



Relation of allelochemicals and nutritive value of mango cultivars *Mangifera indica* to infestation with the scale insect *Aulacaspis tubercularis* (Hemiptera: Diaspididae)

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Abstract

The effect of mango (*Mangifera indica* L.) chemical composition of different cultivars (Keitt, Fagriklan, and Alphonso) on the infestation level with the white mango scale insect *Aulacaspis tubercularis* (Newstead) (Hemiptera: Diaspididae) was evaluated. The highest seasonal abundance of *A. tubercularis* was recorded on 1st May. The number of insects of different instars (Nymph, adult, and gravid) that infested mango leaves were 1200, 748, and 630 individuals/25 leaves for Keitt, Fagriklan, and Alphonso, respectively. Mango cultivars' susceptibility levels could be grouped in the following way: Keitt>Fagriklan>Alphonso. The lowest abundance occurred during mid-January for different cultivars. Analysis of fresh leaf composition on 1st May revealed that *A. tubercularis* numbers that infested mango leaves had a strong positive correlation with leaf protein ($r=+0.997$), Nitrogen (N) ($r=+0.994$) and Potassium (K) ($r=+0.999$). On the contrary, it had a high negative correlation with terpenoids ($r=-0.949$), and a medium negative correlation ($r=-0.442$) with carbohydrates. Phosphorus (P), allelochemicals such as phenols, and flavonoids showed a weak negative correlation. Variations in chemical content among different mango cultivars were also discussed. It can be concluded that nutritive components and some allelochemicals of mango leaves may play a significant role in the susceptibility of mango leaf cultivars to infestation with the white mango scale insect, *A. tubercularis*.

Introduction

Mango (*Mangifera indica* L.) (Family: Anacardice), the "king of fruit," is one of the important fruit crops worldwide, including Egypt. Mango fruits are popular due to their richness in

vitamins A and C, mineral salts, and varying amounts of carbohydrates and protein among different varieties (Abourayya *et al.*, 2011). It plays a crucial role as a major tropical crop for the Egyptian

economy. In 2022, the total cultivated area of mango fruits in Egypt reached approximately 326626 Feddan (Economic Affairs Sector, Egypt). In Egypt, several cultivars such as Keitt, Fagriklan, and Alphonso are disease-resistant and have been successfully grown (Knight *et al.*, 2009). *Aulacaspis tubercularis* (Newstead) (Hemiptera: Diaspididae) (White mango scale insects) is considered one of the most important pests of mango trees around the world (Del Pino *et al.*, 2023) because it produces a lot of pink blemishes on mango fruit, which affecting on the economic value and making them not exportable. It is one of the key insect pests of mango around the world, causing poor blossoming, drying up of young twigs, and devastating losses (Miller and Davidson, 2006; Del Pino *et al.*, 2021, and Fita, 2023). This pest is polyphagous, feeding on a variety of plants from over 37 genera and 23 families, with the majority of reports being on mangos (*Mangifera indica*). In the absence of control, this pest may cause up to 90% yield losses in mango crops and death of the trees. Also, it causes a major problem not only in all mango growing areas of Egypt (Abo-Shanab, 2012), but also in many countries such as East and West Africa, North and South America, Australia, and Pakistan (Khan *et al.*, 2016). Abd Elrahman *et al.* (2006) show that different mango cultivars vary in their susceptibility to infection by *Icerya seychellarum* (Westwood) (Hemiptera: Monophlebidae). Mango trees belonging to the Sultani cultivar are severely damaged and strongly infested with *I. seychellarum*, whereas trees belonging to the Alphonso cultivar are completely free of this

disease. In chemotaxis, feeding, and toxicity assays, a comparison of the two cultivars' leaves and their complete extracts showed that Alphonso cultivar leaf material contained a toxic component, a feeding deterrent, and an *I. seychellarum* repellent.

The mango cultivar Alphonso's leaves had a repulsive effect on the mango shield scale *Kilifia acuminata* (Signoret) (Hemiptera: Coccidae). In the bioassay conducted in the lab. Hexane was used to extract the volatile components of Alphonso leaf, and two separate solvent solutions were used for the two subsequent silica gel column chromatograph fractionations. The chemotaxis assay results and the gas chromatographic/mass spectrometric (GC-MS) examination of the separated fractions revealed that the volatile oils in Alphonso leaves, including α -pinene, β -pinene, and d-limonene, can repel insects. It is proposed that the combined effects of α -pinene, β -pinene, and d-limonene may be partially or entirely the reason for *K. acuminata*'s lack of tendency for the leaves of the Alphonso mango cultivar (Abd Elrahman *et al.*, 2013).

Based on the approaches presented in the above studies, the purposes of this study were to: (1) Quantify and compare the chemical composition of mango leaves from different cultivars of *M. indica* L. proteins, carbohydrates, inorganic ions, and allelochemicals as secondary metabolites were analyzed. (2) Measure the infestation level of mango leaves cultivars with *A. tubercularis* at different stages (Nymph, adult, and gravid). Insects-infested leaves were counted every 15 days throughout the year. (3) Test

which of the leaf components may affect the level of infestation. The correlation coefficient (r) was calculated for the number of insects infested by each cultivar (Keitt, Fagriklan, and Alphonso) and the quantity of each biochemical component.

Materials and methods

1. Field and sampling:

This study was carried out in the Faculty of Agriculture (Cairo University). Three mango cultivars were chosen (Keitt, Fagriklan, and Alphonso) and recognized by Egyptian National Botanical Institute taxonomy specialists (Dokki, Giza, Egypt). Samples were collected twice a month from five infested mango trees for each variety with no use of any pesticides during this study. Selected mango trees were similar in size, height, shape, and infestation during the one-year study from May 2023 to April 2024. Twenty-five infested leaves were collected randomly from four cardinal directions (East, West, North, and South) and the tree core of each tree. Infested leaves were collected separately in paper bags and transferred to the laboratory for inspection using a stereoscopic microscope. Non-infested leaves (Free from any infestation) were randomly collected from different mango cultivars to perform chemical analysis (5 leaves replicated three times for each cultivar). The leaves were collected on the first of May during a high-level infestation of mango trees of Keitt, Fagriklan, and Alphonso cultivars.

2. Apparatus:

Plant samples were homogenized in a refrigerated Teflon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland) for biochemical

analysis. After being homogenized, supernatants were kept in a deep freezer at -20°C until they were required for biochemical testing. A twin-beam ultraviolet/visible spectrophotometer (Spectrophotonic 1201, Milton Roy Co., USA) was used to detect the absorbance of colored substances or metabolic products.

3. Chemical assays:

3.1. Determination of total carbohydrates:

Total carbohydrates were estimated by the phenol-sulfuric acid reaction by Dubois *et al.* (1956). Fill a boiling tube with 100 milligrams of the plant specimen by weight. After adding 10 ml of 2.5 N HCl, hydrolyze by holding it in a boiling water bath for three hours, then cool at ambient temperature. Use sodium carbonate to neutralize it and bring it to effervescence. To prepare the supernatant for analysis, centrifuge and gather it (Sadavivam and Manickam, 1992). Add 0.5 ml of 20% w/v phenol to 100 microliters of the extract in a colorimetric tube. Next, quickly add 5 ml of concentrated sulfuric acid while shaking. At 490 nm, the distinctive orange-yellow hue absorbance is quantified in relation to a blank (Distilled water).

3.2. Determination of total proteins:

Typically, extraction is done using the same buffers as are used for enzyme assays. 500 mg of the plant sample should be weighed and thoroughly ground using a mortar and pestle in 5 milliliters of pH 7 phosphate buffer (0.01). To estimate the amount of protein, centrifuge and use the supernatant. Using Coomassie Brilliant Blue G-250 as the dye, the Bradford (1976) technique was used to calculate the total amount of proteins.

The absorbance at 595 nm was measured after two min. and before one hour later, compared to a blank made with five milliliters of protein reagent and one milliliter of phosphate buffer. The reference protein was bovine serum albumin.

3.3. Determination of phenols:

Extraction was performed as described by Kähkönen *et al.* (1999). Using an electric homogenizer, two batches of ten milliliters of 80% aqueous methanol were extracted from five grams of grounded plant seedlings. After centrifuging the samples for 10 min. at 3000 rpm, the mixed extracts were transferred into tiny conical flasks that had been previously weighed. There was less pressure used to extract the methanol. Weighing the solid residue (Crude extract), 5 ml of Δ H₂O was added to dissolve it. The amount of total phenolics in extracts was determined by the Folin-Ciocalteu method as modified by Singleton and Rossi (1965). Two hundred microliters of plant extracts were introduced into test tubes; 1 mL of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. Absorption at 760 nm was measured against a blank containing everything except the sample. Gallic acid standard (5 gm%) was used.

3.4. Determination of total flavonoids:

Total flavonoid content was measured using the aluminum chloride colorimetric method (Zhishen *et al.*, 1999). A 1 ml volume of distilled water was filled, and ten microliters of the sample were added. Subsequently, 100 uL of sodium nitrite (5% W/V) was added and left to stand for five min. Then, 100 uL of AlCl₃ (10%) was added and incubated for a minute.

Finally, 1M NaOH was added, and the volume was adjusted to 5 mL with distilled water. Following 15 min. of thorough mixing, the absorbance at 510 nm was measured in comparison to the blank. The standard for expressing total flavonoids per gram of material was ug catechin (CE).

3.5. Determination of triterpenoids:

Briefly, 10 mg of each extract was dissolved in 1 mL of methanol. Then, 100 /L of the solution was combined with 500 μ L of perchloric acid solution and 150 μ L of vanillin-glacial acetic acid solution (5% w/v). After being heated to 60°C for 45 min., the sample solution was cooled to room temperature in an ice-water bath. The absorbance of the sample solution was measured at 548 nm using a UV-160A UV-visible-light spectrophotometer (Shimadzu Corporation, Kyoto, Japan) after the addition of 2.25 mL of glacial acetic acid. The standard utilized was ursolic acid (0.025–0.5 mg/mL in methanol).

3.6. Determination of nitrogen (N):

The protein's nitrogen is transformed during digestion by H₂SO₄ into ammonium sulphate. After being steam-distilled, this salt releases ammonia, which is gathered in a solution of boric acid and titrated against standard acid. The nitrogen content of the sample is determined by computation, as 1 milliliter of 0.1 N acid is equal to 1.4 milligrams of N. (Sadasivam and Manickam, 1991).

3.7. Inorganic phosphorus (P) determination:

With the aid of an industrial kit from Spain's Quimica Clinica Applicada S.A., the phosphate ion was found. P and molybdate combine to form phosphor-molybdate, which is then reduced to a molybdenum blue that may be detected by photometry at 650 nm. Results were achieved by

comparing the acquired zero adjustment against the reagent blank to a reference standard (Conc. 4 mg%).

3.8. Potassium (K) determination:

On a radiometer FLM3 flame photometer, measurements of ions were performed. Potassium chloride (5 ± 0.5 mmol/L) and 14 ± 1.4 mmol/L of sodium chloride were kept at ambient temperature ($25\text{ }^{\circ}\text{C}$) in the standard solution. 500 ml of distilled water was mixed with 5 ml of concentrated lithium chloride (300 ± 5 mmol/L) to create a blank for zero adjustment (Chapman and Pratt, 1961).

4. Statistical Analysis:

The collected values were pooled from three replicates. Means and standard deviations were obtained, and the data were analyzed using ANOVA with Costas statistical software (Cohort Software, Berkeley). Duncan's multiple range tests confirmed the relevance of variable treatments ($p < 0.01$). The Correlation coefficient and slope were obtained using Microsoft Corporation. Microsoft Excel [Internet]. 2018. Available from: <https://office.microsoft.com/excel>.

Results and discussion

1. Levels of infestation and density of population of *Aulacaspis tubercularis*:

The densities of the population of *A. tubercularis* on mango cultivars' leaves Keitt, Fagriklan, and Alphonso at the demonstration dates are shown in Table (1). Results showed that the seasonal abundance of *A. tubercularis* on mango trees differed among different cultivars. The highest mean numbers of *A. tubercularis* individuals per 25 leaves were found on the Keitt cultivar (330.42), followed by

Fagriklan (189.58), and then Alphonso (101.91). The susceptibility levels of mango cultivars to *A. tubercularis* could be arranged in descending order as follows: Keitt (highly susceptible) > Fagriklan > Alphonso. The infestation rate of *A. tubercularis* was comparatively low during the winter, summer, and autumn months, while the highest peaks were captured in the spring months. The greatest quantity was noted during 1st May with 1200, 748, and 630 individuals for Keitt, Fagriklan, and Alphonso, respectively. The lowest abundance was recorded during mid-January with 106, 52, and 30 individuals for Keitt, Fagriklan, and Alphonso, respectively. These differences in population densities of the *A. tubercularis* may be related to the variations of the chemical components of the mango leaf cultivars.

2. Main metabolites and secondary metabolites contents of different fresh mango leaves cultivars *Mangifera indica*:

The results showed that mango leaves of different cultivars were more or less different in their nutrients and allelochemical content point of view. Table (2) shows the main metabolite content of different fresh mango leaf cultivars. *M. indica*, protein contents of the studied mango cultivar for Keitt leaves were found to contain significantly higher protein (58 mg/g fresh leaves) than Fagriklan and Alphonso varieties (50 and 46.9 mg/g fresh leaf weight), respectively. On the other hand, the carbohydrate content of the Keitt variety was relatively lower (81.8 mg/g fresh leaf weight) than Fagriklan and Alphonso (124.2 and 87.8, respectively). The Carbohydrate content of the Fagriklan variety was relatively greatly higher (124 mg/g fresh leaves) than other varieties (Figure 1).

Table (1): Half-monthly mean numbers of different stages population density on three mango cultivars at Giza Governorate during 2023-2024 seasons.

Date	Keitt				Fagriklan				Alphonso			
	Nymph	Adult	Gravid	Total	Nymph	Adult	Gravid	Total	Nymph	Adult	Gravid	Total
1-May	522	390	288	1200	318	246	184	748	214	266	150	630
15 May	466	296	60	822	132	90	80	302	90	78	50	218
1-Jun	254	208	20	482	74	64	58	196	50	38	18	106
15-Jun	126	18	98	242	62	16	44	122	38	30	10	78
1-Jul	337	225	138	700	96	84	60	240	56	21	13	90
15-Jul	284	115	161	560	88	73	51	212	20	18	32	70
1-Aug	236	98	126	460	72	85	39	196	24	17	15	56
15-Aug	112	69	39	220	51	41	24	116	38	22	12	72
1-Sep	84	58	18	160	68	78	68	214	44	32	18	94
15-Sep	60	44	26	130	44	58	22	124	20	30	12	62
1-Oct	76	30	61	167	70	118	36	224	38	74	28	140
15-Oct	196	86	50	332	44	102	38	184	22	26	12	60
1-Nov	112	84	58	254	78	38	22	136	32	22	10	64
15-Nov	98	62	42	202	58	158	50	266	16	17	11	44
1-Dec	70	58	36	164	32	34	28	94	24	46	12	82
15-Dec	78	44	26	148	36	24	22	82	20	32	10	62
1-Jan	56	36	20	112	26	20	19	65	10	22	6	38
15-Jan	50	30	26	106	24	16	12	52	6	14	10	30
1-Feb	76	38	13	127	46	36	13	95	18	17	9	44
15-Feb	80	44	24	148	58	46	18	122	22	14	18	54
1-Mar	130	102	80	312	86	64	40	190	28	12	20	60
15-Mar	148	122	104	374	96	70	44	210	40	20	32	92
1-Apr	118	64	50	232	76	58	72	206	58	16	20	94
15-Apr	130	108	98	336	38	56	60	154	64	24	18	106
Total	3899	2429	1602	7930	1771	1675	1104	4550	992	908	546	2446
Avarage	162.45	101.20	66.75	330.4	73.79	69.79	46	189.58	41.33	37.83	22.75	101.91

Table (2): Main metabolites content of different fresh Mango leaves cultivars *Mangifera indica* after infestation.

Mango variety	Total proteins (mg/g fresh weight)	Total carbohydrates (mg/g fresh weight)
Keitt	58±1.8 ^a	81.8±2.3 ^b
Fagriklan	50±2.1 ^b	124.2±3.8 ^a
Alphonso	46.9±2.3 ^b	87.8±3.3 ^b

Information is displayed as mean ± standard deviation. Significant differences are indicated by various superscripts within a column (p < 0.01, ANOVA).

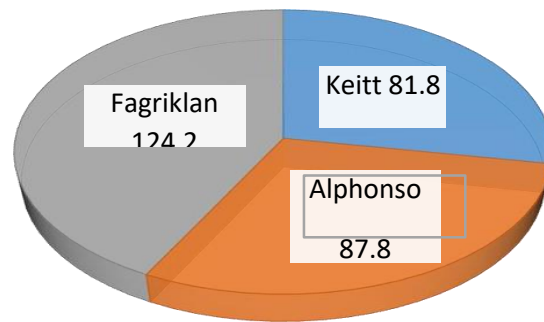


Figure (1): Carbohydrate content of mango fresh leaf varieties, *Mangifera indica* was expressed by mg/g leaf fresh weight.

On the other hand, secondary metabolites (Allelochemicals) values are illustrated in Table (3). The Keitt variety of mango had the lowest level of the total amount of the studied secondary metabolites (phenols,

flavonoids, and terpenoids). Total amounts of these metabolites were 9, 11.1, and 12.9 mg/g of fresh leaves for Keitt, Fagriklan, and Alphonso, respectively (Figure 2).

Table (3): Secondary metabolites composition of different fresh mango leaf cultivars, *Mangifera indica*, after infestation.

Mango variety	Total phenols (mg GA/g fresh weight)	Flavonoids (mg CE/g fresh weight)	Terpenoids (mg acid/g fresh weight)
Keitt	3.42±0.2 ^b	1.4±0.1 ^b	4.2±0.1 ^b
Fagriklan	2.9±0.1 ^b	1.03±0.1 ^c	7.2±0.1 ^a
Alphonso	4.4±0.3 ^a	1.7±0.1 ^a	6.8±0.2 ^a

Information is displayed as mean ± standard deviation. Significant differences are indicated by various superscripts within a column (p < 0.01, ANOVA).

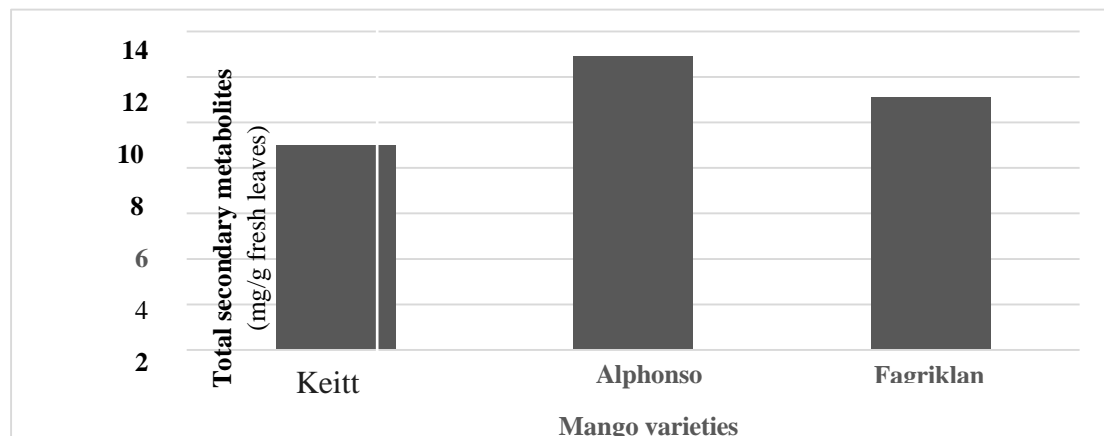


Figure (2): Total secondary metabolites content of mango leaf varieties, *Mangifera indica*.

3. NPK content:

The results (Table 4) revealed that N>K>P content with regard to the inorganic content of *M. indica L.* leaves of different cultivars. The Keitt variety had a significantly high content of N

and K. P showed no significant changes among different varieties. It was 129, 123, and 137 mg/100g fresh leaves for the Keitt, Fagriklan, and Alphonso varieties, respectively.

Table (4): NPK content of different fresh mango leaf cultivars *Mangifera indica* after infestation.

Mango variety	N (mg /100 g)	P (mg /100 g)	K (mg /100 g)
Keitt	943±31 ^a	129±3.6 ^{ab}	263±5.5 ^a
Fagriklan	795±13.6 ^b	123±2.1 ^b	227±2.5 ^b
Alphonso	778±12.6 ^b	137±5.5 ^a	219±3.1 ^b

Information is displayed as mean ± standard deviation. Significant differences are indicated by various superscripts within a column (p < 0.01, ANOVA).

4. Correlation coefficient:

The highest infestation level had a strong positive correlation (Table,5) with the proteins (r= +0.997), N (r= +0.994) and K (r= +0.999) content of mango leaves. On the contrary, the number of infested insects was highly negatively correlated with terpenoids (r= -0.949). Keitt cultivar had the highest amount of proteins, N and K, and relatively low secondary

metabolites (phenols and flavonoids), this may contribute to the higher susceptibility to infestation of Keitt mango cultivar than Fagriklan and Alphonso cultivars. Carbohydrates showed a moderate negative correlation (r= -0.442) with infestation level. The other biochemical components had a weak negative correlation with insect number infested mango leaf cultivars.

Table (5): Correlation coefficient (r) and slope between totally different stages number of *Aulacaspis tubercularis* infested mango cultivars *Mangifera indica* and the quantity of each biochemical component.

Chemical components	Correlation (r)	Slope
Proteins	+0.997	0.018
Carbohydrates	-0.442	-0.034
Phenols	-0.364	-0.0009
Flavonoids	-0.137	-0.0002
Terpenoids	-0.949	-0.005
N	+0.994	0.300
P	-0.276	-0.006
K	+0.999	0.078

The purpose of the present study focused on which of the mango leaf components may have an effect on the level of infestation with *A. tubercularis*. The data clearly showed that the Keitt cultivar is the highly susceptible level to *A. tubercularis* infestation, followed by the Fagriklan and Alphonso cultivars. This somewhat agrees with the data obtained by Salem *et al.* (2006), who revealed that *I. seychellarum*, the Seychelles fluted scale, may be used to rank the mango cultivars susceptibility levels in declining order as follows:

Sultani>Baladi and Hendi>Ewaisi>Alphonso. El-Badawy (2014) also noted that the following descending order might be used to arrange mango cultivars that are most susceptible to *I. seychellarum*: Fagriklan>Alphonso-Naser> Baladi> Hendi> Dabsha.

The infestation rate of *A. tubercularis* was obtained to be relatively low in the winter, summer, and autumn months, whereas the highest peaks were captured in the spring months. The greatest quantity

was noted during 1st May with (1200, 748, and 630 individuals for Keitt, Fagriklan and Alphonso, respectively, and the period with the lowest abundance was in mid-January. These results are consistent with the results obtained by Mokhtar and Moussa (2015), who noticed that the insects under survey had a year-round existence of mango plants. On March 1st, *A. tubercularis* displayed the highest total mean values compared to the other species (260.06 individuals/branch). *P. citri* followed with the highest total mean values on May 1st, (149.28 individuals/branch), *I. seychellarum* on April 1st, (124.58 individuals/branch), and *K. acuminata* peaked on May 15th, 72.78 individuals/branch. In contrast to the data given by Mahgoob (2006), he reported that, on some mango types, the bud mite *Eriophyes mangiferae* had its peak population from September to January or February and its lowest population from March to June.

With respect to some main metabolites, this study shows that Keitt leaves were found to contain significantly higher protein than Fagriklan, and Alphonso while the carbohydrate content of Fagriklan>Alphonso>Keitt cultivars. On the other hand, it was found that the Keitt variety of mango had the lowest level of the total amount of the studied secondary metabolites (phenols, flavonoids, and terpenoids) in addition to having the significant high content of nitrogen (N) and potassium (K) as some inorganic contents of mango leaves. Abd Elrahman *et al.* (2006) mentioned that different mango cultivars could have different nutritional values that affect *I. seychellarum* choice of its host trees. Proteins are the major source of amino acids and nitrogen, and carbohydrates are the main source of energy for insects (Jain *et al.*, 2000), thus high concentration of such

essential nutrients in mango leaves may increase the tree's suitability for *A. tubercularis* infestation. By either pulling into their enemies or repelling them, or by having poisonous effects on them, secondary metabolites aid in keeping plants safe from biotic stresses (Al-Khayri *et al.*, 2023), accordingly, the relatively low level of secondary metabolites contributes to the high susceptibility of Keitt mango cultivars to infestation with *A. tubercularis*. These results are in good agreement with the data obtained by Salem *et al.* (2006), who concluded that, compared to the other four mango cultivars under study, the most sensitive mango cultivar. (Sultani) to *I. seychellarum* had the lowest quantities of total phenolics and tannins and the highest concentrations of both soluble proteins and total carbohydrates.

Moreover, the experiments protruded the correlation coefficient between the totally different stage number of *A. tubercularis* infested the studied mango cultivars and the quantity of each biochemical component. The data underscored the strong positive correlation with proteins, N, and K content of mango leaves and negatively correlated with terpenoids, carbohydrates, and other biochemical components. These results nearly agree with the data obtained by Mokhtar (2022), who stated that a significant positive correlation between *I. seychellarum* and fats, proteins, nitrogen, carbohydrates, and moisture percentage had been discovered, particularly on Balade, Zebda, and Alphonso cultivars. Also, the results are consistent with that of Mahgoob (2006), who determined that the most significant mango varieties' susceptibility to mite infestation was inversely connected with total soluble sugars and carbohydrates and positively correlated with total soluble proteins

and amino acids; however, there was no discernible trend in the phenol content.

It could be concluded that nutritive components (protein and carbohydrates), some allelochemicals (phenols, flavonoids, and terpenoids), and some inorganic contents (N, P, and K content) of mango leaves may play a significant role in vulnerability differences of mango leaf cultivars to infestation with the white mango scale insect, *A. tubercularis*.

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