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ORIGINAL ARTICLE

Physiochemical responses in coconut leaves infected by spiraling whitefly and the associated sooty mold formation

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Abstract

Biotic stressors contribute significantly towards the major loss of coconut (*Cocos nucifera* L.) economy. The objective of this study was to evaluate the influence of spiraling whitefly (*Aleurodicus rugioperculatus* Martin) and sooty mold fungi (*Capnodium* sp.) on photosynthetic efficiency, metabolomics and oxidative stress as well as antioxidative status in leaves of coconut. Although, the leaves showed severe infection of spiraling whitefly and sooty mold fungi, however, hyphal penetration of the fungus into the tissue was not observed in the leaf. Even then, the combined infection by the fly and the fungi significantly reduced Chl *a* fluorescence parameters, such as F_V/F_O (efficiency of water splitting complex), area above the induction curve and PI_{ABS} (performance index of PS II on absorption basis), which could reduce the growth by lowering the photosynthesist in leaves. Reactive oxygen species (ROS) accumulation in the infected leaves induced membrane degradation and at the same time, it boosted the activity of superoxide dismutase (SOD). The accumulation of different metabolites, such as amino acids, phenolics, soluble protein and soluble sugar content, was increased in infected leaves. In conclusion, spiraling whitefly and sooty mold infection did not directly disturb the host plant through hyphal penetration but as these organisms covered up the entire leaves, interruption in photosynthesis occurred and resulted in enhancement of various metabolites accumulation necessary to counter this particular biotic stress.

Keywords Biotic stress · Coconut · Sooty mold · Spiraling white fly

Introduction

Coconut (*Cocos nucifera* L.) cultivation significantly contributes to the Indian agrarian economy as the third-largest producer in the world with a share of 19.20% of the total production. It is cultivated on 1.94 million ha land in India which spread over 19 states and three union territories of the country, with an average productivity of 44.27 nuts/palm/year (Mahapatro 2015). Coconut palm provides food, oil, beverage, medicine, fibre and many other value added products, such as 'neera', a health drink, which increases its commercial value. But the coconut yield is drastically decreasing due to various biotic and abiotic stresses. Susceptibility of coconut palm towards different pathogenic diseases, such as root wilt, bud rot, leaf rot and stem bleeding, are the most frequent problems encountered in its cultivation and production (Rajesh et al. 2018).

Spiraling whitefly (*Aleurodicus rugioperculatus* Martin) is an important polyphagous pest with more than 200 host plants, is found to infect mainly agricultural crops, such as coconut, in the regions of South India (Martin 2004). It is a small-winged insect that feeds the phloem sap and excretes sticky, shiny, sugary, and colourless honey dew which act as an excellent medium for the growth of fungi. The outbreak of the *A. rugioperculatus* and fungal infection affects the growth and yield of the coconut palm. The infected palm leaves are generally seen with black sooty mold on the upper surface of the leaf and the whitefly infestation on the lower surface of leaf (Josephrajkuma et al. 2012). In severe cases of the disease, sooty mold even spreads to the wood of the plant. This sooty mold fungus is disseminated by the air currents or

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rain splash and thus lands on the honeydew produced by the fly, which is a good medium for the fungi to flourish (Nelson 2008). The spread of sooty mold infection limits the light availability to the leaves and that leads to a suboptimal condition for the growth of the host plant. Moreover, whitefly can directly affect the plant by feeding on the phloem sap. Sucking insects can also act as a vector to spread the pathogen infection from one plant to another (Omena et al. 2012).

Biotic stress induces impairment in the physiological functions of the host plants especially in the rate of photosynthesis (Hossain et al. 2018). The metabolic adjustment and the up-regulation in the phytoalexins synthesis associated with the pathogenic infection were reported in different host plants (Halldorson and Keller 2018; Komives and Kiraly 2019). The infestation of the spiraling whitefly in coconut trees resulted in the premature death of leaves and it causes the reduction in the nut yield per tree. Prolonged dry condition and absence of natural enemies help in the multiplication of the fly (Sundararaj and Selvaraj 2017). The other two whitefly species, *Aleurocanthus arecae* and *Aleurodicus dispersus*, affecting coconut are considered as minor pests but *A. rugioperculatus* infection shows more impact on the productivity and health of plant (Kumar et al. 2012). So it was felt important to resolve the physiological and biochemical modifications in coconut leaves by the *A. rugioperculatus* and sooty mold infection.

As the result of spiraling whitefly and sooty mold infection, there may be alterations in the functional biology of the plant. This study investigates the physio-chemical modifications occurring in coconut leaves so as to cope with this particular biotic stress.

Materials and methods

Study materials

The coconut leaflets infested by spiraling whitefly and sooty mold were collected from Thenjipalam, Malappuram district (longitude of 75.876741° E 11.119442° N) of Kerala, India. Healthy leaflets were also collected from the same site which was used as the control in this study. The level of sooty mold infestation was different in the leaves of the coconut palms, thus only severely infested leaves (score 9) were selected for this study. The infection status of the leaves was scored in '0–9' scale (Table 1) according to Naik et al. (1997). The dark mycelia and secretion of the fly were whipped out using tissue paper and further analysis was conducted.

Table 1 '0–9' scale developed for scoring infection severity in coconut leaves

% leaflet area covered by fly and fungus	Severity score
1–10	1
11–20	2
21–30	3
31–40	4
41–50	5
51–60	6
61–70	7
71–80	8
Above 80	9

Calculation of percentage disease intensity (PDI)

$$\text{PDI} = (\text{sum of the score} \times 100)/(\text{number of rating} \times \text{maximum score})$$

To study the sooty mold infection, freehand sections of the infected leaves were prepared (Ahmad et al. 2018), stained with lactophenol cotton blue and was observed under microscope (CH 20i, Olympus, Tokyo, Japan).

Identification of the fly

The fly was directly collected from the field and the morphological characters were observed under the stereomicroscope (mzs900, Magna zoom, USA). The fly was identified according to the procedure of Shanas et al. (2016).

Identification of the sooty mold fungus

The dark mycelium covered on the adaxial side of the leaf was inoculated onto PDA medium as described by Gams et al. (1998) under sterile conditions. The cultures were maintained at 37 ± 1 °C and observed regularly. Mycelia with specific morphological characteristics were transferred to fresh PDA plates. From these isolated cultures, conidial spores were isolated and characters were observed under microscope (CH 20i, Olympus, Tokyo, Japan).

Analysis of host physiochemical features

Total chlorophyll and carotenoid content

200 mg fresh leaf sample was crushed in 80% acetone for the extraction of chlorophyll and carotenoid content. After homogenization, the samples were centrifuged at 5000 rpm for 10 min at 4 °C. The quantification of the total chlorophyll and carotenoids content was performed as per the method of Arnon (1949).

Chlorophyll *a* fluorescence analysis

Chlorophyll *a* fluorescence parameters were analyzed as per the methodology of Strasser et al. 2004, on the upper and lower surface of the infested and non-infested leaves after dark adaptation for a period of 20 min. All measurements were recorded up to 1 s with a data acquisition rate of 10 µs.

ABS/CS_M absorption of photon per excited cross section, TRo/CS_M (excitation energy flux trapped by PSII, measured over a cross section of active and inactive reaction centers), ETo/CS_M (Electron flux transported by PSII, over a cross section of active and inactive reaction centers), DIo/CS_M (the dissipation rate/cross section), Area (area above the fluorescence induction curve), F_V/F_O (efficiency of water splitting complex), PI_{ABS} (performance index of PS II on absorption basis), N (turn over number of Q_A) and t_{FM} (time taken to reach F_M) were the parameters studied to analyse the efficiency of photosynthetic apparatus. Data analysis including radar plot and energy pipeline model deduction was performed with the help of Biolyzer HP3 software (Bioenergetics Laboratory, University of Geneva, Switzerland).

Photosystem I and II activities

Thylakoids from control and infected leaves were isolated as per the methodology of Puthur (2000). Clark-type oxygen electrode (DW1/AD, Hansatech, Norfolk, UK) was used to analyze the photochemical activities of the isolated thylakoids. The PSI and PSII activities were measured by irradiating white light with an intensity of 1800 µmol photons m^{-2} s^{-1} on the thylakoid samples. The activity of photosystems was expressed in terms of µmol of O_2 consumed (PSI)/evolved (PSII) min^{-1} mg^{-1} chlorophyll.

Biochemical parameters

Analysis of protein, free amino acids, soluble sugars, phenolics, flavonoids, ascorbate, glutathione and proline

Total protein 500 mg of leaf tissue was homogenized in 5 ml of 0.1 M phosphate buffer (pH 7) and the protein was precipitated with 10% trichloroacetic acid (TCA). The precipitate was washed twice with cold 2% TCA, 30% perchloric acid, diethyl ether, followed by washing with 80% acetone. Total protein content of the coconut leaves was analyzed using Folin–Ciocalteau reagent (Lowry et al. 1951).

Total free amino acids 500 mg of coconut leaf was homogenized with 5 ml of 80% (v/v) ethanol. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C. Estimation was performed using the protocol of Moore and Stein (1948) with ninhydrin reagent. L-leucine was used as the standard.

Total soluble sugar 500 mg leaf tissue was homogenized in 5 ml of 80% ethyl alcohol. The extract was centrifuged at 8000 rpm for 10 min at 4 °C. The total soluble sugar was estimated using the method proposed by Dubois et al. (1956).

Total phenolics 200 mg of coconut leaf sample was homogenized in 5 ml of 80% ethyl alcohol and the homogenate was centrifuged at 10,000 rpm for 20 min. After the re-extraction of the residue, total phenolics were estimated by Folin–Denis reagent as per the method of Folin and Denis (1915).

Flavonoids 200 mg of fresh leaf samples was homogenized with 5 ml of acidified methanol (methanol: HCl: H_2O in 79:1:20). After 24 h, the estimation of flavonoids content was performed according to the protocol of Mirecki and Teramura (1984).

Ascorbate (ASA) content 500 mg of leaf tissue was homogenized with 5 ml of 5% (w/v) TCA. After centrifugation, the estimation of ASA content was performed as per the method of Chen and Wang (2002).

Glutathione (GSH) content 500 mg of coconut leaf tissue was homogenized with 5 ml of 5% (w/v) TCA. After centrifugation, the GSH content was analyzed as per the method of Chen and Wang (2002).

Proline 500 mg of coconut leaf tissue was homogenized in 5 ml of 3% (w/v) aqueous sulfosalicylic acid by adding a pinch of polyvinyl polypyrrolidone. After centrifugation, the proline content was analyzed according to the protocol of Bates et al. (1973).

Estimation of ROS generated and the scavenging mechanism

Hydrogen peroxide content

200 mg of coconut leaf tissue was homogenized in 5 ml of 0.1% ice-cold TCA and was centrifuged at 12,000 rpm for 15 min. The hydrogen peroxide content was analyzed as per the method of Junglee et al. (2014).

Superoxide (O_2^-) content

200 mg of coconut leaf tissue was cut into pieces (1 mm^2) and immersed in 0.01 M phosphate buffer (pH 7.8) containing 0.05% nitro blue tetrazolium (NBT) and 10 mM NaN_3 (sodium azide) and the superoxide content was estimated as described by Doke (1983).

Malondialdehyde (MDA) content

500 mg of coconut leaf tissue was homogenized in 5 ml of 5% TCA and centrifuged at 12,000 rpm for 15 min at 25 °C. The protocol of Heath and Packer (1968) was used for the estimation of MDA content.

Membrane stability index (MSI)

Membrane stability index (MSI) was analysed as per the protocol of Sairam et al. (1997). 100 mg of coconut leaf tissue was cut into pieces (10 mm^2) and dipped in two tubes containing 5 ml of distilled water. Thereafter, one of the tube was kept at 40 °C for 30 min and using conductivity meter (Eutech, Cyberscan 600) the electric conductivity (c_1) of the sample was measured. While, the electric conductivity of the sample in the second tube was measured after placing the same in boiling water bath at 100 °C for 15 min and the MSI was calculated using the equation,

$$\mathrm{MSI} = [1 - (c_1/c_2)] \times 100$$

Enzymatic antioxidants

Superoxide dismutase (SOD)

500 mg of coconut leaf tissue was homogenized in 5 ml of 50 mM phosphate buffer (pH 7.8) and 100 mg of polyvinyl polypyrolidone was added to it as phenolic binder. The homogenate was centrifuged at 16,000 rpm for 15 min at 4ºC. For the estimation of SOD activity, the method of Giannopolitis and Ries (1977) was used.

Catalase (CAT)

500 mg of coconut leaf tissue was homogenized in 5 ml of 50 mM phosphate buffer (pH 7.0). After filtration, centrifugation was performed at 16,000 rpm for 15 min at 4 °C. The activity of CAT in the samples was determined, following the method of Kar and Mishra (1976).

Ascorbate peroxidase (APX)

Extraction of APX enzyme was done as per the protocol of Zang and Kirkham (1996). 500 mg of coconut leaf tissue was homogenized with 5 ml of extraction medium. APX activity in the samples was analyzed as per the method of Nakano and Asada (1981).

Statistical analysis

SPSS software (SPSS 16.0, Chicago, USA) was used to found the level of significance between the parameters of control and infected plants. Pearson's correlation analysis was performed to evaluate the interaction between the variables obtained in coconut during infection. The data represent mean ± standard error (SE) and the values are the average of three independent experiments, each with three replicates (i.e., $n = 9$).

Results

Assessment of severity of the infection

The calculated percentage of disease intensity (PDI) was 88%. The adaxial side of the infected leaves was covered with dark mycelial mass and abaxial side showed intense white spiral cottony secretion of the fly (Fig. 1). The cross sections of infected leaves revealed that the fungal hyphae of the sooty mold did not penetrate the epidermal layer but was spread over the surface of leaves as a thick layer. Presence of haustoria like structures was not observed in the epidermal and hypodermal cells (Fig. 1).

Identification of the fly

Major morphological characters of the fly include reticulated cuticle on dorsum, compound pores on abdominal segments VII and VIII, corrugations/rugosity on the surface of operculum and acute shape at the apex of lingula. Based on these morphological features of the puparium, the fly was identified as *A. rugioperculatus* Martin (Fig. 2a, b).

Identification of the fungus

The major fungus causing the black sooty mold on the upper surface of host plants was identified as *Capnodium* sp. (Fig. 2). Fungal genus *Capnodium* was characterized by the dark brown thin thallus, which can be removed easily from the infected leaf surface. The thallus consisted of cylindrical hyphae. Superficial hyphae were thin, septate, constricted at the septum, branched, brown to dark brown, with subcylindrical hyphal cells. Colonies were slow growing on PDA, superficial to erumpent, sometimes hyphae growing downwards and penetrate into the media, surface was verrucose and velvety.

Photosynthetic features

Photosynthetic pigments

The photosynthetic pigment content was decreased during the infection of *A. rugioperculatus* and sooty mold in

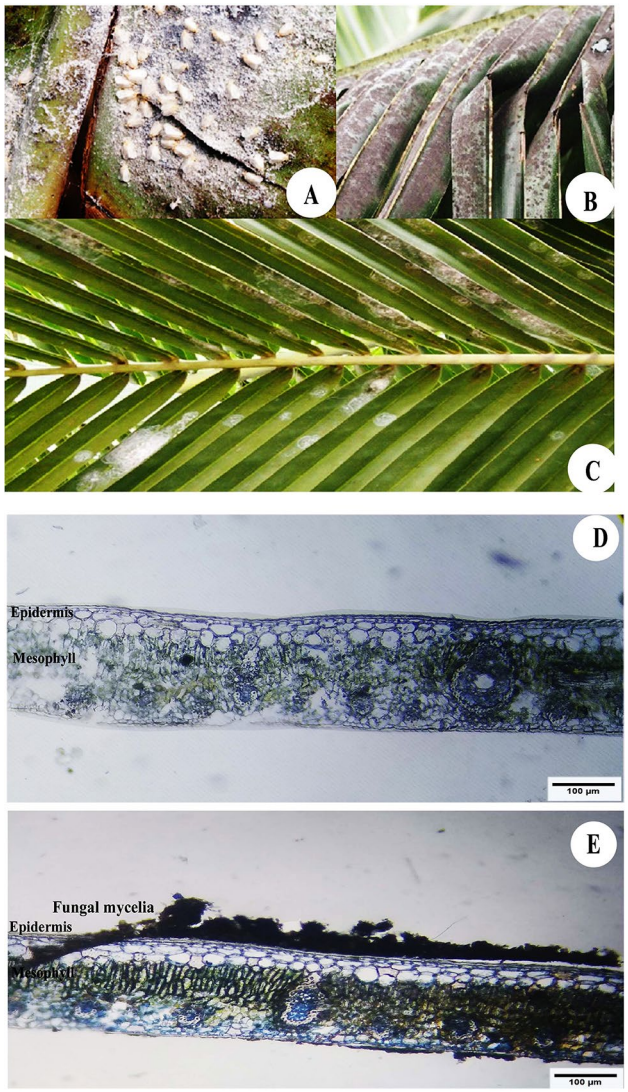

Fig 1 Coconut leaf infested with fly and sooty mold **a**, **b** abaxial side infested with fly **c** adaxial side infested with sooty mold **d** anatomical changes induced by *A. rugioperculatus* and associated sooty mold infection in leaf section **e** section of leaf when sooty mold is wiped off

coconut leaves. The total chlorophyll content showed a decline of 50% and the carotenoid content showed 34% of decrease in the infected leaves as compared to the control (Fig. 3).

Photosystem activities

Activity of photosystem I was less reduced in the infected plants and the reduction was only 12% but the activity of the photosystem II was decreased by 43% when compared to the non-infected leaves (Fig. 3).

Chlorophyll *a* fluorescence parameters

Biotic stress-induced modifications were observed in the phenomenological leaf models of *C. nucifera* and the closure of the reaction centres was the prominent response seen in the infected leaves. Different parameters like ABS/CS_M, TRo/CS_M, ETo/CS_M, and DIo/CS_M were visualized in energy pipeline models and the result indicates that the energy transfer efficiency of photosynthetic apparatus was drastically decreased in the infected leaves as compared to the control (Fig. 4). The reductions of the above described parameters were 78, 89, 94 and 27% on the adaxial side, but these were only 42, 47, 58 and 23%, respectively, on the abaxial side of the infected leaves as compared to the control. The response of an individual active reaction centre towards the pathogen infection was different from that observed in the case of a collection of reaction centres in a cross section. ABS/RC, TRo/RC, and DIo/RC were increased in the abaxial and adaxial surface of the leaf. When ABS/RC showed a threefold increase, DIo/RC increased up to 16-fold on the adaxial surface of the infected leaves. The increase was only to the extent of 28 and 73% in the abaxial side for ABS/RC and DIo/RC, respectively. The ETo/RC was reduced in the infected leaves and the reduction was 50 and 5% on the adaxial and abaxial side, respectively, as compared to the control plant. Area, F_V/F_O and PI_{ABS} were reduced in the infected leaves and the reduction was maximum on the adaxial surface of the infected leaves (Fig. 5). The turnover number (N) was increased in the abaxial side of the leaf and it was decreased in the adaxial side as compared to the control. Another important parameter t_{FM} showed an increase during the infection but significant increase was observed only on the abaxial surface of the infected leaf.

Biochemical parameters

Total protein and total free amino acids

The leaves of *C. nucifera* infected by spiraling whitefly and sooty mold showed an increase in the soluble protein (114%) and amino acid content (126%) when compared to the control (Fig. 6a).

Total sugars and phenolics

The increase of soluble sugars and phenolics content in leaves of coconut was very less on infection. The total sugar content increased by 25% and phenolics increased by 20% in the infected leaves over that of the normal leaves (Fig. 6b).

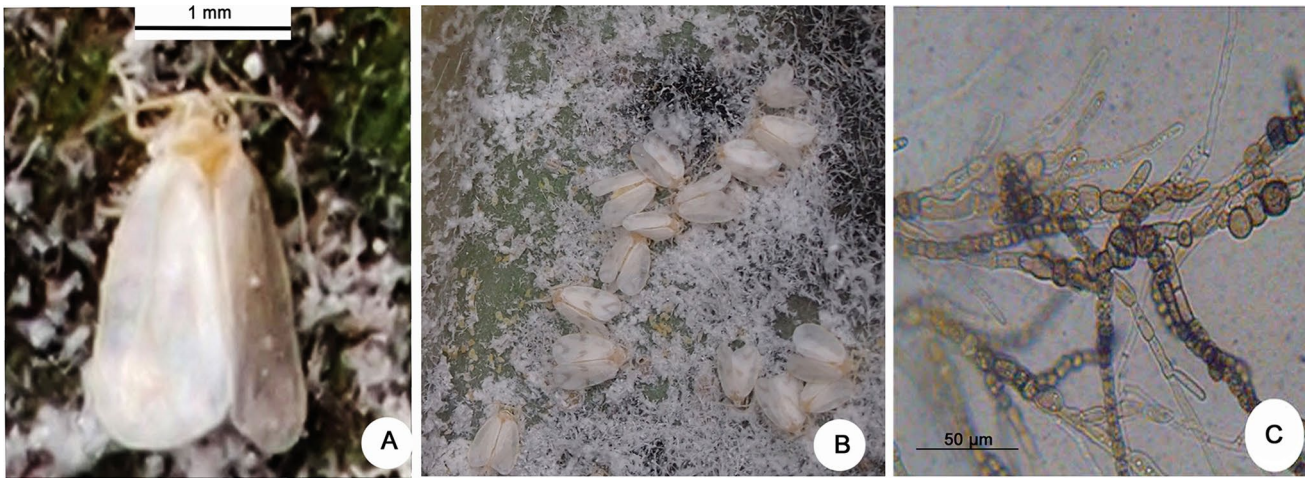

Fig 2 Morphological features of infected fly and fungus **a** *A. rugioperculatus* and **b** *Capnodium* sp. fungi

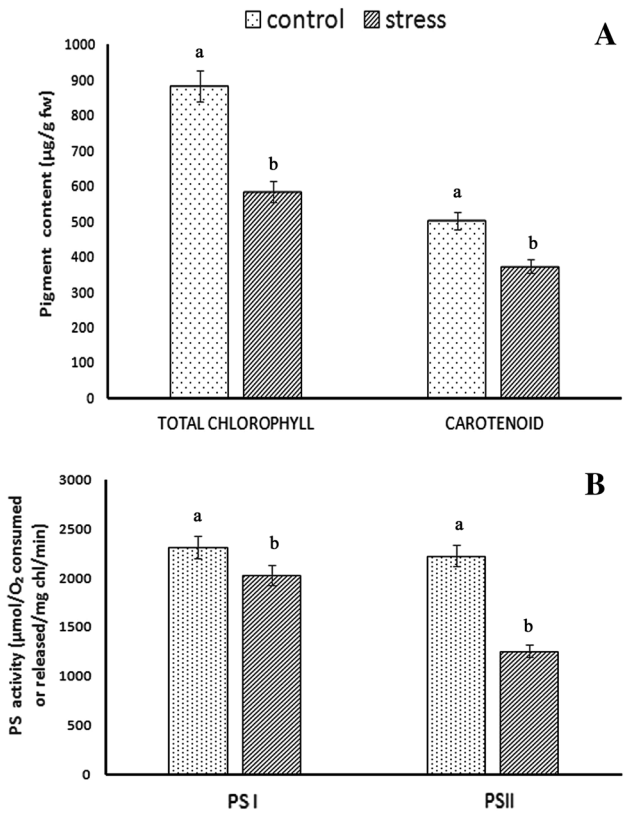

Fig 3 Photochemical response of leaves infested with *A. rugioperculatus* and associated sooty mold **a** comparison of total chlorophyll and carotenoid in the infected and control **b** comparison of photosystem activities in the infected and control

Glutathione and ascorbate

Both the glutathione and the ascorbate got increased in the leaves of infected coconut plant. The increase in glutathione content was 3.4-fold and that of ascorbate was ninefold in infected leaves when compared to the control leaves (Table 2).

Flavonoids and proline

A. rugioperculatus and sooty mold infection induced a slight increase in the accumulation of flavonoids but that of proline was very high in leaves. Only negligible increase (5%) was observed in flavonoids content, but fivefold increase was observed in proline content in the infected coconut leaves as compared to the control (Table 2). A strong correlation was observed between proline content and different parameters of infected coconut leaf (supplementary data Table 1). The correlations were positive with phenolics ($R^2 = 0.994$ and $P \leq 0.01$), sugar ($R^2 = 0.990$ and $P \leq 0.01$), amino acids ($R^2 = 0.995$ and $P \leq 0.01$), SOD ($R^2 = 0.970$ and $P \leq 0.01$), CAT ($R^2 = 0.970$ and $P \leq 0.01$), APX ($R^2 = 0.997$ and $P \leq 0.01$), and MDA ($R^2 = 0.994$ and $P \leq 0.01$). At the same time, the correlation was strongly negative with some other parameters, such as total chlorophyll ($R^2 = -0.997$ and $P \leq 0.01$), carotenoids ($R^2 = -0.996$ and $P \leq 0.01$), PSII activity ($R^2 = -0.991$and $P \leq 0.01$), and MSI ($R^2 = -0.992$ and $P \leq 0.01$) of the infected coconut leaf.

ROS accumulation and scavenging

Hydrogen peroxide content and superoxide (O_2^-) content

ROS accumulation was increased in the infected plants. There was only a slight increase of superoxide content but hydrogen peroxide content increased by 121% in the infected leaves when compared to the non-infected leaves (Table 2).

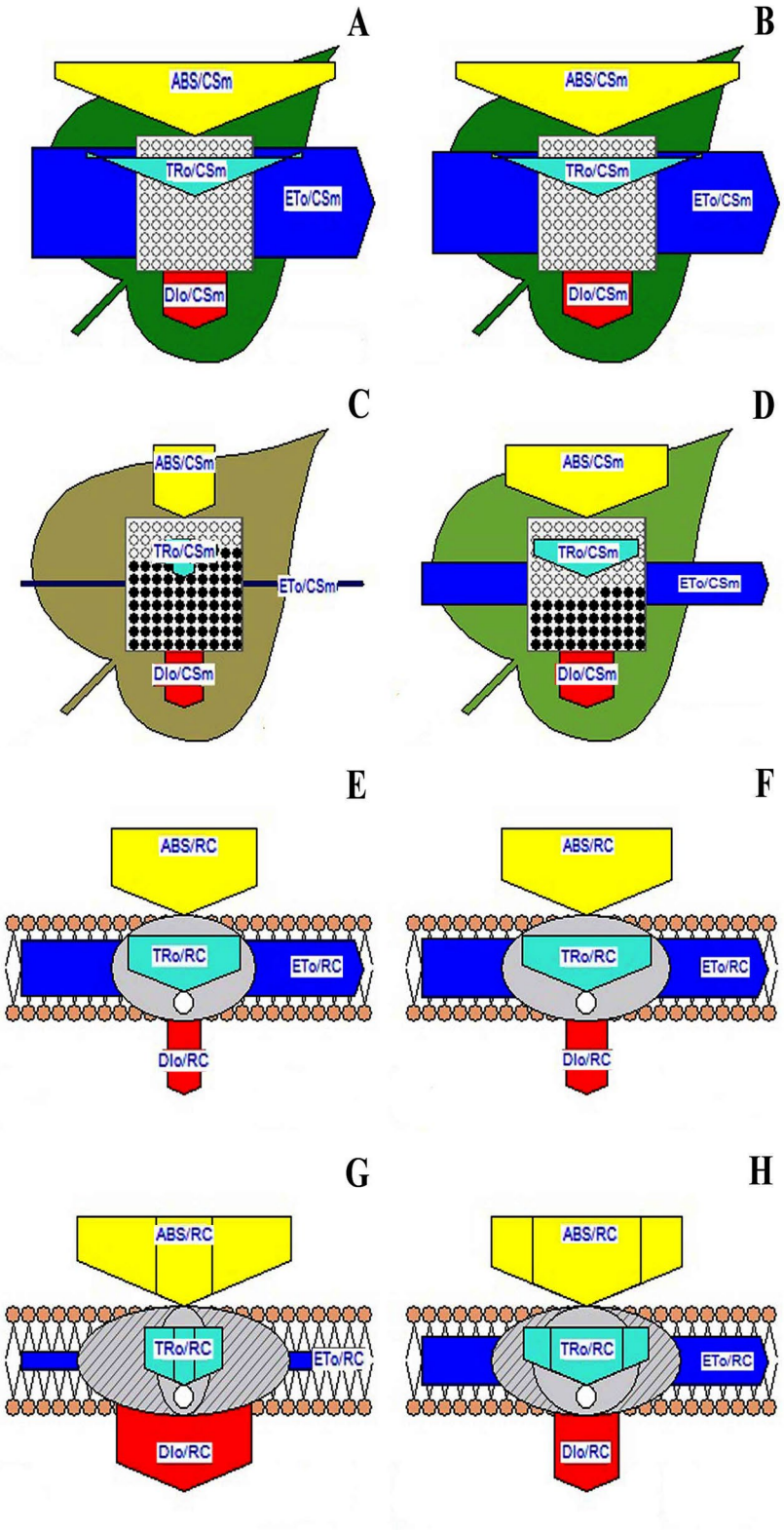

Fig 4 Leaf pipeline model showing the proportion of phenomenological energy flux parameters recorded in the leaves of *Cocos nucifera* affected with sooty mold and spiraling whitefly, **a**, **b** energy pipeline model of adaxial and abxial surface of control plant, **c**, **d** energy pipeline model of adaxial and abaxial surface of infected plant, **e**, **f** specific membrane model of adaxial and abaxial surface of control plant and **g**, **h** specific membrane model of adaxial and abaxial surface of infected leaf. The width of the arrow represents the relative values of each parameter and empty and dark circles represent active and non active reaction centers, respectively

Malondialdehyde content and membrane stability index

Malondialdehyde content of the infected leaves was 94% higher than that of the control and the membrane stability was decreased by 23% in the leaves of infected leaves with respect to control leaves (Table 2).

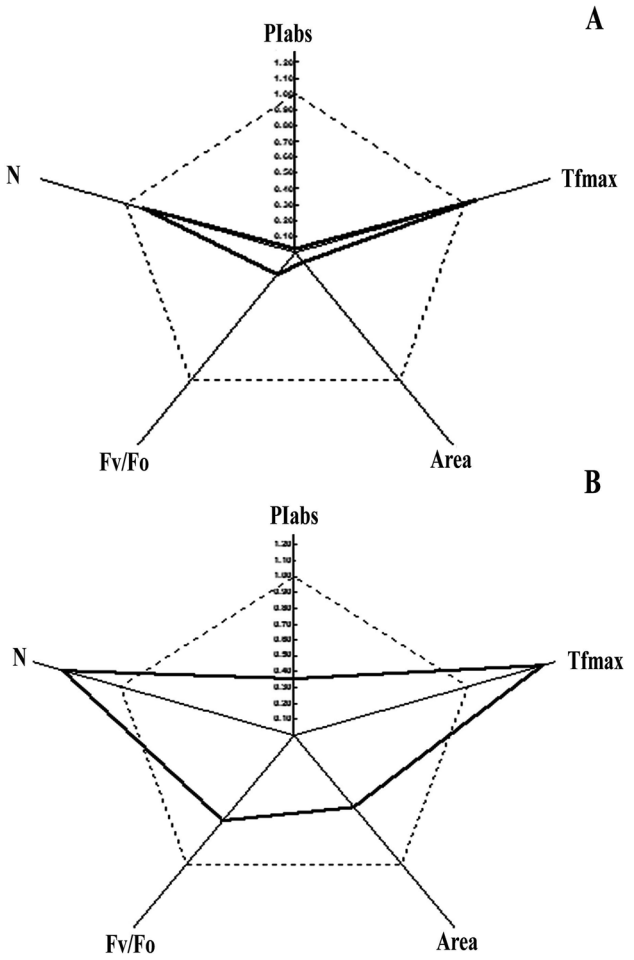

Fig 5 Radar plots of different JIP parameters deduced from chlorophyll *a* fluorescence induction curves **a** Adaxial side **b** Abaxial side of infected coconut leaf and all values are shown as percent of control. The black dotted and continuous lines represents control and infected plants, respectively

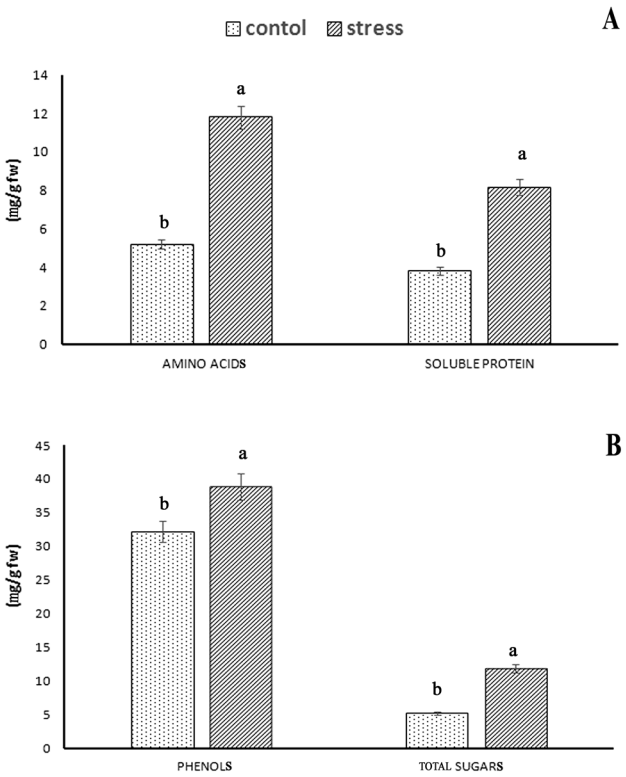

Fig 6 Metabolic alterations observed in the leaves infested with *A. rugioperculatus* and associated sooty mold **a** comparison of total free amino acid and soluble protein in the infected and normal leaf of coconut palm **b** comparison of phenol content and total soluble protein content in the infected and normal leaf of coconut palm

Enzymatic antioxidants

Superoxide dismutase, catalase and ascorbate peroxidase

The activities of enzymatic antioxidants were increased in the infected coconut leaves. Activity of SOD, CAT and APX were increased by 0.8-, 2.5- and 3.7-folds, respectively, in the infected coconut leaves when compared to normal leaves (Fig. 7).

Discussion

Different fungal genera have the potential to cause sooty mold diseases in plants. *Alternaria*, *Cladosporium*, *Aureobasidium*, *Antennariella*, *Limacinula*, *Scorias*, *Meliola* and *Capnodium* are the major fungal genera causing sooty mold and the family *Capnodiaceae* is usually considered as the true sooty mold group (Kim 2016). In the present study, *Capnodium* sp. was identified as the major member of sooty mold which ramified on the adaxial surface of coconut leaves.

The adaxial side of the infected leaves was covered with dark mycelial mass and abaxial side showed intense white spiral cottony secretion of the fly. The fungal hyphae did not penetrate the leaf tissues to acquire nutrients. Hence, it can be assumed that the fungus does not have any direct role in inducing stress conditions but it has an indirect role by covering up the photosynthetically active regions and thus hindering active photosynthesis. The proliferation of the sooty mold is supported by the honeydew produced by the fly (Santos et al. 2013). The fly also does not create any stress condition directly but indirectly it supports the proliferation of the fungus, which ultimately brings about various physio-chemical changes within the leaf tissue, due to its spread over the leaf surface.

Table 2 The changes in content of various metabolites induced by spiraling white fly and sooty mold infestation in the leaves of *C. nucifera*

	Flavonoid (mg/g fw)	Ascorbate (mg/g fw)	Glutathione (mg/g fw)	Proline (µg/g fw)	Superoxide (µg/g fw)	Hydrogen peroxide (µg/g fw)	MDA (µmol/g fw)	Membrane stability (%)
Control	0.138 ± 0.009^{a}	0.0398 ± 0.002^{b}	3.5867 ± 0.003^{b}	200.01 ± 0.001^{b}	15.4016 ± 0.7701^{b}	0.6227 ± 0.0385^{b}	17.7032 ± 0.038^{b}	57.198 ± 0.019^{a}
Stress	0.1459 ± 0.073^{a}	0.3851 ± 0.019^{a}	12.507 ± 0.004^{a}	1002.63 ± 0.001^{a}	17.1762 ± 0.8588^{a}	1.3787 ± 0.0429^{a}	34.3741 ± 0.049^{a}	43.751 ± 0.021^{b}

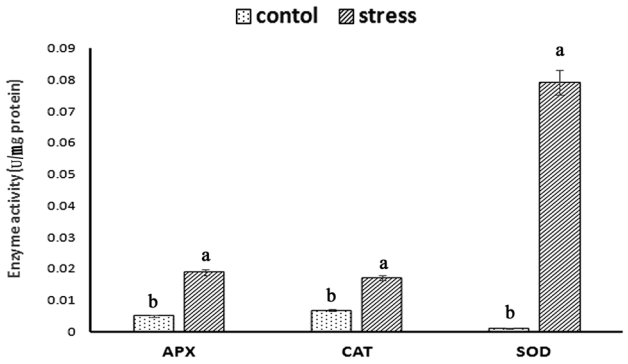

Fig 7 Variations in the activities of different enzymes (APX, SOD and CAT) observed in the coconut leaves infested with *A. rugioperculatus* and associated sooty mold

Photosynthesis

The machinery of photosynthesis including the composition of photosynthetic pigments, photosystems activity, and efficiency of electron transport system were affected due to the spiraling whitefly attack. The fungal cover over the leaves reduced the light availability for absorption by the pigments, causing an inefficient energy harvest. Moreover, the prevalence of such a condition even caused the degradation of the chlorophyll pigments in the infected plant. The reduction in light affects the biosynthesis of photosynthetic pigments (Hudson et al. 1993). The reduction in photosynthetic pigments results in a reduced absorption capacity of both the photosystems (PSI and PSII) and hence photosynthetic capacity was reduced. Barón et al. (2012) reported a decrease in photosynthetic efficiency of plants due to fungal infection. Prolonged infection in the coconut leaves by the sooty mold severely damages the leaf tissue by preventing photosynthesis, turning the cells dead due to dearth of photosynthate and ultimately the infected leaves dry up prematurely.

The carotenoids not only play a role as accessory light-harvesting pigments but they are also act as antioxidants, protecting the infected plants from oxidative damage (Stahl and Sies 2003). Moreover, stabilization of light-harvesting complex proteins as well as thylakoid membrane is an important function of carotenoids (Niyogi et al. 2001). Carotenoids are associated with thylakoid membrane as well as with many other proteins that constitute the photosynthetic apparatus. Zhang and Wen (2008) reported a decrease in the carotenoid content after the infection of the pest *Bemisia tabaci* and sooty mold in the plant *Mikania micrantha*. In the present study, the whitefly and sooty mold infection over the coconut leaves lead to the decrease in the carotenoid content, which could be either due to reduction in the synthesis or faster rate of degradation as observed in the case of chlorophyll content.

In the infected leaves of coconut palms, the fungal and whitefly infection caused a significant decrease in the activities of the photosystem I (PSI) and photosystem II (PSII). Burd and Elliott (1996) reported a reduction in PSI and PSII activities of *Triticum aestivum* infected by Russian wheat aphids. The damage of PSII due to the infection of *Bemisia tabaci* and sooty mold was observed in *Mikania micrantha* (Zhang and Wen 2008). In coconut also, the biotic stress caused by the sooty mold and fly infection results in the decreased activity of photosystems and this could be primarily due to the reduction of energy transfer from the light-harvesting complex as there was insufficient photosynthetic pigment. Long-term acclimation to low light intensity (induced by sooty mold infection) caused the reduction of PSII content and it may be also due to the degeneration of this photosystem in the suboptimal conditions (Kouřil et al. 2013). The higher reduction observed in the case of PSII as compared to PSI could be due to the higher vulnerability of the former photosystem to any kind of stresses as reported by Barth et al. (2001).

Analysis of various Chl *a* fluorescence parameters revealed that the infection of sooty mold and whitefly induced inhibition of the photochemistry in *C. nucifera*. The enhanced ROS content in infected leaves (discussed later) would be a major reason for this inhibitory effects on photosynthesis. As similar to these results, when rice leaves were exposed to oxidative stress, a decline in the photosynthetic yield was observed and it can be attributed to a reduction in electron transfer potential at the oxidizing site of PSII (Strasser 1997). The flux of absorption (ABS/CS_M) and electron transport (ETo/CS_M) were decreased significantly in *C. nucifera* and similar findings were reported by Perez et al. (2014) in wheat cultivars exposed to various biotic stress factors. Even though ABS/RC, TRo/RC, and DIo/RC were increased in abaxial and adaxial sides of the infected leaves, ETo/RC was found to be decreased. This indicates the inefficiency of electron transport which can be correlated with the reduced F_V/F_O. F_V/F_O is the parameter specifying the activity of oxygen evolving complex at the donor side of PSII and

the infection caused a decline in the water splitting and associated electron migration. Similar results were reported in *Physalis Peruviana* seedlings infected with *Fusarium*, where Fv/Fm and electron transport rate were reduced as the rate of infection was increased (Chávez-Arias et al. 2019). The drastic reduction observed in the photosynthetic efficiency at the adaxial side of the infected leaves indicates that sooty mold has a higher inhibitory effect on photosynthesis of crop plants as compared to the abaxial, where fly settles down. This could be due to the fungus proliferation in the adaxial side forming a dark mycelial mass covering up the entire leaf area.

Metabolites

The leaves of coconut plants accumulated more proteins as part of defence against the whitefly and sooty mold infection. There were earlier reports indicating that the increasing levels of proteins helped the plants in maintaining their growth under various stressful environments (Agastian et al. 2000; Ferreira et al. 2007). The increased soluble protein content was evidenced in the increased activity of the antioxidant enzymes, indicating the enhancement in the biosynthesis of different antioxidant enzymes, capable of alleviating the oxidative stress induced by the infection.

Amino acids are the monomers of proteins, and the metabolic variation in the amino acids content aiding the plant to overcome the extreme environment. The degraded proteins can contribute to the total amino acids pool and also the amino acids may be formed afresh by synthesis (Reggiani et al. 1988; Patterson et al. 2009). In many plants, stress acclimation was associated with the increase in the levels of specific amino acids (Hayat et al. 2012). This increase in amino acids aids in the maintenance of the nitrogen metabolism and thus could impart stress tolerance to coconut. Nitrogen has significant role in averting biotic stress as analysed in tomato plants, and the plants grown at low nitrogen availability were less tolerant to the fungal pathogen *Botrytis cinerea* (Hoffland et al. 1999). Moreover, the increased amino acid content potentially regulates the pathogen infection, especially the aspartic acid-derived amino acid biosynthetic pathways play a crucial role in the biotic stress resistance of plants (Zeier 2013).

During a pathogen attack, the sugar molecules can act as signalling molecules inducing defence mechanism (Morsy et al. 2007; Morkunas and Ratajcza 2014). Moreover, the sugar molecules have a crucial involvement in the osmotic adjustment mechanisms where it acts as a compatible solute (Nounjan et al. 2018). The observed increase in total sugar in the infected coconut leaves was not to the same level as in the case of abiotic stresses, such as drought, salinity, metal toxicity, etc. Therefore, it could be presumed that the limited accumulation of sugars could only play the role in signalling and may not have anything to do with the build-up of osmoticum. The phenolics including flavonoids and anthocyanins plays major roles in abiotic and biotic stress responses (Lattanzio 2013). The fungus and fly infection over the leaves and the phloem sap feeding whitefly might have triggered the production of high levels of phenolics in the coconut palms to prevent the further spread of the infection and this is a general strategy adapted by plants to counter the spread of the pathogen. When the leaf of *Zostera marina* was infected with *Labyrinthula zosterae*, the caffeic acid content was increased to 45 mg/g DW and the same secondary metabolite was only 8 mg/g DW in the uninfected leaves and this increase aids to prevent further spreading of the infection (Vergeer and Develi 1997). Similarly, *Vitis vinifera* affected with esca disease showed a prominent increase in phenolic content in the leaves (Lima et al. 2017). However, from the results, it could be well understood that the phenolics accumulation was not sufficient to restrict the spread of the infection. In the present study, an insignificant increase of total flavonoid content was observed in the infected leaves which indicate the minimal role of flavonoids in preventing this specific pathogen attack in coconut leaves. Flavonoid is a group of phenolics. Therefore, the reduction in the increase of flavonoids could be very well correlated with the reduced accumulation of plant phenolics (Treutter 2005; Sulaiman and Balachandran 2012). Ascorbate played an important role in protecting cells against oxidative stress (Horemans et al. 2000; Sruthi and Puthur 2019). The infected plant has more ascorbate content because the oxidative stress induced the production of ROS and consequently ascorbate was produced at higher levels to scavenge the ROS, through its involvement in the ascorbate–glutathione cycle.

There was a fivefold increase in proline content in the infected coconut leaves. Proline, a stress indicator amino acid accumulated in plants, has been reported to occur after pathogen infection, and various abiotic stresses (Siripornadulsil et al. 2002; Palliyath and Puthur, 2018). Stress conditions induced the accumulation of proline inside the cell and this facilitated the optimal water potential and thus maintained ion homeostasis inside the cell (Cushman 2001; Szabados and Savoure 2010). Proline can also act as a molecular chaperon and help proteins to maintain their integrity and thus can maintain the activity of different enzymes. From this study, the potential of this imino acid as a stress indicator was evident from the correlation analysis, where it showed strong negative correlation with most of the growth parameters.

ROS accumulation and scavenging

Infection resulted in the increased production of reactive oxygen species (ROS) including superoxide radical and hydrogen peroxide (H_2O_2). Consequently, lipid peroxidation rate increased, resulting in membrane damage. Generally, MDA content is directly related to the intensity of the oxidative stress and it is the final product of lipid peroxidation (Davey et al. 2005; Yan and Tam 2013). MDA content increased enormously in the leaf tissues of plants infected by whitefly and sooty mold, proving that the leaf tissues encountered with oxidative stress. When the overaccumulated ROS molecules damage the polyunsaturated fatty acid, it led to increase in fluidity as well as permeability of the membrane (Sharma et al. 2012). Exposure to different stressors elicited an increase in peroxidation of membrane lipids, which eventually caused membrane damage, leading to leakage of essential elements (Djebali et al. 2005). The infected plants had less membrane stability than the normal ones. The stress due to the infection might have made the membrane unstable by the action of the reactive oxygen species, arising out of infection. This could be one of the major reasons for the death of the cells and drying up of the leaves after a certain period of infection. The toxic effects of ROS were effectively counteracted by enzymatic as well as non-enzymatic anti-oxidative system, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Antioxidant enzyme SOD, converts the superoxide radicals to hydrogen peroxide and thus protect the plants from the toxic effects of super oxides (Fatima and Ahmad 2005). This enzyme converts super oxides to hydrogen peroxides in cytosol, chloroplasts and mitochondria and therefore plays a crucial role in cellular defence mechanism against the risk of hydroxyl radical formation (Gratao et al. 2005; Thomas and Puthur 2019). The manyfold increase of SOD in leaves of infested plants is a clear indication that the superoxide radicals synthesized are very well taken care by the antioxidative role of SOD. The increased activity of APX as compared to CAT points towards the greater role played by the former in neutralizing the H_2O_2 formed.

APX was involved in the scavenging of hydrogen peroxide using ascorbate as the electron donor. This biocatalyst scavenges the H_2O_2 content accumulated in chloroplasts, where normally CAT activity is absent (Asada 1992). In the infected leaves, the ascorbate content was higher than the control and the increase can be correlated to the increased activity of APX.

Conclusion

The direct impact of spiraling whitefly and sooty mold infection was insignificant in coconut leaves as assessed from the non-formation of hyphae in the inner tissue. But indirectly, it causes photosynthetic inefficiency along with metabolic alterations in the host leaves. The infection caused accumulation of different ROS species and drastic up-regulation of the SOD activity indicates the elicitation of host defence mechanisms. This study revealed the indirect role of *A. rugioperculatus* and sooty mold infection in reducing the rate of photosynthesis and associated yield loss of the coconut palm.

Author contribution statement KA performed the analysis, processed the experimental data and interpreted the results. EJ drafted the manuscript and designed the figures. JJ helped to shape the research and analysis aided in interpreting the results and worked on the manuscript. JTP provided critical feedback and helped shape the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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