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Effectiveness of Antioxidants and Resistance to Diamondback Moth Infestation in Green Mustard Plants Using Ethanol Extracts from *Millettia pachyloba* Drake Leaves: An *In Vitro* and *In Vivo* Evaluation

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Abstract

The growing interest in medicinal plants as sustainable alternatives to synthetic pesticides has led to the exploration of *Millettia pachyloba* (MPDE) for pest management. This study aims to evaluate the effectiveness of MPDE in controlling Diamondback moth (DBM) infestation and enhancing oxidative stress tolerance in green mustard plants. The goal is to determine MPDE's potential to improve pest resistance and mitigate plant oxidative stress. The experiment involved applying MPDE at concentrations of 2, 4, 6, 8, and 10% (w/v) to DBM larvae and green mustard plants, with fipronil 5% SC and water as controls. *In vitro*, the effects of MPDE on DBM larvae were assessed using bioassays, including oviposition, feeding preferences, and insecticidal activity. *In vivo*, MPDE was sprayed on green mustard plants infested with DBM larvae, and plant growth, survival, and yield were monitored. Antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and reducing power assays. The results of the study at $p < 0.05$ showed that MPDE significantly reduced oviposition and feeding by DBM, with higher concentrations demonstrating greater effectiveness. MPDE also increased DBM larval mortality, improved stem bending capacity, and enhanced plant survival, particularly at concentrations of 10%, which were comparable to fipronil. Moreover, MPDE exhibited significant antioxidant activity and reversed DBM-induced inhibition of antioxidant enzymes, reducing oxidative stress. These findings suggest that MPDE is a promising, eco-friendly alternative to chemical pesticides, enhancing pest resistance and oxidative stress tolerance in plants.

Keywords: bio-insecticide; inhibition effect; insect mortality; plant extract; toxicity

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INTRODUCTION

The Diamondback moth (*Plutella xylostella* L.) is a major pest of Brassicaceae crops, including cabbage, cauliflower, and broccoli. Its larvae damage plants by feeding on leaves, flowers, and young shoots, creating entry points for pathogens such as bacteria and fungi. The pest's significant impact is due to its wide host range, high reproductive rate (over 20 generations annually in tropical regions), disruption of natural predators, and genetic resilience (Zhou et al., 2024). Feeding damage by Diamondback moth (DBM) ruptures plant cells, triggering defense responses and generating reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen

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peroxide (H_2O_2), and hydroxyl radicals (OH). These ROS cause oxidative stress, damaging vital biomolecules like lipids, proteins, and DNA, impairing photosynthesis, stunting growth, reducing yield, and increasing susceptibility to diseases (Nhung and Quoc, 2024a). Excessive ROS levels exacerbate cellular damage through lipid peroxidation, protein oxidation, and DNA degradation, further impairing plant resilience and product quality (Deffa and Daikh, 2024).

To counteract these effects, plants produce secondary metabolites such as phenolics, flavonoids, alkaloids, terpenoids, and antioxidants. These compounds are vital for stress adaptation, disease resistance, and natural pest control, providing sustainable alternatives to synthetic pesticides. This is particularly crucial in developing countries, where chemical pesticides pose environmental and health risks. For example, phytohormone-based biostimulants improve plant resilience under stress conditions, aligning with global goals for climate-resilient agriculture (Khetsha et al., 2024). Through coevolution, plants and insects have developed an intricate "arms race" of defense mechanisms and counter-adaptations, with secondary metabolites playing a central role in plant defense systems (Nhung and Quoc, 2024b). However, prolonged DBM infestations can overwhelm these defenses, causing severe oxidative stress and damage in cruciferous plants. Plant extracts rich in secondary metabolites have shown potential in mitigating adverse effects and enhancing plant resilience (Li et al., 2024). For example, *Andrographis paniculata* extract has been found to disrupt the pupal-to-adult transition in DBM, leading to increased mortality and deformities, highlighting its potential as a natural pest control solution (Jagajothi et al., 2024). Similarly, Ferreira et al. (2020) demonstrated that extracts from *Ludwigia* species, such as *L. tomentosa*, *L. longifolia*, and *L. sericea*, effectively reduced larval mortality, fecundity, and egg survival in *P. xylostella*, inhibiting food consumption and disrupting pest development. These findings further support the role of plant extracts as effective, natural pest control agents.

The genus *Millettia* (Leguminosae) comprises approximately 200 species distributed across tropical and subtropical regions in Africa, Asia, the Americas, and Australia. Traditionally, these species have been used in folk medicine to treat conditions such as rheumatoid arthritis, skin disorders, parasitic infections, and insect repellents. Phytochemical studies have identified various bioactive compounds in *Millettia*, including steroids, alkaloids, triterpenoids, and flavonoids (Jena et al., 2020). Among these species, *Millettia pachyloba* Drake (MPDE), a perennial woody vine native to subtropical climates, is particularly known for its medicinal properties. It is used to treat tumors, rheumatoid arthritis, and edema, with its roots offering relief for joint and muscle pain and inflammation (Yan et al., 2019). In addition to its medicinal uses, MPDE exhibits significant insecticidal properties, largely attributed to its high content of bioactive compounds such as flavonoids, saponins, and alkaloids. These compounds disrupt pest nervous systems and hinder development, as demonstrated in the case of the DBM (*P. xylostella*) (Nhung and Quoc, 2024b). Moreover, MPDE possesses antioxidant properties that help mitigate oxidative stress caused by DBM infestations. Its natural compounds neutralize free radicals and ROS generated during pest attacks, thereby aiding plant recovery, strengthening defenses, and enhancing overall plant health (Nhung and Quoc, 2024a). In a similar context, Song et al. (2024) tested 13 essential oils and found that they produced strong electrophysiological responses and effective repellency against DBM. Oils from *Pogostemon cablin*, *Rosmarinus officinalis*, and *Polygonum hydropiper* notably reduced egg numbers, offering safe and effective alternatives for DBM control. These findings complement the natural defense mechanisms of plants, like MPDE, further supporting the potential of bioactive compounds in managing pest infestations.

Despite advances in pest management, sustainably controlling DBM infestations in cruciferous crops remains challenging. Using chemical pesticides carries risks, including environmental pollution, pest resistance, and harm to non-target organisms. While plant secondary metabolites show promise as natural pest control agents and stress relievers, their dual role in protecting plants from pests and mitigating oxidative stress has not been fully explored. MPDE, traditionally known for its medicinal properties, also exhibits insecticidal and antioxidant capabilities. However, its potential for agricultural pest management, particularly against DBM, remains under-researched. Most studies have focused on its medicinal uses, leaving a gap in understanding its role in enhancing plant resilience and combating oxidative stress. This study explores ethanol extracts from MPDE leaves, which are rich in bioactive compounds such as flavonoids, saponins, and alkaloids. Using

both *in vitro* and *in vivo* approaches, the research demonstrates the extract's dual function: directly disrupting DBM development and indirectly enhancing plant defenses through its antioxidant properties. By integrating traditional knowledge with modern agricultural practices, this study offers a novel, sustainable solution for pest control, contributing to sustainable agriculture and global food security.

MATERIALS AND METHOD

Collection of plant materials and preparation of the extracts

MPDE leaves were collected from Da Oai Commune, Da Huoai District, Lam Dong Province, Vietnam, in December 2023. The voucher specimen (MP221223VST) was deposited at the Biotechnology Laboratory, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City. The collected leaves underwent meticulous selection to ensure quality: bright green color, fresh and resilient, free from tears, damage, pests, and diseases, and uniform in size. The selected leaves were washed three times with clean water, followed by distilled water, to remove impurities. They were then dried in a Memmert oven (Germany) at 50 °C until a constant weight was achieved. Subsequently, the dried leaves were ground into a fine powder using an MN300B herbal grinder (Dong Nam Company, Vietnam). The leaf powder was stored in moisture-proof bags at room temperature, protected from light, for further experimental use.

The crude extract was obtained by extracting 500 g of dried MPDE leaf powder three times with 2,000 ml of 70% ethanol for 24 hours at room temperature. The extract was filtered through coarse cloth and Whatman No. 4 filter paper. It was then concentrated under reduced pressure at 40 °C for 16 hours using a rotary evaporator RE301B-T (Yamato, Japan). The resulting extract, named MPDE, had a yield of 3.26% (16.31 g). The extract was stored in a vacuum desiccator at 4 °C until further use.

Screening and quantification of phytochemicals in extracts

Based on the phytochemical screening method described by Tran et al. (2023), ethanol extract of MPDE leaves was investigated for various phytochemical components including alkaloids, flavonoids, polyphenols, steroids, tannins, terpenoids, cardiac glycosides, and saponins using qualitative colorimetric indicators.

Quantification of phenolic content

Total phenolic content was determined using the Folin-Ciocalteu method, where standard solutions of gallic acid at concentrations of 1, 0.5, 0.25, and 0.125 mg ml^{-1} in methanol were prepared. Subsequently, 1 ml of gallic acid standard solution was added to each test tube. Then, 0.5 ml of Folin-Ciocalteu reagent (2 N) and 5 ml of distilled water (diluted 1:20) were added to each tube. After 8 minutes, 2 ml of 7.5% Na_2CO_3 was added, and the volume was adjusted to 10 ml with distilled water. The solution was then kept at room temperature for an additional 30 minutes. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Jenway Model 6500, UK). Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract, following the method described by Nhung and Quoc (2024c) with slight modifications.

Quantification of alkaloid content

Twenty milligrams of quinine alkaloid standard was weighed. The quinine was then dissolved in a small amount of 80% ethanol. The solution was transferred to a 100 ml volumetric flask and 80% ethanol was added to the flask up to the 100 ml mark to obtain a stock solution with a 200 µg ml^{-1} concentration.

The following standard dilutions with concentrations of 0, 10, 20, 30, and 50 µg ml^{-1} were prepared from the stock solution. Afterward, 1 ml of each standard dilution was pipetted into clean test tubes. To each test tube, 1 ml of Dragendorff's reagent was added, and the contents were mixed thoroughly by shaking. The reaction was allowed to proceed for 10 to 15 minutes at room temperature. The absorbance of each solution was measured at 540 nm using a UV-Vis spectrophotometer (Nhung and Quoc, 2024c).

Quantification of saponin content

One gram of dried and finely ground plant material was weighed. The sample was transferred to a sealed container. To the container, 10 ml of 80% ethanol was added. The container was sealed and thoroughly shaken or stirred with a magnetic stirrer for 24 hours at room temperature. After 24 hours, the mixture was filtered through filter paper to remove solid residues. The filtrate was collected in a volumetric flask.

The preparation of the saponin standard solution

The preparation of the saponin standard solution was carried out by accurately weighing

10 mg of the diosgenin saponin standard. It was dissolved in a small amount of 80% ethanol and transferred to a 100 ml volumetric flask. Ethanol was added to the mark to obtain a stock solution with a concentration of 100 µg ml^{-1}. Dilutions of the stock solution were prepared to achieve concentrations of 0, 10, 20, 30, 40, and 50 µg ml^{-1}.

The reaction with vanillin-sulfuric acid

One milliliter of each standard solution was pipetted into clean test tubes. To each test tube, 1 ml of vanillin-sulfuric acid reagent was added, and the mixture was thoroughly shaken. The test tubes were heated at 60 °C for 10 minutes to allow the reaction to occur. The absorbance of each solution was measured at a wavelength of 550 nm using a UV-Vis spectrophotometer (Nhung and Quoc, 2024c).

Experimental design

Treatment formulation

The concentrated extract obtained from solvent evaporation served as the stock solution. This solution was diluted to create five distinct concentrations (2, 4, 6, 8, and 10% w/v), labeled as MPDE2, MPDE4, MPDE6, MPDE8, and MPDE10 groups, respectively. The commercial product fipronil 5% SC (100 ppm) was used as the standard pesticide and positive control (fipronil group). Water was used as the reference treatment and negative control (water group). All treatments were applied for managing DBM in both *in vitro* (laboratory) and *in vivo* (greenhouse) experimental studies.

In vitro experimental design

The experiment was conducted at the Department of Biotechnology, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Vietnam. DBM (*P. xylostella*) larvae were collected from mustard fields in Tan Hiep Commune, Di An District, Binh Duong Province, and brought to the university. DBM was reared following the method by Chi et al. (2024), with minor modifications. The DBM samples were kept in plastic cages (60 cm × 30 cm) with a sponge soaked in water at the bottom to maintain humidity. A green mustard (*Brassica juncea*) leaf plate was placed on the sponge to stimulate oviposition. Adult DBM were fed a 10% honey solution, supplied through a round hole at the end of the cage. Green mustard leaf plates containing eggs were transferred to glass dishes, and maintained under suitable environmental conditions until hatching. The DBM larvae were then reared in the plastic cages until adulthood, with fresh green mustard leaves provided and replaced daily. Pupae were collected and placed in ventilated plastic-capped test tubes, maintained at appropriate ambient temperatures until adult emergence. The entire DBM lifecycle was repeated with the emergence of new adults. The DBM population was maintained at ambient temperatures of 22 to 24 °C, with relative humidity between 65% and 75%, and a natural light cycle of 12/12 hours.

In vivo experimental design

The experiment was conducted in a greenhouse at the biological experimental garden of Mien Dong Company Ltd., located in Tan Hiep Commune, Di An District, Binh Duong Province, Vietnam. The study followed the method described by Escobar-Garcia et al. (2023), with minor modifications. Green mustard plants were grown in plastic pots (30 cm diameter, 25 cm height) using a fertilizer mix of NPK 20-20-15, organic fertilizer, lime powder, and bio-fertilizer (1, 5, 0.5, and 0.2 kg, respectively). This mix provided essential nutrients and growth support for optimal plant development. Plants were regularly watered and cared for until they were 7 weeks old when the experiment began. MPDE leaf extract was applied at concentrations of 2, 4, 6, 8, and 10% (w/v). Fipronil 5% SC (100 ppm) was the standard treatment, and water was used as the negative control. In the experiment, a randomized block design was employed with three replicates for each treatment. Each replicate (block) consisted of 8 plants. Within each pot, plants were spaced 10 cm apart, with 15 cm between rows, to promote optimal growth. Fixed distances of 50 and 75 cm were maintained between treatments and replicates, respectively, ensuring systematic arrangement and alignment with the research objectives. Each plant was infested with seven third-instar DBM larvae, sourced from the Biotechnology Laboratory of Industrial University of Ho Chi Minh City. A 50 ml plastic spray bottle was used to apply MPDE, fipronil, and water to the plants, ensuring each plant received approximately 20 ml of the respective solution.

Assessment of the impact of MPDE solution on DBM larvae in vitro experiments

Choice oviposition bioassays for DBM adults using treated green mustard leaf discs

Choice oviposition bioassays were conducted with adult DBM, maintained in the laboratory without exposure to extracts. Green mustard leaf discs were immersed in different treatments,

air-dried for 20 minutes, and placed in plastic cages. In the test, three pairs of DBM were introduced into a plastic cage (30 cm × 15 cm × 12 cm) containing six treated green mustard leaf discs (4 cm in diameter) arranged in a circle. Each disc was treated with extracts from MPDE, and two discs were treated with controls (distilled water and fipronil). The DBM pairs were kept for four days for oviposition and fed daily with 10% honey solution soaked in cotton. Eggs were counted at 24, 48, 72, and 96 hours using an Olympus optical microscope (Olympus Corporation, Japan), and leaf discs were replaced with freshly treated ones at each interval. The oviposition deterrent index (ODI) by Ferreira et al. (2022) was calculated using Equation 1.

$$\text{ODI (\%)} = \frac{\text{C-T}}{\text{C+T}} \times 100 \quad (1)$$

Where, C is the number of eggs laid on the control discs and T is the number of eggs laid on the treated discs.

Choice bioassays for evaluating feeding preferences of third-instar DBM larvae

For choice bioassays, third-instar DBM larvae were starved for 4 hours. Green mustard leaf discs (4 cm in diameter) were immersed in respective treatments and controls (distilled water and fipronil) for one minute, then air-dried for 20 minutes in a plastic tray. In the choice test, four green mustard leaf discs were placed in a petri dish (9 cm diameter, 1.5 cm height) in a crosswise arrangement, with two discs treated with extracts and two with controls (distilled water as negative control and fipronil 5% SC as positive control). The discs were placed on a moist filter paper (9 cm diameter). Three third instar DBM larvae were introduced into the petri dish and left for 48 hours (Cerda et al., 2019). The formula for calculating the antifeedant activity index (AAI) against DBM is presented in Equation 2.

$$\text{AAI (\%)} = \frac{\text{A-B}}{\text{A+B}} \times 100 \quad (2)$$

Where, A is the amount of leaf area consumed in the control treatment and B is the amount consumed in the treated sample.

Evaluation of MPDE's insecticidal effects on DBM larvae using the leaf-dip bioassay method

The leaf-dip bioassay method described by Zhou et al. (2024) was applied to evaluate the insecticidal effect of MPDE against DBM larvae. Green mustard leaves were washed with distilled water and air-dried for approximately 10 minutes. Fresh green mustard leaf discs (7.5 cm in diameter) were dipped in the test solution for 60 seconds. These treated, air-dried leaves were placed in petri dishes lined with moist filter paper. Each petri dish contained 10 third instar larvae, introduced using a camel hair brush, and allowed to feed for 48 hours. Each treatment was replicated three times with 10 larvae per replicate. Observations were made at 24-hour intervals for larval mortality, pupal mortality, and adult emergence. The mortality rate of DBM larvae was calculated according to Equation 3.

$$\text{Mortality of DBM (\%)} = \frac{\text{Number of insects died}}{\text{Total number of insects released}} \times 100 \quad (3)$$

Impact of MPDE on growth and yield of green mustard

Assessment of stem bending in green mustard plants using a flexible bending frame

Green mustard plants with uniformly straight stems and minimal damage were selected. A flexible bending frame OEM (Vietnam) with adjustable bending force was employed to gently apply pressure to the plant stems, and the frame direction was gradually altered to achieve the desired curvature. Bending pressure was ensured to range at 1.45 kPa to prevent physical or physiological damage to the plants. The bending force was monitored using a digital force measurement device, Tekscan 776-A201-1 (Tekscan, USA), applied to the plant stems. The bending angle of the stems was measured using a TECH 700 DA digital angle gauge (Stabila, Germany), aligned parallel to the stem's bending direction for precise angle measurement. The bending angle of the green mustard stems was recorded immediately after applying MPDE, fipronil, and water, and subsequent measurements were taken at intervals of 20, 25, and 30 days. Stem durability (SD) was calculated using Equation 4.

$$\text{SD}\left(\frac{\text{Ncm}}{\text{rad}}\right) = \frac{\text{M}}{\theta - \text{L}} \quad (4)$$

Where, M (Ncm) is the bending moment, usually calculated from the bending force and distance from the bending axis; θ (rad) is the bending angle of the tree, measured after pressure is applied; and L (cm) is the length of the tree (Hussein et al., 2023).

Monitoring survival rates of green mustard plants

In the low MPDE concentration and water treatment groups, plant death was primarily caused by pest infestation and oxidative stress. These conditions weakened the plants, making them more vulnerable to environmental stressors and reducing their overall survival rates. In the initial observation, the number of green mustard plants in each experimental and control group was recorded. In the periodic monitoring, the number of surviving green mustard plants was counted at 10-, 20-, and 30-day intervals during the experiment. The survival rate (SR) was calculated using Equation 5 described by Hussein et al. (2023).

$$\text{SR (\%)} = \frac{\text{Number of surviving plants}}{\text{Initial number of plants}} \times 100 \quad (5)$$

Assessment of biomass yield and harvest efficiency in green mustard plants

The green mustard plants from each experimental and control group were harvested at the end of the trial period. The plants were thoroughly cleaned to remove soil and debris. The total fresh biomass of the harvested plants was weighed immediately after collection. The plants were placed in a drying oven (Memmert, Germany) at 70 °C until a constant weight was achieved. The dried plants were weighed using a precision scale to determine the dry biomass. To better understand the biomass yield and water content of crops, harvest efficiency (HE) was calculated according to Equation 6 described by Hussein et al. (2023)

$$\text{HE (\%)} = \frac{\text{Dry biomass}}{\text{Fresh biomass}} \times 100 \quad (6)$$

Influence of MPDE on antioxidant activity

In vitro antioxidant activity

Determination of antioxidant activity via DPPH radical scavenging assay: The antioxidant activity of plant extracts against DPPH radicals was determined using the method proposed by Chaves et al. (2020) with additional modifications. A methanolic solution of DPPH at 10^{-4} M was prepared. Aliquots of 1 ml from each methanol extract sample were mixed with 2 ml of the methanolic DPPH solution. The mixtures were kept in the dark at room temperature for 16 minutes, and the absorbance was measured at 517 nm using a UV-30 spectrophotometer (Giorgio-Bormac SRL, Carpi, Italy). A blank was prepared by diluting DPPH in methanol. The standard curve was established using quercetin concentrations of 0.01, 0.02, 0.03, 0.04, and 0.05 mM. The percentage inhibition of DPPH was calculated according to Equation 7.

$$\text{Percentage of inhibition (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad (7)$$

Where, $A_{control}$ is the absorbance value of the DPPH solution without a test sample and A_{sample} is the absorbance value of the DPPH solution containing the test sample.

Determination of antioxidant activity via reducing power (RP) assay: The RP of plant extracts was determined using the method described by Chaves et al. (2020) with additional modifications. Aliquots of 1 ml of each methanol extract were mixed with 2.5 ml of 0.2 mM phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes in a water bath. After incubation, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3,000 rpm for 10 minutes. Then, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. The absorbance was measured at 700 nm using a UV-30 spectrophotometer. A blank was prepared by replacing the extract with an equal amount of methanol. The standard curve was established using quercetin concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mM.

In vivo antioxidant activity

Peroxidase (POD): POD activity was assessed using a modified protocol inspired by Gou et al. (2022). Initially, 1 g of finely powdered leaf tissue was blended with 10 ml of cold phosphate buffer (0.1 M, pH 6.0). Following centrifugation at 12,000 rpm for 15 minutes at 4 °C, the supernatant containing the POD enzyme was carefully isolated and maintained on ice for subsequent analysis. For the enzymatic assay, 0.1 ml of the enzyme extract was combined with a reaction mixture consisting of 2.8 ml of phosphate buffer (0.1 M, pH 6.0), 0.1 ml of hydrogen peroxide (H_2O_2), and 0.1 ml of guaiacol solution. Absorbance readings at 470 nm were taken using a BioMate 160 spectrophotometer (Thermo, USA) every 30 seconds over a 5-minute duration.

Glutathione reductase (GR): The activity of GR was determined using a modified method as described by Nhung and Quoc (2024d). Initially, 1 g of powdered leaf tissue was ground in a mortar

and pestle with 10 ml of cold phosphate buffer (0.1 M, pH 7.5) containing EDTA (1 mM) and Triton X-100 (0.1%, v/v). The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4 °C to obtain a uniform supernatant containing GR enzyme. For the enzymatic assay, 0.1 ml of the enzyme extract was mixed with a reaction mixture comprising 2.6 ml of phosphate buffer (0.1 M, pH 7.5), 0.1 ml of EDTA (1 mM), 0.1 ml of NADPH (2 mM), and 0.1 ml of glutathione (GSH, 10 mM). Absorbance at 340 nm was measured using a BioMate 160 spectrophotometer (Thermo, USA), recording changes every 30 seconds over 5 minutes. Enzyme activity was calculated using the molar extinction coefficient of NADPH (6.22 mM^{-1} cm^{-1}) to convert the absorbance change into units of enzyme activity (µmol of NADPH oxidized per minute per ml of enzyme extract).

Glutathione peroxidase (GPx): GPx activity was measured using a modified protocol from Nhung and Quoc (2024d). One gram of leaf tissue was ground with 10 ml of cold phosphate buffer (0.1 M, pH 7.0) containing EDTA (1 mM) and Triton X-100 (0.1%, v/v). The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4 °C, and the supernatant containing GPx was collected. For the assay, 0.1 ml of the enzyme extract was added to a reaction mixture containing 2.4 ml of phosphate buffer (0.1 M, pH 7.0), 0.1 ml of EDTA (1 mM), 0.1 ml of glutathione (GSH, 10 mM), 0.1 ml of hydrogen peroxide (H_2O_2, 5 mM), and 0.1 ml of sodium azide (NaN_3, 1 mM). Absorbance was measured at 340 nm using a spectrophotometer, with readings taken every 30 seconds for 5 minutes. Enzyme activity was calculated using the molar extinction coefficient of NADPH (6.22 mM^{-1} cm^{-1}) to determine the rate of NADPH oxidation (µmol of NADPH oxidized per minute per ml of enzyme extract).

Statistical analysis

The study's data were obtained from experiments conducted in triplicate, with results reported as mean±SD. Differences among groups were evaluated using one-way ANOVA followed by Dunnett's test. Statistical significance was determined at $p < 0.05$. Data analysis was performed using Statgraphics Centurion XIX software.

RESULTS AND DISCUSSION

Phytochemical screening and quantification of MPDE

Ethanol extraction was employed to analyze the chemical composition of MPDE leaves, revealing the presence of tannins, flavonoids, terpenoids, polyphenols, saponins, steroids, and alkaloids in the extract. Notably, cardiac glycosides were absent. This assessment underscores MPDE as a rich source of bioactive compounds such as saponins, alkaloids, and polyphenols. Quantitatively, saponins were found at 377.58±5.49 µg ml^{-1}, alkaloids at 198.36±3.69 µg ml^{-1}, and polyphenols at 69.42±1.43 mg GAE g^{-1} in the ethanol extract (Table 1).

Mustard greens are cultivated worldwide but are highly vulnerable to various damaging insect pests, significantly reducing crop yields. One of the most prevalent and destructive pests is the DBM. Notably, DBM is the first pest recorded to develop resistance to over 100 types of synthetic pesticides, including Bt toxins from widely used biopesticides, thereby limiting effective control options (Lin et al., 2020). Plant extracts represent environmentally and human-friendly biological control options to manage this common pest species (Soth et al., 2022). Ethanol extract from MPDE leaves, containing chemical compounds such as tannins, flavonoids, terpenoids, polyphenols, saponins, steroids, and alkaloids, has demonstrated efficacy against this harmful moth species in this study.

In MPDE, tannins are recognized for their antioxidant properties, scavenging free radicals, and reducing oxidative stress in plants. This antioxidative capability helps protect plant tissues from damage caused by ROS, enhancing overall plant health and resilience against environmental pressures. Steroids influence plant growth and development, contributing to defense mechanisms against insects by regulating plant physiology, potentially through endocrine modulation or structural modifications to deter feeding. Flavonoids and terpenoids exhibit potent antioxidant activity, shielding plant cells from ROS-induced damage, thereby maintaining redox balance and preventing oxidative stress (Al-Khayri et al., 2023). Saponins, particularly, are reported for their insecticidal properties against various pests, including the DBM. They disrupt cell membranes and interfere with insect digestion, reducing feeding and inhibiting larval development. Additionally, saponins can induce systemic resistance in plants, enhancing their ability to resist pests and diseases (Hussain et al., 2019). Alkaloids possess potent biological activities that deter herbivores by affecting the nervous transmission or metabolic processes in insects. They act as feeding deterrents or toxins, reducing

Table 1. Phytochemical screening and quantification of ethanol extract from MPDE leaves

Phytoconstituents	Test	Observation	Present in MPDE	Quantification of phytochemicals
Tannins	2 ml extract + 2 ml H_2O + 2–3 drops $FeCl_3$ (5%)	Green precipitate	+	NT
Flavonoids	1 ml extract + 1 ml $Pb(OAc)_4$ (10%)	Yellow coloration	+	NT
Terpenoids	2 ml extract + 2 ml $(CH_3CO)_2O$ + 2–3 drops conc. H_2SO_4	Deep red coloration	+	NT
Polyphenols	2 ml extract + 2 ml $FeCl_3$	Bluish-green appearance	+	69.42±1.43 (mg GAE g^{-1})
Saponins	5 ml extract + 5 ml H_2O + heat	Froth appearance	+	377.58±5.49 (µg ml^{-1})
Steroids	2 ml extract + 2 ml $CHCl_3$ + 2 ml H_2SO_4 (conc.)	The reddish-brown ring at the junction	+	NT
Cardiac glycosides	2 ml extract + 2 ml $CHCl_3$ + 2 ml CH_3COOH	Violet to blue to green coloration	-	-
Alkaloids	2 ml extract + a few drops of Hager's reagent	Yellow precipitate	+	198.36±3.69 (µg ml^{-1})

Note: Presence of phytochemicals in MPDE = (+) present and (-) absent; NT = Not tested

plant palatability to pests like the DBM (Xu et al., 2023).

In MPDE, polyphenols function as feeding deterrents, reducing the attractiveness of host plants to DBM larvae. They disrupt crucial physiological processes for larval development such as digestion, metabolism, or endocrine regulation, thereby limiting their growth and development. Certain polyphenols can also disrupt oxidative stress mechanisms, and alter DBM larval behavior affecting mating, oviposition, or feeding, leading to cellular dysfunction and overall reduced reproductive capacity of insects (Singh et al., 2021). Using plant extracts like MPDE not only protects mustard greens from DBM but also enhances overall plant resistance and health in agricultural environments. The compounds in MPDE can improve the overall health of mustard greens, helping them combat oxidative stress and other harmful factors. This approach represents a promising biological method to reduce synthetic pesticide use and safeguard the environment.

Assessment of the impact of MPDE solution on DBM larvae *in vitro* experiments

Choice oviposition bioassays for DBM adults using treated green mustard leaf discs

Table 2 illustrates significant variations in the ODI of DBM across different treatments ($p < 0.05$). The ODI increased progressively over time and with higher concentrations, peaking at 96 hours in the MPDE10 treatment. Notably, ODI values at MPDE concentrations of 2, 4, 6, and 8% (w/v) were significantly higher compared to the water treatment. Fipronil 5% also exhibited the highest ODI at 96 hours.

Oviposition is a critical reproductive behavior activated by the interaction of factors such as biological signals from female DBM, chemical cues from host plants, and environmental conditions. The oviposition process in DBM involves a complex interplay of physiological and chemical factors. Female DBM detects chemical compounds like glucosinolates in cruciferous plants (Brassicaceae) to identify suitable host plants. They then approach the leaf surface, using tactile and chemical sensors on their legs and antennae to assess its suitability. Females lay eggs using their ovipositors to deposit eggs on the leaf surface (Li et al., 2020). In oviposition deterrence trials with ethanol extract from MPDE leaves, *P. xylostella* females primarily laid eggs on control discs, indicating oviposition deterrence effects. MPDE at 10% (w/v) caused the highest deterrence rate in bioassays. When DBM land on leaves, their perception of the leaf's chemical and physical properties is crucial for oviposition decisions (Nhung and Quoc, 2024b).

Table 2. Choice oviposition deterrent bioassays for DBM adults using treated green mustard leaf discs

Treatment	ODI (%)			
	24 hours	48 hours	72 hours	96 hours
Water	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Fipronil	65.18±4.26[f]	63.52±2.07[g]	63.17±1.16[g]	63.79±0.86[g]
MPDE2	19.54±3.98[b]	20.47±2.03[b]	19.69±0.27[b]	19.89±0.20[b]
MPDE4	33.33±4.44[c]	32.91±0.73[c]	33.63±0.51[c]	33.34±0.64[c]
MPDE6	44.44±4.81[d]	43.07±1.52[d]	42.86±0.69[d]	42.96±0.75[d]
MPDE8	57.66±3.81[e]	56.08±1.85[e]	55.79±0.98[e]	55.81±0.91[e]
MPDE10	60.11±3.11[ef]	59.71±1.96[f]	60.63±1.10[f]	60.01±0.79[f]

Note: ODI = Oviposition deterrent index. Values are expressed as mean±SD, and letters (a, b, c, d, e, f, and g) represent the difference between treatments ($p < 0.05$)

Factors contributing to oviposition deterrence include the presence of repellent chemicals, changes in taste, toxic substances, or physiological impacts on DBM that make the plants less attractive for oviposition, reduce survival and reproduction, or disrupt reproductive processes (Ferreira et al., 2022). The presence of plant chemicals in MPDE caused physiological and behavioral changes in adult *P. xylostella*, as evidenced by oviposition responses. Compounds most reported in literature affecting insect oviposition include alkaloids, saponins, flavonoids, terpenoids, and phenols (Li et al., 2020). Another factor is the presence of deterrent substances in the extract, limiting DBM presence on the oviposition substrate (Ferreira et al., 2022). Oviposition deterrence effects have been observed by other authors when evaluating plant extracts from *Miconia albicans* (Sw.) Triana (Melastomataceae) (Padial et al., 2023) and *Ludwigia* spp. (Onagraceae) on *P. xylostella* (Ferreira et al., 2022).

Choice bioassays for evaluating feeding preferences of third-instar DBM larvae

In the AAI assay (Figure 1), the water treatment showed an AAI value of 0%, indicating no inhibition of larval feeding. In contrast, ethanol extract from MPDE leaves at concentrations of 2, 4, 6, 8, and 10% (w/v) exhibited AAI values of 23.59, 33.96, 46.85, 66.15, and 69.88%, respectively, at the 48-hour mark, significantly higher than the water treatment. Notably, the MPDE10 treatment (69.88%) achieved an efficacy comparable to fipronil 5% (70.53%), demonstrating the antifeedant activity of MPDE. Thus, compared to the control, MPDE significantly reduced the leaf area consumed.

The feeding behavior of the DBM is a crucial aspect of its life cycle, directly influencing its survival and reproduction. Chemical compounds such as glucosinolates in cruciferous plants attract DBM larvae. The larvae use their mandibles to efficiently cut through plant material, while digestive enzymes break down plant cell walls,

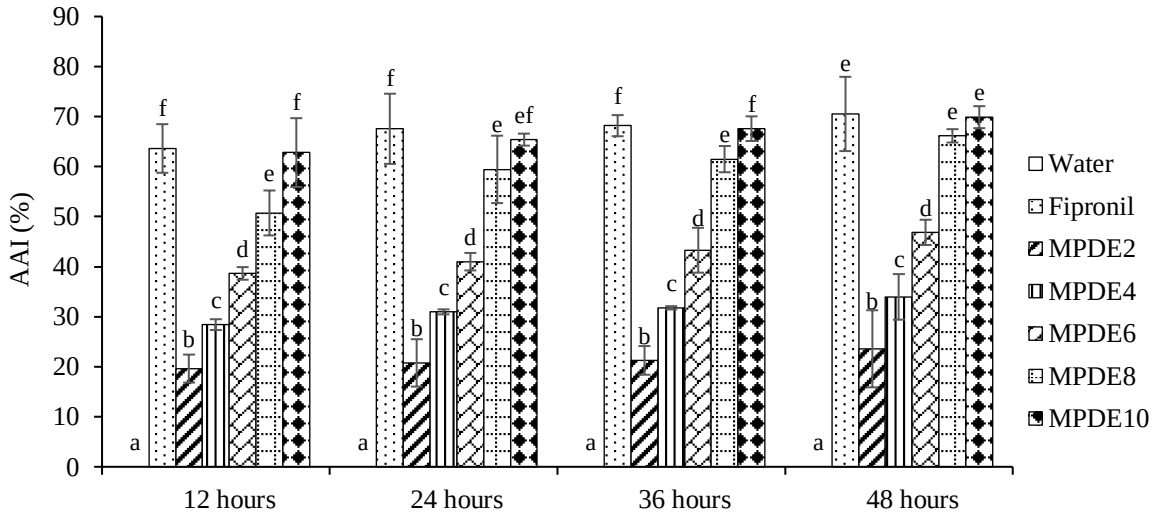

Figure 1. Choice bioassays for evaluating feeding preferences of third-instar DBM larvae

Note: AAI = Antifeedant activity index. Values are expressed as mean±SD, and letters (a, b, c, d, e, and f) represent the difference between treatments ($p < 0.05$)

facilitating nutrient absorption (Han et al., 2023). In the study by Ferreira et al. (2022), ethanol extract of *L. tomentosa* significantly reduced consumption in treated discs compared to control discs, indicating that the extract is largely unattractive to DBM. The reduction in leaf consumption upon exposure to plant extracts is often due to antifeedant properties or sublethal toxicity, preventing feeding and digestion (Ferreira et al., 2022). Similarly, the results of this study show that MPDE extracts performed well in bioassays on feeding preference, leading to reduced leaf consumption - an effective form of control for crops like green mustard, where leaves are the commercial product.

Evaluation of MPDE's insecticidal effects on DBM larvae using the leaf-dip bioassay method

Figure 2 presents the mortality rates of DBM larvae. The data indicate that mortality rates increased significantly with higher concentrations of MPDE and longer exposure durations, compared to the water treatment. Notably, the highest mortality rates were observed with MPDE at 8% and 10%, which were comparable to the efficacy of fipronil at 5%. The mortality rate of DBM larvae is a critical parameter for understanding and managing population dynamics. Various biological and abiotic factors influence DBM mortality, including environmental conditions, natural mortality agents, biological control factors, plant resistance, and cultivation practices (Yang et al., 2021). Ethanol extract from MPDE leaves increases DBM mortality through multiple physiological and biochemical mechanisms. MPDE enhances mortality by combining toxic effects, antifeedant properties, disruption of growth and development, repellency, immune system modulation, and interaction with pathogens.

Compounds such as saponins, alkaloids, and phenolics can disrupt physiological functions, inhibit key digestive and detoxification enzymes, and impede metabolic processes, leading to increased mortality (Singh et al., 2021). Flavonoids and tannins are known to cause antifeedant effects in insects (Al-Khayri et al., 2023). Research observations align with the findings of Shilaluke and Moteetee (2022), who investigated the effects of extracts from *Ekebergia capensis*, *Melia azedarach*, *Trichilia dregeana*, *Turraea floribunda*, and *Turraea obtusifolia* on insect mortality. The biological assays conducted by Shilaluke and Moteetee (2022), combined with the results of this study, suggest that the enhanced mortality observed with MPDE is attributed to its pronounced effects on DBM larvae, significantly increasing their mortality rate.

Assessment of the impact of MPDE solution on DBM larvae *in vivo* experiment

Assessment of stem bending in green mustard plants using a flexible bending frame

Plant's stem sturdiness or SD is a critical indicator of overall plant health, structural integrity, and adaptability. Figure 3 presents the results of evaluating the bending capacity of green mustard stems using a flexible bending frame. At 20, 25, and 30 days, the water treatment group exhibited the lowest SD, with SD values decreasing over time. In contrast, MPDE-treated groups showed significantly higher SD compared to the water treatment. SD increased with both the duration of the survey and the concentration of

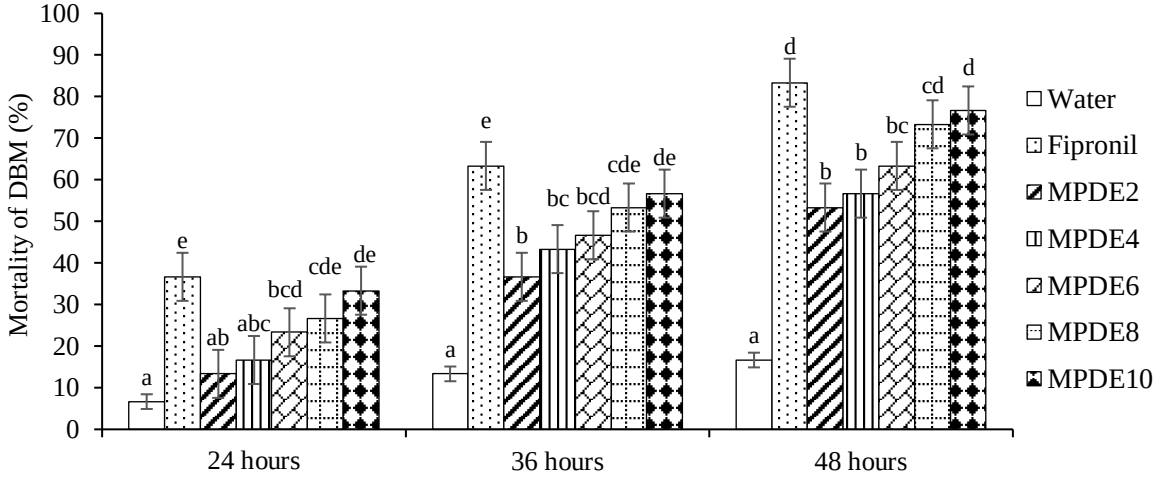

Figure 2. Insecticidal effects of MPDE on DBM larvae using the leaf-dip bioassay technique

Note: Values are expressed as mean±SD, and letters (a, b, c, d, and e) represent the difference between treatments ($p < 0.05$)

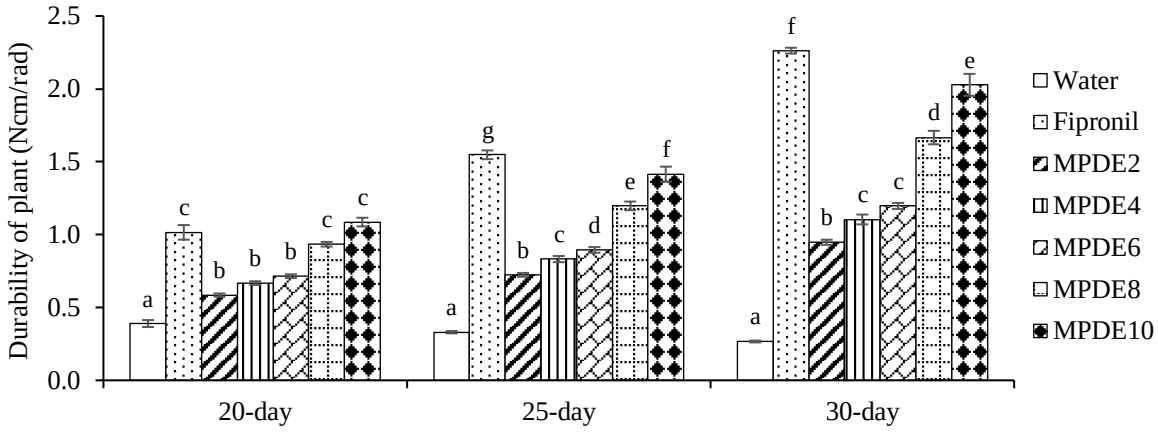

Figure 3. The flexural ability of green mustard plant stems using a flexible frame
Note: Values are expressed as mean±SD, and letters (a, b, c, d, e, f, and g) represent the difference between treatments ($p < 0.05$)

MPDE. While SD differences were minimal among concentrations at 20 days, they became significantly pronounced after 25 and 30 days. These findings suggest that MPDE treatment significantly enhances the sturdiness of green mustard stems in a time- and concentration-dependent manner, indicating improved plant health, structural integrity, and adaptability.

Healthy plant stems provide essential support, enabling the plant to stand upright and withstand wind and other mechanical stresses. Stem strength is often linked to the efficient transport of nutrients and water. Robust stems enhance disease and pest resistance, limit pathogen spread, and contribute to overall plant health and longevity. Stem sturdiness indicates healthier growth, better competition for light, water, and nutrients, and improved environmental adaptability. This increases the plant's resilience to harsh conditions such as strong winds, heavy rain, and mechanical damage caused by animals (Al-Khayri et al., 2023). In this study, treatment with ethanol extract from MPDE leaves significantly enhanced stem sturdiness. The phenolic compounds, terpenoids, saponins, steroids, alkaloids, and antioxidant enzymes in MPDE promote lignin and cellulose synthesis, thereby reinforcing cell wall integrity and protecting plant cells from oxidative stress, which contributes to overall stem strength (Kumar et al., 2023).

Additionally, MPDE exhibits antimicrobial, antioxidant, and insecticidal properties that reduce disease incidence and pest infestation, mitigating stress and allowing the plant to allocate more resources to structural growth and development (Nhung and Quoc, 2024a). The lack of significant differences in SD among MPDE concentrations at 20 days may be attributed to the initial acclimatization period, the time required for active compound accumulation, gradual treatment responses, measurement sensitivity, and inherent biological variability. Significant differences became more apparent with extended treatment duration as the plant's response to the treatments intensified. By days 25 and 30, the pronounced differences in SD among MPDE concentrations were due to the cumulative effects of the treatment, enhanced physiological responses, prolonged exposure, time-dependent sensitivity, developmental stage interactions, and reduced biological variability. These factors collectively contributed to the clearer distinctions in SD across different MPDE concentrations.

Monitoring of SR of green mustard plants

The SR of the plants is detailed in Table 3. Notably, SR in treatments with MPDE and fipronil differed significantly from the water treatment. For plants treated with water and MPDE at concentrations of 2, 4, and 6% (w/v), SR declined progressively over the study period. In contrast, SR remained stable for plants treated with MPDE at 8% and 10% (w/v), showing no significant difference from the fipronil 5% treatment.

The SR of plants is a critical parameter for assessing plant resilience and the effectiveness of interventions designed to improve or maintain plant survival. SR quantifies plant viability, indicating whether a treatment is protective, less harmful, or potentially detrimental to plant health and longevity (Pathirana and Carimi, 2024). In this study, the significant differences in SR between MPDE and fipronil treatments compared to the water control highlight the substantial impact of these treatments on plant survival. This suggests that MPDE and fipronil have notable

Table 3. Monitoring of SR of green mustard plants *in vivo* experiment

Treatment	The SR of the plant (%)		
	10-day	20-day	30-day
Water	83.33±7.22^{a}	75.00±12.50^{a}	66.67±7.22^{a}
Fipronil	100.00±0.00^{b}	100.00±0.00^{c}	100.00±0.00^{d}
MPDE2	95.83±7.22^{b}	83.33±7.22^{ab}	79.17±7.22^{b}
MPDE4	100.00±0.00^{b}	91.67±7.22^{bc}	83.33±7.22^{bc}
MPDE6	100.00±0.00^{b}	95.83±7.22^{c}	91.67±7.22^{cd}
MPDE8	100.00±0.00^{b}	100.00±0.00^{c}	100.00±0.00^{d}
MPDE10	100.00±0.00^{b}	100.00±0.00^{c}	100.00±0.00^{d}

Note: SR = Survival rate. Values are expressed as mean±SD, and letters (a, b, c, and d) represent the difference between treatments ($p < 0.05$)

effects, which can be beneficial or harmful depending on the context and concentration. The gradual decline in SR for plants treated with water and lower concentrations of MPDE (2, 4, and 6% w/v) over time indicates these treatments may be less effective in maintaining plant health, possibly due to insufficient potency or adverse effects. Conversely, the stable SR observed in plants treated with higher MPDE concentrations (8% and 10% w/v), comparable to fipronil 5%, suggests that these concentrations effectively sustain plant health. Thus, the SR data underscore MPDE's potential as a viable alternative to fipronil for enhancing plant survival, especially at higher concentrations, and highlights the importance of carefully considering treatment concentrations and their long-term effects on plant health.

Assessment of biomass yield and HE in green mustard plants

HE serves as a key metric for evaluating the effectiveness of harvesting practices, as illustrated in Figure 4. HE increased progressively with rising concentrations of MPDE, with values recorded at 11.53, 12.44, 13.59, 16.71, and 17.45% for 2, 4, 6, 8, and 10% (w/v) concentrations, respectively. In contrast, the HE for the water treatment was significantly lower at 7.78%, showing a notable difference compared to the MPDE and fipronil treatments. This indicates that higher concentrations of MPDE effectively enhance the efficiency of biomass conversion, highlighting its potential for improving harvest outcomes.

HE is a key metric for evaluating the effectiveness of converting fresh biomass into dry biomass. It provides insights into plant health, productivity, and the effectiveness of cultivation practices. Higher HE values indicate that a greater proportion of fresh biomass is converted to dry biomass, reflecting efficient growth and productivity. HE is inversely related to water content in plants; lower HE suggests higher water content, possibly due to excessive moisture, while higher HE indicates more efficient biomass conversion and lower water content. Analyzing HE helps assess the impact of growth conditions, treatments, and interventions on plant

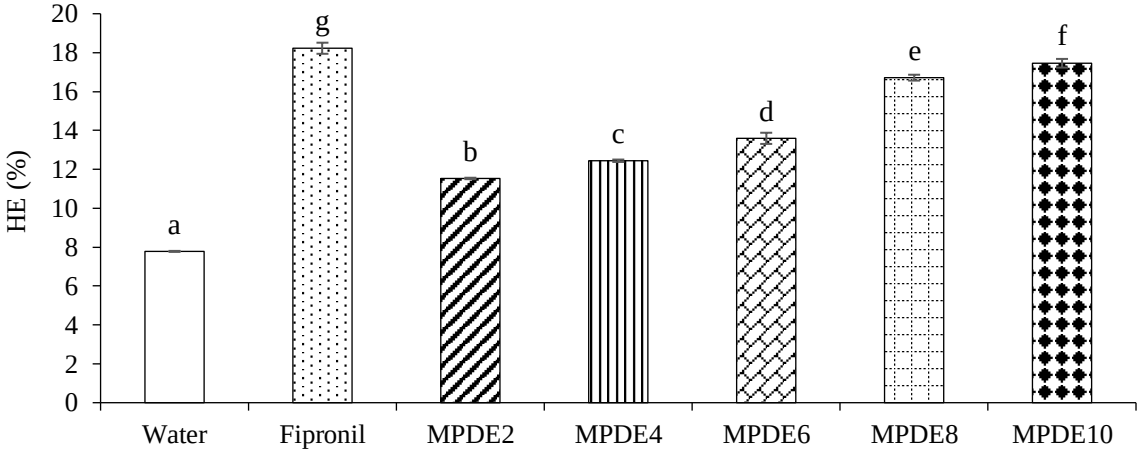

Figure 4. Biomass yield and HE of green mustard plants *in vivo* experiment

Note: HE = Harvest efficiency of plant. Values are expressed as mean±SD, and letters (a, b, c, d, e, f, and g) represent the difference between treatments ($p < 0.05$)

development and biomass accumulation (Burgess et al., 2023). In this study, HE increased with higher concentrations of MPDE, indicating that higher MPDE concentrations are more effective in converting fresh to dry biomass. The maximum HE of 17.45% at 10% MPDE demonstrates the highest conversion efficiency among the tested concentrations. In contrast, the water treatment yielded a significantly lower HE of 7.78%, highlighting its reduced effectiveness in enhancing biomass conversion, underscoring the beneficial effects of MPDE and fipronil treatments on biomass retention and conversion. The progressive increase in HE with higher MPDE concentrations reflects its efficacy in improving plant biomass conversion, suggesting that MPDE enhances the plant's ability to maintain a higher proportion of dry biomass, thus improving overall crop productivity. Overall, the MPDE improves HE in a dose-dependent manner, with higher concentrations providing better conversion of fresh to dry biomass.

Influence of MPDE on antioxidant activity

In vitro antioxidant activity

As shown in Table 4, the ethanol extract from MPDE leaves demonstrated concentration-dependent antioxidant activity, including radical scavenging and RP. The DPPH radical scavenging activity of MPDE increased with higher concentrations, with values of 11.73, 12.88, 15.36, 19.65, and 21.80% for 2, 4, 6, 8, and 10% (w/v) concentrations, respectively, significantly higher compared to the 0% observed in the water treatment. Additionally, the RP was lowest in the water treatment (0.61 mg QE g^{-1} dw) and significantly higher in MPDE treatments, with values of 0.72, 0.75, 0.78, 0.85, and 0.88 mg QE g^{-1} dw for the respective concentrations.

Antioxidants neutralize free radicals through various mechanisms, including donating a hydrogen atom to the radicals to neutralize them or providing electrons to convert them into less reactive forms. The effectiveness of the ethanol extract from MPDE leaves in scavenging free radicals can be assessed using assays such as the DPPH test, which measures the extract's ability to reduce DPPH radicals to a non-radical form (Haran et al., 2024). MPDE's reducing capacity indicates its ability to donate electrons, thereby converting ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This conversion helps prevent oxidative damage since Fe^{2+} is less reactive and less likely to participate in harmful Fenton reactions that generate hydroxyl radicals (Gorman, 2023). The results indicate that the MPDE exhibits significant antioxidant properties. The increase in DPPH radical scavenging activity with higher MPDE concentrations (ranging from 11.73 to 21.80%) demonstrates the extract's effective ability to neutralize free radicals. This increase is statistically significant compared to the water treatment, which showed no scavenging activity, highlighting MPDE as a potent antioxidant with enhanced efficacy at higher concentrations. Furthermore, the higher RP values observed in MPDE treatments (ranging from 0.72 to 0.88 mg QE g^{-1} dw) compared to the water treatment (0.61 mg QE g^{-1} dw) confirm MPDE's effective reduction of Fe^{3+} to Fe^{2+} ions, further supporting its strong antioxidant capacity. Overall, MPDE shows substantial antioxidant activity that increases with concentration, making it a valuable natural source of antioxidants for combating oxidative stress.

In vivo antioxidant activity

In the context of DBM-induced damage, POD, GR, and GPx are crucial antioxidant enzymes that protect green mustard plants from oxidative stress. Green mustard plants infested by pests showed significant inhibition of POD, GR, and GPx activities during underwater treatment, indicating the presence of oxidative stress. In contrast, this inhibition was reversed in the groups treated with MPDE and fipronil (Table 5). POD, GR, and GPx enzyme levels significantly increased in the MPDE and fipronil-treated groups compared to the water-treatment group. This suggests that MPDE can restore the activities of antioxidant enzymes, mitigating oxidative stress-induced damage.

Table 4. Comparative assessment of *in vitro* antioxidant activity using DPPH radical scavenging and RP assays

Treatments	*In vitro* antioxidant activity	
	DPPH (% inhibition)	RP (mg QE g^{-1} dw)
Water	0.00 ± 0.00^{a}	0.61 ± 0.01^{a}
Fipronil	23.78 ± 0.45^{f}	0.91 ± 0.01^{g}
MPDE2	11.73 ± 1.09^{b}	0.72 ± 0.01^{b}
MPDE4	12.88 ± 0.47^{b}	0.75 ± 0.02^{c}
MPDE6	15.36 ± 0.86^{c}	0.78 ± 0.02^{d}
MPDE8	19.65 ± 1.15^{d}	0.85 ± 0.01^{e}
MPDE10	21.80 ± 0.11^{e}	0.88 ± 0.01^{f}

Note: RP = Reducing power. Values are expressed as mean±SD, and letters (a, b, c, d, e, f, and g) represent the difference between treatments ($p < 0.05$)

Table 5. Evaluation of *in vivo* antioxidant activity through enzymatic levels of POD, GR, and GPx

Treatment	*In vivo* antioxidant activity		
	POD (U ml^{-1})	GR (µmol of NADPH oxidized $minute^{-1}$ ml^{-1})	GPx (µmol of NADPH oxidized $minute^{-1}$ ml^{-1})
Water	97.26±0.31[a]	0.09±0.07[a]	0.94±0.01[a]
Fipronil	145.89±0.56[g]	0.18±0.02[b]	1.69±0.09[f]
MPDE2	116.71±0.21[b]	0.12±0.01[ab]	1.22±0.04[b]
MPDE4	121.58±0.16[c]	0.13±0.01[ab]	1.32±0.02[c]
MPDE6	126.44±0.57[d]	0.14±0.02[b]	1.36±0.02[c]
MPDE8	136.16±0.15[e]	0.16±0.15[b]	1.48±0.02[d]
MPDE10	141.03±0.42[f]	0.17±0.01[b]	1.59±0.04[e]

Note: POD = Peroxidase, GR = Glutathione reductase, GPx = Glutathione peroxidase. Values are expressed as mean±SD, and letters (a, b, c, d, e, f, and g) represent the difference between treatments ($p < 0.05$)

When DBM infests green mustard plants, they suffer physical damage and metabolic disruption. The feeding activity of DBM larvae causes cellular damage and increases the production of ROS, such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). These ROS attack lipids in cell membranes, causing lipid peroxidation, which results in membrane integrity and function loss, leading to cellular damage and leakage. ROS can also oxidize proteins, resulting in loss of enzyme activity, structural alterations, and disruption of essential cellular processes. Additionally, ROS induces DNA mutations and strand breaks, compromising genetic integrity and the plant's ability to grow and reproduce (Ali et al., 2024). Plants possess a defense system that includes antioxidant enzymes such as POD, GR, and GPx. These enzymes play a crucial role in detoxifying ROS and protecting cells from oxidative damage. POD catalyzes the reduction of H_2O_2 to water using various electron donors. GR regenerates reduced glutathione (GSH) from oxidized glutathione (GSSG), maintaining cellular redox balance. GPx reduces H_2O_2 and organic hydroperoxides to water and alcohol, respectively, using GSH as a substrate (Nhung and Quoc, 2024a).

In the current study, MPDE was found to significantly enhance the activity of key antioxidant enzymes (POD, GR, and GPx) in green mustard plants under DBM-induced stress. The significant increase in enzyme activity in the MPDE and fipronil-treated groups compared to the water-treated group demonstrates the protective effect of MPDE against oxidative stress. The enhanced antioxidant enzyme activity suggests that MPDE can mitigate oxidative damage, thereby promoting better plant health and resilience to pest attacks. By enhancing these enzyme activities, MPDE aids in detoxifying ROS, reducing oxidative damage, and improving the overall health and resilience of plants against DBM infestation. This indicates that MPDE helps alleviate oxidative stress caused by DBM damage, thereby protecting plants and enhancing their health and resilience. Overall, these findings highlight the potential of MPDE as an effective treatment for improving oxidative stress responses in green mustard plants.

CONCLUSIONS

Ethanol extracts from MPDE leaves significantly improved the ODI and AAI in a concentration-dependent manner, outperforming water treatment. Higher MPDE concentrations increased DBM larval mortality and enhanced the SD of green mustard stems, indicating better structural integrity and resilience. MPDE also maintained higher plant SR at 10% concentrations, comparable to fipronil. Additionally, MPDE improved HE and demonstrated significant antioxidant activity, including radical scavenging (DPPH) and RP. MPDE reversed the inhibition of key antioxidant enzymes (POD, GR, and GPx) caused by DBM, reducing oxidative damage. Overall, MPDE is an effective natural treatment for boosting antioxidant defenses and DBM resistance, offering a sustainable alternative to synthetic pesticides.

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