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Antioxidative responses of Melon Manis Terengganu (MMT) against *Fusarium* sp.

Norhasnita Md. Baharudin[1], Nur Fatin Wahida Abdul Rani[1], Nurul Faziha Ibrahim[2] and Norhayati Yusuf[1,3]*

[1]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia.
[2]Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia.
[3]Biological Security and Sustainability Research Interest Group (BIOSES), Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
Email: yatiyusuf@umt.edu.my

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ABSTRACT

Aims: Melon Manis Terengganu, MMT is one of the economically important fruits in Terengganu, which contains numerous nutritional values and bioactive compounds that benefit human health. The major problem is MMT has been affected by *Fusarium* sp., which is the common fungus in the Cucurbitaceae family resulting in Fusarium wilt disease and lowering melon production. It may also affect the antioxidant value of MMT; however, limited study has been conducted on this issue. Hence, the objective of this study was to determine the non-enzymatic as well as enzymatic activities in response to *Fusarium* sp. (S2 and S4) infection.
Methodology and results: In this study, MMT leaves were incubated in culture filtrate (CF) obtained from Murashige and Skoog (MS) liquid medium. The antioxidative responses were assayed at 0, 1, 3, 5, 7 and 9 days of treatment. In response to *Fusarium* infection, the ascorbic acid, α-tocopherol and carotenoid content were significantly stimulated at the early stages of the experiment and slowly reduced afterward. This current study also demonstrated that the CAT-specific activities were initially induced in S2 CF-treated leaves. Similar APX and gPOD specific activity patterns were observed in both S2 and S4 CFs treatments. The APX and gPOD-specific activities were induced at the later stages of infection in S4 CF-treated leaves.
Conclusion, significance and impact of study: The results revealed that enzymatic and non-enzymatic antioxidants worked together to fight against stress caused by the fungal infection, with the activation of the plant defense system.

Keywords: Antioxidants, culture filtrates, *Fusarium* sp., Melon Manis Terengganu, plant defense mechanism

INTRODUCTION

Melon Manis Terengganu (*Cucumis melo* var. inodorus cv. Manis Terengganu 1, MMT) from the family of Cucurbitaceae is one of the exclusively commercialized melons originated from Terengganu. Although it was newly introduced in 2015, this locally grown salmon-coloured sweet melon has earned good returns for local entrepreneurs and planters. Due to its high demand locally and in international markets, especially in Japan and Singapore, the Terengganu state government, with support from the Federal Agriculture Marketing Authority, FAMA (FAMA, 2016) and the Department of Agriculture (DOA) has committed to opening larger commercial scale for the MMT cultivation. MMT is enriched with many nutritional values, including vitamin C and potassium, and almost 90% of the fruits are composed of water, with lower calories. Even the peel contains high dietary fiber of 49.25 ± 2.05 g/100 g (Ong *et al*., 2022). Existing literature demonstrated that MMT peel consisted of bioactive compounds, vitamins and minerals with health benefits (Ong *et al*., 2021). The tremendous amount of nutrients and antioxidants in MMT can lower the risk of heart disease, diabetes and cancer; thus, it has great potential to be commercialized. The texture is firm, crunchy, sweet and juicy tastes. The average weight is about 1.0 to 1.8 kg/fruit. Thus, MMT is undoubtedly a highly favoured and economically significant fruit plant.

MMT is infected by numerous viral, bacterial, mycoplasmal and fungal organisms, which can affect melons at any plant developmental stage, causing enormous economic losses (Parvin *et al*., 2015). According to Natasya Amirah *et al*. (2020), MMT's most common and serious insect pest is the red pumpkin beetle, RPB (*Raphidopalpa foveicollis*). The leaves and stems of MMT plants were subject to attack by adult RPB, resulting in losses of 35% to 75% for seedlings in cucurbit plants at both the leaf and fruit stages (Rashid *et al*.,

***Corresponding author**

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2016). Melon worm (*Diaphania hyalinata*) was found during the foliage expansion, fruit set and maturation stages and dropped the yield by up to 33% (Panti *et al*., 2016). The melon fruit fly (*Zeugodacus cucurbitae*) is widely regarded as the most damaging pest of melons and is capable of causing up to 100% yield loss (Sohel *et al*., 2019). The attacks are particularly during the fruit ripening stage, where they are attracted to volatile fruit compounds. At this stage, the ripened fruits will undergo changes in various characteristics such as their colour, texture, production of volatile aroma, accumulation of starch and quantity of other organic compounds (Mahmoud *et al*., 2022).

Other common insects are leaf miner fly (*Liriomyza trifolii*), melon thrips (*Thrips palmi*), silver leaf whitefly (*Bemisia tabaci*) and orange colour leafhopper (*Bothrogonia addita*). Natasya Amirah *et al.* (2020) also listed 5 diseases observed in MMT, namely cucurbit yellow stunting disorder virus (CYSDV, 58%), followed by powdery mildew (45%), verticillium wilt (38%), watermelon mosaic virus (WMV) and anthracnose.

The soil-borne fungus is another major problem for growers to keep the MMT in good condition. *Fusarium oxysporum* f. sp. melonis Snyder and Hans. (Fom) represents the most common and harmful causing agent leading to melon's Fusarium wilt disease (Suárez-Estrella *et al*., 2004). This destructive disease causes losses of up to 100% of melon production (Luongo *et al*., 2015). Symptoms of the Fusarium wilt disease include combinations of vein clearing, leaf epinasty and wilting, resulting in loss of their turgor pressure of the vines followed by a yellowing of the leaves, necrosis and abscission (MacHardy and Beckman, 1981; Boughalleb and El Mahjoub, 2006). This fungus becomes a successful pathogen because of its necrotrophic lifestyle, which kills the living cells of its host and then feeds on the dead matter and produces mycotoxins resulting in carcinogenic of the host. In this study, crude culture filtrate (CF) of *Fusarium* sp. was used. According to Ogórek (2016), fungus CF can develop phytotoxic metabolites known as mycotoxin. Members of the genus Fusarium produce a range of phytotoxic compounds, including fusaric acid, fumonisins, beauvericin, enniatin, moniliformin and the trichothecenes (Ismaiel and Papenbrock, 2015). These compounds possess various biological and metabolic effects, including necrosis, chlorosis, growth inhibition, wilting and inhibition of seed germination.

Interaction between the fungal CF and MMT plants is believed to induce oxidative stress in plants by signalling the molecular communication between plants and pathogens, and it starts almost immediately after the pathogen contacts the plant surface (Aoun, 2017). Plants primarily developed defensive mechanisms consisting of several antioxidants as strategies to cope with oxidative stress, reduce the reactive oxygen species (ROS) and restore the ROS balance that arises from the interaction (Jimdjio Kouasseu *et al*., 2023). Non-enzymatic strategies depend on small molecules such as α-tocopherols, ascorbic acid, glutathione, phenolic compounds,

Figure 1: Young and fully expanded MMT leaf.

alkaloids; flavonoids; carotenoids, as well as non-protein amino acids (Hasanuzzaman *et al*., 2020). Enzymatic-dependent mechanisms include superoxide dismutase (SOD), which detoxifies superoxide anions through the formation of H_2O_2 and O_2. Several enzymes with antioxidant properties, such as catalase (CAT), ASC peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR) and guaiacol peroxidase, are capable of metabolizing H_2O_2 thus reducing the toxicity effect (Erofeeva, 2015; Nita and Grzybowski, 2016; Ozyigit *et al*., 2016). In this study, MMT plants' non-enzymatic and enzymatic defense mechanisms were determined in response to the infection with *Fusarium* sp. S2 and S4 CFs.

MATERIALS AND METHODS

Samples

The young MMT leaves (Figure 1) were obtained from GM Peladang Sdn. Bhd., 1678 Lorong Fertigasi Padang Negara Kuala Ibai, 20400 Kuala Terengganu.

Isolation and identification of *Fusarium* sp.

MMT plants exhibiting symptoms of Fusarium wilt were gathered from the field and subjected to the following procedures under aseptic conditions: the infected leaf parts were first washed with tap water, then sliced into small 5 mm × 5 mm segments using a sterilized scalpel blade along the leaf vein. These segments were soaked in 70% (v/v) ethanol for 30 sec, followed by a 10 min immersion in 15% Clorox solution. Afterward, the segments were rinsed thoroughly with sterile distilled water 4-5 times to remove any detergent residue. The surface-sterilized segments were dried with sterile filter paper and immediately placed on Potato Dextrose agar

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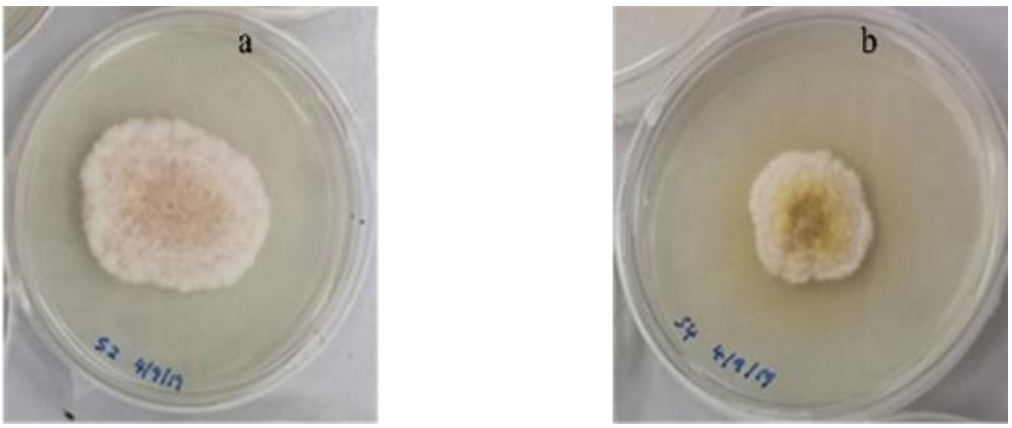

Figure 2: *Fusarium* sp. isolates (a) S2 and (b) S4 in Murashige and Skoog agar.

(PDA). Mix colonies of fungi were isolated and then re-isolated again to obtain pure cultures. The pure cultures were maintained and incubated at 25 ± 2 °C for 5 days. The Fusarium isolates were identified based on their microscopic and macroscopic characteristics, as described in previous studies (Marasas *et al*., 2001; Schroers *et al*., 2009).

Preparation of crude culture filtrate

The *Fusarium* S2 and S4 crude culture filtrates (CFs) were prepared by inoculating two plugs (diameter 1.0 cm) of young mycelium cultures in Murashige and Skoog agar (MSA, Figure 2) into 100 mL of MS liquid medium. The isolates and media were chosen based on the growth performance of the fungi in MS media (data not shown). Cultures were shaken on a rotary shaker at 100 rpm, at 25 ± 2 °C in continuous illumination from fluorescent tubes. The CFs were harvested at 5 days of the incubation period. The mycelium was removed from the CFs by filtering them through the Whatman No. 1 filter paper. The resulting filtrates were collected and sterilized using Millipore filter paper (cellulose acetate/cellulose nitrate type, CA/CN) with a pore size of 0.45 µm.

Surface sterilization and treatment of MMT leaves

Young MMT leaves (3rd leaf stage) were immersed in 70% ethanol for 30 sec, followed by 10 min soaked in 15% Clorox. The leaves were then rinsed 4-5 times with sterile distilled water. Discs of 1.0 cm diameter were then aseptically excised from the surface-sterilized leaves using a sterile cork borer. Three leaf discs were either incubated in CFs or in a basic MS liquid medium for control by placing the discs carefully in sterile plastic Petri dishes. Each experiment was repeated twice with 5 replicates for each treatment. The enzymatic and non-enzymatic antioxidant assays were determined at 0, 1, 3, 5, 7 and 9 days of treatment periods.

Antioxidant assays

Non-enzymatic antioxidant assays

The method of Jagota and Dani (1982) was used to determine the concentration of ascorbic acid. A total of 0.15 g of leaf disc was ground with pre-chilled mortar and pestle in 1.0 mL of 10% trichloroacetic acid (TCA) and clean sand under dim light and in ice-cold conditions. The ground sample was then centrifuged (Universal 32R) at 10,000 rpm for 10 min at 4 °C.

The supernatant obtained (300 µL) was added into 1700 µL distilled water and 200 µL of 10% Folin reagent. The mixture was gently swirled and left on bench under dim light for 10 min. The absorbance was measured at 760 nm using a spectrophotometer (Shimadzu, UV-1601) and the amount of ascorbic acid in the samples was calculated based on the standard curve prepared using ascorbic acid at 0-60 µg/mL.

To determine the level of α-tocopherol, Sies and Murphy's method (1991) was used. A leaf disc weighing 0.15 g was ground with 1.5 mL acetone in a mortar and pestle at 0-4 °C in low light conditions. The mixture was extracted with 0.5 mL hexane, followed by vortexing for about 30 sec. The mixture was then centrifuged at 10,000 rpm for 10 min. After the centrifugation, the top layer was removed, and the hexane extraction was repeated twice. The assay mixture was prepared as per Kanno and Yamauchi's (1977) method. A total of 0.5 mL of the hexane-extract was added into 0.4 mL 0.1% (w/v) PDT (3-(2-pyridyl)-5,6-diphenyl-1,2,4 triazine, prepared in ethanol) and 0.4 mL 0.1% (w/v) ferric chloride (prepared in ethanol). The volume was made up to 3.0 mL with absolute ethanol and the mixture was gently swirled and left for 4 min for colour development. Following this, 0.2 mL of 0.2 M orthophosphoric acid was added to the mixture and allowed to stand for 30 min at room temperature before the absorbance of the mixture was measured at 554 nm. The amount of α-tocopherol in the sample was calculated based on a standard curve produced using α-tocopherol (Sigma, type V) at 0-4 µg/mL.

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Carotenoid content was estimated using Lichtenthaler's method (1987). The leaf disc (0.15 g) was ground up with 3 mL of 80% (v/v) acetone and clean sand in a mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant obtained was measured at 663.2, 646.8 and 470 nm, and 80% acetone was used as a blank.

Enzymatic antioxidant assays

The CAT-specific activity was determined using Havir and McHale's method (1987). A total of 0.15 g of leaf disc was ground with 1.0 mL of 50 mM phosphate buffer (pH 7.4) and clean sand in pre-chilled mortar and pestle at 0-4 °C. The ground sample was then centrifuged at 10,000 rpm at 4 °C for 10 min. The reaction mixture contained 3 mL of reaction buffer (19 mM H_2O_2 in 50 mM phosphate buffer, pH 7.0) and 100 µL of enzyme extract was added to start the reaction. CAT activities were determined based on the rate of H_2O_2 decomposition measured using a spectrophotometer at 240 nm for 3 min at 25 °C. An extinction coefficient of 43.6 $M^{-1}cm^{-1}$ was used to calculate CAT-specific activities.

The APX specific activities were extracted following the method of Nakano and Asada (1981). Leaf disc (0.15 g) was ground up with 1.0 mL of 0.1 M phosphate buffer (pH 7.0) containing 1 mM ascorbic acid and clean sand in pre-chilled mortar and pestle at 4 °C. The homogenate was then centrifuged at 10,000 rpm at 4 °C for 10 min. The reaction mixture consists of 3.0 mL of solution containing 1.5 mL 100 mM potassium phosphate buffer (pH 7.0), 0.5 mL 3 mM ascorbate, 0.1 mL 3 mM EDTA, 0.3 mL distilled water, 0.4 mL crude enzyme extract and 0.2 mL 1.5 mM H_2O_2 to start the reaction. The activities were measured using a spectrophotometer at 290 nm, and extinction coefficients for ascorbate, 2.8 $mM^{-1}cm^{-1}$ were used to calculate the specific activities.

The method of Agrawal and Patwardhan (1993) was used to assay g-POD specific activities. About 0.15 g of leaf disc was ground with 1.0 mL of 100 mM phosphate buffer (pH 7.0) in prechilled mortar and pestle at 0-4 °C. The mixture was centrifuged at 10000 rpm at 4 °C for 10 min. Approximately 100 µL of crude enzyme was added to 3.0 mL reaction mixture containing 1.0 mL of 50 mM phosphate buffer (pH 7.5), 1.0 mL of 20 mM guaiacol and 1.0 mL of 30 mM H_2O_2. The changes in the absorbance were recorded at 470 nm for 3 min using a spectrophotometer. The g-POD-specific activities were measured by the amount of tetra-guaiacol formed using the molar extinction coefficient at 26.6 $mM^{-1}cm^{-1}$.

The protein concentration was calculated according to a standard curve prepared with various concentrations (0.0 to 1.0 mg/mL) of Bovine Serum Albumin (BSA) (Bradford, 1976).

Statistical analysis

Statistical analysis was performed by using Statistical Package for the Social Science (SPSS) software. The two-way ANOVA was used to compare the mean values between the control and treated leaves. The data were expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Effect of *Fusarium* sp. CFs on ascorbic acid, α-tocopherol and carotenoids content

In regular plant cells, there exists a delicate balance between the scavenging of reactive oxygen species (ROS) and their accumulation, which is crucial in maintaining an optimal ROS level for proper redox biology reactions and the regulation of various critical processes in plant growth and development (Mittler, 2017). However, in stressful conditions, particularly during plant-pathogen infections, the production of ROS upsets this equilibrium, causing cellular damage and ultimately resulting in cell death while reducing plant productivity (Raja *et al*., 2017). To combat this, ascorbic acid, an essential antioxidant, plays a vital role in protecting against infections. It performs this task by contributing to the ascorbate-glutathione cycle, which helps scavenge ROS by donating electrons and maintaining its stability. Furthermore, ascorbate assists in regenerating α-tocopherol from tocopheroxyl radical or by scavenging hydroxyl radical ($OH^{\bullet}$) and superoxide radicals ($O_2^{\bullet-}$) (Naz *et al*., 2016; Seminario *et al*., 2017).

In this study, the changes in ascorbic acid concentration of MMT leaf treated with *Fusarium* sp. S2 and S4 in MS media are depicted in Figure 3. Generally, the ascorbic acid concentration in both treated and control MS increased at the early stages of the experiment from 0 to 5 days, except for control S4. The ascorbic acid continuously decreased after that. Leaves treated with S2 and S4 CFs manage to significantly induce ($P<0.05$) the ascorbic acid concentrations compared to control, especially on days 3 and 5 of the experiments. The increments were 1.5-fold higher in both S2 and S4 on day 3, whereas the ascorbic acid concentration shot up to 1.3-fold (S2) and 4-fold (S4) on day 5. The observed increase in ascorbic acid during the early stages of infection in this study may be attributed to the significant accumulation of ROS in plant cells following pathogen attack. This finding is consistent with previous research conducted by Yusuf *et al*. (2016), who observed a similar initial increase in ascorbic acid in *Capsicum annum* infected with *Colletotrichum capsici*. Similarly, an increase in ascorbate levels was also reported in *Brassica rapa* resistant cultivars following infection with *Turnip mosaic virus* (TuMV), as reported by Fujiwara *et al.* (2013).

The ascorbic acid was significantly lowered after 5 days of experiments, indicating the interaction between the *Fusarium* sp. S2 and S4 CFs and the MMT plants. Nganje *et al*. (2004) describe that this pathogenic *Fusarium* sp. colonizes the host as biotrophic fungi and during the necrotrophic stage, they produce toxins and cellulolytic enzymes that are aimed to hijack host secondary metabolic pathways for a better establishment and uptake of host nutrients. Among the *Fusarium* mycotoxins are primarily the trichothecenes, fumonisins

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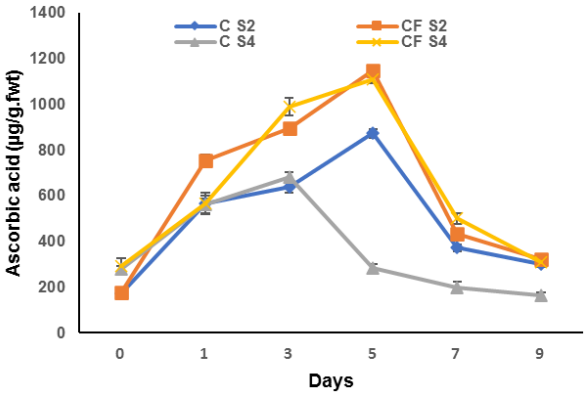

Figure 3: Changes in ascorbic acid concentration (µg/g.fwt) of MMT leaf treated with *Fusarium* isolate S2 and S4 in MS media. Data are means ± SD (n=5).

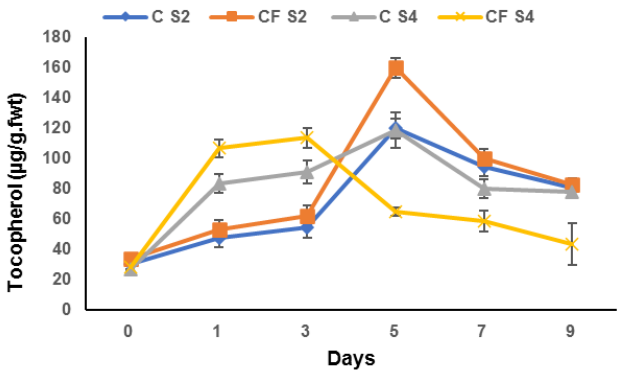

Figure 4: Changes in α-tocopherol concentration (µg/g.fwt) of MMT leaf treated with *Fusarium* isolates S2 and S4 in MS media. Data are means ± SD (n=5).

and zearalenone (Riley *et al.*, 2018). These mycotoxins can induce oxidative stress in plant cells, leading to changes in the ascorbate system, which is a part of the plant's defense mechanism against ROS. This can ultimately result in a decrease in the levels of ascorbic acid in the plants, as was observed in the MMT plants studied. The results obtained were in line with the work by Mozafar (2017). He found that mango infected with *Botryodiplodia* lost almost 100% of its ascorbic acid during a 10-day treatment period, while the control plant lost only 32.6% of its vitamin.

Tocopherols contribute significant roles in plant growth and physiological processes, thus affecting the plants' yield. Due to their antioxidant activity, tocopherols play a vital role in conferring tolerance to several abiotic and biotic stresses. Several reports indicate that stress-tolerant plants exhibit an enhanced level of tocopherols through their ability to scavenge or quench lipid peroxides, oxygen radicals, or singlet oxygen, resulting in the detoxification of ROS (Hasanuzzaman *et al*., 2014; Sadiq *et al*., 2019).

The changes in α-tocopherol concentration of MMT leaf treated with *Fusarium* sp. S2 and S4 CFs are shown in Figure 4. Distinct responses were noted in the α-tocopherol concentrations of S2 and S4 CFs. During the initial 0 to 3 days of the treatment period, the α-tocopherol levels in S4 CF were elevated compared to the control, whereas this increase was not observed in S2 CF. However, after day 3, S2 CF exhibited an increase in α-tocopherol concentration, which was not the case for S4 CF when compared to the control. The upregulation of α-tocopherol appears more rapidly when exposed to S4 CF. These differences in response could be attributed to the distinct substances produced by S2 and S4 CFs. During the later stages of the experimental period, there was a noticeable divergence in the response patterns. In particular, the α-tocopherol concentration in MMT leaves treated with S2 CF showed a substantial increase, reaching a level that was 2.5-fold higher by the fifth day of the experiment. Nevertheless, following this 5-day timeframe, a significant reduction ($p<0.05$) in α-tocopherol concentration was observed in both the treated and control leaves.

A similar trend was observed for the enhanced α-tocopherol in infected *Carthamus tinctorius* L. leaves over control (Mahadik and Mali, 2018) and of *Aloe vera* leaves infected with *C. gloeosporioides* and *F. moniliforme* fungal pathogen (Avasthi, 2014). A widespread of plant secondary metabolites are produced when facing mycotoxins *Fusarium* sp. Apart from killing the pathogen, they either inhibit the production of the toxins or limit the spreading of the pathogen (Atanasova-Penichon *et al*., 2016). Certain secondary metabolites with antioxidant potential, including α-tocopherols and carotenoids, were frequently reported as the primary line of defense against common fungal pathogens (Picot *et al*., 2013).

Carotenoids constitute another important class of antioxidant molecules known to scavenge harmful free radicals and protect light-harvesting complex proteins and thylakoid membrane stability (Zhang *et al*., 2019). Generally, the carotenoids concentration in all MMT leaves decreased significantly after 1 day of the experiment. No significant difference ($p>0.05$) was observed in all treated leaves and control except for leaves treated with S4 CF, which produced significantly ($p<0.05$) higher carotenoids compared to S2 CF on day 3 (Figure 5). The results were compliant with the study by Lobato *et al*. (2010). They also observed the reduction of carotenoids in the common bean plant in the early stage of the experiment after *C. lindemuthianum* infection. Additionally, infection of the tomato plant with *Tobamovirus* directed to a significant decrease in the estimated pigments, including carotenoids (Aseel *et al*., 2019). It is interesting to report that antioxidants are potential inhibitors of *Fusarium* toxins where α-tocopherols blocked the production of fumonisins produced by *F. sacchari*, *F. proliferatum*, *F verticillioides*, *F. subglutinans* and *F. fujikuroi* whereas carotenoids had less or no effect on mycotoxin production.

Higher carotenoids in S4 CF treated leaf are well explained by Perincherry *et al*. (2019) where the activation of the defense-related pathways and severity of

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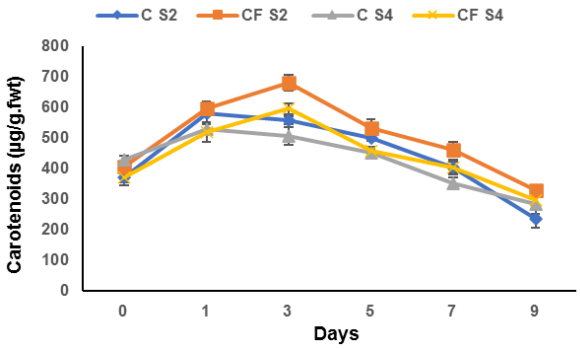

Figure 5: Changes in carotenoids concentration (µg/g.fwt) of MMT leaf treated with *Fusarium* isolates S2 and S4 in MS media. Data are means ± SD (n=5).

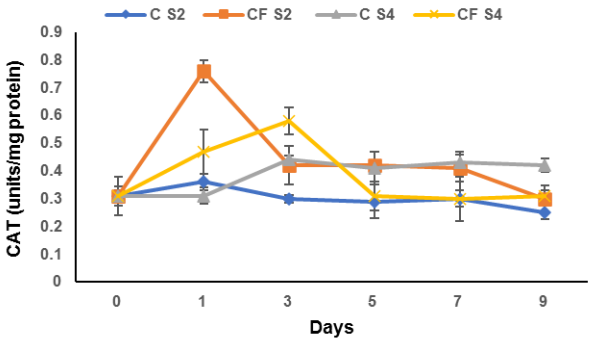

Figure 6: Changes in CAT specific activities (units/mg protein) of MMT leaf treated with *Fusarium* isolates S2 and S4 in MS media. Data are means ± SD (n=5).

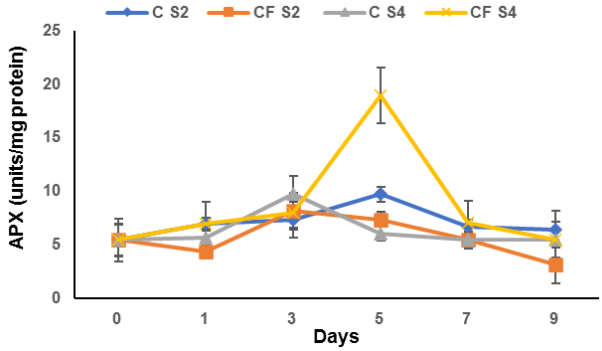

Figure 7: Changes in APX specific activities (units/mg protein) of MMT leaf treated with *Fusarium* isolates S2 and S4 in MS media. Data are means ± SD (n=5).

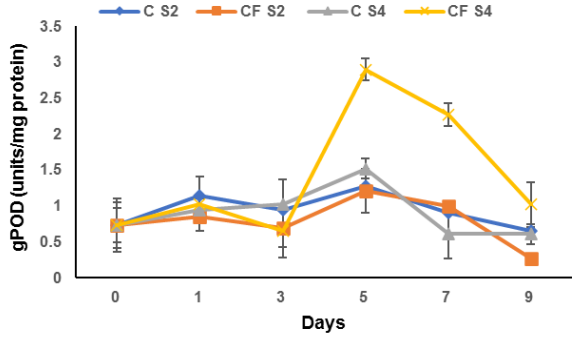

Figure 8: Changes in gPOD specific activities (units/mg protein) of MMT leaf treated with *Fusarium* isolates S2 and S4 in MS media. Data are means ± SD (n=5).

the disease incidence relies completely on the fungal species and strains, as well as the number of mycotoxins produced.

Effect of Fusarium sp. CFs on CAT, APX and gPOD-specific activities

In plants, the enzyme SOD is directly related to stress, which initiates the first line of defense, converting $O_2^{\bullet-}$ into H_2O_2 (Biczak, 2016). This generated H_2O_2 can be further converted into H_2O by the CAT, APX and POD enzymes. CAT-specific activities were in the range of 0.31 ± 0.04 to 0.76 ± 0.03 units/mg protein. At the early stages of the experiment, the CAT-specific activities in S2-treated leaves were significantly higher ($p<0.05$) compared to S4 and their respective controls. The CAT-specific activities in S2 treated leaves were significantly induced ($p<0.05$) to 2.1-fold at 1 day of exposure compared to its control. In contrast, as a response to S4 CF, MMT leaves need a longer incubation period to boost up the CAT-specific activities. No significant difference ($p>0.05$) was observed in these enzyme activities at the later stages of treatment periods (Figure 6).

Increased CAT-specific activities in treated MMT might be due to the activation of the plant defense system against stress build-up by *Fusarium* sp. CFs infection faced by the plant. The results were in line with the study of Kuzniak and Sklodowska (2005) on a tomato that was infected with *B. cineria*. They clearly indicated that initial infection enhanced the CAT activities, indicating antioxidant defense activation was followed by a progressive inhibition concomitant with disease symptom development. Reduction in CAT activities might also be related to the efficiency of other antioxidant enzymes, such as APX and g-POD, which have a higher affinity to the substrate, H_2O_2 than CAT (Rady *et al*., 2020).

S2 and S4 CFs treatments did not significantly alter the APX specific activities in all treated and control plants throughout the experiments except for plants treated with S4 CF on day 5 (Figure 7). APX-specific activities getting higher in S4-treated leaves denote that plant and pathogen contact would rapidly trigger the signal transduction process on the plasma membrane (Shen *et al*., 2017). Hence, the signal will enhance the production of APX. It was postulated that the strong phytotoxicity exhibited by S4 CF can increase the ROS level, reduce

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the activities of antioxidant enzymes like CAT and APX, and induce cell death, as observed in tomato leaves (Singh and Upadhyay, 2014).

Although a significant increase in peroxidase activity in plant-pathogen interactions has been previously reported (Yusuf *et al*., 2016), we did not observe any significant changes in gPOD-specific activities except for MMT leaves treated with S4 CF which spikes to a maximum activity of 2.9 ± 0.3 units/mg protein. Similar to CAT-specific activities, gPOD activities were almost constant among the S2 control leaves (Figure 8). In this current study, APX and gPOD-specific activities were developed at the final stages of experiments, which differ from the CAT activities. Thus, suggesting that MMT preferences to CAT at initial infection stages and later favoured APX and gPOD specific activities to counteract the ROS, particularly H_2O_2 produced during the infection process. Maintaining the peroxidase activity comparable to the control could be explained by the involvement of CAT in H_2O_2 degradation. POD requires a substrate to detoxify the H_2O_2; on the other hand, CAT does not need a reductant to catalyse H_2O_2 dismutation, the reaction of H_2O_2 removal being performed using a very energy-efficient mechanism (Hasanuzzaman *et al*., 2019).

The different affinities of PODs and CATs for H_2O_2 suggest that the function of these two different classes of H_2O_2-scavenging enzymes differs, CATs being involved in the removal of H_2O_2 excess, whereas PODs would be mainly involved in fine modulation of ROS (Noctor and Foyer, 1998). Thus, CAT appears to be the first enzyme involved in H_2O_2 detoxification, and only in systems where CAT is no longer sufficient, H_2O_2 or its derivatives, such as organic peroxides, must primarily be metabolized by POD, which all require reductants (Willekens *et al*., 1997).

CONCLUSION

Fusarium sp. S2 and S4 culture filtrates (CFs) can trigger the non-enzymatic and enzymatic antioxidants in MMT plants, thus activating their antioxidative defense system. However, the extent of antioxidant stimulation depends on the fungal species and strains and the specific toxins present in the CFs. The induction of the plant antioxidant system in MMT is particularly significant in enhancing plant protection against fungal pathogens. A more profound comprehension of the interaction between the fungus and plant could aid in understanding the nature of plant resistance to fungal diseases, which could ultimately limit the loss of MMT. Therefore, conducting a comprehensive investigation into the impact of *Fusarium* on MMT plants is essential for sustainable control of this devastating plant pathogen.

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