

## How Quantitative and Qualitative Traits of Thomson Navel Orange Affected by Citrus Red Mite, *Panonychus citri*

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### ABSTRACT

To examine the effect of Citrus Red Mite (CRM) damage on quantitative and qualitative characteristics of Thomson navel orange *Citrus sinensis* (L.) Osbeck, paired-treatment experiment, multiple treatment experiment, and regression/correlation methods were used. There were significant differences among the treatments in paired-treatment and multiple treatment experiments in terms of the average population per leaf of CRM of different life ages and concentration of chlorophyll in the leaves. Also, there were significant differences among the treatments in multiple treatment experiment in terms of the Total Soluble Solids (TSSs) of the fruit extract. In control treatment of the paired-treatment experiment, the highest concentration of chlorophyll ( $79.13 \pm 1.06$ ) was observed when the population density of CRM was  $0.48 \pm 0.09$  per leaf. With increasing the mean number of CRM to  $10.59 \pm 1.09$  per leaf, chlorophyll content was decreased to  $62 \pm 1.15$ . Furthermore, the results showed that the storage life of the fruits in treatments with mean number of  $6.33 \pm 1.8$  mites per leaf was significantly reduced. The results of regression and correlation analysis on chlorophyll showed significant and negative relationship between these parameters and CRM population density. The results indicated that increasing the population density of CRM to  $10.59 \pm 1.09$  per leaf at the beginning of the season caused fruit drop and dry twigs. In addition, by increasing population density of CRM to  $5.72 \pm 0.43$  per leaf, the storage capability of the fruits was significantly reduced.

**Keywords:** Citrus red mite, Crop loss assessment, Quality loss, Yield loss.

### INTRODUCTION

Herbivorous mite species of the family Tetranychidae, including two-spotted spider mite *Tetranychus urticae* (Koch), red European mite *Panonychus ulmi* (Koch), Citrus Red Mite (CRM) *Panonychus citri* (McGregor), and *Tetranychus kanzawai* (Kishida) not only cause severe damage to agricultural crops but also bring about major human allergies outdoors and lead to asthma and inflammation of the nasal mucosa (Rhinitis) in rural areas (Niu *et al.*, 2011).

CRM is a major pest of citrus in many parts of the world (Gotoh and Kubota, 1997;

Jamieson *et al.*, 2005; Vacante, 2010). This pest feeds on more than 80 species of plants including citrus (Zhang, 2003). CRM feeds on cell contents of the upper part of the citrus leaves. In case of severe infestation of the leaves by this pest, gray or silver spots appear on the leaf surface and reduce the photosynthesis rate of the leaves. This pest causes dieback of branches and reduces growth power of the plant (Prischmann *et al.*, 2005; Albrigo *et al.*, 1981). In addition, the severe damage caused by the pest, the fruits turn pale green in terms of the form and even ripe fruits get the yellow color of the amber. If the severe damage occurs before the fruit ripening, it may cause a complete loss and

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drop of the fruits from the trees (Childers *et al.*, 2007). To prevent the damage caused by CRM in the San Joaquin Valley, pests control is applied when the spring population is below the threshold of 2 adult females per leaf in Thomson navel orange (*Citrus sinensis*) (Hare and Youngman, 1987). Studies conducted by Hare *et al.* (1990) in the United States revealed that the peak population of mites on non-sprayed trees (5 and 8 mature females per leaf) remarkably decreases the crop yield (~10%). However, a slight increase in the average weight of the fruit on control trees will compensate slight reduction in the total yield.

Regarding the fact that CRM is one of the major citrus pests in northern Iran, chemical control operations against it is necessary. As mentioned above, CRM is largely considered an economic pest that feed on the leaf, devastates chlorophyll, reduces leaf photosynthesis, and ultimately decreases the yield of the crop and the growth of the trees. Considering the economic importance of this pest in terms of health and the lack of information on the feeding behavior of the pest on quantitative and qualitative traits of Thomson navel orange in the citrus-growing regions, the present research was conducted to achieve the above-mentioned objectives.

## MATERIALS AND METHODS

### Study Location and Tree Selection

This study was conducted in 2016 at the Horticultural Research Station in Ghaemshahr city, northern Iran. Initially, 15 Thomson navel orange trees upon citrange rootstock were selected. Each selected tree was about 6-7 years old and about 2-2.5 meters of height. In addition, one tree was appointed as marginal.

### Sampling Program

Thomson navel orange leaf was considered as a sampling unit in this study.

Sampling of CRM population was carried out once a week between 9 to 11 AM. Sampling started on May 18 and continued to November 18, 2016 (pre-harvest).

In order to determine the appropriate number of samples, an initial sampling with 20 leaves in each treatment was done. In order to examine the accuracy of the initial sampling, Relative Variation (RV) was calculated according to Hillhouse and Pitre (1974) procedure. RV shows the precision of the initial sampling. To determine the RV, the following equation was used;

$$RV = \left( \frac{SE}{m} \right) \times 100 \quad (1)$$

Where, *SE* is the Standard Error of the mean and *m* is the mean of primary sampling data (Naseri *et al.*, 2009). In this study, the RV value was determined as 2 % (Jarosik *et al.*, 2003; Khodayari *et al.*, 2010). Obviously, if the RV is larger than the acceptable, the number of the initial samples should be increased.

Reliable sample size was determined using the following equation:

$$N = \left( \frac{ts}{dm} \right)^2 \quad (2)$$

Where, *N*= Sample size, *t*= t-student, *s*= Standard deviation, *d*= Desired fixed proportion of the mean and *m*= The mean of primary data (Darbemamieh *et al.*, 2011)

After sampling the treatments at the desired location, the samples were placed into coded plastic bags and transferred to the laboratory. To count and record the number of CRM different life ages in the upper and lower surface of each leaf, a stereoscope with 10X magnification was used. It should be noted that the samples should be collected at random so that each sample has an equal chance of being selected (Pedigo and Buntin, 1994).

### Experimental Design

To examine the effect of CRM damage on quantitative and qualitative characteristics of Thomson navel orange, the paired-treatment and multiple-treatment experiments were

applied. Moreover, in order to evaluate the relationship between CRM populations with other measured characteristics, two statistical methods i.e. linear regression and correlation were used.

### Paired-Treatment Test

In this method, control trees were protected from infestation using the commonly used Fenpyroximate acaricide. However, trees of another treatment were exposed to natural populations of CRM. The purpose of the paired-treatment test was to compare between the measured quantitative and qualitative characteristics in two treatments with 5 replicates of Thomson navel orange trees.

### Multiple-Treatment Test

To perform the experiment, five tree groups were selected for different treatments, which included the treatment 1 with single artificial infestation, treatment 2 with double infestation, treatment 3 with three cases of infestation, treatment 4 with four artificial infestation, and treatment 5 (the control treatment) without artificial CRM infestation. The infestation operation of the treatments was carried out during 4 weeks in April 2016. For this purpose, the CRM infested twigs were cut from the infested orchards and placed on the four directions of trees or the selected treatments (North, South, West and East). In order to obtain different densities from the population of CRM, every week one treatment was removed from the infestation operation. Finally, in the fourth week, only the treatment 4 was infested. Also, in case of observation of infestation after each weekly sampling of CRM in the control treatment, controlling operation was carried out using common acaricides.

### Regression/Correlation

The method of statistical analysis was to examine the relationship between CRM pest

population density as the fixed variable and the measured quantitative and qualitative characteristics as random variables. Using linear regression between the average population of CRM during 19 inventory dates as the independent variable and the average measured as the dependent variable; measured parameters reactions during tests with an average density of the population was studied. Moreover, to determine the correlation between the variables CRM population and the measured, correlation test was used. We used both regression and correlation analysis because, in case the CRM population density is considered as a fixed variable (because of artificial infestation), we should use regression, and if it is considered as a random variable, we should use correlation analysis to reveal the relationship between CRM population density and the qualitative and quantitative characteristics of Thomson navel orange.

### Analysis of Plants' Response

The qualitative and quantitative characteristics of Thomson navel orange including yield, chlorophyll concentration (SPAD), fruit diameter (mm), fruit weight (g), diameter of the skin (mm), the extract of each fruit (mL), the weight of waste (g), Total Acidity or TA (Titratable Acidity, g mL<sup>-1</sup>), pH, soluble solids percentage or TSS (Total Soluble Solids), Conductivity or EC by Siemens/meter (S/m) and vitamin C (mg 100 mL<sup>-1</sup> of water) were separately measured in each treatment and replicate.

After ripening of the fruits, harvesting was carried out on June 16, 2016. To calculate performance of the experimental treatments, weighing and recording of the data were performed using a digital scale simultaneously with the harvest process. Also, after weighing the fruits, two pieces of fruit from different directions of each tree were taken to the laboratory to assess the quantity and quality of the fruits.



Chlorophyll concentration in both experiments was measured using the chlorophyll meter (model SPAD-502). This device shows the relative concentration of chlorophyll in accordance with the amount of light passing through the leaves in two wavelengths chlorophyll absorption varies. It should be noted that the number of SPAD does not specify chlorophyll but provides an estimation of chlorophyll concentration, and this number is highly correlated with the amount of chlorophyll. Measurement of chlorophyll was performed on 20 leaves (in different geographical directions) randomly selected from each treatment. Registration and measurement of chlorophyll concentration were conducted in three different dates, July 3, September 27, and December 20, 2016.

To calculate the weight of each fruit in the experiment, the harvested fruits from each replication were weighed using digital scale in grams. Then, an average for each fruit was calculated.

To calculate the diameter of each fruit in the treatments, a digital caliper was used and the diameter of the fruits was measured in millimeter.

To determine the diameter of each fruit skin in the treatments, the harvested fruits in each replicate and treatment were sliced into half by a sharp knife and then, using a digital caliper, the diameter of the skin of any fruit were taken and calculated in millimeter.

To obtain the extract of the fruits in the experiments, the sliced fruits were extracted by an electric juicer. To measure and record the amount of extract in mL, a scaled cylinder was used.

After the extraction of fruits, the obtained pulps from each treatment and replication were separately weighed and recorded in grams.

In northern Iran, the storage of the citrus fruits after harvest usually lasts for 3 to 4 months in the traditional stores. These warehouses do not follow any particular standard and are usually constructed in

accordance with the financial capacity and facilities of the gardeners. To investigate the effect of CRM damage on the storage of the fruits, five fruits were separately labeled from each treatment and packaged into special plastic bags designed for storing the fruits. Afterwards, the fruits were put into 20-kg baskets and placed in normal conditions of storage for a period of 4 months. The number of healthy and rotten fruits in each treatment and replication were recorded on a monthly basis.

### Analyzing Fruit Extract

To measure the impact of CRM damages on the quality of citrus fruit including the amount of TSS, pH, TA, EC and Vitamin C, extract of harvested fruits in each treatment and replication were separately passed through the filter paper to become completely smooth. Then, the considered qualitative characteristics on the extracts were measured in laboratory environment.

To measure the percentage of TSS in the fruit extract in each treatment and replication, one drop of the filtered extract was placed on a glass plate of the refractometer (Atago-ATC-20E model) and then the rate was read and recorded by the lens of the device.

To measure the pH of the fruit extract, a pH meter device was used. First, we added 10 mL of the extract of each treatment and replication separately into small test tubes. Then, the electrode of the pH meter was placed inside the test tubes after disinfection with distilled water. Afterwards, we recorded the number on the screen of the device. The pH meter indicates the acidity or basicity of the material.

To measure total titratable acidity or TA, 10 mL of fruit extract of each treatment along with 20 mL of distilled water and one drop of 0.1% phenolphthalein with 0.1 normal sodium hydroxide (NaOH) were titrated until a pale pink color appeared (AOAC, 2005). The amount of NaOH used

for titration was noted and the titratable acidity was calculated using the following equation:

$$\text{Titratable acidity (\%)} = \frac{a \times f \times 0.06 \times 100}{\text{sample size}} \quad (3)$$

Where, Sample size= 10 mL, Fixed factor ( $f$ )= 1,  $a$ = mL of used NaOH (0/1N).

To measure EC, EC meter device was used. For this purpose, 10 mL of fruit extract of the treatments was separately poured in small test tubes. The device electrode was put into the test tubes after washing with water, then, the EC of their extracts was recorded. This device displays the electrical conductivity in Siemens /meter (S/m).

To measure ascorbic acid or Vitamin C, titrimetric method was used along with iodine, potassium iodate, and potassium iodide in the presence of starch. The vitamin C content in fruit extract was calculated in mg per 100 mL (Arya, 2000).

### Statistical Analysis

The data obtained were recorded as mean±standard error. Data analysis was done using one-way ANOVA. If the means were significant, the comparison between the means of the data was obtained by Tukey test at 5%, using the Minitab 17.1.0.0 software.

## RESULTS

### CRM Population Density

The analysis of variance of CRM population density in the artificial infestation test showed significant differences in the treatments on the first sampling date (May 18, 2016) (Table 1). The highest population density is associated with the treatment 4 with an average of  $4.30 \pm 1.74$  CRM (different life ages) per leaf and the lowest population density was related to the control treatment with near zero infestation rate (Table 1).

In treatments of artificial infestation, CRM population data analysis before harvest

showed no significant difference in population in the treatments and all treatments were classified in a single group.

During the 19 sampling dates, variance analysis of total population of CRM in the treatments indicated that there were significant differences (Table 1). The highest number of CRM per leaf belonged to the treatment 4 with an average number of  $10.59 \pm 1.09$  and the lowest rate was observed in the control treatment with an average number of  $0.48 \pm 0.09$  CRM/leaf (Table 1).

CRM population analysis in the paired-treatment test showed that, in the first sampling date (May 18, 2016), CRM population was zero. However, analysis of pest population variance before harvest (November 6, 2016) and also the total mean of CRM population (the 17<sup>th</sup> date of sampling) indicated significant differences. The highest pest population at harvest time was related to non-control treatments, with an average number of  $5.93 \pm 1.5$ , and the highest number of CRM in the whole sampling period was related to the control treatment with an average of  $2.26 \pm 0.34$  CRM/leaf (Table 2). Thus, given the significance of CRM population in both tests, we achieved one of our important objectives in this study and continued to examine the impact of CRM damage on quantitative and qualitative characteristics of the fruits.

### Chlorophyll Analysis

Analysis of variance of leaf chlorophyll concentration in the treatments in Multiple-treatment test showed significant differences in treatments. The highest concentration of chlorophyll belonged to the control treatment, with an average of  $79.13 \pm 1.06$  and the lowest related to treatment 4, with an average of  $62.80 \pm 1.51$  (Table 1).

The analysis of leaf chlorophyll concentration in paired-treatments tests showed significant differences and the highest average of concentration of chlorophyll was related to the control

**Table 1.** Comparison of mean ( $\pm$ SE) of quantitative and qualitative parameters of Thomson navel orange, in a multiple-treatment design.<sup>a</sup>

Parameter <sup>b</sup>	Control (0.48 $\pm$ 0.09 CRM/leaf)	Treatment 1 (1.12 $\pm$ 0.3CRM/leaf)	Treatment 2 (5.72 $\pm$ 0.43CRM/leaf)	Treatment 3 (6.33 $\pm$ 1.8CRM/leaf)	Treatment 4 (10.59 $\pm$ 1.09CRM/leaf)	f	df	P
FSORM	0	1.70 $\pm$ 0.37 ab	0.85 $\pm$ 0.35 ab	0.30 $\pm$ 0.19 b	4.30 $\pm$ 1.74 a	4.47	4, 15	0.014
LSORM	0.05 $\pm$ 0.05 a	0.20 $\pm$ 0.11 a	0.25 $\pm$ 0.25 a	0.10 $\pm$ 0.1 a	0	0.61	4, 15	0.663
ATSORM	0.48 $\pm$ 0.05 c	1.12 $\pm$ 0.3 c	5.72 $\pm$ 0.43 b	6.33 $\pm$ 1.8 ab	10.59 $\pm$ 1.09 a	17.88	4, 15	<0.0001
COC	79.13 $\pm$ 1.06 a	71.55 $\pm$ 0.98 b	69.76 $\pm$ 1.3 b	68.83 $\pm$ 0.67 b	62.80 $\pm$ 1.51 c	26.42	4, 15	<0.0001
WOF	242.32 $\pm$ 22.7 a	241.40 $\pm$ 12.5 a	232.93 $\pm$ 8.65 a	214.39 $\pm$ 15.3 a	0	55.43	4, 15	<0.0001
DOF	2008.4 $\pm$ 60.70a	2023.6 $\pm$ 59.69 a	1925 $\pm$ 36.83 a	2058 $\pm$ 25.90 a	0	433.81	4, 15	<0.0001
TOSF	98.81 $\pm$ 3.556 a	93.22 $\pm$ 7.62 a	101.16 $\pm$ 7.62a	78.04 $\pm$ 7.112 a	0	46.13	4, 15	<0.0001
FE	91.38 $\pm$ 5.63 a	98.13 $\pm$ 5.53 a	96.75 $\pm$ 6.1 a	97.00 $\pm$ 3.54 a	0	82.32	4, 15	<0.0001
FW	88.09 $\pm$ 7.33 a	115.74 $\pm$ 9.67 a	98.67 $\pm$ 5.25 a	107.51 $\pm$ 5.25 a	0	54.39	4, 15	<0.0001
S	4.75 $\pm$ 0.25 a	5.00 $\pm$ 0 a	4.25 $\pm$ 0.47 a	2.25 $\pm$ 0.25 b	0	63.09	4, 15	<0.0001
pH	2.80 $\pm$ 0.06 a	2.73 $\pm$ 0.05 a	2.71 $\pm$ 0.06 a	2.83 $\pm$ 0.01 a	0	689.38	4, 15	<0.0001
EC	0.331 $\pm$ 0.006a	0.305 $\pm$ 0.002 b	0.316 $\pm$ 0.005 ab	0.305 $\pm$ 0.007 b	0	683.71	4, 15	<0.0001
TA	0.94 $\pm$ 0.051 b	1.06 $\pm$ 0.051 ab	1.18 $\pm$ 0.037 a	1.00 $\pm$ 0.028 b	0	152.25	4, 15	<0.0001
TSS	9.12 $\pm$ 0.125 b	9.50 $\pm$ 0.289 b	10.87 $\pm$ 0.125 a	10.37 $\pm$ 0.239 a	0	592.14	4, 15	<0.0001
VC	80.96 $\pm$ 2.45 ab	69.52 $\pm$ 5.26 b	81.40 $\pm$ 1.11 ab	85.65 $\pm$ 0.83 a	0	181.38	4, 15	<0.0001

<sup>a</sup> The means followed by different letters in the row are significantly different ( $P < 0.05$ , Tukey), <sup>b</sup> The term has meanings including: CRM/Leaf (Citrus Red Mite number per leaf), FSORM (First Sampling Of CRM, different life ages), LSORM (Last Sampling Of CRM, different life ages), ATSORM (Average Total Samples Of CRM, different life ages), COC (Concentration Of Chlorophyll), WOF (Weight Of Fruit, g), DOF (Diameter Of Fruit, mm), TOSF (Thick Of Skin Fruit, mm), FE (Fruit Extract, mL), FW (Fruit Waste, g), S (Storage), pH (Potential of hydrogen), EC (Electric meter/Siemens/meter), TA (Titratable acidity, g L<sup>-1</sup>), TSS (Total Soluble Solids, %), VC (Vitamin C, Mgr $\times$ 100 mL).

treatment with an average of  $82.41 \pm 0.42$  in (Table 2).

### Quantitative Characteristics of Thomson Novel Orange

The analysis of storage period of the fruits harvested in a multiple-treatment experiment showed that the treatments were significantly different, and the shortest storage period was associated with the treatment 3 with an average number of  $2.25 \pm 0.25$  healthy fruits during 4 months. The control treatment and treatments 1 and 2 were commonly categorized in a single group with average of, respectively,  $4.25 \pm 0.47$ ,  $5.00 \pm 0$ , and  $4.75 \pm 0.25$  healthy fruits (Table 1). It is notable that the yield of the treatment 4 in the citrus orchard was zero. In this treatment, all fruits and leaves

were gradually lost and many twigs dried up.

The analysis of fruit weight, fruit diameter, diameter of the fruit skin, fruit extract and fruit waste weight measured in the multiple-treatment method showed that there were no significant differences in the treatments.

Variance analysis of showed that the treatments were significantly different in terms of fruit weight, fruit diameter and extract, and the weight of fruit waste. Maximum mean weight per fruit, diameter of the fruit, and fruit extract were observed in non-spraying treatment with average of, respectively,  $228.1 \pm 11$  g,  $78.24 \pm 1.2$  mm, and  $98.8 \pm 6.2$  mL. The maximum fruit waste weight per fruit belonged to the control treatment with an average of  $80.5 \pm 6.1$  g. However, the analysis of yield, fruit skin diameter, and storage revealed no significant differences (Table 2).

**Table 2.** Comparison of mean ( $\pm$  SE) of quantitative and qualitative parameters of Thomson navel orange, in a paired-treatment design.

Parameter <sup>a</sup>	No spraying	Sprayed	t (t-value)	df	P-value
FSORM	0	0	0	0	0
LSORM	$5.93 \pm 1.5$	$0.180 \pm 0.092$	3.71	1, 19	0.001
ATSORM	$2.26 \pm 0.34$	$1.012 \pm 0.19$	3.19	1, 30	0.003
COC	$77.83 \pm 1.2$	$82.41 \pm 0.42$	-3.69	1, 23	0.001
WOF	$228.1 \pm 11$	$177.6 \pm 11$	3.30	1, 37	0.002
DOF	$1987 \pm 29$	$1787 \pm 33$	4.63	1, 37	<0.0001
TOSF	$110 \pm 05$	$98 \pm 04$	1.86	1, 31	0.073
FE	$98.8 \pm 6.2$	$78.3 \pm 4.6$	2.66	1, 35	0.012
FW	$51.9 \pm 3.0$	$80.5 \pm 6.1$	-4.20	1, 27	<0.0001
S	$4.350 \pm 0.22$	$4.700 \pm 0.069$	-1.49	1, 16	0.156
Y	$11.1 \pm 4.5$	$9.15 \pm 1.7$	0.41	1, 5	0.699
pH	$2.852 \pm 0.027$	$2.882 \pm 0.024$	-0.83	1, 37	0.410
EC	$0.318 \pm 0.005$	$0.361 \pm 0.006$	-5.52	1, 35	<0.0001
TA	$1.044 \pm 0.023$	$1.0740 \pm 0.021$	-0.96	1, 37	0.342
TSS	$10.275 \pm 0.16$	$9.575 \pm 0.12$	3.48	1, 34	0.001
VC	$79.02 \pm 1.9$	$69.60 \pm 1.9$	3.46	1, 37	0.001

<sup>a</sup> Symbols are defined under Table 1.



### Qualitative Characteristics of Thomson Novel Orange

The analysis of quality including pH, EC, TA, TSS and vitamin C in the multiple-treatment experiment showed no significant difference in the treatments in terms of pH, but the difference was significant in other characteristics (Table1). The highest EC ratio was observed in the control treatment with an average of  $0.331 \pm 0.006$  S/m and the other treatments were in the same group. Comparison of the mean of TA traits showed that the treatments were significantly different and the highest TA was related to the treatment 2 with an average of  $1.18 \pm 0.037$  g L<sup>-1</sup> and the lowest rate was related to the control treatment with an average of  $0.94 \pm 0.051$  g L<sup>-1</sup> (Table1). Comparison of the means of TSS also showed that the treatments were significantly different and the highest TSS was observed in the treatments 2 and 3, with an average of  $10.87 \pm 0.125$  and  $10.37 \pm 0.239$  g L<sup>-1</sup>, respectively, which were jointly classified in a similar group. The control treatment and treatment 1 with an average of  $9.12 \pm 0.125$  and  $9.50 \pm 0.289$  g L<sup>-1</sup>, respectively, were in the similar group (Table 1). Comparison of the means of vitamin C indicated that the treatments were significantly different and the highest vitamin C content was related to the treatment 3, with an average of  $85.65 \pm 0.83$  mg 100 mL<sup>-1</sup> (Table 1).

Analysis of qualitative traits including pH, EC, TA, TSS and vitamin C in the paired-treatment experiment showed that treatments were not significantly different in pH and TA characteristics. However, for other qualitative characteristics, the treatments were significantly different (Table2). Therefore, according to Table 2, the measured EC in the treatments were significantly different and the highest EC was related to the control treatment with an average of  $0.361 \pm 0.006$  S/m and the lowest was related to non-controlled treatment with an average of  $0.318 \pm 0.004$  S/m. Moreover, the highest mean of TSS was observed in the non-controlled treatment ( $10.275 \pm 0.16$ ). Regarding vitamin C, the

highest rate was close to non-controlled treatment, with an average of  $79.02 \pm 1.9$  mg 100 mL<sup>-1</sup> (see Table 2).

### Regression/Correlation

The results of relationship (regression analysis) between CRM population in multiple-treatment test with 19 sampling dates and quantitative and qualitative characteristics of the Thomson navel orange showed a significant negative relationship between the average CRM population (at different life ages) and concentration of chlorophyll ( $b = -1.2895$ ,  $r^2 = 0.774$ ,  $P = 0.031$ ). The negative value of  $b$  indicates that when the CRM population increases, chlorophyll concentration decreases. Also, there was a significant negative relationship between the average population of CRM and storage period of fruits ( $b = -0.4694$ ,  $r^2 = 0.80$ ,  $P = 0.026$ ). The negative value of  $b$  suggests that when CRM population grows, storage life of the fruits decreases (Table 3). Furthermore, there was no significant linear relationship between CRM population and other measured parameters.

The results of examining the means of the CRM population and correlation coefficients between the measured characteristics are shown in Table 3. Among the examined parameters, chlorophyll concentrations ( $r = -0.911$ ,  $P = 0.031$ ) and storage life of fruit ( $r = -0.922$ ,  $P = 0.026$ ) showed a significant negative correlation in the experiment. However, other measured parameters showed no significant correlation with CRM population.

### DISCUSSION

Investigating the population density of CRM on Thomson navel oranges in multiple-treatment and paired-treatment experiments showed that the pest density at the beginning of the season was very important in causing damage. Our findings showed that the treatments with average annual population density of 10.275 per leaf cause severe damage. Therefore, decreasing



the infestation of the treatment to an average of 1.12 CRM/leaf, during the year, the least amount of damage was created.

The comparison of the number of different life ages of CRM in the sampling dates showed that, at the beginning of the season, due to the low population of CRM per leaf, less damage was caused to leaf chlorophyll in the paired-treatment test. In general, regarding the results obtained from the study of the effect of population density of CRM on chlorophyll concentration of Thomson novel orange leaves, in both methods, the time of infestation and the density of different life ages of CRM were effective in the severity of damage to the host leaves. The assessment studies conducted on damage by tetranychid mites on citrus leaves during 1996-1999 in Florida showed a significant negative relationship between leaf life and mortality rate of these mites (Hall and Simms, 2003). Based on these results, the physical damage to the citrus leaves caused by tetranychid mites may be very different according to mite density. In examining the effect of damages caused by the pest mites *Panonychus citri*, *P. ulmi*, *Tetranychus pacificus* McGregory, and *T. urticae* on the leaf gas exchange in almond leaf, researchers found out that nutrition damage by these pests caused significant reduction in the stomatal

conductance, mesophyll conductance, and photosynthesis, compared with the control treatment without pests (Hare and Youngman, 1987).

The results of the present study on fruit yield attributes indicated that the yield was zero at high CRM density (10.59 CRM per leaf at different life ages). Furthermore, the storage life of the fruits in the warehouse was significantly different in the treatments and the minimum storage time was related to the treatment with a mean population of 6.33 CRM per leaf. However, the yield of the product did not significantly decrease. Because of the gradual loss and fall of some of the fruits of the infested trees, other remaining fruits gain weight and, ultimately, compensate for the loss of yield. A review of the studies conducted in this field showed that the study done by Hare *et al* (1990) in the US is in line with our results. They showed that the population peak of CRM per leaf of the tree without spraying was 5-8 mature females. This caused a significant reduction in yield (~10%). The results of the mentioned study showed that the average size of fruits per tree increases with the increase in number of mites (Hare *et al.*, 1990).

The review of the studies also showed that CRM is an indirect pest of citrus fruit and rarely feeds on fruits. This pest feeds on the leaves of host plants and ultimately

**Table 3.** Regression and correlation between the total population of the CRM measured by quantitative and qualitative parameters.

Parameter <sup>a</sup>	a	b	r <sup>2</sup> <sub>reg</sub>	r <sub>cor</sub>	P <sub>reg</sub>
COC	76.672	-1.2895	0.774	-0.911	0.031
WOF	283.37	-20.040	0.509	-0.795	0.108
DOF	2418	-168.0	0.472	-0.777	0.122
TOSF	114.7	-8.35	0.555	-0.816	0.092
FE	114.41	-7.785	0.422	-0.753	0.142
FW	122.15	-8.278	0.380	-0.731	0.160
S	5.5266	-0.4694	0.80	-0.922	0.026
Y	17.734	-1.2528	0.327	-0.704	0.185
pH	3.3288	-0.230	0.458	-0.770	0.127
EC	0.3819	-0.2691	0.504	-0.793	0.110
TA	1.2321	-0.0809	0.324	-0.702	0.186
TSS	11.520	-0.731	0.270	-0.673	0.213
VC	92.7	-6.02	0.308	-0.694	0.194

<sup>a</sup> Symbols are defined under Table 1.



reduces the yield by reducing photosynthesis (Hare and Youngman, 1987). In case of extreme feeding by CRM on leaves, branches and fruits cause the postponement of fruit growth, yield loss, and even loss of tree leaves (Hare and Youngman, 1987). Moreover, CRM feeding on leaf cell contents reduces plant photosynthesis and can potentially decrease the quality of the fruits (Prischmann *et al.*, 2005; Liu, 1992).

The results of qualitative characteristics of the fruits including EC, TA, TSS and vitamin C in our experiments showed that, with annual average increase in CRM population density (above 5.72 per leaf), TSS, TA, and vitamin C increased in fruit extract, but EC declined. The important indices in citrus fruit harvest are the percentage of Soluble Solids (TSS), percentage of Titratable Acidity (TA), and TSS/TA. Thus, in case of a tension caused by CRM on the orange trees, the percentage of TSS of fruits rises and fruits are ready for harvest earlier, but they have shorter storage life compared with the healthy ones.

Direct observations of the fruits showed that the skin of the fruits with a high density of CRM were yellow and ready for early harvest, while the fruit skins in the treatments with lower infestation were yellowish green. The results of research conducted in other citrus-growing parts of the world also showed that, in case of a severe attack of this pest to citrus, quantity and quality (weight, sugar content and appearance) of the citrus crop will be diminished (Liu, 1992).

There was a significant relationship in the regression analysis between the CRM population and chlorophyll concentration ( $r^2= 0.774$ ,  $P= 0.031$ ) and storage life ( $r^2= 0.80$ ,  $P= 0.026$ ), indicating a linear relationship between CRM population mean and these parameters. Thus, the significant linear relationship between variables indicates a severe impact of CRM population on chlorophyll concentration and storage life in the treatments. The results of the correlation analysis between the

mentioned variables were the same as mentioned for the regression analysis. According to the results of regression and correlation, we can conclude that if the population of CRM exceeds 5.72 per leaf, chlorophyll concentration will sharply decline. Consequently, it will reduce the storage life of Thomson navel orange.

Overall, our results revealed that the increase of CRM population with an average number of 10.59 per leaf during the Thomson navel orange growing season causes severe and complete fruit loss. In addition, this will bring about full falling fruits, early fall of the leaves and dieback of the young branches. If the CRM population density increases to the seasonal average of 5.72 per leaf, storage life of fruits and the qualitative characteristics of Thomson novel orange will diminish. Therefore, to prevent the mite-affected fruits from rotting in warehouses, it is suggested that such fruits be offered to the market for sale to be consumed freshly.

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## بررسی تاثیر خسارت کنه قرمز مرکبات روی صفات کمی و کیفی پرتقال تامسون ناول

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### چکیده

کنه قرمز مرکبات (*Panonychus citri* (McGregor)) یکی از آفات مهم مرکبات در جهان است. برای بررسی تاثیر خسارت کنه قرمز بر روی صفات کمی و کیفی پرتقال تامسون ناول *Citrus sinensis* (L.) Osbeck، از آزمایشات زوج تیماری، چند تیماری و روش آماری رگرسیونی و همبستگی استفاده گردید. نتایج تجزیه واریانس داده ها نشان داد که میانگین های جمعیت کنه قرمز در هر برگ و غلظت کلروفیل برگ، انبار مانی میوه ها، درصد ماده خشک و ویتامین ث عصاره میوه تیمارها تفاوت معنی دار دارند. تیمار شاهد در آزمایش زوج تیماری با میانگین  $0.48 \pm 0.09$  عدد مراحل مختلف سنی کنه در برگ، بیشترین میزان غلظت کلروفیل در برگ  $79.13 \pm 1.06$  را داشته و در صورت افزایش جمعیت کنه قرمز به  $10.59 \pm 1.09$  عدد در برگ (آزمایش چند تیماری)، غلظت کلروفیل به میزان  $62 \pm 1.151$  کاهش می یابد. همچنین نتایج نشان داد که میزان انبار مانی میوه ها در تیمارهایی با جمعیت  $6.33 \pm 1.8$  عدد کنه در برگ، کاهش معنی داری دارند. نتایج محاسبات همبستگی و رگرسیونی بر روی غلظت کلروفیل ( $r^2 = 0.774, p = 0.031, r = -0.911$ ) و انبار مانی میوه های تیمارها ( $r^2 = 0.80, p = 0.026, r = -0.922$ ) معنی دار و نشان از رابطه منفی و معنی دار بین این صفات و جمعیت کنه قرمز مرکبات است. نتایج این تحقیق نشان داد که در صورت افزایش جمعیت کنه قرمز به تعداد  $10.59 \pm 1.09$  عدد در هر برگ در ابتدای فصل، باعث ریزش میوه ها، خشک شدن سر شاخه ها و خزان زودهنگام برگ ها می شود. همچنین با افزایش تراکم جمعیت کنه قرمز به  $5.72 \pm 0.43$  عدد مراحل مختلف سنی در هر برگ، انبار مانی میوه ها کاهش معنی داری پیدا می کند. لذا جهت پیشگیری از فاسد شدن میوه های کنه زده در انبارها، پیشنهاد می گردد که این نوع میوه ها را به بازار عرضه کرده تا به صورت تازه خوری مورد استفاده قرار گیرند.