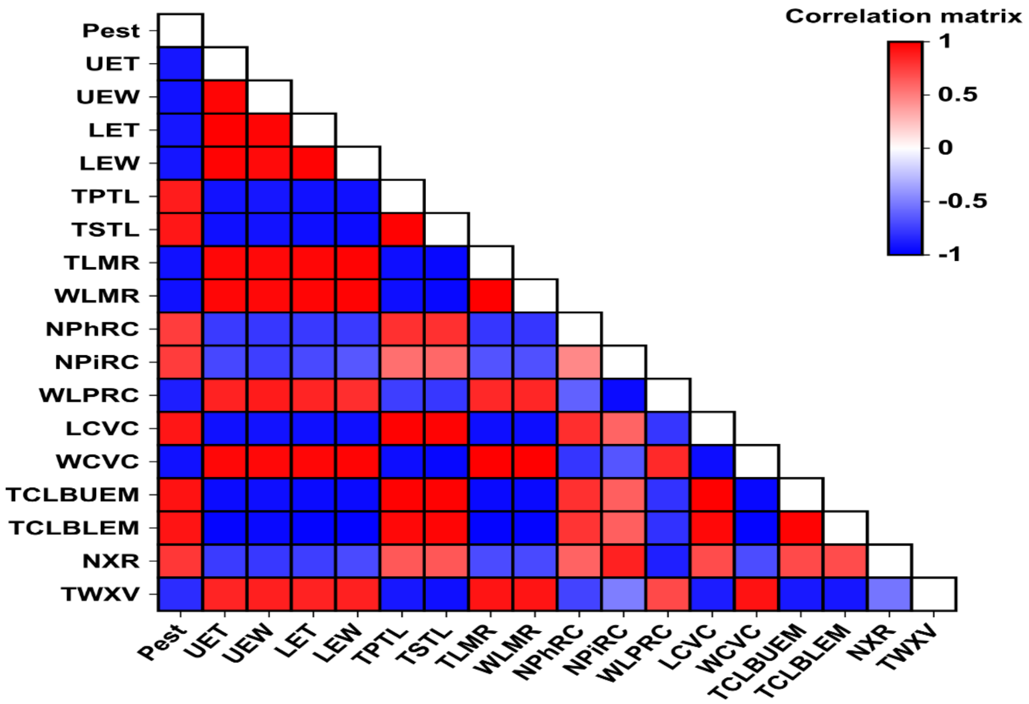

Fig. 3. The correlation matrix between *F. virgata* counts per mango leaf and the anatomical characteristics of certain mango cultivar leaf on a two-year average. The intensity of the color reveals the strength of the relations; the blue color reveals a negative relation, and the red color reveals a positive relation.

There is a strong positive correlation (dark red) between several plant anatomical measurements (Fig. 3). For instance, the thickness of the upper and lower epidermis (UET & LET) and the upper and lower epidermis at the wing (UEW & LEW) strongly positively correlated. If one thickness measure increases, it generally expected that the other would tend to increase as well. In particular, the thickness of the palisade tissue layer (TPTL) and the thickness of the spongy tissue layer (TSTL) seem to be related measurements, exhibiting a strong positive relationship. Additionally, there was a strong positive correlation between the number of phloem resin canals (NPhRC) and the number of pith resin canals (NPiRC). These may represent measurements related to physiological responses produced by the plant. There was evidence indicating that the thickness of the collenchyma layers below the upper and lower epidermis at the midrib (TCLBUEM & TCLBLEM) were also positively strongly correlated, which is an indication of two related measurements (Fig. 3). These strong positive associations exhibited that the pairs of traits tend to increase or decrease together. This information is valuable for understanding the underlying biological relationships.

With a focus on the widespread detrimental effects of mealybug individuals on several other measured characteristics, this heatmap is an effective tool for examining the intricate web of interactions between different mango cultivar attributes.

4- Heat Map and Hierarchical Clustering for Tested Traits on Six Mango Cultivars:

Based on the anatomical parameters examined, this heatmap shows the Euclidean distance in similarity (or dissimilarity) between different mango cultivars. These parameters including upper and lower epidermis layer thickness (UET & LET), upper and lower epidermis at the wing (UEW & LEW), thicknesses of the palisade tissue layer (TPTL), thickness of spongy tissue layers (TSTL) thickness of the leaf midrib (TLMR), width of the leaf midrib (WLMR), the number of phloem resin canals (NPhRC), the number of pith resin canals (NPiRC), width of the largest phloem resin canal (WLPRC), length and width of the central vascular cylinder (LCVC & WCVC), thickness of collenchyma layers below upper and lower epidermis at midrib (TCLBUEM & TCLBLEM), the number of xylem rows in the vascular bundle (NXR), thickness of the widest xylem vessel in the vascular bundle (TWXV), and counts of individual *F. virgata*.

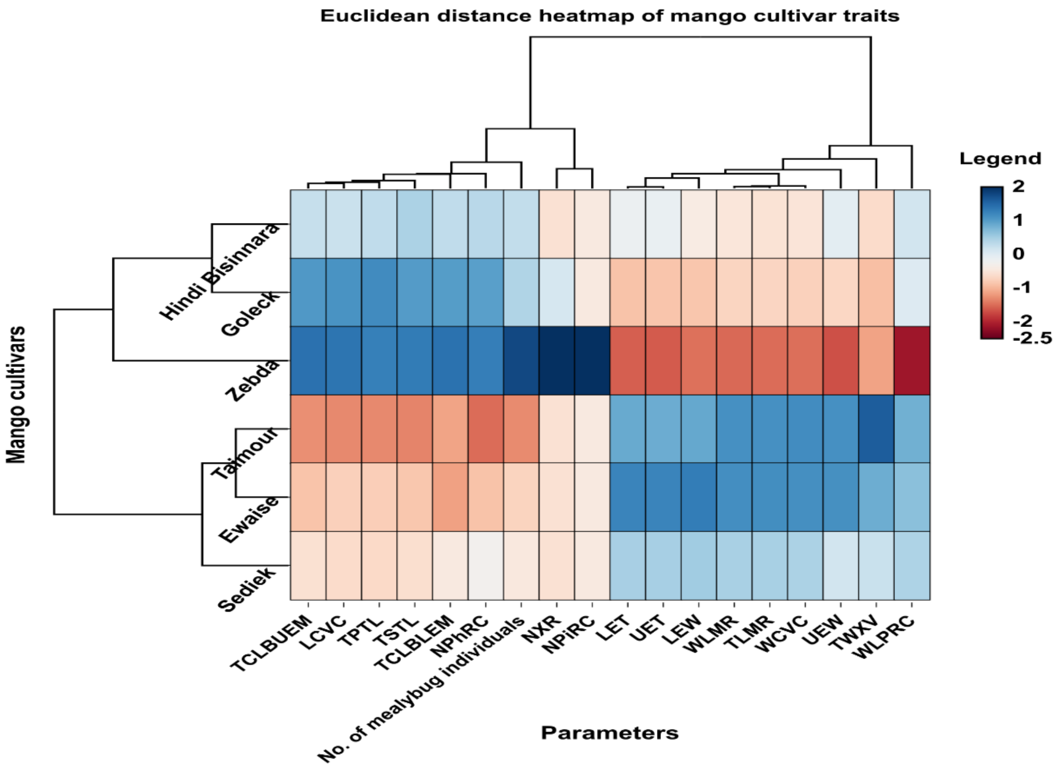

Fig. 4. The heat map represents a similarity matrix for a collection of mango cultivars based on a UPGMA clustering analysis using Euclidean distance. The clustering analysis used anatomical characteristics averaged over two years. The dendrogram clustered the mango cultivars (rows) and the anatomical characteristics to evaluate (columns).The color scale indicates dark red is a negative decrease, while dark blue is a positive increase.

The color intensity reflects the "distance" or difference, with darker red indicating greater similarity (closeness) and darker blue showing greater difference (less similarity). Referring to Figure 4, the hierarchical clustering is shown by the dendrograms on the top (for anatomical traits) and left (for mango cultivars), where clustered items are typically more closely related.

Dendrograms (Tree Structures):

Left Dendrogram (for mango cultivars): This hierarchical clustering represents how similar the mango cultivars are to each other based on all their measures. Cultivars that clustered together with short branches are more similar. For instance, the 'Hindi Bisinnara' and 'Goleck' cultivars closely clustered, suggesting they have many similarly appearing traits. The same is true for the 'Sediek' and 'Ewaise' cultivars. The Zebda cultivar appeared to have formed its own "cluster," and we can say that it has no association with the other cultivars, suggesting it has an entirely different set of traits based on its position in the cultivar dendrogram. The Taimour cultivar also appeared to be "more different" or further away from the other cultivars but not related to other cultivars. This identifies the natural groupings and/or relationships of the mango cultivars.

Top Dendrogram (for Measurements): This hierarchical clustering represents how similar the parameters (traits) based on the variation across the cultivars. For instance, clustered parameters correlated and/or showed similar behavior in the different cultivars. For example, the collenchyma layer thickness below the upper and lower epidermis (TCLBUEM & TCLBLEM) at the midrib, the central vascular cylinder length (LCVC), the palisade tissue layer thickness (TPTL), the spongy tissue layer thickness (TSTL), and the number of phloem resin canals (NPhRC) were clustered together, suggesting these may be correlated or related aspects of plant biological relationships. Additionally, traits including the number of xylem rows in the vascular bundle (NXR) and the number of pith resin canals (NPiRC) grouped together in another group. As well, the traits such as the thickness of the upper and lower epidermis (UET and LET), the upper and lower epidermis at the wing (UEW and LEW), the thickness of the leaf midrib (TLMR), the width of the leaf midrib (WLMR), the number of pith resin canals (NPiRC), the width of the largest resin canal in the phloem (WLPRC), the width of the central vascular cylinder (WCVC), and the thickness of the widest xylem vessel in the vascular bundle (TWXV) are grouped together.

Interpretation of the Heatmap Cells (Cultivar vs. Parameter Interaction):

Red Cells (Low Euclidean Distance/High Similarity): Zebda showed that very dark red for the width of the largest resin canal in the phloem (WLPRC), indicating a decrease in this parameter. At the same time, the Zebda cultivar recorded degrees of red color in the following characteristics: thickness of the upper and lower epidermis (UET and LET), the upper and lower epidermis at the wing (UEW and LEW), the thickness of the leaf midrib (TLMR), the width of the leaf midrib (WLMR), the number of pith resin canals (NPiRC), the width of the central vascular cylinder (WCVC), and the thickness of the widest xylem vessel in the vascular bundle (TWXV).

Taimour showed that very dark red for the number of mealybug individuals, which could indicate that this cultivar exhibited a low number of pest individuals.

Blue Cells (Low Similarity/High Distance): Zebda showed that very dark blue for the number of xylem rows in the vascular bundle (NXR) and the number of pith resin canals (NPiRC), showing an increment in these parameters. Taimour had a dark blue color for the thickness of the widest xylem vessel in the vascular bundle (TWXV), showing an increment in this parameter.

Zebda cultivar recorded degrees of blue color in the following characteristics: the collenchyma layer thickness below the upper and lower epidermis (TCLBUEM & TCLBLEM) at the midrib, the central vascular cylinder length (LCVC), the palisade tissue layer thickness (TPTL), the spongy tissue layer thickness (TSTL), and the number of pith resin canals (NPiRC).

Taimour and Ewaise cultivars showed a relatively high degree of similarity to each other (blue color) in the upper and lower epidermis (UET and LET), the upper and lower epidermis at the wing (UEW and LEW), the thickness of the leaf midrib (TLMR), the width of the leaf midrib (WLMR), the number of pith resin canals (NPiRC), the width of the largest resin canal in the phloem (WLPRC), and the width of the central vascular cylinder (WCVC).

Similar research conducted in Egypt by Shalaby (1998) revealed that the Giza variety of common beans, which had thicker palisade and spongy tissue in microns, was less vulnerable to infestation by *Bemisia tabaci* and Aphis spp., whereas the Bronco variety, which had thinner palisade and spongy tissues, was more susceptible to these insect infestations. According to Koschier *et al.* (2002), *Thrips tabaci* nymphs and adults damage onion plants directly by destroying their epidermal cells while feeding on green leaf tissue. According to Legrand and Barbosa (2003), adults of *Coccinella septumpunctata* were less effective against the aphids *Acyrthosiphon pisum* on pea plants when the plant's morphology was more complex.

Aphid and whitefly population densities found to be negatively connect with palisade and spongy tissues and positively associated with the lower and upper epidermis (Tantawy, 2006). Conversely, the *Thrips tabaci* population had a negative correlation with the upper and lower epidermis and a positive correlation with the palisade and spongy layers. According to Abou-Zaid (2013), the spongy tissue layer had a positive correlation with the population density of *Tetranychus urticae* infesting cucumber plants, while the upper and lower epidermis and palisade layers had a negative correlation. According to Hanafy *et al.* (2014), there were positive and significant correlations between the population densities of pests on cucumber and kidney beans, including aphids, whiteflies, *Bemisia tabaci*, and the spider mite, *T. urticae*, and palisade and spongy layers. However, this relationship between the upper and lower epidermis was substantially negative. In other words, infestation rates of all the pests under study rose as the thickness of the palisade and spongy tissues increased, while the thickness of the upper and lower epidermis decreased. According to Salem *et al.* (2007), the Ewaisi and Alphonoso mango varieties had the lowest population densities of *Icerya seychellarum* (*Hemiptera: Monophlebidae*) among the other Egyptian mango varieties. They also had the toughest leaves and the best pest resistance. Bakry and Abdel-Baky (2020**)** reported that the leaves of infested trees (both light and heavy) had significantly higher thicknesses of spongy tissue, collenchyma layers beneath the upper epidermis at the midrib, the number of xylem rows in the vascular bundle, and the length of the midrib vascular bundle compared to the un-infested ones. However, the leaves of un-infested mango trees had the thickest midrib, largest xylem artery in the vascular bundle, and breadth of midrib vascular bundle, as well as the thickest epidermal layer dimensions (upper and lower), compared to the light and heavy leaves of infested mango trees.

Conclusions:

Plant-pest interactions and mango cultivar resistance can better understand by analysing the anatomical attributes of affected mango cultivar leaves. Therefore, the study aimed to determine the anatomical responses of six mango cultivars to mealybug infestation. Cultivar-specific changes showed by the results, some of which exhibit adaptive features. Choosing pest-resistant cultivars and putting integrated pest management (IPM) techniques into practice depend heavily on these findings.

Declarations:

Ethical Approval: This study did not involve any live animals. It was based solely on experimental laboratory and analysis of plant extracts

Conflict of interest: The authors declare no conflict of interest.

Author's Contributions:Moustafa M.S. Bakry conceived the study, developed the methodology and writing the manuscript.Lamiaa H.Y. Mohamed: conceptualization.Rania M. Taha: Supervision, oversaw the project and anatomical analysis.Eman F.M. Tolba: Data analysis and reviewed the manuscript

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