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Biochemical and anatomical characteristics of some mango tree cultivars infested by two mealybugs, *planococcus citri* (risso) and *icerya seychellarum* (westood) in Egypt

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ABSTRACT

Anatomical and biochemical parameters have been studied for six mango leave cultivars (Balade, Skare, Zebda, Fagri Klan, Alphonso and Ewais) infested with two mealy bugs, *Planococcus citri* (Risso) and *Icerya seychellarum* (Westood). Ammonium humate 3% was used as a bio insecticide to control these pests during the period from April 2020 till March 2021 under field condition. Statistical analysis showed that Ammonium Humate had positive effects on reducing the populations of both *I. seychellarum* and *P. Citri.* No infestation by *P. Citri* was occurred on Alphonso variety while less infested by *I. Seychellarum* was recorded. Leaf anatomy for this mango cultivar indicated the highest number of resin canals as 14 canals. Skare and Ewais Cvs. had no infestation with *I. seychellarum* but *P. citri* was present because a high number of resin canals as 11 and 9-10 canals, respectively. Fagri Klan Cv. had the highest average mean number for *I. seychellarum and p.citri* populations among treated and untreated mango varieties. Strong positive correlation of Fats, Proteins, Nitrogen, Carbohydrates and Moisture % had found with the *I. seychellarum* especially on Balade, Zebda & Alphonso Cvs. Likewise, a strong positive correlation occurred with *p. citri* on Balade and Zebda Cvs. Therefore, Ammonium humate 3% could be used as a bio pesticide in the integrated pest management programs, also, the health of mango crops could be improved according to the biochemical and anatomical parameters of mango plants in this study.

Keywords: Ammonium humate, Mango cultivars, mango leaf anatomy, *Icerya seychellarum*, *Planococcus citri*

INTRODUCTION

Mealybugs are phloem feeders and sap-sucking insects from all plant parts of the order Hemiptera, Family: Pseudococcidae. They are little, delicate bodied coverings with white waxy emissions from which they get their name (Kosztarab and Kozár, 1988). These insects are sucked a great amount of plant fluids resulting in the secretion of honey dew which supports the growth of a black fungus called sooty molt and also, transmit some serious plant diseases (Fisheries and Foresty, 2008). These problems lead to real financial misfortune, especially for the harvested plants of mango trees (Ben-Dov, 1994).

Mangifera Indica L., (Anacardiaceae) is an important fruit crop in Egypt, especially in the Ismailia Governorate, where about 69 known species are grown. Its has a great nutritional value and the richest sources of vitamins, mineral salts, enough amounts of carbohydrates, proteins with different delicious varieties (Abourayya *et al.,* 2011). Unfortunately, the heavily infestation by mealybugs causes a lot of damage for all parts of mango trees and a huge loss in the mango yield.

Various techniques were utilized to decrease and control the number of inhabitants in this vermin. This work meant to utilize one of these strategies. Ammonium humate is an environmentally friendly bio insecticide that applied in this study to a different mango varieties and how this substance effected on the pest population and the anatomy for these mango varieties.

Our target of this study is to highlighting the population dynamics of two common mealybugs infested mango trees: the citrus mealybug, *Planococcus citri* (Risso) and the seychelles mealybug, *Icerya seychellarum* (Westood) associated with different varieties of treated and untreated mango trees (Balade, Skare, Zebda, Fagri Klan, Alphonso and Ewais) with Ammonium humate in Ismailia Governorate at Arab Republic of Egypt (A.R.E.). Also, to explain the relationship between the population dynamic of these pests and the biochemical, anatomical aspects for mango's different Cultivars.

MATERIAL AND METHODS

Ammonium humate extract from compost:

Treat the compost with 1.0 N KOH and 2 N NH4OH. A mixture of 40 g of compost and 800 ml of the solution was shaken in a sealed bottle at 120 rpm for 12 h under N_2 gas atmosphere, then centrifuged at 6000 rpm for 15 min, the supernatant was removed, and the solution was dehydrated by the following method. The pH was adjusted to 7.0 by adding 2 M H2SO4. The neutralized solution was determined for total nitrogen content in ammonium humate by steam distillation (Kjeldahl) (Page *et al.,* 1982) and (Shehata, 2018).

Field assay and sampling:

Six mango varieties (Balade, Skare, Zebda, Fagri Klan, Alphonso and Ewais) were nominated for experiments in Ismailia Governorate (El-Kasasen district orchards) without the use of any pesticides. Before data collection, approximately 400 liters (200 ml/tree) of 3% Ammonium humate were added into the soil of infested mango tree cultivars once a week for one month using the soil soak method.

Samples collected twice a month from four infested mango trees of each variety, as similar as possible in size, shape, height and infestation, during the one-year study period from April 2020 to March 2021 of treated and untreated trees. Twenty-five infested leaves about 15 cm long, 25 cm diameter, and from cardinal directions (north, south, east and west) and tree core of each selected trees were picked up using a garden shears.The collected samples were packaged in paper bags, transported and examined by the aid of a needle using a stereoscopic microscope at the laboratory of the Ismailia Agriculture Research Station.

Live stages of nymph and adult female for *P. citri* (Risso) *and I. seychellarum* (Westood) were counted and recorded. Specimens were enclosed in glass jars (15 cm diameter and 20 cm height). The jar is covered with muslin held in position by a rubber band. The samples were checked daily.

Anatomy parameters:

Treated and untreated fresh sections of the six mango leaf cultivars were selected and kept in formalin acetic acid alcohol solution (F.A.A) for anatomical preparation. Leaves of the study taxa were preserved at F.A.A. embedded in paraffin wax, then serially sectioned at 10-15 µ according to the conventional method (Johansen, 1940).

Sections were stained in crystal violt-erthrosin (saturated in colve oil) combination. Leaf micrometric attributes were examined and recorded using Olympus© CHS Binocular Microscope. Photomicrograph taken with Sony® digital camera mounted on a microscope. The magnification is given automatically by the ImageJ® Tool software (Rasband, 2011). A description of each slide is placed in the Faculty of Botany, Department of Botany, Suez Canal University, Ismailia, Egypt.

Biochemical parameters:

Five biochemical parameters (total Carbohydrate, Protein, Nitrogen, fat and Moisture percentages) were selected to measure their percentages for the six infested untreated mango cultivars. These components's play an important role in the nutrition for both the pest and its host. The measurements were carried out at the Agricultural Research Center of the Central Laboratory of the Egyptian Horticultural Institute as follows:

Carbohydrates %.:

Approximately (0.1 gm) of dried mango variety samples were placed in a test tube, followed by the addition of 1N HCl acid (10 mL). Seal the tube and place in an oven at 100°C for 6 hours. The solution was then filtered and the filtrate was clarified by the leading and deluding method using lead acetate solution (137 gm/L.) and the excess of lead salt was precipitated using N/3 potassium oxalate solution. The extract was measured into a measuring flask (50 mL). The combined filtrate was completed to the mark with distilled water. Total sugars were determined according to the method of (Dubois *et al.,* 1956). Date is expressed as gm/100 gm distilled water.

Nitrogen percentages:

Determine the minerals content by take 0.5 gram of dried sample was digested using the H2SO4 and H2O2 as described by (Cottenie, 1980). Extracted samples were used to determine the following minerals: Nitrogen content (gm./100gm. D.W.) was determined in the digested solution by the modified microkjeldahl method as described by (Plummer, 1971).

Protein percentages:

Multiplying total nitrogen by 6.25 then the proteins proportion is obtained by a sample (A.O.A.C., 1990).

Fats percentages:

A known weight of fat from leaves (5 g) of each mango variety was extracted with petroleum ether in a Soxhlet apparatus for 18 hours. According to (A.O.A.C., 1990), the solvent was evaporated and the residue was dried at 95°C to constant weight. The percentage of oil content is then calculated based on dry weight.

Moisture % :

A dry sample of known weight (2 g) was weighed in a porcelain crucible and placed in a muffle furnace controlled at 55°C for several hours until a constant weight was obtained (A.O.A.C., 2005). Calculate the percentage of moisture in the leaves of each cultivar.

Statistical analysis:

Results were statistically analyzed using analysis of variance (ANOVA), while to differentiated between groups at ($P < 0.05$) using Fisher' s the least significant differences analysis (L.S.D) test. The simple correlation (r) and regression co-efficient value (b) were adopted to clarify the change occur between the biochemical parameters and the mealybugs populations according to SPSS version 16.

RESULTS

Populations dynamics of two mealybugs, *P. citri* (Risso) *and I.seychellarum* (Westood) infesting six mango cultivars.

Tables (1 & 2) recorded the average mean numbers for mealybugs population of six mango cultivars before and after adding 3% ammonium humate during the one-year study. Analysis of variance in Table 3 clarified that the Fagri klan Cv. had the highest mean numbers of I. seychellarum and p.citri populations among the treated and untreated mango cultivars at (101.3 ± 9.6 and 66.8 ± 4.3; 100.8 ± 9.2 and 79.5 ± 9 mean/ per leaf), respectively. Alphonso Cv. had the least infestation with *I. seychellarum* before and after treatment (36.48 ± 7.1 & 17.88 ± 2.2 average / per leaf), while Ewais had the lowest infestation rate against p. citrus during the trial period was (29.7 ± 5.4 & 18.8 ± 3 mean/leaf). Data assured that there were a significant difference between the six mango varieties infested with the *I. seychellarum* prior and then afterward treatment as (F-ratio = 23.9, p-value at 0.05= 0.000001*** and L.S.D = 14.92) , Also Skare and Ewais varieties have no infestation with this mealybug.

On other hand, the normal all out mean for *p.citri* mealybug showed additionally a huge contrasts among treated and untreated mango varieties as (F-ratio = 39.9, p-value = 0.000001*** and L.S.D = 18.1), while Alphonse variety has any infestation.

Table 1. Average numbers of the Seychelles mealybug, *Icerya seychellarum* before and after adding Ammonium humate 3% for six mango cultivars during one year of study.

Sampling dates		Total mean no. for *Planococcus citri*											
		Before adding Ammonium Humate						After adding Ammonium Humate					
		Balade	Skare	Zebda	Fagri klan	Alphons	Ewais	Balae	Skare	Zebda	Fagri klan	Alphons	Ewais
April	1st	165.55	126.15	148.66	192.44	0	86.74	65.55	30.15	65.66	92.44	0	42.74
	15th	102.73	58.08	82.98	124.88	0	35.59	54.73	18.08	42.98	79.88	0	22.59
May	1st	77.22	39.13	55.23	98.81	0	17.18	41.22	29.13	21.23	28.81	0	9.18
	15th	157.76	110.71	135.3	188.24	0	82.17	32.76	55.71	19.3	21.24	0	6.17
June	1st	126.23	81.84	103.11	152.3	0	67.59	45.23	22.84	12.11	56.3	0	6.59
	15th	54.39	18.84	31.27	77.88	0	14.63	14.39	18.84	11.27	77.88	0	14.63
July	1st	33.01	17.61	20.66	63.4	0	13.92	12.01	17.61	20.66	33.9	0	13.92
	15th	26.7	15.65	19.73	57.37	0	12.61	6.7	15.65	19.73	57.37	0	12.61
Aug.	1st	23.31	15.53	18.59	52.37	0	12.79	23.31	15.53	18.59	52.37	0	12.79
	15th	34.12	17.32	20.47	52.24	0	14.08	34.12	17.32	20.47	52.24	0	14.08
Sept.	1st	53.46	18.62	31.35	77.46	0	15.45	53.46	18.62	31.35	77.46	0	15.45
	15th	95.01	41.68	71.67	117.67	0	21	95.01	41.68	41.67	117.67	0	21
Oct.	1st	157.15	105.67	133.32	183.15	0	75.9	157.15	45.67	68.32	183.15	0	35.9
	15th	122.42	83.63	103.35	147.44	0	47.11	122.42	23.63	72.35	147.44	0	27.11
Nov.	1st	74.18	40.21	61.9	97.62	0	26.73	74.18	40.21	61.9	97.62	0	26.73
	15th	37.31	17.83	22.11	60.96	0	15.13	37.31	17.83	22.11	60.96	0	15.13
Dec.	1st	33.81	16.78	21.98	57.05	0	15.05	33.81	16.78	21.98	57.05	0	15.05
	15th	29.41	16.01	19.76	45.3	0	12.59	29.41	16.01	19.76	45.3	0	12.59
Jan.	1st	46.94	36	42	59.04	0	1.31	46.94	36	42	59.04	0	1.31
	15th	54.32	39.71	47.87	65.45	0	3.26	54.32	19.71	15.87	65.45	0	3.26
Feb.	1st	42.27	16.7	28.38	80.73	0	11.36	42.27	16.7	8.38	80.73	0	11.36
	15th	52.3	17.94	24.41	85.28	0	14.66	52.3	17.94	24.41	85.28	0	14.66
March	1st	140.91	83.89	110.96	174.12	0	69.78	140.91	33.89	34.96	174.12	0	69.78
	15th	81.65	37.9	59.39	108.33	0	25.29	81.65	37.9	22.39	108.33	0	25.29
Average total mean		**75.9**	**44.72**	**58.93**	**100.8**	**0**	**29.66**	**56.2**	**25.9**	**30.81**	**79.66**	**0**	**18.7**

Table 2. Average numbers of the citrus mealybugs, *Planococcus citri* before and after adding Ammonium humate 3% for six mango cultivars during one year of stu

Sampling dates		Total mean no. for *Icerya seychellarum*											
		Before adding Ammonium Humate						After adding Ammonium Humate					
		Balade	Skare	Zebda	Fagri klan	Alphons	Ewais	Balade	Skare	Zebda	Fagri klan	Alphons	Ewais
April	**1st**	106.58	0	74.73	126.18	48	0	57.38	0	50.73	87.12	36	0
	15th	91.78	0	71.93	120.92	41.74	0	46.04	0	33.93	65.07	22.74	0
May	**1st**	105	0	28.64	126.09	54.35	0	13	0	8.64	66.32	13.35	0
	15th	213.05	0	198.26	246.62	157.05	0	7.05	0	7.26	99.22	11.05	0
June	**1st**	103.22	0	81.34	145.82	69.11	0	18.22	0	6.34	83.56	5.11	0
	15th	71.25	0	53.07	93.6	28.29	0	12	0	8.07	93.6	6.29	0
July	**1st**	49.34	0	32.04	72.04	17.7	0	25.34	0	12.04	72.04	17.7	0
	15th	43.84	0	18.95	62.99	15.88	0	28.84	0	18.95	62.99	15.88	0
Aug.	**1st**	42.13	0	33.9	64.28	17.68	0	42.13	0	33.9	64.28	17.68	0
	15th	43.46	0	28.04	74.54	17.85	0	43.46	0	28.04	74.54	17.85	0
Sept.	**1st**	83.08	0	63.35	110.7	40.22	0	55	0	33.35	92.7	40.22	0
	15th	52.55	0	29.81	77.26	17.27	0	41.36	0	29.81	77.26	17.27	0
Oct.	**1st**	132.86	0	115.22	166.48	75.97	0	52.12	0	42.22	91	25.97	0
	15th	103.5	0	84.86	134.34	67.93	0	58.02	0	37.86	42.08	37.93	0
Nov.	**1st**	60.96	0	43.08	95.03	18.77	0	41.08	0	43.08	55.18	18.77	0
	15th	43.06	0	29.16	69.57	16.9	0	43.06	0	29.16	40.36	16.9	0
Dec.	**1st**	35.61	0	19.64	65.02	17.12	0	35.61	0	19.64	65.02	10.66	0
	15th	26.84	0	18.75	51.48	14.72	0	26.84	0	18.75	51.48	14.72	0
Jan.	**1st**	43.35	0	38.27	56.67	0.87	0	20.22	0	12.26	17.88	0.87	0
	15th	48.87	0	40.27	58.11	1.33	0	19.18	0	11.22	22.8	1.33	0
Feb.	**1st**	36.95	0	22.24	79.01	15.23	0	25	0	8.24	79.01	9.23	0
	15th	32.98	0	18.94	63.33	13.45	0	23.14	0	7.94	63.33	13.45	0
March	**1st**	72.67	0	47.8	100.72	25.14	0	40.34	0	47.8	72.16	25.14	0
	15th	140.14	0	114.43	170.3	82.98	0	77.02	0	71.43	64	32.98	0
Average total mean		**74.29**	**0**	**54.44**	**101.29**	**36.48**	**0**	**35.47**	**0**	**25.86**	**66.79**	**17.87**	**0**

Table 3. Variation analysis of mealybugs, *Icerya seychellarum* (Westood) and *Planococcus citri* (Risso) among untreated and treated mango cultivars with Ammonium humate 3% in Ismailia Governorate.

Avearage total mean for pest population	Ammonum humate 3% treatment	Mango varieties Mean ± S.E					
		Balade	**Skare**	**Zebda**	**Fagri klan**	**Alphons**	**Ewais**
Icerya seychellarum	Before	74.3 ± 9	0	54.5 ± 8.6	101.3 ± 9.6	36.48 ± 7.1	0
	After	35.5 ± 3.5	0	25.9 ± 3.5	66.8 ± 4.3	17.88 ± 2.2	0
P-value at 0.05* < 0.000001 **							
F-ratio = 23.9							
L.S.D = 14.92							
Planococcus citri	Before	76 ± 9.5	44.7 ± 7.1	59 ± 8.6	100.8 ± 9.2	0	**29.7 ± 5.4**
	After	56.3 ± 8	26 ± 2.4	30.8 ± 3.9	79.5 ± 9	0	**18.8 ± 3**
P-value at 0.05* < 0.000001 **							
F-ratio = 39.9							
L.S.D = 18.1							

Anatomical characteristics:

In this part of study, differences in anatomical features of mango leaf cultivars infested by the two mealybugs that treated and untreated with Ammonum humate are shown. Table 4 and Figure 1 focus on a number of characteristics, notably the cuticle thickness observed upper the adaxial epidermis, number of phloem resin canals and crystals, which play an important role in leaf pest protection and attract beneficial insects as Predators and parasitic wasps.

Balade, Skare and Alphonse cultivars have thick cuticles after treatment, while other breeds have thin cuticles. Skare, Zebda, Fagri Klan, Alphons and Ewais had higher numbers of Phloem resin canals after treatment with 11, 11-13, 9, 14 and 9 -10, respectively, than before biocide application, except for Balade variety, which showed lower numbers. There was a numerous crystal after treatment for Skare, Fagri klan and Ewais cultivars, whereas others have few and solitary crystals. The bundle sheath layer around the xylem and phloem tissues was absent in Zebda, Fagri klan, Aphonse and Ewais varieties before treatment and found in other cultivars.

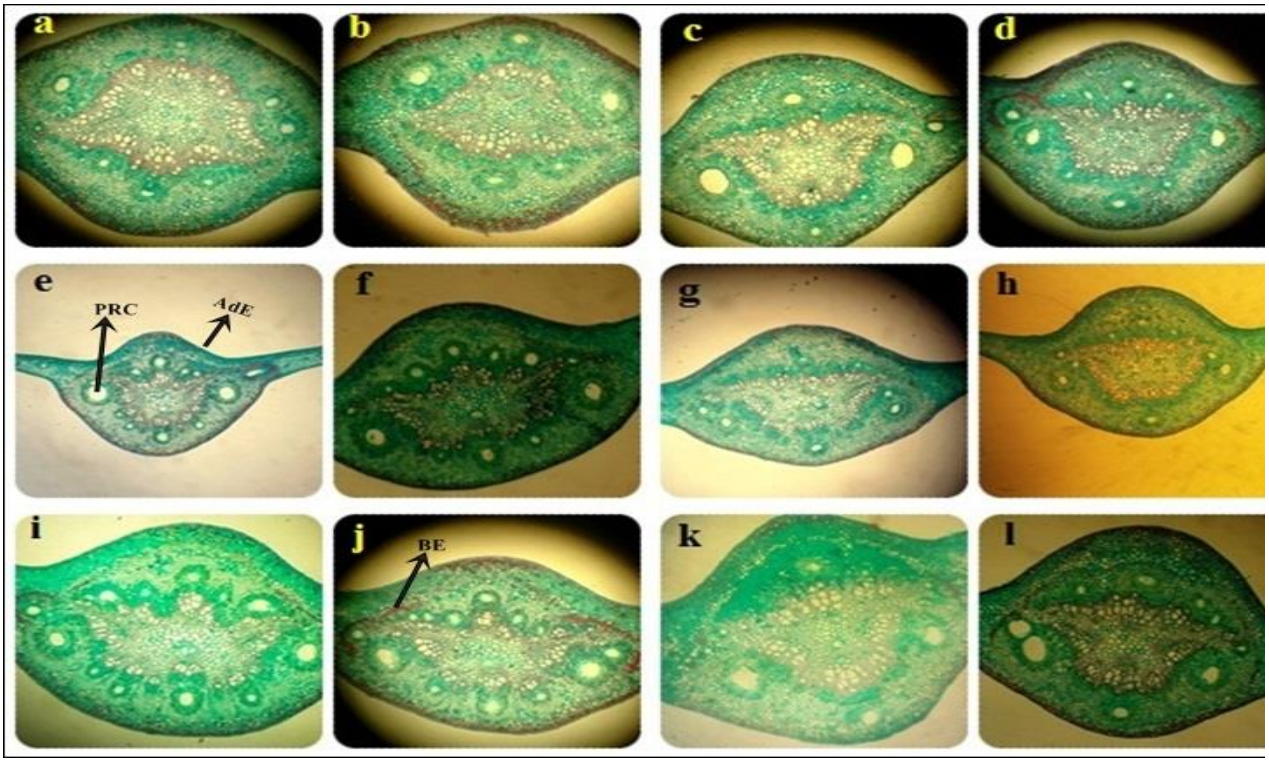

Fig.1. Leaf transverse sections of treated and untreated mango cultivars: Balade (a) untreated(b) treated, Skare(c) untreated(d) treated, Zebda(e) untreated(f) treated, Fegriklan(g) untreated(h) treated, Alphonso(i) untreated(j) treated, Ewais (k) untreated(l) treated. [(AdE) Adaxial epidermis (BS) Bundle Sheath, (PRC) Phloem Resin Canal].

Table 4. Anatomical features comparison between six treated and untreated mango varieties with Ammonium humate 3% infested with *Icerya seychellarum* and *Planococcus citri* mealybugs**.**

Characters		Mango leaf cultivars					
		Balade		Skare		Zebda	
		Untreated	Treated	Untreated	Treated	Untreated	Treated
Anatomy of leaf	Adaxial epidermis	one thin cuticle layer columnal like cells	one thick cuticle layer square like cells	one thin cuticle layer columnal like cells	thick cuticle layer columnal like cells	one thin cuticle layer columnal like cells	one thin cuticle layer square like cells
	Mesophyll layers	one palisade layer spongy layer is wide	one palisade layer spongy layer is regular	one short palisade layer spongy layer wide	one palisade layer spongy layer compact	one palisade layer spongy layer is wide	one palisade layer spongy layer is compact
	central vascular cylinder	Triangle shape bundle sheath present	Triangle shape bundle sheath present	wings shape	wings shape	Zigzag shape No bundle sheath	Zigzag shape bundle sheath absent
	Number of phloem resin canal	8	7	6	11	(9-13)	(11-13)
	Pith region rewin canal	absent	absent	absent	absent	1	absent
	Crystals	Few & solitary	absent	Few & solitary	numerous	absent	Druses & solitary
Characters		Fagri Klan		Alphonso		Ewais	
		Untreated	Treated	Untreated	Treated	Untreated	Treated
Anatomy of leaf	Adaxial epidermis	Thin cuticle layer columnal like cells	Thin cuticle layer columnal like cells	Thin cuticle layer Palisade like cells	Thick cuticle layer Palisade like cells	Thin cuticle layer square like cells	Thin cuticle layer square like cells
	Mesophyll layers	one or two palisade layer one spongy layer	Double palisade layer spongy layer is irregular	Double palisada layer spongy layer irregular shape	one palisade layer spongy layer so wide	Double palisada layer spongy layer irregular shape	one palisade layer spongy layer wide
	central vascular cylinder	Triangle shape No bundle sheath	wings shape bundle sheath present	Zigzag shape No bundle sheath	Zigzag shape bundle sheath present	Triangle shape bundle sheath absent	wings shape bundle sheath present
	Number of phloem resin canal	7	9	12	14	4	(9-10)
	Pith region rewin canal	absent	absent	absent	absent	absent	absent
	Crystals	Few & solitary	Numerous	Few & solitary	Few & solitary	Few & solitary	Numerous

Relationship between biochemical parameters and mealybugs population:

The results in Figure 2 represent the biochemical parameter rates of fat, protein, nitrogen, carbohydrate, and moisture for six mango leaf cultivars before treated with ammonium humate. The maximum values for fat, protein, nitrogen, carbohydrate, and moisture were 6.2 ± 0.9, 21.39 ± 1.2, 3.1 ± 0.25, 16.51 ± 1, and 56.77 ± 3.8% for the Skare, Balade, Ewais, Fagriklan, and Zebda varieties, respectively. The lowest observed rates for fat, carbohydrate, and moisture were 2.36 ± 0.29, 14.58 ± 1.1, and 31.74 ± 1.42%, respectively, in Zebda variety, and 9.58 & 1.44 in Alphonso variety, respectively.

Table 3 analyzes the relationship between the average total mealybug population and biochemical composition of the six mango cultivars. The seychelles mealybug, *Icerya seychellarum* had a strong direct correlation (r = 0.99***, p-value = 0.02) with the fat content of Balade cultivar and a moderate direct correlation with Alphonso, Zebda and Fagriklan cultivars (r = 0.59**, 0.55** and 0.54; p-values = 0.66, 0.63 and 0.71). Protein ratios had very strong direct correlations with mealy bug populations in Alphonso, Zebda and Balade cultivars, namely (r = 0.89***, 0.78*** and 0.99**; p-values = 0.54, 0.58 and 0.5, respectively). The correlation with the Fagriklan variety was very weak (r = 0.21*, p-value = 0.87). A strong direct correlation between this mealy bug and nitrogen percentage was found in Zebda, Fagriklan & Balade varieties (r= 0.98***, 0.99*** & 0.98***; p-value = 0.51, 0.5 and 0.51, respectively), while a medium indirect correlation with Alphonso as (r = - 0.32, p-value = 0.88). There was a strong direct correlation between *I. seychellarum* and carbohydrate value on Alphonso, Zebda &Balade varieties as(r = 0. 8***, 0.9***& 0.96***; p-value= 0.57, 0.5and 0.51), respectively also a medium direct correlation with Fagriklan as (r= 0.55**, p-value= 0.68).

Moisture ratio had a strong direct correlation with mealybug populations on Zebda & Balade cultivars (r = 0.99*** & 0.78*** ; p-value = 0.5, and 0.58, respectively), but a medium direct correlation with Fagriklan (r= 0.62**, p-value= 0.65) and very weak with Alphonso (r= 0.08*, p-value= 0.95). Although not noticed a relationship with the Ewais and Skare breeds.

On the other hand, there was a very strong direct correlation between the citrus mealybug, *Planococcus citri* and the fat content of Balade, Ewais & Skare cultivars, as (r = 0.95***, 0. 89***& 0.92*** ; p-value = 0.12, 0.29 & 0.25, respectively), also with proteins on Zebda & Balade as (r = 0.75*** & 0.9*** ; p-value = 0.58& 0.53, respectively). Likewise with nitrogen % on Zebda, Fagriklan & Balade varieties as (r = 0.99***, 0.99***, & 0.89**; p-value = 0.5, 0.5 and 0.52, respectively) and the carbohydrate on Zebda & Balade cultivars as (r = 0.9*** & 0.85***; p-value = 0.5 & 0.55, respectively). Finally, the moisture on Zebda & Balade varieties as (r = 0.98*** & 0.92***; p-value = 0.5 & 0.53), respectively.

Besides that a medium direct correlation obtained between this mealybug and fats % on Zebda & Fagriklan as (r = 0.51** & 0.36**; p-value = 0.63 & 0.77, respectively). As well, protein on Ewais & Skare as (r= 0.52** & 0.29**, p-value = 0.7 and 0.82, respectively) and carbohydrate on Fagriklan variety as (r = 0.47** ; p-value = 0.72) aside from moisture on Fagriklan & Skare as (r= 0.54** & 0.34**, p-value = 0.68 and 0.8), respectively. In addition to a weak direct correlation with proteins on Fagriklan variety as (r = 0.12*; p-value = 0.92) and nitrogen & carbohydrate on Skare as (r = 0.2* & 0.21*; p-value = 0.87 and 0.87) , respectively. A negative correlation showed on Ewais variety as (r = -0.19, -0.54 & - 0.58; p-value = 0.89, 0.78 and 0.72), respectively with nitrogen %, carbohydrate and moisture. Alphonso cultivar has no relationship with p. citri population and biochemical for this mango leaves cultivars.

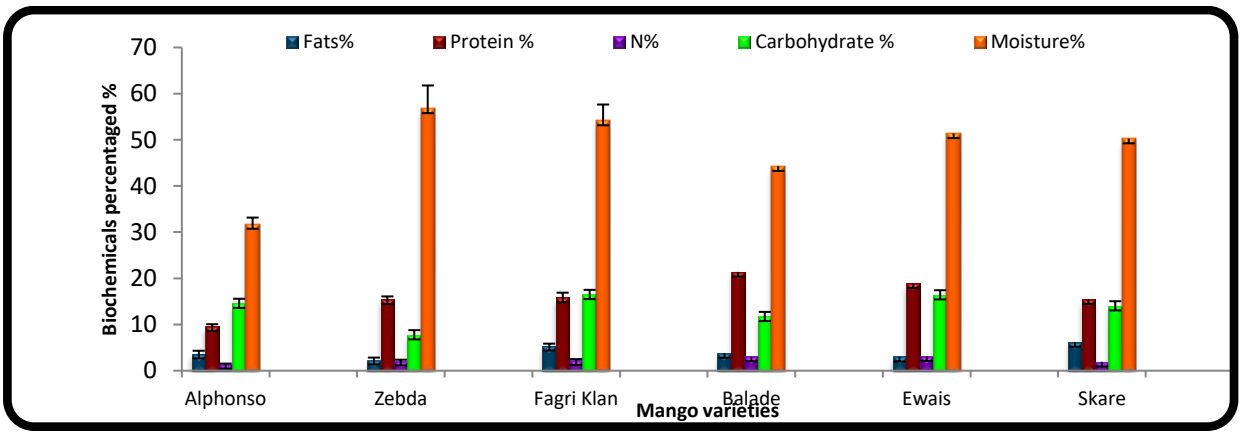

Fig. 2. Biochemical parameters percentage for six untreated mango leave varieties.

Table 5. The correlation co efficiency between biochemical parameters and mealy bugs, *Icerya seychellarum* & *Planococcus citri* for six mango varieties.

Maelybug species	Biochemical composition	Correlation co efficiency	Mango varieties					
			Alphonse	Zebda	Fagri Klan	Balade	Ewais	Skare
Icerya seychellarum	Fats	r	0.59**	0.55**	0.45**	0.99***	0	0
		p-value	0.66	0.63	0.71	0.02	0	0
	Proteins	r	0.89***	0.78***	0.21*	0.99***	0	0
		p-value	0.54	0.58	0.87	0.5	0	0
	Nitrogen %	r	-0.32	0.98***	0.99***	0.98***	0	0
		p-value	0.88	0.51	0.5	0.51	0	0
	Carbohydrates	r	0.8***	0.9***	0.55**	0.96***	0	0
		p-value	0.57	0.5	0.68	0.51	0	0
	Mositure	r	0.08*	0.99***	0.62**	0.78***	0	0
		p-value	0.95	0.5	0.65	0.58	0	0
Planococcus citri	Fats	r	0	0.51**	0.36**	0.95***	0.89***	0.92***
		p-value	0	0.63	0.77	0.12	0.29	0.25
	Proteins	r	0	0.75***	0.12*	0.9***	0.52**	0.29**
		p-value	0	0.58	0.92	0.53	0.7	0.82
	Nitrogen %	r	0	0.99***	0.99***	0.89***	-0.19	0.2*
		p-value	0	0.5	0.5	0.52	0.89	0.87
	Carbohydrates	r	0	0.9***	0.47**	0.85***	-0.54	0.21*
		p-value	0	0.5	0.72	0.55	0.78	0.87
	Mositure	r	0	0.98***	0.54**	0.92***	-0.58	0.34**
		p-value	0	0.5	0.68	0.53	0.72	0.8

DISCUSSION

The purpose of this study focused on how to improve the plant qualities of mango varieties that infected with mealybug insects, and what mango varieties are most tolerant of them? The data clearly show that Fagri Klan is the most infected mango variety, with two mealybugs, *I. seychellarum* and *p. citri* followed by Balade, then Zebda cultivar, (Pheophanh and Ki-Jeong 2016) found nine species of scale insects on mango trees in laos, *I. seychellarum* was among them, also (Hagar *et al.,* 2016) recorded the presence of *I. seychellarum* and *p. citri* on mango trees with different population peaks at Mansoura Governorate , Egypt. Otherwise no infestation was found in Ewais and Skare cultivars with *I. seychellarum* and Alphonso with *p. citri* mealybug. Results recorded by (Abd Elrahman *et al.,* 2006) about the heavily infestation of Sultani Cv. With *I. seychellarum* while Alphonso Cv. Was completely free from this mealybug .

The results underscore the importance of the bio pesticide ammonium humate 3%, which had a positive effect on the populations of two mealybugs on mango cultivars. Whereas Alphonso Cv. has the lowest infestation with *I. seychellarum* followed by Zebda , Balade and Fegri Klan, as well *p. citri* population density showed minimum rates on Ewais, Skare, Zebda, Balade and Fegri Klan varieties, respectively after treatment. Several studies correspond to this part of the study and explain the effects of other bio pesticides, for example (Mangoud *et.al.,* 2012 and Amala et al. 2014) stated that (Biofly, Neem Azal and super Mesrona oil) had medium effects on nymphs & adult females of *I. seychellarum* on mango leaves. In addition (PAN., 2005) this international coalition studied all the pests attack mango trees and how to control them by non-chemical methods especially mealybugs by used plant extracts and soap spray.

Furthermore, the anatomy of mango leaves before and after treatment was examined in detail. Meanwhile, the results estimated that the Alphonso, Zebda, Skare and Ewais cultivars had the strongest defenses against these mealybugs during the experimental period due to the presence of numerous resinous canals and crystals. Agreed, these channels contain toxic compounds that protect plants from pests, as well as volatile phenolic compounds that attract insect predators and parasitic wasps (Joel, 1980; Mitchell, 1990). According to (Aida H. M., 2018) studied the anatomical features for infested and non-infested six mango cultivars with scale insects and the important for these resin canals for mango leaf defense against pests.

Moreover, the experiment protruded the relation between the bio chemical parameters for six untreated cultivars of mango and the mealybugs populations. The data underscore the strong positive correlation between Fats, Proteins, Nitrogen, Carbohydrates and Moisture concentrations and the *I. seychellarum* density especially on Balade, Zebda & Alphonso Cvs. On the other hand, strong positive relation occurred between these parameters and *p. citri* population on Balade and Zebda Cvs. These results nearly to the idea of (Hassan, 1998) that mentioned the relation between the biochemical of Fig acacia and their pests *Hemiberlesia latania* and *Asterolecanium pustulans*. Likewise with (Tobih *et al.,* 2002) concluded that Fat content of infested and uninfested unripe mango fruits were not

significantly different with infestation with mango mealybug,,*Rastrococcus invadens*. But ripe mango fruits contained significantly higher (P<0.05) crude protein than unripe fruits.

CONCLUSION

The results of this study demonstrate the importance of 3% ammonium humate as a bio pesticide in an integrated pest management program for these mealybugs. Alphonso, Ewais and Skare are among the six mango varieties that are very resistant to these pests. Because the presence of a huge number of resin canals and crystals. These canals had a toxic compounds protect the plant from pests also a volatile phenolic compounds that attract a benefits insects as predators and parasitoids.

Beside that biochemical compositions for untreated mango leaves had a strong positive correlation with these mealybugs populations and controlling them for the six mango cultivars. This research focuses on the extent of the effect of ammonium humate anatomically on mango leaves and does it have a role in reducing infection? , in addition to the biochemical part that is more interesting in untreated mango leaf cultivars, that we may have the opportunity in the future to do more specialized research on this part and whether ammonium humate affects chemically on these pests as well.

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الصفات البيوكميائية و التشريحية لستة أصناف من أشجار المانجو المصابة بنوعين من البق الدقيقي في مصر

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البق الدقيقي من الآفات التي تتسبب في أضراراً جسيمة لأشجار المانجو حول العالم وخاصة في جمهورية مصر العربية. فأشجار المانجو ذات أهمية اقتصادية عالية, حيث أن الثمارعالية القيمة الغذائية و الدوائية ايضاً. لذلك تمت دراسة العلاقة بين التغيرات البيوكميائية و التشريحية لستة اصناف من اوراق المانجو المنتشره بجمهورية مصر العربية خاصة بمحافظة الإسماعيلية وهم (بلدي , سكري ، ذبدة ، فجر كلان ، الفونس ، عويس) و نوعين من البق الدقيقي المصاحبة لهم *Planococcus citri* و *Icerya seychellarum*. بجانب دراسة تأثير إحدى المبيدات الحيويه، هيومات الأمونيوم بتركيز 3% على تعداد هاتين الآفاتين بهذه الأصناف خلال العام من ابريل 2020 حتى مارس 2021 تحت الظروف الحقلية .

أظهر التحليل الإحصائي أن هيومات الأمونيوم له تأثير ايجابي على تقليل كثافة تعداد البق الدقيقي *I. seychellarum* بين اصناف المانجو المختلفة حيث ان p-value at 0.05 < 0.000001 and L.S.D = 14.92 وهكذا مع *P. citri* فكانت النتيجة p-value at 0.05 < 0.000001 and L.S.D = 18.1 . وجد أن صنف ألفونس كان أقل الاصناف إصابة ببق *I. seychellarum* مع عدم وجود اي اصابه ببق *P. citri* عندما تم اخذ قطاعات تشريحية لاوراق هذا الصنف وجد احتوائة على عدد كبير من قنوات ريتنج حوالي 14 قناة وهي قنوات تلعب دور مهم في افراز مركبات سامة لحماية النبات. من ناحية أخرى, وجد صنفي السكري و العويس أقل اصابه ببق *P. citri* بينما لا يوجد اي اصابه ببق*I. seychellarum* فوجد من خلال تشريح اوراقهم وجد عدد كبير من قنوات رتينج 11 و 9-10 قناة على التوالي. في حين صنف الفجركلان هو أكثر الأصناف إصابة بكلا الآفتين. من جانب أخر, ابرزت النتائج العلاقة القوية الإيجابية بين نسبة الدهون, البروتينات, النيتروجين, الكربوهيدات و الرطوبة باوراق اصناف المانجو مع تعداد *I. seychellarum* خاصة بأصناف البلدي, الزبدة و ألفونس و ايضاً مع تعداد *P. citri*بأصناف البلدي و الزبدة. اثبتت النتائج أن هيومات الأمونيوم 3% لها دور كبير بتقليل تعداد هاتين الآفتين بأشجار المانجو. من جهة أخرى, مدي اهمية دراسة الجوانب البيوكميائية و التشريحية لأوراق أصناف المانجو التي لها دور مهم بتقليل تعداد هاتين الآفتين حيث يمكن مستقبلاً تتطوير الدراسة بهذا الجزء و التوسع به.

الكلمات المفتاحية: اصناف اشجار المانجا، مبيدات حيوية، قنوات ريتنج، امونيوم هيومات، بق الموالح الدقيقي، بق السيشسلاريم الدقيقي