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# Effect of Phytochemical Components, Morphological and Histological Leaf Structure of Five Tomato Hybrids on *Tetranychus urticae* Koch Infestation

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## ABSTRACT

Experiments were conducted to estimate the susceptibility of five tomato hybrids (Supper-gekal, F1Gs-12, Marwa, El-basha 1077 and Salymia 65010) to infestation with *Tetranychus urticae* Koch and its population fluctuation during the two successive seasons, summer 2013 and 2014, at Giza governorate. Supper-gekal and F1Gs-12 hybrids were the most highly significant susceptible to infestation. It recorded 31.16 (25.10%) & 24.61 (24.82%) and 28.10 (22.63%) & 22.09 (22.28%) moving mite stages / leaflet during 2013 and 2014 seasons, respectively, followed by moderate infestation observed on hybrids (El-basha 1077 and Marwa), being 25.20 (20.29%) & 19.80 (19.96%) for the former; 21.32 (17.17%) & 17.67 (17.82%) for the latter hybrid during the two seasons, respectively. The lowest infestation was observed on Salymia (65010) hybrid which recorded, 18.38 (14.81%) and 15.00 (15.13%) during the two successive seasons, respectively. Susceptibility of five tomato hybrids to infestation with *T. urticae* may be affected by plant leaf morphological and histological characteristics and its chemical contents. The number of leaf trichomes /$cm^2$ in upper and lower surfaces averaged (2606&8606), (3939&10557), (5788&9000), (7030&13870) and (10970&16818) for Supper-gekal, F1Gs-12, El-basha 1077, Marwa and Salymia 65010, respectively, the less number of trichomes the more mite infestation. Also, the mean thickness of lower epidermis was 19.61, 18.85, 27.57, 22.63 and 26.55 µm, while the upper epidermis was 25.64, 28.58, 27.77, 26.78 and 30.50 µm for the aforementioned hybrids, respectively. Positive relationship occurred between mite infestation levels and total carbohydrates in tomato leaves, while a negative relationship were found with alkaloids and total phenolic compounds, total flavonoids and total carotenes. Mite populations reached its peak in the 1st week of October in the first season, while during the second season occurred in the 2nd week of September and the 1st week of October. The high population density of *T. urticae* found in 2013 compared with 2014 might be due to environmental conditions.

**Key words:** Susceptibility, Tomato leaf structure, Population dynamics, *Tetranychus urticae*.

## INTRODUCTION

Tomato, *Solanum lycopersicum* L. Family: Solanaceae, is considered one of the most important vegetable crops. Egypt is the fifth greatest producer of tomatoes in the world, about 531,115 Fadans. of the crop grown annually, with an average productivity of 8105260 tons (FAO 2013). This vegetable crop is often severely damaged by many phytophagous pests (insects and mites). In Egypt, the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) has become one of the most important pests in many greenhouse and filed vegetable corps, especially Solanaceae and Cucurbitaceae on which can build high population densities and cause serious damage to tomato plants. *T. urticae* is highly polyphagous and has been recorded to feed on over 1100 plant species, among them tomato (Dermauw *et al.,* 2012).

Evaluation the susceptibility of tomato hybrids to infestation with *T. urticae* in order to select the most resistant ones is considered important to avoid using more pesticides. Morphological, histological leaf characteristics and chemical contents, 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 infestation with *T. urticae*; (Ibrahim *et al.,* 2008 , Abdallah *et al.*, 2009, El-Saiedy *et al.*, 2011 and Afifi *et al.*, 2013).

Plants respond to herbivory through various morphological, biochemicals, 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 thickness leaves) or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) that either kill or retard the development of the herbivores, (Hanley *et al.,* 2007). Indirect defenses against pests are mediated by the release of a blend of volatiles that specifically attract natural enemies of the herbivores, (Arimura *et al.*, 2009).

Therefore, the present work was conducted to evaluate the susceptibility of five tomato hybrids to *T. urticae* infestation during two seasons and the effect of the morphological, histological leaf characteristics and its phytochemical components on the infestation rate. The population dynamics of the mite throughout the two successive seasons; 2013 and 2014 were also studied.

## MATERIALS AND METHODS

**Experimental procedures:**

Five tomato hybrids; Supper-gekal , F1GS-12, Marwa , El-basha 1077 and Salymia (65010) were cultivated in open field during the two successive early summer seasons. Barnasht village, El-Ayat, Giza governorate was chosen to conduct these experiments during 2013 and 2014 seasons. Five tomato hybrid seeds were sown at the last week of June in the nursery then seedlings were planted in the field at the third week of July and after about one week, samples were taken weekly. The cultivated tomato hybrids received all normal agricultural processes without using pesticides. For population dynamics of *T. urticae*, weekly samples, each of 30 leaves, were randomly collected from every tomato hybrid from the fourth week of July till the last week of October during the two successive seasons (2013 and 2014). Leaf samples were examined using a stereomicroscope and hand lens. Movable stages and eggs of *T. urticae* were counted and recorded.

**Morphological and histological leaf characteristics**

Leaf samples of five tomato hybrids were collected and imaged the upper and lower surface of tomato leaves using the Analytical a Scanning Electron Microscopic Technique (SEM) (Joel jsm.6390LA) at the Central Laboratory of Water Station Fustat, Greater Cairo Water Company using SEM technique according to (Karnowsky, 1965 and Fischer *et al.,* 2012). Density (numbers /$cm^2$) of glandular and non-glandular trichomes were determined from five regions of the upper or lower surface of tomato leaves and averaged for all five spots, (Luczynski *et al.,* 1990). Trichomes were counted using Compu Eye, Leaf & Symptom Area soft were according to (Bakr, 2005). Also, leaf samples of five tomato hybrids imaged the different cells behind the midrib of tomato leaves using the Transmission Electron Microscopy in TEM lab FA-CURP, Faculty of Agriculture, Cairo University - Research Park (CURP) using TEM technique according to (Bozzola and Russell 1999). Semiultrathin sections (1µm) for light microscope (LM) were made using a microtome, then stained with methylene blue.

**Biochemical Studies**

Chemical analysis of leaf samples were carried out during the peak season of infestation. Tomato leaves of the five tomato hybrids were collected and transferred to Acarology Laboratory to dry under room conditions, then transferred to the Faculty of Agriculture Research Park, , Cairo Univ. for chemical analysis.

Some specific chemical constituents of tomato leave hybrids were determined as follow: Total phenol content was determined by Folin- Ciocateu method as modified by Singelton and Rossi (1965).Total carbohydrates were extracted from the plant leaves and prepared for assay according to Crompton and Birt (1967). Total protein was calorically assayed by ninhydrin reagent according to the method described by Lee and Takabashi (1966). Total flavonoids content were determined by Folin-Ciocalteu method according to Hung & Morita (2008). Total carotenoides and chlorophyll were determined colorimetrically according to Holden (1965). Alkaloids were determined titermetrically according to Sabri *et al.,* (1973).Total lipids were determined according to Bligh and Dyer (1959).

Data were analyzed by one-way analysis of variance and mean comparison at 5% level of significance using Fisher's least significant difference (LSD), using the Statistical Analysis System (SAS) software (1988).

## RESULTS AND DISCUSSION

**Susceptibility of different tomato hybrids to *T. urticae* infestation:**

The tested tomato hybrids significantly (P > 0.05) differed in their susceptibility to *T. urticae* infestation. (Table1). During the two successive seasons (2013 &2014), it could be arranged in a descending order as follows: Supper-gekal and F1Gs-12 hybrids were the most highly significant susceptible to infestation. It recorded 31.16 (25.10%) & 24.61 (24.82%) and 28.10 (22.63%) & 22.09 (22.28%) moving mite stages / leaf during the two successive seasons, respectively, followed by moderately infestation observed on hybrids (El-basha 1077 and Marwa), being 25.20 (20.29%) & 19.80 (19.96%) for the former; 21.32 (17.17%) & 17.67 (17.82%) for the latter hybrid during the two seasons, respectively. The lowest infestation was observed on Salymia (65010) hybrid which recorded, 18.38 (14.81%) and 15.00 (15.13%) during the two successive seasons, respectively.

Table (1): Susceptibility of five tomato hybrids to *T. urticae* infestation during 2013&2014 seasons

| Tomato hybrid | Season 2013 | | Season 2014 | |
|---|---|---|---|---|
| | Mean No. | Infestation % | Mean No. | Infestation % |
| Supper-gekal | $31.16^a \pm 4.77$ | 25.10 | $24.61^a \pm 4.01$ | 24.82 |
| F1Gs-12 | $28.10^b \pm 4.34$ | 22.63 | $22.09^b \pm 3.85$ | 22.28 |
| El-basha 1077 | $25.20^c \pm 4.10$ | 20.29 | $19.80^c \pm 3.42$ | 19.96 |
| Marwa | $21.32^d \pm 3.72$ | 17.17 | $17.67^d \pm 3.02$ | 17.82 |
| Salymia (65010) | $18.38^e \pm 3.27$ | 14.81 | $15.00^e \pm 2.60$ | 15.13 |
| LSD value at 0.05 | 1.44 | - | 1.47 | - |

Table (2): Mean densities (number/cm$^2$) of glandular and non-glandular trichomes for five hybrid tomato cultivars

| Tomato hybrid | Non-glandular trichome /cm$^2$ | | Glandular trichome /cm$^2$ | | Total trachomes / cm$^2$ ±SE | |
|---|---|---|---|---|---|---|
| | Upper surface | Lower surface | Upper surface | Lower surface | Upper surface | Lower surface |
| Supper-gekal | 1970 | 8515 | 636 | 91 | 2606±277 | 8606±260 |
| F1Gs-12 | 3515 | 10436 | 424 | 121 | 3939±83 | 10557±245 |
| El-basha 1077 | 3818 | 8909 | 1970 | 91 | 5788±188 | 9000±177 |
| Marwa | 6212 | 13173 | 818 | 697 | 7030±414 | 13870±518 |
| Salymia (65010) | 9182 | 16576 | 1788 | 242 | 10970±316 | 16818±368 |

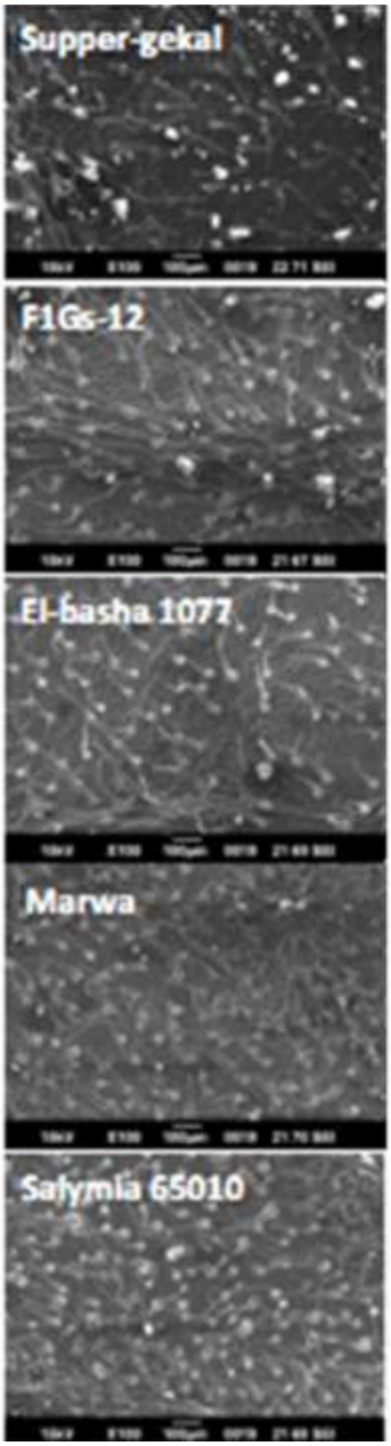



Fig. (1): Scanning electron micrographs of lower surfaces leaf trichomes for five tomato hybrids.

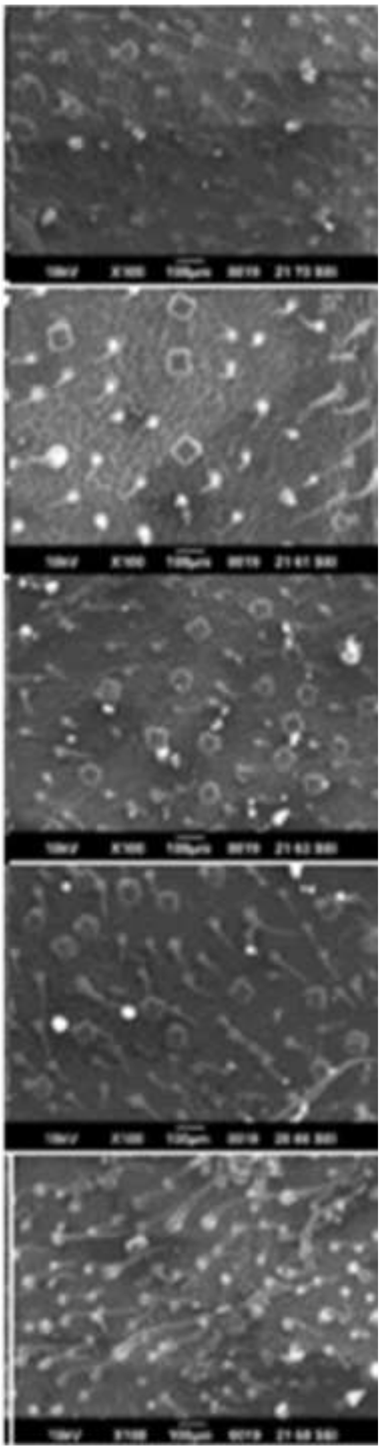


Fig. (2): Scanning electron micrographs of upper surfaces leaf trichomes for five tomato hybrids.

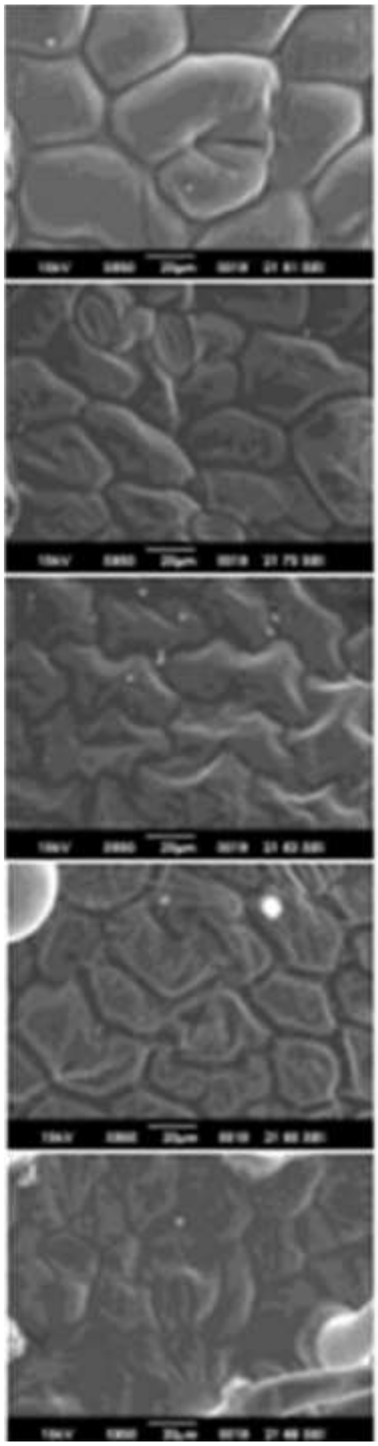


Fig. (3): Scanning electron micrographs of lower epidermal cell surface for leaf of five tomato hybrids.

Table (3): Measurements of some histological characters in transverse sections of tomato leaves

| Tomato hybrids | Mean thickness µm ± SE | | | |
|---|---|---|---|---|
| | Lower epidermis | Mesophyll tissues | | Upper epidermis |
| | | Spongy parenchyma | Palisade parenchyma | |
| Supper-gekal | 19.61±0.72 | 84.01±4.26 | 67.81±1.88 | 25.64±1.75 |
| F1Gs-12 | 18.85±1.72 | 112.40±4.79 | 77.08±4.14 | 28.58±1.66 |
| El-basha 1077 | 27.57±2.59 | 141.56±4.95 | 69.39±3.55 | 27.77±1.68 |
| Marwa | 22.63±22.63 | 200.15±6.43 | 98.44±2.95 | 26.78±1.33 |
| Salymia (65010) | 26.55±1.95 | 127.20±10.43 | 102.73±3.74 | 30.50±1.35 |

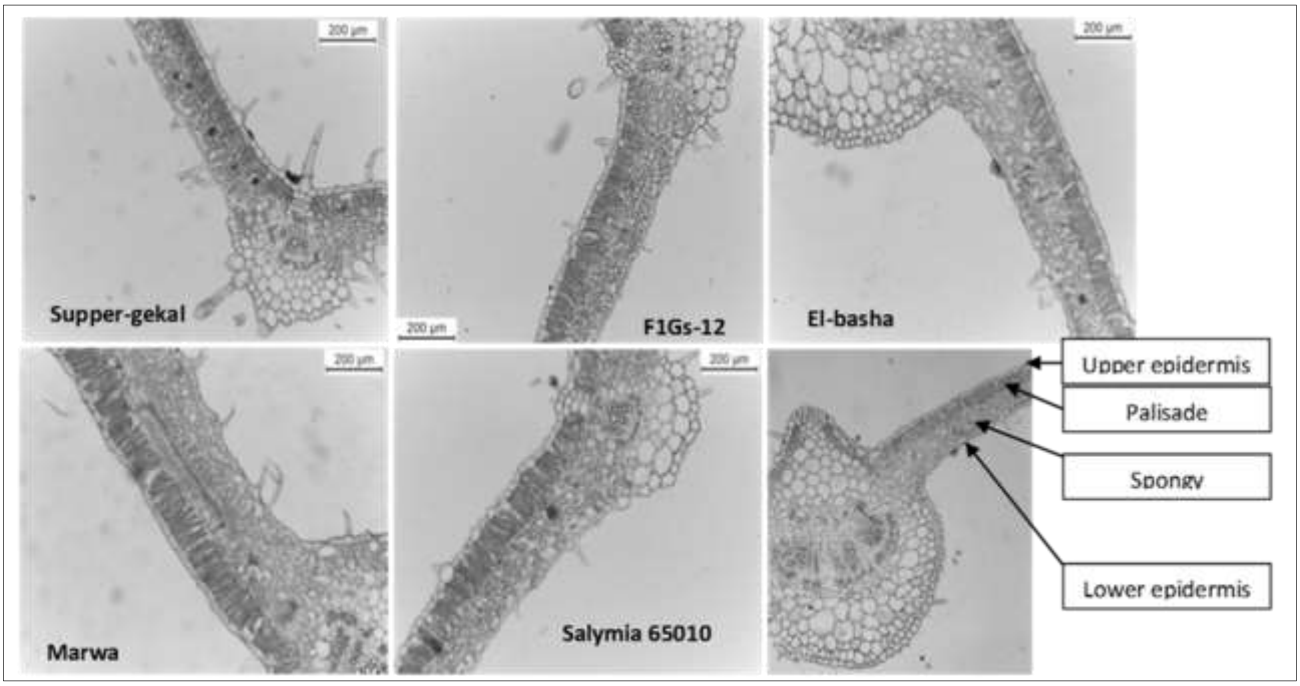
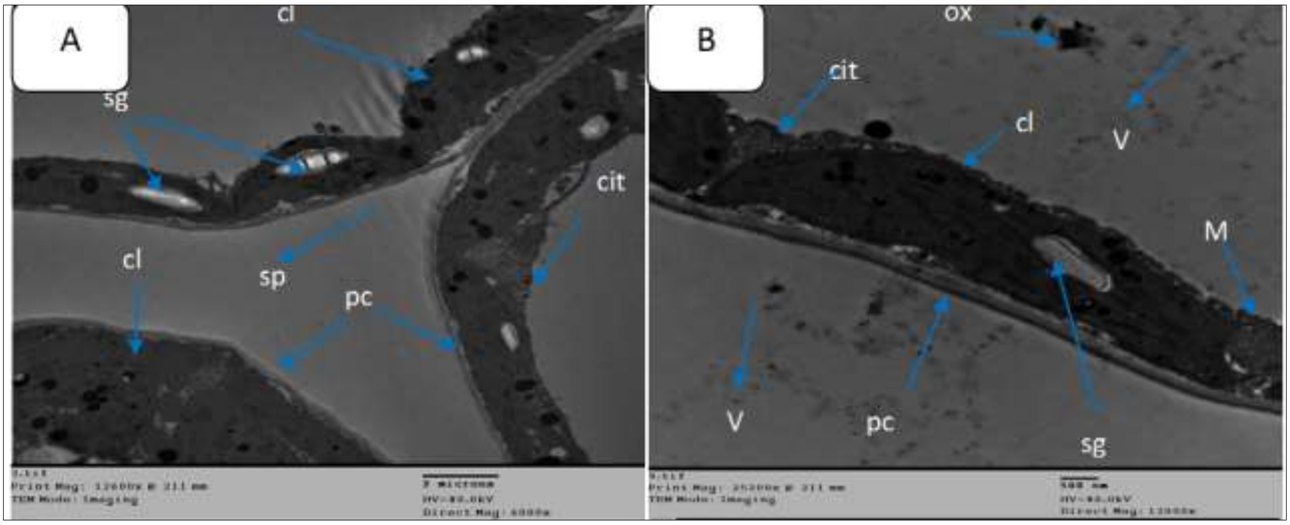



Fig. (4): Light micrographs of cross section in leaves of five tomato hybrids.




abbreviations: A- spongy parenchyma cells, B- palisade parenchyma cells (cit- cytoplasm; cl- chloroplasts; pc- cellular wall; sp- intercellular space; V- vacuole; M- mitochondria; ox- calcium oxalate and sg- numerous starch grains).

Therefore, it could be concluded that all tested tomato hybrids were variably infested with *T. urticae* during early Summer plantation. These results were in agreement with those obtained by (Kilany, 1997, Ibrahim *et al.,* 2008, Abdallah *et al.,* 2009, El-Saiedy *et al.,* 2011, Afifi *et al.,* 2013 and Yassin *et al.,* 2014).

**Effect of morphological and histological leaf characteristics of five tomato hybrids on *T. urticae* infestation**

Susceptibility of tomato hybrids to infestation with *T.* urticae may be affected by plant leaf morphological and histological structure. Therefore, the differences between the five tomato hybrids, Supper-gekal, F1Gs-12, El-basha 1077, Marwa and Salymia (65010) in their leaf structure were studied. The average number of upper surfaces trichomes /cm$^2$ was 2606, 3939, 5788, 7030 and 10970, while those on the lower surfaces were 8606, 10557, 9000, 13870 and 16818 trichomes /cm$^2$ for Supper-gekal, F1Gs-12, El-basha 1077, Marwa and Salymia 65010, respectively. One of the most important factors that may explain the susceptibility or the tolerance of tomato hybrids to *T. urticae* infestation is the shape of epidermal cells, which may be due to the influence the feeding of the red spider mite, *T. urticae* on the contents of cells. In addition, the densities (number/cm$^2$) of glandular and non-glandular upper and lower surfaces trichomes differed with tomato hybrids leaves. (Table 2 and Figs.1&2). In supper-gekal tomato hybrid, the shape of lower epidermal cells was flat and smooth compared with other tomato hybrids, texture was coarse. (Fig. 3).

The Mean thickness µm of lower epidermis was 19.61, 18.85, 27.57, 22.63 and 26.55, that of the upper epidermis was 25.64, 28.58, 27.77, 26.78 and 30.50 for Supper-gekal, F1Gs-12, El-basha 1077, Marwa and Salymia (65010) respectively. (Table 3 and Fig.4). The guild of stylet-feeding arthropods can be divided into two sub guilds, those that feed predominantly on vascular sap, usually phloem sap, and those that feed from cytoplasm only, (Miles, 1972).

The transmission electron micrograph in Fig. (5) showed the effect of infestation rate by *T. urticae* on parenchyma cells components and the differences of this components in sponge and palisade parenchyma cells between the five tomato hybrids. The layer of upper and lower epidermis and parenchyma of the leaf measured using light microscope (LM) are shown (Table 3 and Fig. 4). The number of chloroplasts per cell decreased in Supper-gekal hybrid compared with other tomato hybrids observed with TEM in the parenchyma which might be due to the highest infestation on this hybrid. The epidermal and mesophyll cells became irregular with numerous empty spaces in the mesophyll, (Fig 5).

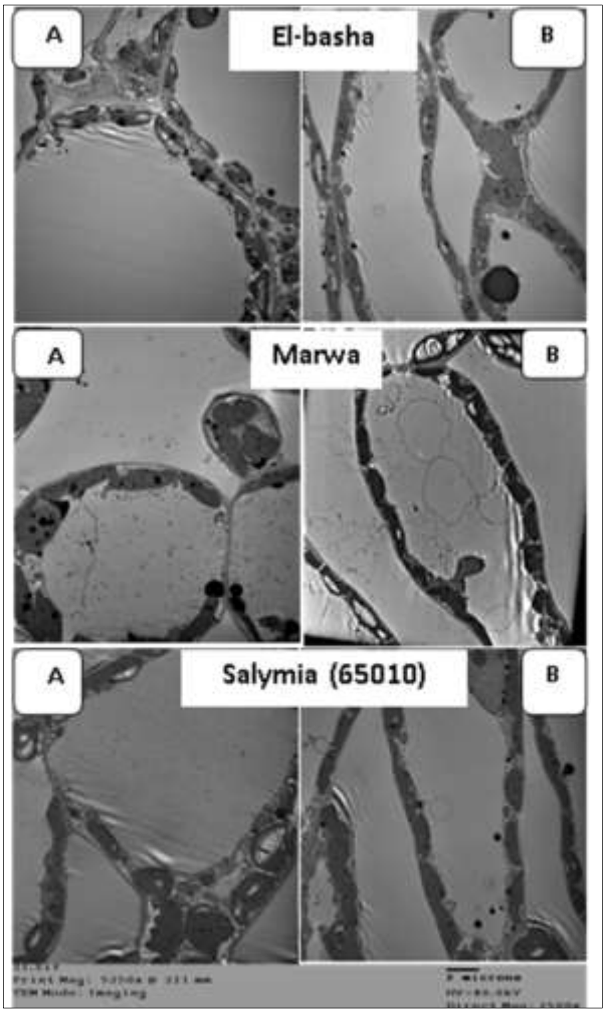



Fig. (5): Transmission electron microscopy using to investigate the differences of parenchyma cells for five tomato hybrids leaves.

Park and Lee (2002) stated that, adult *T. urticae* could feed through the spongy parenchyma and part of the palisade parenchyma of the leaf, while immature *T. urticae* could feed only through the sponge parenchyma. *T. urticae* punctured individual epidermal cells and consumed the contents of the mesophyll cells. Injured leaves had more empty space in the spongy parenchyma and fewer chloroplasts per cell. Jeppson *et al.*, (1975) stated that, the latter applied to spider mites (*Tetranychus* spp.), the adults use stylets of c. 150 µm long for lacerate-and flush feeding on mesophyll cells, predominantly parenchyma, of which they could empty up to 18–22 cells per minute. The stylet length of female *T. urticae* is typically 132±27.0 µm, Sances *et al.*, (1979), and can vary from 103µm (larvae) to 157 µm (adult females) depending on its developmental stages, Avery and Briggs (1968).

**Effect of some phytochemical components on the infestation rate of *T. urticae***

One of the most important factors which play a role in the susceptibility of tomato hybrids to *T. urticae* infestation is leaf phytochemical components.

Table (4): Relationship between phytochemical components of five tomato hybrids leaves and population of *T. urticae*.

| Tomato hybrids | Mean number of *T. urticae* movable stages | phytochemical components | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total carbohydrates mg/g | Total protein % | Total lipids % | Total chlorophyll mg /g | Total phenol mg/g | Total flavonoids mg/g | Total carotenoides mg /g | Alkaloids % |
| Supper-gekal | 53.20 [a] | 13.60 [a] | 17.10 [c] | 7.00 [c] | 107.74 [b] | 0.35 [c] | 25.12 [b] | 8.43 [b] | 4.17 [c] |
| F1Gs-12 | 48.40 [ab] | 14.29 [a] | 15.57 [d] | 9.03 [b] | 107.35 [b] | 0.43 [ab] | 24.12 [b] | 10.08 [a] | 5.00 [b] |
| El-basha 1077 | 46.17 [bc] | 10.25 [c] | 20.03 [a] | 7.40 [c] | 100.20 [c] | 0.39 [bc] | 27.66 [a] | 9.88 [a] | 5.41 [ab] |
| Marwa | 40.80 [cd] | 9.58 [c] | 18.97 [b] | 7.35 [c] | 119.57 [a] | 0.38 [bc] | 24.91 [b] | 7.71 [c] | 5.23 [ab] |
| Salymia (65010) | 36.10 [d] | 11.86 [b] | 16.53 [cd] | 10.23 [a] | 118.84 [a] | 0.45 [a] | 27.05 [a] | 9.82 [a] | 5.75 [a] |
| LSD0.05 | 6.652 | 1.038 | 1.040 | 0.891 | 2.652 | 0.064 | 1.530 | 0.478 | 0.633 |
| Correlation Coefficient (*r*) | | 0.55 | -0.09 | -0.61 | -0.71 | -0.64 | -0.41 | -0.06 | -0.88 |

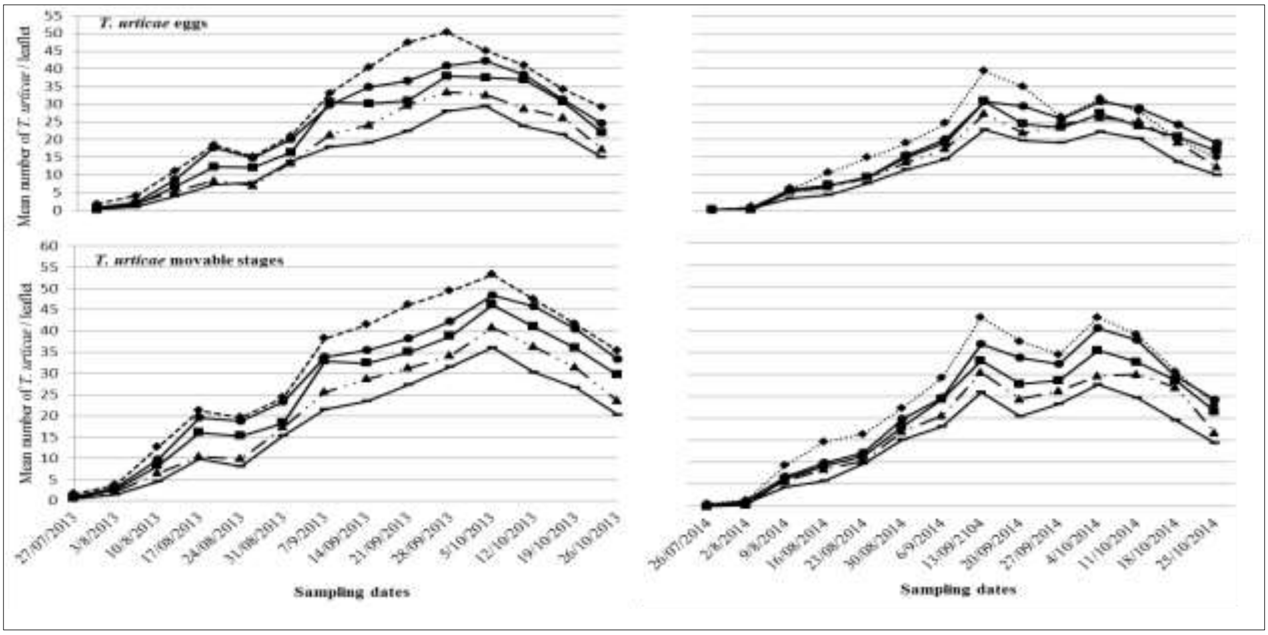



Fig. 6: Population dynamics of *T. urticae* stages on five tomato hybrids at Giza Governorate during seasons 2013 and 2014.

The Obtained data indicated that there is a positive relationship occurring between mite infestation levels and total carbohydrates in tomato leaves, while a negative relationship found with alkaloids and total phenolic compounds, total flavonoids and total carotenes. (Table 4). Tomczyk and Kropczynska (1985) stated that, the concentration of oxganic compounds in the tissue of the plant leaf, i.e., proteins, carbohydrates, phenolic compounds could be influenced by mite feeding, as the reduction of total protein occurred, the injured leaves protein could be degenerated or its synthesis was prohibited. In addition, changes in the concentration of soluble sugars and amino acids not only created better physical conditions for feeding, but also provided a higher nutritive value of the food for spider mites. Also there are many authors discussed this topic; (Ibrahim *et al.,* 2008, Abdallah *et al.,* 2009, Afifi *et al.,* 2009, El-Saiedy *et al.,* 2011 and Afifi *et al.,* 2013).

**Population dynamics of *Tetranychus urticae* on five tomato hybrids**

Population dynamics of the two-spotted spider mite, *T. urticae* were recorded during the two successive seasons 2013 and 2014 from the fourth week of July till the last week of October. The infestation of five tomato hybrids; Supper-gekal, F1Gs-12, Marwa, El-basha 1077 and Salymia (65010) with *T. urticae* started on the 4th week of July then it gradually increased to reach its peaks in the 1st week of October for five tomato hybrids during 2013 season, while in 2014 season, infestation started on the 4th week of July, then gradually increased to reach its peaks in the 2nd week of September, then it dropped till the last week of September, then increased to reach its peaks in the first week of October, then it gradually decreased (Fig. 6).

Finally, the average numbers of *T. urticae* movable stages and eggs after fourteen weeks at the five tomato hybrids; Supper-gekal, F1Gs-12, El-basha 1077, Marwa and Salymia 65010 were 31.16 &28.05; 28.10 &24.48; 25.20 &21.88; 21.32 &17.91 and 18.38& 15.11 during 2013 season, respectively. While it was 24.61 & 19.33; 22.09 & 17.64; 19.80 & 15.92; 17.67& 14.88 and 15.00 &12.11 during 2014 seasons, respectively. Thus, it is clear that Supper-gekal and F1Gs-12 hybrids were more susceptible to the two-spotted spider mite infestation.

The population density of *T. urticae* was higher during 2013 than in 2014 and this might be due to environmental conditions. (Fig. 6). It could be concluded that the numbers of eggs and movable stages of *T. urticae* reached its peaks during September and October in seasons 2013 and 2014, respectively. Similar results were recorded by (Prasad, 2006, Ibrahim *et al.,* 2008 and Yassin *et al.*, 2014).

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## REFERENCES

Abdallah, A. A.; El-Saiedy, E. M. A.; Sholla, Salwa, M. E. and El-Fatih, Monira, M. 2009. Field and Labortory studies to evaluate three Squash cultivars for their relative susceptibility to spider mite *Tetranychus urticae* Koch and three sucking insect species.Minufiya,J. Agric. Res. 34 (5):1913-1926.

Afifi, A. M.; Shahira, M. El-Bishlawy and Mahmoud, Hanady, A. A. 2013. Resistance of two Eggplant cultvars againt the two-spotted spider mite; *Tetranychus urticae* Koch infestation, with notes on its biology. Acarines, 7(2): 23-27.

Arimura GI, Matsui K, Takabayashi J. 2009. Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. Plant Cell Physiol., 50:911-923.

Avery, D. J., and Briggs J. B. 1968. The aetiology and development of damage in young fruit trees infested with fruit tree spider mite, *Panonychus ulmi* (Koch). Ann. Appl. Biol. 61: 277-278.

Bakr, E. M. 2005. A new software for measuring leaf area, and area damaged by *Tetranychus urticae* Koch. Journal of Applied Entomology 129 (3), 173-175.

Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology. 37:911-917.

Bozzola, J.J. and Russell, L.D. 1999. Electron Microscopy, Second Edition. Sudbury, MA: Jones and Bartlett Publishers.

Crompton, M. and Birt, L. M. 1967. Changes in the amounts of carbohydrates. Phoshagen, and related compounds during the metamorphosis of the blowfly, *Lucilia cuprina*. J. Insect Physiol., 13:1575-1595.

Dermauw, W.; Wybouw, N.; Rombauts, S.; Menten, B.; Vontas, J.; Grbic, M.; Clark, R.M. Feyereisen, R. and Van Leeuwen, T. A. F. 2012. A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. Proceedings of the National Academy of Sciences, USA, 110: 112–113.

El-Saiedy, E. M. A.; Afifi, A. M.;Ali, F S. and Ahmed, M. M 2011. Susceptibility of four Watermelon cultivars to infestation with *Tetranychus urticae* Koch. Acarines 5(1): 23-28.

FAO 2013. Food and agricultural organization of the United States.

Fischer, E. R.; Hansen, B. T.; Nair, V.; Hoyt, F. H. , and Dorward, D. W. 2012. Scanning Electron Microscopy. Curr Protoc Microbiol, 76p.

Hanley, M. E.; Lamont, B. B.; Fairbanks, M. M. and Rafferty, C. M. 2007. Plant structural traits and their role in antiherbivore defense. Perspec. Plant Ecol Evol Syst, 8:157-78.

Holden, M. 1965. Chlorophylls: In chemistry and biochemistry of plant pigment. (Ed. Goodwin, T. W.), Academic press, London, p, 88-462.

Hung, P.V. and Morita, N. 2008. Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant Capacities. J. Food Chemistry, 109 (2):325-331.

Ibrahim, M. M. S.; El-Esnawy, B. A. and El-Adawy, A. M. 2008. Imbrications of certain cucurbit crops characteristics with the two-spotted spider mite infestation. J. Acarines. 2: 61-65.

Jeppson, L. R.; Keifer, H. H. and Baker, E.W. 1975. Injurious Tetranychid mites. In: Jeppson LR, Keifer HH, Baker EW, eds. Mites injurious to economic plants. Berkeley, CA, USA: University of California Press, 127–252.

Karnowsky, M. J. 1965. A formaldehyde–glutaraldehyde fixative of high osmolarity for use in electron microscopy. Journal of Cell Biology 27, 137A.

Kilany, S. M. 1997. Ecological aspects and control of the two-spotted spider mite, *Tetranychus urticae* Koch on cucumber plant in the greenhouse in Egypt. Ann. Agric. Sci. Ain-Shams Univ., 42 (1): 277-286.

Lee, Y. P. and Takabashi, T. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. Anal. Biochem., 14:71-77.

Luczynski, A.; Isman, M. B.; Raworth, D. A. and Chan, C. K. 1990. Chemical and morphological factors of resistance against the two-spotted spider mite in beach strawberry. J. Econ. Entomol. 83:564–569.

Miles, P. W. 1972. The saliva of Hemiptera. Advances in Insect Physiology 9: 183–255.

Park, Y.-L. and Lee, J.-H. 2002. Leaf Cell and Tissue

Damage of Cucumber Caused by Twospotted Spider Mite (Acari: Tetranychidae). J. Econ. Entomol., 95(5): 952-957.

Prasad, R. 2006. Occurrence and pest status of phytophagous mites infesting common vegetables. Indian Journal of Entomology, 68(3):235-239.

Sabri, N. N.; El-Masry, S. and Khafaey, S. M. 1973. Phytochemical investigation of Hyoscyamus Desertorum. Planta Medica, 23(1):4-9.

Sances, F. V.; Wyman, J. A., and Ting, I. P. 1979. Morphological responses of strawberry leaves to infestations of two spotted spider mite. J. Econ. Entomol. 72: 710-713.

SAS Institute 1988: SAS / Stat user’s guide, 6.03 ed. SAS institute, Cary, NC.

Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Viticult 16: 144-158.

Tomczyk, A. and Krompczyńska, D. 1985. Effects on the host plant. pp. 317– 329. In: Spider mites: their biology, natural enemies and control, vol 1A. W. Helle and M. W. Sabelis. (Eds.) Elsevier, Amsterdam. 405 pp.

War, A. R. ; Paulraj, M. G. ; Ahmad, T. ; Buhroo, A. A. ; Hussain, B. ; Ignacimuthu, S. and Sharma, H. C. 2012. Mechanisms of plant defense against insect herbivores. Plant Signal Behav. 7(10): 1306–1320.

Yassin, S. A.; Arafat, N. F. and Baiomy, F. A. 2014. Susceptibility of some tomato varieties to some pests and predators. Egypt. Acad. J. Biolog. Sci., 7(1): 1 – 10.