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Study on the trapping effects of *Brassica* allelochemicals on *Plutella xylostella* adults

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The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is a major pest of Brassica crops worldwide. This study explores the application of *Brassica* spp. allelochemicals in trapping diamondback moth adults. The effectiveness of eight commercially obtained *Brassica* spp. volatiles has been investigated, including - (Z)-3-hexenyl acetate, cis-3-hexene-1-ol, β-pinene, sabinene, n-heptanal, allyl isothiocyanate, and Brassica non-volatiles - sinigrin and 4-methylsulfinylbutyl glucosinolate. These allelochemicals were tested at varying concentrations (10 to 90 μg/μL) in laboratory and open-screen cage environment trapping experiments. Cis-3-hexene-1-ol, (Z)-3-hexenyl acetate, sabinene, and β-pinene significantly attracted and trapped more female moths than controls; attraction decreased at higher concentrations. A 1:1 blend of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate displayed optimal attraction in lab and open environment trials for the tested blends. Among the colors tested, green-colored containers proved to be the most effective for trapping. While the plant allelochemicals attracted diamondback moths, signs of non-target cabbage loopers were observed on treated plants. Y-tube olfactometer assays revealed that (Z)-3-hexenyl acetate and cis-3-hexene-1-ol were attractive to both male and female moths. These findings demonstrate the potential of *Brassica* spp. allelochemicals, particularly cis-3-hexene-1-ol and (Z)-3-hexenyl acetate, for trapping diamondback moths.

Key words: Lepidoptera, Integrated pest management, Plant-insect interactions, Host plant cues, Insect behaviour.

INTRODUCTION

Brassica spp. plants in the *Brassicaceae* family are globally cultivated and recognized for their nutritional

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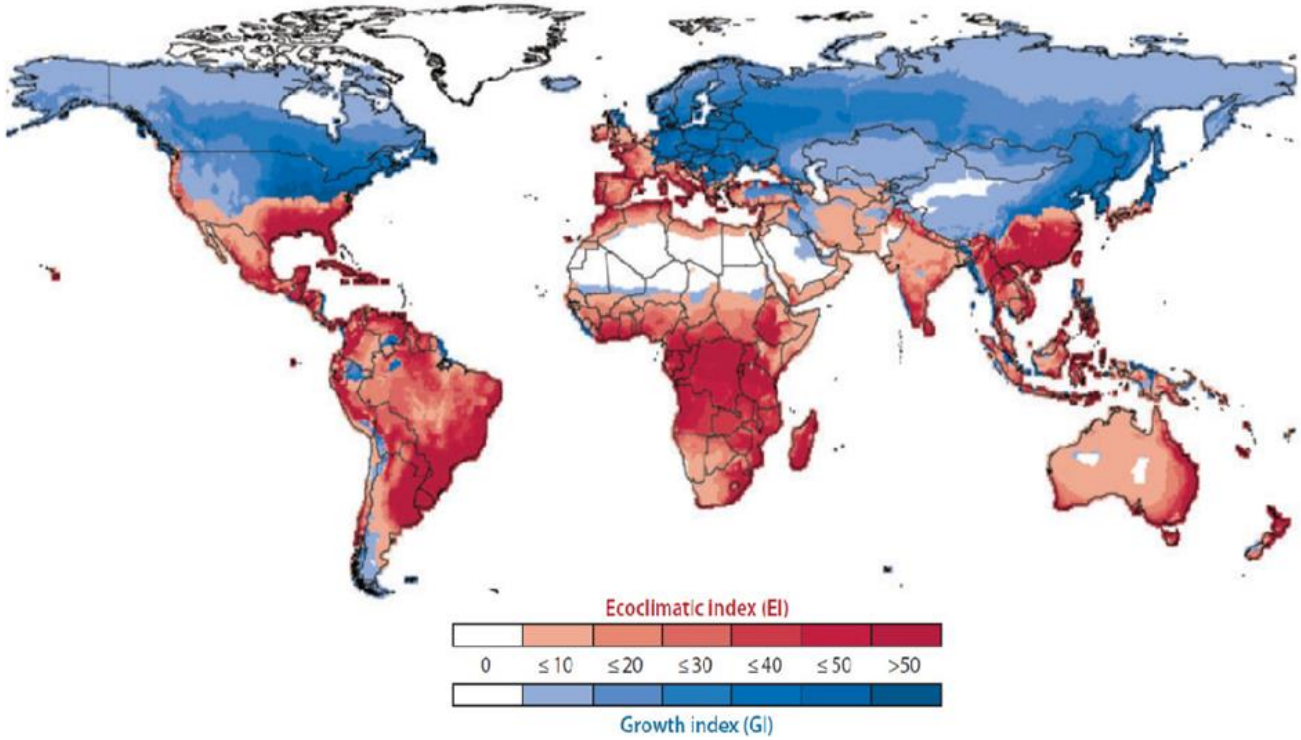

Figure 1. Cosmopolitan distribution of diamondback moth worldwide based on a validated ecoclimatic model. The blue areas indicate regions where the diamondback moth cannot overwinter, while the red regions indicate areas where the diamondback moth's presence is reported year-round.
Source: Furlong et al. (2013).

value and adaptability (Jabeen, 2020). With cross-shaped flowers and over 40 species - including mustard, turnips, cabbage, broccoli, and cauliflower – *Brassica* spp. production and popularity have increased notably in recent years (Zandberg et al., 2022). Beyond culinary applications, Brassica spp. play vital roles in agriculture and food systems due to their health benefits. Rich in essential vitamins, minerals, and fiber (Masarirambi et al., 2020), different *Brassica* spp. vegetables attract both farmers and consumers worldwide.

A key driver of widespread *Brassica* spp. cultivation is their extraordinary adaptability to diverse environments, enabling growth across climate zones and soil types (Biondi et al., 2021). They exhibit resilience to both cold and hot climates, thriving from temperate to tropical regions (Behmer and Joern, 2008), allowing for year-round production to enhance yields (Raza et al., 2019). Additionally, *Brassica* spp. can prosper in sandy, loamy, and clayey soils, permitting agricultural expansion into areas previously unsuitable for conventional crops (Blanco-Canqui et al., 2015). Such flexibility elevates output while diversifying viable farmland. However, despite being an important crop, their production faces a major constraint: the diamondback moth, *Plutella xylostella* (L.) Lepidoptera: *Plutellidae*. This moth is a devastating pest, causing billions in annual management costs due to larval leaf damage that can kill young plants or make older parts unsellable. Diamondback moth infestation can slash cabbage yields by up to 52% (Sarfraz et al., 2005). Recent estimates suggest that the economic impact of damage and management costs associated with diamondback moths reaches approximately US$ 4-5 billion per year (Zalucki et al., 2012).

The diamondback moth stands out in the *Plutellidae* family as a significant global agricultural pest (Jamiołkowska, 2020). This migratory cabbage moth can travel long distances, thought to have originated from Europe or the Mediterranean region of South Africa. Despite uncertain origins, this species has effectively spread worldwide (Figure 1). It is considered the most widely dispersed Lepidoptera, spanning the Americas, Southeast Asia, Australia, and New Zealand (Sarfraz et al., 2005). The diamondback moth possesses a cream-colored band along its dorsal side, forming a distinctive diamond pattern when folded (Htwe et al., 2009). This feature serves as the basis for their common name. When observed from the side, the wing tips exhibit a slight upward curve. Both male and female specimens of this species share similar appearance and dimensions (Liu and Tabashnik, 1997). Adult cabbage moths have a body length of approximately 6 mm, accompanied by a wingspan of about 15 mm. Their forewings, though diminutive, are adorned with lighter front edges, while displaying scattered dark spots (Fu et al., 2022).*Brassica* spp. plants in the *Brassicaceae* family are globally cultivated and recognized for their nutritional value and adaptability (Jabeen, 2020). With cross-shaped flowers and over 40 species - including mustard, turnips, cabbage, broccoli, and cauliflower – *Brassica* spp. production and popularity have increased notably in recent years (Zandberg et al., 2022). Beyond culinary applications, Brassica spp. play vital roles in agriculture and food systems due to their health benefits. Rich in essential vitamins, minerals, and fiber (Masarirambi et al., 2020), different *Brassica* spp. vegetables attract both farmers and consumers worldwide.

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MATERIALS AND METHODS

Materials

Plants

The study followed a standard protocol for cultivating cabbage seedlings (*Brassica* rapa subsp. *pekinensis*). The seedling cultivated in pots and nourished with urea (N ≥ 46%) at the rate of 3g per week to boost growth until they reached 5 weeks of age to allow for the development of fully expanded leaves. These seedlings were a mid-maturing first-generation hybrid variety, purchased from Taishi Lvshuo Seed Industry Technology Company Limited in Xingtai City, Hebei province, China.

Insects

Diamondback moth multiplication followed a modified version of Nguyen's protocol (2014). Larvae were sourced from the Institute of Insect Sciences, College of Agriculture and Biotechnology at Zhejiang University. These larvae were reared in Tupperware® containers at the Urban Entomology laboratory of Zhejiang University in insect rearing sleeve cages at a temperature of 26 ± 2°C, 50-60% relative humidity (RH), and a 16:8 light-dark (L:D) cycle.

Upon adult emergence, diamondback moths were sexed, released in mesh cages (60 × 60 × 60 cm) in mixed-sex groups to allow mating under the same rearing conditions (26 ± 2°C, 50-60% RH, 16:8 L:D cycle) used for larval development. Adults had continuous access to 10% sucrose solution on cotton wicks as a carbohydrate food source. Fresh cabbage leaves were provided daily as an oviposition substrate and replaced after egg collection. Upon hatching, 1st instar larvae were moved to small Tupperware® containers and fed commercial artificial feed containing carrageenan purchased from Keyun Bio in Jiyuan City, Henan Province, until pupation.

For all trapping experiments, 1–2-day old mated adult moths of mixed mating status were tested. Moths were transferred to experimental rooms maintained at 26 ± 2°C, 55 ± 5% RH and ambient indoor lighting. Adults were deprived of sucrose solution for 1 hour prior to assay initiation to standardize motivational state. All tested moths were discarded after trials.

Chemicals

Eight plant volatile and non-volatile chemicals with known purity were procured. Cis-3-hexene-1-ol (purity ≥ 98%), and β-pinene (purity ≥ 95%) were obtained from Shanghai Mindel Biochemical Technology Co., Ltd, Shanghai. (Z)-3-hexenyl acetate (purity ≥ 95%) was acquired from Hangzhou Bangyi Chemical Co., Ltd, Zhejiang. Sabinene (purity ≥ 75%) was purchased from Jiangsu Aikon Biopharmaceutical R&D Co., Ltd, Jiangsu. Allyl isothiocyanate (purity ≥ 94%) was sourced from Alfa Aesar (China) Chemical, Shanghai. N-heptanal (purity ≥ 97%) was secured from Anhui Zesheng Technology, Anhui. Sinigrin (purity ≥ 99%) and 4-methylsulfinylbutyl glucosinolate (purity ≥ 98%) were obtained from Hangzhou Shanghe Biopharm Co., Ltd, Zhejiang.The selection of the 8 plant allelochemicals used in the trapping experiments was based on previous research that identified these eight chemicals as among the most abundant compounds emitted by *Brassica* spp. For instance, cis-3-hexen-1-ol and (Z)-3-hexenyl acetate are abundant green leaf alcohols and acetates emitted by wounded *Brassica* spp.

foliage (Tholl et al., 2006). Meanwhile, β-pinene and sabinene constitute principal monoterpene products of dual biosynthetic pathways in *Brassica* spp. (Chen et al., 2011). The isothiocyanate allyl isothiocyanate was selected, as it arises from the myrosinase-Mediated breakdown of the precursor glucosinolate sinigrin, abundant in B. *rapa* subsp. *pekinensis* tissues (Wittstock et al., 2004). Additionally, 4-methylsulfinylbutyl glucosinolate, another dominant glucosinolate found in *Brassica* spp. that can degrade into a range of volatile isothiocyanate products were procured (Hanschen et al., 2012).

Test sites

The experiments were conducted during the summer of 2022/23 at the Urban Entomology Laboratory and open screen cage environmental conditions within the College of Agriculture and Biotechnology, Zhejiang University - Zijigang campus, located in Hangzhou, Zhejiang Province (30.30° N, 120.10° E).

Methods

Experimental design

All experiments, both in the laboratory and in the open screen cage environmental conditions, except the Y-tube olfactometer test, employed a Randomized Complete Block Design (RCBD) to ensure sound experimental design and reduce potential biases. In the laboratory setting, spatial variation in light intensity and humidity were identified as potential sources of bias that could influence the response of the diamondback moths to the treatments. To control for this variation, the laboratory was divided into homogeneous blocks based on light intensity and humidity readings, and the treatments were randomly assigned within each block. Three independent replicates were utilized in the experiments, enabling statistical analysis and enhancing the credibility of the findings.

Y-tube olfactometer test

The study followed a standardized Y-tube olfactometer assay protocol (Aak et al., 2010; Geier et al., 1999; Ruschioni et al., 2015; Turlings et al., 2004) to investigate the exclusive attraction of plant allelochemicals to diamondback moths, without any visual cues, under ambient indoor lighting. The olfactometer featured two 15 cm long arms connected to a central chamber, with a stem of at least 15 cm in length and 2.5 cm in diameter. The aim was to understand how specific semiochemicals influenced diamondback moths' olfactory preferences shedding light on insect-plant interactions.

Air was filtered through an active charcoal filter and directed through jars containing filter papers soaked in 0.1 g of the commercially purchased semiochemical compounds (in 10 ml of acetone) and then the filter paper was air-dried for 3 min before being introduced into the jars. Cleaned air with the semiochemical compounds was directed into one arm of the Y-tube olfactometer, while the other arm received air passed through a jar with acetone-soaked filter paper as a control.

For each chemical being assessed, 20 (day-old) moths (10 males and 10 females) were individually introduced into the Y-tube at the stem's beginning. Moths were observed and their preferences recorded within the first 10 minutes, but only if they travelled more than 5 cm into the chosen arm. To minimize directional bias, the positions of the arms with treatment scents and controls were switched after every 5 individual insect evaluations. Moths that remained idle for 10 minutes without making a choice were excluded from the results.

After only one use, each stimulus and control filter paper was swapped out with fresh ones for a different individual. Due to nocturnal activity of the adults, all experiments were carried out between 19:00 and 23:00. And three replicates were performed with20 moths for each treatment interval.

Effectiveness of different kinds of allelochemicals to trap adult diamondback moths under laboratory conditions

Eight chemicals were precisely prepared in the lab at various concentrations using acetone as the solvent: Cis-3-hexene-1-ol, β-pinene, (Z)-3-hexenyl acetate, Sabinene, Allyl isothiocyanate, N-heptanal, Sinigrin, and 4-methylsulfinylbutyl glucosinolate were made at concentrations of 10, 30, 50, 70, and 90 μg/μL and each concentration had three replicates. Allelochemicals concentrations ranging from 10 to 90 μg/μL, increasing in 20 μg/μL increments, were chosen deliberately based on earlier studies on optimal allelochemicals release rates thereby standardizing the concentration gaps to minimize potential bias toward any part of the response curve. Lower release rates of 10-30 μg/μL are consistent with baseline volatile emissions from intact *Brassica* plants (Ninkovic et al., 2001). The 50 μg/μL limit corresponds to plant volatile emission levels in *Brassica* crops caused by larval feeding damage (Peng *et al,* 1999). Finally, the 70 and 90 μg/μL concentrations provide supranatural release rates that can be used to test whether greater plant volatile volumes improve trap catch. This range of release rates from sub-damage to supra-damage aids in the construction of a concentration-response curve that quantifies how trap efficiency improves with plant volatile density.

The prepared chemical solutions were dispensed into 15 mL plastic tubes, filling each tube to the 10 mL mark. A 1 mm hole was drilled in the cap of each tube to allow gradual release of the volatile chemicals at doses of 0.1, 0.3, 0.5, 0.7 and 0.9 g (dissolved in 10 ml of acetone). Prior to use, tubes were left uncapped for 10 minutes to allow the acetone solvent to evaporate. Clear plastic containers (20 cm diameter, 10 cm depth) were used as traps, with each container filled halfway with water containing a 0.1% diluted detergent solution. A stand was positioned at the centre of each container to hold one chemical release tube. A potted cabbage plant was positioned 5 cm adjacent to each trap. While the traps themselves contained the test chemicals, having a live *Brassica* spp. host plant nearby was intended to simulate a more naturalistic foraging context for the moths. Treatments were placed at 50 cm apart and replicates were at 100 cm from each other.

The experiment started at 06:00, which was the beginning of the observation period. The testing environment received 600 2-day old adult diamondback moths, maintaining a 1:1 male-to-female ratio. The number of adult diamondback moths caught in each trap was then counted, with data being recorded every 2 h. The experiment was run for a total of 48 h and was repeated three times.

Effectiveness of chemical mixtures to trap adult diamondback moths both in laboratory and open screen cage environment settings

Following the initial experiment, a comprehensive data analysis was undertaken. This analysis unveiled four specific chemicals that outperformed the other test substances. Subsequently, the efficacy of chemical blends incorporating these four superior chemicals was set to be examined in a second experiment. This subsequent experiment aimed to meticulously assess the trapping performance of their blends. This evaluation encompassed both controlled laboratory conditions and open screen cage environmental settings.

The initial experiment's results prompted the selection of four specific chemicals for further investigation. These chemicals, identified as cis-3-hexene-1-ol (purity ≥ 98%), (Z)-3-hexenyl acetate (purity ≥ 95%), sabinene (purity ≥ 75%), and 4-methylsulfinylbutyl glucosinolate (purity ≥ 98%), were labelled as Chemical A, Chemical B, Chemical C, and Chemical D, respectively. To create various chemical blends, these chemicals were diluted to a concentration of10 μg/μL using acetone, maintaining a 1:1 ratio for all test chemicals. The combinations were as follows: A + B, C + D, A + C, A + D, B + C, B + D, and A + B + C + D and each blend had three replicates. A trap with solvent-only was added as a control to isolate effects of the chemical lures themselves in the blend experiments.

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The experiment commenced at 06:00, with the observation period beginning an hour later. In each testing environment (both laboratory and open screen cage conditions), 150 3-day-old adult diamondback moths were introduced, maintaining a 1:1 ratio of males to females. The count of adult diamondback moths attracted and captured in each trap was then recorded at two-hour intervals. To facilitate comprehensive data collection, the experiment continued for 48 hours, ensuring an extended observation period. Additionally, the experiment was repeated three times to enhance reliability and validate the results.

Effect of plant chemicals in different coloured containers to trap the adults of diamondback moth in the open environment

The primary objective of this experiment was to evaluate if trap colour influences the efficacy of the superior cis-3-hexen-1-ol + (Z)-3-hexenyl acetate blend established in the prior assays. The same 10 μg/μL solution of this 2-component lure was used across all trap colours to isolate effects of visual cue changes on trap catch.

The prepared chemical solutions were dispensed into 15 mL plastic tubes, filling each tube to the 10 mL mark. A 1 mm hole was drilled in the cap of each tube to allow gradual release of the volatile chemicals at doses of 0.1 g (dissolved in 10 ml of acetone). Prior to use, tubes were left uncapped for 10 minutes to allow the acetone solvent to evaporate. Clear plastic containers (20 cm diameter, 10 cm depth) were used as traps; with each container filled halfway with water containing a 0.1% diluted detergent solution. A stand was positioned at the centre of each container to hold one chemical release tube. A potted cabbage plant was positioned 5 cm adjacent to each trap to replicate a more natural foraging environment for the moths. Treatments were placed at 100 cm apart and replicates were at 200 cm from each other. The treatments for this experiment encompassed various setups: the chemical blend-in; clear container (control), red container, yellow container, white container, brown container, green container, and blue container. Using transparent traps helped determine the baseline catch of the chemical blend without any colour enhancement.

The experiment commenced at 06:00, marking the onset of the observation phase. 150 2-day old adult diamondback moths were introduced within the testing environment, maintaining an equal male-to-female ratio. The count of adult diamondback moths captured in each trap was recorded at two-hour intervals. To facilitate comprehensive data collection, the experiment spanned 48 h, ensuring extended observation duration and was repeated thrice.

Assessing the potential non-target effects of using allelochemicals traps

The experiment was conducted in an open cabbage field located at the college of agriculture and biotechnology of Zhejiang University. Four chemicals (Z)-3-hexenyl acetate, cis-3-hexene-1-ol, Sabinene, and 4-methylsulfinylbutyl glucosinolate were selected from the top performers in the initial experiments to assess their non-target effects. Each chemical was diluted to a dose of 0.1 g of the chemical in 10ml acetone and dispensed in 15 mL plastic tubes with 1 mm holes in the caps for gradual release.

A stand holding the chemical and control tubes (acetone) was anchored in the soil beside a cabbage plant. The cabbage plant provided some visual cue and surface for non-target insects' verification that oriented to the chemicals. Treatments were placed 100 cm apart and replications at 200 cm apart. The experiment was repeated three times for reliability and run for 96 h.

Data analysis

The gathered data were statistically analysed using SPSS 25.0 software. The analysis used the one-way ANOVA method, followed by Tukey's test to establish the connections between each treatment and the control. The Y-tube bioassay's insect response distribution was analysed through a chi-square test.

RESULTS AND DISCUSSION

Results

Y-tube olfactometer test

Male and female moths exhibited distinct behavioural responses to the allelochemicals emitted by their respective host plants in Y-tube bioassays. Specifically, (Z)-3-hexenyl acetate and cis-3-hexene-1-ol were found to be attractive to both sexes compared to the control, as determined by chi-squire test ($P < 0.05$). Conversely, neither males nor females displayed significant attraction to β-pinene, Sabinene, N-heptanal, 4-methylsulfinylbutyl glucosinolate and Allyl isothiocyanate, based on chi-squire test results ($P > 0.05$) (Figures 2A and B).

Effectiveness of different kinds of allelochemicals to trap adult diamondback moths under laboratory conditions

The study's findings indicate that four chemicals, namely (Z)-3-hexenyl acetate, cis-3-hexene-1-ol, sabinene, and 4-methylsulfinylbutyl glucosinolate, exhibited significant attractiveness to adult diamondback moths. (Z)-3-hexenyl acetate attracted a total of 25.04% of the adult moths, while cis-3-hexene-1-ol attracted 14.5%, sabinene attracted 12.5%, and 4-methylsulfinylbutyl glucosinolate

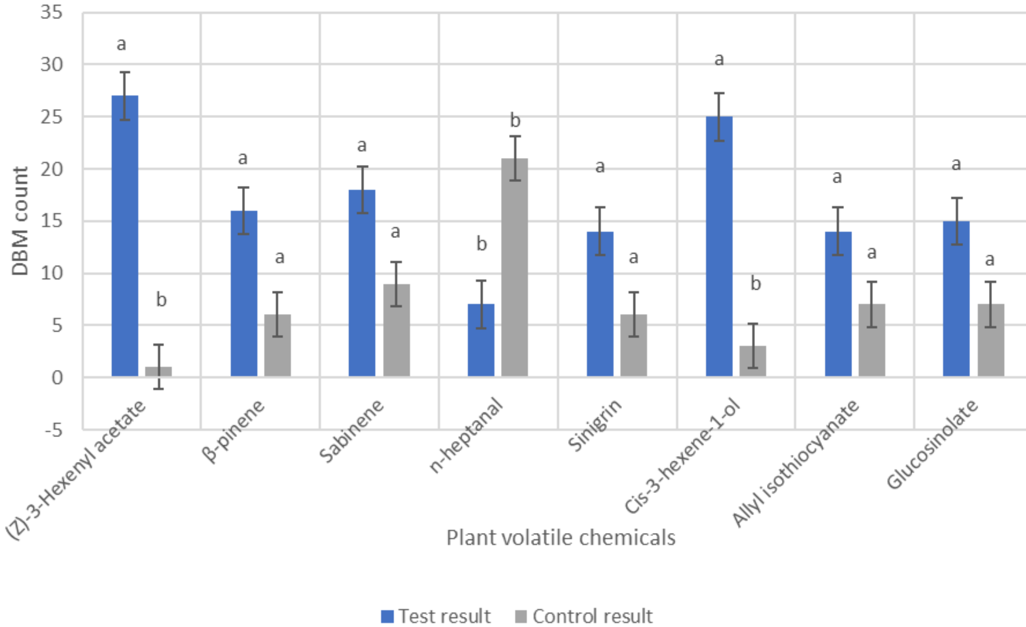

Figure 2a. Y-tube count of behavioural responses of virgin female Plutella xylostella moths to a 10µg/µL dose of plant volatile chemicals. Different lowercase letters indicate significance difference from the control tested with each chemical in each arm based on chi squire test (Chi-sq, 45.645; df, 7)

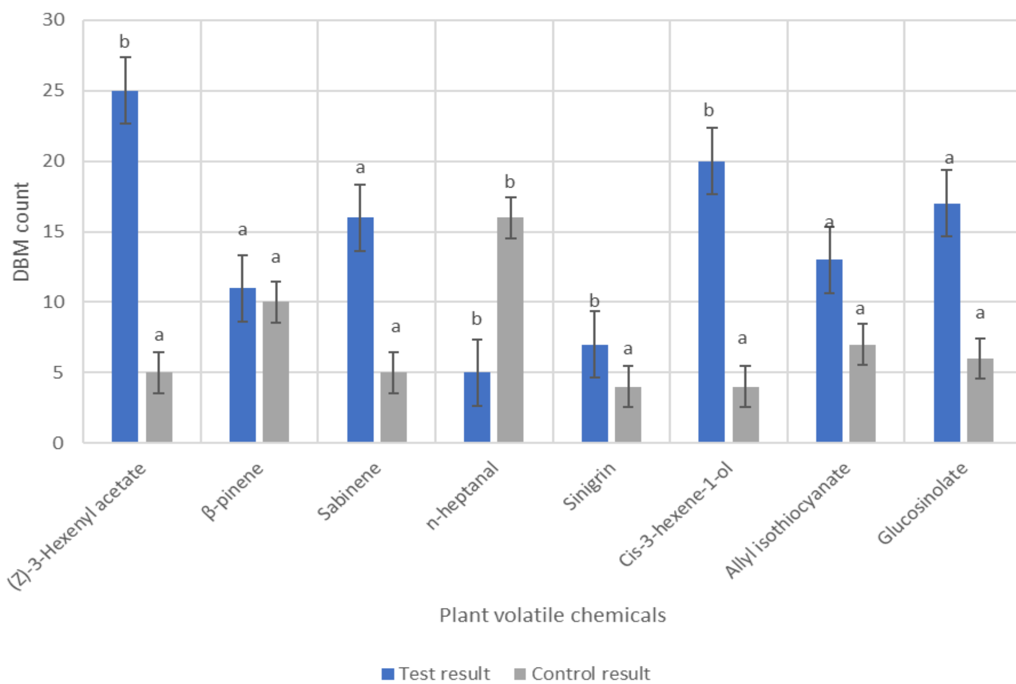

Figure 2b. Y-tube count of behavioural responses of virgin male Plutella xylostella moths to a 10µg/µL dose of plant volatile chemicals. Different lowercase letters indicate significance difference from the control tested with each chemical in each arm based on chi squire test (Chi sq, 41.370; df, 7).

attracted 12.17%. In contrast, the control only attracted 1.67% of the adult moths. These chemicals had a notable attraction and trapping effect on female diamondback moths at the tested concentrations. Statistical analysis confirmed that female diamondback moths were significantly more attracted to these chemicals than the control group ($P \leq 0.01$). Furthermore, the analysis revealed that as concentrations increased, the chemicals' efficacy in attracting moths decreased. However, there were no significant differences in the attraction of male diamondback moths to these chemicals at the tested concentrations ($P > 0.05$) (Table 1).

Conversely, the remaining four chemicals, β-pinene, Sinigrin, N-heptanal, and Allyl isothiocyanate, did not have a significant effect on attracting or trapping diamondback moths at the tested concentrations. They attracted 4.33, 3.67, 3.3, and 3.17% adults, respectively, compared to the total of 1.67% attracted by the control. These chemicals were not found to be attractive to either female or male diamondback moths at the tested concentrations (Table 1).

Effectiveness of chemical mixtures to trap adult diamondback moths both in the laboratory and open environmental settings

Under laboratory conditions, the blend of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate showcased notable attraction to the diamondback moth. This blend attracted a total of 20.6% adults, compared to the 5.3% captivated by the control. Meanwhile, Sabinene + 4-methylsulfinylbutyl glucosinolate attracted 8%, cis-3-hexene-1-ol + Sabinene attracted 5.3%, cis-3-hexene-1-ol + 4-methylsulfinylbutyl glucosinolate enthralled 8%, (Z)-3-hexenyl acetate + Sabinene attracted 8.6%, cis-3-hexene-1-ol + 4-methylsulfinylbutyl glucosinolate attracted 10.6%, and cis-3-hexene-1-ol and (Z)-3-hexenyl acetate + Sabinene + 4-methylsulfinylbutyl glucosinolate collectively attracted 4.6%.

Remarkably, the blend of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate exhibited significant attraction towards female diamondback moth adults in comparison to the control. Nevertheless, in the laboratory setting, there was no notable difference in the attraction of male diamondback moths between the chemical blends and the control ($P \leq 0.01$) (Table 2).

Upon conducting experiments in the open screen cage environment, it was evident that the blend of Cis-3-hexene-1-ol and (Z)-3-hexenyl acetate attracted a notably higher number of diamondback moth adults (both males and females) when compared to both the control and other chemical blends in the open environment setting ($P \leq 0.01$) (Table 3).

In the open screen cage environment, the blend of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate demonstrated robust attraction to the diamondback moth, engaging attracted 25.3%, in contrast to the control's 4% of the adults. Similarly, Sabinene + 4-methylsulfinylbutyl glucosinolate attracted 6%, cis-3-hexene-1-ol + Sabinene enticed 3.3%, cis-3-hexene-1-ol + 4-methylsulfinylbutyl glucosinolate enticed 10%, (Z)-3-hexenyl acetate + Sabinene attracted 4.6%, cis-3-hexene-1-ol + 4-methylsulfinylbutyl glucosinolate attracted 6.6%, and the combination of cis-3-hexene-1-ol, (Z)-3-hexenyl acetate, Sabinene, and 4-methylsulfinylbutyl glucosinolate collectively enthralled 6% (Table 3).

Effect of plant chemicals in different colour containers to trap the adults of diamondback moth in the open environment

When compared to the control group, it was evident that the green container displayed the most pronounced level of attraction, drawing 20% of the adults. Following closely, the yellow container attracted 15.6% while the red container exhibited a moderate level of attraction at 15.6% ($P \leq 0.01$). Notably, no significant distinction in attraction among various colours was observed within the male diamondback moth sample population ($P > 0.05$) as compared to the number of males attracted by the control (Table 4).

Assessing the potential non-target effects of using allelochemicals traps

The experiment revealed that the allelochemicals not only attracted the diamondback moth but also had an impact on attracting additional pests. During the course of the experiment, signs of cabbage looper (*Trichoplusia ni*) herbivory were observed on cabbage plants treated with (Z)-3-hexenyl acetate and cis-3-hexene-1-ol, in contrast to the control. Over the 4-day assessment period, an average of 0.11 ± 0.3 cabbage looper larvae were discovered on (Z)-3-hexenyl acetate treated plants, significantly higher than the 0 larvae found on control plants. Cis-3-hexene-1-ol treated plants contained 0.7 ± 0.2 larvae, also significantly more than controls.

These signs included larvae feeding on the underside of leaves, leaves showing small irregular holes, leaves curling up, and the presence of greenish-brown excrement pellets at the bases of the leaves. All observations were natural oviposition by cabbage loopers.

Discussion

Y-tube olfactometer test

The findings of this study's Y-tube bioassays reveal intriguing and gender-specific behavioural responses of male and female moths to allelochemicals emitted by their respective host plants. The distinct behavioural responses aligned with sexual dimorphism documented in other Lepidopteran species (Landolt and Phillips, 1997), carrying implications for ecological interactions and potential pest management strategies. This divergence underscores the importance of investigating

Table 1. Percentage of captured Plutella xylostella (Percentage ± SD, n = 600) per plant volatile chemical trap in lab trapping experiments.

Treatment	Concentration	Male	Female	Total
Control	-	2.00 ± 0.34[d]	1.33 ± 0.30[d]	1.67 ± 0.44[d]
(Z)-3-Hex	10 μg/μL	8.00 ± 0.65[c]	13.67 ± 0.82[c]	10.83 ± 1.23[c]
	30 μg/μL	5.00 ± 0.52[d]	7.00 ± 0.71[d]	6.00 ± 0.86[a]
	50 μg/μL	2.33 ± 0.31[d]	5.67 ± 0.64[d]	4.00 ± 0.82[d]
	70 μg/μL	3.00 ± 0.39[d]	4.67 ± 1.37[d]	3.83 ± 1.40[d]
	90 μg/μL	0.33 ± 0.12[d]	1.33 ± 0.30[d]	0.83 ± 0.32[d]
β-Pin	10 μg/μL	1.67 ± 0.27[d]	1.33 ± 0.24[d]	1.50 ± 0.35[d]
	30 μg/μL	0.00 ± 0.00[d]	0.33 ± 0.12[d]	0.17 ± 0.12[d]
	50 μg/μL	0.33 ± 0.12[d]	0.00 ± 0.00[d]	0.17 ± 0.12[d]
	70 μg/μL	1.00 ± 0.27[d]	1.67 ± 0.32[d]	1.33 ± 0.41[d]
	90 μg/μL	1.33 ± 0.30[d]	1.00 ± 0.21[d]	1.17 ± 0.36[d]
Sab	10 μg/μL	4.00 ± 0.46[d]	8.00 ± 0.69[a]	6.00 ± 0.88[a]
	30 μg/μL	2.33 ± 0.31[d]	4.33 ± 0.47[d]	3.33 ± 0.61[d]
	50 μg/μL	1.00 ± 0.21[d]	1.00 ± 0.21[d]	1.00 ± 0.29[d]
	70 μg/μL	1.33 ± 0.30[d]	0.67 ± 0.17[d]	1.00 ± 0.34[d]
	90 μg/μL	1.33 ± 0.30[d]	1.00 ± 0.21[d]	1.17 ± 0.36[d]
N-Hep	10 μg/μL	0.33 ± 0.98[d]	0.67 ± 0.25[d]	0.50 ± 0.27[d]
	30 μg/μL	1.33 ± 0.30[d]	1.00 ± 0.21[d]	1.17 ± 0.36[d]
	50 μg/μL	0.33 ± 0.12[d]	1.00 ± 0.27[d]	0.67 ± 0.30[d]
	70 μg/μL	0.33 ± 0.12[d]	0.00 ± 0.00[d]	0.17 ± 0.12[d]
	90 μg/μL	1.00 ± 0.21[d]	0.67 ± 0.17[d]	0.83 ± 0.27[d]
Sin	10 μg/μL	2.00 ± 0.34[d]	2.00 ± 0.29[d]	2.00 ± 0.55[d]
	30 μg/μL	0.33 ± 0.12[d]	0.33 ± 0.12[d]	0.33 ± 0.17[d]
	50 μg/μL	0.00 ± 0.00[d]	0.33 ± 0.12[d]	0.17 ± 0.12[d]
	70 μg/μL	0.67 ± 0.17[d]	0.00 ± 0.00[d]	0.33 ± 0.17[d]
	90 μg/μL	0.33 ± 0.12[d]	1.33 ± 0.35[d]	0.83 ± 0.36[d]
Cis-3-hex	10 μg/μL	5.00 ± 0.55[d]	9.33 ± 0.75[c]	7.17 ± 1.09[c]
	30 μg/μL	3.00 ± 0.39[d]	5.67 ± 0.62[d]	4.33 ± 0.78[a]
	50 μg/μL	1.33 ± 0.30[d]	1.67 ± 0.32[d]	1.50 ± 0.49[d]
	70 μg/μL	1.00 ± 0.21[d]	1.00 ± 0.21[d]	1.00 ± 0.29[d]
	90 μg/μL	0.67 ± 0.17[d]	0.33 ± 0.12[d]	0.50 ± 0.21[d]
Allyl iso	10 μg/μL	3.00 ± 0.39[d]	0.33 ± 0.12[d]	1.67 ± 0.44[d]
	30 μg/μL	0.00 ± 0.00[d]	0.67 ± 0.25[d]	0.33 ± 0.25[d]
	50 μg/μL	0.33 ± 0.12[d]	0.67 ± 0.25[d]	0.50 ± 0.27[d]
	70 μg/μL	0.00 ± 0.00[d]	1.33 ± 0.30[d]	0.67 ± 0.30[d]
	90 μg/μL	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]
Gluc	10 μg/μL	5.00 ± 0.52[d]	8.67 ± 0.68[b]	6.83 ± 0.94[c]
	30 μg/μL	3.33 ± 0.36[d]	5.33 ± 0.61[d]	4.33 ± 0.74[d]
	50 μg/μL	0.00 ± 0.00[d]	0.33 ± 0.12[d]	0.17 ± 0.12[d]
	70 μg/μL	0.33 ± 0.12[d]	1.00 ± 0.21[d]	0.67 ± 0.24[d]
	90 μg/μL	0.33 ± 0.12[d]	0.00 ± 0.00[d]	0.17 ± 0.12[d]

cis-3-hex, cis-3-hexene-1-ol; (Z)-3-hex, (Z)-3-hexenyl acetate; Sin, Sinigrin; sab, sabinene; N-Hep, N-Heptanal; Allyl iso, Allyl isothiocyanate; Gluc, 4-methylsulfinylbutyl glucosinolate. Different lowercase letters indicate significance difference based on one-way ANOVA followed by Tukey's test ($P < 0.05$).

Table 2. Percentage of captured Plutella xylostella (Percentage ± SD, n = 150) attracted per chemical blend trap in lab trapping experiments.

Treatment	Male	Female	Total
Control	5.30 ± 0.24^c	5.30 ± 0.24^c	5.30 ± 0.31^c
Cis-3-hex + (Z)-3-Hex	10.60 ± 0.33^c	30.60 ± 0.71^b	20.60 ± 0.86^a
Sab + Gluc	10.60 ± 0.33^c	5.30 ± 0.24^c	8.00 ± 0.49^c
Cis-3-hex + Sab	4.00 ± 0.21^c	6.60 ± 0.27^c	5.30 ± 0.41^c
Cis-3-hex + Gluc	6.60 ± 0.32^c	9.30 ± 0.43^c	8.00 ± 0.52^c
(Z)-3-Hex + Sab	12.00 ± 0.39^c	5.30 ± 0.24^c	8.60 ± 0.47^c
(Z)-3-Hex + Gluc	8.00 ± 0.29^c	13.30 ± 0.44^c	10.00 ± 0.63^c
(Z)-3-Hex + Gluc + Cis-3-hex + Sab	8.00 ± 0.29^c	1.30 ± 0.12^c	4.60 ± 0.36^c

cis-3-hex, cis-3-hexene-1-ol; (Z)-3-hex, (Z)-3-hexenyl acetate; sab, sabinene; Gluc, 4-methylsulfinylbutyl glucosinolate. Asterisks indicate means difference significance from the control: Different lowercase letters indicate significance difference based on one-way ANOVA followed by Tukey's test (P < 0.05).

Table 3. Percentage of captured Plutella xylostella (Percentage ± SD, n = 150) attracted per each of the chemical blend trap in open screen cage environment trapping experiments.

Treatments	Males	Females	Total
Control	4.00 ± 0.21^c	4.00 ± 0.21^c	4.00 ± 0.29^c
Cis-3-hex + (Z)-3-Hex	18.60 ± 0.41^a	32.00 ± 0.72^b	25.30 ± 0.96^b
Sab + Gluc	8.00 ± 0.29^c	4.00 ± 0.21^c	6.00 ± 0.43^c
Cis-3-hex + Sab	2.60 ± 0.17^c	4.00 ± 0.21^c	3.30 ± 0.32^c
Cis-3-hex + Gluc	6.60 ± 0.27^c	13.30 ± 0.44^c	10.00 ± 0.60^c
(Z)-3-Hex + Sab	5.30 ± 0.24^c	4.00 ± 0.21^c	4.60 ± 0.36^c
(Z)-3-Hex + Gluc	4.00 ± 0.21^c	9.30 ± 0.31^c	6.60 ± 0.36^c
(Z)-3-Hex + Gluc + Cis-3-hex + Sab	5.30 ± 0.24^c	6.60 ± 0.32^c	6.00 ± 0.43^c

cis-3-hex, cis-3-hexene-1-ol; (Z)-3-Hex, (Z)-3-hexenyl acetate; sab, sabinene; Gluc, 4-methylsulfinylbutyl glucosinolate. Different lowercase letters indicate significance difference based on one-way ANOVA followed by Tukey's test (P < 0.05).

Table 4. Percentage of captured Plutella xylostella (Percentage ± SD, n = 150) attracted per chemical trap in different coloured containers in open screen cage environment trapping experiments.

Treatment	Male	Female	Total
Control	2.60 ± 0.17^c	9.30 ± 0.31^c	6.00 ± 0.39^c
Yellow	8.00 ± 0.34^c	22.60 ± 0.38^c	15.60 ± 0.56^a
Green	12.00 ± 0.35^c	28.00 ± 0.54^a	20.00 ± 0.76^b
Blue	4.00 ± 0.21^c	9.30 ± 0.29^c	6.60 ± 0.43^c
Brown	4.00 ± 0.17^c	5.30 ± 0.24^c	4.60 ± 0.29^c
White	5.30 ± 0.21^c	5.30 ± 0.24^c	5.30 ± 0.40^c
Red	14.60 ± 0.41^c	16.00 ± 0.39^c	15.30 ± 0.71^a

Different lowercase letters indicate significance difference based on one-way ANOVA followed by Tukey's test (P < 0.05).

sex-specific insect responses, as males and females often play distinct roles in foraging, mating, and broader ecological interactions. Understanding these differences can significantly impact insect population dynamics and pest management efforts.

One of the most striking findings from the study is the distinct difference in behavioural responses between male and female moths when exposed to different plant volatile chemicals. Females exhibited a stronger attraction to plant volatiles like (Z)-3-hexenyl acetate and cis-3-hexene-1-ol, which are known to be important cues for host plant recognition and oviposition site selection (Ansebo et al., 2004; Hartlieb and Rembold, 1998). This variation emphasizes the significance of examining insect sex-specific responses since they frequently play separate roles in foraging, mating, and overall ecological interactions (Reddy and Guerrero, 2004). Understanding these distinctions can have a big impact on insect population dynamics and pest management.

The plant volatiles (Z)-3-hexenyl acetate and cis-3-hexene-1-ol were found to be attractive to both male and female moths, suggesting potential roles as chemical cues facilitating host plant recognition and selection. These volatiles may contribute to signalling herbivore feeding damage (Karban, 2020) and glucosinolate breakdown products to specialized *Brassica* spp. feeders (Alan and Renwick, 2002). Further research is needed to confirm the potency and specificity of these chemicals before definitive statements can be made about their roles in moth-host plant communication (Marchioro and Foerster, 2011). While the shared attraction implies possible significance in the moths' feeding and reproductive behaviours, extensive field studies are still required to fully elucidate the ecological function of these plant volatiles. This preliminary research provides a starting point for identifying candidate semiochemicals that warrant more rigorous laboratory and field evaluation regarding their potency and utility in integrated pest management applications.

Effectiveness of different kinds of allelochemicals to trap adult diamondback moths under laboratory conditions

Chemical communication, facilitated by allelochemicals, holds significant importance in various insect activities such as host search, mating, and oviposition (Reddy and Guerrero, 2000). While the initial Y-tube olfactometer assays tested individual allelochemical attraction, this subsequent trapping study purposefully retained all chemicals - even those that failed to demonstrate significant activity when screened in isolation. As (Bruce et al., 2005) discuss, odour perception in phytophagous insects frequently involves combinatorial processing, where the integrated signals from multiple components guide attraction rather than singular compounds in isolation. With *Brassica* spp. plants naturally emitting complex allelochemical bouquets, containing up to eight simultaneously released plant volatile and non-volatiles following tissue damage (Reddy et al., 2002), evaluating mixtures was imperative. Compounds inactive alone may still contribute to sensory integration or have concentration-dependent impacts.

The observed attractiveness of (Z)-3-hexenyl acetate, cis-3-hexene-1-ol and Sabinene specifically to female diamondback moths aligns with prior evidence suggesting possible sex-based differences in odour perception (Uefune et al., 2020). As common 'green note' volatiles released by plants, (Z)-3-hexenyl acetate and cis-3-hexen-1-ol can provide orientation cues to DBM for locating host plants (Reddy et al., 2002) (Renwick et al., 2006). These volatile substances, which were derived from green leaf sources, displayed strong allure and showed how useful they might be for managing and monitoring DBM populations (Renwick et al., 2006). These attractants provide useful insights into population dynamics, the evaluation of pest activity, and the application of targeted control strategies by successfully capturing adult moths. The decreasing efficacy of the four significantly attractive chemicals at higher concentrations underscores the importance of optimal allelochemical blends and ratios in mediating insect host finding, as subtle changes can switch phago-stimulation to deterrence and avoidance (Webster et al., 2010).

Notably, the study revealed gender-specific responses, with females exhibiting a stronger attraction to these plant volatiles compared to males. This finding aligns with the well-documented role of plant volatiles in guiding female insects to suitable oviposition sites and host plants for their offspring's development (Ansebo et al., 2004). The heightened sensitivity of females to these cues likely evolved to maximize reproductive success and ensure optimal host plant selection for egg-laying (Renwick and Chew, 1994; Burghardt et al., 2000).

Although 4-methylsulfinylbutyl glucosinolate is widely considered non-volatile, its unexpected attraction for diamondback moths in this study underscores the intricate reality of insect-plant interactions. This occurrence may be due to moths’ possession of sensitive taste receptors, potentially allowing them to directly detect the compound through contact with the tube's contents (Molina and García-Olmedo, 1997). Secondly, minimal volatilization under specific conditions might have produced airborne traces sufficient for the moths' keen sense of smell. Alternatively, 4-methylsulfinylbutyl glucosinolate might act within a "multimodal language" alongside volatile attractants, guiding moths through combined sensory cues (Mdrup et al., 2010). Lastly, the experiment prompts contemplation of extraneous factors such as light, moisture, or even the material of the tube, inadvertently contributing to the modulation of moth behaviour. Ultimately, these findings highlight the limitations of strictly categorizing chemicals as volatile or non-volatile, urging us to delve deeper into the multifaceted communication strategies employed by insects and plants.

Interestingly, it was also observed that attraction reduced as plant volatile chemical concentrations increased. Lower release rates result in more trap captures and more focused flying behaviours (Reddy and Guerrero, 2000a). It appears that there is an ideal range beyond which extremely high plant volatile chemical levels overwhelm receptors and may cause avoidance (Yang et al., 2004). While there are slight changes in attraction between married and virgin females, which are most likely related to physiological variances, both remain significantly stimulated by essential plant volatile chemicals (Zheng et al., 2023). This demonstrates the DBM olfactory systems' fine-tuned sensitivity to these key plant chemicals.

The limited capacity of β-pinene, allyl isothiocyanate, n-heptanal, and sinigrin to attract diamondback moths suggests modest potential for management applications. However, compounds like (Z)-3-hexenyl acetate and cis-3-hexene-1-ol showed levels of attraction that may have promise for future eco-friendly integrated pest management. While further optimization and field testing are necessary to validate their efficacy in agricultural settings, these findings represent a significant step towards the development of sustainable alternatives for diamondback moth control in *Brassica* spp. agriculture.

Examining morphological and neurological differences between male and female moths could provide insight into the observed sexual dimorphism in odour perception (Andersson et al., 2015). Additionally, evaluating behavioural responses to these compounds under natural field conditions is essential, as the highly artificial bioassays lacked key environmental cues influencing host navigation. Controlled on-farm trials are imperative before the attractants can be validated for agricultural use.

Effectiveness of chemical mixtures to trap adult diamondback moths both in laboratory and open environment settings

In the subsequent phase of the research, the aim was to build upon the insights gleaned from the initial experiment. The results of this experiment revealed a distinct combination that displayed notable appeal to diamondback moths. The attraction of both male and female moths to the blend of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate builds on previous evidence that combining host volatiles can enhance orientation effects beyond individual components (Reddy et al., 2002).

Significantly, the blend of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate not only generated a higher level of attraction in both male and female diamondback moths but also surpassed all other tested blends and the control group. These compelling outcomes affirm this particular blend as a promising attractant for diamondback moths, corroborating earlier findings by Li et al. (2012). In addition to Li et al. (2012), various other studies have underscored that cis-3-hexene-1-ol and (Z)-3-hexenyl acetate wield stronger allure for diamondback moths than either compound alone. For instance, Zhu et al. (2018) identified that this blend outperformed a range of commercial attractants in terms of its appeal to diamondback moths. The consistent alignment of findings across distinct studies lends support to the notion that this blend holds considerable potential for practical pest management applications. While still limited, the open screen cage environment expands upon simplified laboratory assays.

Recent studies have further reinforced the significance of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate in diamondback moth management. For example, Uefune et al. (2020) demonstrated that a blend of these compounds, when used in conjunction with the herbivore-induced plant volatile (HIPV) benzyl cyanide, effectively attracted the diamondback moth's natural enemy, the parasitoid wasp Cotesia vestalis. This finding highlights the potential for using plant volatiles not only for direct pest control but also for enhancing biological control strategies.

The attractiveness of this blend in laboratory settings opens the door to more DBM behavioural research. Extensive research into their response mechanisms and behavioural patterns, when exposed to this attractant blend, can help us understand their ecology and provide insights into more effective pest control strategies. For instance, studying the neurophysiological and molecular basis of how DBM perceives and responds to this blend could lead to the development of novel disruption tactics or even genetically engineered crops that manipulate these chemical cues.

Although three-chemical blends would have allowed for a more thorough investigation of possible antagonistic or synergistic interactions, the choice to concentrate on two- and four-chemical blends was mainly motivated by two factors. First, a more thorough evaluation of these blends was possible, offering insightful information about their individual and combined impacts by ranking the most promising combinations according to the results of the first experiment. Second, considering the practical constraints of time and resources, a more focused approach was opted for to ensure a thorough investigation of the most promising combinations while leaving room for future studies to explore three-chemical blends more comprehensively.

Effect of plant chemicals in different colour containers to trap the adults of diamondback moth in the open environment

In this phase of the research, the focus was on investigating the impact of container color on the attraction and capture of diamondback moths in the open screen cage environment, utilizing a blend of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate as the attractant. The primary objective was to discern how diverse container colors influenced the moths' responsiveness to this attractant blend and which colors proved most efficacious in enticing these pests. While primarily active at night, the moths may still use visual cues to help locate plants and resources. Testing colors could reveal if certain wavelengths are more visible or attractive under low light conditions. The moths may have enough residual vision to distinguish coarser color differences. Testing different colors could therefore reveal if certain wavelengths are more visible and attractive in low light conditions. The implications of this study encompass the formulation of targeted and streamlined strategies for pest management in cruciferous crop systems.

The outcomes of the open environment experiment unveiled intriguing insights into diamondback moth preferences regarding container color. Among the array of container colors examined, green containers exhibited the highest appeal to diamondback moths. These green-colored containers notably attracted moths to a significantly greater extent, serving as preferred sites for capture. This observation aligns remarkably with preceding research by Cho and Lee (2012), offering confirmation of the notion that container color wields considerable influence in drawing diamondback moths. The coherence between these findings and previous investigations underscores the proposition that containers in shades of green are remarkably adept at capturing moths' attention.

Moreover, the findings signify that red-colored containers exhibit a diminished appeal to diamondback moths in comparison to their green counterparts. While they did not surpass the allure of green containers, red containers still generated a substantial level of attraction, rendering them a valuable alternative for pest surveillance and management endeavors. Additionally, while not as potent as green or red containers, yellow containers contributed to the overall attractant effect to a certain degree. Their moderate level of allure underscores their potential usefulness when used in conjunction with other attractant strategies or as supplementary monitoring tools.

These findings are significant because of the practical implications for pest management in cruciferous crop systems. Understanding the color preferences of the moths allows agricultural practitioners to improve the design of trapping and monitoring devices, increasing their efficacy in capturing DBM (Adler et al., 2020). Implementing green and red-colored containers, either alone or in combination, can be a highly targeted and efficient method of luring these pests and limiting their population growth (Cho and Lee, 2012).

Moreover, these insights into DBM color preferences contribute to understanding their visual ecology and behavior. The strong attraction to green containers likely stems from an innate association with host plant foliage, while the moderate attraction to yellow containers could be related to the presence of photoreceptors sensitive to longer wavelengths (Kolb and Scherer, 1982). By leveraging these color preferences, researchers and growers can develop more effective and sustainable strategies for monitoring and controlling DBM populations.

Assessing the potential non-target effects of using allelochemicals traps

A critical aspect of this research involved assessing the potential non-target impacts of using allelochemicals traps. This approach provides valuable insights into the broader ecological consequences of employing these trapping techniques. While designed to target diamondback moths, an unintended consequence was the attraction of cabbage loopers (*Trichoplusia ni*), another *Brassica* spp. pest. This non-target attraction presents both risks and opportunities while cabbage loopers contribute to crop damage, their simultaneous monitoring and removal could aid pest management (Sarfraz et al., 2005). However, impacts on beneficial insects must also be considered.

Using plant volatile chemical traps to catch DBM is a powerful weapon that can employed in the armoury of pest management tactics of the pest. This strategy, however, is not without inherent difficulties, as it has the potential to trigger a cascade of non-target effects—unanticipated consequences that could resonate beyond the intended target and ricochet throughout the ecosystem (Gregg et al., 2016). Non-target effects, or unintended outcomes, occur when the deployment of these traps extends their influence to recipients who were not intended. The threat to non-target insect species is one of the most significant concerns. Chemical compounds generated by these traps, which are designed to mimic the scent signals of host plants or mating partners, may mistakenly attract insects other than DBM (Pivnick et al., 1994). These unwitting bystanders, which may include helpful pollinators and natural predators, may inadvertently fall into the traps' clutches, disrupting the ecosystem's delicate balance or maybe additional pests that may contribute to crop loss.

The findings of this study concur with previous studies showing that plant volatiles can attract cabbage looper moths which are consistent with research conducted by Pivnick et al. (1994) which suggested that plant volatiles can also attract the cabbage looper apart from attracting the DBM. So, while plant volatiles may enhance top-down control of diamondback moth by recruiting its natural enemies, they may also make crops more conspicuous or attractive to other herbivores like cabbage looper. This trade-off between pest control and pest attraction needs careful evaluation. Optimizing lure formulations and trap placement can help minimize non-target effects and maximize DBM control. Further research needs to be conducted in actual *Brassica* spp. fields to further explore the non-target effects as according to previous research, plant volatiles have the potential to attract other pests and beneficial insects.

Conclusion

This study provides valuable insights into the chemical ecology of the diamondback moth-*Brassica* spp. crop interaction and the potential of using plant allelochemicals for pest management. Y-tube olfactometer assays and laboratory trapping experiments revealed that (Z)-3-hexenyl acetate, cis-3-hexene-1-ol, sabinene, and 4-methylsulfinylbutyl glucosinolate were significantly attractive to adult diamondback moths, particularly females. A 1:1 blend of cis-3-hexene-1-ol and (Z)-3-hexenyl acetate displayed optimal attraction in both laboratory and open screen cage settings, with green-coloured traps further enhancing trapping efficacy. However, the study also revealed potential non-target effects on cabbage loopers, emphasizing the need for careful evaluation and optimization of these attractants. This research lays the foundation for developing targeted and sustainable pest management strategies in Brassica spp. agriculture, but future studies should focus on field-testing, understanding moth olfactory perception mechanisms, and mitigating non-target impacts to harness the power of chemical ecology for eco-friendly pest control.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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