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Susceptibility of Two Apple Cultivars to Infestation with European Red Mite *Panonychus ulmi* Koch (Acari: Tetranychidae)

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ABSTRACT

Experiments were conducted to estimate the susceptibility of two apple cultivars (Anna and Golden dorset) to infestation with *Panonychus ulmi* Koch (Acari: Tetranychidae) and its population fluctuation during the two successive seasons, 2012 and 2013 at Menoufia governorate. The results showed that, Anna cultivar was the most highly susceptible recording average of 7.34 and 10.5 mite moving stages/leaf during the two successive seasons, respectively. The lower infestation was recorded with Golden dorset cultivar, being 1.6 & 7.49 mite moving stages/leaf during the two successive seasons, respectively. Susceptibility of apple cultivars to infestation with *P. ulmi* may be affected by plant leaf morphological structure and its chemical contents. The density of trichomes of Golden dorset cultivar was higher than that of Anna cultivar, the less density of trichomes the more mite infestation. Also, when the level of total phenolic compounds increased the infestation percentage decreased, while no effect of Tannins occurred. Mite populations reached its peak during May and April in the first and second seasons, respectively, and infestation was lower in the first season.

Key words: Susceptibility; Host plant resistance; Apple cultivars; Population dynamics; *Panonychus ulmi.*

INTRODUCTION

Apple *Malus domestica* Borkh. (Family: Rosaceae) is one of the most common crops in the world. It is usually infested with the European red spider mite; *Panonychus ulmi* Koch (Acari: Tetranychidae). So, evaluation the susceptibility of some apple cultivars to infestation with *P. ulmi* in order to select the most resistant ones is considered important to avoid using more pesticides. Chemical contents and morphological 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 *Tetranychus urticae*; Hanafy (2004) and Ibrahim *et al.,* (2008).

The present work was conducted to determine the level of infestation of two apple cultivars with *P. ulmi* Koch, and its relationship with plant leaf morphological characteristics and certain chemical contents. The population dynamics of the mite throughout the two successive seasons; 2012 and 2013 was also studied.

MATERIALS AND METHODS

Experimental procedures:

Two Apple cultivars Anna and Golden Dorset were chosen for this study. An orchard of 7 years located in Ashmon, El-menoufia governorate was chosen to conduct the experiments during the two successive seasons; 2012 and 2013. The orchard was divided into 20 experimental units (15trees /unit). A row of trees separated the experimental units and the distances between trees was 3 meters.

To study the population dynamics of *P. ulmi*, weekly samples each of 20 leaves were randomly collected from each apple cultivar. Samples were collected from the first week of March till the second week of November during the two successive seasons 2012 and 2013. Leaf samples were transferred to the laboratory for examination and different stages of *P. ulmi* were counted.

The percentages of infested apple cultivars with *P. ulmi* were calculated according to El-Saiedy *et al.* (2011) formula.

Morphological and Biochemical Studies:

1- Morphological Studies

Leaf samples of the tow apple cultivars were collected and imaged the upper and lower surface of apple 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. Samples were washed in 0.1 M phosphate buffer (pH 7) and post-fixed in 2% Osmium tetraoxide (OsO4) (pH 7). Samples were then taken through an alcohol dehydration series (15%, 25%, 40%, 50%, 70%, and 95% ETOH). SEM samples were critical point dried, sputter-coated to 20 nm with gold/paladium, and mounted on aluminum stubs for observation under a Phillips (SEM at 10-15 kV), Karnowsky (1965) and Fischer *et al*. (2012).

2- Biochemical Studies

Some specific chemical constituents of apple leaves were determined as follow:

A. Extraction and determination of phenolic compounds:

a. Extraction:

Ten plant leaves from each replicate (3 replicate for each treatment) were washed with water and placed in an oven to dry at 45ºC for 4 days. Then they were grounded in an electric grinder into fine powder. Extraction was preformed as described by Kahkonen *et al*., (1999). Grounded plant seedlings (5gm) were extracted with 2x10 ml of 80% aqueous methanol using electric homogenizer for 5 min. Samples were centrifuged (10min,3000r.p.m), and combined extracts were poured into pre-weighed small conical flasks. Methanol was removed under reduced pressure. The solid residue (crude extract) was weighed and dissolved in water to a 5 ml volume.

b. Determination

The amount of total phenolic compounds in extracts was determined by Folin–Ciocateu method as modified by Singleton and Rossi (1965). The Folin- Ciocalteu reagent was prepared by adding 100 gm sodium tungstate, 25 gm phosphomolybdic acid, 100 ml HCl, and 50 ml orthophosphoic acid (85%) to 700 ml deionized water in conical flask. The flask was refluxed for 10 hours, cooled and then 150 gm lithium sulphate was added. Few drops of liquid bromine were added to make the solution yellow in color and then the final volume was completed by deionized water to 1 liter. Two hundreds 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. The tubes were mixed and allowed to stand for 30 minutes. Absorption at 760 nm was measured against blank containing everything except the sample. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction. Gallic acid standard was used, and the total phenolic content was expressed as mg/g gallic acid equivalents (GAE).

B. Extraction and determination of tannins:

Tannins were extracted, from samples, using 70 per cent aqueous acetone. The contents were centrifuged (3,000 g at 4°C for 10 min) and the supernatant was taken for determination of tannins. Total phenols and simple phenols were estimated using folin-ciocalteu reagent using tannic acid as a standard (Makkar *et al*., 1993) and net tannins were calculated by difference between total phenols and simple phenols.

RESULTS AND DISCUSSION

Susceptibility of two apple cultivars to *P. ulmi* infestation:

The tested apple cultivars could be arranged in descending order according to their susceptibility to infestation with *P. ulmi* during the two seasons, 2012 and 2013 as follows: Anna cultivar was more significant susceptible to infestation than Golden dorset cultivar. It recorded 2.53 (71%) motile mite stages / leaf during season (2012) and 3.9 (63%) motile mite stages / leaf during season (2013) , The lowest infestation was observed with Golden dorset cultivar, being 0.99 (28.97) and 2.3 (37.10%) for the latter cultivar during the two successive seasons, respectively (Table 1).

Morphological and Biochemical Studies:

1- Morphological Studies

Susceptibility of apple cultivars to infestation with *P. ulmi* may be affected by plant leaf morphological structure. Therefore the differences between the two apple cultivars, Anna and Golden dorset, in their leaf morphological structure were studied. The density of leaf trichomes on lower surfaces of Golden dorset cultivar was higher than that of Anna cultivar. (Figs. 1&2); while the density of leaf trichoms on upper surfaces showed almost the same in the two apple cultivars.

Host plant resistance depends on many factors, including morphological characters; i.e. plant surface (Tingey and Laubengayer, 1982; Juniper and Jeffree, 1983). Morphological characteristics within a plant species may play an important role in host plant preference because it may affect insect feeding (Hoffman and McEvoy, 1985). Warabieda *et al*. (1997) investigated the morphological and anatomical characters of apple leaves associated with cultivar susceptibility to *T. urticae* infestation. The lower (abaxial) epidermis of the leaves seemed to suggest that the layout and thickness of cuticle, rather than the epidermal thickness itself could be a factor determining the susceptibility of apple cultivars to

Table (1): Susceptibility of two apple cultivars to *P. ulmi* infestation during 2012&2013 seasons.

Apple cultivars	Mean number of *P. ulmi* motile stages / leaf ± SE			
	2012 season		2013 season	
	Mean No.	Infestation %	Mean No.	Infestation %
Golden	0.99±0.18	28.98	2.30±0.86	37.10
Anna	2.53±0.79	71.02	3.90±1.36	62.90
LSD 0.05	10.446		21.1	

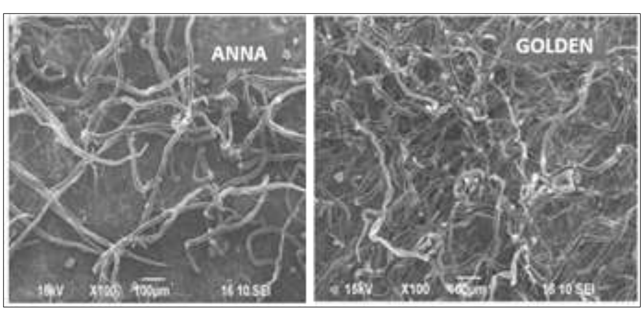

Fig. (1). Scanning electron micrographs of lower surfaces leaf trachoms for two apple cultivars.

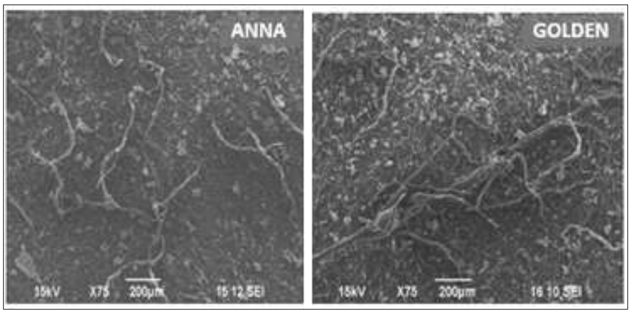

Fig. (2). Scanning electron micrographs of upper surfaces leaf trachoms for two apple cultivars.

Table (2): Relationship between phytochemical components of two apple cultivars leaves and high infestation peak of *P. ulmi*

Apple cultivars	Mean number of *P. ulmi* motile stages	Phytochemical Components	
		Total phenol ug GA/gm fresh weight	Total tannins ug GA/gm fresh weight
Golden	2.30[a]	3076.67[b]	583.67[a]
Anna	3.90[b]	3912.00[a]	575.33[a]
Correlation Coefficient (*r*)		0.9593	0. 0221

Table (3): Population dynamics of *Panonychus ulmi* Koch stages / leaf on two apple cultivars during 2012 season

Sampling dates	Motile stages		Eggs	
	Golden	Anna	Golden	Anna
Mar	0.30	0.48	2.1	4.18
Apr	1.66	4.06	4.33	7.00
May	1.10	7.34	2.13	47.23
Jun	1.26	1.30	0.74	1.89
Jul	0.52	0.67	1.67	2.08
Aug	0.30	0.54	0.88	1.94
Sep	0.93	1.48	0.55	2.68
Oct	1.35	2.98	0.65	5.57
Nov	1.53	3.08	2.65	4.58
Mean	0.99[a]	2.53[b]	1.76[a]	9.47[b]
LSD at 0.05	0.89		7.49	

Table (4): Population dynamics of *Panonychus ulmi* Koch stages / leaf on two apple cultivars during 2013 season

Sampling dates	Motile stages		Eggs	
	Golden	Anna	Golden	Anna
Mar	0.22	1.50	2.22	3.02
Apr	5.00	10.50	8.3	54.00
May	7.49	10.39	9.61	58.84
Jun	0.83	1.68	6.28	14.13
Jul	0.80	1.03	1.3	2.25
Aug	0.53	0.93	0.43	1.25
Sep	1.18	1.83	1.43	2.58
Oct	2.20	3.83	2.38	4.10
Nov	2.50	3.48	4.03	7.75
Mean	2.30[a]	3.90[b]	3.99[a]	16.43[b]
LSD at 0.05	1.13		9.52	

T. urticae. Host plant resistance is based on certain morphological, and/or chemical plant defensive factors (Pedigo, 2006).

2- **Biochemical Studies**

One of the most important factors which may explain the susceptibility or tolerance of apple cultivars to *P. ulmi* infestation is the phytochemical components of their leaves. Therefore, this study was carried out during the peak of season 2013 to determine the relationship between *P. ulmi* infestation at peak season level /leaf and the phytochemical components of the studied apple cultivars. The high mean number of *P. ulmi* motile stages occurred on the leaves of Anna cultivar, which associated with lower levels of total phenolic compounds (Table 2) and this indicated a negative correlation with the population densities; while with tannins indicated no correlation with the population densities as a resultant throughout the growing season.

The Golden dorset cultivar recorded low infestation rate of *P. ulmi* motile stages, and this was associated with higher levels of total phenolic compounds therefore there is a negative correlation occurred between them (Table 2). Generally the obtained data indicated that there was a negative relationship occurred between mite infestation levels and total phenolic compounds in Apple cultivar leaves. In Anna cultivar the average of total phenolic was 3076 (ug GA/gm fresh weight), while in Golden dorset cultivar the average was 3912 (ug GA/gm fresh weight) (Table 2).

These results are in agreement with those recorded by; Pree, (1977) who stated that, the Phenolic compounds, i.e., Phloridzin which was mainly found in high concentration in the foliage of *Malus taxa*, miaht play an important role in insect resistance. Phenolics are important defense chemical compounds. Apple leaves from different genotypes (*Malus* spp.) contain various levels of phenolic compounds such as chlorogenic acid, gallic acid, quercitrin, phloridzin, and phloretin (Garcia *et al*., 1995).

Hanley *et al*. (2007) found that the biochemical mechanisms of defense against the herbivores are wide-ranging, highly dynamic, and are mediated both by direct and indirect defenses. The defensive compounds are either produced constitutively or in response to plant damage, and affect feeding, growth, and survival of herbivores. 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) or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) that either kill

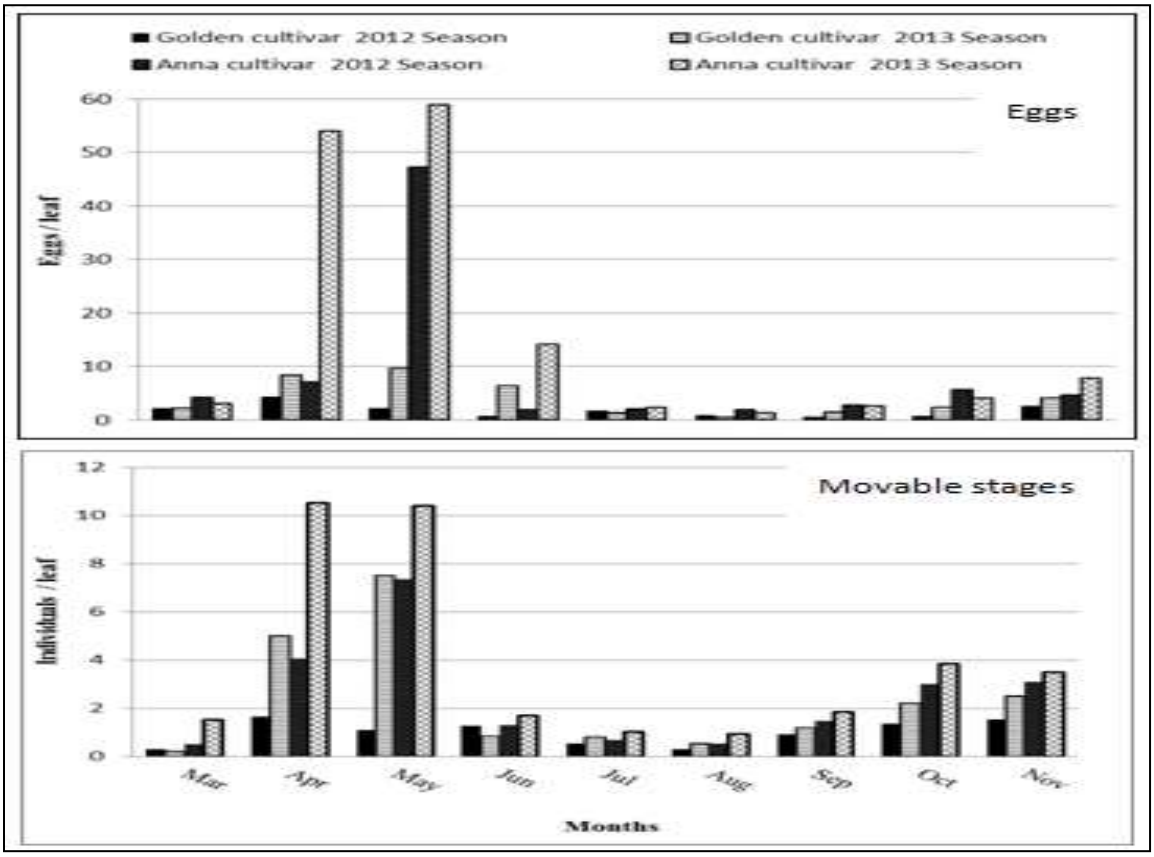

Fig. (3). Mean number of *P. ulmi* motile stages and eggs on two apple cultivars during 2012 and 2013 seasons.

or retard the development of the herbivores.

Population dynamics of *Panonychus ulmi* on two apple cultivars

Population dynamics of European Red Mite, *Panonychus ulmi* Koch were recorded during season 2012 from the second week of March till the second week of November and during season 2013 from the second week of March till the second week of November.

Data in tables (3&4) and illustrated in Fig. (3) revealed that the infestation of apple cultivars Anna and Golden dorset with *P. ulmi* started on March then gradually increased to reach its peak in April for Golden dorset cultivar, and in May for Anna cultivar during 2012 season; while in 2013 season, infestation started in the end of March, then gradually increased to reach its peaks in May for both Anna and Golden dorset cultivars.

Population density of *P. ulmi* motile stages and eggs / leaf of Anna and Golden dorset cultivars averaged (0.48 & 4.18; and 0.30 & 2.10), (1.50 & 3.02 and 0.22 & 2.22) at the beginning of infestation during 2012 and 2013 seasons, respectively; while in the high peak of infestation it averaged (7.34 & 47.23 and 1.66 & 4.33), (10.50 & 58.84 and 7.49 & 9.61 individuals / leaf) during 2012 and 2013 seasons, respectively.

The population of *P. ulmi* started to decrease from Jun in Anna cultivar, and in the end of May in Golden dorset cultivar during 2012 season, while during 2013 season it started to decrease from June in Anna and Golden dorset cultivars.

The mean numbers of *P. ulmi* motile stages and eggs at the two apple cultivars; Anna and Golden dorset averaged 2.44 & 8.57 and 0.99 &1.74 during 2012 season, respectively, while it averaged 3.90 & 16.43; and 2.30 &3.99 individual / leaf during 2013 season, respectively. Thus, it is clear that Anna cultivar was more susceptible to the European red spider mite infestation.

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Finally, it could be concluded that the numbers of eggs and motile stages of *P. ulmi* reached its peak during May in seasons 2012 and 2013. Then it slightly decreased, recorded the lowest numbers at Aug.

Statistical analysis in tables (3&4) showed that there are significant differences between the numbers of *P. ulmi* stages during all two seasons 2012 and 2013. The obtained results revealed that there was significant differences between Anna and Golden dorset cultivars.

These results were in agreement with those recorded by Kitashima and Gotoh (2003) who stated that, the *P. osmanthi* population was bimodal with one peak in spring (May-June) and another in winter (November-January). Also, Baiming *et al*. (2007) stated that, two population peaks appeared in a year in the orchards infested with *P. ulmi*, the first in the last 20 days of June and the next in the beginning of August. The population fluctuation of *P. ulmi* differed during the two studied seasons, but the peak was recorded during May or June in both seasons according to the grapevine cultivars, Mannaa *et al*. (2012).

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