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RESEARCH ARTICLE

REDUCTION OF MAJOR PHOTOSYNTHETIC PIGMENTS INDUCED BY *OLIGONYCHUS COFFEAE* (NIETNER) (ACARI: TETRANYCHIDAE) INFESTING *CAMELLIA SINENSIS* (L) O. KUNTZE

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ABSTRACT

Tea, *Camellia sinensis* (L) O. Kuntze, is one of the most popular beverages in the world. *Oligonychus coffeae* (Nietner) has been recognized as a serious pest of tea. The feeding activity of this mite on the leaves of tea induced drastic reduction in the levels of major photosynthetic pigment, chlorophyll. Chlorophyll is an essential element of photosynthesis and its content in plant leaves indicates their photosynthetic capacity. The present study elucidates the damage potential of *O. coffeae* on tea.

INTRODUCTION

Tea, *Camellia sinensis* (L) O. Kuntze, is one of the most popular beverages in the world. Presently tea is cultivated in 54 countries. India is the world's second largest tea producer accounting for more than 20% of total tea production. The crop suffers from the attack of a number of pests and pathogens, causing significant yield losses. Mites are one of such pests and infest tea plants throughout the world. Tea is found attacked by 12 species of mites and the total yield loss from mites had been reported to reach 18% during periods of severe infestation (Anitha Roy *et al*., 2008). The red spider mite (RSM), *Oligonychus coffeae* (Nietner), is an important pest of tea which causes considerable crop loss in South India (Jeppson *et al*., 1975; Muraleedharan *et al*., 2005; Babu *et al*., 2008) and infestation by mite often lead to 5-15% yield loss in India (Anitha Roy *et al*., 2008). This mite is characterized by a high reproductive capacity, which leads to high population levels in a short time, causing important economic damage (Das, 1959; 1960).

Nymphs and adults of *O. coffeae* (Nietner), normally infests the upper surface of mature tea leaves and lacerate cells, producing minute characteristic reddish brown marks and when severity of infestation increases they move even to the lower surface of older leaves and tender tea shoots. Severe infestation ultimately leads to defoliation (Selvasundaram and Muraleedharan, 2003). Chlorophyll is an essential element of photosynthesis and its content in plant leaves indicates their photosynthetic capacity. The chlorophylls- Chl *a* and Chl *b* are the most important among plant pigments, and are thus virtually essential for the oxygenic conversion of light energy to the stored chemical energy that powers the biosphere (Groff *et al*., 1995). The present experiment was carried out so as to study the effect of *O. coffeae* infestation on the variation in chlorophyll content of tea leaves.

MATERIALS AND METHODS

Random sampling of the leaves of *O. coffeae* was carried out from various localities of Wayanad district of Kerala, India, for a period from December 2013 to February 2014. Leaf samples were collected based on visible damage symptoms like the presence of reddish brown marks. Presence of mites in the leaves was confirmed by using a hand lens. The collected samples were carried to the laboratory for subsequent microscopic observation under a Stereozoom Microscope (Leica, Germany) for recording the presence of various life stages of *O. coffeae*. No other inhabitants could be noticed during field sampling. Nine samples each of experimental

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(heavily infested) and control (uninfested) were used for the quantitative analysis of chlorophyll.

Laboratory breeding

Qualitative and quantitative assessment of the damage induced by *O.coffeae* on the leaves of *C. sinensis* was made by rearing the species in the laboratory under constant temperature and humidity conditions. Live specimens comprising 20-25 adults of *O. coffeae* were transferred to fresh uninfested leaves of *C. sinensis*. Rearing of live mites was carried out in the laboratory by placing these leaf samples containing mite specimens on moistened cotton pads kept in Petri dishes. Regular observation was carried out under the microscope to record the development of feeding symptoms on the leaf samples. Three replicates were maintained for each culture set in order to confirm the results. Laboratory rearing was carried out at a temperature of 30^{o}C and a relative humidity of 65 by keeping the Petri dishes in an incubator.

Estimation of Chlorophyll

Estimation of the pigments was done according to the protocol advocated by Arnon (1949). Fresh leaves of control as well as experimental plants were collected for analysis, washed with water and blotted between sheets of filter paper. To estimate chlorophyll, chilled 80% acetone was used as the extraction medium. Enough precautions were taken to avoid any exposure of the extract to light. 0.1g of fresh leaf sample was weighed in an electronic balance (Sartorius, Germany). It was then powdered using liquid nitrogen, crushed with the help of mortar and pestle in 20 ml of 80% acetone (v/v, Merck, India). Then the homogenate was centrifuged at 5,000 rpm for 10 minutes in a cooling centrifuge at 4^{o}C (Sigma, Germany) and the supernatant was collected in a polypropylene tube (Tarsons, India). The residue was again washed with 80% acetone and centrifuged again. The process was repeated till the pellet became colour less. The final volume of the pooled supernatant was noted. The absorbance was read at 663 nm and 645 nm against the solvent blank (80% acetone) using a UV visible spectrophotometer (Thermo scientific, USA). Then the amount of chlorophyll present in the extract was calculated using the following formulae adopted from Arnon (1949), Manuela *et al*. (2005), Molazem *et al*. (2010) and Khaleghi *et al*. (2012). The concentration of chlorophyll pigments are expressed in mg/g fresh weight of the leaf tissue.

$$\text{Chlorophyll } a \text{ (mg/g)} = \frac{[12.7x(A663)-2.69x(A645)]xV}{(1000\,xW)}$$

$$\text{Chlorophyll } b \text{ (mg/g)} = \frac{[22.9x(A645)-4.68x(A663)]x\,V}{(1000x\,W)}$$

$$\text{Chlorophyll total (mg/g)} = \frac{[20.2x(A645)+8.02x(A663)]x\,V}{(1000\,xW)}$$

Where, W is the fresh weight of the leaf sample taken, V is the total volume of the sample solution.

Statistical analysis

Statistical analysis of the data on chlorophyll loss was carried out using t-test to evaluate the significance.

RESULTS AND DISCUSSION

Results of field studies helped to recognize the infestation by *O. coffeae* on the upper surface of the leaves of *C. sinensis*, near the midrib or veins, as previously mentioned by Roy *et al*. (2012). The observations showed mite infestation was highest on mature leaves as reported by Selvasundaram *et al*. (2003). On binocular stereo zoom microscopic observation, the infested leaves collected from the field disclosed the presence of large number of reddish brown spots due to the feeding activity of the mite. The feeding punctures were often found coalesced to form bronzy areas. Heavy infestation caused acute chlorosis of the leaves forming necrotic areas on the lamina (Figures 1&2).

Figure 1 *Oligonychus coffeae*

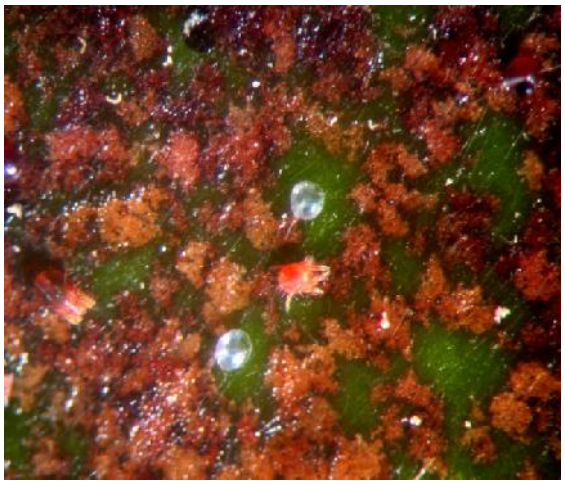


Figure 2 Infested area on leaf

Leaves bearing such symptoms were found to harbour adults, nymphs and larvae of *O. coffeae*. Such heavily infested leaf tissues when subjected to quantitative analysis by the estimation of chlorophyll content revealed drastic reduction in both Chorophyll *a* and Chlorophyll *b* contents (Table 1). As shown in Table 1, chlorophyll *a* content of heavily infested leaves showed a drastic decline ranging from 57.82% to 63.28% and the reduction in chlorophyll *b* ranged from 31.18% to 56.25%. The reduction in total chlorophyll content varied from 9.78% to 45.28%. These data when subjected to statistical analysis (t-test) were found significant at 0.05 levels (Table 2).

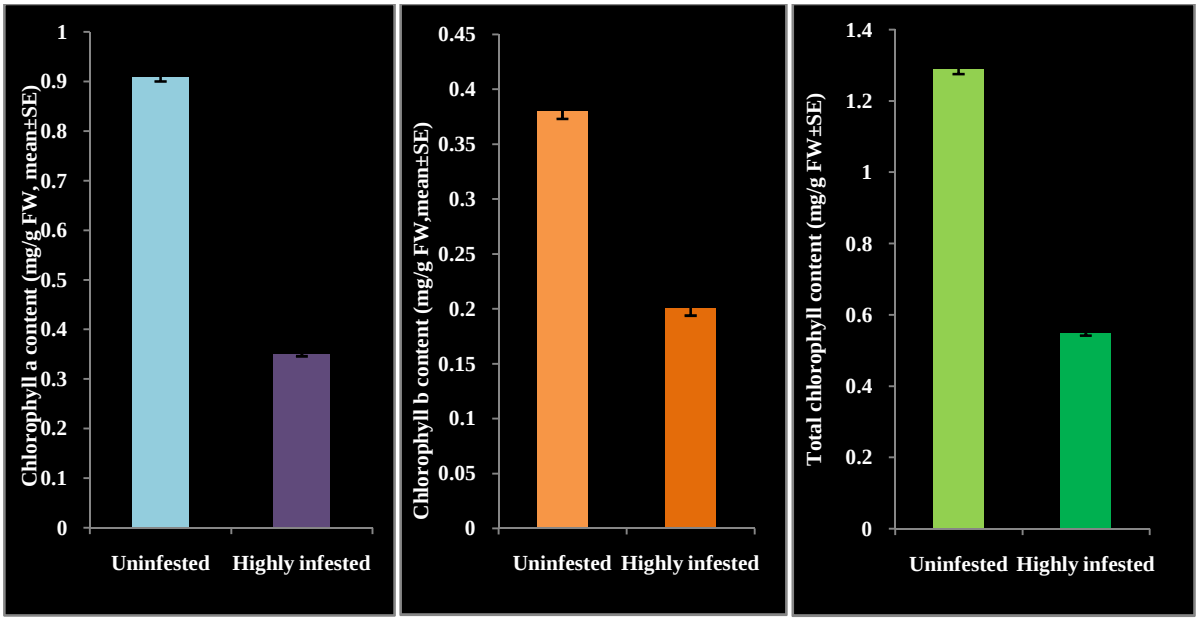
Table 1 Quantitative variation in Chlorophyll *a*, Chlorophyll *b* and total chlorophyll in infested and control leaves.

	Chlorophyll a			Chlorophyll b			Total Chlorophyll		
No	Non infested	Heavily infested	Percentage of reduction	Non infested	Heavily infested	Percentage of reduction	Non infested	Heavily infested	Percentage of reduction
1	0.84	0.33	60.89	0.33	0.20	40.60	2.55	1.68	34.12
2	0.91	0.35	61.84	0.37	0.16	56.25	2.47	2.15	12.96
3	0.84	0.34	60.06	0.38	0.17	54.77	2.2	1.95	11.36
4	0.95	0.37	61.50	0.42	0.20	53.73	2.25	1.88	16.44
5	0.94	0.35	62.40	0.35	0.24	31.18	2.65	1.45	45.28
6	0.91	0.38	57.82	0.40	0.22	44.88	2.29	1.75	23.58
7	0.93	0.34	63.28	0.38	0.23	41.08	2.43	1.51	37.86
8	0.92	0.37	59.70	0.41	0.18	55.22	2.25	2.03	9.78
9	0.93	0.35	62.51	0.36	0.20	43.58	2.6	1.73	33.46
Mean	**0.91**	**0.35**	**61.14**	**0.38**	**0.20**	**47.16**	**2.41**	**1.79**	**25.63**

Table 2 Statistical analysis

	Chlorophyll *a*		Chlorophyll *b*		Total Chlorophyll	
	Mean ± SD	Standard error	Mean ± SD	Standard error	Mean ± SD	Standard error
Uninfested	0.909±0.045	0.015	0.378±0.028	0.009	2.410±0.168	0.056
Heavily infested	0.353±0.017*	0.005	0.200±0.026*	0.008	1.792±0.232*	0.077

*shows significant variation



Picture 3 Graphs showing variations in Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content in infested and control leaves.

Chlorophyll is an important part of chlorophyll protein complexes on the thylakoid membranes. It is the key photosynthetic pigment and its content directly reflects the photosynthetic efficiency and assimilation capacity. The efficiency of light captured to drive photosynthesis is directly correlated to the chlorophyll concentration in the leaf (Netondo *et al*., 2004)

The heavy loss of chlorophyll content as evidenced by the present study revealed the potential of *O. coffeae* to affect harmfully the general health of host plant, *C. sinensis*, thereby leading to decrease in the growth rate and biomass of the plant.

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