Acta entomologica serbica, 2020, 25(1): 21-28

UDC: 632.935:595.784.2(55)
DOI: 10.5281/zenodo.3737858

EFFECT OF DIFFERENT LIGHT SPECTRA ON FECUNDITY OF *EPHESTIA KUEHNIELLA* ZELLER (LEPIDOPTERA: PYRALIDAE)

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Abstract

This paper examines the effects of different light spectra on the number of eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as a general host of biological control agents. Six distinct spectra of light, ranging from 395 nm to 625 nm, were examined in laboratory conditions. Green and red spectra with ranges of 520-525 nm and 620-625 nm caused the highest and the lowest numbers of eggs, respectively. Additionally, the effects of time on the mean egg load of *E. kuehniella* were also found to be significant at five days. The implications of the current results are discussed below and suggest that the application of a green spectrum in insectariums could help in increasing egg production.

KEY WORDS: Fecundity, Mediterranean flour moth, mass rearing, light spectrum

Introduction

Continuous availability of biological control agents through mass rearing is required for the combatting of pests (St-Onge *et al*., 2014). Biological control can be effective, safe and economical, but it depends on the production and trade of biological control agents (Ashouri, 2012). A large number of species have been identified as biological control agents, and 230 of them were applied commercially (Van Lenteren, 2012). The mass rearing of biological control agents is related to the presence of a production line of their host.

The Mediterranean flour moth *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) is mass-reared as a substitute host for different species of biological control agents around the world (Pakyari *et al*., 2016). Daumal *et al.* (1975) described the primary method of mass rearing of the flour moth, which has improved over the years (Bigler, 1986). In order to achieve the maximum potential of mass rearing *E. kuehniella* for the purpose of the mass rearing of parasitoids and predators, all factors that influence host behavior, such as environmental factors, should be studied together.

Light, as an important environmental factor, plays a substantial role in the development and behavior of *E. kuehniella* (Shimoda & Honda, 2013; Fisher *et al*., 2015). The three characteristics of light, intensity, time of exposure and wavelength, could affect the behavior of the insect (Jeon *et al*., 2012). Meanwhile, the effects of wavelength and intensity of light varies for different species of insects (Yang *et al*., 2003). Insects can see different colors of light (Roessingh & Stadler, 1990; Zheng *et al*., 2014) and possess color vision that can distinguish two different spectral compositions of light regardless of their relative intensity (Skorapski & Chittka, 2011). Experiments have demonstrated that both butterflies and moths can distinguish between wavelengths of light (Eguchi *et al*., 1982).

Several studies have shown that light could affect the life cycle and cause the larvae of the *Ephestia* species to diapause (Cox, 1975; Bell, 1977), but no study has been available that focused on the effect of light spectra on its oviposition. Only Cymborowski and Giebultowixz (1976) showed that there was an effect of light on the fertility and development of *E. kuehniella*; however, data about the effect of each spectrum of light on the oviposition of the Mediterranean flour moth is missing.

Oviposition plays a very important part in the female's life and every factor that affects oviposition could affect fecundity. Considering the scientific and economic importance of *E. kuehniella* in laboratory research (Brindley, 1930) and the mass rearing of biological control agents (St-Onge *et al*., 2016), the present study aimed to evaluate different light spectra on the fecundity of *E. kuehniella* as an efficient host for biological control agents.

Materials and Methods

Culture of *Ephestia kuehniella*

The *Ephestia kuehniella* population used in this experiment originated from a population reared at the Ecology and Behavior Laboratory of the University of Tehran in Karaj, Iran. The population has been reared continuously for approximately 25 generations on wheat flour and yeast under laboratory conditions (25±1ºC, 70±10 relative humidity (RH), 16L:8D). One-day-old females were used for all the tests. Light-emitting diodes (LEDs) were provided as the source of light. LEDs were used for their ability to produce various monochromatic light; namely, LEDs are capable of producing light in a narrow range of wavelengths (Shimoda & Honda, 2013). Since the purpose of the present study was to investigate the effect of different spectra on fecundity and it did not include evaluating other attributes of light, all the experiments were carried out with a constant light intensity without swing (1000 $\mu W/cm^2$) and a time of LED exposure of 16L:8D was applied for all. The wavelengths of blue, green, white, yellow, red and violet LED lights are shown in Table I.

Table I. Specifications of LEDs

Color	Wavelength (nm)
Ultraviolet (UV)	395-400
Blue	460-465
Green	520-525
Yellow	585-590
White	450-620
Red	620-625

Experimental design

To evaluate the fecundity of *E. kuehniella* under distinct spectra of light, transparent container (15 cm in height × 2.5 cm in diameter) were used. A hole was made on the upper part of the containers (with a diameter of 2.5 cm, covered with a fine mesh) for air circulation, and LED light was emitted in the same direction, on top and directly to all the containers. There was one diode for all replications in each treatment (spectrum). The bottoms of the containers were covered with 0.2-mm mesh, and under each of the containers, a Petri dish with the same diameter as the bottom of the container was placed. To prevent the egg sticking to the Petri dish, wax paper was placed on the bottom of each dish. The containers were kept in a dark chamber. A constant temperature of 25±1°C, with 70±10 relative humidity (RH) and a light:dark cycle of 16:8 h, were maintained in all replications. A single pair of virgin male and female flour moths of the same age was used. Twenty pairs of adults (replications) were used for each spectrum (treatment). The number of eggs laid by each female was counted daily.

The majority of the female Mediterranean flour moths died after the fifth day, and therefore the best time to check the treatment was from the first to the fifth day. Although the data relating to days after the fifth day were recorded, statistical analysis data was gathered only from the first five days.

Statistical analysis

The statistical software used for data analysis was SPSS (version 23). The effect of spectra on the total number of eggs per female (fecundity) was analyzed using analysis of variance (ANOVA). Tukey's HSD test was performed to compare differences among the mean values. Data were expressed as mean±standard error of the mean (SEM). Linear regression was used to determine the relationship between time and fecundity.

Results

The effects of different LED colors on the mean fecundity of *E. kuehniella* during five days showed significant differences ($F_{5.570}$=3.776, p<0.01) (Table II).

Table II. Fecundity of *E. kuehniella* under distinct spectra of light at different times. The same letters are not significantly different (Tukey's HSD test).

Color/wavelength (nm)	Number of laid eggs (mean±SEM,%)				
	Days				
	1	2	3	4	5
Blue	38.6%±11.07c	30.3±7.23bc	39.65±8.26c	12.4±2.95c	19.1±5.00c
Green	91.1%±15.25a	87.75±10.33a	66.8±5.85a	38.6±3.72a	57.85±5.56a
Red	33.25±6.35c	58.4±8.73b	47.45±7.02bc	12.45±1.88bc	26.9±3.79c
Violet	37.9±11.98c	79.4±14.38a	70.15±12.18a	24.6±4.74a	34.4±6.87b
White	24.4±7.67c	60.55±1.39b	58.9±9.00ab	24.05±4.64b	34.25±4.68b
Yellow	26.2±13.93b	41.6±6.17bc	46.8±8.03bc	11.3±2.05b	34.65±7.41c

Based on the first day, different colored LEDs showed a significant effect on the the number of eggs laid by *E. kuehniella* ($F_{5.119}$=3.16, p<0.01). During 24 h, the maximum eggs produced were under the green LED light (520-525nm) (mean=91.1%±15.25) and the minimum number was produced under the white LED light (mean=24.40±7.67) (Table 2). On the second day, the eggs laid by the flour moth under different colored LEDs revealed significantly different results ($F_{5.119}$=1.1.28, p<0.01). The highest and lowest average numbers of eggs produced were recorded under green and blue LEDs, respectively (87.75±10.23; 30.30±7.23) (Table 2). The result indicated that the number of eggs laid by the flour moth under different colored LEDs was not significantly different on the third day ($F_{5.119}$=0.56, p<0.001). The highest average number of produced eggs was obtained under the violet LED (70.15±12.18), while the blue LED caused the lowest average (39.65±8.26) (Table 2). The number of eggs laid was significantly different on the fourth day ($F_{5.119}$=0.90, p<0.01). The highest average number of eggs produced was recorded under the green LED (57.85±5.56), while the blue LED demonstrated the lowest average (19.10±5.00) (Table 2). Statistical comparison of the eggs laid by the flour moth showed significant differences on the fifth day ($F_{5.119}$=1.30, p<0.01). The green and yellow LEDs caused the highest and lowest average numbers of eggs produced, respectively (38.60±3.72; 11.30±2.05) (Table 2).

A cumulative plot of the average numbers of eggs produced under different spectra of light in five days is shown in Fig. 1, and reveals that the effects of distinct spectra were not sudden and abrupt.

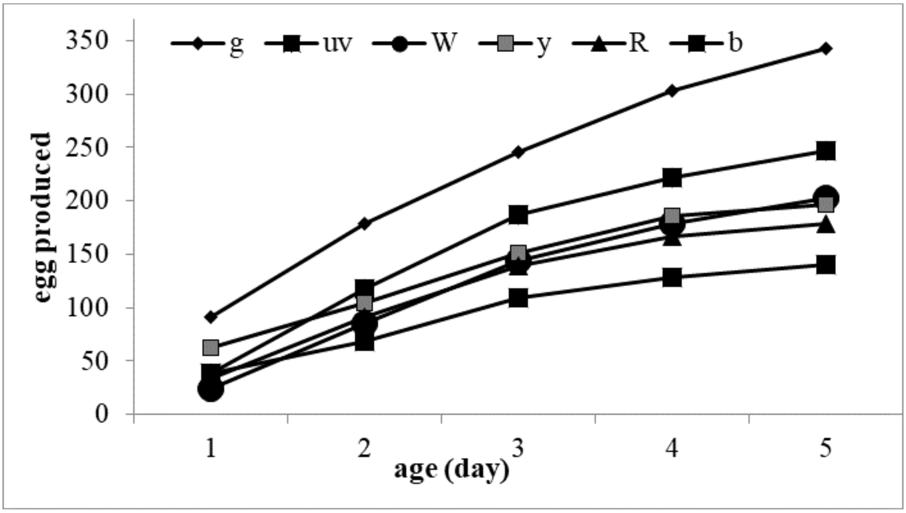

Figure 1. Amount of eggs produced by *E. kuehniella* in five days.

Effect of time on laid eggs of *Ephestia kuehniella* in five days

The regression of time and laid eggs of the flour moth yielded a linear relationship. The highest average was recorded on the second day (56.66%) and the lowest on the fifth day (20.65%) (Fig. 2).

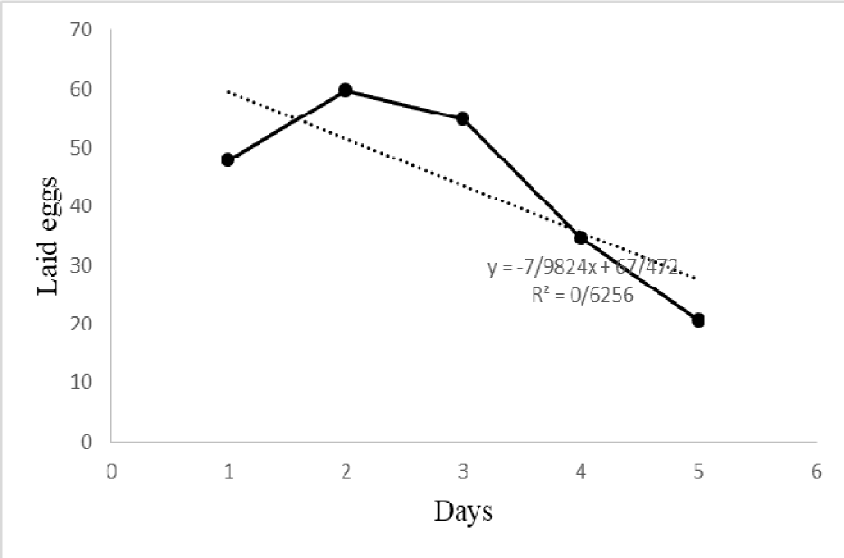

Figure 2. Relationship between time and laid eggs of *E. kuehniella* (%) over five days.

Discussion

The present study shows that *E. kuehniella*, like other members of the Lepidoptera family, can perceive differences among different spectra of light. An increase in the number of eggs produced by *E. kuehniella* was elicited as a result of exposures to distinct spectra of light. These results are debatable from the two viewpoints.

First, the information identified seemed to be useful for producing a line of flour moths. Eguchi *et al*. (1982) studied the spectral sensitivity of 35 Lepidoptera species using electroretinogram (ERG) recordings. In all the studied species, a maximum of three or four was seen in the spectral sensitivity curve figures and two of them belonged to blue and ultraviolet light (383 nm, 460 nm, respectively) and the others were in the range of 500 nm-620 nm. This resulted in the highest number of eggs laid by *E. kuehniella* under the green spectrum (500-525 nm). Electrophysiological studies indicated the presence of three receptors, ultraviolet, blue and green, in the members of Lepidoptera, and noted that these spectra of light compared to the others were preferred in the responses of Lepidoptera (Autrum & Zwehl, 1964). Three types of receptors (ultraviolet, blue and green) have been found in moths, and were reported in *Deilephila elpenor*. Sensitivity in the range of ultraviolet and green was present in *Synanthedon myopaeformis*, while the red receptor was heightened in some moths, including *Spodoptera exempta* and *Mamestra brassicae* (Brehm, 2017).

By using the ERG technique, Gilburt and Anderson (1996) revealed that there were two spectral sensitivity areas in the eyes of *Ephestia cautella*, one of them to yellow-green (around 546 nm) and the other to ultraviolet (around 350 nm). Oviposition patterns and the peak of activities of a large number of stored insects were affected by photoperiod and time of day (Sambaraju *et al.*, 2016). It seems that distinct spectra of light could affect the egg load of *E. kuehniella*. Visual elements, such as color, are used for distinguishing plants and elements in the environment (Jeon *et al.*, 2012). Harris & Miller (1983) described that for *Delia antiqua*, color was one of the influencing variables on pre-oviposition behavior. The results were consistent with those obtained by Roessingh and Stadler (1990) on the oviposition behavior of *Delia radicum*, the cabbage root fly, which found that the most eggs were laid around bright green or yellow light models. Results confirm that the effect of distinct spectra of light on the number of eggs produced by *E. kuehniella* in five days was not abrupt; probably it was a chronic effect. In *Drosophila melanogaster*, egg-laying was rhythmic under constant light conditions (Manjunatha *et al*., 2008). It was also believed that egg-laying was rhythmic in *E. kuehniella* and the spectra of light might have been more effective on egg production than their effect as stimulants. Of course, molecular studies could be helpful in understanding the issue better.

The analyses also indicated that *E. kuehniella* egg production decreased under the red spectrum.

From the second perspective, the results in this paper could be useful in the control of *E. kuehniella* as a storage pest. Considering that *E. kuehniella* is an important storage pest (Trematerra & Gentile, 2010) and the reduced amount of egg load of each female flour moth under the red spectrum (620-625 nm), the use of red light could help control the pest. The use of a LED trap to control the insect as a pest was considered recently (Zheng *et al*., 2014; Park & Lee, 2016). Low temperature, low cost, small size and high reliability are some of the benefits of LEDs (Chen *et al*., 2004). Park and Lee (2016) proved that light traps with a green LED could be a new control strategy against the storage pest *Plodia interpunctella*, because it caused the highest attractive rate compared to the other colored LED. Using the red spectrum could be safe due to the reduced numbers of eggs of storage pests such as *E. kuehniella.*

Taken together, all the results of prior and the present investigations demonstrate that different spectra of light could affect the egg production of *E. kuehniella* females. Finally, the current paper only set out to test the effect of distinct spectra of light on the number of eggs produced by *E. kuehniella*, but it should not be forgotten that egg-laying behavior is a multilayered process and could be affected by several factors, including various light characteristics. The application of green light could probably cause an increase in the numbers of eggs produced by *E. kuehniella*. Further studies need to be conducted on the physiology and molecular causes of these results.

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УТИЦАЈ РАЗЛИЧИТОГ СПЕКТРА СВЕТЛОСТИ НА ФЕКУНДИТЕТ *EPHESTIA KUEHNIELLA* ZELLER (LEPIDOPTERA: PYRALIDAE)

ФАТЕМЕХ ФАРСИ, СЕЈЕД ХОСЕИН ГОЛДАНСАЗ И АХМАД АШУРИ

Извод

У овом раду су испитивани ефекти различитог спектра светлости на број јаја врсте *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) који је честа штеточина. Испитивано је шест различитих таласних дужина спектра светлости, у распону од 395 nm до 625 nm у лабораторијским условима. Зелени и црвени део спектра са распонима од 520-525 nm и 620-625 nm узроковали су највећи и најмањи број јаја. Поред тога, утврђено је да су ефекти времена на средњи број јаја *E. kuehniella* значајни у трајању од пет дана. Ефекти тренутних резултата детаљно су разматрани у раду и сугеришу да би примена зеленог дела спектра у инсектаријумима могла да помогне у повећању производње јаја.

Received: January 14th, 2020
Accepted: March 20th, 2020