

Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jpmp.journals.ekb.eg

Influence of Infestation with the Scale Insect; *Kilifia acuminata* on the Leaves of Mango (*Mangifera indica* L.) Cultivars in Egypt

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ABSTRACT

The response of the infested mango leaves *Mangifera indica* L. different cultivars (Keitt, Alphonso and Fagriklan) to the infestation with the scale insect; *Kilifia acuminata* (Signoret) (Hemiptera: Coccidae) was investigated. At Giza governorate, on mid-May-2023 which is the time of leaves collection for evaluation, Mango leaves were moderately infested by *K. acuminata*. The effect of this infestation on the primary and secondary metabolites of the different host plant cultivars, which were infested with different stages by *K. acuminata* was evaluated. The obtained results indicated a notable reduction in the primary metabolites examined (total proteins and total sugars), particularly in the Keitt variety. With respect to total phenols as a studied secondary metabolite, there was a significant increase in the total phenol content of the Alphonso variety. However, infestation with *K. acuminata* different developmental stages induced oxidative stress in different mango cultivars due to elevation of antioxidants, probably to counteract pest infestation effect. The data demonstrated that the Alphonso variety exhibited a higher level of oxidative stress compared to the Fagriklan and Keitt varieties. Mango cultivars differed in their defensive enzymes' reaction to infestation. It could be concluded that the different mango cultivars differ in either their adversely affected active components or biochemical response to the pest which might be related to the degree of infestation. We anticipate that this information will help us better understand how mango leaves protect themselves from *K. acuminata* infestations and find new, effective IPM solutions that are safe for both humans and the environment.

Keywords: *Mangifera indica* L., *Kilifia acuminata*, biochemical response, infestation effect, oxidative stress.

INTRODUCTION

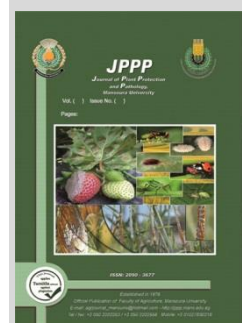
The genus *Mangifera* L. is classified within the family *Anacardiaceae* and the order *Sapindales*, encompassing 69 recognized species. *Mangifera indica* serves as a pivotal tropical agricultural product within the global economy. Furthermore, *Mangifera indica* is a crucial tropical crop for the economy of Egypt. It stands as one of the country's most significant fruit crops and is characterized as a vital component of the National Food Basket (El-Banhawy *et al.*, 2021). In 2022, the total cultivated area of mango fruits in Egypt reached approximately 326626 Feddan (Economic Affairs Sector, Egypt). Mango (*Mangifera indica* L.) is regarded as one of the most prevalent and esteemed fruits, often referred to as the sovereign of fruits due to its delectable taste, alluring aroma, diverse pigmentation, and remarkable nutritional benefits (Abd-Rabou *et al.*, 2012).

Scale insects are frequently regarded as among the most significant pests that infest mango trees across numerous nations globally. The soft scale insect *kilifia acuminata* (Signoret) (Hemiptera: Coccidae) represents one of the primary insect adversaries affecting mango trees and inflicts damage upon both the foliage and the fruit (Awadalla *et al.*, 2017). Plants face numerous biotic and abiotic efforts during their lifespan, with the biotic effort induced by insect pest attacks being one of the most significant threats to plant health, which can ultimately leading to a considerable decrease in potential yield (Sharma *et al.*, 2003). Flora exhibit responses to herbivory via a multitude of morphological, biochemical, and molecular processes aimed at mitigating or

compensating for the consequences of herbivore incursion (War *et al.*, 2013).

The biochemical mechanisms employed for the defense against herbivorous organisms are extensive and are characterized by a high degree of dynamism and are supported by both direct and indirect defense strategies. The phytochemicals involved in plant defense are produced either constitutively or in response to phytotoxic damage, thereby affecting the feeding patterns, development, and survival rates of herbivorous entities. Additionally, plants release volatile organic compounds that function to attract the natural predators of herbivores. These defensive mechanisms may function autonomously or synergistically in their effects (War *et al.*, 2012).

An investigation presented the inaugural empirical evidence of systemic biochemical disruptions in cotton as a consequence of *P. solenopsis* infestation, underscoring significant alterations in the biosynthesis of defensive compounds (phenolics) and protein metabolic pathways, while exhibiting negligible effects on the structural integrity of the photosynthetic machinery (Nagare *et al.*, 2017). Nevertheless, there exists a dearth of literature concerning the biochemical alterations in mango foliage resulting from the infestation of *kilifia acuminata* (Signoret) (Hemiptera: Coccidae). Hence the present study aim of the work was undertaken to evaluate adverse effects of *K. acuminata* on its host plant *M. indica* L. different cultivars, and the response or reaction of the infested leaves to such infestation. Current experiments were done to: 1- evaluate the effect of infestation on main and secondary metabolites of the host plant, 2- detect defensive enzymes response, such as esterases and β -glucosidases, to *K. acuminata*, 3- measure



Article Information
Received 11/ 8 /2025
Accepted 8/ 9/2025

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DOI: 10.21608/jpmp.2025.409938.1366

the possible oxidative stress induced by the pest. Keitt, Alphonso and Fagriklan cultivars were compared from these aspects of view.

MATERIALS AND METHODS

1. Field and Sampling:

This investigation was conducted within the Faculty of Agriculture at Cairo University. Three distinct mango cultivars (Keitt, Fagriklan, and Alphonso) were selected, as identified by taxonomists at the Egyptian National Botanical Institute located in Dokki, Giza, Egypt. Samples were systematically collected bi-monthly from five infested mango trees for each cultivar, ensuring that no pesticides were utilized throughout the duration of this study. The selected mango trees exhibited homogeneity in terms of size, height, morphology, and level of infestation over the one-year research period extending from May 2023 to April 2024. A total of twenty-five infested leaves were randomly harvested from four cardinal orientations (East, West, North, and South) as well as the tree core of each specimen. The infested leaves were individually placed into paper bags and subsequently transported to the laboratory for examination utilizing a stereoscopic microscope. Additionally, non-infested leaves (devoid of any infestation) were randomly collected from various mango cultivars to facilitate chemical analysis, comprising five leaves replicated three times for each cultivar.

2. Plant sample preparation:

Fresh mango leaves (ranging in weight from 0.1 to 0.4 g) were meticulously quantified and subsequently preserved at a temperature of -20 °C, followed by processing in accordance with the methodology delineated in (Ni *et al.*, 2001).

3. Total proteins:

Total proteins were quantified utilizing the methodology established by (Bradford, 1976), employing Coomassie Brilliant Blue G-250 in conjunction with bovine serum albumin as a standard reference protein.

4. Determination of total sugars:

Total sugars were extracted from fresh mango leaves using 80% ethanol and subsequently assessed through the phenol-sulphuric acid reaction as delineated by (Dubois *et al.*, 1956).

5. Determination of Phenols:

Extraction procedure:

The extraction process was conducted in accordance with the protocol established by (Kähkönen *et al.*, 1999). The residual solid (crude extract) was weighed and reconstituted in deionized water to achieve a final volume of 5 ml.

Quantification of total phenols:

The quantification of total phenolic compounds in the extracts was performed utilizing the Folin – Ciocalteu assay as adapted by (Singleton & Rossi, 1965). To the test tubes, two hundred microliters of the plant extracts were added, followed by the incorporation of 1 ml of Folin-Ciocalteu reagent and 0.8 ml of a 7.5% sodium carbonate solution. The contents of the tubes were mixed thoroughly and allowed to incubate for a period of 30 minutes. The absorbance was subsequently measured at 760 nm, using a blank containing all reagents except the sample for calibration. A gallic acid standard (5 g%) was employed, and the total phenolic content was expressed as milligrams of gallic acid per gram of dry weight of the original sample (mg GA/g dw).

6. Determination of Phenol oxidase:

The activity of phenol oxidase was assessed according to a modified procedure of (Ishaaya, 1971). The enzymatic

reaction was initiated by the addition of the catechol solution. After precisely 10 minutes, the optical density was measured, with zero adjustment performed against a sample blank at 405 nm.

7. Quantitative Determination of Peroxidase:

Peroxidase activity was quantified in accordance with the methodology delineated by Hammerschmidt *et al.* (1982). The enzyme activity was formulated as the variation in absorbance per minute per gram of sample.

8. Total antioxidant capacity:

The assessment of antioxidant capacity was conducted following a spectrophotometric technique established by Prieto *et al.* (1999).

9. Alpha esterases:

The determination of alpha esterases (α -esterases) was conducted in accordance with the methodology established by van Asperen (1962), utilizing α -naphthyl acetate as the substrate.

10. β -glucosidase:

The activity of β -glucosidase was quantified by assessing the glucose released through the enzymatic hydrolysis of salicin, as delineated by Lindroth *et al.* (1988). The optical densities were evaluated in comparison to a blank containing the boiled enzyme. The enzyme activity was reported as micrograms of glucose released per minute per milligram of protein.

11. Statistical Analysis:

Data are expressed as the mean $\pm$ standard deviation derived from a minimum of three independent repetitions ($n = 3$). The data obtained for the various parameters under investigation were analyzed utilizing one-way analysis of variance (ANOVA) facilitated by Costats statistical software (Cohort Software, Berkeley). Duncan's multiple range test indicated the presence of statistically significant differences at ($p < 0.01$). Additionally, the data were analyzed for percentage changes (both increases and decreases) in the infested versus control (healthy) leaves, calculated in accordance with the following equation:

$$\% \text{ increase or decrease} = \frac{(\text{Value of healthy plant} - \text{Value of infected plant})}{(\text{Value of healthy plant})} \times 100$$

RESULTS AND DISCUSSION

Results

Fig. (1) illustrates that in Cairo, on mid-May-2023, Mango leaves experienced a moderate infestation by *K. acuminata*. Number of nymphs, gravids and adults infested Keitt cultivars were 3175/ 25 leaves. Total number of insects infested Alphonso and Fagriklan cultivars were 370 and 225 individuals/ 25 leaves, respectively.

The collected leaves of different cultivars were used to examine how infestation with the scale insect; *K. acuminata* affect mango plant. Table (1) illustrates the effect of infestation on main and secondary metabolites of the host plant different cultivars infested with *K. acuminata* different stages. Infestation with *K. acuminata* affected the tested main metabolites, as it caused a significant decrease in total proteins and total sugars. Percentage total protein change of the studied Keitt cultivar infested leaves were found to decrease

significantly (-43.2%) than Fagriklan (-25%) and Alphonso (-13.6) varieties in response to control (uninfested) leaves. On the other hand, total sugars percentage change for Keitt cultivar decreased (-21.8%) more significantly than Alphonso (-1.3%), while it somewhat increased for Fagriklan variety (+0.16%) than control.

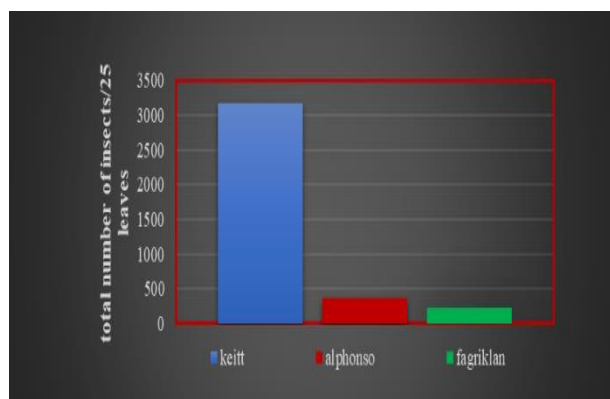


Fig. 1. total number of nymphs, adults and gravid of *Kilifia acuminata* infested different mango leaves *Mangifera indica* L. cultivars in mid-May-2023.

Table 1. Metabolites of different mango leaves *Mangifera indica* L. cultivars infested with the scale insect; *Kilifia acuminata*.

Mango cultivar	Total proteins (mg/g fresh weight)		
	Infested	Control	% Change
Keitt	32.9±0.9 ^b	58±1.8 ^a	-43.2
Alphonso	40.5±2.2 ^a	46.9±2.3 ^a	-13.6
Fagriklan	37.5±0.5 ^b	50±2 ^a	-25
Mango cultivar	Total Sugars (mg glucose /g fresh weight)		
	Infested	Control	% Change
Keitt	31.8±1.6 ^b	40.7±1.2 ^a	-21.8
Alphonso	43.1±0.8 ^a	43.7±1.6 ^a	-1.3
Fagriklan	61.8±2.4 ^a	61.7±1.5 ^a	+0.16
Mango cultivar	Total phenols (mg/ g fresh weight)		
	Infested	Control	% Change
Keitt	2.9±0.2 ^a	3.3±0.1 ^a	-12.1
Alphonso	4.11±0.4 ^a	3.3±0.2 ^b	+24.5
Fagriklan	2.6±0.1 ^a	2.9±0.2 ^a	-10.3

-Data are represented as the mean± SD. -% change = percent decrease or increases as compared to control.- Means within a row for each determination that exhibit distinct superscripts are significantly different ($p < 0.01$, as determined by Duncan's multiple range test).

Total phenols, as a secondary metabolite, was analyzed. The data revealed that, percentage change in total phenols was increased for Alphonso cultivar (+42.5%) but decreased for Keitt (-12.1%) and Fagriklan (-10.3%) cultivars with respect to control leaves. Generally, the studied metabolites were not severely affected by infestation. Keitt cultivar was the most affected variety as compared to the other studied varieties and the total proteins were the highest metabolites that showed significant reduction.

Results (table, 2) showed that infestation with *K. acuminata* different developmental stages caused oxidative stress in different mango cultivars due to elevation of antioxidants, probably to counteract pest infestation effect. The data illustrated that Alphonso variety was the most one that had an observed oxidative stress, it had a significant high percentage change increase in phenol oxidase activity (+59.4%), peroxidase (+81%) and total antioxidant capacity (+66.7%) than Fagriklan and Keitt varieties. Alphonso and Fagriklan had relatively high levels of total antioxidant capacity, might be to get mango leaves able to reduce oxidative damage caused by scale insect infestation.

Mango cultivars differed in their defensive enzymes' reaction to infestation (table, 3). Keitt and Fagriklan showed significant positive increase percentage change of alpha esterases activity response to *K. acuminata* infestation, while Fagriklan and Alphonso had significant increase in β -glucosidases activity as compared to non-infested control.

Table 2. Oxidative stress on different mango leaves; *Mangifera indica* L. cultivars after infestation with the scale insect; *Kilifia acuminata*.

Mango cultivar	Phenol oxidase (m O.D. units/min/g fresh weight)		
	Infested	Control	% Change
Keitt	863±10.6 ^a	710±10 ^b	+21.5
Alphonso	1063±40 ^a	667±15.5 ^b	+59.4
Fagriklan	885±12.5 ^a	731±7.6 ^b	+21
Mango cultivar	Peroxidases (Δ O.D./min/g fresh weight)		
	Infested	Control	% Change
Keitt	40.8±1.5 ^a	36.8±1 ^a	+10.9
Alphonso	51.4±0.9 ^a	28.4±1.2 ^b	+81
Fagriklan	51.7±1.6 ^a	29.8±1.8 ^b	+73
Mango cultivar	Total antioxidant capacity (mg AAE/ g fresh weight)		
	Infested	Control	% Change
Keitt	5.4±0.1 ^b	6.5±0.4 ^a	-16.9
Alphonso	9±0.2 ^a	5.4±4 ^b	+66.7
Fagriklan	8.1±0.1 ^a	6.5±0.2 ^b	+24.6

Data are represented as the mean± SD. -% change = percent decrease or increases as compared to control. - Means within a row for each determination that exhibit distinct superscripts are significantly different ($p < 0.01$, as determined by Duncan's multiple range test). - AAE: ascorbic acid equivalent.

Table 3. Defensive response of different mango leaves; *Mangifera indica* L. cultivars to infestation with the scale insect ; *Kilifia acuminata*.

Mango cultivar	α - esterases (ug α - naphthol/min/g fresh weight)		
	Infested	Control	% Change
Keitt	163±3.8 ^a	132±3 ^b	+24.3
Alphonso	129±3.8 ^a	123±3 ^a	+4.8
Fagriklan	168.8±1.5 ^a	138±2 ^b	+22.8
Mango cultivar	β -glucosidases (ug glucose/min/g fresh weight)		
	Infested	Control	% Change
Keitt	4.6±0.3 ^b	6±0.5 ^a	-23.3
Alphonso	8.8±0.3 ^a	5.4±0.4 ^b	+63
Fagriklan	10.7±0.2 ^a	8.1±0.3 ^b	+32

-Data are represented as the mean± SD. -% change = percent decrease or increases as compared to control. - Means within a row for each determination that exhibit distinct superscripts are significantly different ($p < 0.01$, as determined by Duncan's multiple range test).

Discussion

The primary focus of this paper is to investigate the impact of infestation with the scale insect; *K. acuminata* on the biochemical contents of mango leaves different cultivars. The results demonstrated that there was a significant decrease in the studied main metabolites (total proteins & total sugars) specially for the Keitt variety, and with respect to total phenols as a studied secondary metabolite, there was a significant increase in the content of total phenols in Alphonso variety but there was a decrease in both Keitt and Fagriklan varieties as compared to uninfested control leaves.

The data acquired are in alignment with the findings of previous research that indicated that the infestation of woolly apple aphids (*Eriosoma lanigerum*) led to a reduction in the content of soluble sugars, proteins, and amino acids in Fuji apple twigs; conversely, the total phenolic compounds, which constitute a significant category of defensive metabolites against aphids, exhibited an increase of 30.5% and 6.0% in mechanically injured twigs of Qinguan and Zhaojin 108, respectively, while there was a decline of 21.7% and 16.1% in aphid-infested twigs of Red Fuji and Red General, respectively (Zhou *et al.*, 2013). Furthermore, (Ojimelukwe *et al.*, 1999) documented a significant reduction in the concentrations of protein, starch, and soluble sugars in cowpea seeds infested by the pulse beetle (*Callosobruchus maculatus*).

The infestation by the aphid species *Aphis gossypii* (Hemiptera: Aphididae) and the jassid species *Amrasca devastans* (Hemiptera: Cicadellidae) resulted in a decrease in the starch and protein levels of all five cotton cultivars examined under field conditions (Amin et al., 2016). Aphids assimilate amino acids from the phloem tissue to meet their nitrogen demands, thereby leading to a reduction in protein levels (Douglas, 2003). A decline in protein content was noted in rice genotypes infested by the Leaf folder (*Cnaphalocrocis medinalis*) according to the findings of (Punithavalli et al., 2013). (Bhimrao et al., 2011) reported a reduction in total reducing sugars in most mulberry (*Morus alba* L) cultivars as a consequence of leafroller (*Diaphania pulverulentalis* Hampson) infestation.

Conversely, (Chen et al., 2009) posited that an increase in the amount of protein is a widespread response in plants to harm caused by insects, serving as a defense mechanism. An investigation into the biochemical alterations in cotton plants (*Gossypium hirsutum* L.) due to infestation by the cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) revealed an elevation in total protein and total phenol contents following infestation compared to healthy specimens, although no significant difference in total soluble sugar levels was detected (Nagrare et al., 2017).

A notable rise in the amount of soluble protein and phenolic compounds was documented as a result of the infestation by *H. armigera* and *A. craccivora* in groundnut relative to the control group (War et al., 2013). Infestation by the Leaf folder (*Cnaphalocrocis medinalis*) led to a substantial elevation in the concentrations of phenolic compounds when compared to uninfested rice specimens (Punithavalli et al., 2013).

An imbalance between free radicals and antioxidants causes oxidative stress, which damages cells. Our data revealed that, generally, there is a significant increase in the percentage change of phenol oxidase, peroxidase and total antioxidant capacity over the uninfested leaves for all the tested mango cultivars which may be an indicator of higher oxidative stress and an attempt to protect the cells from damage occurred as a result of the *K. acuminata* infestation. These findings closely resemble those of (Anusha et al., 2016) who reported that five mango hybrids at two phases of leaf maturity (new flush and old leaves) following leafhopper infestation had a strong induction of peroxidase, glutathione reductase, and polyphenoloxidase.

Moreover, the data clearly showed an increase in the % change of the tested defensive enzymes (α -esterases & β -glucosidases) over the control leaves after infestation of *K. acuminata* which reflects a superior of mango leaves resistance system. Regretfully, there are not enough references on the impact of *K. acuminata* insect pest infestation on the defensive enzymes assessed on mango leaves to be included in the discussion of the current results.

It could be concluded that the different mango cultivars differ in either their adversely affected active components or biochemical response to the pest which might be related to the degree of infestation. We hope that this information will help us better understand how mango leaves protect themselves from *K. acuminata* infestations and find new, effective IPM solutions that are safe for both people and the environment.

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تأثير الإصابة بحشرة المانجو القشرية الرخوة *Kilifia acuminata* على اصناف اوراق اشجار المانجو *Mangifera indica L.* في مصر

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الملخص

تمت دراسة مدى استجابة الأصناف المختلفة (كيت؛ ألفونس؛ فجر كلان) لأوراق أشجار المانجو *Mangifera indica L.* المصابة بحشرة المانجو القشرية الرخوة *Kilifia acuminata* في محافظة الجيزة، في منتصف مايو ٢٠٢٣ وهو وقت جمع الأوراق للتقييم، كانت أوراق المانجو مصابة بشكل معتدل بهذه الحشرة. تم تقييم تأثير الإصابة على المستقلات الرئيسية والثانوية للنبات المضيف لأصناف مختلفة مصابة بـ *K. acuminata* في مراحل مختلفة. وأظهرت النتائج أن هناك انخفاضاً كبيراً في المستقلات الرئيسية المدروسة (إجمالي البروتينات وإجمالي السكريات) خاصة بالنسبة لصنف كيت. وفيما يتعلق بإجمالي الفينولات كمستقبل ثانوي تمت دراسته، كانت هناك زيادة كبيرة في محتوى إجمالي الفينولات في صنف ألفونسو. ومع ذلك، فإن الإصابة بـ *K. acuminata* في مراحل نمو مختلفة تسببت في إجهاد تأكسدي في أصناف مختلفة من المانجو بسبب ارتفاع مضادات الأكسدة، ربما لمقاومة تأثير الإصابة بالافات. أوضحت البيانات أن صنف ألفونسو كان الأكثر تعرضاً للإجهاد التأكسدي الملحوظ مقارنة بأصناف فجر كلان وكيت. اختلفت أصناف المانجو في تفاعل إنزيماتها الدفاعية مع الإصابة. ويمكن الاستنتاج أن أصناف المانجو المستخدمة في الدراسة تختلف إما في مكوناتها النشطة المتأثرة سلباً أو في استجابتها الكيميائية الحيوية للآفة والتي قد تكون مرتبطة بدرجة الإصابة. نأمل أن تساعدنا هذه المعلومات على فهم أفضل لكيفية حماية أوراق المانجو لنفسها من الإصابة بـ *K. acuminata* وإيجاد حلول جديدة وفعالة للإدارة المتكاملة لهذه الآفة تكون آمنة لكل من الناس والبيئة.