Host-Plant Volatiles Enhance the Attraction of Cnaphalocrocis medicals (Lepidoptera: Crambidae) to Sex Pheromone

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Research Article

Keywords: Cnaphalocrocis medicals, Sex pheromone, Host-plant volatiles, EAG, Wind tunnel, Field trapping

Posted Date: May 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-484527/v1

Abstract

Cnaphalocrocis medinalis Guenée (Lepidoptera: Pyralidae) is a notorious pest of rice, *Oryza sativa* L.(Poaceae). Sex pheromone and host-plant volatiles can trap *C. medinalis* separately. To improve the trap efficiency of sex pheromone, we first tested the synergistic effect of 8 host-plant volatiles, including 2-Phenylethanol, 1-Hexanol, 1-Heptanol, (*Z*)-3-Hexenal, (*E*)-2-Hexenal, Octanal, Valeraldehyde, and Methyl Salicylate on the attraction of *C. medinalis* to the female-produced sex pheromone in electroantennography. The addition of (*E*)-2-Hexenal, Methyl Salicylate, Valeraldehyde, and (*Z*)-3-Hexenal increased electroantennogram response of *C. medinalis* to sex pheromone. Further testing of the mixtures of these four compounds and sex pheromone in wind tunnel experiments indicated that additive (*E*)-2-Hexenal or Methyl Salicylate stimulated the landing behaviors of both male and female *C. medinalis* compared with sex pheromone alone. Field evaluations showed that mixtures of sex pheromone and (*E*)-2-Hexenal or Methyl Salicylate resulted in significantly higher catches to male moths than sex pheromone alone. Using 1:1 and 1:10 combinations of the sex pheromone and (*E*)-2-Hexenal, showed a synergistic effect of 95% and 110%, respectively. Furthermore, 1:1 and 1:10 mixtures of the sex pheromone and Methyl Salicylate exhibited a synergistic effect of 69% and 146%, respectively. These results may provide the basis for developing efficient pest management strategies against *C. medinalis* using host-plant volatiles and insect sex pheromones.

Introduction

Most insects use odors present in the external environment released by conspecifics, prey, natural enemies, or host plants to find food, communicate with their mates, and identify suitable oviposition sites (Badeke et al. 2016; Bruce and Pickett 2011; Dupuy et al. 2017). Insect sex pheromones are chemical substances used for intraspecific identification and communication (Badeke et al. 2016). So far, the majority of moth sex pheromones identified are released by females used efficiently to attract the males and locate mate sites (Roelofs et al. 2002). The control of moths in the field through synthetic sex pheromone blend is feasible by population monitoring, mating disruption, and mass trapping (Mayer 2019). However, sex pheromones mixed up with other volatile compounds under natural conditions, including those released by surrounding vegetation. If insects in natural settings recognize signals from food or plants from which biologically active odorants emanate, human-made lures based solely on sex pheromone are not likely to be competitive when placed in the field (Deng et al. 2004a; Kawazu et al. 2000).

Host-plant volatiles are low molecular weight, lipophilic character, and high volatility compounds (Loreto and Schnitzler 2010; Pichersky et al. 2006) full of natural environments (Dicke et al. 2009; Pinto et al. 2008). In natural conditions, host-plant volatiles plays an essential role in guiding phytophagous insects to feeding, mating, and oviposition sites (Von Arx et al. 2012). Increasing evidence suggests that host-plant volatiles and insect sex pheromone are interactive under field conditions (Reddy and Guerrero 2004). Certain moths acquire or sequester host-plant compounds and use them as a sex pheromone, sex pheromone precursors, or particular host plant cues for producing or releasing sex pheromone (Landolt and Phillips 1997; McNeil and Delisle 1989; Nishida 2014). It has been confirmed that host-plant volatiles enhances sex pheromone responses in some species of Lepidopterans moths. One field test showed that a mixture of *Holotrichia parallela* sex pheromone and (*E*)-2-hexenyl acetate could increase sex pheromone baited trap captures in the field, thereby improving current uses of sex pheromones (Ju et al. 2017). E-β-ocimene and E-β-farnesene, two of the prominent volatiles of certain *Grapholita molesta* hosts, significantly raised the capture of *G. molesta* male moths than traps baited only with sex pheromone in the field (Xiang et al. 2019). An efficient sex pheromone attractant is still missing for the management of *Ectomyelois ceratoniae* because the major pheromone component is unstable. Interestingly, the combination of female sex pheromone and volatiles from cracked pomegranates have great potential for application in baited trap capture of carob moth (Hosseini et al. 2017). Host-plant volatiles combined with female sex pheromone, have broad application prospects in integrated pest management (IPM) strategies.

The rice leaffolder, *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae), is a migratory rice pest that causes significant damage to rice, *Oryza sativa* L. (Poaceae), in Asia, Oceania, and Africa (Khan et al. 1988). Recently, *C. medinalis* has increased in major rice-growing regions of Asia and caused significant economic losses (Ren and Xian 2017; Wan et al. 2015; Wang et al. 2009). Sex pheromone components of *C. medinalis* has been identified that including (*Z*)-11-octadecenal, (*Z*)-13-octadecenal, (*Z*)-11-octadecen-1-ol, and (*Z*)-13-octadecen-1-ol (Kawazu et al. 2000; Rao et al. 1995; Yao et al. 2011) and the synthetic sex pheromone blend is widely used for *C. medinalis* moths flight monitoring in the paddy field (Kawazu et al. 2009; Kawazu et al. 2005; Yao et al. 2011). However, using sex pheromone alone can't effectively control insects in the field (Cherif et al. 2018; Kwadha et al. 2017). Electroantennogram response of *C. medinalis* moths to host-plant volatiles compounds in our laboratory indicated that 2-Phenylethanol, 1-Hexanol, 1-Heptanol, (*Z*)-3-Hexenal, (*E*)-2-Hexenal, Octanal, Valeraldehyde, and Methyl Salicylate could elicit higher EAG responses compared with control treatment (Cheng et al. 2016). Based on this study, we hypothesized that these host-plant volatiles compounds could be candidate products to enhance the attraction of *C. medinalis* moths to sex pheromone. Here we first tested the electroantennogram responses elicited by these host-plant volatiles compounds in combination with sex pheromone. Subsequently, we conducted wind tunnel and field tests to determine if these compounds enhance sexual communication in *C. medinalis*. The main objective is to facilitate the development of more efficient lures for monitoring and controlling this insect pest.

Materials And Methods

Insects. C. medinalis used in this study came from an experimental plot in Yangzhou University, Yangzhou, China (119° 43 E; 32° 39 N). Larvae were reared on rice seedlings in an insectary (27 ± 1°C, 75 ± 5% RH, 16:8 L:D photoperiod) until pupation. Pupae were separated as males and females into 35 x 25 x 25 cm cages. Three-day-old unmated moths were used in all bioassays. Healthy moths were used only once and starved for 12 hr before testing.

Synthetic Compounds. C. medinalis synthetic sex pheromone compounds ((*Z*)-11-octadecenal, (*Z*)-13-octadecenal, (*Z*)-11-octadecen-1-ol and (*Z*)-13-octadecen-1-ol at a ratio of 60:500:60:120 (Yao et al. 2011)) were purchased from Pherobio Technology Co,.Ltd (Beijing, China). Host-plant volatiles 2-Phenylethanol, 1-Hexanol, 1-Heptanol, (*Z*)-3-Hexenal, (*E*)-2-Hexenal, Octanal, Valeraldehyde, and Methyl Salicylate were all purchased from Sigma- Aldrich Co. and Aldrich chemical Co. (Sigma-Aldrich Co. St Louis USA; Sigma-Aldrich Chemie Steinlheim Germany).

Electroantennogram Recordings. For bioassays, the tested chemicals were dissolved individually in normal hexane. The sex pheromone and host-plant volatiles were mixed at ratios of 1:1, 1:10, 1:100, and 1:1000. The concentration of the sex pheromone solution was 50 μl/L for all tests. Solely sex pheromone was used as a control. These solutions were used for EAG as well as for behavior assays.

EAG responses of 3-day-old unpaired male/female moth antennae were measured after excised from its base, and the distal part of the terminal segment was cut off. The antenna was fixed on the PR electrode with SpectraR360 conductive adhesive at the outlet of the glass tube. In general, 10 μl of the liquid sample was applied to both sides of a filter paper piece. Meanwhile, the filter paper was inserted into a glass tube. An airstream of 0.5-sec duration was blown over the antennae, and EAG responses were recorded. The stimulation interval was 60 s using a flow rate of 1 L/min for both stimulants and purge airflow.

One antenna from each individual was tested with three repeated stimulations. Six antennae were tested for each stimulant. The EAG system used in our experiment is IDAC-232 produced by Syntech (Hilversum, The Netherlands). EAG responses from isolated antennae were recorded according to the method described by Hao (Hao et al. 2006). Data were analyzed using EAG2000 for Windows (SYNTECH, Hilversum, The Netherlands).

Wind Tunnel Experiments. The assays were performed in a dark room (27 ± 1°C, 75 ± 5% RH, 0.3 lux (red light)) using a wind tunnel (flight section 63 × 63 × 200 cm, made by order), 0.3 lux (red light). All trials were run between 10:00 and 18:00 (daylight hours), during which period female pheromone emissions and male activity are reported to be high (Charlton and Cardé 1982; Tobin et al. 2009).

Mixtures of the sex pheromone and host-plant volatile at ratios of 1:1, 1:10, 1:100, and 1:1000 were tested. Solely sex pheromone was used as a control. Lures were formulated by applying 10 μl of liquid sample applied to each filter paper before the bioassays. The lure was then pinned to a steel jack for testing. Each lure was used once. 3-day-old moths were transferred individually to test tubes and then introduced into the tunnel individually. The airspeed was 30 cm/sec. Moths were permitted 5 min to respond and scored for the following behaviors: activation, take off, partial flight (50cm, 100cm, 150cm), and landing on the cage containing the lure. Each treatment consisted of 60 moths, which were divided into three groups on average.

Field Bioassays. After the laboratory evaluation, (*E*)-2-Hexenal and Methyl Salicylate were selected as possible candidates to enhance the attractivity of the *C. medinalis* sex pheromone in the field. To assess the trapping efficiency of single host-plant volatiles, sex pheromone, and the mixture of sex pheromone and host-plant volatiles at a different ratio, field trials were conducted in paddy fields in Yizheng, China (119° 18 E; 32° 27 N) from 22 July to 21 August 2016. Treatments were as follows: (1) (*E*)-2-Hexenal; (2) Methyl Salicylate; (3) Sex pheromone; (4) Sex pheromone + (*E*)-2-Hexenal (at a ratio of 1:1); (5) Sex pheromone + (*E*)-2-Hexenal (at a ratio of 1:10); (6) Sex pheromone + (*E*)-2-Hexenal (at a ratio of 1:100); (7) Sex pheromone + Methyl Salicylate (at a ratio of 1:1); (8) Sex pheromone + Methyl Salicylate (at a ratio of 1:10); (9) Sex pheromone + Methyl Salicylate (at a ratio of 1:100). The concentration of the single host-plant volatiles solution was 50 μl/L and the sex pheromone solution was 50 μl/L for all tests.

The trap-loaded lures were placed about 20 cm above the plant canopy. The dispenser was a capillary with 90 mg of chemical attractants, which were stored in a freezer before use. The layout of the trial followed a completely randomized block design. The distance between traps was about 25m to minimize interference. Lures were replaced every four days. Insects captured in the traps were removed every two days, counted, sexed, and cataloged. Each treatment was replicated six times in six blocks.

Statistical Analysis. EAG values and trapping data from experiments conducted in the rice paddy were statistically evaluated by one-way ANOVA followed Tukey tests (P<0.05). The responding percentage in wind tunnel experiments was analyzed using a $\chi 2$ test.

Result

Effect of Host-Plant Volatiles on EAG Response to Sex Pheromone. Different EAG responses of male or female antennae to the mixtures of sex pheromone and various host-plant volatiles compounds were observed. The EAG values of *C. medinalis* female moths to the mixtures of sex pheromone and (*E*)-2-Hexenal increased by 834% and 646% at ratios of 1:100 and 1:1000 compared with sex pheromone alone ($F_{4,25}$ = 7.91; P = 0.0003; Table 1), indicating the strongest enhancing effects among the combination of all the tested host-plant volatiles and sex pheromone. Furthermore, the EAG values of *C. medinalis* female moths increased by 312% and 83% for sex pheromone and (*Z*)-3-Hexenal mixture at a ratio of 1:1000 ($F_{4,25}$ = 9.039; P = 0.0001; Table 1) and for sex pheromone and Valeraldehyde mixture at a ratio of 1:100 ($F_{4,25}$ = 3.058; P = 0.0351; Table 1) compared with sex pheromone alone. Significantly stronger EAG responses of male moths were observed for sex pheromone and (*E*)-2-Hexenal mixtures at ratios of 1:100 and 1:1000 ($F_{4,25}$ = 5.747; P = 0.02; Table 2) compared with sex pheromone alone. The EAG values increased by 267% and 305%, respectively. The mean response value of males to sex pheromone and Methyl Salicylate mixture at a ratio of 1:100 ($F_{4,25}$ = 12.361; P = 0.0001; Table 2) was 97% higher than the response to sex pheromone alone.

Wind Tunnel Bioassay under Laboratory Conditions. Based on the results obtained from EAG tests, (*Z*)-3-Hexenal, (*E*)-2-Hexenal, Valeraldehyde, and Methyl Salicylate were selected as candidates for wind tunnel bioassay. For activation and take-off behavior, all mixtures of sex pheromone and (*E*)-2-Hexenal or Methyl Salicylate (at ratios of 1:1, 1:10, 1:100, and 1:1000) significantly increased the response level of females compared with the sex pheromone alone (P < 0.05; Table 4). When mixed with sex pheromone, Valeraldehyde also had a significant enhancing effect on the proportion of females activation (at ratios of 1:1 and 1:10) and take off (at ratios of 1:10 and 1:1000) (P < 0.05; Table 4). (*E*)-2-Hexenal and Methyl Salicylate added to sex pheromone raised the proportion of females partial flight (P < 0.05; Table 4) expect the percentage of 100cm partial flight and 150cm partial flight at a ratio of 1:1000 (P > 0.05; Table 4). However, Valeraldehyde mixtures only had a significant enhancing effect on the proportion of females short flight (50cm: at ratios of 1:1, 1:10 and 1:100; 100cm: at ratios of 1:10 and 1:1000) compared with the sex pheromone alone (P < 0.05; Table 4). Compared with using sex pheromone alone, only the mixtures of sex pheromone and (*E*)-2-Hexenal or Methyl Salicylate at ratios of 1:1, 1:10, and 1:100 can significantly increase the proportion of females landing on (P < 0.05; Table 4).

Mixtures of sex pheromone and (*Z*)-3-Hexenal treatments caused significantly fewer males to fly upwind and land on the source than the sex pheromone alone ($P < 0.05$; Table 5). When the mixtures of sex pheromone and Valeraldehyde were used, the response level of all behavioral categories by males was significantly decreased at a ratio of 1:100, and the percentage of activation was declined at all concentrations compared with sex pheromone alone ($P < 0.05$; Table 5). Specifically, the response level of males activation achieved 100 percent when mixtures of sex pheromone and (*E*)-2-Hexenal or Methyl Salicylate at ratios of 1:10 and 1:100. At the same time, in the presence of (*E*)-2-Hexenal (at a ratio of 1:100) and Methyl Salicylate (at ratios of 1:10 and 1:100), the response level of land on by males was significantly raised compared with the sex pheromone alone ($P < 0.05$; Table 5). Across all treatments in wind tunnel assays, only (*E*)-2-Hexenal mixtures and Methyl Salicylate mixtures increased the attractiveness of both males and females to sex pheromone.

Field Trapping Efficiency of Different Compounds. Wind tunnel assays revealed that the mixtures of sex pheromone and (*E*)-2-Hexenal or Methyl Salicylate increased the proportion of males and females landing on the cage containing the lure compare with sex pheromone alone. Therefore, sex pheromone plus various ratios of these two host-plant volatiles were evaluated in the field. The data recorded once every two days was recorded 15 times in total. Among them, traps baited with a combination of sex pheromone and Methyl Salicylate at ratios of 1:1 and 1:10 were significantly raised male moths catches compare with sex pheromone alone 13 times and 8 times respectively. The quantity of male moths caught by the mixtures of sex pheromone and (*E*)-2-Hexenal at ratios of 1:1 and 1:10 was significantly higher than that of sex pheromone alone 5 times and 13 times respectively (Fig. 1).

In the surveyed field, traps baited with the mixtures of sex pheromone and (*E*)-2-Hexenal at ratios of 1:1 and 1:10 significantly increased the total number of male moths catch by 94.5% and 109.8%, respectively, compared with traps baited with sex pheromone alone ($F_{4,15} = 27.079$; $P = 0.0001$; Fig. 2). Male moths caught by the mixtures of sex pheromone and (*E*)-2-Hexenal between the ratios of 1:1 and 1:10 showed no significant difference. Similar to (*E*)-2-Hexenal, traps baited with the mixtures of sex pheromone and Methyl Salicylate at ratios of 1:1 and 1:10 both captured significantly more male moths than the number caught in traps baited with sex pheromone alone (increased by 69% and 146%) ($F_{4,15} = 37.588$; $P = 0.0001$; Fig. 2). However, the combination of sex pheromone and Methyl Salicylate at a ratio of 1:1 traped fewer male moths than the mixtures of sex pheromone and Methyl Salicylate at a ratio of 1:10. Traps baited with a combination of sex pheromone and (*E*)-2-Hexenal or Methyl Salicylate at a 1:100 ratio did not raise catches compared with traps baited with sex pheromone alone. Unexpectedly, few female moths are trapped by lures. Moreover, only the lures with (*E*)-2-Hexenal or Methyl Salicylate almost can not attract *C. medinalis* moths.

Discussion

Our study demonstrates that (*E*)-2-Hexenal increased responses of *C. medinalis* to sex pheromone in EAG, wind tunnel, and field trapping experiments. (*E*)-2-hexenal has been reported to enhance the attraction of some other Lepidoptera to their sex pheromones. Deng *et al.* (2004b) have shown that traps baited with (*E*)-2-hexenal and sex pheromone enhanced *Spodoptera exigua* catches by 38.8% compared to traps baited with sex pheromone alone in field tests. Solely (*E*)-2-hexenal was not attractive to *Leguminivora glycinivorella* males at any dose tested but showed a strong synergistic effect when blended with sex pheromone in the field (Hu et al. 2013). Our data indicated that the ratio between sex pheromone and (*E*)-2-hexenal could have an impact on the synergistic effect. When blended at ratios of 1:1 and 1:10, the mixtures increased the attraction of *C. medinalis* male moths to the sex pheromone in field experiments. It is worthwhile mentioning that in the EAG and wind tunnel experiments, the response concentration is not consistent with that of the field tests. This discrepancy could be attributed to different dosages, backgrounds, and bait carriers used. The ratio of host-plant volatiles should deserve attention when combing with sex pheromone.

We observed that Methyl Salicylate also had a synergistic effect on *C. medinalis* sex pheromone. Deng *et al.* (2004a) reported that a combination of Methyl Salicylate and sex pheromone elicited stronger EAG responses from *Helicoverpa armigera* male antennae than sex pheromone alone. Besides, Von Arx *et al.* (2012) have shown that Methyl Salicylate affected only the initial behavioral responses of *Lobesia botrana* to sex pheromone in wind tunnel tests. Similarly, low doses (at ratios of 1:1 and 1:10) of Methyl Salicylate enhance the attraction of *C. medinalis* to sex pheromones, while high doses (at a ratio of 1:100) may not affect the *C. medinalis* male moths response. Our results and previous studies suggest that (*E*)-2-hexenal and Methyl Salicylate enhance the response of *C. medinalis* male moths and some other Lepidoptera to their sex pheromones, and these two compounds may be used as synergists with sex pheromone baits for controlling Lepidoptera pests.

So far, the enhancement of attraction to sex pheromone by host-plant volatiles has been frequently reported in male moths trapping, but there are only a few reports in female moths. Field studies indicate that traps baited with sex pheromone + (*Z*)-3-hexenyl acetate + (*Z*)-3-hexen-1-ol + allyl isothiocyanate has a stronger attraction to both males and females *Plutella xylostella* compare with sex pheromone alone (Dai et al. 2008). A possible explanation for the field trapping results is those female moths may locate feeding and oviposition sites by responding to host-plant volatiles, and male moths may locate host sites as well as an area of possible female activity by responding to host-plant volatiles and sex pheromone (Bruce et al. 2005). Another view is that male moths use defensive host-plant volatiles as a sex pheromone, which recognition and preference by female moths (Nishida 2014). The combined results of EAG and wind tunnel showed that the mixtures of sex pheromone and (*E*)-2-hexenal or Methyl Salicylate could increase the response of *C. medinalis* females to sex pheromone, but almost no females were trapped in the field. We assumed that it is difficult for female moths to recognize an individual (E)-2-hexenal and Methyl Salicylate in natural conditions due to the high background noise in the field.

The olfactory system plays an essential role in insect behavior, such as detecting food, mates, oviposition sites, and avoiding enemies (Sato and Touhara 2008). Insects mainly rely on olfaction-related proteins to recognize and percept semiochemicals from the external environment (Li et al. 2019). The soluble odorant-binding proteins (OBPs) are thought to facilitate the transport of odorant molecules through the sensillum lymph. On the olfactory receptor neurons (ORNs) dendritic membrane, odorant receptors (ORs) linked with the complex of OBP/odors to detect and discriminate distinct odorants (Leal 2013). Ligand-binding experiments using N-phenyl-naphthylamine (1-NPN) as a fluorescent probe demonstrated that trans-11-tetradecen-1-yl acetate, the sex pheromone component of *Loxostege sticticalis*, and (*E*)-2-Hexenal, the most abundant plant volatiles in essential oils extracted from host plants, had high binding affinities to LstiGOBP2 (Yin et al. 2012). *Grapholita molesta* OBP12 (GmolOBP12) displayed a higher expression level in male antennae than in female antennae. Fluorescence binding assays exhibited that GmolOBP12 had strong binding affinities to the sex pheromone (*Z*)-8-dodecenyl alcohol and host-plant volatile (*E*)-2-hexenal (Li et al. 2019). Furthermore, it has been reported that *Epiphyas postvittana* OR1 (EpOR1) is closely related to the pheromone receptors (PR) of other lepidopterans and sensitive to Methyl Salicylate, recognizing this odorant to a low concentration of 10-15M (Zhang et al. 2017). We speculated that the synergism between host-plant volatiles and sex pheromones might be related to the olfactory protein that responds to both odors. It needs further study to confirm our speculation.

In summary, we have demonstrated that the addition of (*E*)-2-Hexenal or Methyl Salicylate to the sex pheromone bated traps can increase trap catches of *C. medinalis* male moths in the field. We believe that apart from looking for the molecular mechanism of the olfactory system, future research should explore food-based synthetic attractant trapping female moths because of their significant role in oviposition. These findings may provide the basis for developing efficient pest management systems against *C. medinalis*.

Declarations

Acknowledgments

This research was financially supported by the National Key Research Program (2018YFD0300802), the Key Research Program of Jiangsu Province (BE2018355), and the Jiangsu Agricultural Scientific Self-innovation Fund (No. CX[18]1003).

Funding

Funding for this study was provided by the National Key Research Program (2018YFD0300802), the Key Research Program of Jiangsu Province (BE2018355), and the Jiangsu Agricultural Scientific Self-innovation Fund (No. CX[18]1003).

Conflicts of Interest/Competing Interests

The authors declare no conflicts of interest.

Availability of Data and Material

Not applicable.

Code Availability

Software packages and codes used for statistical analyses in this study are available from the corresponding author.

Authors' contributions

Not applicable.

Ethics approval

All the procedures performed in studies involving insects were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Consent to Participate

All authors consent to participate.

Consent for Publication

All authors approved of the submission of the manuscript.

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Tables

Table 1. EAG responses of *C. medinalis* female moths to sex pheromone in combination with different host-plant volatiles compounds

Host-plant volatiles	Value of EAG（mv）				
	SP	SP:HPV (1:1)	SP:HPV (1:10)	SP:HPV (1:100)	SP:HPV (1:1000)
Phenylethanol	2.29±0.18a	2.32±0.28a	2.51±0.23a	2.40±0.28a	2.16±0.27a
1-Hexanol	1.86±0.25a	1.79±0.21a	2.43±0.40a	2.68±0.38a	2.86±0.39a
1-Heptanol	1.44±0.25a	1.79±0.25a	1.92±0.25a	2.03±0.18a	1.94±0.19a
(*Z*)-3-Hexenal	2.14±0.21b	2.29±0.24b	2.60±0.29b	3.07±0.28b	8.81±2.05a
(*E*)-2-Hexenal	1.58±0.17b	2.06±0.33b	2.16±0.38b	14.76±4.07a	11.78±2.86a
Octanal	1.82±0.28a	2.26±0.27a	2.50±0.31a	2.98±0.46a	3.08±0.50a
Valeraldehyde	2.41±0.15b	2.65±0.15ab	2.68±0.21ab	4.41±0.94a	3.26±0.29ab
Methyl Salicylate	1.73±0.29a	2.02±0.37a	2.20±0.45a	2.29±0.37a	2.08±0.37a

Note：*SP* sex pheromone, *HPV* host-plant volatiles; Treatment with different letters in the same line indicates cases in which means for the sex pheromone + host-plant volatiles treatments and sex pheromone alone were significantly different (Tukey test, $P < 0.05$).

Table 2. EAG responses of *C. medinalis* male moths to sex pheromone in combination with different host-plant volatiles compounds

Host-plant volatiles	Value of EAG（mv）				
	SP	SP:HPV (1:1)	SP:HPV (1:10)	SP:HPV (1:100)	SP:HPV (1:1000)
Phenylethanol	2.67±0.29a	2.76±0.42a	3.30±0.51a	2.35±0.34a	2.00±0.27a
1-Hexanol	3.32±0.77a	3.41±0.68a	3.64±1.000a	4.15±1.25a	3.29±0.79a
1-Heptanol	2.70±0.32a	2.93±0.49a	2.79±0.44a	2.31±0.15a	2.67±0.31a
(*Z*)-3-Hexenal	2.30±0.41a	2.87±0.75a	2.67±0.65a	2.77±0.54a	7.32±3.30a
(*E*)-2-Hexenal	2.73±0.41c	3.12±0.53bc	2.98±0.49bc	10.07±1.82ab	11.05±3.36a
Octanal	2.75±0.44a	2.67±0.49a	2.75±0.62a	3.39±0.80a	2.68±0.32a
Valeraldehyde	3.32±0.43a	3.56±0.39a	3.72±0.48a	4.24±0.56a	2.98±0.23 a
Methyl Salicylate	2.68±0.49bc	5.29±0.73a	4.77±1.26ab	1.82±0.26c	1.14±0.07c

Note：*SP* sex pheromone, *HPV* host-plant volatiles; Treatment with different letters in the same line indicates cases in which means for the sex pheromone + host-plant volatiles treatments and sex pheromone alone were significantly different (Tukey test, $P < 0.05$).

Table 3. Statistics from the analysis for EAG responses of *C. medinalis* adults to different matched compounds

Female			Male		
Treatments	*F* value	*P* value	Treatments	*F* value	*P* value
SP + Phenylethanol	0.27	0.8945	SP + Phenylethanol	1.675	0.1872
SP + 1-Hexanol	2.033	0.1204	SP + 1-Hexanol	0.147	0.9626
SP + 1-Heptanol	1.048	0.4025	SP + 1-Heptanol	0.4	0.8067
SP + (*Z*)-3-Hexenal	9.039	0.0001	SP + (*Z*)-3-Hexenal	1.783	0.1638
SP + (*E*)-2-Hexenal	7.91	0.0003	SP +(*E*)-2-Hexenal	5.747	0.002
SP + Octanal	1.918	0.1386	SP + Octanal	0.294	0.8792
SP + Valeraldehyde	3.058	0.0351	SP + Valeraldehyde	1.18	0.3437
SP + Methyl Salicylate	0.328	0.8568	SP + Methyl Salicylate	12.361	0.0001

Note: *SP* sex pheromone**.**

Table 4. Responses of *C. medinalis* female moths to different lures in a wind tunnel.

Treatments	Ratio of SP:HPV	Activation	Take off	50cm	100cm	150cm	Land on
Sex pheromone	0	36.7	26.7	23.3	20.0	20.0	16.7
Sex pheromone + (*Z*)-3-Hexenal	1:1	50.0	50.0	30.0	30.0	30.0	30.0
	1:10	53.3	50.0	40.0	30.0	30.0	20.0
	1:100	56.7	50.0	30.0	20.0	10.0	10.0
	1:1000	43.3	30.0	23.3	13.3	6.7	6.7
Sex pheromone + (*E*)-2-Hexenal	1:1	86.7***	76.7***	63.3**	60.0**	43.3*	43.3*
	1:10	86.7***	86.7***	70.0***	66.7***	60.0**	50.0**
	1:100	76.7**	76.7***	76.7***	66.7***	50.0*	46.7*
	1:1000	76.7**	70.0***	50.0*	40.0	40.0	30.0
Sex pheromone + Valeraldehyde	1:1	66.7*	50.0	50.0*	40.0	40.0	36.7
	1:10	70.0**	70.0***	56.7**	50.0*	40.0	20.0
	1:100	50.0	50.0	40.0	40.0	40.0	30.0
	1:1000	60.0	60.0**	60.0**	53.3**	36.7	33.3
Sex pheromone + Methyl Salicylate	1:1	90.0***	60.0**	50.0*	50.0*	46.7*	46.7*
	1:10	83.3***	83.3***	66.7***	63.3***	56.7**	40.0*
	1:100	96.7***	93.3***	73.3***	56.7**	56.7**	40.0*
	1:1000	90.0***	70.0***	53.3*	33.3	23.3	23.3

Note：*SP* sex pheromone, *HPV* host-plant volatiles; Data are the percentages of behavioral items carried out within 5 mins; Asterisks among a behavioral response in the same line indicate differences between the blend and individual sex pheromone ($P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$***).

Table 5. Responses of *C. medinalis* male moths to different lures in a wind tunnel.

Treatments	Ratio of SP:HPV	Activation	Take off	50cm	100cm	150cm	Land on
Sex pheromone	0	96.7	90.0	76.7	73.3	53.3	40.0
Sex pheromone + (*Z*)-3-Hexenal	1:1	76.7*	56.7**	43.3**	43.3*	40.0	40.0
	1:10	66.7**	53.3**	36.7**	33.3**	23.3*	20.0*
	1:100	66.7**	43.3***	20.0***	16.7***	16.7**	16.7*
	1:1000	86.7	53.3**	20.0***	20.0***	20.0**	20.0*
Sex pheromone + (*E*)-2-Hexenal	1:1	83.3	83.3	40.0**	36.7**	36.7	36.7
	1:10	100.0	100.0	70.0	50.0	50.0	50.0
	1:100	100.0	96.7	70.0	66.7	66.7	56.7*
	1:1000	83.3	70.0	63.3	50.0	40.0	40.0
Sex pheromone + Valeraldehyde	1:1	80.0*	66.7*	60.0	60.0	40.0	40.0
	1:10	80.0*	80.0	60.0	56.7	53.3	50.0
	1:100	73.3*	53.3**	43.3**	40.0**	20.0**	20.0*
	1:1000	80.0*	80.0	46.7*	33.3**	30.0	23.3*
Sex pheromone + Methyl Salicylate	1:1	96.7	90.0	56.7	53.3	33.3	33.3
	1:10	100.0	93.3	63.3	56.7	56.7	56.7*
	1:100	100.0	96.7	96.7*	80.0	73.3	70.0**
	1:1000	86.7	76.7	66.7	60.0	53.3	43.3

Note: *SP* sex pheromone, *HPV* host-plant volatiles; Data are the percentages of behavioral items carried out within 5 mins; Asterisks among a behavioral response in the same line indicate differences between the blend and individual sex pheromone ($P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$***).

Table 6. The Chi-square analysis of the flight behavior of *C. medinalis* female moths to different lures in a wind tunnel.

Treatments	Ratio of SP:HPV	Activation			Take off			50cm			100cm			1
		χ2	df	*P*	χ2	df	*P*	χ2	df	*P*	χ2	df	*P*	χ
Sex pheromone + (*Z*)-3-Hexenal	1:1	1.086	3	0.2974	3.4548	3	0.0631	0.3409	3	0.5593	0.8	3	0.3711	0
	1:10	1.6835	3	0.1945	3.4548	3	0.0631	1.9255	3	0.1652	0.8	3	0.3711	0
	1:100	2.4107	3	0.1205	3.4548	3	0.0631	0.3409	3	0.5593	0	3	1	1
	1:1000	0.2778	3	0.5982	0.0821	3	0.7745	0	3	1	0.48	3	0.4884	2
Sex pheromone + (*E*)-2-Hexenal	1:1	15.8637	3	0.0001	15.0167	3	0.0001	9.7738	3	0.0018	10	3	0.0016	3
	1:10	9.7738	3	0.0018	11.2792	3	0.0008	4.5933	3	0.0321	2.8571	3	0.091	2
	1:100	9.7738	3	0.0018	15.0167	3	0.0001	17.0667	3	0	13.3032	3	0.0003	5
	1:1000	15.8637	3	0.0001	21.991	3	0	13.125	3	0.0003	13.3032	3	0.0003	1
Sex pheromone + Valeraldehyde	1:1	5.406	3	0.0201	3.4548	3	0.0631	4.5933	3	0.0321	2.8571	3	0.091	2
	1:10	6.6964	3	0.0097	11.2792	3	0.0008	6.9444	3	0.0084	5.9341	3	0.0149	2
	1:100	1.086	3	0.2974	3.4548	3	0.0631	1.9255	3	0.1652	2.8571	3	0.091	2
	1:1000	3.2703	3	0.0705	6.7873	3	0.0092	8.2971	3	0.004	7.177	3	0.0074	2
Sex pheromone + Methyl Salicylate	1:1	18.3732	3	0	6.7873	3	0.0092	4.5933	3	0.0321	5.9341	3	0.0149	4
	1:10	13.6111	3	0.0002	19.4613	3	0	11.3805	3	0.0007	11.5886	3	0.0007	8
	1:100	24.3	3	0	27.7778	3	0	15.0167	3	0.0001	8.5311	3	0.0035	8
	1:1000	18.3732	3	0	11.2792	3	0.0008	5.7109	3	0.0169	2.8571	3	0.091	0

Note: *SP* sex pheromone, *HPV* host-plant volatiles.

Table 7. The Chi-square analysis of the flight behavior of *C. medinalis* male moths to different lures in a wind tunnel.

Treatments	Ratio of SP:HPV	Activation			Take off			50cm			100cm			150
		χ2	df	*P*	χ2	df	*P*	χ2	df	*P*	χ2	df	*P*	χ2
Sex pheromone + (*Z*)-3-Hexenal	1:1	5.1923	3	0.0227	8.5227	3	0.0035	6.9444	3	0.0084	5.5543	3	0.0184	1.07
	1:10	9.0167	3	0.0027	9.9316	3	0.0016	9.7738	3	0.0018	9.6429	3	0.0019	5.71
	1:100	9.0167	3	0.0027	14.7	3	0.0001	19.2881	3	0	19.4613	3	0	8.86
	1:1000	1.9636	3	0.1611	9.9316	3	0.0016	19.2881	3	0	17.1429	3	0	7.17
Sex pheromone + (*E*)-2-Hexenal	1:1	2.963	3	0.0852	0.5769	3	0.4475	8.2971	3	0.004	8.1481	3	0.0043	1.68
	1:10	1.0169	3	0.3132	3.1579	3	0.0756	0.3409	3	0.5593	3.4548	3	0.0631	0.06
	1:100	1.0169	3	0.3132	1.0714	3	0.3006	0.3409	3	0.5593	0.3175	3	0.5731	1.11
	1:1000	2.963	3	0.0852	3.75	3	0.0528	1.2698	3	0.2598	3.4548	3	0.0631	1.07
Sex pheromone + Valeraldehyde	1:1	4.0431	3	0.0444	4.8118	3	0.0283	1.9255	3	0.1652	1.2	3	0.2733	1.07
	1:10	4.0431	3	0.0444	1.1765	3	0.2781	1.9255	3	0.1652	1.8315	3	0.176	0
	1:100	6.4052	3	0.0114	9.9316	3	0.0016	6.9444	3	0.0084	6.7873	3	0.0092	7.17
	1:1000	4.0431	3	0.0444	1.1765	3	0.2781	5.7109	3	0.0169	9.6429	3	0.0019	3.36
Sex pheromone + Methyl Salicylate	1:1	0	3	1	0	3	1	2.7	3	0.1003	0.108	3	0.0667	2.44
	1:10	1.0169	3	0.3132	0.2182	3	0.6404	1.2698	3	0.2598	1.8315	3	0.176	0.06
	1:100	1.0169	3	0.3132	0	3	1	5.1923	3	0.0227	0.3727	3	0.5416	3.58
	1:1000	1.9636	3	0.1611	1.92	3	0.1659	0.7387	3	0.3901	1.2	3	0.2733	0

Note: *SP* sex pheromone, *HPV* host-plant volatiles.

Table 8. The variance analysis of the field test of *C. medinalis.*

(*E*)-2-Hexenal		Methyl Salicylate	
F value	*P* value	*F* value	*P* value
27.079	0.0001	37.588	0.0001

Figures

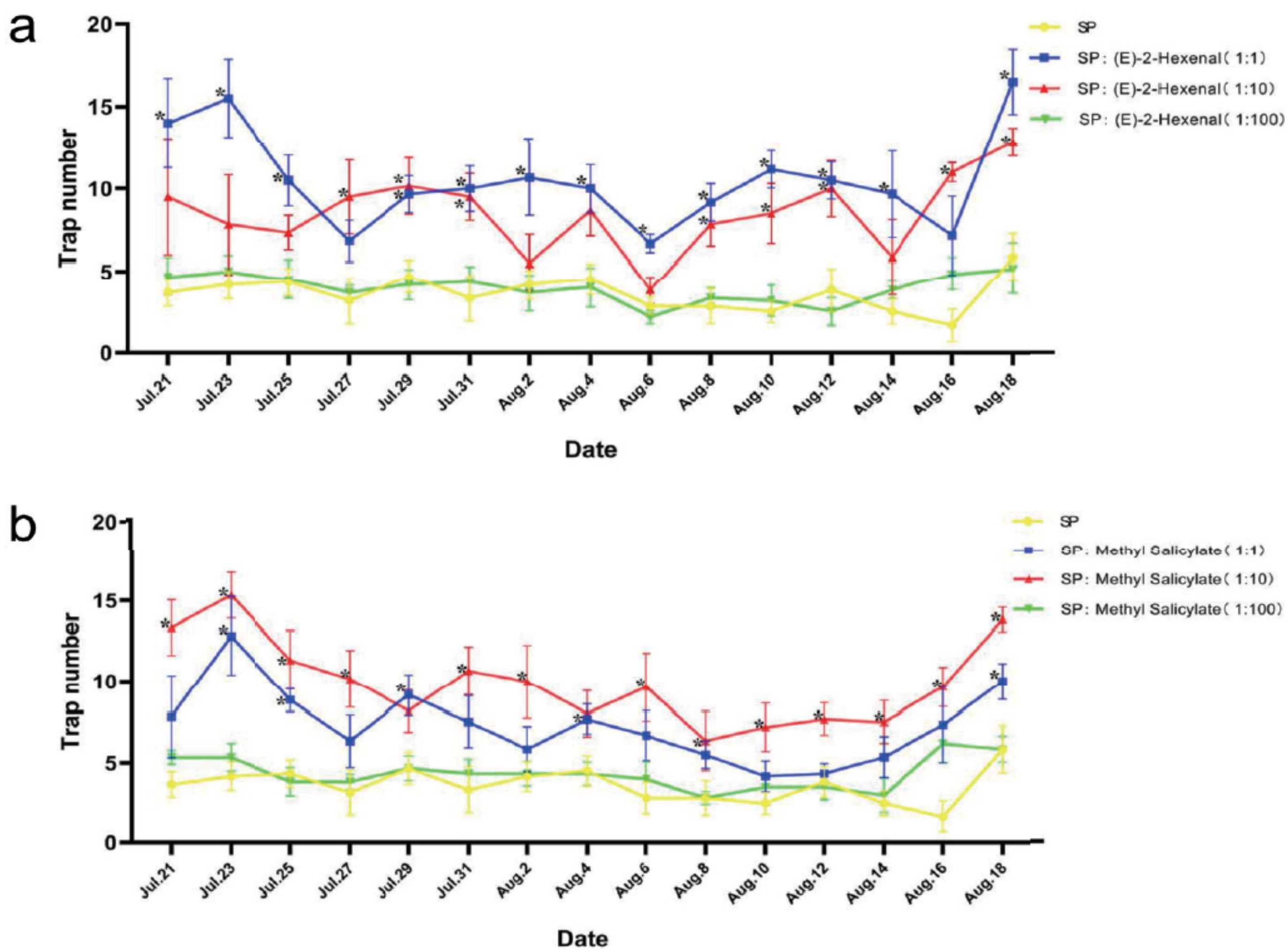

Figure 1

The attraction of C. medinalis male moths to sex pheromone (SP) in combination with the individual (E)-2-Hexenal (a) or Methyl Salicylate (b) in a rice paddy. Synthetic sex pheromone combined with (E)-2-Hexenal (a) or Methyl Salicylate (b) at different concentrations (at a ratio of 1:1, 1:10, and 1:100). The mean number of C. medinalis captured in different traps under field conditions. Treatments were replicated six times in trials. We recorded the number of moths captured by each lure every two days. Asterisks indicate statistically significant differences between mixtures and sex pheromone alone (Tukey test; * $P < 0.05$).

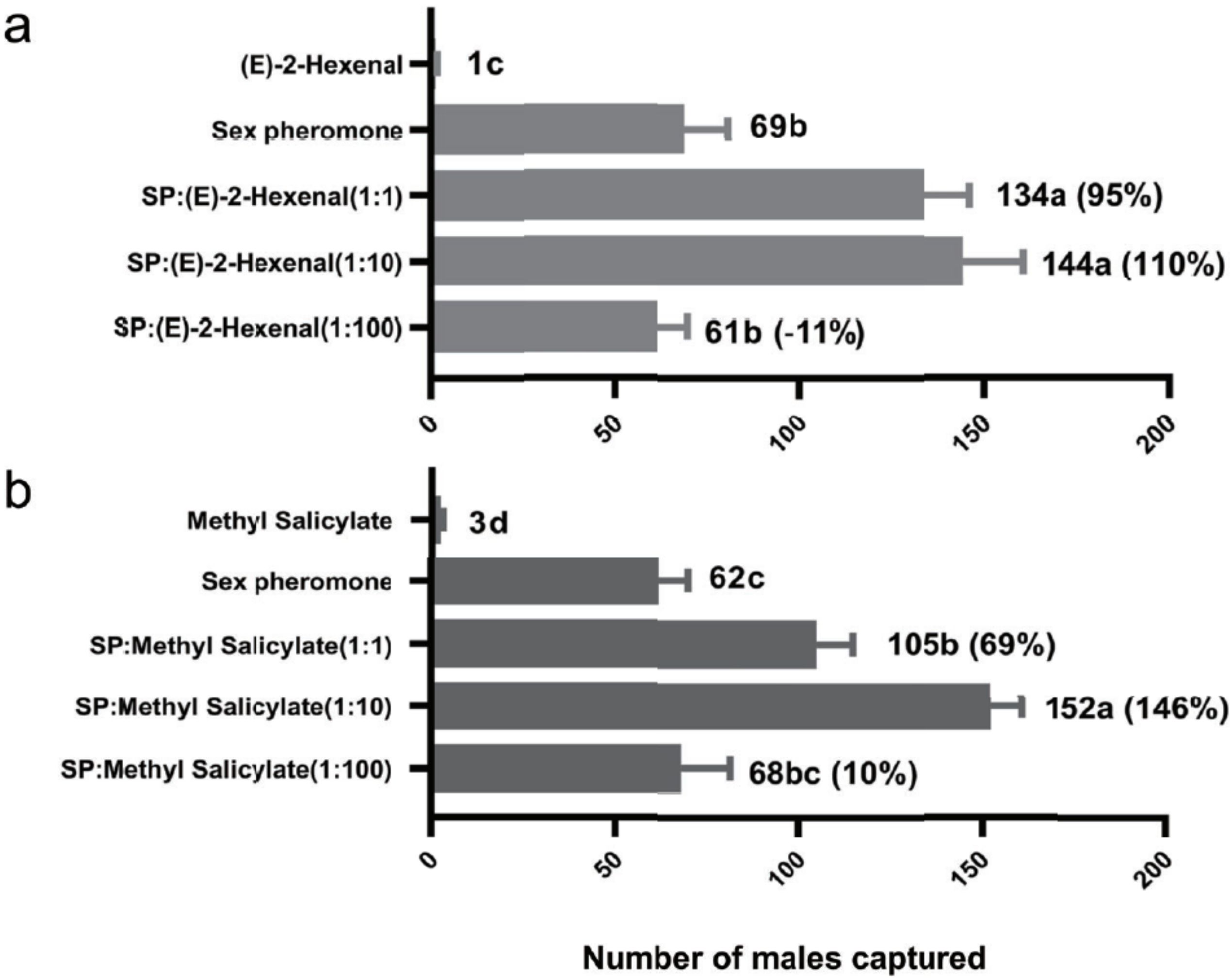

Figure 2

Total number of C. medinalis male moths captured in traps with mixtures of the sex pheromone (SP) in combination with (E)-2-Hexenal (a) or Methyl Salicylate (b) at different concentrations (at a ratio of 1:1, 1:10, and 1:100) in a rice paddy. Significant differences between different treatments were analyzed by one-way ANOVA followed by Tukey tests ($P < 0.05$). Significant differences are marked with different letters.