Zagazig Journal of Plant Protection Research 209-224

Zagazig J. Agric. Res., Vol. 43 No. (1) 2016

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

EFFECT OF SOME PHYSICAL PROPERTIES AND CHEMICAL CONTENTS OF DIFFERENT CITRUS SPECIES AND VARIETIES ON INFESTATION WITH CITRUS LEAFMINER *Phyllocnistis citrella* Stainton (LEPIDOPTERA: GRACILLARIIDAE) IN EGYPT

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ABSTRACT

This investigation was carried out to study the relationship between citrus leafminer (CLM) *Phyllocnistis citrella* Stainton and some factors *i.e.* flushes (growth cycles), leaf area, leaf age, citrus varieties, some chemical contents in leaves and storage fruits of six citrus varieties and species at El-Kassasien District, Ismailia Governorate and Minia El-Kamh District, Sharikia Governorate orchards for three successive seasons 2013, 2014 and 2015. The results could be summarized as follows: infestation started to appear in small leaves (0.4-0.9, 1.8-2.7, 1.1-1.7 cm^2 for orange varieties) and (0.3, 0.9, 0.8 cm^2 for mandarin) in different emerged flushes (spring, summer and autumn), respectively during seasons 2013 and 2014. Also, leaf blade was not infested when area reached more than 3.2- 3.8, 6.5- 7.6 and 6.5- 7.0 cm^2 in orange varieties as well as 1.6, 2.9 and 2.0 cm^2 in mandarin through spring, summer and autumn flushes, respectively. The young leaves less than three days old were subjected more to highly infestation rate than old ones. Percentage of infestation, significantly decreased in descending order (autumn, summer and spring flushes cycle, respectively). The new sprouted growth in spring flush (March) were least damaged and escaped from CLM infestation. The tested citrus species and varieties showed significant differences in infestation rates with CLM, where navel orange recorded the highest followed by valencia orange, mandarin, sweet orange, baladi orange and sour orange. The effect of certain chemical contents (volatile oil contents, phenols, total carbohydrates, total protein and pH level) in some citrus varieties leaves on infestation rate of CLM was investigated. Total content of volatile oils in citrus leaves was affected by diversity of varieties. Differences of citrus infestation rates with CLM depend on total content of volatile oils in some citrus leaves of tested varieties and other varieties have no relation with volatile content. The present study demonstrates the effect of phenols content on insect infestation rate. Meanwhile, high total carbohydrate in leaves of citrus varieties showed a significantly lower infestation with CLM. Total proteins and pH level in citrus leaves showed no relation with CLM infestation. Fruits storage on trees after the normal date of harvesting increased significantly CLM infestation, where infestation in navel orange reached 42.88 and 47.44% after storage 90-120 days compared with 31.72 and 34.83% in normal date of harvesting, Also, infestation in mandarin reached 38.66 and 41.55% after storage compared with 24.00 and 27.33% in normal harvesting date.

Key words: Citrus leafminer, leaf age, leaf area, flushes, citrus varieties, fruits storage on trees, chemical consituents.

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INTRODUCTION

Citrus are represented as one of the most important fruit crops in Egypt, the cultivated area of citrus (more than 340000 faddans) has been rapidly increased specially in newly reclaimed lands. Yet, in recent years citrus has been infested by citrus leafminer (CLM) *Phyllocnistis citrella* Stainton, caused extensive damage to new leaf flushes with a noticeable reduction in the annual yield. Infestation was found in all citrus orchards with heavy infestation in nurseries all over the year (Mogahed *et al.*, 2013).

CLM during its larval stage, mines the newly formed leaves and stems of citrus trees and sometimes fruits (Legaspi and French, 1996), causing roll up of the leaves and reducing chlorophylls (Abo-Sheaesha, 2003). The heavy infestation can affect the growth of the young planted trees and reduce yield (Knapp *et al.*, 1994; Khalil *et al.*, 2001; Gobran, 2002; Habibur-Rahman and Jahan., 2005).

The CLM infestation is recorded in leaves at the period extended from early March and mid April until it disappeared in early December (Mogahed, 1999; El-Saadany *et al.*, 2002; Kheder *et al*., 2002; Elkady, 2005).

Many factors play an important role concerning citrus trees infestation with CLM causing a serious damage with noticeable reduction either in tree growth or in the annual fruit yield year after year such as weather factors (Patel *et al.*, 1994; Katole *et al*., 1997; Bene and Landi, 1999; Kheder *et al.*, 2002; El-Dessouki *et al.*, 2005; Habibur- Rahman and Jahan, 2005).

Many authors investigated the effect of flushes (growth cycles) such as: Batra *et al*. (1998), Pena (1998), Khalil *et al.* (2001), El-Saadany *et al.* (2002), Kheder *et al.* (2002), Shivankar and Rao (2003), Bernet *et al*. (2005) and Mogahed *et al.* (2013) and citrus varieties: Shevale and Pokharkar (1992), Jacas *et al*. (1997), Batra *et al*. (1998), Mogahed (1999); Khalil *et al.* (2001), El-Saadany *et al*. (2002), Kheder *et al*. (2002), Hu-JunHua *et al.* (2004), Elkady (2005) and Mogahed *et al.* (2013).

This paper is concerned mainly with studying the effect of leaf area, leaf age, flushes, susceptibility of some citrus varieties and leaf citrus contents (volatile oils, phenols, carbohydrates and proteins) on infestation with CLM, and assessing the effect of on-tree storage fruits in relation to CLM infestation.

MATERIALS AND METHODS

This study was carried out in two orchards, the first one at Minia El-Kamh district, Sharkia Governorate during two successive seasons (2013- 2014 and 2014- 2015) and the second one at El-Kassasien district, Ismailia Governorate during two successive seasons (2013 and 2014) on some citrus species and varieties. Ten trees, homogenous in size and age (10 years old) were randomly selected from each variety. The experimented varieties namely: Navel orange (*Citrus sinensis*), Baladi orange (*Citrus sinensis* var. baladi), Sweet (succary) orange (*Citrus sinensis*), Valencia orange (*Citrus sinensis*), Baladi Mandarin (*Citrus reticulata*) and Narang (sour orange) (*Citrus aurantium*). The selected citrus trees received all normal agricultural practices and left free from insecticides application during the period of investigation.

Effect of Citrus Leaf Area (cm^2) and Leaf Age (days) in Relation to Percentage of Infestation with CLM

The leaf area (cm^2) in different collected leaves was calculated according to the equation of Chou (1966), 2/3 length x width. Average of leaves/shoot length (cm^2) were also considered. For this purpose, five trees from each variety were selected in the starting of growth cycle and samples of leaves (100 leaves/age) were randomly taken from 1-7 days old, also leaves had two and three weeks old. The percentage of infestation in leaves of different ages of each variety were estimated to calculate the relation between leaf age of different varieties in each flush (growth cycle) and percentage of infestation in both seasons of study 2013 and 2014.

Effect of Flushes (Growth cycles) on the Percentage of CLM Infestation

The selected trees from each species /varieties gave three distinct cycles (flushes) through the year round on adult fruiting trees (observed in the last year of 2013), under conditions of the experimented orchard at El-Kassasien District, Ismailia Governorate. From such flushes the first flush (spring flush) started from early Mar. to late April, while the second (summer flush) started from early June to early Sept. and the third flush (autumn flush) started from late Sept. to the end of growing season. To estimate the growth cycle vigour, four branches more than 5 cm length at different directions were labeled in each tree (replicate) of the studied varieties (five trees/each variety) and in each season, number of sprouted shoots was counted then recorded in different emerged cycles per year. Yet, the number of the new produced leaves per shoot were also recorded and examined weekly up to the end of growing season. The numbers of infested leaves by CLM and percentage of infestation were also recorded, during each flush (growth cycle).

Susceptibility of the Tested Citrus Varieties to Infestation with CLM

Monthly samples of 500 leaves/30 trees for each of the six varieties from tender branches were taken at random from each flush and examined to calculate percentage of infestation in selected varieties.

Effect of On-tree Storage of Fruits on Infestation with CLM

Twenty trees were picked at normal date of harvesting (late Nov. or early Dec.) and the other 20 trees were left for storage period. Mandarin and navel orange 90-120 days from late Nov. up to early April. Samples of 400 leaves /20 trees in different flushes/year at random different locations were examined.

The effect of on-tree storage of fruits occurred during 2013 and 2014 on CLM infestation was estimated at the following seasons of 2014 and 2015, respectively (as residual effect of on-tree storage of fruits). The number of alive mines (which contain alive larvae or pupae) were counted in each sample.

Chemical Analysis

The leaves of citrus trees were collected from six varieties of citrus namely navel orange, valencia orange, sweet orange, baladi orange, sour orange (narang) and mandarin grown in El-Kassasien District, Isamilia Governorate, Egypt.

Volatile oil extraction

Fresh leaves materials were subjected to hydro-distillation (500 ml distillated for 3hr.) using a Clevenger- type apparatus according to the method recommend by British (1988).

Determination of phenols

Ten plant leaves from each replicate (3 replicates for each treatment) were washed with ΔH_2O and placed in an oven to dry at 45 ºC for 4 days. Then they were ground in an electric grinder into fine powder. Extraction was performed as described by Kâhkônen *et al.* (1999).

The amount of total phenolics in extracts was determined by Foin-Cocateu method as modified by Singelton and Rossi (1965). The total phenolic content was expressed as mg gallic acid per mg dry weight of original sample (mg GA/g dw).

Determination of crude protein

The crude nitrogen content was determined by micro-kjeldahl method, crude protein content was calculated as percentage of the dry weight bases by multiplying crude nitrogen percentage by the conversion factor of 6.25 (AOAC, 2000).

Quantitative analysis of carbohydrates and/or glycosides

A sample of 2 mg of powdered whole leaf was extracted with 50% ethanol and tested for the presence of carbohydrates and/or glycosides using Molischs test. Total carbohydrates content was determined according to the methods of Balbaa *et al.* (1976).

pH determination in leaves of citrus varieties

pH determination in leaves of some citrus varieties was carried out according to the method described by AOAC (1970).

Statistical Analysis

Data were tabulated and analyzed by analysis of variance using LSD 5% and 1% according to Snedecor (1970).

RESULTS AND DISCUSSION

Effect of Leaf Area (cm^2) and Leaf Age (days) on Citrus Leafminer (CLM) Infestation

Data recorded in Tables 1 and 2 show the effect of leaf area (cm^2) and leaf age in different flushes on percentage of infestation by CLM on some citrus varieties at El-Kassasien District, Ismailia Governorate during 2013 and 2014 seasons.

Leaf area (cm^2)

Leaf area differed according to age which were 1.1 – 1.7; 3.0 – 4.0 and 6.0 – 7.0 cm^2/leaf during autumn flushes on orange varieties while they were 0.8, 1.8 and 2.0 cm^2/leaf during the same growth flushes in mandarin for leaf ages 1-7 and 8-14 and 15-21 days, respectively. For example, during autumn flushes when leaf area of orange group was 1.1 - 1.7 and 2.0 cm^2/leaf in mandarin, infestation was reached to 88.5 and 89.3% while it was decreased to 14.7 and 21.8% in 2013 and 2014 seasons, when leaf area was 6.0 - 7.0 and 2.0 cm^2/leaf, respectively.

Leaf age (day)

Young leaves (1-7 days old) in all orange and mandarin varieties were the most susceptible to CLM infestation in all flushes (growth cycles). For example in young leaves (1-7) days old CLM infestation were 2.7, 47.7, 88.5% (during 2013) and 4.2, 56.2, 89.3% (during 2014) for growth cycles (flushes) spring, summer and autumn, respectively.

Generally it can be concluded that, young size of tender leaves aged nearly about 1-7 days old appeared to be the most susceptible to infest with *Phyllocnistis citrella* in different emerged flushes and the percentage of infestation (at the same age and leaves area) significantly and gradually increase from spring flush, followed by the summer flush then autumn flush in ascending order.

However, the leaves aged 2-3 weeks old less infested with CLM and leaves more than three weeks old rarely infested in the most cases.

These results are in harmony with the findings of Mogahed (1999) in Egypt, who reported that the new leaves (1-5 days old) of all tested citrus varieties appeared to have highest infestation with CLM; while, the citrus leaves aged (11-15 days/old) appeared to be tolerant against the infestation.

In addition, Caleca *et al.* (1996) reported that the greatest number of early instar larvae was recorded on leaves 1-3 cm long, whereas 78% of pupae were found on leaves > 5 cm long.

Susceptibility of Some Citrus Varieties to Infestation with CLM in three Flushes (Growth cycles)

Flushes (growth cycles)

Data presented in Tables 3 and 4 show the number of new sprouted growth per each flush in both studied seasons. Yet, all studied citrus varieties, at El-Kassasien District, Ismailia Governorate, gave three flushes /year *i.e.* spring (Mar.–May), summer (June – Aug.) and autumn (Sept. -Nov.). In addition, spring flush was the vigour one (according to the number of sprouted growth /tree) followed by summer flush then autumn flush.

The obtained data also show that the young leaves of spring flush (4.38 and 7.10) less attacked by CLM as compared with summer (15.66 and 19.66) came after spring flush and / or autumn one (35.89 and 38.40) the heaviest infestation in 2013 and 2014 seasons, respectively. Moreover, months of April, Aug. and Nov. recorded the highest percentage of infestation in spring, summer and autumn flushes (in ascending order), respectively. In this concern, the infestation with CLM showed low level in March. It appeared that, summer months showed gradually increments in attacked leaves with CLM, reached its peak in August and the other peak was found in Oct., of autumn leaves in both seasons of study.

These results are in agreement with many investigators such as Masheshwari and Sharma (1986), Berkani *et al*. (1996), Caleca *et al.* (1996), Costa-Comelles *et al.* (1997), Batra *et al.* (1998), Pena (1998), Alkhateeb *et al*. (1999), Gobran (2002) and Kheder *et al*. (2002).

However, Rao and Shivankar (2002) and Shivankar and Rao (2003) disagreed with the obtained results.

It could be concluded that CLM insect pest not able to attack the new sprouted shoots of citrus in spring flush in March and flushes were least damaged and escaped from CLM infestation on all studied varieties, owing to unfavorable atmosphere conditions, such as temperature, relative humidity or other climatic factors. The results obtained herein are in accordance with Batra *et al.* (1998), Pena (1998), Khalil *et al*. (2001), El-Saadany *et al*. (2002),

Table 1. Effect of leaf area (cm^2) and leaf age (day) in different flushes on infestation percentage by *Phyllocnistis citrella* Stainton in the tested citrus varieties at El-Kassasien District, Ismailia Governorate during 2013 season

Flushes	Age of citrus leaf/day	Mean leaf area/cm^2 for orange varieties	Mean leaf area/cm^2 for mandarin variety	Infestation (%)							
				Navel orange	Valencia orange	Sweet orange	Baladi orange	Sour orange	Mandarin	General Total	General Mean
	1 – 7	0.4 - 0.9	0.3	4.4	3.0	1.9	1.4	1.1	4.4	16.2	2.7
Spring	8 - 14	1.9 – 2.5	1.2	1.9	1.5	0.6	0.7	0.8	1.9	7.4	1.2
	15 – 21	3.2 – 3.8	1.6	1.6	1.2	0.5	0.5	0.5	1.1	5.4	0.9
Mean				2.6	1.9	1.0	0.9	0.8	2.5	9.7	1.6
	2.23										
LSD 5%	0.63										
	0.396										
	3.192										
LSD 1%	0.90										
	0.564										
	1 - 7	1.8 – 2.7	0.9	66.0	46.5	45.5	43.0	36.5	49.0	286.5	47.7
Summer	8 - 14	3.7 – 4.0	2.1	31.0	30.5	22.0	16.0	12.5	23.0	135.0	22.5
	15 – 21	6.5 – 7.6	2.9	10.0	8.0	7.0	8.5	6.0	8.5	48.0	8.0
Mean				35.7	28.3	24.8	22.5	18.3	26.8	156.4	26.1
	2.133										
LSD 5%	2.202										
	0.562										
	3.051										
LSD 1%	3.15										
	0.803										
	1 - 7	1.1 – 1.7	0.8	100	100	100	75.0	56.0	100	531	88.5
Autumn	8 - 14	3.0 – 4.0	1.8	88.5	77.0	55.5	55.0	40.0	60.5	376.5	62.7
	15 - 21	6.0 –7.0	2.0	25.5	18.0	12.5	14.5	5.5	12.5	88.5	14.7
Mean				71.3	65.0	56.0	48.2	33.8	57.7	332	55.3
	4.58										
LSD 5%	4.624										
	3.933										
	6.393										
LSD 1%	6.617										
	5.663										

Table 2. Effect of leaf area (cm^2) and leaf age (day) in different flushes on percentage infestation by *Phyllocnistis citrella* Stainton in the tested citrus varieties at El-Kassasien District, Ismailia Governorate during 2014 season

Flushes	Age of citrus leaf/day	Mean leaf area/cm^2 for orange varieties	Mean leaf area/cm^2 for mandarin variety	Infestation (%)							
				Navel orange	Valencia orange	Sweet orange	Baladi orange	Sour orange	Mandarin	General Total	General Mean
	1 - 7	0.4- 0.9	0.3	6.1	4.1	5.7	2.5	1.9	5.0	25.3	4.2
Spring	8 - 14	1.9 – 2.5	1.2	2.0	3.1	0.9	1.8	0.7	2.0	10.5	1.7
	15 – 21	3.2 – 3.8	1.6	1.6	1.5	0.7	1.0	0.5	1.8	7.1	1.2
Mean				3.2	2.9	2.4	1.8	1.0	2.9	14.2	2.4
	2.671										
LSD 5%	1.071										
	0.894										
	4.684										
LSD 1%	1.53										
	1.278										
	1 - 7	1.8 – 2.7	0.9	72.0	52.0	45.0	61.0	37.5	69.5	337	56.2
Summer	8 - 14	3.7 – 4.0	2.1	36.5	46.0	31.0	20.0	12.5	30.5	176.5	29.4
	15 - 21	6.5–7.6	2.9	19.0	18.0	14.0	7.0	6.0	13.0	77.0	12.8
Mean				42.5	38.7	30.0	29.3	18.7	37.7	196.9	32.8
	4.465										
LSD 5%	6.372										
	3.921										
	6.39										
LSD 1%	9.126										
	5.612										
	1 - 7	1.1 – 1.7	0.8	100	100	100	77.5	58.5	100	536	89.3
Autumn	8 - 14	3.0– 4.0	1.8	91.5	75.0	54.0	59.5	48.5	75.5	404	67.3
	15 - 21	6.0 –7.0	2.0	39.0	23.5	17.0	21.5	5.5	24.5	131	21.8
Mean				76.8	66.2	57.0	52.8	37.5	66.7	357	59.5
	7.867										
LSD 5%	7.757										
	5.636										
	11.2										
LSD 1%	11.1										
	8.064										

Kheder *et al*. (2002) and Bernet *et al*. (2005). They reported that, the availability of tender growth of flushes appeared to be the most important factor affecting pest incidence in citrus varieties and species.

Citrus species and varieties

Tables 3 and 4 reveal the significant differences concerning percentage of infestation among the tested citrus varieties in different emerged flushes in both seasons of study. As such, navel orange variety was considered the most infested with CLM in spring flush (8.0 , 10.0%), followed by valencia orange (5.3 , 9.3%) then mandarin variety (5.0 , 8.0%), sweet orange (3.3 , 7%) and balady orange (2.4 , 5.3%) Meanwhile, sour orange was the least infested variety (2.3 , 3.0%) in the first and second seasons, respectively.

The same trend was noticed in summer and autumn flushes with significant differences also between evaluated citrus varieties in both seasons.

Anyhow, total percentage of infestation through the years round, revealed that navel orange variety was the most preferable variety (27.20 and 30.00%) and most susceptible compared with other citrus varieties.

Meanwhile, valencia orange and mandarin came after recording 21.92, 26.11% and 19.64, 23.00% while sweet orange, balady orange and sour orange were the least infested varieties exhibiting 16.82, 20.43% ; 14.99, 18.30% and 11.30, 12.43% in 2013 and 2014 seasons, respectively with significant differences between the tested citrus varieties.

Variation between evaluated citrus varieties might be attributed to flush vigour (number of sprouted growth/cycles), date of emerged growth in every cycle and leaf anatomy *i.e*. cut in thickness and leaf volatile oil content.

Accordingly and on these bases, sour orange, balady orange varieties showed that number of emerged new growths received the maximum in spring flush (less infested with CLM) and emerged new growths in summer and autumn were the lowest numbers as compared with other varieties and consequently reflected to the percentage of infestation. Many investigators came to the same results: Singh *et al.* (1988), Verma (1989), Batra *et al.* (1992), Padmanaban (1994), Mogahed (1999), Arora *et al*. (2000), Gobran (2002), Elkady (2005) and Mogahed *et al*. (2013).

It is proved that sour orange, sweet orange and baladi orange varieties were the least susceptible varieties to CLM infestation as compared with other citrus species and varieties. The obtained result demoumstrated that navel orange variety was the most susceptible variety. These results are in pararell with those of some investigators (Mogahed, 1999; El-Saadany *et al.,* 2002, Gobran, 2002; Mogahed *et al.*, 2013).

Effect of Volatile Oils, Phenols, Carbohydrates, Proteins and pH Level on Infestation by CLM in Different Varieties and Species at El-Kassasien District

Volatile oils content in different citrus varieties and its relation with CLM infestation

Data in Table 5 show wider differences between investigated citrus varieties for their susceptibility to infestation with CLM. On the basis of percent leaf infestation and total content of volatile oils of varieties, they could be classified into two groups: first group are represented by the highest infested citrus varieties such as navel orange (51.3% and 356 mines), valencia orange (45.7% and 304 mines) and mandarin (41.7 and 244 mines). While the highest content of volatile oils were recorded in sour orange (0.46%), navel orange (0.38%), valencia orange (0.36%) and mandarin (0.40%). The second group includes the least susceptible varieties of citrus infestation with CLM such as sour orange (23.3% and 100 mines), baladi orange (32.3% and 209 mines) and sweet orange (36% and 232 mines), however, the least content of volatile oils were obtained in baladi orange (0.30%) and sweet orange (0.32%).

The results showed that there is no distinic relationship between total content of volatile oils obtained in the leaves of different citrus varieties and percent infestation of citrus leaves with CLM, except in case of sour orange variety.

Table 3. Total infestation with *Phyllocnistis citrella* Stainton in different flushes of citrus varieties and species at El-Kassasien District, Ismailia Governorate during 2013 season

Citrus varieties	Average No. of new shoots/ tree			Infestation in different flushes (cycles) (%)															General Mean of total infestation
				Spring					Summer					Autumn					
	Spring	Summer	Autumn	Mar.	April	May	Total	Mean	June	July	Aug.	Total	Mean	Sept.	Oct.	Nov.	Total	Mean	
Navel orange	1166	175	115	2.0	9.0	13.0	24.0	8.0	16.6	20.6	29.6	66.8	22.3	40.6	73.5	39.8	153.9	51.30	27.20
Valencia orange	487	109	90	1.2	4.6	10.2	16.0	5.3	14.5	18.6	19.9	53.0	17.7	33.6	61.8	32.9	128.3	42.77	21.92
Mandarin	1612	236	112	1.2	4.1	9.7	15.0	5.0	14.0	17.0	15.0	46.0	15.3	32.8	61.6	21.5	115.9	38.63	19.64
Sweet orange	830	133	78	1.1	2.2	6.5	9.8	3.3	12.0	17.0	15.0	44.0	14.7	32.6	49.4	15.4	97.4	32.47	16.82
Baladi orange	690	125	85	1.0	1.5	4.7	7.2	2.4	8.3	17.0	14.8	40.1	13.4	32.1	41.0	14.4	87.5	29.17	14.99
Sour orange	350	150	45	1.0	1.3	4.7	7.0	2.3	7.2	10.9	13.8	31.9	10.6	16.8	32.2	14.0	63.0	21.00	11.30
Total				7.5	22.7	48.8	79.0	26.3	72.6	101.1	108.1	281.8	93.9	188.5	319.5	138.0	646.0	215.3	
Mean				1.25	3.78	8.13	13.16	4.38	12.1	16.85	18.02	46.97	15.66	31.41	53.25	23.0	107.66	35.89	
LS D 5%						1.472					2.593					3.248			
LSD 1%						2.101					3.711					4.646			

Table 4. Total infestation with *Phyllocnistis citrella* Stainton in different flushes in citrus varieties and species at El-Kassasien District, Ismailia Governorate during 2014 season

Citrus varieties	Average No. of new shoots/tree			Infestationin different flushes (cycles) (%)															General Mean of total infestation
				Spring					Summer					Autumn					
	Spring	Summer	Autumn	Mar.	April	May	Total	Mean	June	July	Aug.	Total	Mean	Sept.	Oct.	Nov.	Total	Mean	
Navel orange	1105	169	126	2.0	10.0	18.0	30.0	10.0	29.1	28.7	28.2	86.0	28.7	35.8	76.4	41.8	154.0	51.3	30.00
Valencia orange	466	93	73	1.3	9.0	17.7	28.0	9.3	23.5	27.5	19.0	70.0	23.3	30.6	65.6	40.8	137.0	45.7	26.11
Mandarin	1644	205	107	1.0	7.0	16.0	24.0	8.0	21.0	18.4	18.6	58.0	19.3	28.5	62.3	34.2	125.0	41.7	23.00
Sweet orange	682	124	64	1.0	4.5	15.5	21.0	7.0	20.5	16.1	18.4	55.0	18.3	27.7	58.0	22.3	108.0	36.0	20.43
Baladi orange	577	115	77	1.0	2.2	12.8	16.0	5.3	19.5	14.8	17.7	52.0	17.3	24.0	52.0	21.0	97.0	32.3	18.30
Sour orange	340	110	40	1.0	1.5	6.5	9.0	3.0	11.0	8.0	14.0	33.0	11.0	17.2	32.3	20.5	70.0	23.3	12.43
Total				7.3	34.2	86.5	128.0	42.7	124.6	113.5	115.9	354.0	118.0	163.8	346.6	180.6	691.0	230.3	
Mean				1.22	5.70	14.42	21.34	7.1	20.76	18.91	19.31	59.0	19.66	27.3	57.76	30.10	115.20	38.40	
LSD 5%						2.811					2.811					3.572			
LSD 1%						4.024					4.024					5.111			

Table 5. Effect of volatile oils, phenols, carbohydrates, proteins and pH in relation to infestation by *Phyllocnistis citrella* Stainton in different citrus varieties and species at El-Kassasien District during 2014 season

Citrus varieties	Volatile oils (%)	Phenols (mg GA/g dw)	Total carbohydrates (%)	Total proteins (%)	pH	Infestation (%) in autumn	Mean No. of mines	Mean No. of larvae	Mean No. of pupae
Navel orange	0.38	21.9	13.65	7.944	2.13	51.3	356.0	141.0	47.0
Valencia orange	0.36	13.7	13.51	7.569	2.18	45.7	304.0	107.0	32.0
Mandarin	0.40	19.6	15.12	8.781	2.06	41.7	244.0	82.0	22.0
Sweet orange	0.32	23.2	14.00	8.013	2.17	36.0	232.0	78.0	20.0
Baladi orange	0.30	23.2	14.12	8.169	2.15	32.3	209.0	69.0	15.0
Sour orange	0.46	26.6	13.23	8.838	2.17	23.3	100.0	39.0	8.0
LSD 5%	0.06	1.349	0.055	0. 184	0.042	3.572			
LSD 1%	0.087	1.929	0.087	1.15	0.078	5.111			

In the present study the least susceptibility of sour orange, baladi orange and sweet orange to citrus leafminer is in agreement with the findings of Mogahed (1999 and 2005), Elkady (2005) and Mogahed *et al.* (2013). Most of the commercial cultivars of baladi orange, sweet orange, sour orange and mandarin were found to be less susceptible. They were resistant to CLM on basis of leaf infestation and could be exploited commercially in the management programmers to control the citrus leafminer. The present study may suggest the role and importance of total content of volatile oils in the variation of insect infestation of citrus varieties.

The data are in harmony with the findings obtained by Khalil *et al.* (2001) and Mogahed *et al.* (2013) who mentioned that the infested leaves were lower in volatile oils content than the non-infested leaves.

Phenols content in leaves of some citrus varieties and its relation with CLM infestation

Data in Table 5 show that a relation was found between the susceptibility of tested citrus species and varieties to infestation by CLM and phenols content. The lowest the phenols content the highest the infestation occured and the reverse was true. Sour orange, sweet orange and baladi orange were less susceptible to CLM infestation and their phenols content was relatively high 26.6, 23.2 and 23.2%, respectively.

The findings of the present study may suggest the role and importance of phenols content in the varieties against insect infestation of citrus varieties where highly infested leaves were lower in phenols content.

The present data are in agreement with those of Munakata (1975), who found that after detecting phenol compounds, CLM larvae stop nourishing themselves immediately due to anti-feeding effects. Jhonson *et al.* (2002) added that phenolic compounds were believed to be the most important plants chemical resistance. In addition, Thoison *et al*. (2004) stated that flavonoides showed anti-feeding activity and larval growth inhibitors. Also, Bouzouina *et al.* (2012) reported that polyphenols play a part in the chemical defenses of plants and protect them against herbivorous insects. They added that phenols of orange leaves present toxic and antifeedant activity against CLM larvae.

Total carbohydrates and proteins

Data shown in Table 5 represent the susceptibility of different citrus species and varieties to the infestation by the citrus leafminer. These results show that navel orange was the most susceptible to the infestation by CLM compared with the other varieties (51.3%). According to the degree of infestation, the other tested varieties can be arranged in the following descending arrangement; valencia orange was the more susceptible variety (45.7%), then mandarin (41.7%), sweet orange (36.0%), baladi orange (32.3%) and sour orange (23.3%).

There are highly significants differences between the infestation percentage of CLM on the former three citrus varieties (navel orange, valencia orange and mandarin) and those found on the last three ones (sweet orange, baladi orange and sour orange). These results indicate that the first two orange varieties (navel orange and valencia orange) and mandarin were highly susceptible. In the present work, it can be concluded that a kind of relationship was found between the carbohydrates content (Table 5) of leaves and the infestation (%) of CLM. The results presented in (Table 5) show that the lowest carbohydrates content the highest infestation percentage of CLM, occurs, except in case of sour orange.

Also, the protein content of the tested trees was tested as possible factor related to the CLM infestation percentage. From Table 5, it appears that no obvious correlation relationship was found between protein content and the level of infestation. Statistical analysis revealed no significant differences among total proteins in different varieties excepting in case of both navel orange and valencia orange which significantly differed from the other tested varieties.

pH in leaves of different citrus varieties

Data in Table 5, appears that no relation was found between pH level in leaves of citrus varieties and percentage of infestation with CLM. Statistical analysis showed that no significant differences among levels of pH in leaves of different citrus varieties.

Effect of On-tree Storage Fruits on Percentage of Infestation with CLM in Navel Orange and Mandarin Varieties at Minia El-Kamh District

As seen from Table 6 the fruit storage on tree after the normal time of harvesting caused

Table 6. Effect of fruit storage on trees of navel orange and mandarin varieties on percentage of infestation by *Phyllocnistis citrella* Stainton at different flushes during 2013/2014 and 2014/2015 seasons at Minia El-Kamh District, Sharkia Governorate

Varieties		Navel orange				Mandarin			
		2013/2014		2014/2015		2013/2014		2014/2015	
Treatments	Flushes	Infestation (%)	No. of alive mines	Infestation (%)	No. of alive mines	Infestation (%)	No. of alive mines	Infestation (%)	No. of alive mines
Control	Spring	3.50	25	5.00	36	1.50	11	3.00	22
	Summer	32.00	208	36.00	228	26.50	182	28.66	113
	Autumn	59.66	412	63.50	458	44.00	271	50.33	295
	Average	31.72		34.83		24.00		27.33	
On-tree storage fruits	Spring	14.00	60	15.66	69	12.00	48	14.33	66
	Summer	43.00	330	51.00	361	38.00	240	40.00	315
	Autumn	71.66	488	75.66	515	66.00	453	70.33	478
	Average	42.88		47.44		38.66		41.55	
LSD 5%		3.082		2.653		3.093		3.235	
LSD 1%		4.410		3.794		4.426		4.630	

more susceptibility on the following flushes of year for infestation with citrus leafminer. For example, the percentage of infestation in navel orange tree harvested in normal time (November - December) in spring flushes and trees used for storage fruits (90 – 120 days) until April in the following year raised the infestation to about (14.00 and 15.66%) compared with control (3.50 and 5.00%) in 2013/2014 and 2014/2015 seasons, respectively.

The same trend was noticed in both summer and autumn flushes recording 43.00 and 51.00% in summer flush, 71.66 and 75.66% in autumn flush in the first and second seasons, respectively.

Also, significant differences were shown between flushes in both treatments (control and on-tree storage fruits in both seasons.

Mandarin in this concern appeared the same trend found in navel orange tree, where the percentage of infestation significantly increased by increasing storage period on tree (up to April) of different flushes. Significant differences were detected between flushes of both treatments in 2013/2014 and 2014/2015 seasons.

It can be concluded that storage of the fruits on tree either in navel orange or in mandarin, significantly increased infestation in new leaves allover the year. The increments in percentage of infestation in trees used for fruit storage may be due to that storage causing delays exit of the flushing so that the new flushes friendly insect injury or caused weakness in trees used in the storage of fruits which gives new flushes valid tender of insect injury time for exited insects. The available literature in this concern is very scarce.

Average number of alive mines

As shown in Table 6, number of alive mines show variables either per leaf and or per flush emerged throughout the grown season, which reached to the maximum in infested leaves sprouted in autumn ones, while the minimum values of mines sharply increased in infested leaves of on-tree storage fruits as compared with control trees. For example, average number of mines 25, 208 and 412 in control against 60, 330 and 488 mines in on-tree storage fruits in spring, summer and autumn flushes in navel orange in the first season. The same trend was noticed in the second season.

In mandarin the same trend was noticed where the average number of alive mines clearly increased in on-tree storage as compared with control trees in different flushes.

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تأثير بعض الخصائص الفيزيائية والمحتويات الكيمائية المختلفة لأصناف وأنواع الموالح على الإصابة بصانعة أنفاق أوراق الموالح *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) في مصر

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أُجرى هذا البحث لدراسة علاقة حشرة صانعة أنفاق أوراق الموالح ببعض العوامل التى تؤثر عليها مثل صفات الأوراق (مساحة وعمر الورقة) وقوة دورة النمو بالاضافة إلى تقييم حساسية ستة أصناف من الموالح هى البرتقال أبو سرة والصيفي والبلدي والسكري والنارنج بالإضافة إلى اليوسفي البلدي) وذلك للإصابة بصانعة أنفاق أوراق الموالح، وذلك فى موسمي متتالين (٢٠١٣ و٢٠١٤) في مركز القصاصين، محافظة الإسماعلية، كما تضمنت الدراسة تأثير عملية تخزين الثمار on-tree storage fruits بعد الميعاد العادي لجمع الثمار على هذه الأشجار وذلك في صنفى البرتقال أبو سرة واليوسفي بمنطقة منيا القمح|، محافظة الشرقية فى موسمي متتالين (٢٠١٣ - ٢٠١٤ و٢٠١٤ - ٢٠١٥)، وقد أوضحت النتائج المتحصل عليها ما يلى: تبدأ مظاهر الإصابة بصانعة أنفاق أوراق الموالح في مختلف دورات النمو على الأوراق الصغيرة الحديثة عندما كانت مساحتها ٠.٤ - ٠.٩ ، ١.٨ - ٢.٧ ، ١.١ - ١.٧سم[٢] كمتوسط في أوراق أصناف البرتقال كما كان متوسط المساحة ٠.٣ ، ٠.٩ و ٠.٨سم[٢] في اليوسفي في دورات الربيع والصيف والخريف على التوالى، كما أوضحت نتائج الدراسة أن أوراق أنواع ذ و أصناف الموالح بعمر أطول ثلاثة أسابيع نادراً ما تصاب بحشرة صانعة أنفاق أوراق الموالح والأوراق صغيرة الحجم والتى يترواح عمرها بين ١-٧ أيام هي معظم الأوراق التى تصاب بالحشرة وذلك في مختلف دورات النمو، كما لوحظ وجود علاقة بين قوة دورة النمو (عدد النموات الحديثة/دورة) والنسبة المئوية للإصابة بالحشرة، حيث أوضحت النتائج أن دورة الخريف أشد الدورات إصابة يليها دورة الصيف ثم الربيع حيث تستطيع نموات الربيع الهروب من الإصابة، كما أوضحت النتائج أيضاً أن صنف البرتقال أبو سرة هو أكثر الأصناف المختبرة حساسية للإصابة بالحشرة حيث سجل أعلى إصابة في موسمي الدراسة يليه البرتقال الصيفي واليوسفي والبرتقال السكري والبرتقال البلدي وآخيرا النارنج الأقل إصابة، وأيضا تم دراسة تأثير بعض المكونات الكيميائية في أوراق بعض أصناف الموالح وعلاقتها بنسبة الإصابة بصانعة أنفاق أوراق الموالح مثل الزيوت الطيارة والفينولات والكربوهيدرات الكلية والبروتينات الكلية ودرجة pH، حيث أوضحت النتائج أن الزيوت الطيارة في أوراق الموالح تختلف باختلاف الأصناف ولوحظ أن هناك بعض الأصناف يظهر فيها علاقة بين محتواها من الزيوت الطيارة ونسبة الإصابة بصانعة أنفاق أوراق الموالح وبينما الأصناف الأخرى لم يتضح فيها هذه العلاقة، تلعب الفينولات دوراً هاما في الدفاع وحماية النباتات ضد الحشرات ويتضح هنا دور الفينولات الهام في تقليل الإصابة بصانعة أنفاق أوراق الموالح، أما فيما يخص تأثير الكربوهيدرات على الإصابة بصانعة أنفاق أوراق الموالح في بعض أصناف الموالح فقد شوهد تأثيرها في تقليل الإصابة بصانعة الأنفاق إلى أقل حد، أما بالنسبة لتأثير البروتينات الكلية ودرجة pH في الأوراق على صانعة أنفاق أوراق الموالح لم يُلاحظ علاقة واضحة، أما فيما يتعلق بتأثير تخزين ثمار البرتقال أبو سرة واليوسفي على أشجارها بعد نضجها لمدة ٩٠ – ١٢٠ يوماً أوضحت النتائج في المواسم التالية (تأثير متبقى Residual effect) أن عملية تخزين الثمار على أشجارها تؤدي إلى حدوث زيادة معنوية في نسبة الإصابة بحشرة صانعة أنفاق أوراق الموالح ويرتبط ذلك أيضا بقوة النمو وتوقيت دورة النمو في المواسم التالية للتخزين حيث سُجلت النسبة المئوية للإصابة في الأشجار المخزن عليها الثمار في البرتقال أبوسرة أعلى من الكنترول وكذلك الحال مع اليوسفي المخزن على الأشجار خلال موسمي الدراسة.

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