Kuwait J. Sci. **47 (4)** pp. 49-56, 2020

الصفات الكيميائية الحيوية لحَفّار أنفاق أوراق الحمضيات وبعض متطفلاتها على طول تدرج المناخ المحلي للنباتات الحمضية

[1]محمد دبليو. عباس، [1]أبو بكر م. رازا، [2]أسلي داجر، [1]محمد أرشد، [3]محمد أ. خان، [1]محمد آي. الله، [1]محمد أفضل

[1]قسم الحشرات، جامعة Sargodha، باكستان

[2]قسم البيولوجيا الجزيئية وعلم الوراثة، جامعة Necmettin Erbakan، تركيا

[3]قسم علم الحشرات، جامعة الزراعة، فيصل أباد- باكستان

الملخص

يوضح البحث أن الصفات الورقية والمناخ المحلي في الواقع المتباينة للنبات العائل غالباً ما تؤثر على اختيار العائل وسلوك الحشرات آكلة العشب. كما تشير الدراسة الحالية إلى علاقة الصفات الكيميائية الحيوية للأوراق والاصابة بحَفّار أنفاق أوراق الحمضيات (CLM) *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) على ستة أصناف من الحمضيات: حامض ليمون Meyer (هجين من الليمون ومندرين اليوسفي) وكيناو Kinnow وفيوترل Feutrell (حمضيات الماندرين)، وفيرتشيلد Fairchild (اليوسفي)، سوكاري Succari وموسامبي (سيننسيس *Sinensis*). افترض البحث أيضًا أن ضرر CLM يزداد من الظُّلّة السفلي للنبات إلى الظُّلّة العليا. لاحظ البحث وجود أعلى مستوى للإصابة في الظُّلّة العليا للنباتات الحمضية يتبعها الظُّلّة الوسطى والسفلى في حالة كافة أصناف الحمضيات. تَنوع تلف CLM أيضاً بين أصناف الحمضيات الستة وسجل حامض ليمون ماير المستوى الأعلى بنسبة 24.24% وسجلت أصناف كيناو 21.42% بينما كان موسامبي أقل الأصناف تأثراً بتغذية CLM بنسبة إصابة 9.31%. أشار البحث، أنه من ضمن صفات الأوراق، أظهر النتيروجين ومحتويات الرطوبة ارتباط مهم وايجابي مع الإصابة ب CLM في جميع الأصناف الحمضية. لم يُظِهر إجمالي المعادن في الأوراق الارتباط المهم ($P > 0.05$) ضررًا مع CLM في كافة الأصناف الحمضية. أظهرت البروتينات الخام ارتباطًا مهماً مع CLM فقط في حالة صنف حامض الليمون لماير. تقترح نتائجنا أن مستوى الرطوبة ومحتوى النيتروجين في الأوراق هي عوامل مهمة لتحسين نشاط CLM ويمكن أن يؤثر هذا التفاعل على نمو وانتاجية النباتات الحمضية.

Consequences of leaf biochemical characters for Citrus Leafminer, *Phyllocnistis citrella* stainton (Lepidoptera: Gracillariidae) along the microclimatic gradient of Citrus plants

Muhammad W. Abbas[1], Abu Bakar M. Raza[1], Asli Dageri[2], Muhammad Arshad[1,*], Muhammad A. Khan[3], Muhammad I. Ullah[1], Muhammad Afzal[1]

[1]*Dept. of Entomology, University of Sargodha, 40100, Sargodha, Pakistan*

[2]*Dept. of Molecular Biology and Genetics, Necmettin Erbakan University, 42090, Meram, Konya, Turkey*

[3]*Dept. of Entomology, University of Agriculture Faisalabad, Pakistan*

[*]*Corresponding author: makuaf@gmail.com*

Abstract

The leaf traits and microclimate in different positions of a host plant often influence the host choice and performance of herbivore insects. In the present study, the relation of leaf biochemical characters with the infestation of citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) on six citrus cultivars; Meyer Lime (lemon × mandarin hybrid), Kinnow, and Feutrell's Early (*Citrus mandarins*), Fairchild (*C. tangerines*), Succari and Musambi (*C. sinensis*) was determined. It was also hypothesized that the CLM damage increases from lower to upper canopy of the plant. The highest level of infestation was observed on the upper canopy of citrus plants, followed by a middle and lower canopy in the case of all citrus cultivars. The CLM damage also varied between six citrus cultivars with the highest level of 24.24% for Meyer Lime and 21.42% for Kinnow cultivars and the least affected cultivar by CLM feeding was Musambi with 9.31% infestation. Among the leaf traits, nitrogen and moisture contents showed significant and positive relation with CLM infestation in all citrus cultivars. Total minerals in leaves didn't show the significant ($P > 0.05$) relation with CLM damage in all citrus cultivars. Crude proteins showed significant relation with CLM only in the case of the Meyer Lime cultivar. Our findings suggest that the moisture level and nitrogen contents in the leaves are important factors for enhancing the activity of CLM and this interaction can impact the growth and productivity of citrus plants.

Keywords: Citrus orchard; insect-plant interaction; infestation; *Phyllocnistis citrella*; plant canopy

1. Introduction

The defense mechanisms in plants are important considerations in integrated pest management (IPM) programs for sucking insect pests (Tsai & Wang, 2001). Herbivore pressure leads to the evolution of different defense mechanisms in plants, such as physical or mechanical defenses, like thorns, trichomes (Fürstenberg-Hägg *et al.* 2013), and chemical defenses, like the production of defense proteins and toxins that target the physiological processes in the insect body (Trigo *et al*. 2012).

Additionally, the feeding of herbivorous insects may be affected by the nutritional quality of leaf tissues like nutrients, toughness or defense compounds (Cárdenas *et al*. 2014). The amount of nitrogen in leaves is the main predictor for herbivory levels. For example, a low level of water and nitrogen in leaves may reduce the performance of chewing insects (Loaiza *et al.* 2011). Herbivory increases with leaf nitrogen, in terms of high insect density and survival rates, shorter development time and high fecundity rate (Bentz & Townsend, 2001; Huberty & Denno, 2006). The relationship between insect population and the nutrient quality of plants has raised the interest of ecologists. Insects attack the plants to pursue nutrients for growth and development (Joern *et al.* 2012) and the effects on the plants depend on their defense against insect herbivory. The insect-plant interactions vary depending on the insect species involved and the defensive ability of the attacked plants (Gurevitch *et al*. 2006). The relationship between pest reproductive potential and the nutritional quality of host plants is important for IPM in the modern agro-ecosystems (Zarasvanda *et al.* 2013). Further, leaf quality among the vertical gradient of the plants may affect the herbivory. Previously, researchers have reported the higher performance of herbivory on the upper canopy of the plants due to a higher level of nitrogen in the leaves (Fortin & Mauffette, 2002).

Miners are the endophagous insects and many species have been found to be selective feeders (Trier & Mattson, 1997). Previously, authors have reported that miners prefer tissues that are nutritionally rich (Connor & Taverner, 1997; Trier & Mattson, 1997). Little attention has been paid to citrus leafminer (CLM) *Phyllocnistis citrella* Stainton, (Lepidoptera, Gracillariidae) that is one of the major insect pests of the citrus crop worldwide (Mahmood *et al.* 2014; Arshad *et al.* 2019). CLM larvae make serpentine mines and suck the cell sap from the leaves and mostly attack young leaves (Mafi & Ohbayashi, 2004). About 32.0 to 58.0 percent of leaf damage caused by CLM larvae has been reported in Pakistan (Mustafa *et al*. 2014). It also enhances the citrus canker disease by providing an entry hole to the bacterium *Xanthomonas axonopodis* pv. *citri* (Gottwald *et al.* 1997). The intensity of the citrus canker disease increased with the increase in CLM mines on the leaf surface (Jesus *et al.* 2006).

It has been reported that the population density of CLM on host plants is closely linked to the presence of young flushes (Heppner, 1993). Therefore, it is possible that the female CLM oviposits on young leaves containing the highest nutrients that lead to an increase in its population. No comprehensive study is available about the microclimatic gradient of plants and CLM infestation; therefore, a study was proposed to determine the level of CLM damage (leaf damage in terms of a number of mines on leaf surface) on six citrus cultivars. We compared the leaf damage between different microclimatic gradients (upper, middle and lower canopy) of mature citrus plants. The relation of CLM damage was also determined with leaf traits such as nitrogen level, moisture content, total minerals, and crude protein.

2. Materials and methods

2.1. Citrus plants

This study was conducted in a citrus field located at the Post Graduate Agricultural Research Station (31°23’40.9”N 73°01’08.9”E), University of Agriculture, Faisalabad, Pakistan. Based on economic importance, six citrus cultivars; Meyer Lime (lemon × mandarin hybrid), Kinnow, and Feutrell’s Early (*Citrus mandarins*), Fairchild (*C. tangerines*), Succari and Musambi (*C. sinensis*) were selected in the present study. About a 1-acre plot for each cultivar was selected and each plot was further divided into 3 blocks. The plants were 5-7 years old. No insecticides were applied during the experiment and the plants were irrigated at a weekly interval. The experiment was conducted during the summer flush (April-May 2015). The average rate of climatic conditions in the study area was; 32.5 ºC temperature, 23% relative humidity (R.H.), 9.95 kmph wind speed and 5.78 mm rainfall (Pakistan Meteorological Department, 2015).

2.2. CLM infestation

Ten plants from each block were randomly selected and each plant was considered a replicate. The plant’s canopy represented a microclimatic gradient of plants for variation in CLM damage. To record the CLM population, the plant was divided into 3 canopy levels: upper, middle and lower. Four shoots (one from each quadrant) from each canopy level were selected randomly and the total number of leaves and mined leaves were recorded. The total number of mines including all larval stages, pupae, and empty mines was considered as infested leaves. Data were recorded at weekly intervals during the month of May and April. The percent CLM infestation was recorded by the following formula (Mustafa *et al.* 2014):

$$CLM\ infestation = \frac{No. of\ infested\ leaves}{Total\ no. of\ leaves} \times 100$$

2.3. Leaf traits

Healthy and young leaves with no herbivorous damage were randomly collected from each canopy level of selected plants from each cultivar. Leaves in plastic sheets were put into ice boxes and transferred to Horticultural Pomology Laboratory. The leaves were washed twice with distilled water and kept at room temperature for six hours. The leaves were then oven dried at 70 ºC for 12 hours. The oven dried material was ground into powder using an electrical blender and kept in dried polythene bags for further use. The recommended methods of the Association of Official Analytical Chemists (AOAC) were followed in determining the leaf traits. Ten samples for each cultivar were prepared to determine the moisture level, amount of nitrogen, total minerals and crude protein. The moisture contents in the leaves were determined by the following formula:

$$\text{Moisture content (\%)} = \frac{A - B}{A} \times 100$$

Where A = weight of fresh leaves and B = weight of dried leaves

About 2 g leaf powder of each cultivar was placed in a boron-free fused silica crucible. The samples were burnt to ashes in a muffle furnace at 600 °C for 5 hours. The dried material after combustion was weighed. The samples were again put in a silica crucible at the same conditions until converted into white ashes at a constant weight. Total minerals were calculated using the formula suggested by Ranganna (1977);

$$\text{Total mineral (\%)} = \frac{a}{B} \times 100$$

Where a = weight of the ash, B = weight of dried leaves

The quantity of nitrogen in the leaves was determined by the Kjeldahl method (Winkleman *et al.* 1986). The crude protein was determined by the method (nitrogen percent × 6.25) suggested by Winkleman *et al.* (1986).

2.4. Data analysis

Data for the percent CLM infestation were analyzed using analysis of variance (ANOVA) to check the significance for microclimatic conditions of citrus plants. Data for leaf traits were also analyzed to check the significance of cultivars. Means were separated by Tukey's HSD all-pairwise comparison test. Correlation was performed to check the relation of each selected leaf trait with percent infestation of CLM on six citrus cultivars. All the analyses were performed by Minitab 17.0 software.

3. Results

CLM infestation was found significantly different on plant canopy levels (F = 129.0 for upper, F = 82.7 for middle and F = 62.2 for lower canopy at $P < 0.001$) among six citrus cultivars. Across three canopy levels, the infestation was significantly ($P < 0.001$) higher on the upper canopy of each citrus cultivar. CLM infestation was found higher on the upper canopy of Kinnow (31.8%) followed by Meyer Lime (29.3%) then other cultivars.

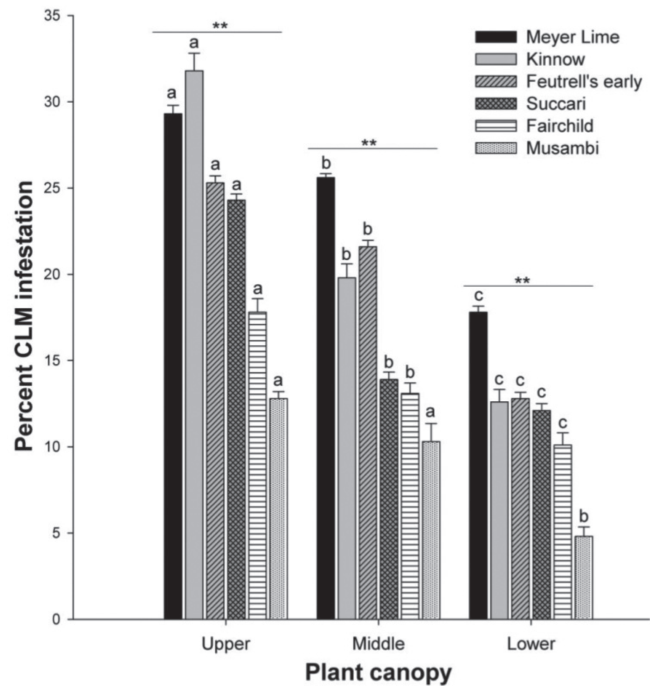

Fig. 1. Percent CLM infestation (means±SE) on different plant positions (canopy levels) in six citrus cultivars means sharing similar letters for each cultivar across plant canopy is not significantly different at $P > 0.05$, ** shows the significance ($P < 0.05$) for each plant canopy across different citrus cultivars.

Similar results for higher infestation were observed on these cultivars at the middle and lower canopy levels. However, the least affected cultivar was Musambi with 12.8% infestation on upper, 10.3% on middle and 4.80% on lower canopy (Figure 1).

Overall, the average rate of infestation was found higher on Meyer Lime (24.4%) and Kinnow (21.4%) and lower was observed on Musambi (9.31%) cultivar. A significant ($P < 0.001$) difference was found in all selected leaf traits of six citrus cultivars. Moisture contents in leaves of Meyer Lime (69.3%) and Feutrell's Early (68.5%) was found to be higher than other cultivars. The total mineral percentage was greater (10.50% and 10.47%) in Kinnow and Meyer Lime, respectively. The percentage of nitrogen (2.56%) in Meyer Lime and crude protein (15.2%) in Fairchild was found higher as compared to other cultivars (Table 1).

Table 1. Mean (±SE) values of CLM infestation and leaf traits of six citrus cultivars

Cultivars	Leaf biochemical characters (%)				CLM infestation (%)
	Moisture content	Total Mineral	Nitrogen	Crude protein	
Meyer Lime	69.3±0.227a	10.47±0.065ab	2.56±0.033a	13.78±0.117d	24.24±0.224a
Kinnow	69.1±0.191a	10.50±0.067ab	2.32±0.043b	14.66±0.113b	21.42±0.361b
Feutrell's early	68.5±0.074ab	10.39±0.035ab	2.23±0.034b	14.09±0.089c	19.87±0.228b
Succari	67.5±0.132b	10.28±0.017bc	2.24±0.034b	14.72±0.023b	16.79±0.164c
Fairchild	62.9±0.203c	10.14±0.024c	2.19±0.036bc	15.22±0.057a	13.65±0.322d
Musambi	61.8±0.234c	10.60±0.034a	2.08±0.039c	13.27±0.029e	09.31±0.351e
F value	*174.0*	*7.77*	*22.7*	*89.5*	*212.0*
P value	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*

P < 0.001 shows the significance, means sharing similar letters within columns are not significantly different at *P > 0.05*

Table 2. Correlation of leaf biochemical characters with CLM feeding on six citrus cultivars

	Leaf biochemical characters (%)							
	Moisture content		Total minerlas		Nitrogen		Crude protein	
Cultivars	r-value	*P-value*	r-value	*P-value*	r-value	*P-value*	r-value	*P-value*
Meyer Lime	0.937	*0.0001*	-0.180	*0.6187*	0.898	*0.0004*	0.693	*0.0262*
Kinnow	0.884	*0.0007*	-0.227	*0.5288*	0.938	*0.0001*	-0.262	*0.4648*
Feutrell's early	0.850	*0.0018*	-0.081	*0.8235*	0.892	*0.0005*	-0.510	*0.8895*
Succari	0.835	*0.0026*	0.304	*0.3935*	0.885	*0.0007*	0.019	*0.9569*
Fairchild	0.888	*0.0006*	-0.132	*0.7166*	0.943	*0.0000*	0.185	*0.6094*
Musambi	0.801	*0.0053*	-0.3284	*0.3541*	0.859	*0.0015*	0.369	*0.2931*

P < 0.001 shows the significance

A positive and significant *(P < 0.05)* relation of moisture contents with CLM infestation was found in all citrus cultivars. No significant (*P > 0.05*) relation of total minerals in leaves with CLM infestation was found in all citrus cultivars. Nitrogen content in the leaves showed significantly (*P < 0.05*) positive relation with CLM infestation in the case of all citrus cultivars. Similar to total minerals, the crude protein didn't show a significant relation with CLM activity in the case of all cultivars, except Meyer Lime, in which the relation was positively significant (Table 2).

4. Discussion

CLM is one of the most important insect pests of Pakistan's citrus nursery stock as well as mature orchards. Among the pattern of CLM damage to microclimates of citrus plants, the infestation was higher on upper canopy than middle and lower. The branches from all four quadrants in the case of each canopy level were selected. A possible explanation for selection may be the differential exposure of flushes at canopy level to the sunlight intensity. Higher infestation of CLM on the east and upper part of the plants' canopy might be due to greater sunlight. Sétamou *et al.* (2016) reported the higher density of citrus psylla *Diaphorina citri* Kuwayama (Liviidae: Hemiptera) on the south oriented canopies of trees than the north oriented canopies due to a slight increase in the temperature. It could be possible that flush growth patterns react to the changing conditions of sunlight. In the selected locality, the east and upper sides of the north-to-south oriented rows were exposed to higher intensity of sunlight all day. This difference in sunlight exposure may lead to more flush growth on the east and upper side of the trees resulting in higher infestation of CLM because it prefers the young leaves to the older (Richardson *et al.* 2011). Leaves from the upper canopy showed higher palatability for CLM damage. Rowe and Potter (1996) also reported that the adult Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) feed more heavily in the upper canopy of linden (*Tilia cordata* L.) plants than in the lower parts. The difference in herbivory across the canopy level varies in direction and magnitude depending on tree species. This complicates overviews about the patterns of herbivory and the response of herbivore insects to different microclimates on the same host plant (Stiegel *et al.* 2017).

Among the citrus cultivars tested against CLM, the infestation rate was higher on Meyer Lime and Kinnow than other cultivars. A possible explanation of variation in damage level may be due to the difference in leaf thickness and certain anatomical modifications, various metabolic changes or due to different chemical compounds in citrus cultivars that act as repellents or attractants for CLM (Smith & Boyko, 2007). Variation in a number of young flushes is another factor influencing the host preference of CLM. Sétamou *et al.* (2016) reported that lemon had more flushes per year than the other Rutaceae plants; thus, chances of *D. citri* oviposition increase. In our study, the Musambi was the cultivar least affected by CLM, and the results are in accordance with Musatafa *et al.* (2014), who reported the least activity of CLM on Musambi, due to the very compact surface of the leaves.

Limited research is available about the relation of leaf traits to CLM infestation. We studied the selected leaf traits such as nitrogen content, moisture content, total minerals and crude proteins in citrus plants and their relation with CLM activity. Our findings showed a strong and positive relation of leaf moisture content and nitrogen amount with CLM damage in almost all citrus cultivars. However, the total minerals and crude protein showed no relation to CLM damage. The amount of nitrogen was higher in the Meyer Lime cultivar that led to higher infestation of CLM. Previously, Bi *et al.* (2001) reported the highest number of *Bemisia argentifolii* on the leaves that had the highest nitrogen level. In this study, the lowest CLM performance was observed on the Musambi cultivar, where the number of mines on the leaf surface were the lowest. In the chemical analysis, we found that this cultivar possesses the lowest amount of nitrogen and crude protein. This low nitrogen level in the leaves can be the cause of lower attraction of CLM. A higher level of nitrogen content in the leaves is an important factor for the growth and development of phloem-feeding insects (Douglas, 2006).

Nutrition has a significant impact on both the fecundity and fertility rate of insects. Nitrogen is one of the prominent elements that plants need in large quantities and a low amount of it negatively affects the phytophagous insects feeding on those plants (Hartley & Jones, 1997). Retarded insect development with a significantly lower number of pupae was seen in the insects feeding on the plants with limited nitrogen contents (Larbat *et al.* 2014). Besides having major effects on fecundity and fertility, nitrogen has an impact on the reproductive strategies of insects by influencing life span, host preferences, etc. (Leather, 2018). The reproductive performance of leafhoppers, *Dicranotropis hamata* Boheman*, Ebmana sulphurella* (Zetterstedt) and *Euscelis incisus* (Kirschbaum) reared on the grass, *Holcus lanatus* L.with high nitrogen levels was found to be remarkably higher than that of insects fed on plants with a limited nitrogen level (Prestidge, 1982). Similarly, *Pieris rapae* (L.) (Pieridae: Lepidoptera) feeding on nitrogen-rich collard (*Brassica oleracea*) leaves performed better than the insects feeding on the leaves with a poor nitrogen level (Loader & Damman, 1991). In the present study, nitrogen demonstrated a positive and strong relationship with CLM infestation, implying that citrus leaves with the high nitrogen levels positively affect the reproduction and development of the CLM.

Similarly, the leaf moisture content also influenced the CLM damage in all citrus cultivars. The plants with high water and nitrogen content positively affected the growth rate of *Papilio polytes* L. (Shobana *et al.* 2010). Zia *et al.* (2011) also noticed the significantly positive relation of leaf moisture contents with the whitefly population. Roslin *et al.* (2006) reported that leaf size and water content affected the performance of the leaf-miner, *Tischeria ekebladella* (Bjerkander). Our findings are well supported by Mustafa *et al.* (2014) who reported the positive relation of leaf moisture content to CLM larval population. Thus, an increase in herbivory damage is not only due to the increase in the food availability in terms of more host plants, but it could also be due to host quality (Maoela *et al.* 2016). In contrast, in the leaf traits discussed above, there were two other traits, crude protein and minerals, that showed no significant relationship with CLM damage. Minerals other than N are perhaps the least-studied elements in the matter of insect nutrition, while some studies reported that insects may also respond to these elements (Linhart *et al.* 2001). Mustafa *et al.* (2014) reported that the association of CLM damage varies with leaf minerals Ca, Mg and K that mainly depend on the age of citrus plants.

5. Conclusion

In conclusion, our study highlights the importance of leaf nutritional quality, especially moisture content and nitrogen level, in the infestation of CLM on mature citrus plants. CLM damage was higher on the upper canopy of citrus plants. The citrus cultivar Meyer Lime seems to be the nutritionally superior host for CLM, followed by Kinnow and Feutrell's Early, whereas Musambi is the most inferior host plant. It was likely to be expected that the higher CLM damage was due to favourable leaf traits (high nitrogen and moisture levels) of citrus plants compared to the understory.

ACKNOWLEDGEMENTS

The data used in this manuscript is the part of Ph.D. research work of first author to fulfill the prerequisite for the award of Ph.D. degree. The authors are thankful to the Department of Pomology, University of Agriculture Faisalabad for providing facilities for the experiment.

Running title

Role of leaf biochemical characters in citrus leafminer infestation

Conflict of interest

The authors declare that they have no conflict of interest.

References

Arshad, M., Ullah, M.I., Afzal, M., Khalid, S., Raza, A.B.M. & Iftikhar, Y. (2019). Evaluation of plant extracts for the management of citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Kuwait Journal of Science **46**(1): 58-67.

Bentz, J. & Townsend, A.M. (2001). Leaf element content and utilization of maple and elm as hosts by the potato leafhopper (Homoptera: Cicadellidae). Environmental Entomology **30**: 533-539.

Bi, J.L., Ballmer, G.R., Hendrix, D.L., Henneberry, T.J. & Toscano, N.C. (2001). Effect of cotton nitrogen fertilization on *Bemisia argentifolii* populations and honeydew production. Entomologia Experimentalis et Applicata **99**: 25-36.

Cárdenas, R.E., Valencia, R., Kraft, N.J.B., Argoti, A. & Dangles, O. (2014). Plant traits predict inter- and intraspecific variation in susceptibility to herbivory in a hyperdiverse Neotropical rain forest tree community. Journal of Ecology **102**: 939-952.

Connor, E.F. & Taverner, M.P. (1997). The evolution and the adaptive significance of the leaf-mining habit. Oikos **79**: 6-25.

Douglas, A.E. (2006). Phloem-sap feeding by animals: problems and solutions. Journal of Experimental Biology **57**: 747-754.

Fortin, M. & Mauffette, Y. (2002). The suitability of leaves from different canopy layers for a generalist herbivore (Lepidoptera: Lasiocampidae) foraging on sugar maple. Canadian Journal of Forest Research **32**: 379-389.

Fürstenberg-Hägg, J., Zagrobelny, M. & Bak, S. (2013). Plant defense against insect herbivores. International Journal of Molecular Sciences, **14**: 10242-10297.

Gottwald, T.R., Graham, J.H. & Schubert, T.S. (1997). An epidemiological analysis of the spread of citrus canker in urban Miami, Florida, and synergistic interaction with the Asian citrus leafminer. Fruits, **52**: 383-390.

Gurevitch, J., Scheiner, S.M. & Fox, G.A. (2006). The Ecology of Plants. (2nd ed.) Sunderland, MA: Sinauer Associates, Inc.

Hartley, S.E. & Jones, C.G. (1997). Plant chemistry and herbivory, or why is the world green? In: Plant Ecology, edited by M. Crawley, Blackwell Science, Oxford, London. Pp. 284-324.

Heppner, J.B. (1993). Citrus leafminer, *Phyllocnistis citrella*, in Florida. Tropical Lepidoptera Research **4**: 49-64.

Huberty, A.F. & Denno, R.F. (2006). Consequences of nitrogen and phosphorus limitation for the performance of two plant hoppers with divergent life-history strategies. Oecologia **149**: 444-455.

Jesus, Jr.W.C., Belasque, Jr.J., Amorim, L., Christiano, R.S.C., Parra, J.R.P. & Filho, A.B. (2006). Injuries caused by citrus leafminer (*Phyllocnistis citrella*) exacerbate citrus canker (*Xanthomonas axonopodis* pv. *citri*) infections. Fitopatologia Brasileira, **31**: 277-283.

Joern, A., Provin, T. & Behmer, S.T. (2012). Not just the usual suspects: insect herbivore populations and communities are associated with multiple plant nutrients. Ecology, **93**: 1002-1015.

Larbat, R., Paris, C., Le Bot, J. & Adamowicz, S. (2014). Phenolic characterization and variability in leaves, stems and roots of Micro-Tom and patio tomatoes, in response to nitrogen limitation. Plant Science **224**: 62-73.

Leather, S.R. (2018). Factors affecting fecundity, fertility, oviposition, and larviposition in insects, In: Insect Reproduction, CRC Pres, Boca Raton. Pp. 143-174.

Linhart, Y.B., Mooney, K.A. Snyder, M.A. & Swoboda-Colberg, N. (2001). Phloem chemistry: effects of genotype and environment and implications for nutritional ecology. International Journal of Plant Science, **162**: 1009-1016.

Loader, C. & Damman, H. (1991). Nitrogen content of food plants and vulnerability of *Pierisrapae* to natural enemies. Ecology, **72**: 1586-1590.

Loaiza, V., Jonas, J.L. & Joern, A. (2011). Grasshoppers (Orthoptera: Acrididae) select vegetation patches in local-scale responses to foliar nitrogen but not phosphorus in native grassland. Insect Science, **18**: 533-540.

Mafi, S.A. & Ohbayashi, N. (2004). Seasonal prevalence of the citrus leaf miner *Phyllocnistis citrella* Stainton and its parasitoids in controlled and uncontrolled citrus groves in Ehime Prefecture, Japan. Applied Entomology and Zoology, **39**: 597-601.

Mahmood, R., Rehman, A. & Ahmad, M. (2014). Prospects of biological control of citrus insect pests in Pakistan. Journal of Agricultural Research, **52**: 229-244.

Maoela, M.A., Roets, F., Shayne, J.M. & Esler, K.J. (2016). Restoration of invaded cape floristic region riparian systems leads to a recovery in foliage-active arthropod alpha- and beta-diversity. Journal of Insect Conservation, **20**: 85-97.

Mustafa, I., Aslam, M., Arshad, M., Muhammad, S., Ullah, M.I., Mustaqeem, M., Bokhari, S.A., Asif, S., Khan, M.R., Waqas, A. & Ahmed, H. (2014). Association of citrus leaf miner *Phyllocnistis citrella* (Lepidoptera: Gracillariidae: Phyllocnistinae) with leaf biochemical factors (Ca+ 2 K+ and Mg+ 2) in Kinnow leaves of district Sargodha Punjab Pakistan. Pakistan Journal of Zoology, **46**: 953-958.

Prestidge, R.A. (1982). Instar duration, adult consumption, oviposition and nitrogen utilization efficiencies of leafhoppers feeding on different quality food (Auchenorrhyncha: Homoptera). Ecological Entomology, **7**: 91-101.

Ranganna, S. (1977). Manual of analysis of fruit and vegetable products. Center for Food Technology Research Institute Mysore Tata McGraw Hill Publ. Co. Ltd., New Delhi.

Richardson, M.L., Westbrook, C.J., Hall, D.G., Stover, E., Duan, Y.P. & Lee, R.F. (2011). Abundance of citrus leafminer larvae on citrus and citrus-related germplasm. HortScience, **46**: 1260-1264.

Roslin, T., Gripenberg, S., Salminen, J.P., Karonen, M., B. O'Hara, R., Pihlaja, K. and Pulkkinen, P. (2006). Seeing the trees for the leaves–oaks as mosaics for a host-specific moth. Oikos, **113**(1): 106-120.

Rowe, W.J. & Potter, D.A. (1996). Vertical stratification of feeding by Japanese beetles within linden tree canopies: selective foraging or height per se? Oecologia, **108**: 459-466.

Sétamou, M., Simpson, C.R., Alabi, O.J., Nelson, S.D., Telagamsetty, S. & Jifon, J.L. (2016). Quality matters: Influences of citrus flush physicochemical characteristics on population dynamics of the Asian citrus psyllid (Hemiptera: Liviidae). PlosOne **11**: e0168997.

Shobana, K., Murugan, K. & Kumar, A.N. (2010). Influence of host plants on feeding, growth and reproduction of *Papilio polytes* (The common mormon). Journal of Insect Physiology, **56**: 1065-1070.

Smith, C.M. & Boyko, E.V. (2007). The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomologia Experimentalis et Applicata, **122**: 1-16.

Stiegel, S., Entling, M.H. & Mantilla-Contreras, J. (2017). Reading the leaves' palm: leaf traits and herbivory along the microclimatic gradient of forest layers. PlosOne **12**: e0169741

Trier, T.M. & Mattson, W.J. (1997). Needle mining by the spruce budworm provides sustenance in the midst of privation. Oikos **79**: 241-246.

Trigo, J.R., Pareja, M. & Massuda, K.F. (2012). O papel das substâncias químicasnas interações entre plantas e insetos herbívoros, In Ecologia das interações planta-animais: uma abordagem ecológico-evolutiva, edited by K. Del-Claro, and H. M. Torezan-Silingardi, Pp.69–88. Technical Books, Rio de Janeiro.

Tsai, J.H. & Wang, J.J. (2001). Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera: Aphididae). Environmental Entomology, **30**: 44-50.

Winkleman, G.R., Amin, R., Rice, W.A. & Tahir, M.B. (1986). Methods: Manual Soils Laboratory, Barani Agriculture Research and Development Project, Pp.30-33. PARC, Islamabad (Pakistan).

Zarasvanda, A.A., Allahyarib, H. & Fattah-Hosseini, S. (2013). Effect of nitrogen fertilization on biology, life table parameters and population abundance of greenbug; *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). Archives of Phytopathology and Plant Protection, **46**: 882–889.

Zia, K., Ashfaq, M., Arif, M.J. & Sahi, S.T. (2011). Effect of physico-morphic characters on population of whitefly *Bemisia tabaci* in transgenic cotton. Pakistan Journal of Agricultural Sciences, **48**: 63-69.

Submitted : 23/11/2019

Revised : 12/02/2020

Accepted : 03/03/2020