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ORIGINAL PAPER

# The effects of non-host plant extracts on electroantennogram responses, behavior and egg hatching of codling moth, *Cydia pomonella*

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## Abstract

The codling moth (*Cydia pomonella* L.) is a significant pest of pome fruit throughout the world. Behavioral and ovicidal activities of five non-host plant extracts (*Arctium lappa*, *Bifora radians*, *Humulus lupulus*, *Verbascum songaricum*, *Xanthium strumarium*), synthetic sex pheromone, (*E*,*E*)-8,10-dodecadienol (codlemone), and the plant volatile lure, (2*E*,4*Z*)-2,4-decadienoate (pear ester) were evaluated against the codling moth, *C. pomonella* L. Codlemone elicited the greatest electroantennogram (EAG) response (6.2 ± 1.2 mV) of the compounds tested from male *C. pomonella* while pear ester elicited 1.7 ± 0.1 mV EAG response in female moths. Codlemone attracted 34.5% of male *C. pomonella* in olfactometer studies, and it was followed by the *X. strumarium* extract with 24.8%. There was a significant difference between the behavior of unmated and mated females. *V. songaricum* extract was the most active extract, attracting 25.4% of unmated females. However, mated *C. pomonella* females exhibited greatest attraction to pear ester. In a wind tunnel bioassay, combining *X. strumarium* with codlemone significantly increased the response of male upwind flight and source contact as compared with codlemone alone. All plant extracts, except for *V. songaricum*, significantly reduced the number of eggs laid. The plant extracts exhibited some toxic effects to eggs, and hatching rate of eggs was reduced as compared with the control. Our results indicate that some of the plant extracts tested are potential candidates for practical use after elucidation and characterization of active compound(s).

**Keywords** Codlemone · Plant extract · Pear ester · *Arctium lappa* · *Bifora radians* · *Humulus lupulus* · *Verbascum songaricum* · *Xanthium strumarium*

## Key message

- New biologically active compounds are needed to improve the control of codling moth and discovery of non-host plant extracts holds potential.
- The tested plant extracts had both behavioral and toxic effects on codling moth eggs, males, and females.
- Some of the tested plant extracts could be further developed as management tools for codling moth.

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## Introduction

The codling moth (CM), *Cydia pomonella* L. (Lepidoptera: Tortricidae), is a cosmopolitan pest of deciduous fruits and causes heavy damage on apple (*Malus domestica* Borkh) and pear (*Pyrus communis* L.) in many parts of the world (Vickers and Rothschild 1991; Beers et al. 1993). The management of this pest heavily relies on the application of insecticides, ranging from organophosphates (OPs) to synthetic pyrethroids, neonicotinoids, and insect growth regulators (IGRs) (Croft and Reidl 1992; Dunley and Welter 2000; Reuveny and Cohen 2004; Mota-Sanchez

et al. 2008). Due to increasing codling moth resistance to certain insecticide groups, e.g., organophosphorous, pyrethroids, oxadiazines, diacylhydrazine insecticides (Stara and Kocourek 2007; Mota-Sanchez et al. 2008) alongside with tight regulation of EPA and EU laws, new control methods to reduce the apple industry reliance on broad-spectrum insecticides have been explored (Varela et al. 1993; Knight et al. 1994; Witzgall et al. 2008). Several control strategies have been postulated and some of them, e.g., mating disruption, attract and kill technology, and biological control, have produced some promising results. Among these methods, mating disruption of CM appears to be the most promising one with a successful application under different climate conditions, despite reports of some failures with this method (Trimble 1995; Stelinski et al. 2007; Witzgall et al. 2010).

Host finding in the codling moth is largely guided by plant volatiles released from host plants, and these plant volatiles have been characterized extensively (Landolt and Guedot 2008). A multi-front attack strategy with using plant volatile compounds for improving CM management has been suggested (Light et al. 2001; Mitchell et al. 2008). This approach could increase the level of efficacy of pheromone mating disruption (Knight et al. 2005) and improve the efficacy of insecticides (Light and Knight 2011; Schmidt et al. 2008). Terpenoids may play an important role in host finding by the CM (Vallat and Dorn 2005). Masking of these terpenoids or reducing their concentration in the air could increase the success of the "push–pull" strategy that has been suggested in the CM management (Cook et al. 2007). Presence of particular non-host plants or presence of a diversity of plants could offer a new dimension in protecting plants from insect attack (Light et al. 1993; Bender et al. 1999). Our previous studies indicate that certain non-host extracts are toxic and behaviorally active, e.g., antioviposition, attractant against important lepidopteran pest species (e.g., oblique banded leafroller, *Choristoneura rosaceana* Harris, red-banded leafroller, *Argyrotaenia velutinana* Walker and grape berry moth, *Paralobesia viteana* Clemens) (Gökçe et al. 2005, 2006, 2010). Preliminary studies with plant extracts used in this study showed that these plants extracts had some biological activities, e.g., attractant, antioviposition against the CM. Further studies with these extracts against the CM may help us to explore full potential for using them in different control strategies, e.g., mating disruption, attract and kill and push–pull strategies of non-host plant extracts.

The objectives of this study were to determine: (1) the effects of the non-host plant extracts, pheromone, and pear ester on behavioral and antennal responses of mated and unmated CM; (2) the impact of non-host plant extracts on CM female oviposition rate; and (3) the ovicidal activity of non-host plant extracts.

## Materials and methods

### Insects

CM pupae were obtained in corrugated cardboard strips from the colony maintained at the USDA-APHIS Wapato, WA. Pupae were sorted by sex as described in Peterson (1965) into plastic (32.5 × 32.5 × 32.5 cm) Bugdorm cages (the BugDorm Store, http://bugdorm.megaview.com.tw) and held in Percival growth chambers (Percival Scientific, Perry, IA, USA) under environmental conditions of 24 °C, 60% relative humidity (RH), and 16:8 Light:Dark (L:D) photoperiod until emergence. Emerged moths were provided with 5% sucrose solution dispensed via dental wicking material sticking through the lids of 1.0 oz SOLO portion cups.

### Tested materials

Plant extracts tested in behavioral studies are given in Table 1. These plants extracts were prepared in the laboratory as described previously in Gökçe et al. (2005). Plant materials were collected in Tokat, Turkey (43.4°N, 36.5°E), dried at room temperature and subsequently ground into a fine powder. Each plant sample (50 g) was soaked in 500 ml of methanol (Sigma) in a 1000 ml Erlenmeyer flask for 24 h, and then, the suspension was filtered through two layers of cheese cloth. Excess methanol was evaporated in a rotary evaporator, and the resulting residue was eluted with acetone to yield 20% (w/v) plant suspensions. The pear ester [ethyl(*E*,*Z*)-2,4-decadienoate] and codlemone [(*E*,*E*)-8,10-dodecadien-1-ol] were > 98% isomerically pure (Bedoukian Co, Danbury, CT, USA) and used as a chemical standard in behavioral tests.

**Table 1** Name of species, family, plant tissue, and collection location of plants used in the study

| Scientific name | Family name | Tissue used | Collection location |
|---|---|---|---|
| *Arctium lappa* | Asteraceae | Whole Plant | Tokat |
| *Bifora radians* | Apiaceae | Whole Plant | Tokat |
| *Humulus lupulus* | Cannabaceae | Cone | Tokat |
| *Verbascum songaricum* | Scrophulariaceae | Leaves | Tokat |
| *Xanthium strumarium* | Asteraceae | Fruit | Tokat |

## Electroantennogram (EAG) recordings

The electroantennogram (EAG) system and test protocols were performed as described previously (Stelinski et al. 2003). A data acquisition interface board (Type IDAC-02) and a universal single-ended probe (Type PRS-1) from Syntech (Hilversum, The Netherlands) were used. The recording and different electrodes were comprised of silver wire in a 10 µl glass micropipette filled with a 0.5 M KCl solution. A computer equipped with an interface card and software (PC-EAG version 2.4) from Syntech was used to record data. EAG cartridges were produced by pipetting 0.25 mg of each plant extract (Table 1) in 25 µl of acetone or 5 mg codlemone and 5 mg pear ester in 25 µl of hexane onto 1.4 × 0.5 cm strips of Whatman No. 1 filter paper. To evaporate the solvent, filter papers were set in a fume hood for 15 min prior to testing. Treated strips were then inserted into disposable glass Pasteur pipettes. Using live insect preparations, 1 ml puffs of air were elicited through EAG cartridges to measure EAG response as maximum amplitude of depolarization.

Male and female codling moths were 2–4 days old when used for electroantennograms. Insects were mounted on a wax-filled, 3.5-cm diameter Petri dish with a clay strip (10 × 3 mm) covering their thorax and abdomen. A recording electrode was positioned above the apex of the antennae while the reference electrode was inserted near the base of the head in close proximity to the antenna. EAGs were recorded for 10 insects of each sex for all chemicals. Filter paper soaked with 20 µl of acetone solvent or hexane was delivered prior as well as after each stimulus presentation as reference control stimulations. For each replicate of moths, two puffs of each volatile treatment and control were applied (12 s apart) to the antenna to produce duplicate depolarization amplitudes. The experiment was performed in a randomized complete block design using chemical odor and CM sex as factors.

## Olfactometer

The olfactometer system and test protocols as described by Gökçe et al. (2005) were used in the experiment. Males and females of CM used in this study were 3 days old adults. The sticky liners of pheromone traps (LPD Scenturion Guardpost, Suterra, Bend, OR) were cut to make 55-mm diameter disks for catching the moths. A 20-mm diameter Whatman Number one filter paper disk was placed centrally on top of each 55-mm sticky disk and then transferred into a sterile 90-mm disposable Petri dish prior to insertion in the olfactometer. Twenty-five milligrams of each plant extract was diluted in 250 µl acetone, and then, 25 µl of the plant extract suspension was applied to the central filter paper disk. In the control treatment, 25 µl of acetone was applied to the disk. In addition to these control disks, the CM pheromone and pear ester were also used as a standard. The treated disks were left to dry for 15 min in a fume hood prior to performing the assays. Using clean forceps, the disks (five plant extracts and one control) and rubber septa with pheromone and pear ester were transferred into an eight-arm olfactometer (Gökçe et al. 2005). The wheel olfactometer was connected to a vacuum pump set at 100 mmHg, which pumped clean air through a hydrocarbon filter into the olfactometer.

In the unmated CM experiment, male and female moths were tested separately. Ten-unmated codling moths were inserted into the central release point of the olfactometer and incubated at 24 °C under a 16:8 h L:D photoperiod. Counts of CM in each olfactometer arm were made after 24 h. The experiment was repeated on six different days, and for statistical analysis, the data were blocked by days. In the experiment with mated CM females, moths were allowed to mate for 24 h prior to the experiment. All preparation for extracts and other chemicals and experimental protocol was similar to those described above. After mating, 10 males and 10 females were released into the olfactometer and incubated at 24 °C under 16:8 h L:D photoperiod. When the experiment was completed, the females were examined for their mating status as described in Mantey and White (1975). Only mated females ($n = 71$) were recorded for statistical analysis.

## Wind tunnel

Based on male CM behavioral data in the olfactometer, further bioassays were conducted in a Plexiglas flight tunnel (Stelinski et al. 2004) to evaluate male response to pheromone and pheromone + *Xanthium strumarium* extract. Male CM (2–3 days old) were collected 30 min prior to assay in aluminum wire mesh cylinder-shaped cages, 8 cm long × 8 cm diameter, with removable plastic Petri dish serving as lids on the open ends. Moths were caged in pairs of the same sex and held in the flight tunnel room maintained at 50–70% RH and 16–18 °C for 30 min to acclimate the conditions. Red rubber sleeve stoppers, (#S0511-Plasticoid) containing 5 mg codlemone and 20 mg plant volatile extract, were placed into the flight tunnel upwind of the cages. Stoppers were pinned 1 cm above a horizontal yellow index secured on the arm of a ring stand 25 cm above the wind tunnel floor. A release cage containing one moth was placed into the airflow at 2 m directly downwind of the stopper. Response of moths was recorded for 3 min until moths stopped movement. Moth behaviors were recorded as either: No Response, Wing Fanning—whereby moths flutter their wings rapidly, Upwind Orientation—flying toward the volatile source, contacting the source, or No Orientation—where the moths either did not respond or flew immediately to the back of the wind tunnel. For each replicate, 10 insects were used, and the whole experiment was repeated three different times for a total of 30 insects per treatment.

### Oviposition bioassay

The protocol for testing antioviposition effects of plant extracts was conducted as previously described in Gökçe et al. (2005). One liter bioassay cups 140 mm in height and 110 mm in diameter were used. Four windows (30 × 30 mm), 90° apart around its circumference, were cut into each bioassay cup and covered with fine mesh. Wax paper strips were cut into 50 × 100 mm strips that were wiped with acetone prior to applying plant extracts. One hundred µl of acetone suspended plant extract (20% w/v) was applied to each side of the wax paper strips and spread with a sterile bent glass rod "hockey stick." In the control treatment, 100 µl of acetone was applied to each side of the wax paper. The treated wax papers were left to dry for 15 m in a fume hood. Each cup contained one acetone-treated wax paper and one plant extract-treated wax paper, arranged 30 mm from the edge of cups and suspended by strings from the top of the cup, as a choice test. A 5% sucrose solution was provided within bioassay cups to act as food sources. Five female and five male CM adults (1–3 days old) were transferred into each bioassay cup. Freshly treated wax paper was replaced daily. The number of individual eggs per wax paper strip was counted for 7 days. A randomized complete block design was used in this study, with each block consisting of four treatment–control cups and one control–control cup, and the entire experiment was replicated six times.

Ovicidal activity of the plant extracts was also quantified in this experiment. The eggs, obtained in antioviposition study, were incubated at 24 °C with 16:8 h L:D photoperiod for 10 days. Hatch rate of eggs both in the treatment and in the control was recorded, and percentage of egg hatch was calculated for each treatment.

### Statistical analysis

Analysis of variance (ANOVA) ($\alpha = 0.05$) was conducted on EAG data, and differences in pairs of means between treatments were separated using Tukey's multiple comparisons test ($\alpha = 0.05$) (Minitab Release 16, McKenzie and Goldman 2011). In the olfactometer tests, the number of male or female insects arriving at each treatment arm was expressed as a percentage of the total number of insects tested in each replicate. The resulting preference values for the treatments resulted in 100%. The data were transformed and normalized using arcsine transformation (Zar 1999) and were then analyzed with ANOVA and followed by Tukey's test to detect difference between treatments. Two-sample *t* tests (Minitab Release 16) were performed to determine whether moth response to plant extracts and pheromone varied significantly between the sexes.

For the oviposition choice test, the number of eggs counted on each treatment was presented as a percentage. Within replicates, the cumulative number of eggs laid on each treatment was divided by the total number of eggs laid. The resulting preference values for the treatments resulted in 100%. The data were transformed to normalized using arcsine transformation (Zar 1999) and were then subjected to paired *t* tests ($\alpha = 0.05$) (Minitab Release 16, McKenzie and Goldman 2005).

The percentage of egg hatch was calculated by dividing the number of hatched eggs by the total number of egg on each treatment. Data were arcsine transformed and subjected to paired *t* tests ($\alpha = 0.05$) for detecting difference between the treatment and the control (Minitab Release 16, McKenzie and Goldman 2005). The wind tunnel assay data were recorded as a percentage for each designated behavior, transformed into arcsine, and subjected to *t* tests ($P < 0.05$) for differentiating differences between treatments.

## Results

### Electroantennogram

Male and female CM exhibited varying EAG responses to the tested chemicals and the control (Table 2). EAG responses of male CM to pheromones (Table 2) were nearly four times higher ($F = 18.63$, $df = 7.79$, $P < 0.01$) than those of females. However, females showed a significantly higher response ($P < 0.01$) to pheromone (Table 2) as compared with the control. The chemical standard, pear ester, caused high EAG responses from both sexes of CM as compared with the control; however, there was no significant difference between them. All plants extracts, except *Arctium lappa* and *Humulus lupulus*, elicited significantly higher EAG response

**Table 2** Electroantennogram responses of male and female codling moth (*Cydia pomonella*) to various plant extracts, pheromone, and pear ester

| Treatment | EAG responses (mV ± SE)[a] upon stimulation with 1 ml of air through stimulus cartridge | | |
|---|---|---|---|
| | Males | | Females |
| Blank | 0.2 ± 0.03d | NS | 0.2 ± 0.03c |
| Pheromone | 6.2 ± 1.2a | * | 1.6 ± 0.1a |
| Pear ester | 2.0 ± 0.2b | NS | 1.7 ± 0.1a |
| *B. radians* | 1.7 ± 0.1b | * | 0.7 ± 0.1b |
| *X. strumarium* | 2.3 ± 0.2b | * | 0.8 ± 0.1b |
| *H. lupulus* | 1.7 ± 0.2b | * | 0.3 ± 0.1c |
| *A. lappa* | 0.6 ± 0.2c | NS | 0.2 ± 0.03c |
| *V. songaricum* | 1.4 ± 0.2b | * | 0.8 ± 0.04b |

[a]Means within columns followed by the same letter are not significantly different ($P = 0.01$, Tukey's multiple comparisons test). Paired values within rows marked with an asterisk are significantly different ($\alpha = 0.05$), and NS indicates lack of significance

from female CM than the control ($F$'s = 27.41, $df$ = 7.79, $P < 0.01$) (Table 2). The greatest EAG responses from male CM were observed to *X. strumarium* extract (Table 2), while for female CM, the greatest EAG response was observed with *Verbascum songaricum* and *X. strumarium* among the tested plant extracts (Table 2).

## Olfactometer study

Behavioral of male CM in the olfactometer differed significantly in response to the tested non-host extracts, pear ester, pheromone, and the control ($F$ = 8.43, $df$ = 7.40, $P < 0.01$). The greatest percentage of male moths (34.5%) was attracted to the pheromone (codlemone) treatment (Fig. 1). This response was similar to that recorded for the *X. strumarium* extract (24.8%), and there was no significant difference between these two treatments with respect to the total number of males captured. The *Bifora radians* extract and pear ester captured 8.4 and 7.5% of males, respectively, but these treatments were not significantly different from response to the control ($P > 0.05$) (Fig. 1). The fewest moths were captured in *H. lupulus*, *V. songaricum,* and *A. lappa* treatments with 2.7, 2.7, and 4.6% of males captured, respectively (Fig. 1).

The responses of female CM adults between treatments were also statistically different in the olfactometer assays ($F = 9.53$, $df = 7.47$, $P < 0.01$). *V. songaricum* and *A. lappa* treatments attracted most moths, capturing an average of 25.4 and 22.9% of the released females, respectively (Fig. 2). About 14.5% of the released moths were captured with the pear ester treatment, and this was followed by *B. radians* extract with 11% of CM females captured (Fig. 2). These four treatments were significantly different from the control treatment ($P < 0.05$). CM sex pheromone and *X. strumarium* captured only 7.2 and 5.6% of female CM, respectively, and these were statistically similar to the control captures.

In the mated CM experiment, although varying numbers of moth were captured among different treatments, there was no significant difference between them ($F = 1.52$, $df = 7.47$, $P = 0.19$). Pear ester captured the most mated females with 18.4%, and it was followed by *V. songaricum* extract, which captured 14.9% of the females. Approximately 12.9% of the females were found captured by the *A. lappa* extract treatment. The other treatment captured varying percentage of females ranging from 7.1% for the sex pheromone to 12.6% for *H. lupulus*.

## Wind tunnel assay

The plant extract, *X. strumarium*, was further investigated in the wind tunnel assay. There was significant difference between pheromone and pheromone + *X. strumarium* in all categories, except for non-oriented flight, and it appeared that the plant extract + pheromone caused greater response than pheromone alone (Fig. 3). The increase was clearer in upwind flight and touching on the

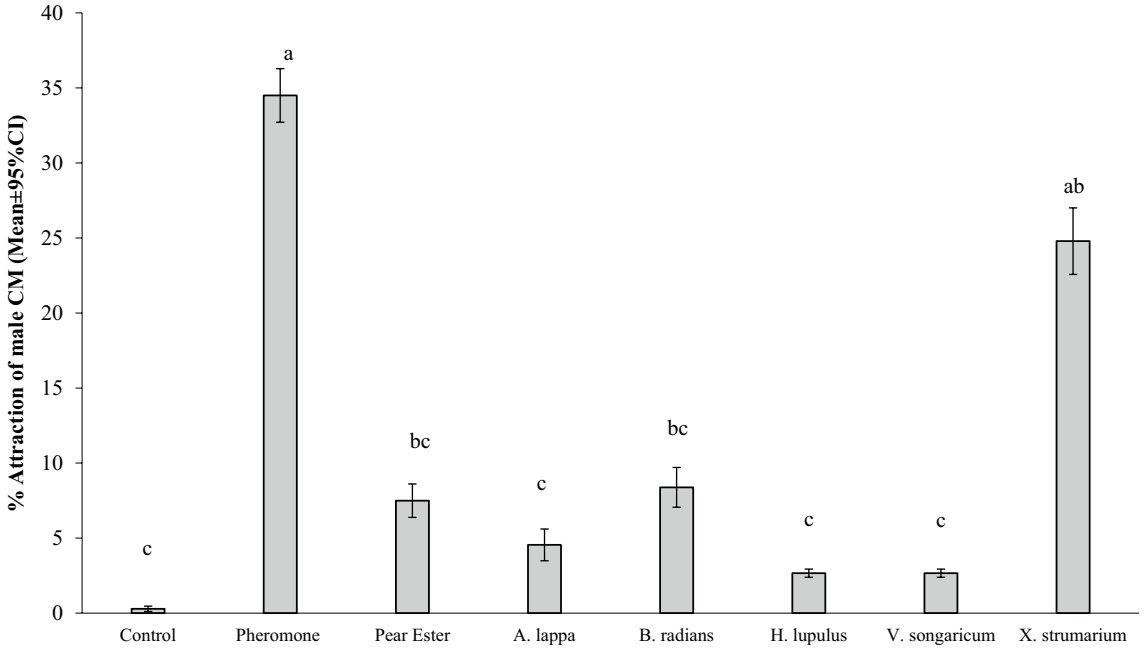


**Fig. 1** Mean number of male codling moth (*Cydia pomonella*) attracted to various non-host plant extracts, pheromone, pear ester, and control. Bars indicated by the same letter are not significantly different (ANOVA followed by Tukey's test, $\alpha = 0.05$). Error bars indicate ± 95% confidence intervals

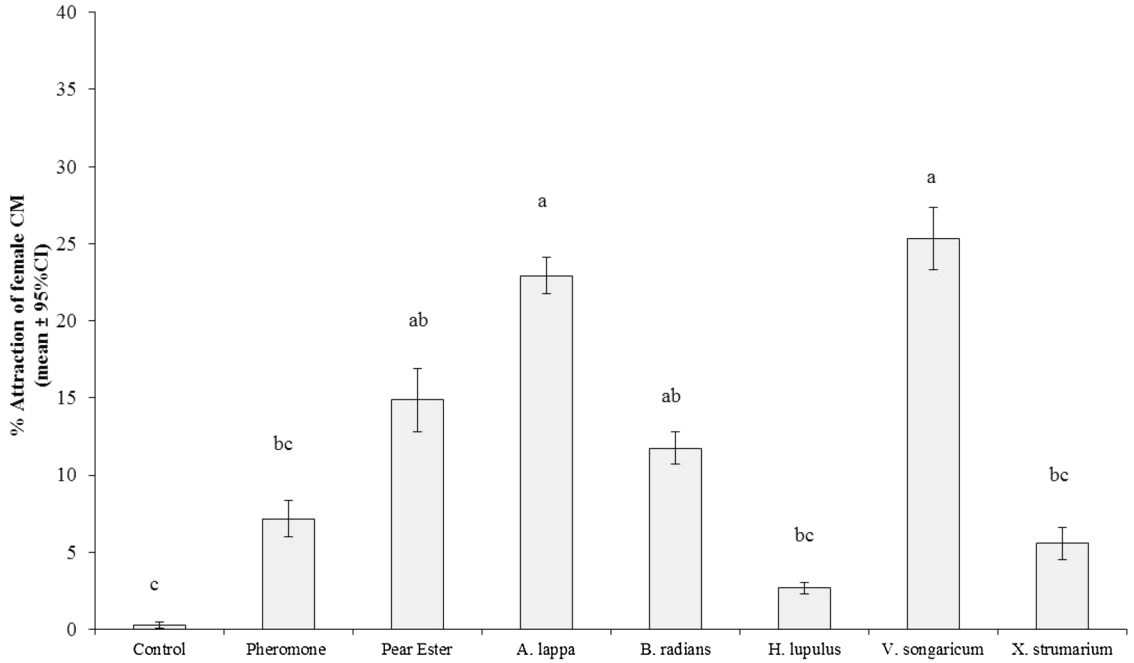


**Fig. 2** Mean number of female codling moth (*Cydia pomonella*) attracted to various non-host plant extracts, pheromone, pear ester, and control. Bars indicated by the same letter are not significantly different (ANOVA followed by Tukey's test, $\alpha = 0.05$). Error bars indicate ± 95% confidence intervals

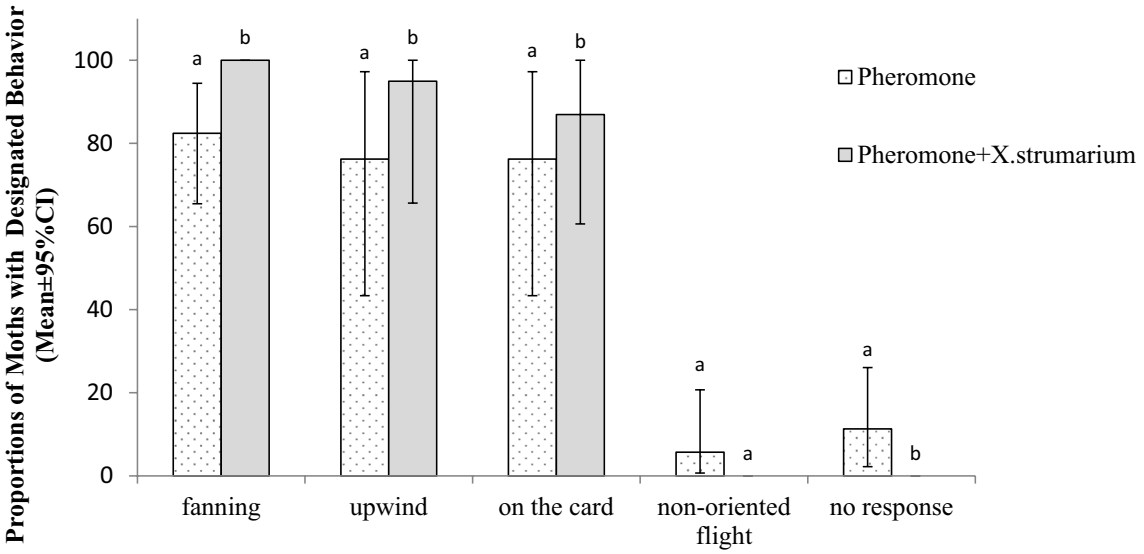


**Fig. 3** Mean (± 95% CI) proportion of behavioral responses exhibited by male codling moth (*Cydia pomonella*) to pheromone and pheromone + *X. strumarium* treatments. Different lower case letters represent statistically significant differences between treatments according to the *t* test ($\alpha = 0.05$)

source categories. Significantly, more moths performed upwind flight (94.9%) in response to pheromone + *X. strumarium* ($t = -4.89$, $df = 2$, $P < 0.05$) than in response to pheromone alone (76.3%). Pheromone + *X. strumarium* also increased contact with the card containing the

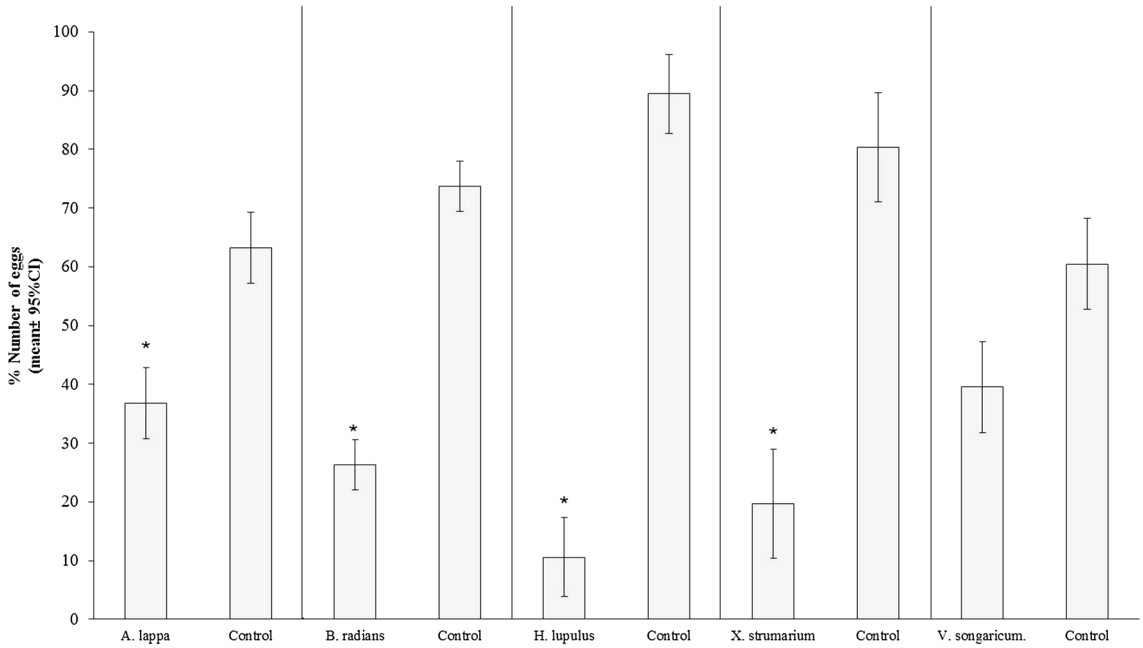


**Fig. 4** Mean number of eggs laid by female codling moth (*Cydia pomonella*) on plant extract versus control group in a choice assay. Bar indicated by an asterisk is significantly different from the control treatment (Paired *t* tests, $\alpha = 0.05$). Error bars indicate ± 95% confidence intervals

odor source (86.9%) as compared with pheromone alone (76.2%) ($t = -5.16$, $df = 2$, $P < 0.05$).

### Oviposition bioassay

All tested non-host plant extracts reduced the number of eggs laid by female CM (Fig. 4). The most pronounced effect was observed with *H. lupulus* extract, and only 10.6% of eggs was laid on the wax paper treated with this extract. This was significantly lower ($t = -9.95$, $df = 5$, $P < 0.01$) than for the control (89.4%) (Fig. 4). Similar reduction was also observed on wax paper treated with *X. strumarium*, where 19.6% of deposited eggs was recorded which was significantly lower ($t = -6.20$, $df = 5$, $P < 0.01$) than in the control (80.4%) (Fig. 3). *B. radians* (26.3%) and *A. lappa* (36.3%) treatments caused significant reduction in deposited eggs as compared with the control ($P < 0.05$). Although wax paper treated with *V. songaricum* extract contained 39.5% of total laid eggs, this was not statistically different from the control treatment ($t = -1.67$, $df = 5$, $P = 0.16$) (Fig. 4).

Egg hatch was significantly reduced by the plant extracts (Fig. 5) ($P < 0.05$). Similar to the oviposition pattern, the lowest egg hatch rate was recorded with *H. lupus* (21.5%) and *X. strumarium* (21.9%) extracts. The greatest egg hatch rate was observed with *V. songaricum* extract (50%). The other two extracts, *B. radians* ($t = -9.84$, $df = 5$, $P < 0.05$) and *A. lappa* ($t = -10.73$, $df = 5$, $P < 0.05$), also significantly reduced egg hatch rate as compared with the control (Fig. 5).

## Discussion

CM is a serious pest of pome fruits and a key pest of apples worldwide. Control strategies primarily rely on insecticide applications and mating disruption with pheromone (Knight 2008; Witzgall et al. 2008, 2010). These technologies generally provide good control of this pest and keep its population below injury thresholds (Witzgall et al. 2008). However, as reported by Trimble (1995) and Witzgall et al. (2008), the use of pheromone for mating disruption is only effective at low population densities of this pest and has failed to control CM at higher population densities. In recent years, plant volatiles have been admixed with sex pheromone (codlemone) in an effort to improve against mating disruption and monitoring of CM (Knight et al. 2005; Schmera and Guerin 2012). Neuroethological investigations have also elucidated that processing of sex pheromone signals and plant volatile cues are coded together in the CM central nervous system (Trona et al. 2013).

Pear esters, β-farnesene, and (*E*,*E*)-farnesol are main plant volatiles used to increase the attraction of both male and female CM. The pear ester attracted both male and female CM moths in combined numbers that were comparable to the attractiveness of conspecific sex pheromone. A series of structure–activity tests were conducted in orchard trials to determine CM attraction specificity to the pear ester kairomone (Doughlas and Knight 2005). Admixing pear ester (3 mg) with codlemone (3 mg) was reported to increase male CM captures in monitoring traps (Knight

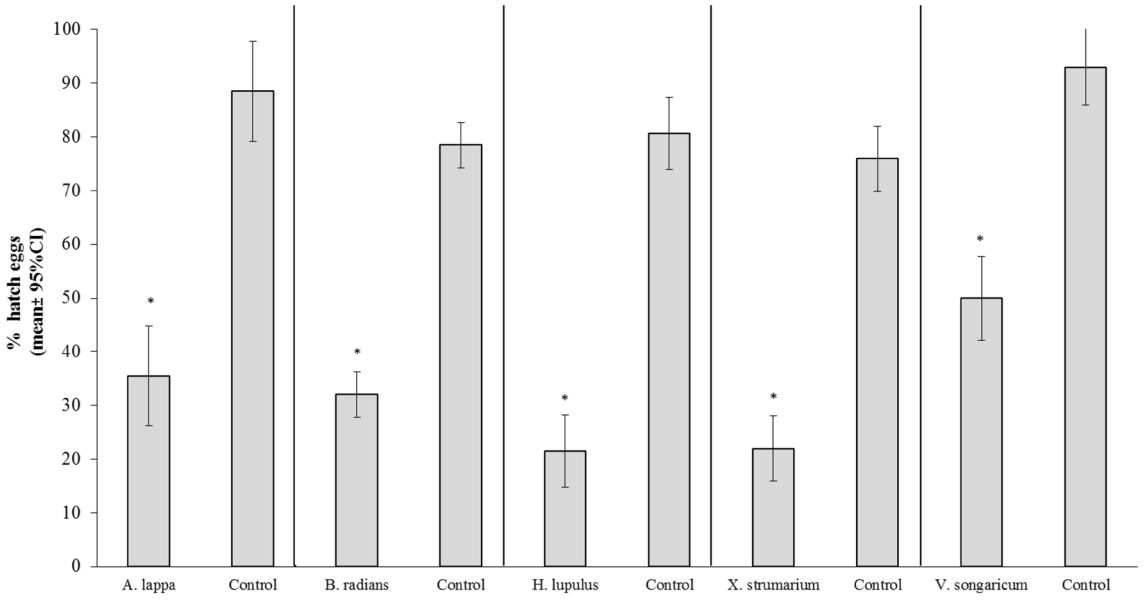


**Fig. 5** Ovicidal activity (± 95% CI) of plant extracts to codling moth (*Cydia pomonella*) eggs. Bar indicated by an asterisk is significantly different from the control treatment (Paired *t* tests, $\alpha = 0.05$). Error bars indicate ± 95% confidence intervals

et al. 2005). However, Knight et al. (2012) reported that simultaneously releasing of codlemone and pear ester did not significantly increase capture male CM as compared with codlemone alone in both Washington and Michigan field tests. In the current study, pear ester caused moderate EAG responses from male moth antennae, which were lower than those recorded in response to codlemone and *X. strumarium* extract. A similar trend with behavioral response was observed in the olfactometer study.

CM pheromone released with *X. strumarium* extract resulted in greater capture of male CM than pheromone alone. *X. strumarium* is known to release sesquiterpene lactones (xanthanolides), which are responsible for most of the biological activities associated with *Xanthium* species (Kamboj and Saluja 2010). *X. strumarium* fruits contain various bioactive compounds, e.g., 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol-thiazine-3,5-dione, chlorogenic acid, ferulic acid, and formononetin (Han et al. 2006). High terpenoid and acid contents of *X. strumarium* fruit extract may explain its possible combined effect with codlemone on CM behavior. Further elucidation and characterization of possible behaviorally active compounds, e.g., antioviposition and attractant from *X. strumarium* fruit extract, are planned to understand interactions between the extract and codlemone.

Green leaf volatiles play important role in host finding, especially for egg-laying females (Light et al. 1993; Pinero and Dorn 2007). Aldehydes, alcohols, and acetates are the main green leaf volatiles found among most plant species. Their proportion is believed to be key factor for host recognition and selection for herbivores. Generally, plant volatiles are more important in highly specialized insect species than polyphagous species. In the current study, female CM showed preference for *V. songaricum* in both the olfactometer and the antiovipoisition bioassays. Many saponins, iridoids, phenylethanoid glycosides, monoterpene glucosides, neolignan glucosides, flavonoids, steroids, and spermine alkaloids were isolated and characterized from *V. songaricum* extracts (Tatli and Akdemir 2004). These green leaf volatiles could play a role in CM female response to *V. songaricum* extract. This plant extract also elicited EAG responses in both *A. velutinana* and *C. rosaceana* and attracted significantly more female *C. rosaceana* than any other treatments (Gökçe et al. 2005).

*Xanthium strumarium* and *H. lupulus* extracts caused high levels of both antioviposition and ovicidal activity against female CM. These results are congruent with previous investigations (Gökçe et al. 2005, 2006), where similar activities of these plant extracts against other lepidopteran moths were reported. Both of these extracts are rich in secondary metabolites especially alkaloids, acids, and terpenoids (Zanoli and Zavatti 2008; Kamboj and Saluja 2010). Repellent and insecticidal effects of these compounds against many insect species were documented in previous studies (Ulubelen et al. 2001; Badgujar et al. 2011). Developing new tools for CM management using non-host volatiles to push female CM away from host trees and also reducing egg deposition and hatch rate could be useful additions to current management programs. *X. strumarium* and *H. lupulus* extracts should be further explored by refining these extracts or isolating and characterizing the active compounds. Bioassay-guided

characterization with associated chemical identification is underway for elucidation and characterization of active compounds from these plant extracts.

In the current study, the effects of several non-host plant extracts on CM behavior and oviposition were examined. Certain extracts showed potential for modifying CM behavior and possible population reduction. Field studies with the promising extracts are underway, and these will help to better understand the role of non-host plant extracts for management of CM.

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**Author contribution** AG, LLS, and MEW designed the research. AG, LLS, and MEW conducted the experiments. AG and LLS analyzed the data. AG, LLS, and MEW wrote the manuscript. All authors read and approved the current manuscript.

## Compliance with ethical standards

**Conflict of interest** All authors declare no conflict of interest.

**Ethical approval** This manuscript does not contain any studies involving human participants and/or animals.

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