CHLOROPHYLL LOSS PERCENTAGE AS INDICATOR FOR CALCULATING ECONOMIC THRESHOLD AND ECONOMIC INJURY LEVELS OF *PARLATORIA ZIZIPHI* (LUCAS) ON NAVEL ORANGE LEAVES

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ABSTRACT: *This study was carried out in Scale Insects and Mealybugs Department, Plant Protection Research Institute, Sharkia Branch, ARC, Egypt from May to September 2017 on navel orange leaves infested with the Parlatoria black scale, Parlatoria ziziphi (Lucas) to calculate economic threshold and economic injury levels depending on the loss of leaves chlorophyll content. Simple correlation and partial regression were calculated between numbers of insect and chlorophyll loss percentage in infested and uninfested units (inch^2). Positive highly significant correlation was recorded between numbers of insect and percentage of chlorophyll loss. Chi-square (X^2) was used to calculate economic threshold and economic injury levels of P. ziziphi that causes economic damage in disk chlorophyll content which were 9 and 14 individuals per inch^2, respectively.*

Key words: *Sap-sucking, hemipterans, Parlatoria black scale, chlorophyll, Simple correlation, partial regression*

INTRODUCTION

Scale insects are some of the most unusual hexapod known. Their common name derives from the frequent presence of a protective covering or 'scale' or from the appearance of the insects themselves. Scale insects or coccoids (Coccoidea) are sap-sucking hemipterans insects. They are most important as agricultural pests of perennial plants and can cause serious damage to fruit trees, woody ornamentals, forest vegetation, greenhouse plants, and house plants. Main damage is usually caused by removal of plant sap, but also may be caused by plant pathogens transmission, injection of toxins and the production of large quantities of honeydew with resultant growth of sooty mold fungi that cover leaf surfaces and reduce photosynthesis. More damages are distortion of foliage, discoloration of flowers, galls and tumors formation, distorted blossoms, reduction in the general vigor of the trees, reduction in quality and salability of fruits, dieback of twigs and sometimes the death of the entire plant (Elhendawy, 2018).

Herbivore caused leaf chlorosis in growing plants should be studied in detail, as chlorophyll fluorescence might prove useful as an indicator of plant responses to stressors including insect damage (Haile *et al.*, 1999). Chlorophyll content is one of the most important parameters in the relationships between plants and herbivores. Chlorophyll levels change during plant development (Costa *et al.*, 2001), and can alter in response to a wide variety of stresses (Fanizza *et al.*, 1991, Samdur *et al.*, 2000, Lawson *et al.*, 2001).

Leaf feeding by sap feeding insects causes chlorosis and necrosis, leading to significant crop loss worldwide. Chlorophyll catabolism is equal to global chlorophyll synthesis, and can be reduced by insect feeding, nutritional

deficiencies and pathogen infections (Ni *et al.*, 2001 &2002).

Few studies have been conducted to assess the effect of feeding insects on chlorophyll levels such as aphids and scale insects (Golawska *et al.*, 2010, Nabil and El-Shafiey, 2011 and Nabil, 2013). Whoever, till now no similar studies have been conducted to assess the effect of feeding by Parlatoria black scale, *Parlatoria ziziphi* (Lucas) on chlorophyll levels, this research represents an initial effort to characterize the effect of *P. ziziphi* on chlorophyll *a* and *b* loss in navel orange leaves.

This work aimed to determine the concentrations of photosynthetic pigment (chlorophyll *a* and *b*) in infested and uninfested navel orange leaves to calculate economic threshold and economic injury levels for *P. ziziphi* on navel orange leaves. Economic threshold and economic injury level numbers were very important numbers because between those chemical control must be took place.

MATERIALS AND METHODS

Experiments were carried out at Scale Insects and Mealybugs Department, Plant Protection Research Institute, Sharkia Branch, ARC, Egypt on navel orange, *Citrus sinensis* (L.) to determine the loss of chlorophyll content in navel orange leaves by infestation with the Parlatoria black scale, *Parlatoria ziziphi* (Lucas).

The study was carried out from May to September 2017, in an area of about one feddan of navel orange. For sampling, mature leaves were picked up randomly and put in black polyethylene bags and transferred into the laboratory. The leaves were divided into disks (square inch) were examined in the same day by the aid of stereomicroscope. The adult females of *P. ziziphi* were counted and recorded (Nabil and El-Shafiey, 2011 and Nabil, 2013).

The chlorophyll content average (chlorophyll *a* and chlorophyll *b*) in 25 disks (inch^2) of navel orange leaves for every level of infestation and uninfested disks as control were extract and determine spectrophotometrically according to (Moran, 1982) as following:

The immersion leaflet disks in 5 ml of N, N-Dimethyl formamide at least 48 hrs. Concentration of chlorophyll *a* and *b* were determined spectrophotometricaly from absorbance at 647 and 664 nm, respectively. The pigment concentrations were calculated using the following equations:-

Chlorophyll *a* (mg/ inch^2) = 12.64 × E664 – 2.99 × E647

Chlorophyll *b* (mg/ inch^2) = 23.26 × E647 – 5.6 × E664

Total chlorophyll (mg/ inch^2) = Chlorophyll *a* + Chlorophyll *b*

Simple correlation (r) between number of insects per disk and percentage of chlorophyll loss was studied using COSTAT Computer Program (2005).

According to Little and Hills (1972), Salem and Zaki (1985), Hassan (1998), Nabil and El-Shafiey (2011) and Nabil (2013) the partial regression formula "C-multipliers" independent variance (X) represent the scale insects per disk, the dependent variance (Y) represent percentage of chlorophyll loss per disk. Simple correlation (r) and simple regression (b) were calculated. The slope (b) of the straight regression line was carried out to obtain the corrected values for the percentage of chlorophyll loss.

Chi-square (X^2) was used to calculate economic threshold and economic injury levels for *P. ziziphi* that causes economically damage in disk chlorophyll content.

RESULTS AND DISCUSSION

Data presented in Table (1) and Fig. (1) revealed that percentage of chlorophyll

content loss per unit was significantly declined with increasing of insect numbers. There was positive highly significant difference between number of insects per disk and percentage of chlorophyll loss where (r) value was 0.960[**].

Simple correlation and partial regression and chi square were used to calculate economic threshold and economic injury levels, Chi-square analysis was applied for mathematic determination of the point at which the increase of insect number caused a significant effect on chlorophyll loss percentage. Results revealed that economic threshold and economic injury levels were 9 and 14 insects per disk, respectively.

Results showed that there was positive highly significant between number of females and percentage of chlorophyll loss.

Table (1): Numbers of insect, *Parlatoria ziziphi* (Lucas) and percentage of chlorophyll loss on navel orange leaves per disk and chi square values

No. of females/ inch^2 (X)	Chlorophyll content (mg / 2 inch^2)	Chlorophyll loss (mg / inch^2) (Y)	% loss (Y$_{Ob}$)	Calculated (Y$_{Ex}$) %	$\dfrac{(Y_{Ob} - Y_{EX})^2}{Y_{EX}}$	$\sum X^2$
1	43.374	3.775	8.01	16.37	4.26	4.26
2	42.860	4.289	9.10	17.54	4.06	8.32
3	41.241	5.908	12.53	18.70	2.04	10.36
4	40.242	6.907	14.65	19.87	1.37	11.73
5	38.174	8.975	19.04	21.03	0.19	11.92
8	35.300	11.849	25.13	24.53	0.01	11.93
9	32.336	14.813	31.42	25.69	1.28	13.28
14	31.278	15.871	33.66	31.52	0.15	13.36
16	27.878	19.271	40.87	33.85	1.46	14.82
21	25.389	21.760	46.15	39.67	1.06	15.88
25	21.521	25.628	54.36	44.33	2.27	18.15
31	18.380	28.769	61.02	51.32	1.83	19.98
39	14.569	32.580	69.10	60.64	1.18	21.16
53	13.600	33.549	71.16	76.95	0.44	21.60
57	12.265	34.884	73.99	81.60	0.71	22.31
61	9.576	37.573	79.69	86.26	0.50	22.81
Control	47.149					

r = 0.960**   Y= 15.21 + 1.16 X

The chart shows a line graph with Y-axis labeled "Percentage of chlorophyll loss" ranging from 0 to 100, and X-axis labeled "No. of adult females / square inch" ranging from 0 to 70. The equation displayed is:
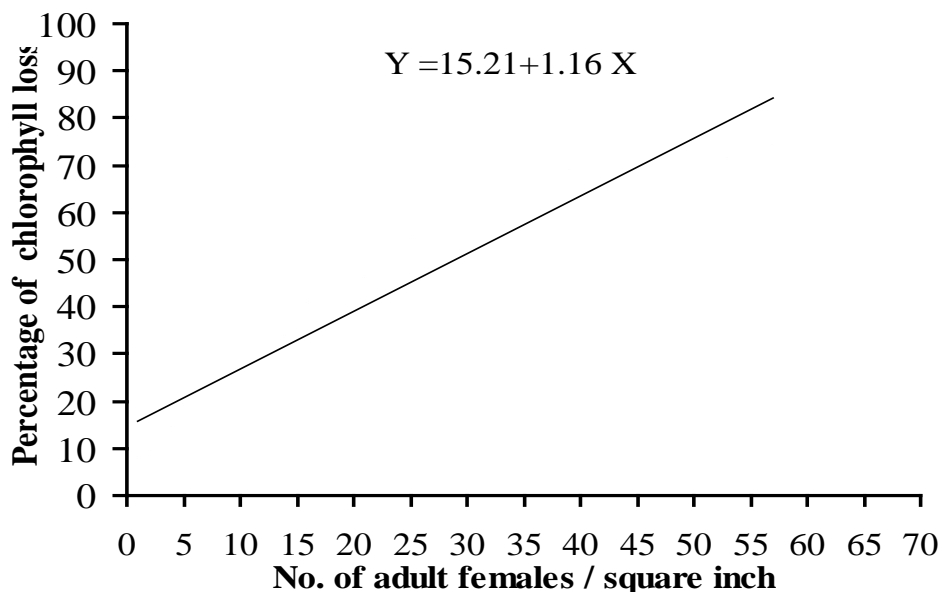
$$Y = 15.21 + 1.16\ X$$

Fig. (1): Relationship between insect numbers of *Parlatoria ziziphi* (Lucas) per disk and percentage of chlorophyll loss.

This result was agreed with those of Golawska *et al.* (2010) who determined the chlorophyll *a* and *b* levels in uninfested leaves and in leaves after 7 and 17 days of aphid infestation in four Fabaceae species (*Pisum sativum* L., *Vicia faba* L., *Trifolium pretense* L, *Medicago sativa* L.). Feeding by pea aphids *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) caused significant loss of chlorophyll *a* and *b* in the infested plants. Uninfested leaves on both short and long infestation plants had significantly higher chlorophyll *a* and *b* than infested leaves. In this study the amount of chlorophyll differed between infested and uninfested navel orange leaves. The chlorophyll *a+b* concentration in uninfested leaves was significantly higher than infested ones.

Nabil and El-Shafiey (2011) studied the reduction on chlorophyll concentration between infested and uninfested mango leaves with white mango scale insect, *Aulacaspis tubercularis* (Newstead), the uninfested leaves had higher levels of chlorophyll in comparison with infested ones. Economic threshold and economic injury levels were determined depended on the loss percentage of total chlorophyll on mango leaves were 3 and 5 insects per inch^2, respectively.

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النسبة المئوية للفقد فى الكلوروفيل كمؤشر لحساب الحد الاقتصادى الحرج وحد الضرر الاقتصادى لحشرة (Lucas) *Parlatoria ziziphi* التى تصيب أوراق البرتقال أبوسرة

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الملخص العربي

أجريت هذه الدراسة بقسم بحوث الحشرات القشرية والبق الدقيقى بمعهد بحوث وقاية النباتات فرع الشرقية بمركز البحوث الزراعية فى الفترة من مايو وحتى سبتمبر ٢٠١٧م على اوراق البرتقال أبوسرة والمصاب بحشرة *Parlatoria ziziphi* (Lucas) لحساب الحد الاقتصادى الحرج وحد الضرر الاقتصادى اعتماداً على النسبة المئوية للفقد فى الكلوروفيل.

حيث تم عمل علاقة ارتباط وانحدار بين اعداد الحشرة والنسبة المئوية للفقد فى الكلوروفيل فى وحدات (بوصة مربعة) مصابة وأخرى غير مصابة بالحشرة وقد وجد أن هناك علاقة طردية عالية المعنوية مابين اعداد الحشرات ونسبة الفقد فى الكلوروفيل.

ومن خلال استخدام مربع كاى تم حساب الحد الاقتصادى الحرج وحد الضرر الاقتصادى وسجلا ٩ ، ١٤ حشرة/ بوصة مربعة على الترتيب.