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Biochemical Changes in Coconut Leaves infested by exotic Whitefly Species (Aleyrodidae: Hemiptera)

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ABSTRACT: Invasive white flies of neotropical origin is an emerging problem in coconut ecosystem. Whitefly infestation impacts the host biochemical components. However, information regarding changes occurring in biochemical constituents after infestation of whiteflies in coconut remain limited. Hence, the present study was focused on analysing the changes in photosynthetic pigments, total soluble sugars, total proteins, total phenols and proline content in healthy and infested leaves of three coconut cultivars *viz.*, COD, WCT and T × D Hybrid. The findings indicated that the photosynthetic pigments including Chl*a*, Chl *b* and total Chlorophyll were significantly declined in all the cultivars after infestation. The other metabolic compounds analysed were significantly increased in all the tested cultivars. The buildup of various metabolites like protein, phenols, soluble sugars and proline content may play a role in coconut cultivars against the whitefly species infestation.

Keywords: Whitefly species, chlorophyll, biochemical components, Coconut.

INTRODUCTION

The Coconut palm, *Cocos nucifera* L. is a major cash crop cultivated throughout the tropical and subtropical zones of India. Coconut cultivation makes a considerable contribution to the Indian economy with 19% production share (Mahapatro, 2015). However, because of vulnerability of coconut palm to multiple biotic stressors like pests and diseases, the production of coconut is considerably declining. There are almost 900 pest species are known to infest on coconut palms. Of these, exotic whiteflies of neotropical origin have emerged as the most devastating pest in recent years in major coconut growing areas of Tamil Nadu.

The incidence of exotic rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin was reported on coconut palms from Pollachi, Tamil Nadu and Palakkad, Kerala during 2016 (Srinivasan *et al*., 2016; Sundararaj and Selvaraj, 2017) followed by two species of nesting whitefly, *Paraleyrodes bondari* and *Paraleyrodes minei* were reported during 2018 (Chandrika *et al*., 2019). In South India, it is currently observed that all the three exotic whiteflies are coexist on coconut. These types of whiteflies cause damage to the host plant by draining sap from leaves and by producing sticky, sugary and colourless honeydew, that adheres the lower surface of leaves and facilitates the development of sooty mould fungus. If sooty mould grows on the upper surface of a leaf, might potentially hinder a plant's ability to synthesise oxygen through photosynthesis.

The physiological activities of the plants, particularly the rate of photosynthesis may be harmed as a result of whitefly species infestation (Hossain *et al*., 2019). It is still unclear that how the whitefly infestation in coconut influences the host physiology and the biochemical components that are involved in it. Hence, the objective of the current investigation was intended to ascertain the biochemical changes triggered by the infestation of exotic whiteflies in coconut leaves.

MATERIALS AND METHODS

Plant samples. The healthy and whitefly infested leaflets of different coconut cultivars *viz.,* Tall (WCT), Dwarf (COD) and Hybrid (T×D) were collected from TNAU Coconut nursery, Coimbatore district of Tamil Nadu. The collected leaflets were brought to the laboratory and using tissue paper the fly's secretion and dark mycelia present in infested leaves were whipped out and further biochemical analysis was conducted.

Analysis of photosynthetic pigments

Chlorophyll. Chlorophyll contents as chlorophyll a, chlorophyll b and total chlorophyll was analysed as per the method followed by Arnon (1949). From each cultivars of coconut, 100 mg fresh leaf samples were macerated with 10 ml of 80% acetone. After maceration, the samples were centrifuged at 5000 rpm for 10 min. The supernatant was collected in test tubes, and using a spectrophotometer the concentrations of chlorophyll a, b, and total chlorophyll were determined by measuring the intensity of the green colour at 645 nm, 663 nm, and 652 nm, respectively.

Analysis of biochemical parameters

Total soluble sugars. The method suggested by Hedge *et al*. (1962) was used to estimate the total soluble sugar in coconut leaves. About 500 mg fresh leaf samples from each cultivars were crushed in 5 - 10 ml of 80% ethanol. The supernatant was collected in test tubes after that the homogenate mixture was centrifuged at 8000 rpm for 10 minutes at 4°C. Aliquots of 0.1 ml of each sample were pipetted out separately in different test tubes and the volume was made upto 1 ml by adding distilled water. The test tubes were heated in a boiling water bath after 4 ml of Anthrone reagent had been added to each tubes using a burette after one minute. After eight minutes, the tubes were removed and cooled under running tap water. A blank test was run without the sample, following the other steps and at 630 nm the absorbance of dark green coloured solution was measured. Glucose solution was used as working standard. The amount of sugar present in the sample was determined with the help of standard graph and expressed as mg of sugars/g fresh wt of sample.

Total protein. The approach proposed by Lowry *et al*. (1951) was used to assess the total protein content of the coconut leaves. 500 mg of leaf tissue from each coconut cultivars were taken and homogenized with 5-10 ml of 0.1 M phosphate buffer (pH 7). The supernatant was obtained after centrifuging the homogenate at 10,000 rpm for 10 minutes at 4°C. Each sample was divided into aliquots of 0.2 ml and pipetted out separately into various test tubes. The volume made up to 1 ml using distilled water and the tube with 1 ml of distilled water alone was taken as blank. After that, 5 ml of freshly prepared Alkaline copper solution was added to each tube including blank and allowed to stand for 10 minutes. After 10 minutes, 0.5 ml of FCR was added and left undisturbed at room temperature in the dark for 30 minutes. After 30 minutes, the intensity of blue colour was measured at 660 nm. The standard curve was plotted using different concentration of Bovine serum albumin.

Total phenols. According to the procedure followed by Malik and Singh (1980), the total phenol content in coconut leaves was estimated. From each cultivars of coconut, 500 mg of leaf tissue was taken and blended with 10 ml of 80% ethanol and centrifuged at 10,000 rpm for 20 min and the supernatant was then collected. The residue was once again re-extracted and the supernatants were pooled and the volume made upto 15 ml. Aliquots of 0.1 ml of each sample were pipetted out separately in different test tubes and the volume was then adjusted to 6 ml using distilled water and the tube containing only 6 ml of distilled water was used as blank. After that, 0.5 ml of Folin Ciocalteau reagent was added to each tube including blank and the tubes were allowed to stand for 3 mins. After 3 minutes, 2 ml of 20% Sodium carbonate solution was added and stirred. The concentration of phenol was measured at 650 nm against blank. Pyrocatechol was used at various concentrations to create a standard curve. The amount of phenols in the leaf samples were calculated from the standard curve and given as mg phenols/g of sample.

Proline. The Bates *et al*. (1973) protocol was followed for the analysis of proline content in leaves of different coconut cultivars. In 10 ml of 3%aqueous sulfosalicylic acid 500 mg of coconut leaf tissue was homogenized. The homogenate was centrifuged at 1500 rpm for 10 min and the supernatant was filtered through Whatmann No. 2 filter paper. Following filtration, 2 ml of the filtrate was placed in each test tube, along with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin. For one hr the test tubes were heated in boiling water bath. After one hour, the tubes were transferred to ice bath. After termination of reaction, 4 ml of toluene was added to each tubes and stirred well for 20-30 sec. Toluene layer was separated and the intensity of red colour was measured at 520 nm. The standard curve was created using various concentration of Proline.

Statistical analysis. For each parameter the data collected from the experiments were analysed separately. To determine the degree of significance between the parameters of control and infested plants, SPSS software (IBM SPSS version 22) was used. The Tukey's HSD test was performed to separate the sample means.

RESULTS AND DISCUSSION

The impact of plant biochemical constituents of different coconut cultivars against invasive whitefly species was studied and presented. All biochemical components (total proteins, total soluble sugars, total phenols and Proline) and photosynthetic pigments (total chlorophyll, chlorophyll a, chlorophyll b) showed a variation in whitefly infested coconut leaves compared to healthy leaves.

Photosynthetic pigments. The photosynthetic pigments content decreased in the infested leaves as compared to the healthy leaves (Fig. 1). The amount of total chlorophyll decreased most (62.11%) and least (45.91%) in the leaves of the Tall and Dwarf types, respectively. The total chlorophyll content in the whitefly infested leaves of Dwarf cultivar was 0.671 mg/g whereas healthy leaf contained 1.771 mg/g. The Tall, Dwarf and hybrid cultivars with whitefly infestation had lower chlorophyll-a content than healthy leaves by 45.22%, 63.02% and 54.83% respectively. The amount of chlorophyll-b was decreased in the range of 47.29% to 60.26% in whitefly infested leaves compared to healthy leaves. There is no significant difference between COD and Hybrid cultivars. Coconut leaves with spiralling whitefly infestations showed a 25% decline in the total chlorophyll content (Arun *et al*., 2021). Similarly, Reduced levels of photosynthetic pigments were observed in okra plants infected by whitefly and yellow vein mosaic virus (Amiteye *et al*., 2021). The amount of light available for the pigments to absorb was decreased by the fungal cover over the leaves. Chlorophyll pigments in the affected plants may have degraded and the biosynthesis of photosynthetic pigments is impacted by the reduction of light(Hudson *et al*., 1993). The sooty mould causes serious harm to the coconut leaves when it infects for an extended period of time because it prevents photosynthesis, kills the cells, and finally causes the leaves to dry up too soon.

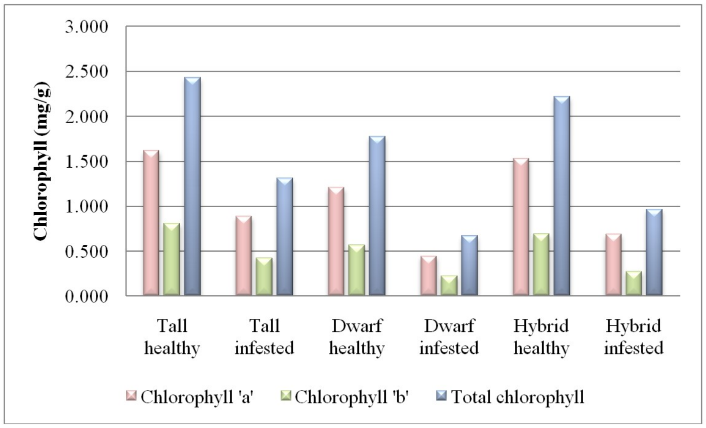

Fig. 1. Comparison of photosynthetic pigments of healthy and whitefly infested plants of different coconut cultivars.

Biochemical parameters

Total soluble sugars. The soluble sugars in leaves of coconut was increased after infestation by whitefly. The soluble sugar content was increased in whitefly infested leaves by 18.84%, 12.17% and 17.82% in Tall, Dwarf and hybrid cultivars respectively (Fig. 2). Tall cultivar recorded more total sugars of 32.96 mg/g and it is increased to 39.18 mg/g after infestation. There is no significant difference in the per cent increase of sugar content in tall and Hybrid cultivars after infestation. According to studies, the soluble sugars in the spiralling whitefly infested leaves of mulberry V1 variety increased by 3.03 % (Mahadeva, 2016). Manzoor *et al*. (2022) recorded the increase of sugar contents in Red Palm Weevil infested date palms over healthy leaves. The accumulation of sugars in the infected leaves may have a function in signalling but may not have any impact on the development of osmaticum because the rise in sugar content was not as similar as in the case of abiotic stress.

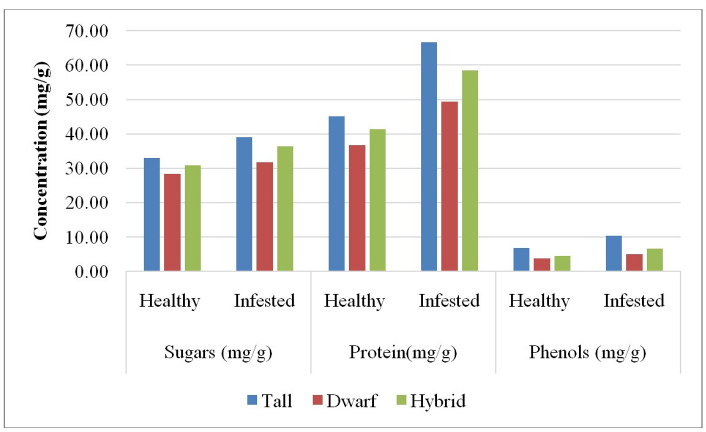

Fig. 2. Comparison of total sugars, total protein and total phenol contents in healthy and whitefly infested leaves of coconut cultivars.

Total protein. The overall protein content in all the three coconut cultivars increased after whitefly infestations. It was minimum (34.42%) in Dwarf cultivar and maximum (47. 61%) in the leaves of Tall cultivar (Fig. 2). The total protein content of whitefly infested Tall variety is 66.60 mg/g whereas the healthy leaves had 45.12 mg/g. The protein content of infested Dwarf variety is 49.45 mg/g whereas the control leaves had 36.8 mg/g. From the present study it is evident that, the tall coconut cultivar accumulated more proteins than Dwarf and Hybrid cultivars as a form of defence against the whitefly and sooty mould infection. The results are in accordance with Vasquez *et al*. (2016) observed that protein content increased in Jamaican Tall, Malayan Yellow Dwarf, and a hybrid JT × MYD coconut cultivars, in response to *Raoiella indica* feeding. According to earlier reports, the plants can able to grow under various adverse conditions by increased protein levels (Agastian *et al*., 2000; Ferreira *et al*., 2007). The increased soluble protein content may contribute to the production of several antioxidant enzymes, which can reduce the oxidative stress brought on by the infestation.

Total phenols. The total phenols estimated in the leaves of different coconut cultivars showed considerable differences among the cultivars (Fig. 2). In all the cultivars, total phenols content was increased in the infected leaves over that of healthy leaves. In the leaves of west coast tall the increase was highest (53.25%) and in chowg hat orange dwarf it was minimum (37.87%). In the current investigation, the total phenols of healthy plants from all three cultivars varied significantly. However, in whitefly infested leaves total phenols were considerably greater in leaves of WCT but there was no noticeable difference in the total phenols of Dwarf and hybrid cultivars.

Phenolic compounds are considered to have a role in the defense in plants and their greater accumulation following an infestation may be connected to the host's defence mechanism (Nicholson *et al*., 1992). In the present study, all the evaluated cultivars acquired more phenolics in the host, but among the three WCT showed highest accumulation of phenols which confer resistance to the tall varieties. The whitefly that feeds on phloem sap may have caused the coconut trees to produce large amounts of phenolics to stop the further spread of infection.

Proline. Whitefly infestation and sooty mould development increased the accumulation of Proline in leaves. In the present study, there was significant difference in the Proline content of healthy and infested plants of all three cultivars (Fig. 3). The increase in proline content was maximum in WCT (64.77%) and minimum in COD (44.46%). Similar increase in the proline content was observed in coconut leaves infested by spiralling whitefly (Arun *et al*., 2021). Pest infestation and other unfavourable abiotic conditions have been linked to the accumulation of proline, an amino acid that denotes stress in plants (Palliyath and Puthur, 2018). Stress related accumulation of Proline inside the cellfacilitates to maintain the cell's ideal water potential and ion homeostasis (Szabados and Savoure 2010).

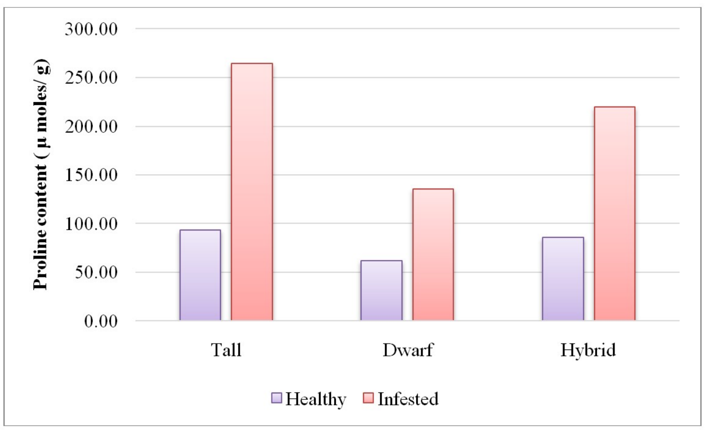

Fig. 3. Comparison of proline content in healthy and whitefly infested leaves of tested coconut cultivars.

CONCLUSION

The attack of exotic whiteflies on coconut leaves has been shown to cause imbalances in the biochemical and photosynthetic pigments. The growth of black sooty mould on the upper surface of the coconut leaves driven by whitefly infestation had a negligible direct effect. But it also affects the host leaves metabolism and photosynthetic properties indirectly. The accumulation of several metabolites caused by the infestation implies that the host defence mechanisms were activated. According to this study, sooty mould and whitefly infestations indirectly slow down photosynthesis.

FUTURE SCOPE

Under the mechanisms of host plant resistance, biochemical components in plants act as major line of defense against insects. So, information regarding the biochemical parameters which are involved in defense against exotic whiteflies will be helpful in breeding of resistant cultivars in coconut.

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Conflict of Interest. None.

REFERENCES

Agastian, P., Kingsley, S. J., & Vivekanandan, M. (2000). Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica*, *38*(2), 287-290.

Amiteye, S., Appiah, A. S., Boateng, F., Kutufam, J. T., & Amoatey, H. M. (2021). Physiological changes associated with Okra mosaic virus infection in field grown okra plants.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology*, *24*(1), 1.

Arun, K., Janeeshma, E., Job, J., & Puthur, J. T. (2021). Physiochemical responses in coconut leaves infected by spiraling whitefly and the associated sooty mould formation. *ActaPhysiologiaePlantarum*, *43*(3), 1-13.

Bates, L. S., Waldren, R. P., &Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*(1), 205-207.

Chandrikamohan, Josephrajkumar, A., Babu, M., Krishna, A., Prathibha, P. S., Krishnakumar, V., & Hegde, V. (2019). Non-native Neotropical nesting whitefly, ParaleyrodesmineiIaccarino on coconut palms in India and its co-existence with Bondar's nesting whitefly, ParaleyrodesbondariPeracchi. *Current Science (00113891)*, *117*(3).

Ferreira, R. B., Monteiro, S. A. R. A., Freitas, R., Santos, C. N., Chen, Z., Batista, L. M., & Teixeira, A. R. (2007). The role of plant defence proteins in fungal pathogenesis. *Molecular plant pathology*, *8*(5), 677-700.

Hedge, J. E., Hofreiter, B. T., & Whistler, R. L. (1962). Carbohydrate chemistry. *Academic Press, New York*, *17*, 371-80.

Hossain, M., Veneklaas, E. J., Hardy, G. E. S. J., & Poot, P. (2019). Tree host–pathogen interactions as influenced by drought timing: linking physiological performance, biochemical defence and disease severity. *Tree Physiology*, *39*(1), 6-18.

Hudson, A., Carpenter, R., Doyle, S., & Coen, E. S. (1993). Olive: a key gene required for chlorophyll biosynthesis in Antirrhinum majus. *The EMBO journal*, *12*(10), 3711-3719.

Lowry, O. H. (1951). Protein measurement with the Folin phenol reagent. *J boil Chem*, *193*, 265-275.

Mahadeva, A. (2016). Changes in biochemical constituents and photosynthetic pigments in spiraling whitefly (*Aleurodicus disperses*) infected mulberry foliage. *Int. Res. J. Biol. Sci*., 5: 1-7.

Mahapatro, G. K., & Kumar, S. (2015). Review on the incidence and management of coconut termites. *Indian Journal of Entomology*, *77*(2), 152-159.

Malik, C. P., & Singh, M. B. (1980). Plant enzymology and histo-enzymology.

Manzoor, M., Yang, L., Wu, S., El-Shafie, H., Haider, M. S., & Ahmad, J. N. (2022). Feeding preference of Rhynchophorusferrugineus (Oliver) (Coleoptera: Curculionidae) on different date palm cultivars and host biochemical responses to its infestation. *Bulletin of Entomological Research*, 1-8.

Nicholson, R. L., & Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. *Annual review of phytopathology*, *30*, 369-389.

Palliyath, S., & Puthur, J. T. (2018). The modulation of various physiochemical changes in *Bruguiera cylindrica* (L.) Blume affected by high concentrations of NaCl. *Acta Physiologiae Plantarum*, *40*(9), 1-18.

Srinivasan, T., Saravanan, P. A., Josephrajkumar, A., Rajamanickam, K., Sridharan, S., David, P. M. M., & Shoba, N. (2016). Invasion of the rugose spiralling whitefly, Aleurodicusrugioperculatus Martin (Hemiptera: Aleyrodidae) in Pollachi tract of Tamil Nadu, India.

Sundararaj, R., & Selvaraj, K. (2017). Invasion of rugosespiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae): a potential threat to coconut in India. *Phytoparasitica*, *45*(1), 71-74.

Szabados, L., & Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends in plant science*, *15*(2), 89-97.

Vásquez, C., Dávila, M., Méndez, N., Jiménez, M. A., Sandoval, M. F., &Alcalá, F. J. (2016). Oxidative Enzymes in Coconut cultivars in response to *Raoiella indica* feeding. *African Journal of Biotechnology*, *15*(33), 1755-1762.

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