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SUSCEPTIBILITY OF THREE SQUASH VARIETIES TO TWO SPOTTED SPIDER MITE INFESTATION AND THEIR EFFECTS ON ANATOMICAL AND HISTOLOGICAL STRUCTURE OF INFESTED LEAVES

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Abstract

Squash is one of the most important plants, because it has many benefits to humans, it contains many nutrients important to humans. This crop infested by *Tetranychus urticae* Koch, which causes numerous injuries to leaves that may reach at sometimes to lose the productivity of the crop. The susceptibility of three squash varieties to infestation with *T. urticae* and its population during two successive seasons, 2017 and 2018 were investigated. Eskandarani was the most highly susceptible to infestation followed by moderate and low infestation on Hytech and Milet, respectively. Susceptibility of three squash varieties to infestation may be affected by trichomes on the leaves whereas, the variety with lowest number of trichomes was highly susceptible to *T. urticae.* Mite populations in Eskandarani and Milet varieties had two peaks in the same time, in 2nd week of May and 2nd week of June during 2017 season, but in 2018 season in 4th week of April and 4th week of May. The third variety Hytech had two peaks in the 3rd week of May and 1st week of June during 2017 season, but in 2018 season, in 1st week of May and 4th week of May. Light microscope investigation showed significant changes in anatomical structure of three squash varieties leaves such as decrease of upper and lower epidermis and an increase in mesophyll tissue thickness compared with healthy one. Ultrastructure changes include cell wall degradation, severe damage in chloroplast, malformation in mitochondria and nucleus was observed by electron microscope.

Key words: Squash, *Tetranychus urticae*, Susceptibility, Population and Light & Electron microscope.

Introduction

Squash plants (*Cucurbita pepo* L.) has been cultivated for its edible vegetables for thousands of years and remains a crop plant of great economic importance today, It is the important economic crop in Egypt for local consumption (Shehata *et al*., 2009). It is good source of several minerals like iron, zinc, phosphorus and potassium and also, it contains anti-oxidant, vitamin C and vitamin A (Whitaker and Bemis, 1976). It can be produced almost as year-round crop (Abou-Zaid *et al*., (2019). The estimated areas cultivated with squash in Fayoum governorate reached about 3507, 2604, 2424 and 1357 thousand Fadden, yield about 18.55, 11.29, 10.97 and 5.97 thousand ton, in 2015, 2016, 2017 and 2018 respectively (according to statistical data, Ministry of Agriculture and Land Reclamation, Egypt, 2019). *Tetranychus urticae* Koch is one of the dangerous pest attacking squash and have a large economic danger in a wide range of outdoor and protected crops worldwide (Hayam, 2020). It is extremely polyphagous; it can feed on hundreds of plants including most vegetables and food crops especially *Solanaceae* and *Cucurbitaceae* (Fatma *et al.,* 2015). *T. urticae* is a serious pest of many plants and feeds on the lower leaf surface, the symptoms of feeding are appear as a small white or yellow spots on lower leaf surface (Mutthuraju, 2013). Response of squash varieties to infestation with *T. urticae* in order to select the most resistant ones is considered important to avoid using more pesticides. Morphological and histological leaf

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characteristics which normally vary from plant variety to another, may affect the population levels of herbivores. There were several studies on the host plant resistance to the infestation with *T. urticae* (Ibrahim *et al.*, 2008; Abdallah *et al.*, 2009; El-Saiedy *et al.*, 2011; Afifi *et al.*, 2013 and Abou-Zaid *et al.*, 2019). Plants respond to herbivores through various morphological, bio-chemicals and molecular mechanisms to counter/offset the effects of herbivore attack (War *et al.*, 2012). Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants *e.g.*, hairs, trichomes, thorns, spines and thicker leaves (Hanley *et al.*, 2007). Therefore, the present work was conducted to evaluate the susceptibility of three squash varieties to *T. urticae* infestation, its population dynamics during two seasons 2017 and 2018, effect of trichomes on susceptibility of three squash varieties to *T. urticae* and its effect of feeding on anatomical, histological leaf structure.

Materials and Methods

Ecological studies

These experiments were conducted at Dar EL-Ramad farm, Agriculture faculty, Fayoum University during two successive seasons 2017 and 2018. Three squash varieties with five replicates for each variety using split plot design were cultivated in experimental field during the two successive seasons. These varieties were planted in the field at the second week of February and after about one month, samples were taken weekly. The cultivated squash varieties received all normal agricultural processes without using pesticides. For population dynamics of *T. urticae*, samples were randomly collected weekly whereas, twenty five leaves of each squash variety were put in tightly closed paper bags and transferred to laboratory where the observed pests were counted by the aid of carton stereomicroscope (NSW-40Fseries). Movable stages and eggs of *T. urticae* were counted and recorded, from the third week of March till the second week of June.

Scanning Electron Microscopy (SEM)

Leaf samples of three squash varieties were collected and imaged using a Scanning Electron Microscopy (SEM) (Joel jsm. 5200LA) at the applied center for Entomonematodes (ACE), Faculty of agriculture Cairo University. The SEM technique used is according to (Karnowsky, 1965 and Fischer *et al.*, 2012). Density (numbers /cm^2) of trichomes were determined from three squash varieties of lower surface of leaves (Luczynski *et al.*, 1990 and Bakr, 2005).

Light microscopy (LM)

Healthy and infested leaf samples of the three squash varieties were examined by light microscope. Squash leaves were cut into small pieces about 1-2 mm specimens which killed and fixed for at least 48 hrs. in Formalin acetic acid glacial alcohol (F.A.A) which contained of 10ml formalin, 5 ml glacial acetic acid, 35 ml distilled water and 50 ml ethyl alcohol 95%. The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 micron, double stained with Erythrosin and crystal violet, cleared inxylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Slides were analyzed microscopically and photomicrographed.

Transmission Electron Microscopy (TEM)

The effect of *T. urticae* on the ultrastructure of three squash varieties were studied by using electron microscope according to (Bozzola and Russell 1999). Pictures slice tissue sampled into~1mm slices. Slice tissue was processed for TEM by fixation in glutaraldehyde and osmium tetroxide, dehydrated in alcohol and embedded in an epoxy resin. Microtome sections prepared at approximately 500-1000μm thickness with a LeciaUltracut UCT ultramicrotome. Thin sections were stained with tolodinblue (1X) then sections were examined by camera lica ICC50HD. Ultra-Thin section prepared at approximately 75-90μm thickness and were stained with uranyl acetate and lead citrate, then examined by transmission electron microscope JEOL (JEM-1400TEM) at the candidate magnification. Images were captured by CCD camera model AMT, optronics camera with 1632×1632 pixel formate as side mount configuration. This camera uses a 1394 fire wire boared for acquision. This work was done on faculty of Agriculture, Cairo University Research Park (CURP).

Statistical analysis

All collected data for various parameters were statistically analyzed according to the technique of analysis of variance for split-plot arranged in randomized complete block design using the Info Stat computer software package (version, 2012). The differences among treatment means were compared by LSD as a post hoc test at d" 5% level of significance (Gomez and Gomez, 1984).

Results

Susceptibility of different squash varieties to *T. urticae* infestation

The tested three squash varieties differed in their susceptibility to *T. urticae* infestation table 1 during the

Table 1: Susceptibility of three squash varieties to *T. Urticae* infestation during 2017&2018 seasons.

Squash varieties	Egg 2017	Egg2018	Season 2017		Season 2018	
	Mean No.		Mean No.	Infestation%	Mean No.	Infestation%
Eskandarani	98.46a±28.64	69.98a±8.27	70.09a±18.08	45.50	37.00a±8.27	54.35
Hytech	82.51ab±22.61	43.89b±9.75	53.26a±14.34	34.58	16.37b±3.39	24.05
Milet	55.08b±15.92	38.75b±6.67	30.68b±8.36	19.92	14.71b±3.02	21.61
LSD (5%)	29.44	16.25	19.29	-	10.35	-

Means with a common letter are not significantly different (P>0.05)

two successive seasons (2017 & 2018). It could be arranged in a descending order as follows: Eskandarani variety was the most highly significant susceptible to infestation, it recorded 70.09 (45.50%) & 37.00 (54.35%) moving mite stages/leaf (Susceptibility) during the two successive seasons (2017 & 2018), respectively, followed by the moderately infestation was observed on Hytech variety being 53.26 (34.58%) & 16.37 (24.05%) during the two seasons, respectively. The lowest infestation was observed on Milet variety which recorded, 30.68 (19.92%) and 14.71 (21.61%) during the two successive seasons, respectively. These results indicated that three squash varieties were variably infested with *T. urticae*.

Population dynamics of *T. urticae* on three varieties of squash

Population dynamics of the two-spotted spider mite, *T. urticae* were recorded during the two successive seasons 2017 and 2018 from the 3rd week of March till the 2nd week of June. The infestation of three squash varieties with *T. urticae* started on the third week of March. Eskandarani and Milet varieties had two peaks in the same time, in 2nd week of May and 2nd week of June during 2017 season, but in 2018 season in 4th week of April and 4th week of May. The third variety Hytech had two peaks in the 3rd week of May and 1st week of June during 2017 season, but in 2018 season, in 1st week of May and 4th week of May Fig. 1. Finally, the average of *T. urticae* movable stages and eggs after thirteen week at the three squash varieties Eskandarani, Hytech and Milet were (70.09 & 98.46; 53.26 & 82.51 and 30.68 &

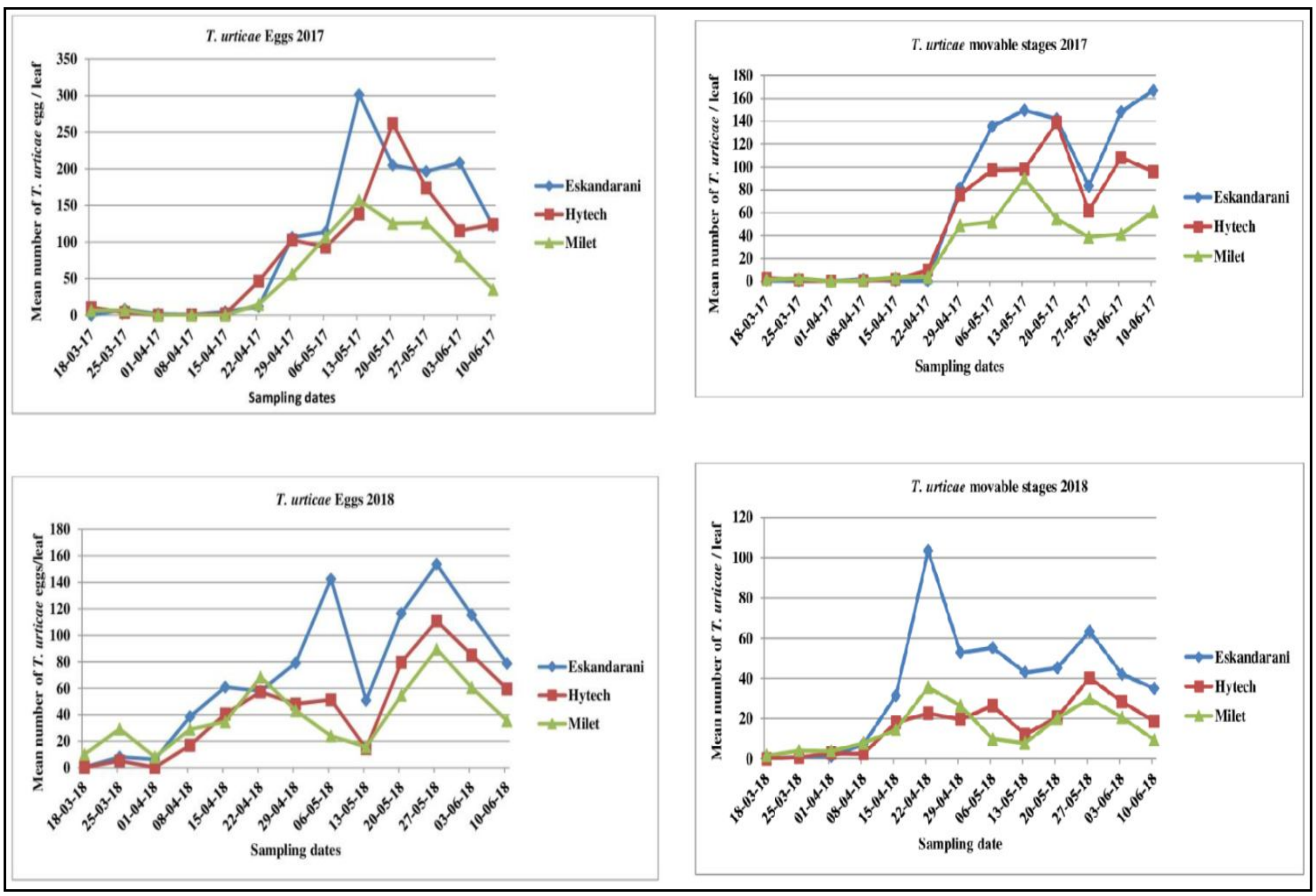

Fig. 1: Population dynamics of *T. urticae* stages on three squash variety at Fayoum Governorate duringseasons 2017 and 2018.

Table 2: Mean densities (number/ cm^2) of trichomes of the lower surface for three squash varieties.

Squash varieties	Trichomes / cm^2 ±SE
Eskandarani	3232.05±79.41
Hytech	3524.89±68. 81
Milet	4017.93±89.34

55.08) during 2017 season, respectively. While during 2018 seasons were (37.00 & 69.98; 16.37 & 43.89 and 14.71 & 38.75), respectively table 1.

Effect of *T. urticae* infestation on anatomical and histological leaf characteristics of three squash varieties

(A) Scanning Electron Microscopy (SEM)

The anatomical and histological structure of squash leaves may be affected by feeding of *T. urticae*. Therefore, the differences between the three squash varieties, Eskandarani, Hytech and Milet in their leaf structure were studied. The average number of the lower surfaces trichomes/cm^2 (densities) varied in different squash varieties leaves. It was 3232.05 ± 79.41, 3524.89 ± 68.81, 4017.93 ± 89.34 in Eskandarani, Hytech and Milet respectively table 2 and Fig. 2. Therefore the most infested variety (Eskandarani) has the lowest number of trichomes compared with other squash varieties.

(B) Light microscopy (LM)

The changes in anatomical structure of three squash varieties due to the infestation by *T. urticae* were studied by light microscopy. The results indicated that, infestation with *T. urticae* induced significant changes on anatomical structure of leaves of three squash varieties leaves as shown in table 3 and Fig. 3. These changes were led to decrease in mean thickness of lower epidermis of Eskandarani , Hytech and Milet by -30.13%, -28.59 % and -30.35%, respectively. On the other hand mean thickness of palisade tissues in three varieties were increased by +14.34, +24.72 and +33.61, respectively. Also the mean thickness of spongy tissues were increased by +29.81, +153.45 and +88.88 in Eskandarani, Hytech and Milet respectively. Finally, decrease in mean thickness of upper epidermis was observed in three varieties by -16.11, -23.04 and -26.42.

(C) Transmission Electron Microscopy (TEM)

The ultra-structural changes in cells of both healthy and infested squash leaves varieties were recognized by TEM revealed aberrations in leaf tissue. The investigation resulted in significant abnormal changes of infested plants. Many abnormal changes in cell wall, palisade parenchyma, spongy parenchyma, nucleus, chloroplast and mitochondria in Eskandarani variety as shown in Fig. 4

Table 3: Effect of feeding of *T. Urticae* on the anatomical structure of three squash varieties leaves.

Mean thickness µm ± SE					
Squash varieties		Lower epidermis	Mesophyll tissues		Upper epidermis
			spongy parenchyma	palisade parenchyma	
Eskandarani	Healthy	6.04±0.34	81.76±2.07	77.26±1.19	21.67±0.57
	Infested	4.22±0.22	106.13±3.81	88.34±1.72	18.18±0.88
Changes %		-30.13	+29.81	+14.34	-16.11
Hytech	Healthy	9.83±0.42	52.55±0.71	67.27±1.33	16.53±1.54
	Infested	7.02±0.45	133.19±4.66	83.90±2.72	12.72±0.62
Changes %		-28.59	+153.45	+24.72	-23.04
Milet	Healthy	8.50±0.39	57.74±1.35	80.12±1.47	22.75±0.79
	Infested	5.92±0.27	109.06±7.14	107.05±3.80	16.74±1.07
Changes %		-30.35	+88.88	+33.61	-26.42

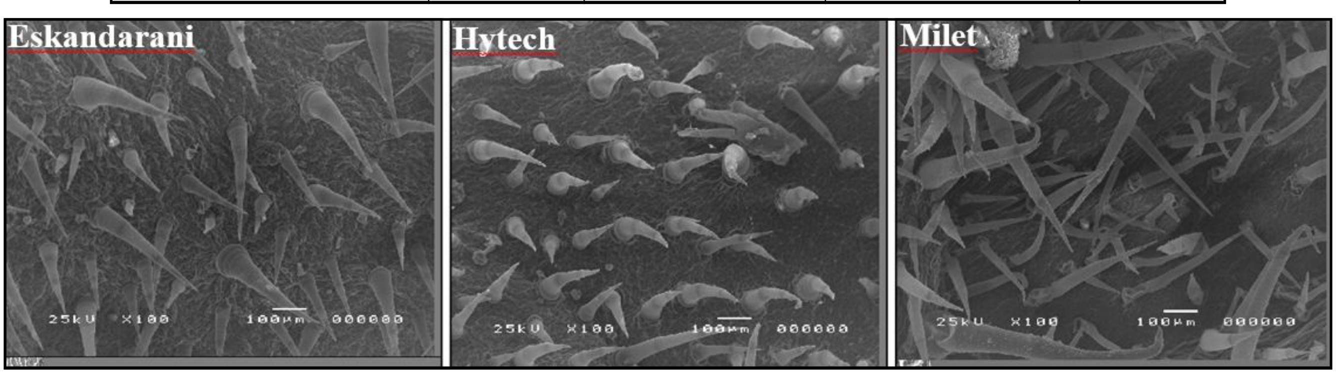

Fig. 2: Scanning electron micrographs of lower surfaces leaf trichomes for three squash varieties.

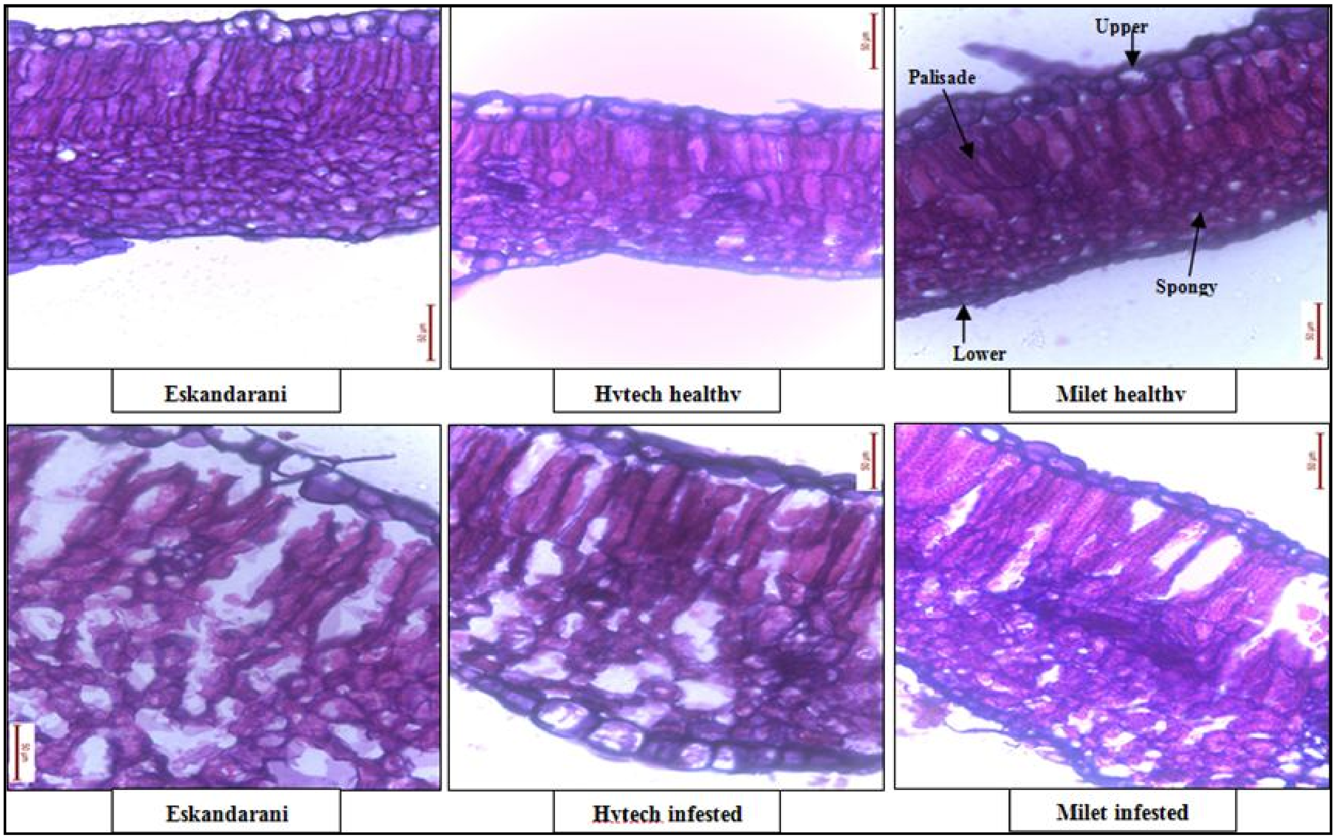

Fig. 3: Light micrographs of cross section in leaves of three squash varieties (Healthy and Infested).

were observed. The cells of palisade parenchyma lost their columnar shape and became irregular and large spaces between cells were founded, spongy parenchyma became irregular and a complete decomposition in cells contents, gradual degradation and dissociation in cell wall was observed, infestation with *T. urticae* led to severe damage in chloroplasts including thylakoids in grana, Intracellular starch accumulation was observed in the chloroplasts of infested leaves compared with healthy one.

Discussion

Our results indicated that three squash varieties were variably infested with *T. urticae*, Eskandarani was the most highly susceptible to infestation followed by moderate and low infestation on Hytech and Milet, respectively. These results were in agreement with those obtained by (Abdallah *et al.*, 2009) indicated that the infestation by spider mite species, *T. urticae* was significantly different among the three different squash cultivars. Eskandarani was the most susceptible harbored, While Hytech cultivar was intermediate and the lowest average number of spider mite infestations was recorded in case of American cultivar. Abou-Zaid *et al.*, 2019 studied the susceptibility of four squash varieties to infestation with *T. urticae* and reported that Andro 174 variety was the most susceptible during the both seasons, while Sama 740 and Arkam varieties were the most tolerant during the investigated period. Aiad *et al.*, 2014 studied the susceptibility of three muskmelon cultivars to infestation with *T. urticae*. Galia2 cultivar was the most highly susceptible followed by Shahd2 cultivar, the lowest infestation was recorded on ananas during the investigated period. Also (Kilany, 1997) who studied the susceptibility of cucumber varieties to infestation by *T. urticae* and revealed that Katia, Celebrity and Afdal varieties harbored a high level of infestation followed by Cordinate, while the varieties Maram and Rawa were least infested. In addition, El-Saiedy *et al.*, 2011 studied the susceptibility of four watermelon cultivars to infestation by *T. urticae* and reported that Aswan cultivar was the most highly susceptible followed by the moderately infested cultivars (Daytona) and the lowest infestation was recorded on Molokai and Giza-1 cultivars. Afifi *et al.*, 2013 studied the susceptibility of two eggplant cultivars to infestation by *T. urticae* and revealed that the most susceptible harbored was Black baity cultivar and the lowest Baity cultivars.

In our study *T. urticae* populations in three squash varieties had two peaks during 2017 & 2018 seasons, Eskandarani and Milet varieties had two peaks in the same time, in 2nd week of May and 2nd week of June during 2017 season, but in 2018 season in 4th week of

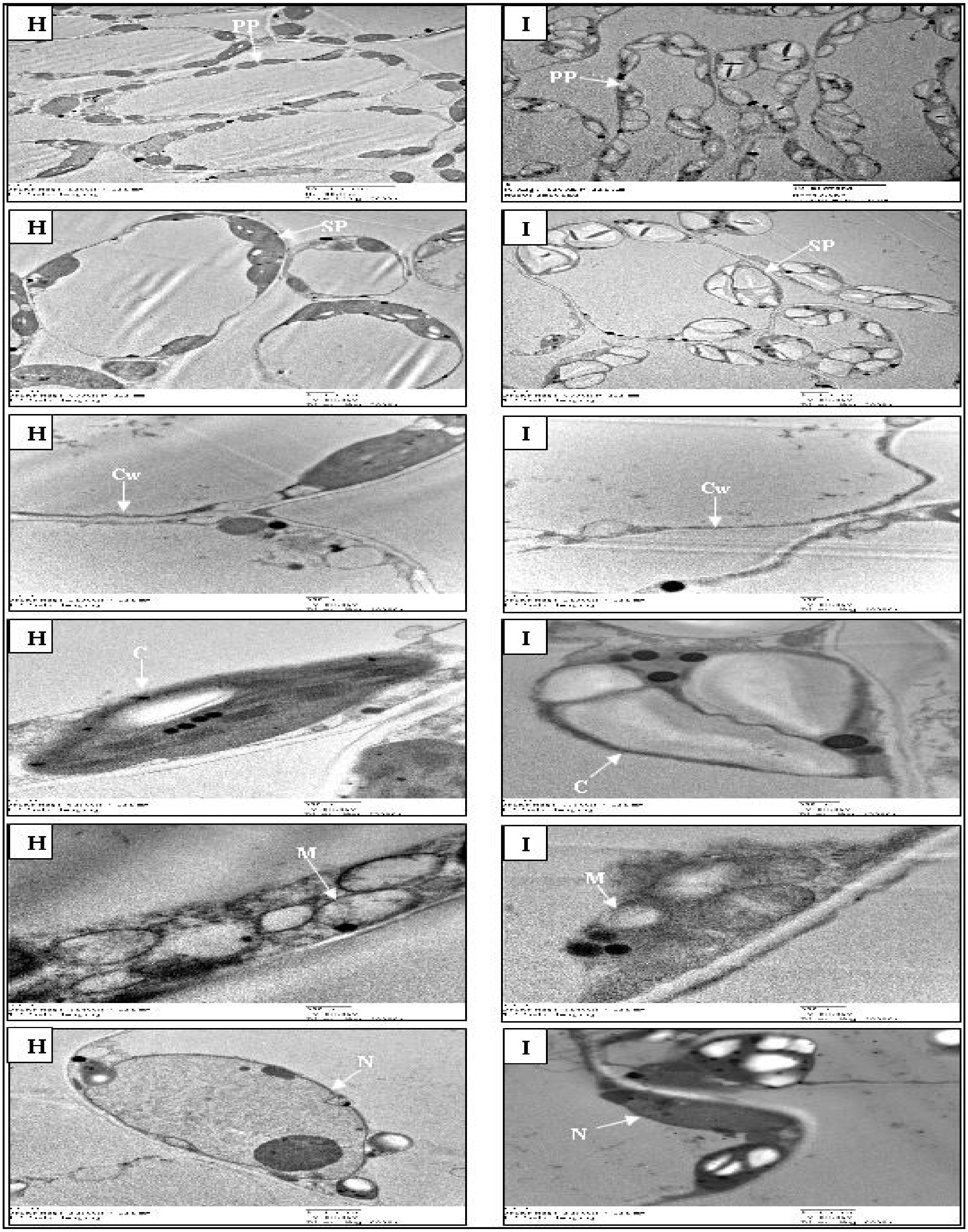

Fig. 4: Transmission electron microscopy using to investigate changes in Eskandarani squash variety leaves.
Abbreviations: H= healthy leaves; I= Infested leaves; PP: palisade parenchyma; SP: spongy parenchyma; CW: cell wall; C: chloroplast; M: mitochondria and N: nucleus.

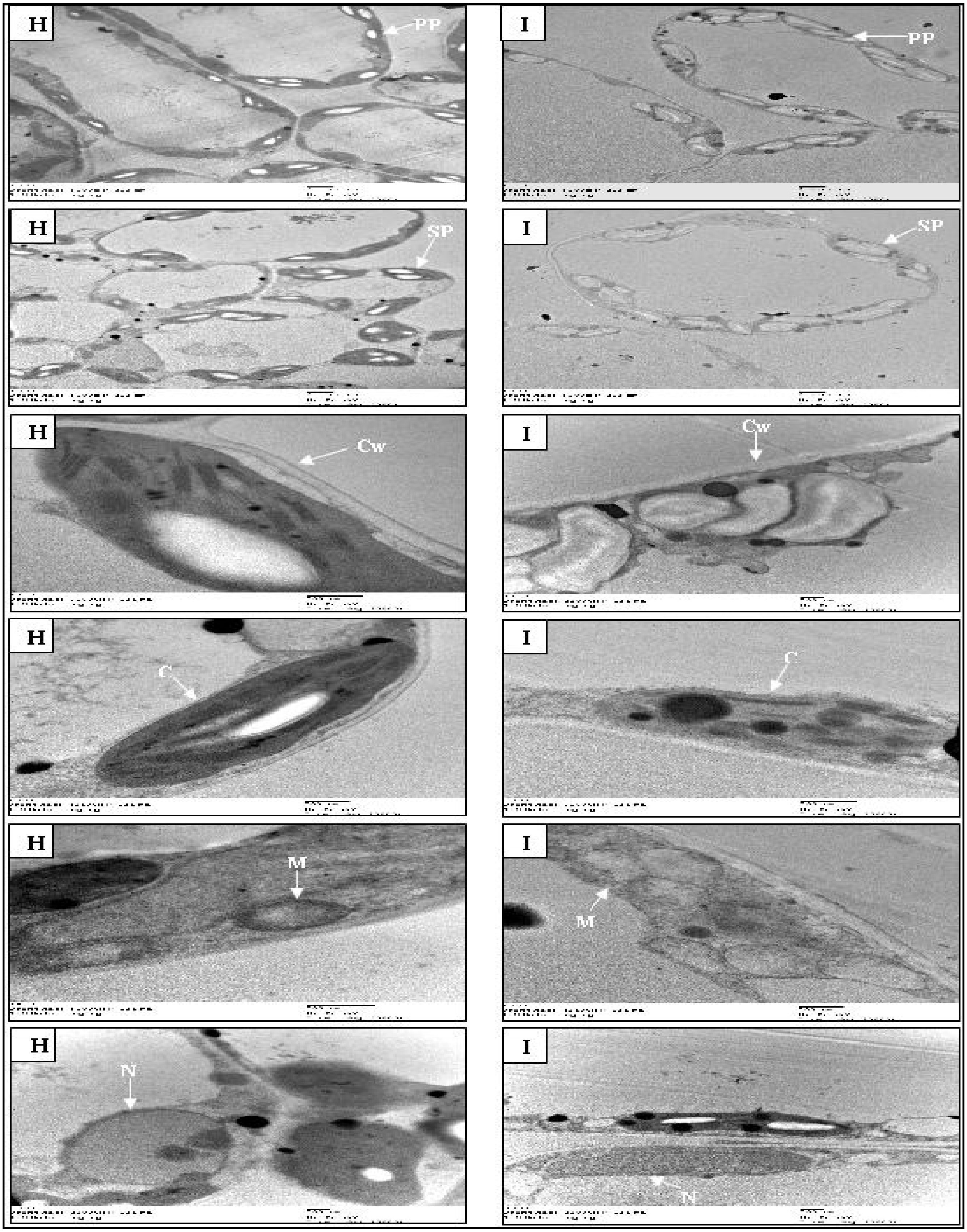

Fig. 5: Transmission electron microscopy using to investigate changes in Hytech squash variety leaves.

Abbreviations: H= Healthy leaves; I= Infested leaves; PP: palisade parenchyma; SP: spongy parenchyma; CW: cell wall; C: chloroplast; M: mitochondria and N: nucleus.

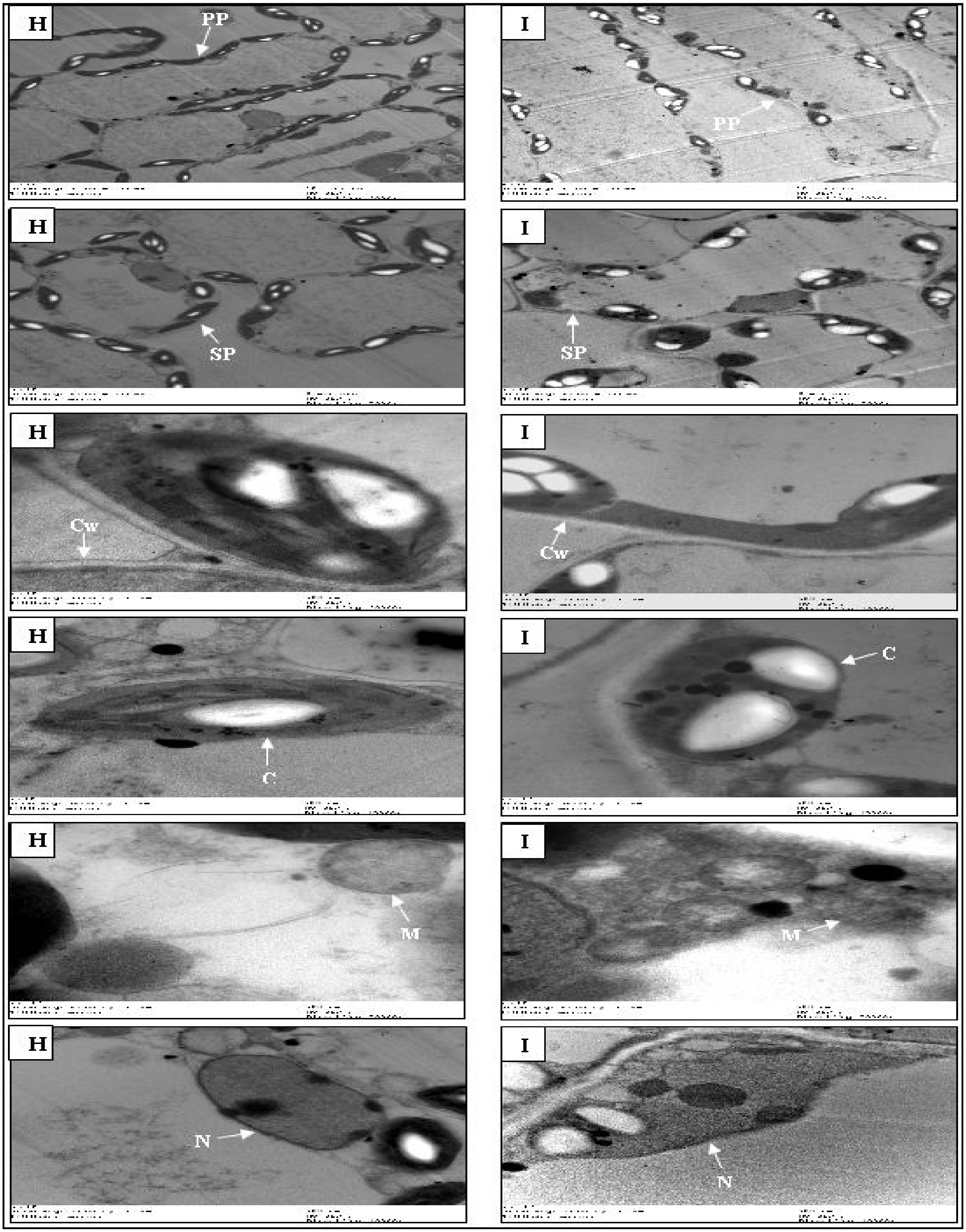

Fig. 6: Transmission electron microscopy using to investigate changes in Milet squash variety leaves.

Abbreviations: H= Healthy leaves; I= Infested leaves; PP: palisade parenchyma; SP: spongy parenchyma; CW: cell wall; C: chloroplast; M:mitochondria and N: nucleus.

April and 4th week of May. The third variety Hytec had two peaks in the 3rd week of May and 1st week of June during 2017 season, but in 2018 season, in 1st week of May and 4th week of May Similar results were recorded by (Gamila *et al.,* 2016) showed the activity period of *T. urticae* had two peaks, the first one occurred in the 2nd week of April, the second peak occurred in the 4th week of April. Also (Abou-Awad *et al.,* 2017) results showed that the infestation of four melon cultivars by *T. urticae* reached their peaks in the third week of April (watermelon) and the first week of May (muskmelon) in the open field conditions during two successive seasons. Also, (Hanafy *et al.,* 2014) stated that *T. urticae* had two peaks of infestation on Cucumber in May in 2011 & 2012.

In our study, the Eskandarani variety had the lowest number of trichomes and it was highly susceptible to *T. urticae*. These results agreed with (El-Saiedy *et al*., 2011) reported that, Infestation with *T. urticae* may be affected by plantleaf morphological structure (trichomes), the cultivar with the less number of trichomes (Aswan) the more mite infestation in four watermelon cultivars. Dense trichomes affect the herbivory mechanically and interfere with the movement of arthropods on the plant surface, thereby, reducing their access to leaf epidermis (Agrawal *et al*., 2009). Trichomes play an imperative role in plant defense against many arthropods whereas it involves both barrier and deterrent effects (Chamarthi *et al*., 2010 and Handley *et al*., 2005). Trichomes are a good defense mechanism against piercing-sucking arthropods, like spider mites. When hairs are dense, they form a physical barrier that makes it very difficult for this kind of herbivores to reach the plant's epidermis (Marit, 2014).

Our results indicated that the mean thickness of mesophyll tissues was increased and the mean thickness of upper and lower epidermis was decreased in Eskandarani, Hytech and Milet. These results were in agreement with Grinberg *et al.,* 2005 who reported that mesophyll cells of the infested cucumber leaf appeared larger and undifferentiated and the leaf was about twice as thick as the control, this increase in thickness resulted from increases in cell volume. Park and Lee, 2002 reported that *T. urticae* Koch, feeding on cucumber on the abaxial leaf surface, *T. urticae* could feed through the spongy parenchyma and part of the palisade parenchyma of the leaf; *T. urticae* punctured individual epidermal cells and consumed the contents of the mesophyll cells. Injured leaves had more empty space in the spongy parenchyma. The abnormal differentiation and increase in cell volume and proliferation are similar to those observed by Nemesthoty *et al*., 1982. Also, Albrigo *et al*., 1981 reported that *T. urticae* led to small pockets of mesophyll cells being evacuated although some starch grains or cell debris often remained.

It was noticed by using TEM, that there were abnormal changes in cell contents of infested leaves of three squash varieties due to the results of *T. urticae* feeding. This phenomenon has also been reported by Grinberg *et al*., 2005 who reported that deformations of the cell walls were observed and starch grains were apparent among the chloroplast grana in cucumber. Also destruction and deformation of the grana of the chloroplast occurred as the result of feeding of *T. urticae* according to (Carmi and Shomer, 1979; Tanigoshi and Davis 1978 & Crawford and Wilkens, 1996). Kielkiewicz, 1999 showed that there is morphological and ultrastructural distortion in mesophyll cells tomato leaves and degenerative changes in the morphology of chloroplasts. In our study severe damage in mitochondria was occurred and the nucleus lost its spherical shape and shrink. Similar changes in ultra-structural of other two varieties cells were observed but low in severity. These agree with (Mothes and Seitz) reported that there is a deformation of nuclei, chloroplast and of other cell organelles must have occurred by feeding of mites.

Conclusion

Eskandarani variety was more susceptible to *T. urticae* infestation; it contains the lowest number of trichomes. Eskandarani, Hytech and Milet had two peaks in May and June during 2017. But in 2018 Eskandarani and Milet had two peaks in April and May; Hytech had two peaks in May in 2018. Light microscopy showed large changes in the anatomical structure of three varieties of squash leaves. Ultrastructure changes in cell contents were noticed by Transmission Electron Microscopy.

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