Journal of Sustainability Science and Management
Volume 19 Number 11, November 2024: 77-93
eISSN: 2672-7226

ANTIOXIDATIVE AS SELF-DEFENSE DURING RED PALM WEEVIL INFESTATION IN COCONUT CULTIVAR "MAWA"

NORHAYATI YUSUF[1,2]*, SHAIDATUL LIYANA ABDUL TALIB[1], NURUL FATIN AZMA AZMAN[1], RABIATUL AISHAH RESEP[1] AND WAHIZATUL AFZAN AZMI[1]

[1]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.*
[2]*Biological Security and Sustainability Research Interest Group, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.*

**Corresponding author: yatiyusuf@umt.edu.my*
http://doi.org/10.46754/jssm.2024.11.005
Received: 14 February 2024 *Accepted: 3 June 2024* *Published: 15 November 2024*

Abstract: The coconut industry ranks fourth in economic importance but the Red Palm Weevil (RPW) creates a major danger, decreasing coconut production. The "MAWA" cultivar, favoured by farmers faces this challenge. This study investigated the plant's defence mechanisms during RPW infestation, focusing on enzymatic and non-enzymatic antioxidants. Infested leaves of "MAWA" showed lower CAT, APX, and gPOD activities in front and middle fronds but higher in end fronds than controls. α-tocopherol and ascorbic acid increased significantly in the front and middle fronds of infested plants, respectively while carotenoids rose in the middle fronds. Infested leaves exhibited lower MDA, H_2O_2, and ion leakage, suggesting enzymatic and non-enzymatic antioxidants' defensive roles during RPW infestation. MDA, H_2O_2, and ion leakage could be early RPW infestation markers in coconuts. This study sheds light on how "MAWA" cultivars deploy enzymatic and non-enzymatic antioxidant defences during RPW attacks, potentially aiding in the early detection of infestations.

Keywords: Reactive oxygen species, defence mechanism, antioxidants, oxidative stress, "MAWA".

Introduction

Coconut palm or *Cocos nucifera* is one of the most significant commercial crops after oil palm, rubber, and rice (Yahya & Mohd Zainal, 2014). Every year, there is a growing demand for goods manufactured from coconuts. This industrial crop in Malaysia generated RM72.8 million or 0.06% of the nation's agricultural export revenue in 2020 (DOA, 2021). In 2020, Malaysia exported products made from coconuts, including charcoal, activated carbon, dried coconut, processed coconut water, and fresh coconuts. Activated carbon accounted for 10.7%, coconut milk accounted for 11.5%, and items made from coconut oil accounted for 60% of the total RM1.36 billion in export value of these goods (Comtrade, 2020). The main coconut cultivars planted in Malaysia are "Malayan tall" (92.2%), "MATAG" (4.3%), "MAWA" (1.7%), "*aromatic Pandan*" (1.7%), and the "Malayan Dwarf" (0.2%) (Zainol *et al.*, 2023). "MATAG" is a newly released hybrid coconut from the "Yellow Dwarf" X "Tagnanan" coconut (Philippines) while "MAWA" is a hybrid of "Malayan Dwarf" X "West African Tall" coconut. In 2018, the Malaysian Agricultural Research and Development (MARDI) introduced six new coconut hybrids known as "Marleca", "Myleca", "Careni", "Careca", "Marena", and "Mylag" (Rozeita & Zulkafli, 2018).

Similar to other palms, there is no exception for a coconut from being attacked by pathogens and pests, which will affect the production rate. Pests and diseases present a notable challenge to palm cultivation significantly diminishing crop yield by competing with the palm for essential elements and causing plant injury (Maluin, 2020). One of the most well-known insect pests is the Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Figure 1). El-Lakwah *et al.* (2011)

(a) (b)

Figure 1: Red Palm Weevil (RPW), larva (a) and adult (b)
Source: Weche (2019)

stated that RPW is the greatest threatening and deadly pest of palm species, which includes coconut, oil palm, sago, and other palm species. During the growth and development of RPW within the tree trunks, the insects feed on the cabbage of the trees, destroying the vascular system and causing the death of the plant. Therefore, the number of productive trees would decrease and finally, the productivity of healthy coconut trees would be affected, leading to a reduction in coconut fruit production. Moreover, heavy infestations frequently result in the collapse of trees, which adds up to the total loss of yield due to the declining number of productive trees (Wahizatul *et al*., 2013).

In Malaysia, the initial appearance of RPW was discovered in 2007 (DOA, 2016) and until 2016, the pest was found in five states: Perlis, Kedah, Kelantan, Penang, and Terengganu. The Department of Agriculture Malaysia (2016) reported that the total coconut plantation in Malaysia is 85,799 ha and 465 ha of coconut trees were infested, mainly in Terengganu and Kedah due to the RPW infestations (DOA, 2016). Therefore, earlier detection of infestation and defence mechanisms in the coconut trees are crucial. Many studies regarding RPW infestations on palm plantations including date and coconut palms have been reported. However, there is a lack of information on the earlier detection and defence mechanisms, especially during the RPW infestation. Earlier detection of infestation would enable pest management at a very early stage and proper treatment may be applied to reduce the severe impact and total loss of the coconut plantations.

Naturally, plants produce a signal of cellular metabolites during stress or infestation as inevitable by-products (Thakur *et al*., 2019). For example, salinity, drought, extreme temperatures, metal toxicity, excessive pesticide use, and pathogen infections all cause the release of Reactive Oxygen Species (ROS), resulting in oxidative stress in plants (Foley *et al*., 2016; Xie *et al*., 2019; Meo & Venditti, 2020). ROS known for their high reactivity, interfere with various cellular, biochemical, and physiological processes in plants. They cause oxidation of carbohydrates, peroxidation of lipids, denaturation of proteins, and degradation of DNA, RNA, enzymes, and pigments, all contributing to plasma membrane damage. Consequently, these activities result in lower agricultural yield and quality (Bhuyan *et al*., 2020; Sachdev *et al*., 2021). Previous research revealed that ROS production was likely to be overproduced during RPW infestations on coconut plants, which led to oxidative stress.

Therefore, infested coconut trees developed a defence mechanism to overcome the overproduction of ROS (Norhayati *et al*., 2016). Moreover, stresses also trigger oxidative stress markers such as hydrogen peroxide (H_2O_2), lipid peroxidation, and ion leakage as the first strategy to activate the defensive metabolites in plants. It was reported that the enzymatic and non-enzymatic antioxidants work synergistically to minimise the damaging impact of ROS (Hasanuzzaman *et al*., 2020). Consequently, the research sought to identify the early oxidative stress markers (H_2O_2, lipid peroxidation, and ion leakage), enzymatic (CAT, APX, and gPOD), and non-enzymatic antioxidants (α-tocopherol, ascorbic acid, and carotenoids) in the coconut, "MAWA" cultivar in response to RPW infestation.

Materials and Methods

Plant Materials

Samples of infested and healthy leaves from 20-years-old "MAWA" coconut cultivars were collected from coconut palms at Agriculture Department of Rhu Tapai in Setiu, Terengganu. Sampling was conducted three times between June and July 2018. This location was chosen because the coconut trees within this plantation area exhibited significant infestation by the Red Palm Weevil (RPW) (DOA, 2016). Three replicates of healthy and infested trees were used. Three pieces of coconut leaflets at the front, middle, and end parts of the same fronds were taken, respectively (Figure 2). Samples were separately placed in a labelled sealed plastic bag and kept in an icebox filled with crushed ice before the extraction process in the laboratory.

Early Oxidative Stress Markers Assay

The H_2O_2 concentration was evaluated following the method outlined by Velikova *et al*. (2000). Leaf sample was extracted using 0.1% Trichloroacetic Acid (TCA). The mixture was centrifuged at 10,000 rpm (Eppendorf 5840R) for 10 minutes at 4°C. The reaction mixture (consisting of the supernatant, potassium phosphate buffer, and freshly prepared potassium iodide, KI) was left for 10 minutes and absorbance was recorded at 390 nm (Shimadzu UV 1601).

MDA levels were determined to assess lipid peroxidation (Heath & Packer, 1968). Samples were ground with 0.1% TCA and centrifuged at 10,000 rpm for five minutes. The supernatant was mixed with 0.5% TBA in 20% TCA, heated at 95°C for 30 minutes, and rapidly cooled. After a 10,000 rpm centrifugation for 10 minutes, the absorbance was measured at 532 nm, and corrected for non-specific absorption at 600 nm. MDA concentration was expressed as nmol MDA per gram fresh weight.

To determine ion leakage, leaf samples were cleaned and placed in double-distilled water at 40°C for 30 minutes to measure initial

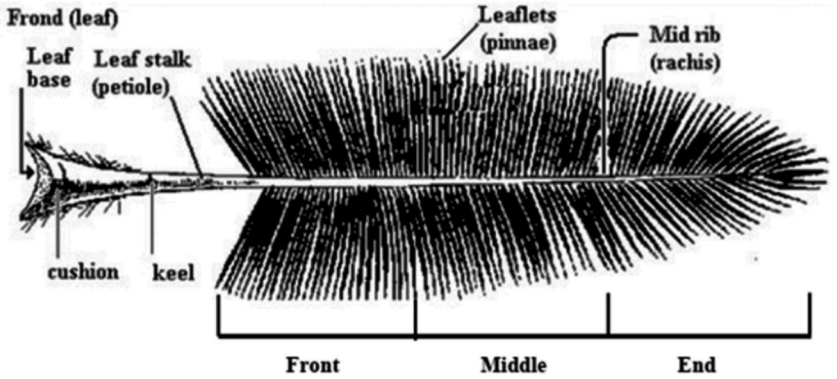

Figure 2: The coconut leaflets with three different parts (front, middle, and end parts) taken from the same side of a frond. Illustration modified from Arbeit (1985)

conductance (C1). After boiling at 100°C for 10 minutes and cooling, the final conductance (C2) was evaluated. The ion leakage percentage was calculated as outlined by Correia *et al.* (2013).

Enzymatic Antioxidant Assays

The Clairborne (1985) approach was applied to analyse the CAT-specific activities. Samples were ground with extraction buffer (pH 7.4) and centrifuged at 4°C, 10,000 rpm for 10 minutes. Enzyme extract was added to a reaction mixture (19 mM H_2O_2 in 50 mM phosphate buffer, pH 7.0). Absorbance changes were observed at 240 nm for three minutes using a spectrophotometer. The CAT-specific activity was expressed as µmol of H_2O_2 consumed per minute per mg protein.

The APX-specific activities were performed using the method of Nakano and Asada (1981). Samples were ground with 1 mM ascorbic acid in 100 mM phosphate buffer (pH 7.0) at 0°C to 4°C. After centrifugation (10,000 rpm, 10 minutes, and 4°C), the reaction mixture (including 100 mM phosphate buffer, 3 mM ascorbic acid, 3 mM EDTA, distilled water, and 1.5 mM H_2O_2), with enzyme extract was monitored for absorbance changes at 290 nm for three minutes.

The gPOD-specific activities were assayed based on the method of Agrawal and Patwardan (1993). The samples were ground with extraction buffer (pH 7.0) in a prechilled mortar and pestle at 0°C to 4°C. The mixture was centrifuged at 10,000 rpm at 4°C for 10 minutes. The enzyme extract was added to the reaction mixture (50 mM phosphate buffer (pH 7.5), 20 mM guaiacol, and 30 mM H_2O_2). Changes in absorbance were monitored at 470 nm for three minutes.

Protein Content

Coomassie Brilliant Blue (G-250) was dissolved in 95% ethanol to make the Bradford reagent. The combination was then added to 100 mL of concentrated phosphoric acid and diluted to 1 L of distilled water. The mixture was filtered and kept in light-proof bottles at room temperature. Roughly 3 mL of Bradford's reagent was mixed with 100 µL of enzyme extract. After 10 minutes, the absorbance was measured at 595 nm (Bradford, 1976). Protein content was determined using a standard curve constructed from BSA (Bovine Serum Albumin).

Non-enzymatic Antioxidant Assays

The extraction of α-tocopherol was carried out using the Hodges *et al*. (1996) method. Fresh samples were crushed with acetone and clean sand at 0°C to 4°C under low light. The second extraction was repeated twice with hexane. Hexane extracts were combined with 0.1% PDT and 0.1% ferric chloride. The mixture was swirled and allowed to develop colour for four minutes. Then, 0.2 mM orthophosphoric acid was added and the mixture stood at room temperature for 30 minutes. Absorbance was measured at 554 nm (Kanno & Yamauchi, 1997).

The extraction process for ascorbic acid followed Jagota and Dani's (1982) guidelines. The sample was crushed in 10% trichloroacetic acid (TCA) in an ice-cold solution using a prechilled mortar and pestle under low light. After centrifugation at 5,320 rpm for 10 minutes at 4°C, the supernatant was mixed with 10% Folin reagent and distilled water. Absorbance was measured at 760 nm.

Carotenoid content was assayed based on the method proposed by Lichtenthaler (1987). Under dim light and over ice, 0.02 g leaf tissue was ground with 3.0 mL of 80% acetone. After centrifugation at 10,000 rpm for 10 minutes, absorbances at 470, 646.8, and 663.2 nm were measured with 80% acetone as the blank.

Statistical Analysis

Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) software version 20. Three replicates of each treatment were employed in the triplicate experiment. All data were expressed as means ± standard error. Data were analysed using two-way ANOVA to evaluate the differences in early oxidative stress responses and activity of

enzymatic and non-enzymatic antioxidants in three-leaf parts (front, middle, and end of fronds) of infested and healthy "MAWA". Values were considered significant at $p \leq 0.05$.

Results and Discussion

Changes in the Early Oxidative Stress Markers

Lipid peroxidation, electrolyte leakage, and malondialdehyde (MDA) concentration are key indicators of oxidative stress in plants, including coconut palms, under various environmental stresses such as insect infestations like the Red Palm Weevil (RPW). Monitoring these markers can provide valuable perspectives on the physiological responses of coconut palms to insect infestations and assist in developing management strategies to mitigate their impact on palm health and productivity. Results in this study showed that H_2O_2 concentration in infested and control leaves was significantly ($p < 0.05$) lower at the end relative to the other part of the fronds. The H_2O_2 concentrations in the infested front fronds significantly decreased relative to their controls. However, no notable difference was observed in H_2O_2 concentrations between the infested and control groups in the middle part of the fronds [Figure 3 (A)]. The elevated levels of H_2O_2 in the frontal region may be attributed to the infestation site, situated within the plants' cabbage part (soft tissue).

Chandrashekar *et al*. (2020) observed that RPW adults commonly deposit their eggs in wounds or crevices near the crowns of coconut palms. Upon hatching, the larvae bore into the palm tissues, including the trunk, crown, and leaf bases where they feed on the soft inner tissue. The observed decrease in H_2O_2 production in the infested front part compared to its control might be because of the action of higher levels of scavenging metabolites such as α-tocopherol and ascorbic acid in the leaves closer to the infestation site [Figures 5 (A) and (B)], respectively, suggest an intricate signal transduction network involved in response to herbivory (Hossain *et al*., 2015), particularly in this case, the infestation by the RPW. When faced with an abrupt pathogen invasion, plants activate various defence mechanisms, including physical and chemical deterrents.

Physical defences such as spines, trichomes, and cuticle layers work in tandem with chemical defences, including Secondary Metabolites (SMs) and Volatile Organic Compounds (VOCs) to thwart the attack (Mostafa *et al*., 2022). These responses entail local and systemic signalling pathways, which detect, recognise, and counteract invading pathogens (Chen *et al*., 2018). When faced with herbivore-feeding activities, plants initially respond with cell wall modification (Kloth *et al*., 2019). Signals emitted by the insect are sensed by receptors, triggering the activation of the plant's defence system, particularly involving compounds like α-tocopherol and ascorbic acid, as observed in this current study. Recognition of Herbivore-Associated Molecular Patterns (HAMPs) by Pattern Recognition Receptors (PRRs) on the plant cell surface sets off PAMP-Triggered Immunity (PTI), prompting defensive reactions designed to hinder pathogen colonisation (Zhang *et al*., 2022). Subsequently, plant cell membranes undergo ion fluxes, inducing disparities in plasma membrane potential (Vm). This cascade leads to the production of secondary messengers such as calcium ions ($[Ca^{2+}]$ cyt) and ROS (Gandhi *et al*., 2020).

Additionally, there is a rapid surge in phytohormones like jasmonic acid, salicylic acid, and ethylene initiating signal transduction and prompting the release of VOCs and SMs, including terpenoids (Reymond, 2021). The observed elevation in scavenging metabolites such as α-tocopherol and ascorbic acid in the infested leaves indicates an adaptive response aimed at mitigating the potentially deleterious outcomes of ROS accumulation (Dvořák *et al*., 2021). The infestation process frequently triggers signalling pathways that induce the production of ROS, notably H_2O_2. This sudden increase in ROS levels (oxidative burst) constitutes an early response to pathogen infestation, initiating events that activate defence genes, and facilitate the synthesis of defence compounds (Wojtaszek,

1997). In this study, the leaves nearer to the infestation site likely detect the presence of the RPW through mechanical damage or chemical cues released by the insects during feeding. This detection triggers signal transduction that induces the accumulation of H_2O_2 at the site of RPW feeding and acts as signalling molecules to activate defence-related genes and pathways.

The accumulation of H_2O_2 in the leaf of infested end fronds compared to its respective controls might also serve as a stimulus to initiate both the enzymatic and non-enzymatic antioxidative responses (Kapoor *et al*., 2015). Moreover, the feeding activity of RPW larvae within the palm tissues can weaken the tree's structural integrity and disrupt the passage of water and essential elements. The combination of mechanical damage, nutrient deprivation, and RPW infestation-associated oxidative imbalance can accelerate the onset of premature senescence in affected palms. Symptoms such as wilting and yellowing of leaves are characteristic of senescence and reflect the decline in physiological function associated with the Programmed Cell Death (PCD) pathways (Nousis *et al*., 2023). The typical reaction of vulnerable plants to various stressors, including biotic stress, involves a disturbance in the balance between the production of ROS and their removal by antioxidative defence mechanisms, leading to the scavenging of free radicals (Caverzan *et al.*, 2016). H_2O_2 is one of the ROS that can be lethal in higher concentrations in plants and insects and it that can trigger antioxidative enzymes that eliminate H_2O_2. H_2O_2 can also induce membrane lipid peroxidation, resulting in damage to the reaction centres of chloroplasts. This process involves the breakdown and disintegration of lipids, leading to the simultaneous production of aldehydes and alcohols (Golan *et al*., 2013).

Deprivation of membrane lipids in plant cells generates free fatty acid that starts oxidative deterioration by generating a substrate for the enzyme lipoxygenase and triggering membrane lipid peroxidation (Galaris *et al*., 2019; Valgimigli, 2023). The consequences of lipid peroxidation can be detrimental to plant cells and tissues. Oxidative damage to membrane lipids compromises membrane integrity and fluidity, affecting the selective permeability of membranes and disrupting cellular processes such as ion transport, signal transduction, and protein trafficking. Additionally, lipid peroxidation by-products such as MDA and 4-hydroxynonenal (HNE) are cytotoxic and can further exacerbate cellular damage by reacting with proteins, DNA, and other biomolecules (Catalá & Diaz, 2016). Based on the results in Figure 3 (B), MDA concentration was significantly lower in the infested front and end parts compared to their respective controls. RPW infestation in the front and end parts may elicit the antioxidant mechanism to eliminate the ROS. Infection triggered by insect suckling can stimulate antioxidative defence mechanisms in plants (Allison & Schultz, 2004).

San *et al*. (2022) also stated that infestation by herbivorous insects results in the build-up of protective substances through physiological, morphological, and biochemical adjustments in plant systems aimed at defending against invaders. ROS generated during herbivore infestation can initiate lipid peroxidation, leading to oxidative degradation. This leads to the development of lipid peroxides and ultimately MDA, a prominent marker of lipid peroxidation. The disruption of membrane integrity due to lipid peroxidation compromises cellular structure and function, exacerbating the damage caused by herbivore feeding (Su *et al*., 2019). Insect herbivory induces plant cell metabolic changes, affecting respiration and photosynthesis. These alterations include reduced photosynthetic efficiency, chloroplast structure disruption, and electron flow imbalance. Consequently, electrons leak onto molecular oxygen (O_2), leading to increased release of ROS, and heightened oxidative stress within chloroplasts (Zhou *et al*., 2015). Similarly, during pathogen attacks, the escalated respiration rate and ROS production disturb the cellular redox balance, further amplifying ROS production via feedback mechanisms (Sachdev *et al.*, 2021). These responses are part of

plants' defence mechanisms against invaders. However, excessive ROS production can harm plant cells, underscoring the delicate balance between defence mechanisms and oxidative stress tolerance in plants during pathogen attacks (Gill *et al*., 2010).

Generally, the RPW feeding did not significantly alter the percentage of ion leakage in all parts of the front. In contrast, percentages of ion leakage in the control front and end parts of fronds were significantly higher than in infested fronds [Figure 3 (C)]. Again, it may be postulated that ROS scavenging activities work efficiently in lessening the effect of oxidative damage by ROS in the infected plant. Thus, lower ion leakage was observed in both plant parts. Ranf *et al.* (2011) postulated that higher ion leakage in control plants may happen due to normal activities of the plant cells. Regular metabolic activities such as cellular respiration and photosynthesis generate ROS as a by-product. ROS also serve as signalling molecules in numerous cellular pathways, regulating processes such as cell proliferation, differentiation, and apoptosis (Guo *et al*., 2023), leading to higher ROS production as observed in control leaves in this current study.

In this study, a decrease in H_2O_2 might lead to decreases in lipid peroxidation and ion leakage. With the increasing concentration of H_2O_2, the plants exhibited an upregulated oxidation state in the tissues; thus, increasing lipid peroxidation and enhancing the ion leakage in the infested plant cell (Khan *et al.*, 2016). The antioxidative defence mechanism is accountable for scavenging them in plant cells to regulate lipid peroxidation and ion leakage. So, it lowers the MDA content and ion leakage in plant cells to prevent any harmful effects on the cellular membrane (Spiteller, 2003). In contrast, a study by Farouk and Osman (2009) reported that in the common beans infested with two-spotted spider mites, H_2O_2 and the lipid membrane peroxidation increased and consequently disturbed its permeability in plant tissues by increasing electrolyte leakage.

Changes in the Enzymatic Antioxidant Activities

RPW infestation stimulates and reinforces antioxidant defence mechanisms, aiding in the detoxification of ROS to uphold manageable, stable levels of ROS, and consequently restrict cell damage. Plants initiate the signalling cascade under oxidative stress by inducing the activity of defensive enzymes SOD, POX, CAT, APX, and metabolites such as phenols, vitamins, and low molecular weight antioxidants as a survival strategy (Hussain *et al*., 2022). In this study, CAT and APX-specific activities followed a similar trend as the activities were higher in the control front and middle fronds than in the infested parts. In contrast, the infested end part of the frond showed higher activities of CAT, APX, and gPOD enzymes (Figure 4). Higher specific activities were found in infested leaves rather than in control, as reported by Arutselvi *et al*. (2012) in their study on turmeric leaves infested by serious pests, *Panchaetothrips indicus* and *Udaspes folus*. Thus, specific activities are proven to increase significantly due to the disproportionation of excess H_2O_2 under stress (Gajweska *et al*., 2006).

Contrast results were obtained in this study whereby CAT, APX, and gPOD-specific activities in healthy front and middle fronds were significantly higher compared to infested fronds (Figure 4). Heng-Moss *et al.* (2004) also found that the decline of CAT-specific activities was due to insect feeding. Compared to healthy plants, they reported a reduction of CAT-specific activities in buffalo grass by chinch bugs. Similarly, in this study, gPOD-specific activities in healthy front fronds were higher. This was aligned with the findings from Golan *et al.* (2013), which showed a nearly 17-fold decrease in peroxidase activity compared to the control group. Moreover, they also found that strongly colonised fern, *Nephrolepis biserrata* by scale insects showed similar drops of POD and CAT-specific activities below the controlled levels. Enhanced or depleted enzyme activities depend on the duration, intensity, and type of environmental stresses. This study shows that the CAT, APX, and gPOD-specific activities

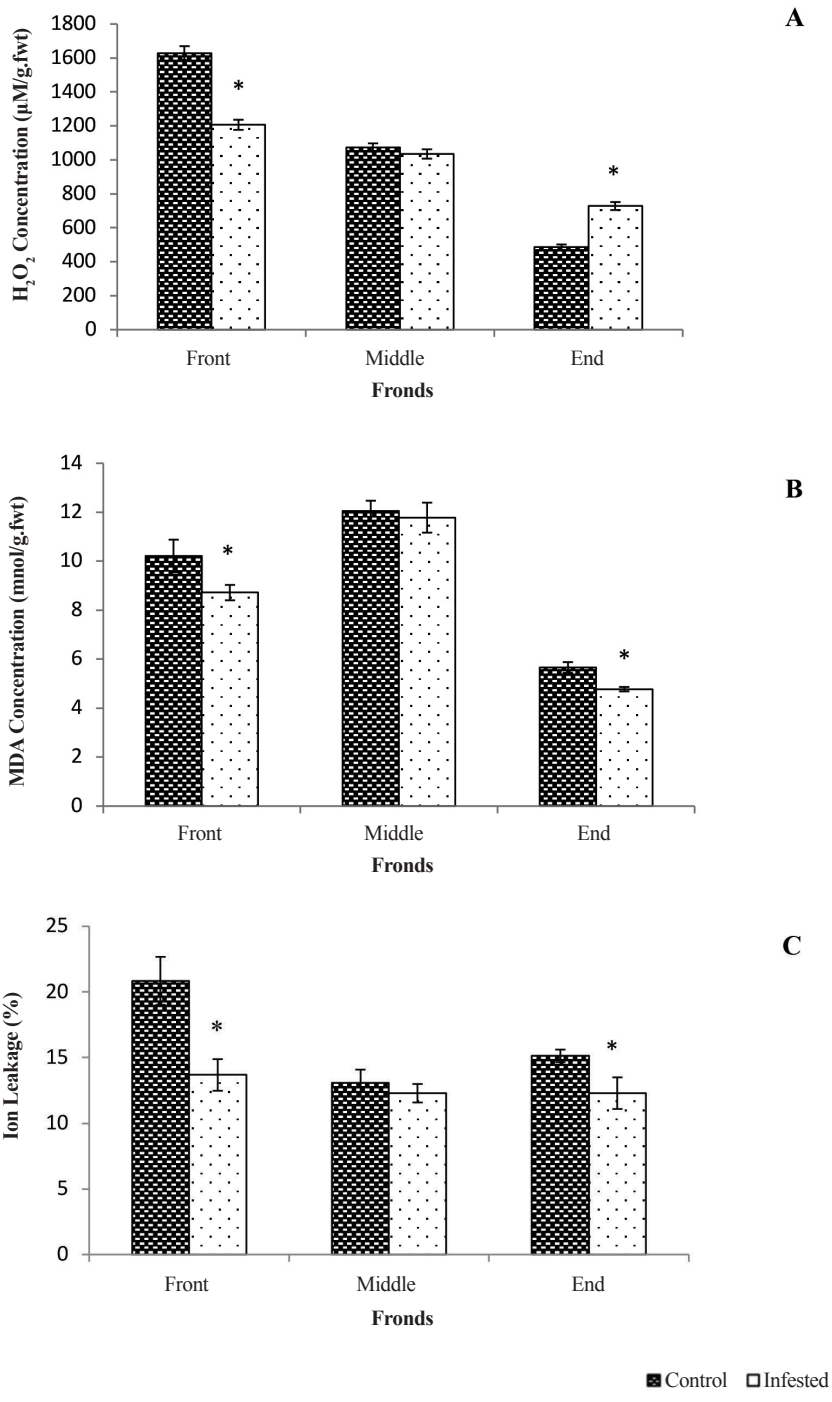

Figure 3: (A) H_2O_2, (B) MDA, and (C) ion leakage in front, middle, and end parts of healthy and infested MAWA fronds. The results shown are means ± standard errors (n = 9)

Note: * = significantly differs between the control and infested group at $p < 0.05$

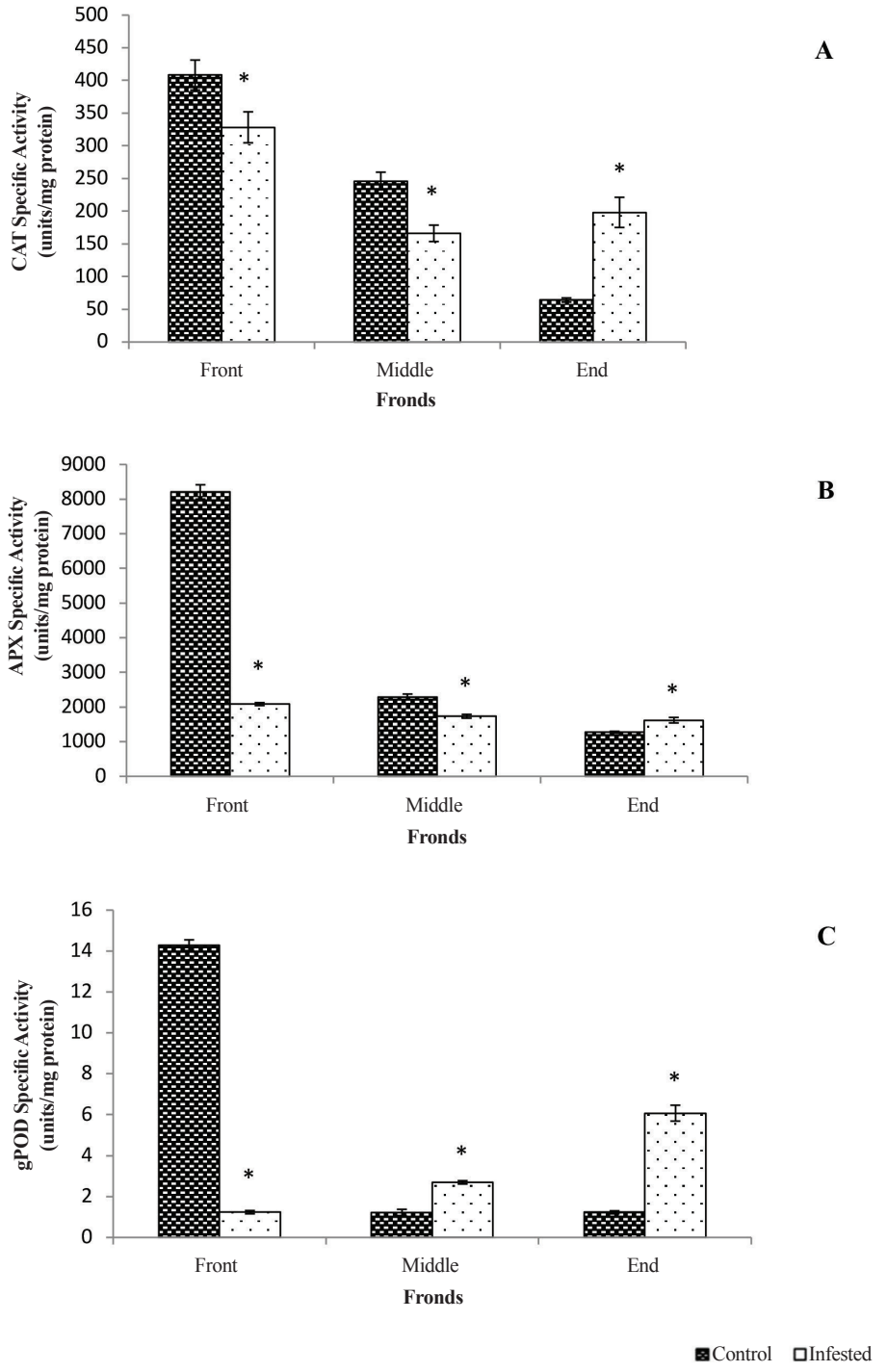

Figure 4: (A) CAT, (B) APX, and (C) gPOD-specific activities in three different parts of healthy and infested MAWA fronds. Data are means ± standard errors (n = 9)

Note: * = significantly differs between the control and infested group at $p < 0.05$

dropped as the infested leaves were depleted despite being enhanced (Moussa & Abdel-Aziz, 2008; Han *et al.*, 2009).

As for specific activities in different parts of fronds, the front parts were higher in the total of CAT, APX, and gPOD-specific activities followed by the middle parts. The highest specific activities of infested front "MAWA" leaves are due to the feeding behaviour of RPW which is known to first attack coconut shoots and straight to the cabbages attached to the front part of leaves (Wahizatul *et al*., 2013). Thus, enzymes will be expressed more in the front part than the middle and end parts in combating ROS production. However, interestingly, the end parts of the frond showed higher specific activities of CAT, APX, and gPOD than the middle parts but lower than the front parts making them astray from the trend. This might happen as the end parts are the outermost part being exposed. Hence, the end parts might be easily influenced by other stresses rather than being only infested by RPW, resulting in more specific activities than the middle parts. The exposure of end parts of fronds, for example, to the high temperature of sunlight might have induced ROS production, reduction of growth, water loss, and change in photosynthetic efficiency (Hasanuzzaman *et al*., 2013). Thus, ROS productions of infested end leaves have resulted from infestations and other stresses, including exposure to sunlight, enabling higher specific activities in infested rather than healthy leaves.

Changes in the Non-enzymatic Antioxidant Activities

The amount of α-tocopherol in infested "MAWA" leaves was significantly higher in the front part compared to its control [Figure 5 (A)]. Increases in α-tocopherol were due to stress response and it will inhibit lipid peroxidation to reduce the ROS levels; thus, preventing oxidative damage (Munne-Bosch, 2005). Shao *et al.* (2007) observed elevated levels of α-tocopherol and ascorbic acid in tomatoes after triazole treatment. This increase may aid in safeguarding membranes against oxidative harm, thereby, enhancing chilling tolerance in tomato plants. This proved that increased *α*-tocopherol levels react to environmental stressors such as pathogen assaults, injuries, and water stress.

Ascorbic acid can regenerate α-tocopherol; thus, increasing the amount of tocopherol as the level of ascorbic acid is higher in the infested plant. Under typical physiological circumstances, ascorbic acid predominantly exists in its reduced form within chloroplasts and leaves (Smirnoff, 2000). Figure 5 (B) shows that ascorbic acid in infested fronds was higher than control for the front and middle parts. This is because when the ROS is higher, the ascorbic acid is also higher to protect the plant. Ascorbic acid can regenerate α-tocopherol from tocopheroxyl radical, thereby, offering membrane protection for the plant (Thomas *et al.*, 1992). Dias *et al.* (2011) confirmed that ascorbic acid serves as the primary precursor of oxalic acid in susceptible and resistant cacao (*Theobroma cacao* L.) infected by the hemibiotrophic fungus, *Moniliophthora perniciosa*. In plant-pathogen interaction, oxalic acid can help in the synthesis of H_2O_2 and function in impeding the growth of biotrophic pathogens. Thus, it helps to prevent plants from being infected by necrotrophic pathogens. This showed that the antioxidant activity of ascorbic acid is embedded with resistance to oxidative stress and longevity in plants (Taqi *et al.*, 2011).

The middle part of the infested frond contains higher carotenoid content compared to the control [Figure 5 (C)]. Carotenoids shield the plant from photo-oxidative harm by extinguishing the triplet state of chlorophyll (Young, 1991), scavenging singlet oxygen and acting as a plant response mechanism. Accumulation of carotenoids in parsley leaves surrounding the Septoria blight lesion implies that these pigments may play a role in the plant's response to pathogen outbreaks. A similar observation was also noted in parsley against *Septoria petroselini* infestation (Rafal *et al.*, 2005). The middle part also might face other environmental stresses such as high temperature

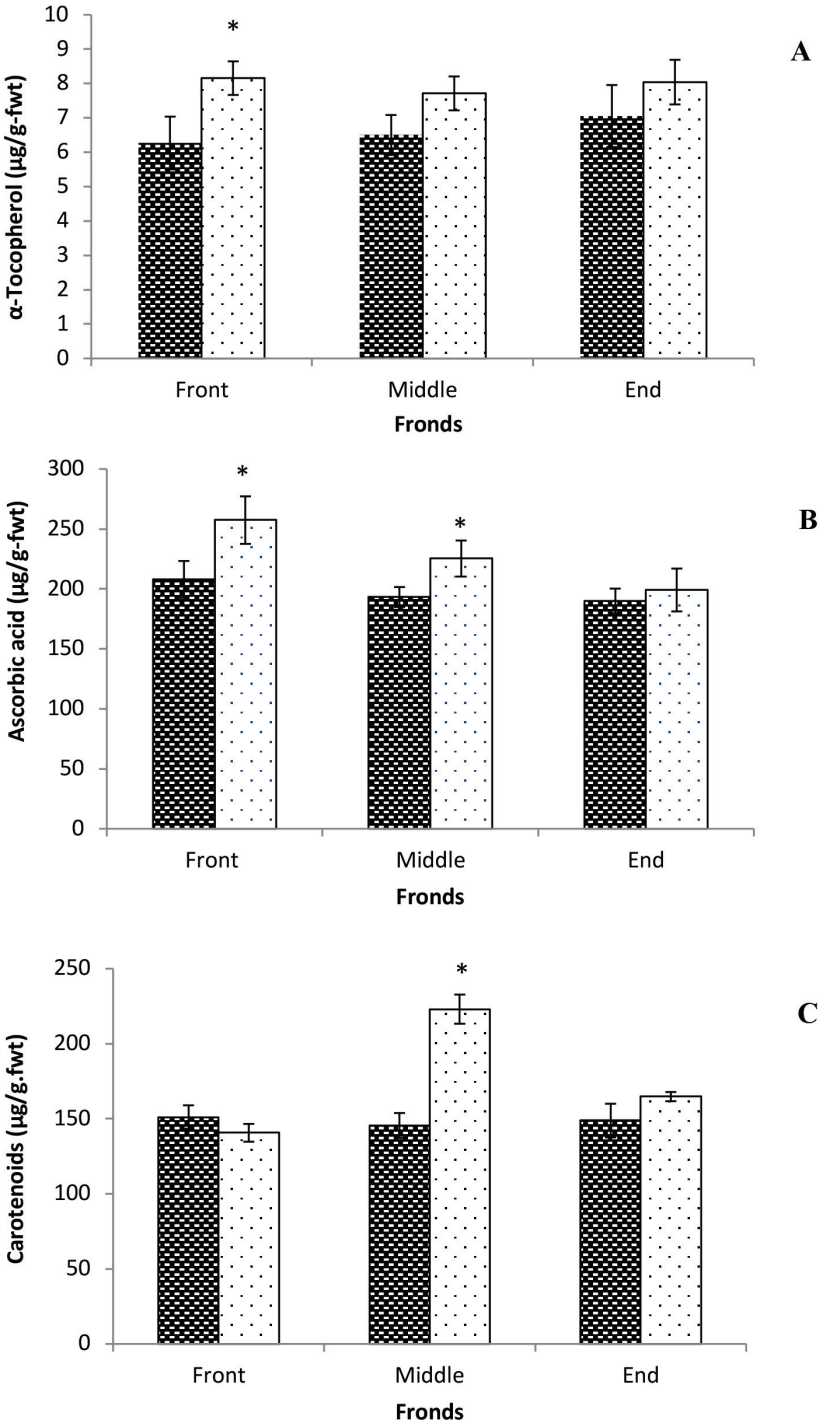

Figure 5: (A) α-tocopherol, (B) ascorbic acid, and (C) carotenoids in three different parts of healthy and infested MAWA cultivar. Data are means ± standard errors (n = 9)

Note: * = significantly differs between the control and infested group at $p < 0.05$

and receiving higher amounts of sunlight as we collect the sample during the dry season. Carotenoids in the infested front part were lower than in control, as this part was sheltered from sunlight. Carotenoids accumulate higher in the leaves that are more exposed to sunlight than the shaded leaves (Matsubara *et al.*, 2009). Thus, the control fronds contain more carotenoids than infested leaves at the front part as some of the carotenoids in infested front fronds might be damaged due to infestation.

Conclusions

This study revealed that RPW infestation can lead to oxidative stress and cause vital damage to the "MAWA" cultivar. The H_2O_2, MDA, and ion leakage were induced in certain parts of the fronds. Healthy fronds exhibited higher CAT, APX, and gPOD-specific activities than infested leaves except for the end part. APX showed significantly higher specific activities in all parts of infested fronds compared to CAT and gPOD. Hence, it can be concluded that "MAWA" had expressed APX the most in combating infestation. Therefore, specific activities of APX can be used as an indicator to differentiate between healthy and infested trees. Results also indicated that the infested "MAWA" cultivar contained a higher amount of α-tocopherol, ascorbic acid, and carotenoids especially in the front and middle parts. The strong antioxidant capacity to neutralise ROS toxicity has been associated with enhanced plant tolerance to this pathogen attacks. The "MAWA" cultivar appears to favour utilising ascorbic acid to combat the infestation, evidenced by the higher levels of ascorbic acid in infested fronds compared to other antioxidants. This study offers valuable insights that could enhance our understanding of the antioxidant defence mechanisms in coconut trees, thereby assisting farmers in mitigating ongoing economic losses by opting for more tolerant cultivars.

Acknowledgements

The authors would like to thank the Ministry of Higher Education (MOHE), the Malaysian Government for funding the project (FRGS 59343) and the Department of Agriculture, Ajil, Terengganu for providing the "MAWA" cultivar.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

Agrawal, R., & Patwardhan, M.V. (1993). Production of peroxidase enzyme by callus cultures of *Citrus aurantifolia. Journal Science Food Agriculture*, *61*, 377-378.

Allison, S. D., & Schultz, J. C. (2004). Differential activity of peroxidase isozymes in response to wounding, gipsy moth, and plant hormones in northern red oak (*Quercus rubra* L.). *Journal of Chemical Ecology*, *30*(7), 1363-1379.

Arbeit, W. (1985). *What are fronds for?* University of Hawaii Press.

Arutselvi, R., Balasaravanan, T., Ponmurugan, P., & Muthu, S. P. (2012). Comparative study of enzyme activity of leaves of turmeric varieties. *Journal of Pharmacy Research*, *5*(4), 2137-2140.

Bhattacharjee, S. (2005). Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal. *Current Science*, *89*, 1113-1121.

Bhuyan, M. B., Hasanuzzaman, M., Parvin, K., Mohsin, S. M., Al Mahmud, J., Nahar, K., & Fujita, M. (2020). Nitric oxide and hydrogen sulfide: Two intimate collaborators regulating plant defense against abiotic stress. *Plant Growth Regulation*, *90*, 409-424.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Catalá, A., & Díaz, M. (2016). Editorial: Impact of lipid peroxidation on the physiology

and pathophysiology of cell membranes. *Frontiers in Physiology*, *7*. https://doi.org/10.3389/fphys.2016.00423

Caverzan, A., Casassola, A., & Brammer, S. P. (2016). Antioxidant responses of wheat plants under stress. *Genetics and Molecular Biology*, *39*(1), 1-6.

Chandrashekar, G. S., Maheswarappa, H. P., Manjunath Hubballi, Jilu, V. S., Sudarshan, G. K., & Basavaraju, T. B. (2020). Evaluation of chemical insecticides against the red palm weevil, *Rhynchophorus ferrugineus* Olivier. *Journal of Pharmacognosy and Phytochemistry*, *9*(6), 2313-2317.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204-7218. https://doi.org/10.18632/oncotarget.23208

Claiborne, A. L. (1985). Catalase activity. In Grenwald, E. A. (Ed.), Handbook of method for oxygen radical research (pp. 283-284). CRS Press.

Comtrade. (2020). Available online: http://www.comtrade.org (accessed on 13 February 2024).

Correia, B., Valledor, L., Meijón, M., Rodriguez, J. L., Dias, M. C., Santos, C., & Pinto, G. (2013). Is the interplay between epigenetic markers related to the acclimation of cork oak plants to high temperatures? *PLOS ONE, 8*(1), e53543.

Dias, C. V., Mendes, J. S., Santos, A. C., Pirovani, C. P., Gesteira, A. D., Micheli, F., Gramacho, K. P., Hammerstone, J., Mazzafera, P., & De Mattos Cascardo, J. C. (2011). Hydrogen peroxide formation in cacao tissues infected by the hemibiotrophic fungus *Moniliophthora perniciosa*. *Plant Physiology and Biochemistry*, *49*(8), 917-922.

Department of Agriculture Malaysia (DOA). (2016). *Report on the current status attack of the red palm weevil*, *Rhynchophorus ferrugineus*, *in Malaysia*. Government Press.

Department of Agriculture Malaysia (DOA). (2021). *Booklet statistik tanaman 2021*. Khazanah Research Institute.

Dvořák, P., Krasylenko, Y., Zeiner, A., *Šamaj*, J., & Takáč, T. (2021). Signaling toward reactive oxygen species-scavenging enzymes in plants. *Frontiers in Plant Science*, *11*, 618835. https://doi.org/10.3389/fpls.2020.618835

El-Lakwah, F. A. M., El-Banna, A. A., El-Hosary, R. A., & El-Shafei, W. K. M. (2011). Impact of certain factors and agricultural practices on infestation of date palm trees by the red palm weevil (*Rhynchophorus ferrugineus* (Oliver). *Egypt Journal of Research*, *89*(3), 1119-1127.

Farouk, S., & Osman, M. A. (2009). Induction of resistance in common bean plants *Phaseolus vulgaris* L. using different plant elicitors against spider mite *Tetranychus urticae* Koch infestation. *Journal of Agricultural Science*, *34*(12), 11399-11419.

Foley, R. C., Kidd, B. N., Hane, J. K., Anderson, J. P., & Singh, K. B. (2016). Reactive oxygen species play a role in the infection of the necrotrophic fungi, *Rhizoctonia solani* in wheat. *PLOS ONE*, *11*, e0152548. https://doi.org/10.1371/journal.pone.0152548

Gajweska, E., Sklodowska, M., Slaba, M., & Mazur, J. (2006). Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. *Biology Plantarum*, *50*, 653-659.

Galaris, D., Barbouti, A., & Pantopoulos, K. (2019). Iron homeostasis and oxidative stress: An intimate relationship. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1866*(12), 118535. https://doi:10.1016/j.bbamcr.2019.118535

Gandhi, A., Kariyat, R. R., Chappa, C., Tayal, M., & Sahoo, N. (2020). Tobacco hornworm (*Manduca sexta*) oral secretion

elicits reactive oxygen species in isolated tomato protoplasts. *International Journal of Molecular Sciences*, *21*, 8297. https://doi.org/10.3390/ijms21218297

Gill, R. S., Gupta, A. K., Taggar, G. K., & Taggar, M. S. (2010). Role of oxidative enzymes in plant defences against insect herbivory. *Acta Phytopathologica et Entomologica Hungarica*, *45*, 277-290.

Golan, K., Rubinowska, K. & Górska-Drabik, E. (2013). Physiological and biochemical responses of fern *Nephrolepis biserrata* (Sw.) Schott. to *Coccus hesperidum* L. infestation. *Acta Biologica Cracoviensia Series Botanica*, *55*(1), 93-98.

Guo, W., Xing, Y., Luo, X., Li, F., Ren, M., & Liang, Y. (2023). Reactive oxygen species: A crosslink between plant and human eukaryotic cell systems. *International Journal of Molecular Sciences*, *24*(17). https://doi.org/10.3390/ijms241713052

Han, C., Liu, Q. & Yang, Y. (2009). Short-term effects of experimental warming and enhanced ultraviolet-B radiation on photosynthesis and antioxidant defense of *Picea asperata* seedlings. *Plant Growth Regulation*, *58*(2), 153-162.

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal Molecular Science*, *14*, 9643-9684.

Hasanuzzaman, M., Borhannuddin Bhuyan, M. H. M., Zulfiqar, F., Raza, A., Mohammad Mohsin, S., Al Mahmud, J., Fujita, M., & Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, *9*, 681. http://dx.doi.org/10.3390/antiox9080681

Heng-Moss, T. M., Baxendale, F. P., Riordan, T. P., Yojng, L. J., & Lee, K. (2004). Chinchbug-resistant buffalograss: An investigation of tolerance, antixenosis and antibiosis. *Journal of Economic Entomology*, *96*, 1942-1951.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, *125*(1), 189-198.

Hodges, D. M., Andrews, C. J., Johnson, D. A., & Hamilton, R. I. (1996). Antioxidant compound responses to chilling stress in differentially sensitive inbred maize lines. *Physiologia Plantarum*, *98*(4), 685-692.

Hossain, M. A., Bhattacharjee, S., Armin, M., Qian, P., Xin, W., Li, Y., Burritt, D. J., Fujita, M., & Tran, S. P. (2015). Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: Insights from ROS detoxification and scavenging. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00420

Hussain, A., Rizwan-ul-Haq, M., & AlJabr, A. M. (2017). Susceptibility, antioxidant-defense, and growth inhibitory response of *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) against the virulence of *Metarhizium anisopliae* isolates. *Universal Journal of Plant Science*, *5*, 17-23.

Jagota, S., & Dani, H. (1982). A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. *Analytical Biochemistry*, *127*(1), 178-182.

Kanno, C., & Yamauchi, K. (1997). Application of a new iron reagent, 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine, to spectrophotometric determination of tocopherols. *Agricultural and Biological Chemistry*, *41*(3), 593-596.

Kapoor, D., Sharma, R., Handa, N., Kaur, H., Rattan, A., Yadav, P., Gautam, V., Kaur, R., & Bhardwaj, R. (2015). Redox homeostasis in plants under abiotic stress: Role of electron carriers, energy metabolism mediators and proteinaceous thiols. *Frontiers in Environmental Science*, *3*, 132074. https://doi.org/10.3389/fenvs.2015.00013

Khan, M. I. R., Khan, N. A., Masood, A., Per, T. S., & Asgher, M. (2016). Hydrogen peroxide alleviates nickel-inhibited photosynthetic responses through increase in use-efficiency of nitrogen and sulfur, and glutathione production in mustard. *Frontiers in Plant Science*, *7*, 44. http://doi.org/10.3389/fpls.2016.00044

Kloth, K. J., Abreu, I. N., Delhomme, N., Petřík, I., Villard, C., Ström, C., Amini, F., Novák, O., Moritz, T., & Albrectsen, B. R. (2019). PECTIN ACETYLESTERASE9 affects the transcriptome and metabolome and delays aphid feeding. *Plant Physiology*, *181*(4), 1704-1720. http://doi.org/10.1104/pp.19.00635

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In Packer, I., & Douce, R. (Eds.), *Methods in enzymology* (pp. 350-382). Academic Press.

Maluin, F. N., Hussein, M. Z., & Idris, A. S. (2020). An overview of the oil palm industry: Challenges and some emerging opportunities for nanotechnology development. *Agronomy*, *10*, 356. http://doi:10.3390/agronomy10030356

Matsubara, S., Krause, G. H., Aranda, J., Virgo, A., Beisel, K. G., Jahns, P., & Winter, K. (2009). Sun-shade patterns of leaf carotenoid composition in 86 species of neotropical forest plants. *Functional Plant Biology*, *36*(1), 20-36.

Meo, S. D., & Venditti, P. (2020). Evolution of the knowledge of free radicals and other oxidants. *Oxidative Medicine and Cellular Longevity*, *2020*, 9829176. https://doi.org/10.1155/2020/9829176

Mostafa, S., Wang, Y., Zeng, W., & Jin, B. (2022). Plant responses to herbivory, wounding, and infection. *International Journal of Molecular Sciences, 23,* 7031. https://doi.org/10.3390/ijms23137031

Moussa, R., & Abdel-Aziz, S. M. (2008). Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Australian Journal of Crop Sciences*, *1*(1), 31-36.

Munné-Bosch, S. (2005). The role of α-tocopherol in plant stress tolerance. *Journal of Plant Physiology*, *162*(7), 743-748.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, *22*(5), 867-880.

Norhayati, Y., Afzan, W. A., Jannah, S. N. S., & Wahidah, N. M. (2016). Antioxidative responses of *Cocos nucifera* against infestation by the Red Palm Weevil (RPW), *Rhynchophorus ferrugineus*, a new invasive coconut pest in Malaysia. *Sains Malaysiana*, *45*(7), 1035-1040.

Nousis, L., Kanavaros, P., & Barbouti, A. (2023). Oxidative stress-induced cellular senescence: Is labile iron the connecting link? *Antioxidants*, *12*(6). https://doi.org/10.3390/antiox12061250

Rafal, B., Malgorzata, B., & Hartwig, S. (2005). Changes in carotenoid content and distribution in living plant tissue can be observed and mapped in situ using NIR-FT-Raman spectroscopy. *Planta*, *222*(3), 448-457.

Ranf, S., Eschen-Lippold, L., Pecher, P., Lee, J., & Scheel, D. (2011). Interplay between calcium signalling and early signalling elements during defence responses to microbe- or damage-associated molecular patterns. *The Plant Journal*, *68*(1), 100-113.

Reymond, P. (2021). Receptor kinases in plant responses to herbivory. *Current Opinion in Biotechnology*, *70*, 143-150. https://doi.org/10.1016/j.copbio.2021.04.004

Rozeita, L., & Zulkafli, I. (2018). The role of R&D in transforming Malaysia's coconut industry. *Proceedings of National Coconut Conference (NCC) 2018: Empowering Coconut Industry*, 7-9th August 2018, Perak, Malaysia.

Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., & Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, *10*(2), 277. https://doi.org/10.3390/antiox10020277

San, S. H., Sagar, D., Krishnan, V., Awana, M., Singh, A., Bhowmik, A., Singh, R., & Chander, S. (2022). Effects of *Helicoverpa armigera* (Hubner) infestation on metabolic sensors dynamics in chickpea. *Allelopathy Journal*, *57*(1), 83-108. https://doi.org/10.26651/allelo.j/2022-57-1-1407

Shao, H. B., Chu, L. Y., Wu, G., Zhang, J. H., Lu, Z. H., & Hu, Y. C. (2007). Changes of some antioxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. *Colloids and Surface B: Biointerfaces*, *54*,143-149.

Smirnoff, N. (2000). Ascorbic acid: Metabolism and functions of a multifaceted molecule. *Current Opinion in Plant Biology*, *3*(3), 229-235.

Spiteller, G. (2003). The relationship between change in the cell wall, lipid peroxidation, proliferation, senescence and cell death. *Physiology Plantarum*, *119*, 5-8.

Su, J., Zhang, H., Gomez, H., Murugan, R., Hong, X., Xu, D., Jiang, F., & Peng, Y. (2019). Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxidative Medicine and Cellular Longevity*, 5080843. https://doi.org/10.1155/2019/5080843

Taqi, A. K., & Mohd Mazid Firoz, M. (2011). Role of ascorbic acid against pathogenesis in plants. *Journal of Stress Physiology & Biochemistry*, *7*(3), 222-234.

Thakur, M., Bhattacharya, S., Khosla, P. K., & Sunil, P. (2019). Improving production of plant secondary metabolites through biotic and abiotic elicitation. *Journal of Applied Research on Medicinal and Aromatic Plants*, *12*, 1-12.

Thomas, C. E., Mclean, L. R., Parker, R. A., & Ohlweiler, D. F. (1992). Ascorbate and phenolic antioxidant interactions in prevention of liposomal oxidation. *Lipids*, *27*(7), 543-550.

Valgimigli, L. (2023). Lipid peroxidation and antioxidant protection. *Biomolecules*, *13*, 1291. https://doi.org/10.3390/biom13091291

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science*, *151*(1), 59-66.

Wahizatul, A. A., Zazali, C., Abdul Rahman, A. R., & Nurul Izzah, A. G. (2013). A new invasive coconut pest in Malaysia. *The Planter*, *89*(1043), 97-110.

Weche, M. J. (2019, March 14). *Using sensor technology to tackle red palm weevils*. King Abdullah University of Science and Technology. https://www.kaust.edu.sa/en/news/using-sensor-technology-to-tackle-red-palm-weevils

Wojtaszek, P. (1997). Oxidative burst: An early plant response to pathogen infection. *Biochemical Journal*, *322*(3), 681-692. https://doi.org/10.1042/bj3220681

Xie, X., He, Z., Chen, N., Tang, Z., Wang, Q., & Cai, Y. (2019). The roles of environmental factors in regulation of oxidative stress in plant. *BioMed Research International*, 9732325. https://doi.org/10.1155/2019/9732325

Yahya, S., & Mohd Zainal, I. (2014). Design and performance of young coconut shaping machine. *Journal of Tropical Agricultural and Food Science*, *42*(1), 19-28.

Young, A. J. (1991). The photoprotective role of carotenoids in higher plants. *Physiologia Plantarum*, *83*(4), 702-708.

Zainol, F. A., Arumugam, N., Daud, W. N. W., Suhaimi, N. A. M., Ishola, B. D., Ishak, A. Z., & Afthanorhan, A. (2023). Coconut value chain analysis: A systematic

review. *Agriculture*, *13*, 1379. https://doi.org/10.3390/agriculture13071379

Zhang, J., Li, Y., Bao, Q., Wang, H., & Hou, S. (2022). Plant elicitor peptide 1 fortifies root cell walls and triggers a systemic root-to-shoot immune signaling in Arabidopsis. *Plant Signaling & Behavior*, *17*, e2034270. https://doi.org/10.1080/15592324.2022.2034270

Zhou, S., Lou, R., Tzin, V., & Jander, G. (2015). Alteration of plant primary metabolism in response to insect herbivory. *Plant Physiology*, *169*(3), 1488-1498. https://doi.org/10.1104/pp.15.01405