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Effect of Different Nutritional Conditions on The Activity of Citrus Leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) on Citrus Trees in Northern Iran Orchards

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Abstract

Citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is one of the most important pests of citrus in nurseries and young orchards of the world and Iran. In this study, two separate experiments, the effects of nutrients-nitrogen, phosphorus, potassium, and calcium compounds-on the activity of citrus leaf miner moths were investigated during the 2018–2019 and 2019–2020 growing seasons in Mazandaran province, Iran. The experimental design was randomized complete block design with 4 and 6 treatments, and 4 replications that was carried out in Bessat citrus orchard in the city of Sari. The results of the first experiment showed that in the first year, effect of different treatments was signified in the number of leaf buds, total leaves, and uninfested leaves and the highest numbers were related to soil application and foliar spraying with averages of 6.24, 5.79 and 5.77, respectively. The results obtained in the second year also showed that effect of different treatments on the number of branches was not significant. However, the highest number of leaf buds, total leaves and uninfested leaves were obtained by soil application + foliar spraying with averages of 6.12, 6.01, and 5.85, respectively. In addition, the results of the second experiment showed that in the first year, the highest number of leaf buds, the total leaves and uninfested leaves with averages of 2.52, 12.59, and 12.34, respectively, were observed in calcium nitrate treatments. In the second year, the results showed that the highest values of the growth traits were obtained in calcium nitrate treatment with averages of 19.51, 96.14, and 94.29, respectively.

1. Introduction

Citrus is one of the most important horticultural crops in the world, belonging to the genus *Citrus*, family Rutaceae, and subfamily Aurantioidea, and is one of the most important subtropical fruits (Fifaei and Ebadi, 2019). Iran ranks tenth in the world with a production of nearly 4.2 million tons of citrus fruits in 2023 (FAO, 2024). In 2024, the production of citrus fruits in Iran was more than 5.97 million tons, and among citrus producing provinces, Mazandaran province (northern Iran) was the largest producer of these products in Iran with a production of more than 3.11 million tons of citrus fruits (Anonymous, 2024). Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is one of the major pests of citrus trees in most parts of the world including Africa, Australia, Middle East, Caribbean Islands, Central, North and South America (Damavandian and Kiaeian Moosavi, 2014; Diez et al., 2006; Legaspi et al., 2001). Since 1994, this pest appeared in the citrus orchards of Mazandaran province (northern Iran), and in less than a year, it spread to all citrus growing regions in the north of Iran. This situation has occurred not only in Iran but also in the countries of the Near East and most citrus orchards in the world. The larvae of CLM mine immature foliage and leaf mining causes severe curling of the leaves and leaf chlorosis, necrosis and leaf drop, which ultimately results in a reduction in photosynthetic capacity of citrus trees (Amiri-Besheli, 2008; Heppner and Fasulo, 2016; Sarada et al., 2014). These serpentine mines will also increase the sensitivity of the leaves to plant pathogens such as *Xanthomonas axopodis* pv. *citri*, causing citrus bacterial canker (Das, 2003; Gottwald et al., 1997; Jesus Junior et al., 2006). Severe infestations can result in a significant reduction in fruit production (Pena et al., 2000). This pest mostly damages the young citrus foliage on the nursery trees and prevents the development of young leaves and sometimes results in their fall (Diez et al., 2006). Trees five years of age or less are especially to CLM damage. The presence of 2 or more mines per leaf can severely damage the young trees, and may result in delayed maturity of 1 to 2 years (Lapointe et al., 2014; Sarada et al., 2014). A wide range of synthetic pesticides have been used to control CLM (Beattie et al., 1995). On the one hand, hiding CLM larvae in the mines and not exposing them to the pesticides has led to the continuous and long-term use of these chemicals, unsuccessful control of CLM and the outbreak of other pests (Damavandian and Kiaeian Moosavi, 2014). On the other hand, environmental pollution caused by the use of pesticides has resulted in the interference in biological control by the natural enemies of citrus pests, which play an important role to control of this pest (Damavandian, 2007). Therefore, the use of these synthetic insecticides should be replaced by safe and low-risk control strategies in order to protect active natural enemies and provide effective control against CLM. Patsias (1996) mentioned cultural practices as a complementary method to control CLM. Based on the principles of pest control, the use of suitable cultural practices is one of the effective strategies to prevent or reduce the damage caused by pests (Abbas and Fares, 2009; Belasque et al., 2005). Proper maintenance of soil fertility and attention to plant nutritional requirements is at the heart of an effective IPM or Plant Health Care program. Fertilizing trees with appropriate fertilizers at the proper time can play a valuable role in reducing or avoiding damage caused by pests (Boman and Obreza, 2002; Futch et al., 2009). Mahmoodi and Alavi (2005) stated that foliar application of potassium nitrate, potassium sulphate, and nitrogen reduced the damage percentage of CLM larvae on the trees treated.

In Mazandaran province (northern Iran), the CLM strats acting since mid June with the increase in temperature, and damages the young summer flushes on citrus trees, while in early spring, despite the flushing of young foliages, it is practically inactive due to unsuitable temperature (Jafarzadeh, 2000). Considering the importance of citrus trees and the impact of the CLM damage on its growth and fruit yield, and considering moderate efficacy of only a few common insecticides in the region (imidacloprid and abamectin) and the repeated sprayings and the emergence possibility of resistance in this pest. This study aimed to investigate the effect of proper nutritional management on activity of CLM on the citrus trees, as a result of a proper feeding plan, maximum flushing of young leaves occurs in the spring when CLM are not able to damage them.

2. Materials and Methods

This study was designed and conducted in two separate experiments:

2.1. Effect of nitrogen, phosphorus, and potassium fertilizers on CLM activity

This experiment was carried out in the Besat 1 citrus orchard (affiliated with Fajr Agriculture and Horticulture Company) located in Sari City, Mazandaran province, Iran, for 2 consecutive years, 2018 and 2019. The study was performed as a randomized complete block design with four treatments in four replications. Each replication included eight trees in a row. Replications were spaced two trees apart. The trees in this orchard were 3-year-old pre-ripe Japanese mandarin trees (*Citrus unshiu* cv. *miyagawa*) and 1.5 m in height with 5 m spacing between and within planting lines. The studied treatments were explained below:

1- The control (without fertilization),

2- Optimal use of nitrogen, phosphorus, and potassium nutrients by soil application at a rate of 140, 100, and 200 g tree^{-1}, respectively,

3- The use of 20:20:20 (N: P: K) fertilizer by foliar spraying at a concentration of 1.5 L 1000 L^{-1},

4- The application of 20:20:20 (N: P: K) fertilizer by soil application (150 g tree^{-1}) and foliar spraying at a concentration of 1.5 L 1000 L^{-1}.

Before applications soil samples were taken from 0-30 and 30-60 cm depths of the soil of the desired orchard to analyze its physical and chemical attributes. Based on the results of the soil analysis, the required fertilizers (NPK) were used at the appropriate and recommended time.

In fertilizing by soil application for the first time, after weighing and mixing, the fertilizers were poured inside the furrow with a 10 cm depth created in surrounding the trunk till the external end of canopy shade at the second week of March 2018 and 2019. Next time, only nitrogen fertilizer (ammonium sulphate) was used in the recommended amount in the same method at an interval of one and two months after the first time. In foliar spraying method, 20:20:20 fertilizer (Omex Bio 20; produced by Omex Co., UK) was selected and sprayed at a concentration of 1.5 L 1000 L^{-1} of water using a 20-liter back pump sprayer three times at a 10-day interval from May 5 to 25 after the beginning of leaf flushing in spring. In soil application + foliar spraying treatment, 20:20:20 fertilizer was used in a combination of soil application and foliar spraying methods three times. For sampling, in the first year, two branches in length of 20 cm were randomly selected on the trees, and in the second year, four branches in length of 20 cm were selected from the main directions (north, east, south, and west). The number of new branches, the number of leaf buds and leaves that emerged on marked branches were counted and recorded every two weeks. In total, 96 branches on treated trees were counted and examined over two years. Sampling started in the middle of May and continued until the end of October.

2.2. Effect of calcium and potassium compounds on CLM activity

This survey was carried out in the Besat 1 Citrus orchard (affiliated with Fajr Agriculture and Horticulture Company) in Sari city, Mazandaran province, Iran, for two consecutive years, 2019 and 2020. This orchard consisted of 3-year-old pre-ripe Japanese mandarin trees (*Citrus unshiu* cv. *miyagawa*). First, samples were taken from the soil, and then the amount of micronutrients required for treatments was determined based on the soil analysis results. This experiment was conducted as a randomized complete block design with six treatments in eight replications. Each tree presented as a replication, and replications for each treatment (i.e., four trees in total) were located in two adjacent rows. The treatments included:
1- Foliar spraying of calcium chloride at a concentration of 5 L 1000 L^{-1} of water three times,
2- Foliar spraying of calcium nitrate at a concentration of 6.6 L 1000 L^{-1} of water three times,
3- Foliar spraying of potassium nitrate at a concentration of 6 L 1000 L^{-1} of water three times,
4- Foliar spraying of potassium chloride at a concentration of 3.5 L 1000 L^{-1} of water three times,
5- Foliar spraying of potassium sulphate at a concentration of 2.4 L 1000 L^{-1} of water three times,
6) Control.

No application of fertilizer was done for the control treatment. The first application of fertilizers was done at the beginning of leaf flushing in spring. The second and third application was done at a 7-day interval with the first and second applications, respectively.

With the beginning of the spring, four branches on each tree in different directions were randomly selected and numbered. Sampling started when new buds appeared and continued until early October at an interval of every two weeks. The number of buds, the total number of leaves, and the number of healthy and infested leaves on four branches (A total of 96 branches) were counted and recorded on each time. In addition, to prevent CLM damage, all trees studied were treated with imidacloprid (Confidor® 35%SC, Ariashimi Co., Iran) by pouring the solution into the soil surrounding of the tree trunk.

2.3. Statistical analyses

Before data analysis, Shapiro–Wilk and Levene's tests were conducted to determine normality and homogeneity of variance, respectively. If data did not meet these assumptions, square-root transformation was applied to normalize and standardize, then was analyzed through one-way analysis of variance (ANOVA) using SAS software (SAS Institute, 2017). Means were separated by using Duncan's Multiple range test at $p = 0.05$.

3. Results and Discussion

3.1. Effect of nitrogen, phosphorus, and potassium fertilizers on CLM activity in 2018

The results of the analysis of variance showed that there was a significant difference among the treatments in the number of leaf buds, the total number of leaves appearing on each seedling, and the number of uninfested leaves, while the difference in the number of branches was not significant (Table 1). As it is clear from Table 1, although the highest number of leaf buds was related to the soil application of fertilizers, there was no significant difference between this method and soil application+foliar spraying. On the other hand, the lowest number of leaf buds was counted for the control, which was significantly different from other treatments (Table 1). According to the results of the mean comparison, foliar spraying treatment followed by soil application + foliar spraying produced the highest total number of leaves and the number of uninfested leaves, while the control yielded the lowest values in these characteristics (Table 1). Data in Table 2 clearly indicated that the highest number of leaf buds, total number of leaves, and uninfested leaves were significantly produced in the first three months of sampling that is when the pest was not present.

3.2. Effect of nitrogen, phosphorus, and potassium fertilizers on CLM activity in 2019

The results in the second year indicated that the seedlings treated by soil application + foliar spraying of fertilizer significantly produced the highest number of leaf buds, uninfested leaves, and

Table 1. Mean comparison of number of branch, leaf buds and uninfested leaves and total number of leaves on trees treated by macronutrients and control in the first year (2018).

Treatments	Mean ± SE†			
	No. branch	No. leaf buds	Total No. leaves	No. uninfested leaves
Soil application	2.49±0.01 a	6.24±0.01 a	4.02±0.01 c	4.00±0.02 c
Foliar spraying	2.59±0.01 a	5.54±0.01 b	5.79±0.02 a	5.77±0.02 a
Soil appl.+foliar spray.	2.54±0.03 a	5.99±0.02 a	5.31±0.04 b	5.30±0.04 b
Control	2.52±0.02 a	5.01±0.02 c	3.48±0.05 d	3.41±0.06 d
F value	0.11^{ns}	8.60**	14.17**	15.27**

†Different letters in each column indicate statistically significant differences between treatments.
(ns)= non-significant difference between the treatments at p>0.05; (**)= significant difference between the treatments at p<0.01.

Table 2. Mean comparison of number of branch, leaf buds and uninfested leaves and total number of leaves on the trees treated by macronutrients before and after emerging of the pest in the first year (2018).

Period*	Treatments	Mean ± SE†			
		No. branch	No. leaf buds	Total No. leaves	No. uninfested leaves
BE	Soil application	4.65±0.05 a	9.72±0.07 a	6.03±0.10 c	6.02±0.05 c
	Foliar spraying	4.87±0.07 a	8.48±0.06 b	8.67±0.05 a	8.66±0.07 a
	Soil appl.+foliar spray.	4.73±0.05 a	9.22±0.07 a	7.94±0.05 b	7.94±0.06 b
	Control	3.55±0.04 b	5.42±0.08 c	4.23±0.09 d	4.21±0.06 d
	Total mean (all treatments)	4.45±0.05 A	8.21±0.06 A	6.72±0.07 A	6.71±0.06 A
AE	Soil application	0.33±0.02 d	2.76±0.05 d	2.01±0.04 e	1.97±0.04 f
	Foliar spraying	0.31±0.03 d	2.60±0.05 d	2.90±0.06 e	2.87±0.06 e
	Soil appl.+foliar spray.	0.35±0.03 d	2.75±0.04 d	2.68±0.05 e	2.66±0.05 e
	Control	1.48±0.05 c	4.60±0.07 c	2.72±0.05 e	2.60±0.05 e
	Total mean (all treatments)	0.62±0.04 B	3.18±0.06 B	2.58±0.05 B	2.53±0.05 B
F value		21.18**	7.23**	8.31**	6.81**

* BE: Before emerging; AE: After emerging.
†Different lower and upper case letters in each column indicate statistically significant differences between treatments at two periods and between total means at two periods, respectively.
(**)= significant difference between the treatments at p<0.01.

Table 3. Mean comparison of number of branch, leaf buds and uninfested leaves and total number of leaves on trees treated by macronutrients and control in the second year (2019).

Treatments	Mean ± SE†			
	No. branch	No. leaf buds	Total No. leaves	No. uninfested leaves
Soil application	2.53±0.04 a	5.56±0.04 b	4.54±0.03 c	4.18±0.04 c
Foliar spraying	2.62±0.02 a	5.63±0.04 b	5.23±0.04 b	5.22±0.04 b
Soil appl.+foliar spray.	2.51±0.02 a	6.12±0.03 a	6.01±0.04 a	5.85±0.05 a
Control	2.46±0.03 a	5.32±0.05 c	3.18±0.03 d	2.41±0.03 d
F value	1.61^{ns}	5.14**	6.86**	4.41**

†Different letters in each column indicate statistically significant differences between treatments.
(ns)= non-significant difference between the treatments at p>0.05; (**)= significant difference between the treatments at p<0.01.

the total number of leaves, with statistically significant differences compared to the control treatment (Table 3). The mean comparison of the traits measured in two periods (before and after the emergence of the pest) also showed that the highest number of leaf buds, uninfested leaves, and the total number of leaves were produced before the emergence of the pest. It is clearly noticed in Table 4 that the number of uninfested leaves on the trees in these two periods did not differ significantly (Table 4).

3.3. Effect of calcium and potassium compounds on CLM activity in 2019

The results in year 2019 showed that there was a statistically significant difference between the studied treatments in all the examined characteristics (p<0.05) (Table 5). Based on the mean comparison results, the highest number of leaf buds were related to calcium nitrate with 2.52 buds per four branches, which was not significantly different from potassium nitrate, potassium chloride, and potassium sulphate. On the other hand, the lowest number of leaf buds was counted for the control with 1.61 buds per four branches, which was not significantly different from other treatments except for calcium nitrate. Also, the highest total number of leaves counted belonged to calcium nitrate with 12.59 leaves per four branches, which was no significantly different from potassium nitrate and potassium chloride. The lowest total number of leaves was recorded for control with 6.18 leaves per four branches, which was not significantly different from the calcium chloride and potassium sulphate. The results showed that the highest infested leaves were obtained from the control with 1.01 leaves per four branches, and the difference between control and other treatments was significant. Also, no infested leaves were observed on the trees treated

Table 4. Mean comparison of number of branch, leaf buds and uninfested leaves and total number of leaves on the trees treated by macronutrients before and after emerging of the pest in the second year (2019).

Period*	Treatments	Mean ± SE†			
		No. branch	No. leaf buds	Total No. leaves	No. uninfested leaves
BE	Soil application	4.59±0.05 b	9.03±0.06 b	6.68±0.07 c	6.54±0.07 c
	Foliar spraying	4.81±0.07 a	9.35±0.09 b	7.85±0.07 b	7.85±0.06 b
	Soil appl.+foliar spray.	4.55±0.06 b	10.04±0.07 a	9.29±0.08 a	9.20±0.08 a
	Control	3.74±0.04 c	5.85±0.07 c	3.52±0.05 d	3.43±0.06 d
	Total mean (all treatments)	4.42±0.05 A	8.57±0.07 A	6.84±0.07 A	6.76±0.07 A
AE	Soil application	0.47±0.03 e	2.08±0.05 e	2.39±0.06 e	1.82±0.05 f
	Foliar spraying	0.43±0.04 e	1.90±0.05 e	2.60±0.05 e	2.59±0.03 e
	Soil appl.+foliar spray.	0.47±0.05 e	2.20±0.04 e	2.73±0.06 e	2.49±0.05 e
	Control	1.18±0.04 d	4.78±0.07 d	2.84±0.05 e	1.38±0.04 g
	Total mean (all treatments)	0.64±0.04 B	2.74±0.05 B	2.64±0.05 B	2.07±0.04 B
F value		7.14**	5.64**	6.63**	7.69**

* BE: Before emerging; AE: After emerging.
†Different lower and upper case letters in each column indicate statistically significant differences between treatments at two periods and between total means at two periods, respectively.
(**)= significant difference between the treatments at $p<0.01$.

Table 5. Mean comparison of number of leaf bud, infested and uninfested leaves and total number of leaves on trees treated by different fertilizers and control in the first year (2019).

Treatments	Mean ± SE†			
	No. leaf bud	Total No. leaves	No. infested leaves	No. uninfested leaves
Calcium chloride	1.71±0.01 b	6.67±0.04 c	0.14±0.003 d	6.53±0.03 cd
Calcium nitrate	2.52±0.02 a	12.59±0.07 a	0.25±0.00 c	12.34±0.06 a
Potassium nitrate	2.08±0.05 ab	10.88±0.06 ab	0.45±0.002 b	10.43±0.06 ab
Potassium chloride	2.41±0.02 ab	10.83±0.07 ab	0.19±0.01 cd	10.64±0.05 ab
Potassium sulphate	2.18±0.02 ab	7.98±0.05 bc	0.00±0.003 e	7.98±0.04 c
Control	1.61±0.02 b	6.18±0.03 c	1.01±0.001 a	5.17±0.04 d
F value	2.04*	2.83*	1.49*	2.63*

†Different letters in each row indicate statistically significant differences between treatments
(*)= significant difference between the treatments at $p<0.05$

Table 6. Mean comparison of number of leaf bud, infested and uninfested leaves and total number of leaves before and after emerging of the pest in the first year (2019).

Period*	Treatments	Mean ± SE†			
		No. leaf bud	Total No. leaves	No. infested leaves	No. uninfested leaves
BE	Calcium chloride	2.94±0.05 b	9.84±0.07 d	0.00±0.00 b	9.84±0.07 d
	Calcium nitrate	4.03±0.06 a	17.44±0.08 a	0.00±0.00 b	17.44±0.08 a
	Potassium nitrate	3.34±0.05 b	15.19±0.09 b	0.00±0.00 b	15.19±0.09b
	Potassium chloride	4.03±0.04 a	14.25±0.07 b	0.00±0.00 b	14.25±0.07 b
	Potassium sulphate	3.81±0.07 a	11.00±0.08 c	0.00±0.00 b	11.00±0.08 c
	Control	3.28±0.06 b	7.06±0.06 e	0.00±0.00 b	7.06±0.06 e
	Total mean (all treatments)	3.57±0.06 A	13.13±0.08 A	0.00±0.00 B	13.13±0.08 A
AE	Calcium chloride	0.48±0.03 d	3.50±0.06 g	0.27±0.03 b	3.23±0.05 g
	Calcium nitrate	1.00±0.02 c	7.73±0.05 e	0.50±0.03 b	7.23±0.07 e
	Potassium nitrate	0.82±0.04 c	6.57±0.06 ef	0.89±0.04 b	5.68±0.05 f
	Potassium chloride	0.79±0.02 c	7.41±0.04 e	0.38±0.02 b	7.03±0.06e
	Potassium sulphate	0.55±0.04cd	4.96±0.07 f	0.00±0.00 b	4.96±0.07 f
	Control	0.14±0.03 d	5.29±0.06 f	2.02±0.05 a	3.27±0.06 g
	Total mean (all treatments)	0.63±0.03 B	5.24±0.05 B	0.34±0.04 A	4.90±0.06 B
F value		11.58**	23.03**	5.40**	25.69**

* BE: Before emerging; AE: After emerging.
†Different lower and upper case letters in each column indicate statistically significant differences between treatments at two periods and between total means at two periods, respectively.
(**)= significant difference between the treatments at $p<0.01$.

with potassium sulphate, and there was a significant difference between this treatment and other treatments. Based on the results, the highest number of uninfested leaves counted were related to calcium nitrate with 12.34 leaves per four branches, which was not significantly different from the potassium chloride and potassium nitrate. Also, control and calcium chloride yielded the lowest number of uninfested leaves with 5.17 and 6.54 leaves per four branches, respectively, and there was no significant difference between them (Table 5). Mean comparison of the traits before and after CLM emergence showed that the number of leaf buds and the total number of leaves appeared before CLM emergence were significantly higher compared to those after CLM emergence. Although the number of infested leaves counted after CLM emergence was higher than those before CLM emergence, but this difference was not significant (Table 6).

Table 7. Mean comparison of number of leaf bud, infested and uninfested leaves and total number of leaves on trees treated by different fertilizers and control in the second year (2020).

Treatments	Mean ± SE†			
	No. leaf bud	Total No. leaves	No. infested leaves	No. uninfested leaves
Calcium chloride	12.89±0.06 bc	65.26±0.18 b	3.43±0.03 a	61.83±0.15 b
Calcium nitrate	19.51±0.10 a	96.14±0.36 a	1.85±0.01 b	94.29±0.22 a
Potassium nitrate	13.14±0.07 b	76.69±0.16 b	4.1±0.03 a	72.59±0.19 b
Potassium chloride	15.88±0.09 b	74.78±0.21 b	4.13±0.02 a	70.65±0.11 b
Potassium sulphate	13.67±0.08b	66.72±0.10 b	1.76±0.01 b	64.96±0.14 b
Control	9.72±0.07 c	45.92±0.12 c	4.51±0.03 a	41.41±0.10 c
F value	5.85**	3.98**	2.09*	3.75**

†Different letters in each row indicate statistically significant differences between treatments.
(**) and (*) indicate significant difference between the treatments at p<0.01 and p<0.05, respectively.

Table 8. Mean comparison of number of leaf bud, infested and uninfested leaves and total number of leaves before and after emerging of the pest in the second year (2020).

Period*	Treatments	Mean ± SE†			
		No. leaf bud	Total No. leaves	No. infested leaves	No. uninfested leaves
BE	Calcium chloride	21.20±0.32 d	92.91±0.52 c	0.68±0.07 d	92.23±0.51 c
	Calcium nitrate	30.97±0.27 a	148.09±0.66 a	0.57±0.08 d	147.52±0.53 a
	Potassium nitrate	21.70±0.25 d	118.75±0.61 b	0.82±0.07 d	117.93±0.61 b
	Potassium chloride	27.33±0.30 b	117.20±0.57 b	0.83±0.09 d	116.37±0.47 b
	Potassium sulphate	24.78±0.19 c	120.15±0.55 b	0.34±0.06 d	119.81±0.43 b
	Control	17.20±0.15 e	77.76±0.60 d	5.69±0.12 b	72.07±0.39 d
	Total mean (all treatments)	23.86±0.36 A	112.48±0.78 A	1.49±0.07 B	110.99±0.57 A
AE	Calcium chloride	4.58±0.06 g	37.60±0.47 ef	6.18±0.11 b	31.42±0.39f
	Calcium nitrate	8.05±0.08 f	44.18±0.38 e	3.13±0.08 c	41.05±0.35 e
	Potassium nitrate	4.58±0.05 g	34.63±0.43f	7.38±0.09 a	27.25±0.26 fg
	Potassium chloride	4.42±0.04 g	32.35±0.40 f	7.43±0.07 a	24.92±0.32 g
	Potassium sulphate	2.55±0.05 h	13.28±0.29 g	3.18±0.05 c	10.10±0.12 h
	Control	2.23±0.04 h	14.08±0.22 g	3.33±0.10 c	10.75±0.14 h
	Total mean (all treatments)	4.40±0.04 B	29.35±0.49 B	5.11±0.09 A	24.24±0.34 B
F value		12.23**	18.78**	15.22**	10.01**

* BE: Before emerging; A.: After emerging.
†Different lower and upper case letters in each column indicate statistically significant differences between treatments at two periods and between total means at two periods, respectively.
(**)= significant difference between the treatments at p<0.01.

3.4. Effect of calcium and potassium compounds on CLM activity in 2020

The results of variance analysis showed that the studied treatments had statistically significant differences with each other in all attributes (p<0.05). Mean comparison of number of leaf buds showed that the trees treated with the calcium nitrate produced the highest leaf buds with 19.51 buds per four branches, which was significantly different from other treatments (Table 7). Also, the highest total number of leaves was related to the calcium nitrate with 96.14 leaves per four branches, and this difference was significant from the other treatments. The lowest total number of leaves with 45.92 leaves per four branches was recorded for the control treatment that there was a significant difference between control and others. Based on the obtained results, the highest number of infested leaves was recorded for the control with 4.51 leaves per four branches that was not significantly different from potassium chloride, potassium nitrate, and calcium chloride treatments. On the other hand, the lowest infested leaves with 1.76 leaves per four branches belonged to the potassium sulphate treatment, which was not significantly different from calcium nitrate. The mean comparison of the number of uninfested leaves also showed that the highest and lowest number of uninfested leaves with 94.29 and 41.41 leaves per four branches related to calcium nitrate and control treatments, respectively, and their difference from other treatments was significant (Table 7). Based on the results presented in Table 8, the number of leaf buds and the total number of leaves on the trees treated with the fertilizers before CLM emergence were significantly higher than after CLM emergence, and also the low infestation level was observed on the leaves produced after CLM emergence due to the less production of young leaves in this period.

In the principles of control, one of the effective ways to reduce the damage of pests is to use suitable agricultural methods to avoid damage caused by harmful factors. Feeding trees with the suitable fertilizers at the appropriate time can play a valuable role in reducing or avoiding damage caused by pests (Abbas and Fares, 2009; Belasque et al., 2005). The investigations show that the use of macro fertilizers for citrus trees in Mazandaran province is usually recommended by experts and consumed by gardeners from the end of February, while the citrus trees need a lot of macro fertilizers, especially nitrogen during the flowering time in late April and later. In Mazandaran province, the use of fertilizers that have a high solubility in water such as Nitrogen before rainfalls in late winter or early spring caused that these fertilizers was removed from the root zone, and not only does not have much effect on the vegetative and reproductive growth of the trees but also causes an increase in production costs and even environmental pollution (it is visual observation). Hence, special attention to this point can help in the optimal and balanced consumption of fertilizers and the appropriate vegetative and reproductive growth of the trees. In the first experiment, the optimum consumption of macronutrients by soil application+ foliar spraying showed a significant difference compared to the control and produced the highest leaf buds in the spring. Due to the inactivity of CLM at this time, all leaves and young shoots produced until late June 2018 and late July 2019 were not damaged by this pest. According to the results obtained in the first experiment, applying fertilizer by soil application+foliar spraying produced more leaf buds and leaves than other treatments. On the other hand, the mean comparisons showed that the macronutrients should be applied before the emergence of the pest in the spring season.

The results of the second experiment showed that the highest number of leaf buds and leaves produced from April 21 to June 21 was protected from the damage by CLM due to the absence of the pest during this period. According to the results obtained (unpublished data), the highest number of infested leaves in the first and second years was observed in the middle of August and from September 23 to October 22, when the ambient temperature increased and leaf buds and leaves were less produced on the trees. Mean comparison of the characteristics in the two periods, before and after the emergence of CLM showed that if the micronutrients are used before the emergence of the pest in the spring, it will produce the highest young leaves and leaf buds before the pest starts its activity.These results show the fact that applying macro- and micronutrients in the spring season and as a result, produce more leaf buds and leaves at this time when CLM is not active, and fewer leaf buds and leaves are exposed to damage by the pest when it is active. Therefore, it is possible to minimize CLM damage through proper nutrition management that will lead to the reduction in the use of chemical pesticides.

The results of the second experiment in 2020 showed that the highest number of uninfested leaves was counted on the trees treated with calcium and potassium nitrate (91.63 and 72.60, respectively). Calcium and potassium nitrate are easily soluble in water and absorbed by plants. Inherent nitrate content enables many nutrients including calcium and potassium to be taken by plants and increases resistance to pests (El-Enien et al., 2017). Accordingly, these two nutrients are multi-functional in physiological processes in the plant and effective in vegetative growth.

Patsias (1996) recommended control methods of CLM in Cyprus, which included increasing the amount of chemical fertilizers, especially nitrogen in early February, light irrigation during the two months (from January 20 to March 20), pruning citrus trees in early February, light irrigation during the summer and autumn and reducing the use of chemical fertilizers in these seasons. All of the mentioned cases aimed to reduce the production of leaf buds and leaves on trees during CLM activity, which is consistent with the findings of the present research. Mahmoodi and Alavi (2005) stated that spraying potassium nitrate and potassium sulphate caused an increase in the number of leaves and the length of new branches on citrus trees and the percentage of damage caused by the larvae of CLM was also reduced by the management of the micronutrients, which is in agreement with the results obtained in the present study.

Mustafa et al. (2014) evaluated the relationship between the level of CLM damage and the biochemical changes of citrus leaves (Ca^{2+}, K^{+}, and Mg^{2+}) caused by the use of chemical fertilizers during the growing season in Punjab, Pakistan. They reported a negative correlation between the CLM damage and potassium in the one-year orchard, while the results obtained in the two-year and three-year orchards showed a positive correlation between the CLM damage and potassium and calcium. These results clarify the effect of mineral nutrients on the level of damage caused by this pest. Concerning other types of fertilizers, research also showed that organic fertilizers such as different composts at 0.5 kg plant^{-1} concentration reduced the CLM infestation by up to 55 and 39% during fall and summer, respectively (Ullah et al., 2019). El-Enien et al. (2017) reported that potassium and calcium compounds had a significant effect on the CLM infestation of Valencia orange seedlings so that potassium nitrate, potassium silicate, and calcium nitrate as soil application and foliar spraying caused up to 50% reduction in CLM infestation. Our results are in line with the findings obtained by El-Enien et al. (2017) and El-Sayed and Ennab (2008) found that spraying potassium sulphate at 2% decreased the CLM infestation on citrus trees. In addition, Dito and Lewis (2013) indicated that foliar spraying of potassium silicate on young citrus seedlings significantly reduced CLM damage.

In general, nutrients can directly or indirectly affect plants so that they become a susceptible host against pest or pathogen attacks. Nutrients can reduce or increase the severity of the damage, influence the environment to attract or deter the pest or pathogen, and induce resistance or tolerance in the host plant (Agrios, 2005; Zambolim et al., 2001). However, mineral nutrients also affect plant growth and reproduction by influencing plant resistance or sensitivity to pathogens and pests (Spann and Schumanu, 2010).

Although plant resistance to pests and diseases is controlled genetically, environmental factors also play a significant role in this process (Bairwa et al., 2014). Some genes responsible for the pest resistance are activated only by environmental stimuli. Nutrients are one of the environmental factors that can significantly affect the management of agricultural systems (Ochola et al., 2014).

Several studies have reported a negative effect of fertilizer application on the population of various insect pests in fields and greenhouses. Chávez-Dulanto et al. (2018) stated that the foliar application of microelements such as calcium, magnesium, zinc, copper, iron, manganese and boron caused a significant reduction in the populations of *Panonychus citri* McGregor and *Phyllocoptruta oleivora* Ashmead, as key pests of citrus crop in Peru. Karim (2013) reported that the use of various manures and fertilizers, including cow-dung, triple superphosphate and micronutrients decreased the populations of aphid (28.02%), fruit borer (35.76%), whitefly (43.30%) and leafhopper (53.75%) in the tomato field in Bangladesh. Karungi et al. (2006) showed that the application of NPK fertilizer in the soil caused a reduction in *Aphis fabae* Scopoli population in *Phaseolus vulgaris* fields in Uganda. Moursy et al. (2021) reported that the sequential application of humic acid in foliar and soil methods resulted in the lowest population density of *Aphis gossypii* Glover, *Bemisia tabaci* Gennadius and *Tetranychus urticae* Koch, which are three main pests on eggplant in greenhouse conditions in Egypt. Oeller et al. (2025) revealed that the organic chicken manure applied to quinoa (*Chenopodium quinoa*) caused the largest reduction in the cowpea aphid (*Aphis craccivora* Koch) and *Lygus* sp. populations due to the lowest survival. In Iran, Olyaie Torshiz et al. (2017, 2020) stated that the pomegranate trees treated with both biofertilizers and humic acid showed the lowest fruit infestation (18.75-28.67%) with *Ectomyelois ceratoniae* Zeller in pomegranate orchards. Yardım and Ewards (2003) also reported that the population of aphids on tomatoes treated with organic fertilizer was lower than on those treated with the synthetic fertilizer as well as the control.

Mineral nutrients affect the primary resistance mechanism in two ways; First, the formation of mechanical barriers, which is mainly through forming a thicker cell wall, and second, making natural defence compounds, including phytoalexins, antioxidants, and flavonoids, which protect the plant against pests (Yadollahpour et al., 2015). Anyway, the role of nutrients in plant-pest interaction is well established. Identifying these interactions is useful for controlling and eradicating pests using the fertilizing program.

4. Conclusions

The CLM is one of the important citrus pests in the citrus growing regions of the world and Iran. The results of this research showed that the use of tested chemical fertilizers by foliar spraying in early spring to stimulate the trees to produce more leaves and branches and to accelerate the vegetative growth of new shoots to avoid pest damage is suitable and economical. The results of the present study showed that the use of macro fertilizers in the spring season by the method of soil application along with foliar spraying as well as the use of calcium chloride and calcium nitrate micronutrient fertilizers at the same time lead to more bud and leaf production before the appearance of CLM. Considering that the CLM damages the young leaves, therefore, when the pest starts to act, the young leaves are out of their sensitive stage and are not damaged by the pest. Considering the adverse environmental effects caused by the use of pesticides on natural enemies and even the outbreak of some pests such as mites due to the excessive use of these chemicals in citrus orchards. Therefore, the damage can be reduced by proper nutrition management using macro and micro fertilizers, and minimize the use of pesticides.

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