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ORIGINAL ARTICLE

Assessment of the insecticidal efficacy of ethanol extract of *Millettia pachyloba* Drake leaves against *Plutella xylostella* Linnaeus moth

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Abstract

Plutella xylostella (L.), a menacing threat to cruciferous crops, exhibits cross-resistance to various chemical agents. The exploration of plant-derived insecticides emerges as an intervention strategy for the successful management of *P. xylostella*. *Millettia pachyloba* Drake is renowned as a traditional remedy for diverse health issues and has insecticidal properties. Experimental investigations in both laboratory and greenhouse settings utilized *M. pachyloba* extract (EMPE) at concentrations ranging from 2 to 10% (w/v). The objectives included inducing toxicity and controlling *P. xylostella* larvae effectively, assessing nutritional impacts through parameters like relative consumption rate (RCR), relative growth rate of larvae (RGR), efficiency of conversion of digested food (ECD), efficiency of conversion of ingested food (ECI), approximate digestibility (AD), and assimilation ratio (AR), and evaluating leaf damage inflicted by *P. xylostella* larvae on *Brassica juncea*. In the laboratory, the application of EMPE on *P. xylostella* larvae and pupae for 24, 48, and 72 hours yielded markedly higher mortality rates than the water-treated control ($p < 0.05$). Significant reductions in RGR, RCR, ECD, ECI, AD, and AR were evident throughout the larval stage ($p < 0.05$). In the greenhouse, EMPE treatments demonstrated notable differences from the water control treatment. On the 15th day of treatment, the EMPE treatment at 10% (w/v) exhibited the highest mortality rate ($p < 0.05$). Significantly reduced leaf damage was observed with EMPE treatments, displaying an inverse correlation with escalating concentrations. Particularly, the highest enhancement across all surveyed parameters was observed in the EMPE 10% (w/v) treatment, which was comparable to the positive control with fipronil ($p > 0.05$). Noteworthy differences in damage reduction percentage (DRP) were identified between EMPE contact treatments and the water control group ($p < 0.05$), indicating the promising potential of *Millettia pachyloba* extract for pest control.

Keywords: biopesticide, *Brassica juncea*, *Millettia pachyloba*, pest management, *Plutella xylostella*

Introduction

Plutella xylostella (L.), commonly known as the diamondback moth (DBM), emerges as a prominent global menace to cruciferous crops. This pest poses a substantial challenge on a global scale, introducing complexities to agricultural management and imposing noteworthy financial burdens annually (Neto-Bandeira *et al.* 2013). The prevailing strategy for controlling moth larvae in gardens is currently centered on the extensive use of chemical insecticides. However, extended application has resulted in a pressing issue of insecticide resistance, particularly against widely utilized chemical insecticides and insect growth regulators. These moth species demonstrate an exceptional capacity to adapt to chemical insecticides, exhibiting conspicuous cross-resistance among commonly employed chemical counterparts. Consequently, the

immediate priority is to identify plant-derived insecticides as a strategic intervention to hinder the development of resistance and proficiently manage *P. xylostella* (Fan *et al.* 2023).

The *Millettia* genus, belonging to the *Fabaceae* family, encompasses over 200 plant species thriving in tropical and subtropical regions worldwide. Traditional uses of *Millettia* species span diverse applications, including antibacterial, anti-tumor, insecticidal, insect-repelling, anti-spasmodic, anti-cancer, joint pain alleviation, and anti-inflammatory properties for arthritis. Various phytochemicals, such as flavonoids, phenolic compounds, phytosterols, saponins, alkaloids, polysaccharides, terpenoids, and resins, have been identified as secondary metabolites across different *Millettia* species. Prominent members within the *Millettia* genus encompass *Millettia extensa* (Benth.) Baker, *Millettia pachycarpa* Benth, *Millettia pinnata* (L.) Panigrahi, *Millettia ovalifolia* Kurz, *Millettia auriculata* Brandis, *Millettia speciosa* Champ., *Millettia laurentii* De Wild., and various other species, each possessing diverse pharmacological properties. Specifically, the stems and bark of *M. conraui* are employed as insecticides and nematocides. *M. pachycarpa* seeds combat tapeworm infections, and the stems and leaves of *M. auriculata* act as insecticides and anthelmintics. Moreover, the bark and roots of *M. dura* demonstrate efficacy against insects and larvae, while the branches and leaves of *M. duchesnei* find utility as fish poison and insect repellent (Jena *et al.* 2020). *Millettia pachyloba* Drake, a member of the Millettia genus within the Leguminosae family, is a perennial evergreen woody plant mainly distributed across India, Malaysia, Indonesia, Vietnam, and specific African regions. Historically, *M. pachyloba* has been employed as a traditional remedy for various health issues, including gynecological conditions, constipation, cardiovascular diseases, abdominal pain, rheumatoid arthritis, and dermatological ailments. Local communities often utilize the plant's stems in herbal medicine to address concerns such as tumors, joint pain, and edema. Phytochemical analysis of *M. pachyloba* highlights the presence of flavonoids, phenolic compounds, phytosterols, saponins, alkaloids, polysaccharides, and terpenoids (Yan *et al.* 2019). As part of a systematic research endeavor focused on evaluating the insecticidal potential of *M. pachyloba*, this study aimed to assess the efficacy of ethanol extracts from *M. pachyloba* leaves against the larvae and pupae of *P. xylostella*. Given the formidable challenge posed by *P. xylostella* larvae and their substantial impact on global cruciferous crop production, the implementation of early-stage control measures becomes imperative to minimize damage to cabbage crops across diverse regions.

Materials and Methods

Collection of plant material and preparation of the extract

Collection of plant material

In April 2023, *M. pachyloba* leaves were gathered in Đạ Oai commune, Đạ Huoai district, Lâm Đồng province, Vietnam. A designated voucher specimen (code MP090423) was archived in the specimen repository of the Department of Biotechnology, Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry, Vietnam. Following a meticulous selection process to exclude afflicted or deteriorated leaves, freshly harvested leaves underwent a thorough washing and were air-dried for 3–5 days under shaded conditions. Post-drying, the material was pulverized into powder using an herbal grinder machine (TMND-A01, Tan Minh Mechanical Company, Vietnam) and stored in moisture-resistant bagsat room temperature for subsequent experimental use.

Preparation of extract

The preparation of *M. pachyloba* leaf extract involved homogenizing 10 g of leaf powder in 100 ml of 70% ethanol for 16 hours at 28°C, employing a mechanical stirrer. The resulting slurry underwent filtration through Whatman No. 4 filter paper and additional clarification using a vacuum filtration system (LF32, Cường Thịnh Co. Ltd., Vietnam). The filtrate was collected, and the solvent was evaporated under reduced pressure utilizing a rotary evaporator (WEV-1010, Daihan, South Korea) at 60°C. The resultant extract (2.08 g), denoted as EMPE was stored in moisture-resistant containers at 4°C until utilized in subsequent experiments.

Screening and quantification of phytochemicals in ethanol extract of *Millettia pachyloba* leaves

Screening of phytochemicals in extracts

Preliminary phytochemical screening of the ethanol extract of *M. pachyloba* leaves was conducted using established procedures (Tran *et al.* 2023), as outlined in Table 1.

Quantification of phytochemicals in extracts

The methods and techniques, as detailed by Ilukho *et al.* (2022), were employed to quantify total phenols, alkaloids, and tannins in the ethanol extract of *M. pachyloba* leaves.

Table 1. Preliminary phytochemical tests for extract

Phytoconstituents	Test	Observation	References
Tannins	2 ml extract + 2 ml H_2O + 2–3 drops $FeCl_3$ (5%)	green precipitate	Tran *et al.* (2023)
Flavonoids	1 ml extract + 1 ml $Pb(OAc)_4$ (10%)	yellow coloration	Tran *et al.* (2023)
Terpenoids	2 ml extract + 2 ml $(CH_3CO)_2O$ + 2–3 drops conc. H_2SO_4	deep red coloration	Tran *et al.* (2023)
Saponins	5 ml extract + 5 ml H_2O + heat	froth appears	Tran *et al.* (2023)
Steroids	2 ml extract + 2 ml $CHCl_3$ + 2 ml H_2SO_4 (conc.)	reddish brown ring at the junction	Tran *et al.* (2023)
Cardiac glycosides	2 ml extract + 2 ml $CHCl_3$ + 2 ml CH_3COOH	violet to blue to green coloration	Tran *et al.* (2023)
Alkaloids	2 ml extract + few drops of Hager's reagent	yellow precipitate	Tran *et al.* (2023)
Phenolic compound	2 ml extract + 2 ml $FeCl_3$	bluish-green appearance	Tran *et al.* (2023)

Total phenol content was determined employing the spectrophotometric approach

The sample underwent boiling with 50 ml of ether for a 15-minute extraction of phenolic constituents. Subsequently, 5 ml of the extract was carefully transferred into a 50 ml volumetric flask, and 10 ml of distilled water was added. To this mixture, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were introduced. After labeling, the samples were allowed to react for 30 minutes to facilitate color development. The measurement was conducted at a wavelength of 505 nm.

Alkaloid quantification using the Harborne method

This method involved weighing five grams of the sample into a 250 ml glass beaker and adding 200 ml of 10% acetic acid in ethanol. The mixture was covered and left undisturbed for 4 hours before being filtered. The resulting filtrate was concentrated in a water bath until it reached 1/4 of the original volume. Concentrated ammonium hydroxide was added drop by drop to the extract until complete precipitation occurred. After settling, the entire solution was filtered, and the precipitate was collected and washed with dilute ammonium hydroxide. The remaining residue, representing alkaloids, was dried and then weighed.

The quantification of tannins, following the Van-Burden and Robinson method

This method commenced by weighing 500 mg of the sample into a 50 ml plastic flask. Distilled water (50 ml) was then added, and the mixture was subjected to mechanical shaking for an hour. Afterward, the solution underwent filtration into a 50 ml volumetric flask, which was subsequently adjusted to the 50 ml mark. Next, 5 ml of the filtered solution was transferred to a test tube and combined with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorption was measured at a wavelength of 120 nm over a 10-minute duration.

Treatment formulations and rearing *Plutella xylostella* larvae

Treatment formulations

Following the condensation process, the concentrated extract resulting from solvent evaporation served as the stock solution. This solution was then diluted to create five distinct concentrations (2, 4, 6, 8, and 10%, w/v), denoted as $EMPE_2$, $EMPE_4$, $EMPE_6$, $EMPE_8$, and $EMPE_{10}$ treatments, respectively. This study employed a commercial product, fipronil 5% SC (100 ppm) (fipronil treatment), as a positive control. Water was utilized as the reference toxicity treatment (water treatment). All of these treatments were implemented to manage *P. xylostella* in laboratory and greenhouse-based experimental studies.

Rearing *Plutella xylostella*

This experiment was conducted in the Department of Biotechnology, Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry, Vietnam. *P. xylostella* was reared using the method described by Cerda *et al.* (2019). Adult *P. xylostella* specimens were collected from *Brassica juncea* fields belonging to Mien Dong Co. Ltd., in Tan Hiep commune, Di An district, Binh Duong province, Vietnam. The specimens were placed in plastic cages (60 × 30 cm) with a sponge soaked in water to maintain appropriate humidity inside the cage. A filter paper disc and a *B. juncea* leaf disc were placed on a sponge to stimulate the egg-laying behavior of *P. xylostella*. Adult specimens were provided with a 10% honey solution prepared and inserted into a circular hole at the cage's end. Leaf discs with eggs were transferred to glass dishes daily, where they remained until *P. xylostella* hatched. Larvae were kept in these containers until pupation and *B. juncea* leaves were always changed daily with fresh leaves. Pupae were collected in sealed test tubes with air-permeable plastic caps. Pupal development was sustained at an ambient temperature until the emergence of adults, at which point they

were relocated to fresh enclosures. The entire life cycle was reiterated following the emergence of adults. The population was upheld within an ambient temperature range of 20–25°C, with a relative humidity fluctuating between 60 and 80%, following the natural light cycle.

Experimental investigations under laboratory conditions

The experimental procedures were conducted in the Laboratory of Biotechnology, Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry, Vietnam, maintaining a controlled environment with a temperature of 30 ± 2°C, relative humidity set at 60 ± 2%, and a 12-hour light-dark cycle.

Toxicity of larvae

The method of leaf-dipping bioassay was employed to evaluate larval toxicity (Neto-Bandeira *et al.* 2013). *B. juncea* leaf discs with a diameter of 8 cm were submerged for 10 seconds in 50 ml of ethanol extract of *M. pachyloba* leaves (EMPE) of various concentrations. Subsequently, the treated leaf discs were air-dried and individually transferred onto Petri dishes (9 cm in diameter) containing a filter paper disc (8 cm in diameter) soaked in distilled water. Ten 1st instar larvae (less than 12 hours old) were introduced onto each leaf disc. Following the sealing of the Petri dishes, they were enveloped with Parafilm to prevent larvae from escaping. Concentrations varied between 2, 4, 6, 8, and 10% (w/v) for EMPE, and 100 ppm for fipronil 5% SC, with three replicates for each treatment. Gently tapping or directly exposing light to the larvae, if devoid of any discernible response, may be indicative of a deceased state. Mortality rates were assessed after 24, 48, and 72 hours of feeding on treated or controlled *B. juncea* leaf discs. The mortality rate of larvae (MR) was calculated using the following formula:

$$\text{MR (\%)} = \frac{\text{NDL}}{\text{TIL}} \times 100,$$

where: MR – the mortality rate of larvae, NDL – the number of deceased larvae, and TIL – the total initial larvae count.

Toxicity of pupae

The mortality ratio of pupae was assessed following the protocol outlined by Neto-Bandeira *et al.* (2013). Young 1st instar larvae were cultivated on *B. juncea* leaf discs treated with different concentrations of EMPE for 72 hours. Following this, untreated *B. juncea* leaf discs were introduced into the cage, with a renewal of fresh discs occurring every 48 hours, adhering to the previously outlined procedure, until the larvae reached adulthood. The negative control cohort received treatment solely with water, while the positive control cohort was subjected to fipronil treatment. Concentrations of the extract varied from 2 to 10% (w/v). The experiment was iterated three times. The experiment was initiated with around 35 larvae per treatment, aiming for a minimum of 20 healthy larvae that successfully pupated per treatment. Subsequently, the pupated larvae were individually relocated to Petri dishes for the assessment of mortality rates within 24, 48, and 72 hours after entering the pupal stage. The absence of movement or responsiveness to stimuli such as gently touching the pupae may serve as an indicative sign of a deceased state. Pupal mortality rate (PR) was calculated using the following formula:

$$\text{PR (\%)} = \frac{\text{PD}}{\text{TIP}} \times 100.$$

In this study, PR was the pupal mortality rate, PD – the number of pupae deceased, TIP – the total initial pupae count.

The nutritional indices of the larvae

In the larval stage, larvae undergo a significant process of growth and development, relying on various nutritional strategies for sustenance. The nutritional indices of the larvae were calculated based on the description provided by Shannag *et al.* (2015):

1. Relative consumption rate (RCR):

$$\text{RCR } (\text{g} \cdot \text{day}^{-1}) = \frac{\text{WIF}}{\text{WLE} \times \text{NED}},$$

where: RCR – the relative consumption rate, WIF – weight of ingested food, WLE – the average weight of larvae during the experimental period, and NED – the number of experimental days.

2. Relative growth rate of larvae (RGR):

$$\text{RGR } (\text{g} \cdot \text{day}^{-1}) = \frac{\text{LG}}{\text{LWE} \times \text{DEP}},$$

where: RGR – the relative growth rate of larvae, LG – larval growth, WLE – mean larval weight during the experimental period, and DEP – duration of the experimental period.

3. The conversion of ingested food was represented by the approximate digestibility (AD). The approximate digestibility (AD):

$$\text{AD (\%)} = \frac{\text{IFW} - \text{WEM}}{\text{IFW}} \times 100,$$

where: the key parameters under consideration included: AD – approximate digestibility, IFW – ingested food weight, and WEM – weight of excreted matter.

4. The efficiency of conversion of ingested food (ECI):

$$\text{ECI (\%)} = \frac{\text{WGR}}{\text{IFW}} \times 100,$$

where: ECI – the efficiency of conversion of ingested food, WGR – weight gain rate, and IFW – ingested food weight.

5. The efficiency of conversion of digested food (ECD):

$$\text{ECD (\%)} = \frac{\text{WGR}}{\text{IFW} - \text{FMW}} \times 100,$$

where: ECD – the efficiency of conversion of digested food, WGR – weight gain rate, IFW – ingested food weight, and FMW – fecal matter weight.

6. The assimilation ratio (AR):

$$\text{AR (\%)} = \text{RCR} \times \text{AD},$$

where: AR – the assimilation ratio, RCR – relative consumption rate, and AD – approximate digestibility.

Experimental studies in a greenhouse environment

Greenhouse experimental design

Experiments were conducted in the greenhouse facilities located within the biological experiment garden of Mien Đong Co. Ltd., situated in Tan Hiep commune, Di An District, Binh Duong Province, Vietnam. The cultivation of *B. juncea* seedlings was undertaken in plastic pots (diameter 30 cm, height 25 cm) utilizing a diverse fertilizer blend. This blend constituted a comprehensive array of distinct elements, including extended-release fertilizers with a nutrient delivery duration spanning 3–9 months, iron nutrients, soil moisture-retaining agents, mineral components, and soil additives. The primary aim was to orchestrate a synergistic integration of essential nutrients and supportive agents, facilitating optimal growth conditions for the *B. juncea* plants. The fertilizer mixture was composed of composted bark, Osmocote Plus 8-9 months (2 kg), Osmocote Plus 3–4 months (1 kg), Nutricote 7 months (2 kg), coated iron (1.3 kg), SaturAid (1.2 kg), Polimite (1.2 kg), and Osmoform (1.3 kg). The plants underwent regular irrigation, and upon reaching the age of 7 weeks, they were integrated into the experimental setup. Leaf extracts derived from *M. pachyloba* (EMPE) were employed, exhibiting diverse concentrations ranging from 2, 4, 6, 8, to 10% (w/v). Serving as a positive control, the application of 100 ppm fipronil 5% SC was implemented, with water being utilized as the negative control. The experimental configuration adhered to a randomized approach incorporating both block design and each treatment method, consisting of three replications (corresponding to three blocks) with eight plants per treatment method. In each pot (corresponding to each treatment method), the spacing between plants was consistently set at 10 cm, with a row-to-row spacing of 15 cm, creating optimal growth conditions. The deliberate distances of 50 and 75 cm between treatments (within each experimental model) and research blocks (corresponding to each replicate model) were methodically established, ensuring a systematic arrangement to meet the study's specific objectives. Each plant, hosting seven third-instar *P. xylostella* larvae, was sourced from controlled laboratory cultivation. Subsequently, a 50 ml plastic spray bottle was employed to administer EMPE, fipronil, and water to the plants, ensuring an even application of approximately 20 ml of the solution per plant (Purwatiningsih *et al.* 2012).

Mortality rate of larvae

The number of surviving larvae was counted on days "0" and 5, 10, 15 of the experimental process. The larval mortality rate (MRL) was calculated based on the number of live larvae before and after treatment using the formula:

$$\text{MRL (\%)} = \frac{\text{LAT}}{\text{LBT}} \times 100 \quad \text{(Purwatiningsih } et\ al. \text{ 2012)},$$

where: MRL – the larval mortality rate, LAT – the number of larvae alive after treatment, and LBT – the number of larvae alive before treatment.

Efficacy of Millettia pachyloba leaf extract in controlling *Plutella xylostella* larvae

Monitoring and quantification of larval populations in each pot were conducted both before treatment initiation and post-treatment. The efficacy (H%) in eradicating *P. xylostella* larvae by EMPE was assessed utilizing the formula delineated by Henderson and Tilton (1955), expressing the effectiveness in the following manner:

$$\text{H (\%)} = (1 - \frac{\text{Ta x Cb}}{\text{Ca} - \text{Tb}}) \times 100,$$

where: H represents the efficacy of the extract/pesticide (%), Ca – denotes the count of surviving individuals in the control after treatment, Cb – signifies the count of surviving individuals in the control before treatment, Ta – indicates the count of surviving individuals after the application of the pesticide/extract, and Tb – corresponds to the count of surviving individuals before the treatment after the application of the pesticide/extract.

Impact assessment of *Plutella xylostella* larvae on *Brassica juncea* plants

The assessment of damage inflicted by *P. xylostella* larvae on *B. juncea* plants involved counting the total number of leaves on each plant and the number of leaves damaged by *P. xylostella* larvae. This procedure was carried out for each *B. juncea* plant within each treatment method. The damage ratio caused by *P. xylostella* larvae on *B. juncea* plants (DRP) was calculated using the following formula:

$$\text{DRP}\,(\%) = \frac{\text{LED}}{\text{NTL}} \times 100 \quad \text{(Sinhouenon } et\ al. \text{ 2019)},$$

where: DRP – the damage ratio caused by *P. xylostella* larvae on *B. juncea* plants, LED – the number of leaves exhibiting damage, NTL – the total number of evaluated leaves.

Statistical analysis

Experimental data from laboratory and greenhouse trials were subjected to statistical scrutiny, employing the One-Way analysis of variance (ANOVA) method for a robust statistical evaluation, unveiling subtle differences that may have otherwise eluded detection. The distinctions between means were assessed utilizing the least significant difference (LSD) test, with a significance level set at $p < 0.05$, adding a layer of precision to our analysis and enabling the identification of meaningful variations in the experimental outcomes.

Results

Screening and quantification of phytochemicals in ethanol extract of *Millettia pachyloba* leaves

The botanical screening of *ethanol extract of M. pachyloba leaves* (EMPE) revealed the presence of various constituents, including alkaloids, tannins, saponins, polyphenols, steroids, terpenoids, and flavonoids, while cardiac glycosides were not detected (Table 2). Quantitative analysis of plant compounds in EMPE indicated a total flavonoid content of 41.64 ± 3.13 mg · 100 g^{-1}, total tannin content of 72.54 ± 4.12 mg · 100 g^{-1}, and total polyphenol content of 25.34 ± 2.21 mg · 100 g^{-1} (Table 3). These values provide detailed information on the nutritional and antioxidant content of EMPE, serving as a crucial foundation for further investigation into potential medicinal properties of this extract.

Experimental investigations in the laboratory environment

Toxicity of larvae

The impact of *M. pachyloba* leaf extract (EMPE) on 1s^t instar *P. xylostella* larvae exhibited significant variation ($p < 0.05$, Fig. 1). Treatment of 1st instar larvae for 24, 48, and 72 hours resulted in a markedly higher mortality rate than the control treated with water ($p < 0.05$). Specifically, at the 72-hour interval, the mortality rate (MR) indices were 53.17 ± 7.34%, 68.89 ± 5.37%, 78.33 ± 7.49%, 85 ± 2.89%, and 91.67 ± 6.22% for EMPE concentrations of 2, 4, 6, 8, and 10% (w/v), respectively, significantly surpassing the water control treatment (20.83 ± 7.72%) ($p < 0.05$). Importantly, the mortality rate of larvae in the $EMPE_{10}$ treatment was comparable to that of the fipronil treatment ($p > 0.05$) at the 24, 48, and 72-hour treatment points. The results indicate that the EMPE had a significant and dose-dependent toxic effect on 1s^t instar *P. xylostella* larvae. Over 72 hours, the mortality rate increased significantly with higher concentrations of EMPE. Notably, the highest concentration ($EMPE_{10}$ treatment) demonstrated a mortality rate comparable to that induced by the commonly used insecticide fipronil. These findings suggest the potential insecticidal efficacy of *M. pachyloba* leaf extract against *P. xylostella* larvae, highlighting its possible application as a biopesticide or as a source of environmentally friendly insect control agents.

Toxicity of pupae

The toxicity results of pupae after prior exposure of larvae to the ethanol leaf extract from *M. pachyloba* (EMPE) are depicted in Fig. 2. Treatment of 1st instar larvae with EMPE for 24, 48, and 72 hours

Table 2. Qualitative screening of phytochemicals in ethanol extract of *Millettia pachyloba* leaves

Phytochemicals	Present in EMPE	Phytochemicals	Present in EMPE
Alkaloids	+	cardiac glycosides	–
Tannins	+	steroids	+
Saponins	+	terpenoids	+
Polyphenols	+	flavonoids	+

Presence of phytochemicals in EMPE: (+) – present and (–) – absent

Table 3. Quantification of flavonoid, alkaloid, and tannin contents in ethanol extract of *Millettia pachyloba* leaves

Sample	Total flavonoid content [mg · 100 g^{-1}]	Total tannin content [mg · 100 g^{-1}]	Total polyphenol content [mg · 100 g^{-1}]
EMPE	41.64 ± 3.13	72.54 ± 4.12	25.34 ± 2.21

Concentration amount (mean ± SD) in mg per 100 g of the extract

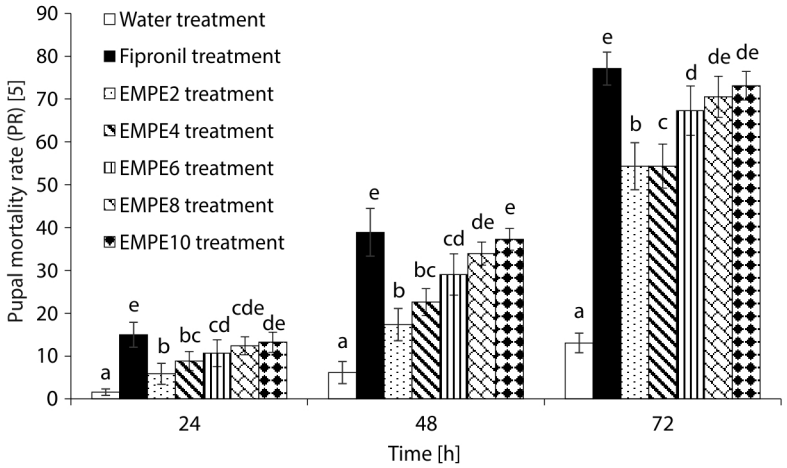

Fig. 1. Toxicity assessment of ethanol extract of *Millettia pachyloba* leaves on *Plutella xylostella* larvae fed treated *Brassica juncea* leaves. The time points of 24, 48, and 72 hours were designated as the treatment durations for 1st instar larvae with EMPE. Values are expressed as Mean ± SD, letters (a, b, c, d, and d) represent the differences between treatments ($p < 0.05$)

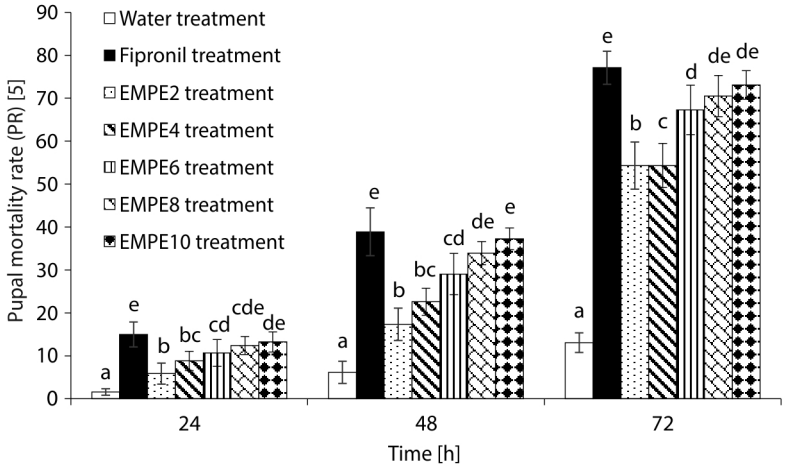

Fig. 2. Toxicity assessment of ethanol extract of *Millettia pachyloba* leaves on *Plutella xylostella* pupae. The time points of 24, 48, and 72 hours were designated as the treatment durations for 1st instar larvae with EMPE. Values are expressed as Mean ± SD, letters (a, b, c, d, and e) represent the differences between treatments ($p < 0.05$)

resulted in a heightened mortality rate in the ensuing pupae compared to the water control treatment ($p < 0.05$). The mortality rates for subsequent pupae were 54.31 ± 5.47%, 54.31 ± 5.14%, 67.26 ± 5.76%, 70.51 ± 4.77%, and 73.08 ± 3.33% following prior larval exposure to 2, 4, 6, 8, and 10% (w/v) EMPE, respectively. The water control treatment exhibited the lowest mortality rate (13.05 ± 2.29%) ($p < 0.05$). Following ingestion of the extract, subsequent pupae exhibited significantly higher mortality rates than the control treatment ($p < 0.05$) at all concentrations. At the highest concentration (10%, w/v), the mortality rate of subsequent pupae was comparable to that induced by fipronil ($p > 0.05$). The results suggest that the EMPE significantly influenced the toxicity of subsequent pupae, following prior exposure of larvae. The observed dose-dependent effect, where higher concentrations of EMPE led to higher mortality rates, highlights the potential insecticidal properties of the extract. Notably, at the highest concentration (10%, w/v), the mortality rate of subsequent pupae was comparable to that induced by the standard insecticide fipronil. These findings underscore the potential of *M. pachyloba* leaf extract as a biopesticide with efficacy against pupae, providing insights for further exploration in pest control applications.

The nutritional indices of the larvae

This investigation explored the impact of *M. pachyloba* ethanol leaf extract (EMPE) on the relative growth rate (RGR) and nutritional indices of *P. xylostella* larvae across their entire post-treatment developmental stage, utilizing various concentrations detailed in Table 4. A significant reduction in computed RGR

(grams of tissue gained per gram of larva per day) was observed throughout the larval stage, particularly at higher EMPE concentrations ($p < 0.05$). Conversely, larvae exposed to water exhibited a notable increase in RGR ($p < 0.05$). The RGR of larvae fed a diet treated with 10% (w/v) EMPE displayed a significant decrease, akin to the impact observed with fipronil ($p < 0.05$). Higher concentrations of EMPE also induced a statistically significant reduction in Relative Consumption Rate (RCR) compared to the water control ($p < 0.05$) (Table 4).

EMPE's impact on the efficiency of conversion of ingested food (ECI) into larval biomass in *P. xylostella* showed a statistically significant difference in experimental trials compared to the water control ($p < 0.05$) (Table 4). The decrease in the conversion efficiency of ingested food was 12.31 ± 0.16% for the $EMPE_{10}$ treatment, comparable to 11.57 ± 1.37% for the fipronil treatment ($p > 0.05$). A parallel pattern was also evident in the efficiency of larvae in converting digested food into growth (ECD) (Table 4).

Results from Table 4 indicate a significant impact of the majority of EMPE treatment methods on food assimilation, as denoted by assimilation ratio (AR) ($p < 0.05$). Additionally, EMPE influenced apparent digestibility (AD), leading to a noteworthy reduction in larvae concentration when treated with EMPE compared to the water control ($p < 0.05$). However, no significant difference was observed in the percentages of AD and AR in the EMPE-treated at a concentration of 10% (w/v) compared to the standard fipronil treatment ($p > 0.05$).

The application of EMPE significantly influenced various key indicators related to the growth and development of *P. xylostella* larvae. The observed reductions in RGR, RCR, ECD, ECI, AD, and AR collectively suggest that EMPE has the potential to impact the growth and development capabilities of these larvae. This implies that EMPE could be considered as a potential agent for application in the management and control of *P. xylostella* based on its biological performance.

Experimental studies in a greenhouse environment

Mortality rate of larvae

The outcomes presented in Table 5 underscore a substantial escalation in the mortality rate of *P. xylostella* larvae, both temporally and across different treatment concentrations. This indicates the efficacy of both EMPE and fipronil in diminishing the population of viable larvae. Significantly discernible variations were observed in treatments involving EMPE

Table 4. Impact of varied concentrations of ethanol leaves extract from *Millettia pachyloba* on consumption, digestion, and growth parameters of *Plutella xylostella* larvae as assessed by nutritional indices

Treatments	RCR [g · day^{-1}]	RGR [g · day^{-1}]	AD [%]	ECI [%]	ECD [%]	AR [%]
Water	1.08 ± 0.08 f	0.38 ± 0.05 e	70.96 ± 2.18 e	21.75 ± 2.75 e	28.15 ± 1.20 d	76.69 ± 7.15 e
Fipronil	0.47 ± 0.07 a	0.11 ± 0.01 a	45.83 ± 1.81 a	11.57 ± 1.37 a	15.52 ± 1.30 a	21.31 ± 2.18 a
$EMPE_2$	0.81 ± 0.06 e	0.31 ± 0.01 d	62.20 ± 4.03 d	17.44 ± 1.16 d	24.54 ± 0.88 c	50.36 ± 4.48 d
$EMPE_4$	0.72 ± 0.07 de	0.27 ± 0.02 c	59.69 ± 4.64 cd	16.61 ± 1.81 cd	22.83 ± 0.71 c	42.86 ± 3.52 c
$EMPE_6$	0.63 ± 0.03 cd	0.23 ± 0.02 c	54.90 ± 3.40 bc	15.42 ± 0.72 bc	20.18 ± 0.80 b	34.55 ± 1.57 b
$EMPE_8$	0.57 ± 0.05 bc	0.19 ± 0.01 b	51.96 ± 1.70 ab	14.03 ± 0.87 ab	18.89 ± 1.02 b	29.57 ± 1.27 b
$EMPE_{10}$	0.49 ± 0.01 ab	0.13 ± 0.01 a	46.81 ± 5.31 a	12.31 ± 0.16 a	16.67 ± 0.79 a	22.94 ± 2.64 a

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatments ($p < 0.05$). RCR – relative consumption rate, RGR – relative growth rate of larvae, AD – the approximate digestibility, ECI – the efficiency of conversion of ingested food, ECD – the efficiency of conversion of digested food, AR – the assimilation ratio (AR)

Table 5. Influence of ethanol extract of *Millettia pachyloba* leaves on the mortality rate of *Plutella xylostella* larvae

Treatments	Mortality rate of larvae (MRL) [%]		
	5 days	10 days	15 days
Water	4.17 ± 1.03 a	11.80 ± 1.01 a	27.47 ± 2.15 a
Fipronil	19.64 ± 1.79 e	37.03 ± 0.71 e	77.63 ± 2.31 e
$EMPE_2$	9.52 ± 1.03 b	26.33 ± 1.45 b	62.52 ± 1.77 b
$EMPE_4$	11.90 ± 1.03 c	29.73 ± 1.05 c	67.34 ± 2.81 bc
$EMPE_6$	13.69 ± 1.03 cd	31.73 ± 1.42 cd	70.69 ± 1.99 cd
$EMPE_8$	15.48 ± 1.03 d	33.11 ± 1.61 d	72.69 ± 4.14 cde
$EMPE_{10}$	18.45 ± 1.03 e	35.78 ± 1.73 e	75.16 ± 6.27 de

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

and fipronil compared to the water control, pointing to the potential impact of these substances in elevating mortality rates. On the 15th day of the treatment regimen, the peak mortality rate reached 75.16 ± 6.27% in the EMPE treatment with a concentration of 10% (w/v), closely resembling the fipronil treatment (77.63 ± 2.31%). This suggests that EMPE may exhibit effectiveness comparable to fipronil in controlling larval survival rates. These findings posit EMPE as a promising avenue for regulating the survival rates of *P. xylostella* larvae, demonstrating efficacy almost on par with the widely-used insecticide fipronil. The increasing significance of EMPE's effectiveness over time and treatment concentration underscores its noteworthy potential in controlling this insect species.

Efficacy of *Millettia pachyloba* leaf extract in controlling *Plutella xylostella* larvae

The effectiveness of *M. pachyloba* leaf extract (EMPE) in managing *P. xylostella* larvae is depicted in Figure 3. The results unequivocally illustrate EMPE's efficacy in restraining the development of *P. xylostella* larvae. The substantial increase in control efficacy following the extract's application is a positive indicator of EMPE's potential to impede larval growth. A notable improvement in control efficacy was evident with escalating concentrations of EMPE. Specifically, from a concentration of 2 to 10% (w/v), control efficacy rose from 24.36% to 53.82%. This emphasizes EMPE as a promising choice for controlling *P. xylostella* larvae, with efficacy proportionally escalating with dosage. In comparison to the water control group, EMPE consistently demonstrated significantly higher control efficacy across all concentrations, with a marked statistical difference observed ($p < 0.05$). The study findings underscored the ability of EMPE to control *P. xylostella* larvae, with efficacy exhibiting an upward trend with increasing treatment concentrations. This highlights the potential of EMPE as an effective pest control measure in a research setting.

Impact assessment of *Plutella xylostella* larvae on *Brassica juncea* plants

The evaluation of the impact of *P. xylostella* larvae on *B. juncea* plants employed the damage ratio percentage (DRP) (Fig. 4). The DRP, indicative of the extent

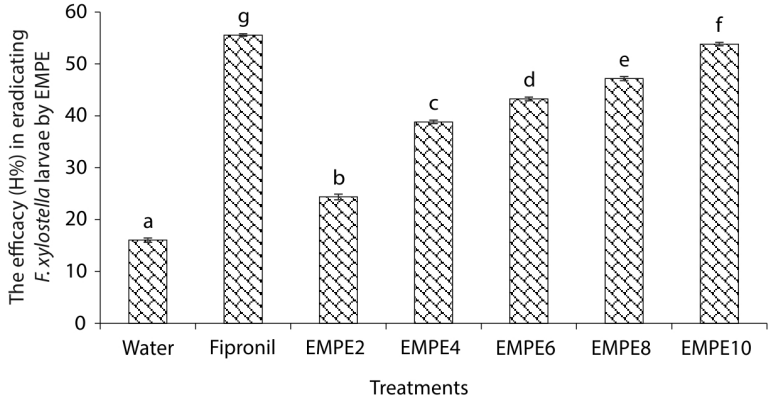

Fig. 3. Efficacy of ethanol extract of *Millettia pachyloba* leaves in controlling *Plutella xylostella* larvae. Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the differences between treatments ($p < 0.05$)

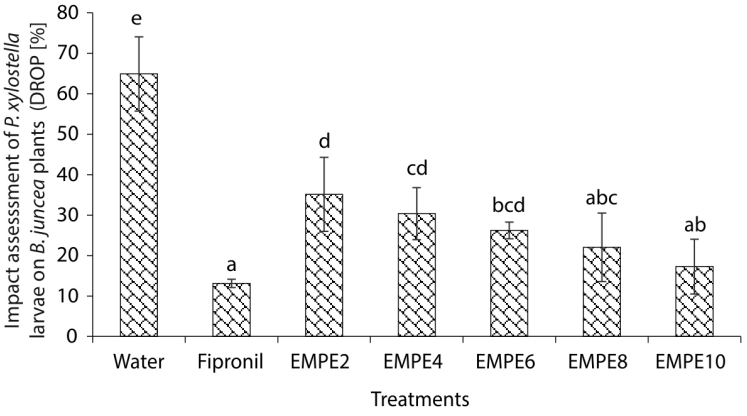

Fig. 4. The damage ratio caused by *Plutella xylostella* larvae on *Brassica juncea* plants. Values are expressed as Mean ± SD, letters (a, b, c, d, and e) represent the differences between treatments ($p < 0.05$)

of damage inflicted by *P. xylostella* larvae on *B. juncea* plants, demonstrated a notable reduction in leaf damage with EMPE treatment ($p < 0.05$). Following treatments with various concentrations of EMPE, the number of leaves damaged by the larvae exhibited an inverse correlation with escalating treatment concentrations. Specifically, at EMPE concentrations of 2, 4, 6, 8, and 10% (w/v), the proportion of damaged leaves per plant was 35.12%, 30.36%, 26.19%, 22.02%, and 17.26%, respectively. A significant difference in DRP was observed between the EMPE-treated and the water control treatment ($p < 0.05$). The study findings indicate that EMPE effectively mitigated the damage caused by *P. xylostella* larvae to *B. juncea*, as evidenced by the reduction in DRP.

Discussion

Plutella xylostella (L.) (Lepidoptera: Plutellidae) stands out as one of the most economically significant insect pests, causing severe damage to cruciferous crops worldwide. Conventional chemical methods have proven ineffective in controlling this pest due to its development of resistance to most available synthetic insecticides. Hence, alternative sources such as plants may serve as a rich reservoir of secondary bioactive molecules with insecticidal properties. Over the past two decades, considerable efforts have been directed toward plant screening to identify their biological activities against *P. xylostella*. Understanding the growth of inhibitory activity in plant compounds may be crucial for sustainable pest management strategies (Rattan and Sharma 2011). In our study, we presented novel findings demonstrating the substantial inhibitory effectiveness of *M. pachyloba* leaf extract against *P. xylostella* larvae, a pest species known to be detrimental to *B. juncea* plants.

Secondary metabolites in plants, encompassing alkaloids, tannins, saponins, polyphenols, steroids, terpenoids, flavonoids, among others, play a crucial role in inherent defense mechanisms against pathogens, insects, and abiotic stress factors. As natural products, these compounds possess biodegradability, lower toxicity to humans, and engage in diverse biological activities (Lyubenova *et al.* 2023). Terpenoids in plants function as insecticidal agents, attract pollinators, and regulate plant hormones (abscisic acid, gibberellin), among other roles. Alkaloids induce toxicity in insects by impacting the nervous system, DNA replication, protein synthesis, and enzyme activity (Lyubenova *et al.* 2023). Both terpenoids and alkaloids emerge as promising candidates for highly effective insecticidal effects due to their ability to inhibit and deter feeding behavior (Rattan 2010). Phenolic compounds exert a direct toxic effect on insects by disrupting their growth and development. Polyphenols, flavonoids, and tannins can generate bitter or unpleasant-tasting substances for *P. xylostella*, inhibiting their feeding ability and directly impacting the cellular structure of *P. xylostella*, causing damage and inhibiting their developmental processes (Rattan 2010). These compounds demonstrate their ability to engage in complex interactions with proteins through intricate mechanisms, encompassing hydrophobic bonding, Van der Waals forces, and hydrophilic interactions. These interactions induce structural alterations and modify the functionality of proteins. Furthermore, polyphenols, flavonoids, and tannins also exert an influence on functional groups within proteins, eliciting changes in spatial structure and activity characteristics (Hayat *et al.* 2020). Furthermore, these compounds possess antioxidant capabilities, hindering cellular pollution processes and inflicting damage on *P. xylostella* cells. They intervene in the hormonal system of *P. xylostella*, inducing alterations in their developmental and reproductive processes. Simultaneously, they may influence the activity of certain vital enzymes within the body of *P. xylostella*, disrupting metabolic processes and biological functions (Rattan 2020). However, in specific scenarios, they possess the ability to inhibit the aggregation of disease-causing proteins (Pengelly 2020). The secondary metabolites present in the leaf extract of *M. pachyloba* exhibit a multifaceted impact on various biological aspects of *P. xylostella*. Additionally, they contribute to diminishing the adaptability of the insect to its environment.

The 1st instar larvae of *P. xylostella*, known as the initial migratory larval stage, are small in size, often forming a compact cluster of recently hatched eggs. They are light white or yellowish in color and are adorned with minute black or red lines and dots. The larvae possess a basic body structure without the intricate characteristics observed in subsequent developmental phases. Their primary dietary source involves consuming plant leaves, resulting in foliage damage and lesion formation. Despite their capability of movement, their motility is generally sluggish, primarily oriented toward foraging for sustenance or relocating to an optimal developmental site. This developmental stage is characterized as non-feeding and notably responsive. *P. xylostella* pupae are found within delicate silk cocoons, typically formed on the lower or outer surface of leaves, with the yellowish cocoons measuring between 7 to 9 mm (Saran and Genç 2021). The eradication mechanism of *P. xylostella* larvae and pupae by *M. pachyloba* extract may involve diverse effects, collectively impacting the control of this insect species. The chemical constituents present in EMPE may give rise to harmful compounds or adversely affect the growth and development of larvae,

such as 8-prenylmilldurone, durmillon, millesianin C, 3,9-dihydroxypterocarp-6a-en, dehydromaackiain, flemichapparin B, (-)-sativin, and others. Antioxidative compounds in EMPE might influence the larvae's antioxidative system, causing cellular damage and reducing survival capabilities (Yan *et al*. 2019). These compounds can exert negative impacts on the growth and development of P. xylostella, including inhibiting metabolic processes and disrupting crucial chemical pathways essential for growth and development. Additionally, they may influence the endocrine system of *P. xylostella*, altering hormone production and affecting its developmental processes. Simultaneously, these compounds also can directly impact the cellular structure of *P. xylostella*, causing damage and reducing cellular functionality (Sharma *et al*. 2014). The extract's secondary metabolites can impact the activity of crucial enzymes in the larvae's body, disrupting metabolic processes and biological functions (Touzout *et al*. 2023). Furthermore, these compounds can directly influence the physiological activities of larval cells, diminishing their survival capabilities (Sangavi and Edward 2017).

The nutritional indices of larvae play a pivotal role in their development and metamorphosis. During the larval stage, substantial growth and development occur, necessitating a diverse range of nutrients for sustenance, including essential proteins, carbohydrates, and fats derived from their diet. The balance and quality of these nutrients profoundly impact larval health and fitness, influencing their transition to the subsequent pupal stage. A meticulous understanding of the nutritional dynamics during the larval phase is crucial for comprehending the life cycle and reproductive success (Dmitriew and Rowe 2011). The relative consumption rate (RCR) offers insights into larvae feeding rates, aiding in understanding nutritional requirements and the interaction between larvae and their food source. The relative growth rate of larvae (RGR) quantifies their growth rate, facilitating an assessment of growth and development under specific experimental conditions. Approximate digestibility (AD) indicates the extent of a larvae's ability to digest and absorb nutrients from their food, which is crucial for evaluating the efficiency of the digestion process. The efficiency of conversion of ingested food (ECI) measures the efficiency of converting ingested food into body biomass, shedding light on how larvae utilize energy from their diet. The efficiency of conversion of digested food (ECD) gauges the efficiency of converting digested food into body biomass, providing information on nutrient utilization. The assimilation ratio (AR) expresses the degree of nutrient absorption from food compared to total intake, serving as a critical indicator of food utilization efficiency. These indices contribute to a comprehensive understanding of nutritional needs, growth capabilities, and larvae interactions with their food environment, laying the foundation for management and control strategies (Medrano and Gall 1976). The current study revealed a significant reduction in key parameters, including RGR, RCR, AD, AR, ECI, and ECD, throughout the entire developmental stage of *P. xylostella* larvae when exposed to EMPE. The pronounced decrease is more evident at higher EMPE concentrations, particularly at elevated levels, suggesting a dose-dependent efficacy of EMPE. Larvae exposed to water exhibited a noteworthy increase in several indices, indicating a substantial impact of the aquatic environment on their development. Results demonstrated a substantial decline in the indices of larvae fed a diet treated with 10% (w/v) EMPE, comparable to the observed effects of fipronil, a commonly used chemical in pest control. Consequently, EMPE has the potential to significantly influence the growth and development of *P. xylostella* larvae, underscoring its viability as a pest control agent.

In the realm of pest control, a high mortality rate is a favorable indicator, signifying the effective reduction of pest survival and potential harm to crops or resources. An increasing mortality rate of *P. xylostella* larvae reflects the impact of treatment factors on larval survival. Thus, larvae mortality rate serves as a crucial metric for evaluating control method efficacy, guiding decisions in pest management, whether in research or agriculture (Bertolaccini *et al.* 2011). This study underscores the effectiveness of both EMPE in decreasing the population of *P. xylostella* larvae, showcasing EMPE's potential as a pest control agent comparable to the chemical pesticide fipronil. A substantial difference was evident in EMPE treatment compared to the water control, indicating a strong influence on larval survival. On the 15th day of treatment, the highest mortality rate occurred in the EMPE treatment at a concentration of 10% (w/v), closely resembling the fipronil treatment. This compelling evidence supports EMPE's capability in controlling larval populations, nearly on par with a commonly used chemical insecticide. These findings affirm EMPE's potential as an effective pest control agent, presenting an alternative or complement to fipronil. The comparable efficacy between EMPE and fipronil is a positive aspect in considering and developing safe and efficient pest control methods.

The extract's efficacy in controlling larvae underscores its potential for inhibiting and managing damage caused by *P. xylostella* larvae, particularly in crops like *B. juncea*. Assessing the extract's effectiveness aims to safeguard and uphold the crop's health, specifically *B. juncea*, against *P. xylostella* larvae damage. This positive outcome suggests that the extract could be considered and applied as a viable option in pest control strategies, providing valuable insights for the research and development of natural and environmentally friendly management methods. Effectively controlling

this pest contributes to minimizing crop damage, creating a more favorable environment for crop growth and production (Iamba and Malapa 2020). The current study revealed a significant enhancement in control efficacy with increasing EMPE concentration, indicating a positive correlation between the extract's concentration and its ability to control *P. xylostella* larvae. The findings emphasize that higher EMPE concentrations resulted in increased control efficacy, thereby improving the ability to inhibit and manage larval development. These results hold important implications for refining and optimizing EMPE dosage within pest control strategies. The outcomes provide essential insights for the development of natural and sustainable pest control methods in agriculture and environmental management.

The damage ratio (DRP) serves as a quantitative measure to assess the impact of *P. xylostella* larvae on *B. juncea* plants, providing valuable insights into the severity of pest-induced damage. This metric is crucial for researchers and agriculturists in evaluating the overall health and vitality of *B. juncea*, with higher DRP values indicating greater damage levels. Utilizing DRP facilitates informed decision-making in pest control, intervention effectiveness assessment, and adjustment of management practices. In scientific research, DRP contributes significantly to understanding the dynamic interaction between pests and plants, playing a pivotal role in developing efficient pest management strategies to minimize damage and optimize plant health (Andrahennadi and Gillott 1998). This study unveiled a noteworthy inverse relationship between the concentration of *M. pachyloba* leaf extract (EMPE) and the damage ratio inflicted by *P. xylostella* larvae on *B. juncea* leaves. As EMPE concentration increased, the damage ratio decreased, indicating the inhibitory effect of EMPE on *P. xylostella* larvae activity. A substantial difference in DRP was evident between EMPE-treated groups and the water control, emphasizing the positive impact of EMPE in reducing crop damage compared to the control treatment. These findings underscore the influence of EMPE on *P. xylostella* larvae in *B. juncea* plants, showcasing its potential in mitigating pest-induced crop damage. The decreasing DRP values with increasing EMPE concentration provide compelling evidence of its effective control capabilities, suggesting EMPE as a viable option in integrated pest management and crop protection strategies.

Conclusions

In the laboratory, treating *P. xylostella* larvae and pupae with ethanol leaf extract of *M. pachyloba* (EMPE) led to a significantly higher mortality rate than the water control. The efficacy of EMPE exhibited a dose-dependent relationship, with notable reductions in larval nutritional indices (RGR, RCR, ECD, ECI, AD, and AR) observed, particularly at higher concentrations. At 10% (w/v) concentration, the impact of EMPE on *P. xylostella* larvae was comparable to that of fipronil. In the greenhouse, EMPE treatment demonstrated significant differences compared to the water control, with peak efficacy observed at 10% (w/v) concentration. Increasing EMPE concentrations resulted in a notable enhancement of control efficacy against larvae and a significant reduction in damage to *B. juncea* leaves. The substantial reduction in larval developmental indices, coupled with high efficacy in greenhouse experiments, positions EMPE as a promising solution for pest management in *B. juncea* crops.

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