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Exploring the therapeutic efficacy of ethanol extracts from *Melia azedarach* Linn. fruit-seeds against oxidative stress induced by melon flies on bitter melon

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Abstract The application of ethanol extract of fruit-seeds *Melia azedarach* (FSMA) resulted in a significant, concentration-dependent reduction in malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels ($p < 0.05$), indicating a marked attenuation of oxidative stress. Enzymatic assays demonstrated that FSMA treatments, particularly at 125 and 652 ppm, significantly enhanced the activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). A strong inverse correlation was observed between the levels of oxidative stress biomarkers and the activities of these antioxidative enzymes, suggesting a mechanistic role of FSMA in modulating redox homeostasis. The findings underscore the efficacy of FSMA in mitigating oxidative damage through the activation of endogenous antioxidant defense systems. These results support the potential use of FSMA as a sustainable and natural alternative to synthetic pesticides, contributing to the development of eco-friendly strategies for oxidative stress management in agricultural systems.

Keywords: Antioxidant properties, Oxidative stress, Pest-induced oxidative damage, Therapeutic potential

Introduction

Momordica charantia L. (*M. charantia*, bitter melon, family Cucurbitaceae) is a climbing plant bearing elongated fruits that transition from greenish to yellow-orange upon ripening. Renowned for its culinary use and traditional medicinal applications, bitter melon has been utilized to address various health issues such as gout, kidney stones, hemorrhoids, and arthritis (Raina *et al*., 2016). However, the production of bitter melon faces significant challenges due to the substantial loss caused by insect pests, with approximately 30-40% of fruits affected annually. Among these pests, *Bactrocera cucurbitae* (*B. cucurbitae*) (Coquillett) (Diptera: Tephritidae) stands

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out for its particularly damaging impact on bitter melon plants. Research by Kaur *et al*. (2014) highlights severe losses in bitter melon production, with infestations by *B. cucurbitae* causing up to 90% fruit damage in a single season. *B. cucurbitae* disrupts fruit tissues, leading to lesions and inflammation that interfere with oxygen and nitrogen metabolic processes. The pest's invasion triggers an overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), overwhelming the plant's antioxidant defense system (Chaki *et al*., 2020). The imbalance in redox homeostasis damages essential biomolecules such as membrane lipids, proteins, and nucleic acids, accelerating tissue degeneration (Somegowda *et al*., 2021). Bitter melon plants possess defense mechanisms against *B. cucurbitae*, but the pest's impact causes ROS accumulation, intensifying oxidative stress and leading to cellular damage (Aljohi *et al*., 2016). When ROS levels exceed the plant's capacity for neutralization, oxidative stress escalates, damaging vital components like DNA and proteins, impairing cellular signaling, and ultimately causing cell death (Choudhary *et al*., 2020). Even though bitter melon plants have developed protective mechanisms, the excessive production of ROS overwhelms defenses, leading to the plant's death. Thus, enhancing the antioxidant system of *M. charantia* to bolster resistance and recovery from oxidative stress becomes imperative (Kerchev and Breusegem, 2022).

Melia azedarach L. (*M. azedarach*), belonging to the Meliaceae family, is a deciduous tree that typically reaches up to 45 meters in height, with a spreading crown and branched limbs. The bark to a greenish-brown color, often showing cracks and a grayish tint. Its alternately arranged leaves are pinnate, ranging from 20 to 40 cm long. The fruits are small, and yellow, containing 4 to 5 black seeds, each about 15 mm in diameter. These seeds are encased in hard shells measuring 3.5 mm by 1.6 mm (Kar *et al*., 2022). *M. azedarach* has many medicinal properties, including antiviral, antimalarial, antifungal, antibacterial, insecticidal, anthelmintic, and anticancer effects (Touzout *et al*., 2023). Extracts from *M. azedarach* fruits and seeds exhibit significant antioxidant and antibacterial activities due to the presence of phenolic compounds. Ethyl acetate extracts from the leaves of *M. azedarach* have also shown potential in iron chelation assays (Azhar *et al*., 2022), and the leaf extracts of *M. azedarach* have demonstrated protective effects against oxidative stress in erythrocytes (Ahmed *et al*., 2008). Despite extensive research into the antibacterial, antioxidant, and anti-inflammatory properties of *M. azedarach* leaves and fruit seeds, the role of ethanol extracts from the fruit-seeds in mitigating oxidative stress induced by *B. cucurbitae* in bitter melon plants has yet to be explored.

This study aimed to investigate the antioxidant potential of ethanol extracts from *M. azedarach* fruit-seeds in alleviating *B. cucurbitae*-induced oxidative stress in Bitter Melon, for protecting the fruit.

Materials and methods

Chemicals and reagents

Chemicals were procured from Sigma-Aldrich (St. Louis, MO, USA). These chemicals were employed as standard agents throughout the experiments.

Collection of material and preparation of the extract

In July 2023, *M. azedarach* fruit-seeds were collected in Dam Bri commune, Bao Loc city, Lam Dong province, Vietnam. A voucher specimen (MA180723VST) was submitted to the herbarium of the Department of Biotechnology, Ho Chi Minh City University of Industry. After collection, ripe and healthy fruits were selected, damaged or diseased ones were discarded, and the fruits were thoroughly washed with clean water. They were then sliced and dried in a Memmert UN 110 drying cabinet (Germany) at 40 °C until the moisture content dropped below 12%. The dried material was ground into a fine powder using a DLF-20 herbal grinder (Vietnam) and stored in moisture-proof bags at room temperature for future research.

The powder from *M. azedarach* fruit-seeds was first soaked in 75% ethanol at a 1:12 ratio for 0.5 hours, followed by extraction for 1.5 hours. The powder was then extracted twice more with 75% ethanol at 1:10 and 1:8 ratios, each for 1 hour. The filtrates from all three extractions were combined, vacuum-filtered, and concentrated using a rotary evaporator RV10 Digital V-C (IKA, Germany) at 45 °C. The ethanol extraction yield of the solution (name FSMA) was calculated using the formula: Extraction yield (%) = (weight of 75% ethanol extract solution) / (weight of powder) × 100%, yielding 15.35%. The extract was stored in a desiccator at 4 °C for subsequent experiments.

Phytochemical screening and quantitative analysis in the ethanol extract of M. azedarach fruit- seeds

Phytochemicals play a vital role in protecting plants against oxidative stress and exhibit insecticidal properties, enhancing plant defense mechanisms. The screening and quantification of compounds in the ethanol extract of *M.*

azedarach fruit-seeds (FSMA) followed standard methods described by Nhung and Quoc (2023) and are detailed in Table 1.

Table 1. Preliminary phytochemical analysis of ethanol extract from *M. azedarach* fruit-seeds

Name of test	Procedure	Observation
Flavonoids test Alkaline reagent test for flavonoids	With the test solution + Few drops of NaOH solution	Yellow color
Phenolic compounds Ferric chloride test	Test solution + a few drops of neutral 5% $FeCl_3$	Green color
Tannins test Braymer's Test	Test solution + 2 mL H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate
Saponins test Foam's Test	Test solution + 5 mL H_2O + heat	Froth appears
Glycosides test Liebermann's Test	Test solution 2 mL $CHCl_3$ + 2 mL CH_3COOH	Violet to Blue to Green coloration
Alkaloids test Hager's test	2 mL extract + a few drops of Hager's reagent	Yellow precipitate
Steroids test Salkowski test	2 mL extract + 2 mL $CHCl_3$ + 2 mL H_2SO_4 (conc.)	The reddish brown ring at the junction

Collection, rearing, and biological material of the insect

Larvae of *B. cucurbitae* infesting bitter melon were collected from experimental fields at Mien Dong Co. Ltd. in Tan Hiep commune, Di An District, Binh Duong province, Vietnam. The collection and rearing of larvae followed the methods of Nhung and Quoc (2024) with slight modifications. Infected fruits were brought to the Animal Biotechnology Laboratory, Faculty of Biotechnology, Ho Chi Minh City University of Industry, placed in containers filled with sterilized sand, and sifted through a fine mesh until the larvae reached the pupal stage. The sand was sterilized in an oven at 155 °C for 1.2 hours. Pupae were then transferred to Petri dishes within wooden cages until adult emergence. Newly emerged *B. cucurbitae* adults were fed an artificial diet of Dextrose-L mixture and hydrolyzed protein (Protinex, Pfizer Ltd.) in insect-rearing cages made of steel mesh. Rearing conditions were kept at 65-85% relative humidity, 25 ± 2 °C, and a 12-hour light/dark cycle. Adults were provided with a sugar-yeast mixture and water through a wet cotton swab. A green water-soaked paperboard was used to stimulate egg-laying. Eggs were collected with a feather brush, and rearing continued until the adults were 30 days old. Eggs were placed on an artificial diet in trays covered with a thin

cloth. Moist-sterilized sand (0.65 kg) was placed beneath the trays to support pupation.

Design experiments in a greenhouse environment

The experimental greenhouse area was established at Mien Dong Co. Ltd., with the model constructed based on the description by Nhung and Quoc (2024), along with implementing some detailed adjustments for better suitability. Each bitter melon plant was planted in a pot with a volume of 17.67 mL (diameter 30 cm, height 25 cm), and each pot was placed in a specific area within the greenhouse separated by shading nets. Bitter melon plants, after growing 2-3 true leaves, were buried in a soil mixture consisting of topsoil supplemented with a substrate (sawdust) and organic fertilizer (cow dung or worm castings) in a ratio of ¼ soil, ½ substrate, and ¼ organic fertilizer. To create optimal conditions for bitter melon growth, soil pH and moisture were maintained at levels ranging from 6-7.1 and 70-80%, respectively. When the plants reached the stage of 3-5 true leaves, the first round of fertilization was carried out using liquid organic fertilizer such as Panga TC fish fertilizer (Hoang Lien Son Agriculture Co. Ltd). At the flowering preparation stage, the second round of fertilization was applied using inorganic fertilizers such as NPK Phu My 15-5-20 + TE (Petrochemical Fertilizer and Chemical Corporation). When the plants reached a height of 25-30 cm, stakes or trellises were used to support vine growth and stabilize the plants. Bitter melon plants underwent a 5-week cultivation period in the greenhouse under optimal conditions for growth (temperature 26 ± 1 °C, relative humidity (RH) 70-80%, 12/12 light/dark cycle), entering the flowering and fruiting stage. *B. cucurbitae* flies were collected from the rearing environment within the laboratory, quantified, and monitored for survival parameters. The transmission of *B. cucurbitae* flies to the cucumber growing area was carried out by releasing cages containing flies (50 males and females/cage/cucumber plant). Following the transmission of *B. cucurbitae* flies, additional methods involving the application of FSMA extract and Sofri protein 10 DD insecticide were initiated to control melon fly infestation. The experiment was designed with 5 treatment groups, utilizing FSMA at corresponding concentrations (1, 5, 25, 125, and 625 ppm) and Sofri protein 10 DD (1.2 L/ha) as the standard fly control agents, with clean water serving as the negative control. FSMA extract and Sofri protein solution were applied at a rate of 40 mL per plant on days 0, 5, and 10 after fly release. The plants were maintained for 8 weeks under standard greenhouse conditions.

Measurement of oxidative stress mediators

Hydrogen Peroxide (H_2O_2) measurement: H_2O_2 levels in *M. azedarach* fruit-seeds were assessed using a UV-VIS spectrophotometer (U-2900, Hitachi, Japan) at 390 nm. A 0.5 g sample was ground in liquid nitrogen, mixed with 5 mL of cold 0.1% TCA, and centrifuged at 12,000 × g for 15 minutes at 4 °C. The supernatant (0.5 mL) was combined with 0.5 mL of 100 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI, then incubated in the dark for 1 hour (Haddidi *et al*., 2020).

Malondialdehyde (MDA) measurement: Lipid peroxidation was evaluated by homogenizing 0.2 g of the sample in 5 mL of 0.1% TCA and centrifuging at 10,000 × g for 5 minutes. The supernatant (1 mL) was mixed with 4 ml of 20% TCA containing 0.5% TBA, heated at 95 °C for 15 minutes, and cooled. MDA was quantified by absorbance at 532 nm, reported in µmol (Haddidi *et al*., 2020).

Measurement of antioxidant enzyme activity

Quantification of catalase (CAT) content: Tissue samples were homogenized in 0.1 M sodium phosphate buffer (pH 7.2) with 100 g PVPP per gram of tissue. Catalase activity was assessed by mixing 20-100 µL enzyme with 8 mL of 67 mM phosphate buffer (pH 7.0) and 50 µL of 240 mM hydrogen peroxide, followed by absorbance readings at 240 nm. Activity was expressed as units per milligram of protein, indicating the enzyme's ability to decompose 1 µM H_2O_2 per minute at 25 °C (Somegowda *et al*., 2021).

Quantification of peroxidase (POD) content: About 100 g of tissue was homogenized in 10 mL of phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 minutes. POD activity was determined by mixing 1.5 mL of 67 mM phosphate buffer (pH 6.0) with 0.5 mL of the sample, followed by the addition of 0.2 ml each of 1.7 mM ABTS and 0.8 mM hydrogen peroxide. Absorbance was measured at 405 nm, with one unit defined as the enzyme increasing absorbance by 0.01 per minute (Somegowda *et al*., 2021).

Quantification of polyphenol oxidase (PPO) content: PPO activity was evaluated by combining 1.5 mL of 40 mM catechol and 2.3 mL of 0.1 M phosphate buffer (pH 6.5), incubating for 5 minutes, and adding 0.2 mL of crude enzyme. The change in absorbance at 420 nm was recorded to determine activity, defined as an increase of 0.001 per minute (Somegowda *et al*., 2021).

Quantification of superoxide dismutase (SOD) content: SOD activity was measured by adding 50 µL samples to a mixture of 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, and 0.1 mM

EDTA. After 10 minutes of illumination, absorbance at 560 nm was recorded, with activity expressed as units per milligram of protein, indicating the enzyme amount required to inhibit 50% of NBT reduction (Somegowda *et al*., 2021).

Quantification of glutathione S-transferases (GSTs): GST activity was measured using a Sigma-Aldrich kit. The reaction mixture contained 980 µL phosphate buffer, 10 µL reduced L-glutathione, 10 µL CDNB, and 2 µL plant extract. Absorbance was recorded at 340 nm over 5 minutes, with activity expressed as µmol/ml/min, using an extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ for the CDNB conjugate (Haddidi *et al*., 2020).

The correlation

The correlation between oxidative stress intermediates and antioxidant enzymes was evaluated based on the description provided by Dandana *et al*. (2011). Measurement of oxidative stress mediators and antioxidant enzymes from tissue samples was conducted. Statistical methods were employed to determine the correlation coefficient between variables using the Spearman correlation coefficient (SPSS 15.0). Based on the obtained correlation coefficient, the degree of correlation between oxidative stress mediators and antioxidant enzymes was assessed. A positive correlation coefficient signifies a direct relationship, meaning both variables increase together, while a negative correlation coefficient indicates an inverse relationship, where one variable increases as the other decreases.

Statistical analysis

Data analysis were conducted using Statgraphics Centurion XIX. Experimental results were expressed as mean ± standard deviation (SD). Statistical comparisons were made using either t-tests or ANOVA (Student's method). The Spearman correlation coefficient (SPSS 15.0) was applied to assess the relationship between oxidative stress intermediates and antioxidant enzymes. A p-value of ≤ 0.05 was considered statistically significant.

Results

Screening and quantitative analysis of phytochemical compounds in the ethanol extract of M. azedarach fruit-seeds

Qualitative analysis of plant phytochemical compounds in the ethanol extract of *M. azedarach* fruit-seeds (FSMA) revealed the presence of alkaloids,

tannins, saponins, polyphenols, steroids, terpenoids, and flavonoids, while cardiac glycosides were absent (Table 2). The quantitative results of flavonoid, polyphenol, and tannin contents in FSMA, with total flavonoid content were 45.66 ± 2.89 mg QE/g, total tannin content of 73.56 ± 2.55 mg CE/g, and total polyphenol content of 74.73 ± 3.45 mg GAE/g (Table 3). The analysis of phytochemical components in FSMA provided essential information about antioxidant compounds, including flavonoids, polyphenols, and tannins. The presence of these compounds may play a crucial role in protecting bitter melon plants from oxidative stress induced by melon flies.

Table 2. Qualitative analysis of phytochemicals in the ethanol extract of *M. azedarach* fruit-seeds

Phytochemicals	Present in FSMA	Phytochemicals	Present in FSMA
Alkaloids	+	Cardiac glycosides	-
Tannins	+	Steroids	+
Saponins	+	Terpenoids	+
Polyphenols	+	Flavonoids	+

Note: Presence of phytochemicals in FSMA: (+) present and (-) absent.

Table 3. Quantification of flavonoids, polyphenols, and tannins contents in the ethanol extract of fruit-seeds of *M. azedarach*

Sample	Total flavonoid content (mg QE/g)	Total tannin content (mg CE/g)	Total polyphenol content (mg GAE/g)
FSMA	45.66 ± 2.89	73.56 ± 2.55	74.73 ± 3.45

Note: GAE: Gallic acid equivalents, QE: Quercetin equivalents, CE: Catechin equivalents.

Measurement of oxidative stress mediators

Malondialdehyde (MDA) contents: Melon fly infestation was significantly increased malondialdehyde (MDA) levels in the flowers, fruits, and leaves of bitter melon plants, indicating oxidative damage (Figure 1). Untreated plants (water group) showed a substantial rise in MDA, particularly in fruit tissues, which had the highest MDA content, while leaf tissues exhibited the lowest ($p < 0.05$). In contrast, MDA levels decreased in plants treated with ethanol extracts of *M. azedarach* fruit-seeds (FSMA), with reductions inversely related to FSMA concentration ($p < 0.05$). Notably, at a concentration of 652 ppm, FSMA treatment reduced MDA levels similarly to the standard drug, Sofri protein ($p > 0.05$). These results indicated that FSMA may effectively mitigate oxidative damage from melon fly infestations. Therefore, managing melon fly infestations and utilizing FSMA could be promising strategies to alleviate oxidative stress in bitter melon plants.

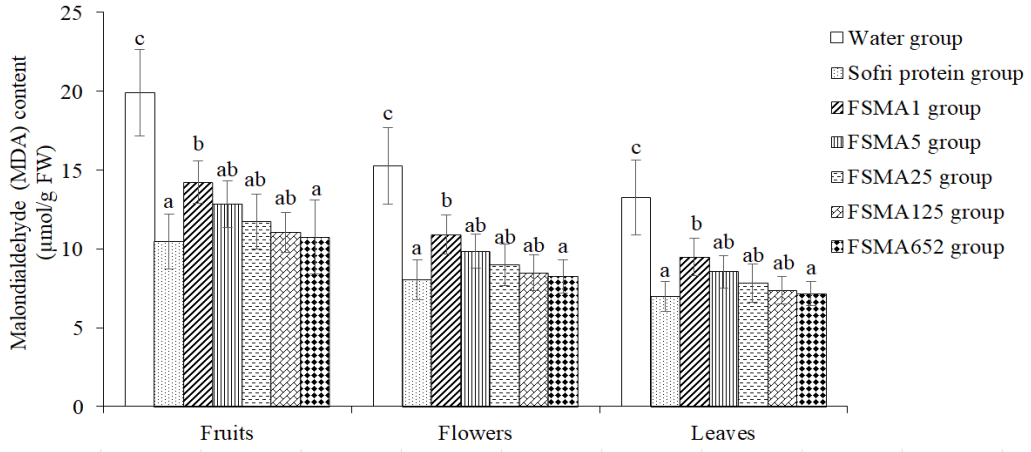

Figure 1. The investigation into the impact of the ethanol extract of *M. azedarach* fruit-seeds (FSMA) on the malondialdehyde (MDA) content in bitter melon fruits, flowers, and leaves aimed to mitigate the oxidative stress induced by melon fly infestation. The findings are expressed as Mean ± SD, with letters (a, b, and c) denoting significant differences among treatments ($p < 0.05$).

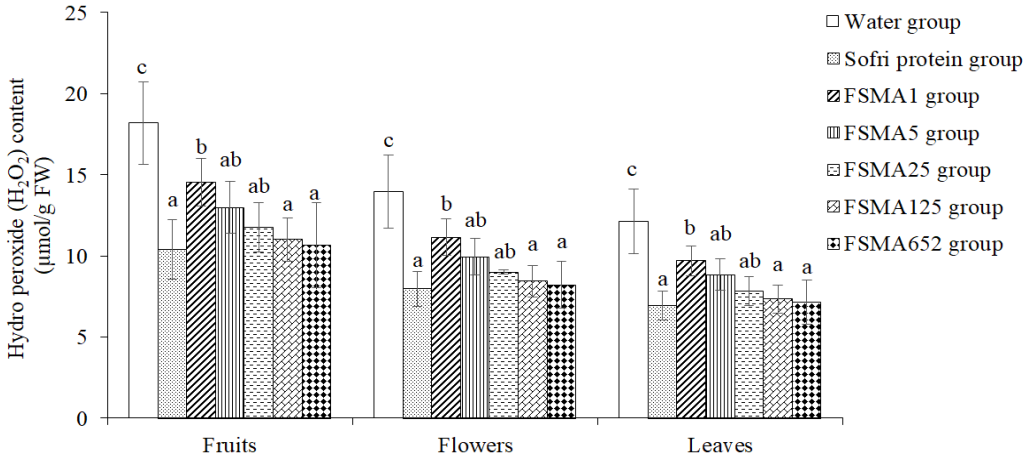

Figure 2. The impact of the ethanol extract of *M. azedarach* fruit-seeds (FSMA) on the hydrogen peroxide (H_2O_2) content in bitter melon fruits. flowers. and leaves was investigated to alleviate the oxidative stress caused by fly melon infestation. The findings are expressed as Mean ± SD, with letters (a, b, and c) denoting significant differences among treatments ($p < 0.05$).

Hydrogen peroxide (H_2O_2) contents: Hydrogen peroxide (H_2O_2) is a significant reactive oxygen species (ROS) that contributes to oxidative stress and cellular damage. It showed that fly melon infestation notably increased

H_2O_2 levels in bitter melon flowers, fruits, and leaves (18.19 ± 2.55, 13.95 ± 2.26, and 12.14 ± 1.98 μmol/g FW, respectively; $p < 0.05$) (Figure 2). Treatment with *M. azedarach* fruit-seeds extract (FSMA) reduced H_2O_2 concentrations, with the highest reduction observed at 652 ppm (10.69 ± 2.62, 8.21 ± 1.45, and 7.14 ± 1.38 μmol/g FW, respectively), similar to the sofri protein control (10.39 ± 1.84, 7.97 ± 1.06, and 6.93 ± 0.89 μmol/g FW; $p > 0.05$) (Figure 2).

Measurement of antioxidant enzyme activity

Quantification of catalase (CAT) and superoxide dismutase (SOD) content

The catalase (CAT) and superoxide dismutase (SOD) activities were assessed across three organs of bitter melon plants (fruit, flower, and leaf) under the influence of *B. cucurbitae* (Table 4). Significantly enhanced activities of CAT and SOD were observed in the negative control group (water group), particularly in the fruit organ. CAT and SOD levels demonstrated a consistent decline in enzyme activities across all three organs - fruit, flower, and leaf of bitter melon plants in the negative control group ($p < 0.05$).

These results highlighted the vulnerability of bitter melon plants to *B. cucurbitae*, via the notable increase in CAT and SOD activities in the negative control group. It indicated a potential stress response induced by the presence of the pathogen. The observation declined in enzyme activities across all plant organs highlights the widespread effect of the stressor on the enzymatic processes essential for plant defense and metabolic regulation. Notably, treatment with the ethanol extract from *M. azedarach* fruit-seeds (FSMA) and sofri protein resulted in a significant increase in enzyme activities ($p < 0.05$). At concentrations of 125 and 652 ppm, FSMA demonstrated efficacy comparable to that of the standard drug, sofri protein ($p < 0.05$). This marked enhancement in enzyme activities after treatment with FSMA and sofri protein indicates their potential as effective agents for mitigating stress caused by *B. cucurbitae*. These findings highlight the complex interaction between plant defense mechanisms and external stressors.

Quantification of polyphenol oxidase (PPO) and peroxidase (POD) content

Polyphenol oxidase (PPO) and peroxidase (POD) are essential enzymes involved in various physiological functions in plants, particularly in defense against pathogens and environmental stresses, as well as in regulating growth and development. The variations in PPO and POD concentrations in the flower,

fruit, and leaf tissues of bitter melon plants were shown in Table 5. Treatment with the ethanol extract from *M. azedarach* fruit-seeds (FSMA) was significantly increased in both PPO and POD levels ($p < 0.05$), indicating FSMA's potential effectiveness in mitigating stress caused by melon fly infestations. Notable differences in enzyme concentrations were observed between FSMA-treated groups and the water control group ($p < 0.05$), highlighting the plant's response to fly melon stress and its ability to adjust enzyme activity in reaction to external threats. The restoration of PPO and POD levels in the FSMA-treated groups, particularly at concentrations ranging from 1 to 652 ppm, demonstrates the stress-relieving properties of FSMA. Importantly, at concentrations of 125 and 652 ppm, FSMA's efficacy was comparable to that of the standard drug sofri protein ($p > 0.05$). This similarity suggested that FSMA could serve as a sustainable alternative for pest management in agriculture, promoting the development of environmentally friendly pest control strategies.

Table 4. The impact of ethanol extract from *M. azedarach* fruit-seeds (FSMA) on the levels of catalase (CAT) and superoxide dismutase (SOD) in the fruit, flowers, and leaves of bitter melon under oxidative stress induced by the effects of melon fly infestation

Groups	CAT (EU/mg protein/min)			SOD (EU/mg protein/min)		
	Fruits	**Flowers**	**Leaves**	**Fruits**	**Flowers**	**Leaves**
Water group	6.84 ± 0.77^{a}	5.71 ± 0.69^{a}	9.09 ± 0.95^{a}	7.27 ± 0.74^{a}	6.07 ± 0.62^{a}	8.56 ± 0.92^{a}
Sofri protein group	12.99 ± 1.89^{d}	10.85 ± 1.44^{d}	17.27 ± 2.58^{d}	13.81 ± 2.36^{d}	11.53 ± 2.04^{d}	16.26 ± 2.17^{d}
FSMA1 group	8.89 ± 0.63^{ab}	7.42 ± 0.59^{ab}	11.82 ± 1.74^{ab}	9.45 ± 1.46^{ab}	7.89 ± 0.84^{ab}	11.13 ± 1.04^{ab}
FSMA5 group	9.12 ± 1.13^{ab}	8.28 ± 1.09^{bc}	13.18 ± 1.88^{bc}	10.54 ± 1.58^{bc}	8.81 ± 0.92^{bc}	12.41 ± 1.25^{bc}
FSMA25 group	10.94 ± 1.33^{bc}	9.14 ± 1.24^{bcd}	14.54 ± 1.62 ^{bcd}	11.63 ± 1.28^{bcd}	9.71 ± 1.13^{bcd}	13.69 ± 1.48^{bcd}
FSMA125 group	11.97 ± 2.18^{d}	9.99 ± 1.56^{cd}	15.91 ± 2.26 ^{cd}	12.72 ± 1.91^{cd}	10.62 ± 1.55^{cd}	14.98 ± 2.22^{cd}
FSMA652 group	12.64 ± 2.19^{d}	10.56 ± 1.67^{d}	16.82 ± 2.74^{d}	13.45 ± 2.36^{d}	11.23 ± 1.88^{cd}	15.83 ± 2.36^{d}

Note: The values are expressed as Mean ± SD, where the letters (a, b, c, and d) indicate differences between treatments ($p < 0.05$).

Table 5. The impact of ethanol extract from *M. azedarach* fruit-seeds (FSMA) on the levels of polyphenol oxidase (PPO), peroxidase (POD) in the fruit, flowers, and leaves of bitter melon under oxidative stress induced by the effects of melon fly infestation

Groups	PPO (1/µg/ml)			POD (1/µg/ml)		
	Fruits	Flowers	Leaves	Fruits	Flowers	Leaves
Water group	1.38 ± 0.23^{a}	1.25 ± 0.12^{a}	1.04 ± 0.04^{a}	1.05 ± 0.01^{a}	0.95 ± 0.02^{a}	0.79 ± 0.02^{a}
Sofri protein group	2.62 ± 0.44^{c}	3.38 ± 2.01^{b}	1.98 ± 0.16^{d}	2.01 ± 0.23^{d}	1.81 ± 0.18^{d}	1.49 ± 0.12^{e}
FSMA1 group	1.79 ± 0.43^{ab}	1.63 ± 0.39^{a}	1.35 ± 0.24^{ab}	1.37 ± 0.27^{ab}	1.24 ± 0.18^{ab}	1.03 ± 0.05^{b}
FSMA5 group	1.99 ± 0.48^{abc}	1.81 ± 0.18^{a}	1.51 ± 0.15^{bc}	1.53 ± 0.13^{bc}	1.38 ± 0.07^{bc}	1.13 ± 0.05^{bc}
FSMA25 group	2.21 ± 0.44^{bc}	2.01 ± 0.33^{ab}	1.66 ± 0.19^{bcd}	1.68 ± 0.33^{bcd}	1.52 ± 0.29^{bcd}	1.26 ± 0.16^{cd}
FSMA125 group	2.42 ± 0.43^{bc}	2.19 ± 0.36^{ab}	1.82 ± 0.14^{cd}	1.84 ± 0.27^{cd}	1.66 ± 0.15^{cd}	1.38 ± 0.14^{de}
FSMA652 group	2.55 ± 0.52^{c}	2.31 ± 0.44^{ab}	1.92 ± 0.35^{d}	1.94 ± 0.31^{cd}	1.76 ± 0.26^{d}	1.46 ± 0.13^{e}

Note: The values are expressed as Mean ± SD, where the letters (a, b, c, d, and e) indicate differences between treatments ($p < 0.05$).

Quantification of glutathione S-transferases (GSTs)

Glutathione S-transferases (GSTs) constitute a group of enzymes crucially involved in the detoxification and elimination of harmful or toxic compounds. They facilitated the conjugation of glutathione (GSH) with toxic or xenobiotic substances, resulting in polar reactions that aid in their easy removal from the body. Upon the infestation by *B. cucurbitae* was significantly increased in GSTs concentrations in the fruit, flower, and leaf organs of bitter melon plants was observed ($p < 0.05$) (Figure 3). The significant increased in GSTs concentrations across the fruit, flower, and leaf organs were enhanced detoxification mechanism activated by the plant in response to the presence of the pathogen. However, following exposure to ethanol extract from *M. azedarach* fruit-seeds (FSMA), the concentrations of GSTs gradually decreased ($p < 0.05$), inversely correlating with the dosage of FSMA treatment. No significant difference in treatment efficacy was observed between sofri protein and FSMA at concentrations of 125 and 652 ppm ($p > 0.05$) (Figure 3). This finding implies that FSMA treatment may have a suppressive effect on GST activity in bitter melon plants. The gradual decrease in GST concentrations suggested that FSMA could aid in modulating the plant's detoxification mechanisms. Notably, the effectiveness of FSMA at both 125 and 652 ppm was

similar to that of sofri protein, highlighting FSMA's potential as an alternative or complementary option for managing stress from *B. cucurbitae* infestation.

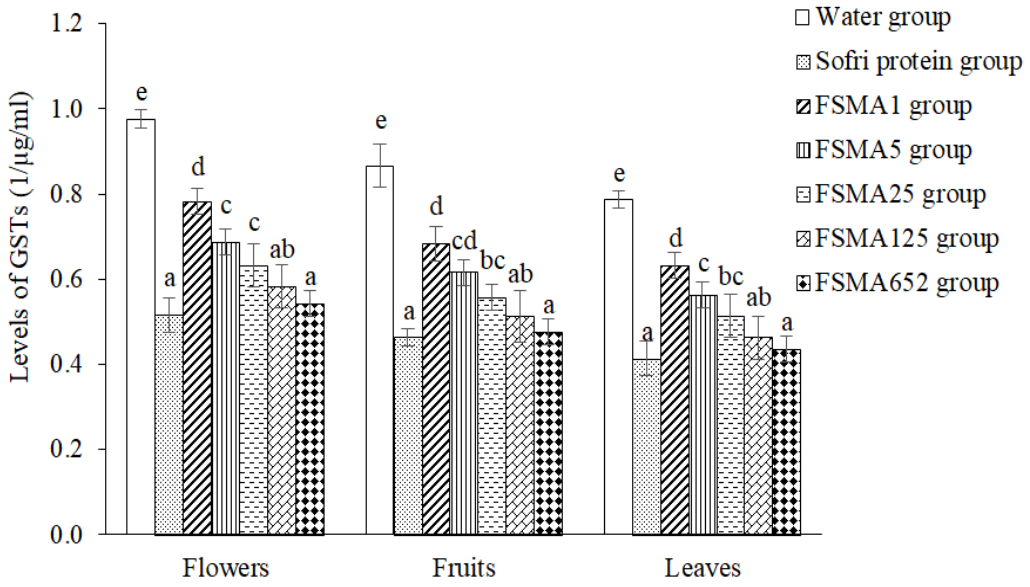

Figure 3. The impact of ethanol extract from *M. azedarach* fruit-seeds (FSMA) on the levels of glutathione S-transferases (GSTs) in the fruit, flowers, and leaves of bitter melon under oxidative stress induced by the effects of melon fly infestation. The findings are expressed as Mean ± SD, with letters (a, b, c, d and e) denoting significant differences among treatments ($p < 0.05$).

Assessing the correlation between oxidative stress mediators and antioxidant enzymes

The evidence of a significant inverse correlation between oxidative stress mediators (MDA and H_2O_2) and antioxidant enzymes (CAT, SOD, PPO, POD, and GST) were shown in Tables 6 and 7. The notable correlation between oxidative stress mediators and antioxidant enzymes in bitter melon plants indicated that as oxidative stress levels increased, the activity of antioxidant enzymes tended to decrease, and vice versa. However, after treatment with FSMA and sofri protein, a positive correlation occurred between oxidative stress mediators (MDA and H_2O_2) and antioxidant enzymes (CAT, SOD, PPO, POD, and GST). These findings underscored the supportive role of FSMA and sofri protein in maintaining cellular homeostasis in bitter melon plants under oxidative stress conditions. FSMA and sofri protein enhanced the activity of antioxidant enzymes such as CAT, SOD, PPO, POD, while reducing GST

activity, as outlined above, thereby reducing the levels of oxidative stress mediators such as MDA and H_2O_2. It supported the minimization of cellular damage and the preservation of intracellular environment stability in bitter melon plants during oxidation processes.

Table 6. Analyzing the correlation between oxidative stress mediators (MDA) and antioxidant enzymes (CAT, SOD, and POD)

Groups	Parameters	PPO (1/µg/ml)		
		Fruits	Flowers	Leaves
Water group	CAT	r = - 0.99, p = 0.0132	r = - 0.98, p = 0.0241	r = - 0.97, p = 0.0156
	SOD	r = - 0.94, p = 0.0035	r = - 0.97, p = 0.0024	r = - 0.96, p = 0.0019
	POD	r = - 0.88, p = 0.0175	r = - 0.93, p = 0.0216	r = - 0.91, p = 0.0144
Sofri protein group	CAT	r = 0.97, p = 0.0372	r = 0.95, p = 0.0416	r = 0.98, p = 0.0224
	SOD	r = 0.89, p = 0.0025	r = 0.91, p = 0.0034	r = 0.95, p = 0.0043
	POD	r = 0.92, p = 0.0162	r = 0.96, p = 0.0229	r = 0.89, p = 0.0192
FSMA1 group	CAT	r = - 0.94, p = 0.0234	r = - 0.98, p = 0.0315	r = 0.96, p = 0.0178
	SOD	r = - 0.92, p = 0.0034	r = - 0.99, p = 0.0017	r = - 0.97, p = 0.0028
	POD	r = - 0.87, p = 0.0114	r = - 0.88, p = 0.0177	r = - 0.88, p = 0.0136
FSMA5 group	CAT	r = - 0.99, p = 0.0431	r = - 0.93, p = 0.0359	r = - 0.95, p = 0.0287
	SOD	r = - 0.88, p = 0.0026	r = - 0.94, p = 0.0033	r = - 0.93, p = 0.0018
	POD	r = - 0.94, p = 0.0245	r = - 0.85, p = 0.0356	r = - 0.96, p = 0.0216
FSMA25 group	CAT	r = - 0.94, p = 0.0243	r = - 0.97, p = 0.0266	r = - 0.95, p = 0.0347
	SOD	r = - 0.89, p = 0.0024	r = - 0.92, p = 0.0013	r = - 0.97, p = 0.0031
	POD	r = - 0.82, p = 0.0263	r = - 0.93, p = 0.0172	r = - 0.86, p = 0.0258
FSMA125 group	CAT	r = 0.93, p = 0.0188	r = 0.95, p = 0.0164	r = 0.96, p = 0.0236
	SOD	r = 0.95, p = 0.0023	r = 0.92, p = 0.0042	r = 0.98, p = 0.0037
	POD	r = 0.95, p = 0.0138	r = 0.87, p = 0.0244	r = 0.99, p = 0.0263
FSMA652 group	CAT	r = 0.95, p = 0.0413	r = 0.94, p = 0.0342	r = 0.97, p = 0.0273
	SOD	r = 0.94, p = 0.0037	r = 0.89, p = 0.0021	r = 0.96, p = 0.0014
	POD	r = 0.86, p = 0.0227	r = 0.88, p = 0.0263	r = 0.93, p = 0.0155

Table 7. Investigation of the correlation between oxidative stress mediators (H_2O_2) and antioxidant enzymes (PPO and GSTs)

Groups	Parameters	Hydrogen peroxide (H_2O_2)		
		Fruits	Flowers	Leaves
Water group	PPO	r = - 0.81, p = 0.0267	r = - 0.79, p = 0.0345	r = -0.83, p = 0.0193
	GSTs	r = - 0.84, p = 0.0031	r = -0.88, p = 0.0043	r = -0.9, p = 0.0017
Sofri protein group	PPO	r = 0.94, p = 0.0195	r = 0.86, p = 0.0246	r = 0.91, p = 0.0317
	GSTs	r = 0.92, p = 0.0024	r = 0.086, p = 0.0026	r = 0.83, p = 0.0019
FSMA1 group	PPO	r = - 0.77, p = 0.0341	r = - 0.82, p = 0.0265	r = - 0.93, p = 0.0413
	GSTs	r = - 0.77, p = 0.0026	r = -0.73, p = 0.0028	r = -0.85, p = 0.0022
FSMA5 group	PPO	r = - 0.93, p = 0.0441	r = - 0.91, p = 0.0359	r = - 0.85, p = 0.0178
	GSTs	r = - 0.74, p = 0.0016	r = -0.84, p = 0.0024	r = -0.87, p = 0.0029
FSMA25 group	PPO	r = - 0.86, p = 0.0264	r = - 0.92, p = 0.0352	r = - 0.97, p = 0.0224
	GSTs	r = - 0.78, p = 0.0033	r = -0.85, p = 0.0024	r = -0.96, p = 0.0011
FSMA125 group	PPO	r = 0.92, p = 0.0335	r = 0.94, p = 0.0139	r = 0.81, p = 0.0244
	GSTs	r = 0.89, p = 0.0026	r = 0.96, p = 0.0014	r = 0.99, p = 0.0034
FSMA652 group	PPO	r = 0.84, p = 0.0266	r = 0.89, p = 0.0374	r = 0.93, p = 0.0283
	GSTs	r = 0.88, p = 0.0028	r = 0.092, p = 0.0024	r = 0.97, p = 0.0036

Disscusion

The melon fly (*B. cucurbitae*) constitutes a significant pest to the bitter melon plant (*M. charantia*) by inducing oxidative stress. Its assault not only inflicts physical damage but also initiates oxidative stress. thereby compromising the plant's resistance and impeding its growth and development. By depositing eggs and feeding on the leaves. flowers. and fruits of the bitter melon plant. the melon fly inflicts physical injuries and structural deformities. creating opportunities for bacteria. fungal. and other microbial invasions. These agents' assaults can trigger oxidative reactions within the plant. leading to oxidative stress. The melon fly secretes peroxidase enzymes onto the bitter melon plant's wounds. intensifying oxidative reactions. generating free radicals. and inducing oxidative stress stimulants. The feeding and movement behaviors of the melon fly result in cellular damage to the plant's leaves and fruits. These damaged cells undergo oxidative stress as their defense mechanisms activate to counter the melon fly's intrusion (Dhillon *et al*., 2005). The growth and development of the fly melon occur optimally at a temperature of 24 °C. Consequently. despite the increased levels of antioxidants and secondary metabolites in bitter melon plants. it does not impede the growth and development of the melon fly. When the cells of bitter melon plants are subjected to a substantial amount of invasive agents. causing a reduction in the plant's ability to defend against oxidative stress. it creates favorable conditions for the escalation of oxidative stress within the plant (Somegowda *et al*., 2021). In such circumstances. the support provided by secondary metabolites in the ethanol extract of *M. azedarach* fruit-seeds (FSMA) has aided in enhancing the plant's resilience against oxidative stress induced by the fly melon. These secondary metabolites act as agents against pathogen invasion and minimize oxidative stress (Jan *et al*., 2021).

Secondary metabolites are crucial for plants. Key compounds such as alkaloids, tannins, saponins, polyphenols, steroids, terpenoids, and flavonoids enhance plant resilience against oxidative damage. Flavonoids, polyphenols, and terpenoids are especially noted for their strong antioxidant properties, interacting with free radicals and mitigating cellular damage due to oxidative stress. Alkaloids, saponins, and tannins possess antimicrobial and insecticidal properties, helping to shield plants from infections caused by microorganisms and pests. Flavonoids and saponins activate the plant immune system, bolstering its defenses against oxidative stressors. The insecticidal effects of saponins and alkaloids deter harmful insect attacks, thus reducing the potential for oxidative damage (Yeshi *et al*., 2022). Flavonoids, polyphenols, and tannins are recognized for their ability to stimulate the plant's natural antioxidant defense mechanisms, curbing the growth of detrimental microorganisms and

alleviating oxidative stress. These reduce oxidative stress by neutralizing free radicals and other reactive species, thereby preventing cellular and DNA damage. They also enhance the activity of critical antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase, which are vital for eliminating free radicals and other oxidative agents, ensuring plant stability and protection. Additionally, the antibacterial, antifungal, and insecticidal properties of flavonoids, polyphenols, and tannins further diminish the risk of infections, thereby promoting plant health and resilience against oxidative stress (Saleem *et al*., 2022).

Oxidative stress mediators are compounds that play a crucial role in oxidative stress processes within biological systems. These include reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and lipid peroxides like malondialdehyde (MDA). An imbalance in these mediators can lead to oxidative damage, contributing to various diseases and pathological conditions (Shohag *et al*., 2022). In bitter melon plants infested by melon flies, increased levels of MDA and H_2O_2 indicate significant oxidative stress caused by the pests. The flies damage the plants by laying eggs and feeding on leaves, flowers, and fruits, resulting in both physical and biological harm. This damage triggers oxidative reactions, enhancing the production of ROS such as H_2O_2, which degrade cell structures and lead to tissue damage. Additionally, the infestation deprives the plants of nutrients and water, further impairing their ability to combat oxidative stress and prompting a cascade of oxidative reactions. Melon flies may also stimulate the production of peroxidase enzymes in bitter melon, contributing to ROS generation (Somegowda *et al*., 2021). The ethanol extract of *M. azedarach* fruit-seeds (FSMA) has been shown to effectively reduce MDA and H_2O_2 levels in bitter melon plants experiencing stress from melon fly infestation. FSMA enhances the plant's intrinsic antioxidant defense system, thereby reducing ROS generation and maintaining cellular integrity. This extract contains various antioxidant compounds, including flavonoids, polyphenols, and terpenoids, which help mitigate ROS production, particularly H_2O_2. Moreover, FSMA increases the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and peroxidase, facilitating the removal of ROS and inhibiting MDA and H_2O_2 formation. As a result, FSMA preserves the structural integrity of plant cells, alleviating oxidative stress-induced damage.

Antioxidant enzymes are essential for protecting biological systems from oxidative stress. They neutralize reactive oxygen species (ROS) and free radicals, which can harm cells and tissues. By catalyzing reactions, these enzymes convert harmful ROS into less reactive forms, thus preventing oxidative damage and maintaining cellular balance (Lei *et al*., 2016). Key antioxidant enzymes include catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), peroxidase (POD), and glutathione S-transferases

(GSTs). CAT decomposes hydrogen peroxide (H_2O_2) into water and oxygen, preventing lipid degradation and malondialdehyde (MDA) formation. SOD converts superoxide radicals into H_2O_2 or oxygen, reducing MDA formation risk. PPO produces quinones from phenolic compounds, which can inhibit MDA formation. POD helps to reduce H_2O_2 levels through the oxidation of phenolic compounds. GSTs detoxify harmful substances by conjugating them with glutathione (GSH), making them easier to eliminate (Durak *et al*., 2021). The infestation of Bitter melon plants by *B. cucurbitae* can induce oxidative stress, leading to decreased concentrations of CAT, SOD, PPO, and POD while increasing GST levels in the plant's fruit, flowers, and leaves. The damage from *B. cucurbitae* creates an oxidative stress environment, prompting an upregulation of CAT and SOD to eliminate free radicals. However, this response may not sufficiently counter the free radicals generated, resulting in reduced enzyme activity. Additionally, *B. cucurbitae* may increase phenolic compound production, enhancing PPO and POD activity as part of the plant's defense. Nonetheless, excessive quinones can lead to enzyme inactivation (Sule *et al*., 2022). *B. cucurbitae*'s attack on Bitter melon creates structural damage and an oxidative stress environment, generating ROS like H_2O_2 and superoxide radicals. Plants respond by activating GSTs to detoxify ROS and xenobiotics through conjugation with GSH, thus minimizing cellular damage and oxidative stress (Liu *et al*., 2022). The ethanol extract of *M. azedarach* fruit-seeds (FSMA) can aid Bitter melon plants in managing oxidative stress induced by *B. cucurbitae*. FSMA enhances the activity of CAT, SOD, PPO, and POD while reducing GST activity, creating conditions that help the plant cope with oxidative stress. FSMA contains antioxidant compounds that stimulate these enzymes, minimizing cellular damage caused by ROS. Additionally, by lowering GST activity, FSMA reduces the detoxification of harmful compounds, preventing prolonged oxidative stress.

The interaction between oxidative stress mediators and antioxidant enzymes illustrates how plants naturally adjust to oxidative stress. When exposed to oxidative stress, levels of intermediates like malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) rise. In response, plants activate antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), peroxidase (POD), and glutathione S-transferases (GST) to mitigate damage. These enzymes convert harmful oxidative agents into less detrimental forms, thereby safeguarding cellular integrity. However, if oxidative stress persists, these antioxidant enzymes can become saturated or lose efficacy, leading to increased levels of harmful agents and diminished protective capabilities. Consequently, antioxidant enzyme activity may decline, resulting in more significant cellular damage. This dynamic emphasizes the delicate balance that must be maintained between oxidative stressors and antioxidant enzymes to protect cells and support plant growth, particularly in

fluctuating and challenging environments (Olufunmilayo *et al*., 2023). In the current study, a negative correlation was observed between oxidative stress mediators and antioxidant enzyme activity, indicating that as oxidative stress levels rise, antioxidant enzyme activity decreases. This decline may be due to the inability of antioxidant enzymes to sufficiently counteract the accumulation of damaging intermediates such as MDA and H_2O_2 during oxidative stress. Such a reduction disrupts the oxidative balance and weakens the plant's protective capacity against oxidative damage. After treatment with the ethanol extract of *M. azedarach* fruit-seeds (FSMA) and sofri protein, a positive correlation emerged between oxidative stress mediators and antioxidant enzymes. This suggests that these treatments have restored balance to the plant's antioxidant system, enhancing its defense against oxidative stress-related damage by boosting antioxidant enzyme activity. This improvement indicates that reestablishing equilibrium between oxidative stress mediators and antioxidant enzymes can bolster the plant's resilience and reduce cellular damage under oxidative stress conditions.

In conclusion, this study highlights the increased oxidative activity and tissue damage in the flowers, fruits, and leaves of bitter melon (*M. charantia*) due to melon fly (*B. cucurbitae*) infestation. While this infestation negatively affects the plants, treatment with ethanol extract from *M. azedarach* fruit-seeds (FSMA) significantly reduces oxidative damage and enhances the activity of antioxidant enzymes. These results indicate that FSMA could be an effective strategy for alleviating the harm caused by melon flies in crops, while also decreasing the dependence on synthetic pesticides. This emphasizes the importance of exploring and utilizing natural extracts like FSMA in modern agriculture, promoting biodiversity and environmental health.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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