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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

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Abstract.- Leaf samples of kinnow were taken from November 2012 to April 2013 from three different orchards (with different ages) of district Sargodha for the study of correlation of citrus leaf miner (CLM) with leaf biochemical factors (Ca, K, Mg). Damage due to CLM ranged from 32-58%. Maximum damage (58%) was observed in the month of December with a decline in percentage damage from January to March (32%) but again an increase in April. One year old orchard showed negative correlation of citrus leafminer with potassium, while two and three year old orchards showed positive correlation of CLM with potassium and calcium. In one year old and three year old orchard CLM was positively correlated with magnesium, while two year old orchard showed negative correlation of CLM with magnesium.

Key words: Citrus leaf miner, Kinnow, leaf biochemical factors, *Citrus reticulate*.

INTRODUCTION

Citrus is the main fruit crop of Pakistan grown on the area of 160,000 hectares with annual production of 1.5 million metric tons. It is grown in all four provinces of Pakistan but Punjab produces over 95% of the crop. Kinnow (*Citrus reticulata*) is also a type of citrus it is a crossbreed of king and willow leaf and classified as kinnow mandarin (Altaf and Khan, 2008). Pakistan is the 10th larger producer of kinnow in the world with 2 million tons per annum (Syed, 2007). Citrus leaf miner (CLM) *Phyllocnistis citrella*, is one of the major pests of citrus. The main damage which is caused by its larvae make mines in immature foliage of nursery plants (Beattie and Hardy, 2004). Larvae develop into pupae after 20 days (Happner, 1993) margin. Adults are small moths (2mm) that appear within the mines in a special pupal cell at the leaf white and silvery. Adults live for few days. CLM mines in the upper and lower surfaces of newly formed leaves during its larval stage (Mafi and Ohbayashi, 2004). Curling of leaves is the main symptom of this pest and it also retards the growth of young plants. Severe infestation occurs in late spring, summer and early fall (Cardwell *et al.*, 2008).citrus leaf miner also enhance the attack of other pests on infected leaves such as it support the attack of snails on citrus leaves which further cause severe damage to the leaves by sucking the fluids (Mustafa *et al*., 2013).

Citrus plants require large amount of minerals such as Ca^{++}, K^{+} and Mg^{++} which play an important

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role in their development. Calcium is important for root development, cell division, chromosome packing and immune system (Zekri and Obreza, 2002). Its deficiency causes stunted growth, less yield, leaf chlorosis and premature leaf fall (Chapman, 1934). Potassium plays an important role in the synthesis of starch, protein and sugars and also control the rate of photosynthesis (Zekri and Obreza, 2002). Its deficiency slows the rate of photosynthesis, yellowing of leaves and increase in fruit splitting (Embleton *et al.*, 1973). Magnesium is the basic element of chlorophyll and takes part in photosynthesis and metabolism of carbohydrates (Zekri and Obreza, 2002). Magnesium deficiency causes yellow spots on leaves (Camp *et al.*, 1949) and it makes trees more susceptible to cold injury (Lawless and Camp, 1940).

The present study was designed to determine correlation of CLM with leaf mineral content (Ca^{++}, K^{+} and Mg^{++}).

MATERIALS AND METHODS

Sample collection and damage assessment

Three orchards of kinnow (*Citrus reticulata*), of one, two and three years of age were selected in different areas of district Sargodha. One and three years old orchards were selected from south branch (125 SB), while the two year old orchard was selected from north branch (43 NB). No chemical spray was done during the whole period of study from November 2012 to April 2013.

For sample collection, 12 plants were selected and tagged in each orchard. These plants were selected in such a way that four plants were selected from three different rows. Then each plant was divided into four quadrants and 10 leaves were taken randomly from each quadrant. In this way forty leaves were taken from each plant. Leaves from each quadrant were placed in plastic bags separately, transported to laboratory and damage was examined with naked eye and microscope. For damage assessment the number of infected and normal leaves were counted and percentage of damage on each plant was calculated.

Biochemical estimation

For estimation of minerals the normal and infected leaves of each plant were oven dried at 70ºC for two days, 1g of each sample was digested with 4ml sulfuric acid and 8ml hydrogen peroxide this process takes place on hot plate until the digest became clear colorless. It was then filtered and diluted up to 50 ml with distilled water.

Leaf samples were analyzed for minerals (Ca^{++}, K^{+}, Mg^{++}) in Shimadzu (AA-6300) atomic absorption spectrophotometer (AAS). The concentrations of calcium, potassium and magnesium were estimated using standard curves of these minerals; concentration of minerals were taken in ppm (parts per million).

Statistical analysis

Using SPSS software version 20th correlation test was applied to correlate the damage of CLM with leaf minerals (Ca, K and Mg). Analysis of variance (ANOVA) was applied to check the variation of damage and minerals during the whole period of study.

RESULTS AND DISCUSSION

Damage done by CLM ranged from 35-54% in one year old orchard, 38-58% in two years old orchard and 32-56% in three years old orchard (Table I). There was a decline in percentage damage from January to March but it again increased in April. In all the trees, maximum damage was observed in December and the highest value was registered on 2 years-old orchard (58%) and minimum damage was observed in March and the lowest value was registered on three years old orchard (32%).

Damage done by CLM in kinnow leaves ranged between 32-58%. Many other researchers such as Lara *et al.* (1998) reported 12-85 percent damaged. Zaib *et al.* (2011) reported 2-55% total leaf damage. Maximum damage due to CLM was observed in December and April. Similar results were reported by Legaspi *et al.* (2001). It was observed that there was a decline in percentage damage from January to March. It is in agreement with Pena *et al.* (1996) who reported lowest population density during January and winter season.

Table I.- **Mean values (Mean±SE) of percentage damage due to CLM and concentration of minerals during the whole period of study.**

Months	Mean±SE of percentage damage	Concentration (Mean±SE)		
		K^+	Ca^{++}	Mg^{++}
One year old orchard				
November	44.16±2.38	11.89±2.27	50.11±4.56	10.43±.06
December	46.25±1.79	17.40±2.41	90.70±10.21	10.71±.08
January	44.58±2.08	20.60±1.04	110.23±5.39	10.69±.05
February	37.08±4.44	17.53±1.97	47.76±3.01	10.63±.06
March	35.00±3.81	20.67±2.60	112.20±1.16	10.50±.11
April	54.16±4.45	17.72±1.33	97.76±7.00	10.62±.10
Two years old orchard				
November	47.91±4.05	21.62±0.56	53.61±10.73	10.68±.07
December	58.91±2.17	21.30±1.18	40.35±7.84	10.71±.05
January	57.91±3.12	19.56±1.91	74.88±6.63	10.65±.05
February	38.16±5.71	19.76±1.74	68.01±6.50	10.79±.04
March	38.83±3.75	16.33±1.79	64.81±7.04	10.71±.04
April	48.75±3.80	18.48±1.07	57.17±9.08	10.76±.06
Three years old orchard				
November	45.41±2.69	14.47±3.72	62.33±8.82	10.64±.06
December	56.66±2.55	17.03±2.12	55.30±15.25	10.69±.08
January	53.33±4.40	21.12±1.72	112.82±3.71	10.66±.05
February	37.75±1.72	19.35±.87	62.57±12.48	10.74±.02
March	32.08±3.50	17.75±0.94	100.69±13.90	10.65±.09
April	43.75±3.69	26.62±3.0	114.21±1.56	10.66±04

Correlation test revealed that in one year old orchard the damage by CLM was negatively correlated with potassium (r = -.130), but positively correlated with calcium (r = .117) and magnesium (r =.390) (Table II). In two years old orchard the damage by CLM showed weak positive correlation with potassium (r = .127), weak negative correlation with calcium (r = -.193) and magnesium (r = -.100) (Table II). In three years old orchard damage percentage again showed weak positive correlation with potassium (r = .097), weak negative correlation with calcium (r = -.001) and weak positive correlation with magnesium (r = .193) (Table II). These results revealed that small orchard showed negative correlation of CLM with potassium and calcium, while large orchards showed positive correlation of CLM with potassium and calcium. In one year and three years, old orchards CLM there was positive correlation with magnesium while two years old orchard showed negative correlation of CLM with magnesium.

Correlation of minerals with each other was also noted. Minerals showed different correlation with each other with respect to the age of orchards and location of orchards. Significant positive correlation was observed between potassium and calcium and one (r = .422) and three (r = .380) years old orchards. While in two years old orchard potassium and calcium showed weak negative correlation (r = -.040) with each other (Table II).

In the same way potassium and magnesium showed weak positive correlation with each other in one (r = .063) and three (r= .104) years old orchards and weak negative correlation in two years old orchard (r = -.009). As the one and three years old orchards were present in the same area so due to the similar soil composition they showed similar results of correlation of potassium with calcium and magnesium (Table II).

Calcium and magnesium showed negative correlation with each other in all the three orchards. The value of r for one two and three years old orchards are (r = -.066) (r = -.112) and (r = -.078) respectively (Table II). Negative correlation of

Table II.- Correlation of damage percentage of CLM with concentration of minerals (Ca, K, and Mg) in infected leaves in different orchards.

Minerals		Damage (%)	K	Ca	Mg
One year old orchard					
Damage (%)	Pearson Correlation	1	-.130	.117	.390*
	Sig. (2-tailed)		.451	.497	.019
	N	36	36	36	36
K^+	Pearson Correlation	-.130	1	.422*	.063
	Sig. (2-tailed)	.451		.010	.714
	N	36	36	36	36
Ca^{++}	Pearson Correlation	.117	.422*	1	-.066
	Sig. (2-tailed)	.497	.010		.702
	N	36	36	36	36
Mg^{++}	Pearson Correlation	.390*	.063	-.066	1
	Sig. (2-tailed)	.019	.714	.702	
	N	36	36	36	36
Two years old orchard					
Damage (%)	Pearson Correlation	1	.127	-.193	-.100
	Sig. (2-tailed)		.460	.259	.563
	N	36	36	36	36
K^+	Pearson Correlation	.127	1	-.040	-.009
	Sig. (2-tailed)	.460		.815	.959
	N	36	36	36	36
Ca^{++}	Pearson Correlation	-.193	-.040	1	-.112
	Sig. (2-tailed)	.259	.815		.517
	N	36	36	36	36
Mg^{++}	Pearson Correlation	-.100	-.009	-.112	1
	Sig. (2-tailed)	.563	.959	.517	
	N	36	36	36	36
Three years old orchard					
Damage (%)	Pearson Correlation	1	.097	-.001	.193
	Sig. (2-tailed)		.573	.997	.259
	N	36	36	36	36
K^+	Pearson Correlation	.097	1	.380*	.104
	Sig. (2-tailed)	.573		.022	.545
	N	36	36	36	36
Ca^{++}	Pearson Correlation	-.001	.380*	1	-.078
	Sig. (2-tailed)	.997	.022		.653
	N	36	36	36	36
Mg^{++}	Pearson Correlation	.193	.104	-.078	1
	Sig. (2-tailed)	.259	.545	.653	
	N	36	36	36	36

*Correlation is significant at the 0.05 level (2-tailed).

calcium was found with magnesium Similar results were reported by Fudge and Fehmerling (1940). They reported repressing effect of calcium on magnesium when calcium is present in high concentration it suppress the absorption of magnesium.

In one year old orchard the concentration of calcium was highest in March (112.20 ppm) and minimum in November (50.11 ppm) this showed negative correlation of damage percentage of CLM with concentration of calcium as the damage of CLM increased the concentration of calcium in infected leaves decreased. Maximum concentration of potassium was found in March (20.67 ppm) and minimum concentration (11.89 ppm) in November. Maximum concentration of magnesium was observed in December (10.71 ppm) and minimum (10.43 ppm) in November, while, its concentration remained same during all other months (Table I).

In two years old orchard the concentration of calcium was highest during the month of January (74.88 ppm) and its lowest concentration (40.35 ppm) was found in December. The concentration of potassium was highest (21.62 ppm) in November and damage caused by CLM was also high during these months. It indicates positive correlation of potassium with CLM damage. As the damage increased the concentration of potassium also increased. The concentration of magnesium was lowest during January (10.65 ppm) and it showed negative with calcium (Table I).

In three years old orchard, concentration of calcium was highest (114.21 ppm) in April. Potassium was highest in April (26.62 ppm) and lowest during November (14.47 ppm). Magnesium was highest (10.74 ppm) in February but remained almost constant all other months (Table I).

Soil composition could be a factor for this variation in correlation results. All the three orchards were present in different location one in north branch and two orchards were
present in south branches. Orchard location may also influence the correlation.

Calcium showed significant positive correlation with potassium in southern orchards and week negative correlation in northern orchard. Smith and Reuther (1950) also reported negative correlation of calcium and potassium. They noted that high potassium depress the concentration of calcium in orange leaves.

So it is concluded that citrus leafminer has week correlations with leaf minerals (Ca, K and Mg) and it was influenced by soil composition, abundance of citrus leafminer, environmental factors such as temperature and location of orchards.

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