

Research Article

Integration of pheromone and light traps for mass trapping of leopard moth, *Zeuzera Pyrina* (Lepidoptera: Cossidae) in walnut orchards

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Abstract

Leopard moth, *Zeuzera pyrina* L., is a dangerous wood borer pest of the walnut trees in different parts of the world. The larvae bore deep into twigs, branches and trunks, and its chemical, biological and mechanical control is difficult, therefore use of mass trapping is an efficient method to reduce the pest population. The purpose of this study was to increase the efficiency of mass trapping by combining the light and pheromone baited traps in walnut orchards. The study was conducted based on the randomized complete block design with three treatments (pheromone trap, solar-powered insect light trap and combined pheromone trap and light) and six replicates during 2014-2015. Pheromone traps were installed one meter below the apical point of tree canopy before the starting of the adult emergence (May 10th). Observation was done weekly during the moth flight period and the number of captured moth was counted and compared in different treatments. Based on the results, in all sampling dates, the highest number of captured males (65.6 ± 4.38 and 79.2 ± 1.25 in 2014 and 2015, respectively) observed in combined pheromone trap and light, whereas the lowest (27.6 ± 1.28 and 27.6 ± 3.78 in 2014 and 2015, respectively) observed in the light trap treatment. Combining of light with pheromone trap significantly increased the efficiency of *Z. pyrina* mass trapping in the walnut orchards.

Key words: Non-chemical control; Wood borer; Walnut; Sex pheromone; Light; Captured moth

تلفیق تله‌های فرمونی و نوری برای شکار انبوه کرم خراط *Zeuzera Pyrina* (Lepidoptera: Cossidae) در باغ‌های گردو

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چکیده

پروانه فری یا کرم خراط، *Zeuzera pyrina* یکی از خطرناک‌ترین آفات چوبخوار گردو در ایران و سایر نقاط جهان به شمار می‌رود. از آنجایی که لاروهای آفت درون سرشاخه، شاخه‌های اصلی و فرعی و تنه درخت زندگی می‌کنند، کنترل آنها مشکل است. بنابراین به کارگیری تله‌های فرمونی برای شکار انبوه حشرات نر، یکی از مهم‌ترین روش‌ها برای کاهش جمعیت آفت می‌باشد. هدف از این تحقیق تلفیق تله‌های فرمونی و نوری به منظور افزایش شکار انبوه آفت در باغ‌های گردو بوده است. آزمایش در قالب طرح بلوک‌های کامل تصادفی شامل سه تیمار (تله فرمونی، تله نوری تغذیه شده با سلول‌های خورشیدی و تلفیق تله فرمونی و نور) و شش تکرار در سال‌های ۱۳۹۳-۱۳۹۴ اجرا شد. تله‌های فرمونی در تاریخ ۲۰ اردیبهشت ماه (قبل از ظهور حشرات کامل)، در ارتفاع ۱ تا ۲ متر پایین‌تر از نقطه انتهایی تاج درخت نصب شدند. آماربرداری و بازدید از تله‌ها به صورت هفتگی در دوره پرواز شب‌پره‌ها انجام شد و تعداد شب‌پره‌های شکار شده در تیمارهای مختلف مقایسه شد. نتایج نشان داد، بیشترین میزان شکار حشرات نر در تیمار تلفیق تله فرمونی با نور (به ترتیب $65.6/60 \pm 4.38$ و $79.2/70 \pm 1.25$ در هر تله) و کمترین شکار در تیمار تله نوری (به ترتیب $27.6/60 \pm 1.28$ و $27.6/70 \pm 3.78$ در هر تله برای سال ۱۳۹۳ و ۱۳۹۴) مشاهده شد. تلفیق تله فرمونی و نور به طور معنی‌داری شکار انبوه پروانه فری را در باغ‌های گردو افزایش داد.

واژه‌های کلیدی: کنترل غیرشیمیایی، کرم خراط، گردو، فرمون جنسی، نور، شکار پروانه.
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Introduction

Walnut is one of the valuable and important fruit trees, which is cultivated in different parts of the world. The Leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae) is a dangerous wood borer insect which is considered as the most important pest of the walnut and pome fruit trees in middle east, north Africa and south Europe (Katsoyannos, 1992; Radjabi, 2002; Kutinkova *et al.*, 2006). The pest attacks over 150 plant species from 20 genera (Carter, 1984; Gratwick, 1992; Kutinkova *et al.*, 2006). Among the fruit trees, the pest causes significant damage and yield loss on apple, pear, olive and walnut (Sarto-Monteys, 2001; Hegazi, 2016; Saeidi, 2020). The larvae of *Z. pyrina* cause a serious damage to the trees by boring into the twigs, branches and trunks, weakening and sometimes killing them. The damage can be particularly extensive in the nurseries and young trees of apple and olive bearing a fruit load which may cause complete death of them (Esmaili, 1991; Hegazi *et al.*, 2016).

Different control methods were reported for the management of *Z. pyrina*. Regular irrigation and appropriate nutrition of the trees, pruning of infested branches, injection of the fumigant insecticides into the active holes (Esmaili, 1991; Radjabi, 2002), killing the larvae inside the galleries using screw elastic wire and biological control using the green lacewing predator, *Chrysoperla carnea* (Stephens) (Merghem & Ahmad, 2017) and entomopathogenic nematodes (Ashtari *et al.*, 2011) were suggested to be effective against the pest in walnut and olive orchards. Kolyaee & Hasani (2014) suggested combining of the mentioned methods with mass trapping of the males in the walnut orchards. Mass trapping of the males using the sex pheromones reported as a successful method for controlling of the pest in hazelnuts (Isart *et al.*, 1997), olive (Hegazi *et al.*, 2009), apple (Almanoufi *et al.*, 2012) and walnut orchards (Kolyaee & Hasani, 2014; Saeidi, 2020).

Several attempts have been made to increase the efficiency of *Z. pyrina* mass trapping. The color, type and size of the traps reported as important factors for increasing efficiency of *Z. pyrina* mass trapping (Kutinkova *et al.*, 2009; Ardeh *et al.*, 2014; Besharatnezhad *et al.*, 2016). Study of Kolyaee & Hasani (2014) showed that application of 16 delta traps per hectare significantly increased the number of captured males compare to 12 and 8 traps. Installation height of the traps also reported as another important factor which significantly influenced the number of captured moth (Saeidi, 2020). Installation of the traps at the height of one meter above the canopy in apple (Pasqualini & Natale, 1999) and olive orchards (Hegazi *et al.*, 2009), and one meter below the apical point of canopy in walnut trees (Saeidi, 2020) significantly captured more number of males compare to the other heights.

Since the *Z. pyrina* larvae bore deep into twigs, branches and trunks, and the chemical, biological and mechanical control of the larvae is extremely difficult and do not have the desired results in many cases, therefore the use of mass trapping method to capture the adults, is an efficient and appropriate method to control of this pest. Considering the use of both light and pheromone baited traps which were recommended for mass trapping of the pest, the purpose of this study was to increase the efficiency of mass trapping by combining the light and pheromone baited traps in walnut orchards.

Materials and Methods

The studied area

The experiments were conducted in the Hossain Abad orchard, Saman, Chaharmahal va Bakhtiari Province, Iran. The studied area is located at an elevation of 2075 meters above the sea level and at latitude 32° 28' 12" to 32° 28' 30" N and longitude 50° 54' 36" to 50° 54' 54" E. The orchard covers about 100 ha area and is one of the most infested to *Z. pyrina*. Trees were approximately 25-30 years old, 13-14 m height and planted at 10×8 m distances between and along the rows. No chemical was applied on experimental plots during the experimental period.

Pheromone, trap and installation height

The pheromone dispensers, type of trap and installation height were followed according Saeidi (2020). Delta traps (triangle-shaped houses, 21 × 18 cm in base and 11 cm in height) were made of plastic (2 mm diameter) (Nafis Cartonplast Ind., Iran). A sticky insert (21 × 18 cm in length and width, respectively) was placed inside of each trap. One pheromone dispenser of *Z. pyrina* (PH-990-1PR, Russell IPM Company, UK) was placed in the center of sticky insert. The dispensers were polyethylene containers loaded each with 10 mg of *Z. pyrina* pheromone; 95% (E, Z)-2, 13-octadecenyl acetate and 5% (E, Z)-3, 13-octadecenyl acetate. Delta traps were installed at a height of 12 m above ground level (about 1 meter below the apical point of tree canopy) on the outer edge of the tree canopy.

Designing of the light trap

A solar-powered insect light trap was designed (Figure 1). The main components of the trap were including the insect collecting unit, light source, rechargeable battery, solar panel and electrical control unit. The light source (lamp) was designed to turn on after sun set (at 7:30 p.m.) and turn off before sun rise (at 5:30 a.m., local time).

1. Collecting unit: consists of a funnel-shaped trap constructed from galvanized iron sheet with top outline diameter of 50 cm, height of 25 cm and insect circular exit hole with diameter of 5 cm. Three sheet blades (deflectors) built from galvanized iron sheet (10×10×20 cm for top width, bottom width and height, respectively) were arranged around the lamp that moths hit them and fall into collecting net (material: polypropylene plastic, hole size: 3 mm

in diameter). A hood made from a thick transparent plastic (60 cm in diameter, 1.5 mm thickness, iron frame) was placed on top of the collecting unit for protecting the components from rain, dust and direct sunlight.

2. Light source: A light emitting diodes bulb (LED) (20 W, 220V, 50/60 Hz) which hanged in the middle of sheet blades

3. Rechargeable battery (model: Saba, 12 V, 60 MA) to store the electrical energy produced by solar cell.

4. Solar panel (model: ZT20-18-P, ZYTECH, the dimensions were 510×355×25 mm) for collecting the energy from the sun and generating electricity.

5. Electrical control unit included a programmable microprocessor (model: ATMEGA 128) which installed on one of the sheet blades and programmed to adjust turn on/off the light source (LED) at specific hours.

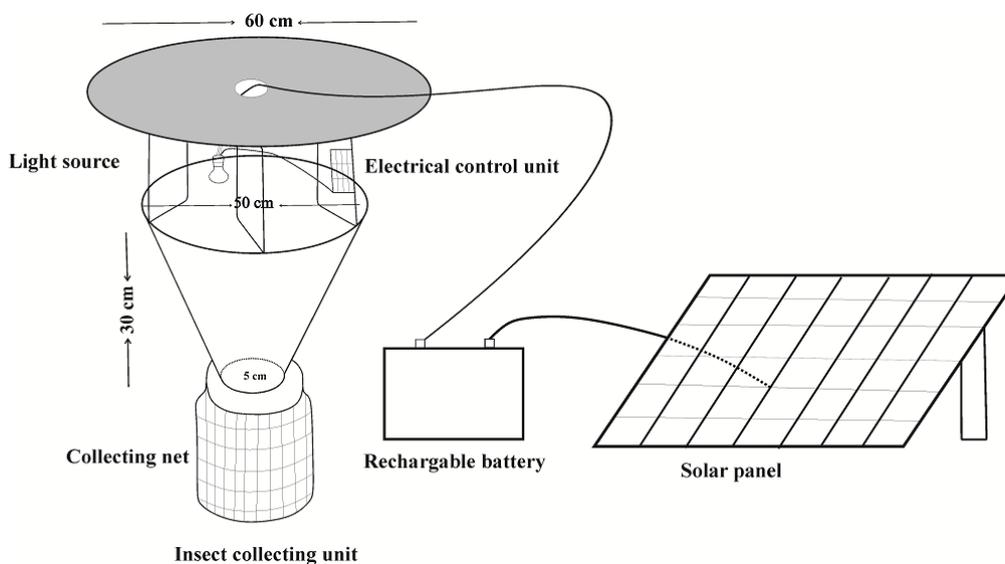


Fig. 1. A schematic diagram of solar-powered insect light trap which was designed to capture *Zeuzera pyrina* males.

Field experiment

A randomized complete block design was conducted with three treatments and six replicates during 2014-2015. Treatments were: pheromone trap, light trap and combined pheromone trap and light. Combined treatment consists of a pheromone trap and a source of light (LED bulb) which hanged 1 meter apart from delta trap to avoid untargeted insects (which attracted to the light) trapped by sticky sheets. The distance between two adjacent blocks was 100 m, whereas the distance between two adjacent traps within the same block was 50 meters to avoid interference between treatments. All traps were hanged at 1 meter below the apical point of trees canopy and leaves and branches were removed around their entrances. The traps were set up in each orchard from May 10 (before the emergence of adult

males) to July 15 (the end of the adults' flight). The numbers of captured leopard moths were recorded weekly. The sticky sheets and pheromone dispensers were replaced every two and four weeks, respectively.

Statistical analysis

Statistical analysis was performed using SAS program (SAS 9.1 Institute Inc.) and SPSS (version 22) software. Proc GLM (General linear model) was performed to identify significant differences among the treatments and means compared using LSD test at $P=0.05$. Data normality was assessed using Kolmogorov–Smirnov test and, data conversion was performed using the formula $\sqrt{x + 1}$, if required.

Results

Analysis of variance on mean number of captured male moths at different sampling times showed significant differences among treatments in both studied years (Table 1). The highest numbers of captured males observed in combined pheromone trap and light, whereas the lowest in light trap treatment (Figs. 1 and 2). In 2014, the first capture was observed on 18th of May, thereafter the population of *Z. pyrina* continuously increased and reached to the maximum density on June 12th and then decreased to minimum value on July 15th. At the peak time of population density (June 12th), the number of captured males in light trap, pheromone trap and combined pheromone trap and light ($F_{2, 15} = 36.22$; $P < 0.0001$) were recorded 27.6 ± 1.28 , 54.2 ± 3.24 and 65.6 ± 4.38 moths/trap/week, respectively (Fig. 2). During the pest flight period (from May 18 to July 16), the total number of captured males in the light trap, pheromone trap and combined treatment (pheromone trap and light) were 122.7 ± 7.33 , 198.40 ± 18.59 and 314.10 ± 24.95 , respectively. Therefore, the efficiency of the combined treatment increased 61% and 31% compared to light and pheromone traps, respectively.

Table 1. Analysis of variance of mean number of captured *Zeuzera pyrina* L. males in different treatments at different sampling dates in both years of study (2014 and 2015).

Source of Variation	df	Mean square											
		May 18	May 23	May 28	May 31	Jun 6	Jun 12	Jun 20	June 26	July 3	July 9		
Year	1	26.69*	2.56**	140.03ns	560.11**	3287.11**	93.50**	536.69**	36.00*	4.69 ns	2.93*		
Replication	10	1.62 ns	12 ns	63.76 ns	97.54 ns	56.34 ns	9.50 ns	19.19 ns	103.02**	3.94 ns	1.36 ns		
Trap	2	56.36**	456.08**	2641.36**	2511.19 **	6352.86**	4671.58**	3692.69**	445.02**	53.08**	9.33**		
Year*Trap	2	6.86*	50.08**	12.86 ns	18.69 ns	125.03 ns	95.58**	30.52 ns	0.58 ns	5.53 ns	0.45 ns		
Error	20	1.57	6.68	39.64	42.87	44.44	14.18	29.61	6.80	1.77	3.53		
CV (%)		41.48	25.01	26.82	13.76	15.40	7.58	14.96	18.78	23.84	48.53		

***, * and ns: significant at 1% probability level, significant at 5% probability level and none significant, respectively

In 2015, similar to the first year of study, mean number of captured males showed significant differences among the treatments in all sampling dates with the highest in the combined treatment (pheromone trap and light). At the peak time ($F_{2, 15} = 93.17$; $P < 0.0001$) of population density (June 6th), the number of captured males were recorded 27.6 ± 3.78 , 51.6 ± 2.34 and 79.2 ± 1.25 moths/trap/week in light trap, pheromone trap and combined pheromone trap and light, respectively (Fig. 3). During the moth flight period, the total number of captured males in light trap, pheromone trap and combined treatment (pheromone trap and light) were calculated 154.7 ± 11.58 , 238.2 ± 17.94 and 381.3 ± 20.91 , respectively. Therefore, the efficiency of the combined treatment increased 59.5% and 37.5% compared to light and pheromone traps, respectively.

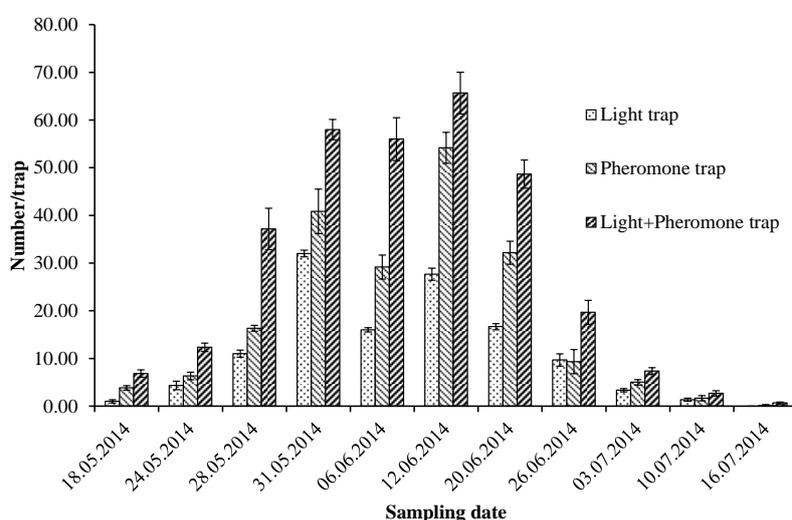


Fig. 2. Mean (\pm SE) number of captured *Zeuzera pyrina* L. males in different treatments at different sampling dates in 2014.

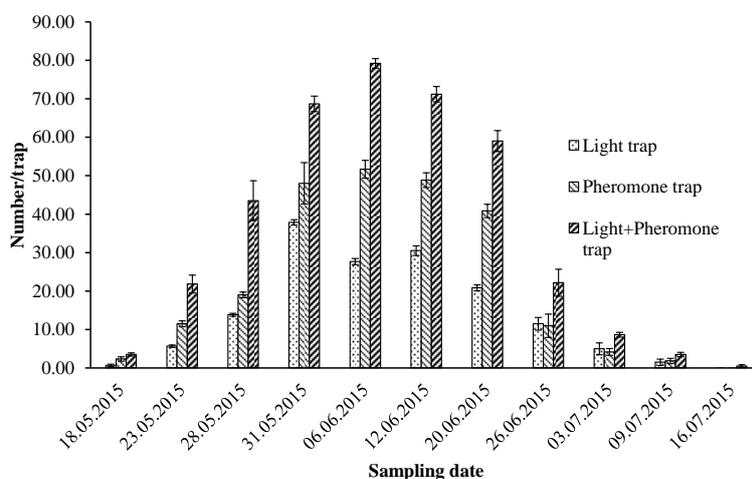


Fig. 3. Mean (\pm SE) number of captured *Zeuzera pyrina* L. males in different treatments at different sampling dates in 2015.

Discussion

Various methods have been suggested for management of *Z. pyrina* in different parts of the world. In the current study we used the sex pheromone traps which was reported as the most effective method for mass trapping (Isart *et al.*, 1997; Hegazi *et al.*, 2009) and mating disruptions of *Z. pyrina* male insects (Sarto-Monteys, 2001; Hegazi *et al.*, 2010). To obtain the desired results in mass trapping technique, it is necessary to consider those factors which influence the efficiency of pheromone traps and increase the number of captured *Z. pyrina* males. The type of pheromone (Saeidi, 2020), color and design of trap (Ardeh *et al.*, 2014), location and installation height of pheromone trap (Saeidi, 2020), the number of traps per hectare (Kolyaee & Hassani, 2014) were reported as the most important factors in mass trapping of *Z. pyrina* males.

In the current study, the pheromone dispensers of *Z. pyrina* was used according to Saeidi (2020) who reported that *Z. pyrina* pheromone dispensers significantly captured more number of males compared to *Synanthedon tipuliformis* (Clerck) pheromone dispensers. Delta trap used in this study, were reported as an effective trap for capturing of *Z. pyrina* males by different researchers (Pasqualini & Natale, 1999; Hegazi *et al.*, 2009; Ardeh *et al.*, 2014; Besharatnezhad *et al.*, 2016; Saeidi, 2020). The traps were installed at the height of 12 m above ground level (in the studied orchards, it was equal to one meter below the apical point of trees canopy). According to Saeidi (2020), the number of trapped moth at this height in the walnut orchards (Saman Chaharmahal va Bakhtiari Province, Iran), was 11.6 and 6.59 times greater than those installed at 4 and 6 meters above the ground levels, respectively.

Since *Z. pyrina* is the most destructive pest of walnut trees and the use of pheromone traps alone might not be effective enough to capture adult males in mass trapping technique, therefore it is necessary to combine this technique with other control methods to reduce the pest population below the economically injurious level. On the other hand, the use of light traps has been reported as another effective method in collecting the adults and reducing the *Z. pyrina* population (Saeidi & Nourbakhsh, 2010). The important finding of this study was increasing the efficiency of pheromone traps by combining them with the light. Our results showed that combining of light with pheromone traps significantly increased the number of captured moth (2.71 and 1.67 times more than pheromone and light trap treatments, respectively). The obtained results were consistent with Hegazi *et al.* (2009) who designed a UV-light-pheromone sticky trap for mass trapping of *Z. pyrina* in olive orchards. According to Mohammad *et al.*, (2018) light traps were useful tools for monitoring and mass trapping of insect pests, which had strong positive photo taxis response. Our observation showed that adult males of *Z. pyrina* had stronger photo taxis response compare to the females and during two- years of study (2014-2015) only the adult males were captured in the light traps. Hegazi *et al.* (2009 & 2106) reported that females of leopard moths were rarely trapped in the UV-

light-pheromone sticky trap, because the females are heavy-bodied and might be extremely poor fliers (Radjabi, 2002; Satro- Monteys, 2015; Hegazi *et al.*, 2016).

Due to the cryptic behavior of *Z. pyrina* larvae which live and feed inside the twigs, branches and trunks of walnut trees, the chemical control of this pest is very difficult, therefore mass trapping of the adult males using the sex pheromone traps, is the alternative way to reduce the damage and population density of *Z. pyrina* (Isart *et al.*, 1997; Hegazi *et al.*, 2009; Patanita & Vargas, 2005; Almanoufi *et al.*, 2012; Kolyaee & Hasani, 2014; Saeidi, 2020). Our results indicated that combining of light and pheromone traps was an effective method in mass trapping of *Z. pyrina* males. Light attracted insects from around, and when they entered the active space of sex pheromone, they are attracted to the pheromone trap and captured by the sticky sheet.

Supply the energy and turn on/off times were two major limitations of light traps in the orchards which solved successfully in this study using solar cells and a programmable microprocessor, respectively. The solar light trap is transferable and provides an efficient insect pest control resource in areas where electricity is unavailable (Mohammad *et al.*, 2018). Attracting of non-target organisms (specially natural enemies such as lace wings,...) is another limitation of light traps. This limitation was solved using an insect proof net with larger holes (3 mm in diameter) instead of insect collecting bucket, which allow the small and medium size insects to escape. According to our results, combining the light and pheromone baited traps is a useful way to increase the efficiency of mass trapping of *Z. pyrina* and decrease damage and pest population density in walnut orchards. Of course, further investigations are suggested to optimize the light emitted from different sources and test different wavelengths to select those with greater effect on photo-taxis response of *Z. pyrina* adults and minimize attraction of non-target insects.

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