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RESEARCH ARTICLE

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Positive Phototaxis of Pulse Weevil, *Callosobruchus chinensis* under the Influence of Artificial Light

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ABSTRACT

Background: Managing insect pests with less environmental hazards is one of the biggest challenges of this century; therefore, new, eco-friendly alternative ways such as electric traps, bait traps or bio-pesticides are gaining great attention. Most economic and effective method is managing agriculture pest by using light trap.

Methods: In this study, the phototactic response of *Callosobruchus chinensis* towards different wavelengths (red 620 nm; green 520 nm; blue 470 nm) of light (LED) at different intensities (25, 50, 100 lux) and durations (2, 6, 12, 24 h) was investigated in a Y maze chamber. Phototactic responses were also observed for IR (730 nm) and UV (365 nm) at different durations (2, 6, 12, 24 h).

Result: The rate of insect attraction was different for different wavelengths of light. The duration of the intended light also showed a positive response to the attraction rate of the insect, as in every case, 24 hours of duration showed the maximum attraction. The green light showed the highest attraction rate (77.78%) at an intensity of 50 lux for 24 h duration, followed by blue (74.44%) and red (65.56%) light at an intensity of 25 lux for 24 h duration (each). UV (65.56%) also showed a positive attraction at 24 h duration, but IR (52.22%) showed the least attraction. These results suggest that green and blue wavelengths can be most useful for monitoring and mass trapping of *C. chinensis*.

Key words: *Callosobruchus chinensis*, Phototaxis, Visible spectrum, Visual stimulus.

INTRODUCTION

Callosobruchus chinensis (Coleoptera : Bruchidae) is one of the major agricultural pest species that infest various stored legumes (like cowpea, chickpea and rajma *etc.*) causing damage up to 60-100 per cent during storage (Fahd *et al*., 2011). Present distribution of this pest is not limited to Asia and Africa only, but throughout the tropical and sub-tropical regions of the world. All stages of life including larvae and pupae (which develop inside the kernels of whole grain) cause a huge damage by reducing quality and quantity of the grains.

Several conventional methods like biological, cultural, physical and chemical control have been used to combat pulse weevil and other stored pests (Upadhyay and Ahmad, 2011) but the chemical controlling method still remains most frequent and popular among them (Asif Shaheen and Khaliq, 2005). All the insecticides and fumigants have undesirable effects on the environment and public health, for this, the uses of major disinfestants has been restricted from 2015 under the policy of Montreal Protocol (United Nations Environment Programme, 1998). Moreover due to long term repeated application most stored pests had gained resistant against fumigants like phosphine (Aurélio Guerra Pimentel *et al*., 2008). Therefore development of an effective but safe method is need of hours. Controlling methods by using different baited traps with various semiochemicals or pheromones gaining popularity (Bray *et al*., 2020) but the most simple method to build a trap is using light which is more cost effective and easy to use for various stored grain insect pests (Abbas *et al*., 2019; Kim *et al*., 2019; Meiswinkel and Elbers, 2016).

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Phototaxis in insects is well known and it depends on three major characteristics of light: 1) wavelength, 2) intensity and 3) duration (Jeon *et al.,* 2012; Shimoda and Honda, 2013). Insects generally respond to light ranging from 350-700 nm and the relative response varies over this range (Land, 1997). Attraction of light over various insects like *Eucespes postfaciatus*, *Plodiainter punctella*, *Phlebotomus papatsi* etc. had been observed (Hoel *et al*., 2007; Nakamoto and Kuba, 2004; Sambaraju and Phillips, 2008). Still phototactic response of *C. chinensis* has not been studied yet. Therefore, the aim of our study is to investigate the phototactic response of *C. chinensis* to various wavelengths (UV-365 nm, blue-470 nm, green-520 nm, red-620 nm and IR-730 nm) at different intensities (25, 50, 100 lux) and durations (2, 6, 12, 24 h) under laboratory condition using LED (Light emitting diode) as light source and evaluate the most attractive wavelength for development of an effective light trap.

MATERIALS AND METHODS

The whole experiment was performed from February, 2021 to April, 2022 at the Ecology Research Laboratory, Department of Zoology, Maulana Azad College (West Bengal, India). Adult *C. chinensis* were collected from the local storehouses containing desi chickpea or Bengal gram (*Cicer arietinum*) seeds of Kolkata. They were maintained in glass jars (1L) covered with fine-mesh nylon nets at 27±1°C temperature, 65±10% relative humidity (RH) and 12L:12D photoperiod in a 'Biological Oxygen Demand' incubator. After three generations newly emerged 4th generation males and females were separated from the stock cultures. Equal number of adult males and females were used for the behavioral tests (90 for each bioassay).

Light source

The LEDs used as light source were near infrared (IR) (730nm), red (620 nm), green (520 nm), blue (470 nm) and ultra-violet (UV) (365 nm). Eighty LEDs of each wavelength were embedded into five different circuit boards. Those circuit boards were attached with the test chamber consecutively as per experimental need. Modules were connected with a power source via a control unit to increase or decrease intensity of light as per needed (Electronic parts and LEDs were purchased from Shenzhen Chanzon Technology Co., Ltd. China). The intensity of particular wavelength (visible) was checked by luxmeter (Hanna, India).

Y-shaped test chamber

The test chamber for investigating the phototactic response of *C. chinensis* was constructed using a modified Y maze phototactic chamber designed by Oh and Lee (2010). The Y maze test chamber had opaque acrylic stem and two arms (length 45 cm and width 15 cm) and each of the arms were equipped with transparent wall at the end. The transparent walls of light arm remained as it were but the transparent walls on the dark arms were covered with an opaque cap. A hole was present at the stem for insect entrance, covered by nylon netted cap. The light source (LEDs) was installed on the outside of the light arm of the Y maze chamber (Fig 1). The whole system was maintained in total darkness.

Experimental design

Y-maze test

The phototactic response of *C. chinensis* to LEDs was investigated in the Y maze chamber under different wavelengths, intensity and duration of light. *Callosobruchus chinensis* adults were collected using a tiny brush and they were released into the Y maze chamber through the insect entrance arm. To determine the phototactic response, the number of chickpea weevils in both light and dark arm were counted and recorded. Insects which did not respond by selecting any arm of the Y-maze were not included in the analyses. The attractive effects of different wavelengths were investigated at various light intensities (25, 50 and 100 lux) and light duration (2, 6, 12 and 24 h). The results were examined to elucidate the optimum time and intensity for each colour of light attracting *C. chinensis*. Attraction for UV and IR were also tested for 2, 6, 12 and 24 h duration.

Two-choice test

After the detection of optimum condition for each separate light colour, insects were tested again in the Y-maze by using different optimum condition of light (except UV and IR) with each other to determine which condition is most attractive for the insects. All experiments were repeated three times.

Statistical analysis

In phototactic bioassays, test insects (male and female) were analyzed by a $\chi 2$ test on the null hypothesis that the probability of scores for the particular light (every intensity and time interval individually) or dark (no light) is equal to 50% (Koschier *et al.,* 2000; Mukherjee and Makal, 2021). Insects that did not respond by selecting any arm of the Y-maze were not included in the analyses.

One way ANOVA was done first between the preferences of insect choice for specific intensity of a wavelength with different time intervals and again between specific time intervals with intensities of different wavelengths. Tukey's Honest Significant Difference test was also conducted followed by ANOVA for comparing the variables with others in SPSS16 (SPSS Inc., IL, USA).

For the two-choice test again, $\chi 2$ test were done on the same null hypothesis as previous that the probability of scores for the two test subjects (*i.e.* two different wavelengths of lights) is equal to 50% (Koschier *et al.* 2000; Mukherjee and Makal, 2021).

RESULTS AND DISCUSSION

A wide range of variations were observed in the phototactic behavior of *C. chinensis* during the study towards red (620 nm), green (520 nm) and blue (470 nm) colour lights at different intensities (25, 50 and 100 lux) and different time intervals (2, 6, 12 and 24 h).

At 25 lux during 2,6,12 and 24 h, attractive effect of red light arm were 53.33%, 57.78%, 61.11% and 65.56%. When with the same light durations, the intensity of red light was increased to 50 lux the attractions were 47.78%, 54.44%, 56.67% and 61.11% respectively. Further increasing the intensity to 100 lux, attractions were 45.56%, 50%, 53.33% and 57.78% for each time interval. That is increment of red light intensity (from 25 lux to 100lux) attraction of insects reduces significantly ($F_{3,8}$=14.44, P=0.001; $F_{3,8}$=16.667, P=0.001; $F_{3,8}$=10.833, P=0.003) but increase projection duration (2,6,12 and 24h) of red light, attractions increases significantly ($F_{2,6}$=7.800, P=0.021; $F_{2,6}$=7.400, P=0.024; $F_{2,6}$=5.256, P=0.047; $F_{2,6}$=12.33, P=0.007). From this we can conclude that the optimum attraction for red light was at 25 lux intensity and 24-hours duration (Table 1).

Attractions of insects towards green light at different intensities were little different than red light. Here attractions for green light at 25 lux intensity were 63.33%, 66.67%, 70% and 74.44% for the time duration of 2,6,12 and 24h respectively. When the green light intensity was increased to 50 lux attraction were 66.67%, 72.22%, 73.33%, 77.78% and for 100 lux intensity the response of the insects were 62.22%, 67.78%, 67.78% and 68.89% respectively *i.e.* for increase of time duration for every intensity of green light a significant ($F_{3,8}$=7.300, *P*=0.011; $F_{3,8}$=4.833, *P*=0.033; $F_{3,8}$=7.333, *P*=0.011) increase in insect attraction were observed. Further, increasing green light intensity from 25 lux to 50 lux insects attraction increases significantly only for 24h duration on the contrary, in 100 lux intensity the attraction reduces ($F_{2,6}$=1.857, *P*=0.236; $F_{2,6}$=1.909, *P*=0.228; $F_{2,6}$=2.714, *P*=0.145; $F_{2,6}$=16.333, *P*=0.004) . From this we can infer that the optimum condition for green light was at intensity of 50lux and 24 h time interval (Table 1).

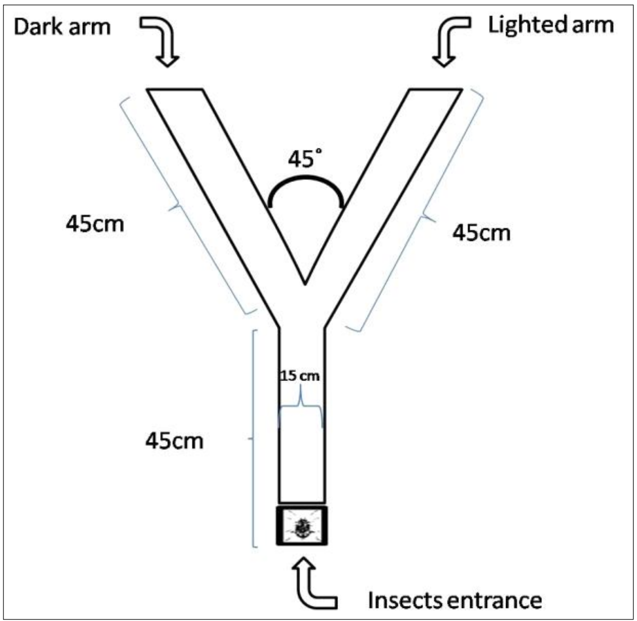


Fig 1: Schematic diagram of modified Y-maze for preference response of weevil to LEDs.

While attraction of insects for blue light at 25 lux and 2,6,12 and 24 h duration were 61.11%, 62.22%, 70% and 74.44% respectively. When the intensity was increased to 50 lux and then to 100 lux for the same time durations attraction rate were 55.56 %,56.67%,62.22%, 67.78% and 57.78%,61.11%,66.67% and 70% respectively i.e. upon increase of duration attraction of insects increases significantly ($F_{3,8}$=21.944 , *P*<0.0001; $F_{3,8}$=8.556, *P*=0.007; $F_{3,8}$=12.167, *P*=0.002) for each intensity. When intensity were increased from 25 lux to 100 lux the attraction of insects were increased significantly only for 2h and 24h duration ($F_{2,6}$=6.333, *P*=0.033; duration $F_{2,6}$=5.600, *P*=0.042) but remain nearly same for 6h and 12 h ($F_{2,6}$=4.200, *P*=0.072; $F_{2,6}$=2.846,*P*=0.135). From this we can conclude that for blue light the optimum attraction was at 25 lux and 24 h interval (Table 1).

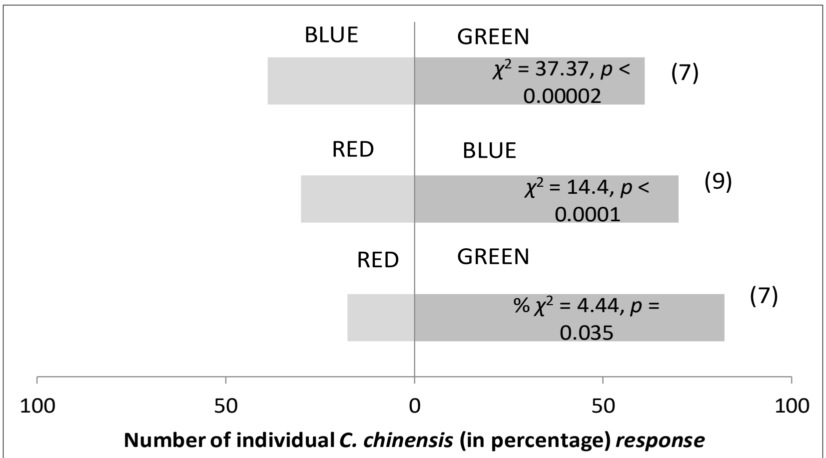
The attraction of the insects for UV and IR light were also determined for 2,6,12 and 24 h duration (as increasing intensity can hamper normal activity of insect so intensity used was 2 μmol m^{-2} s^{-1} on average). Insects attraction was very low for IR light *i.e.* 50%, 51.11%, 52.22% and 52.22% for 2,6,12 and 24 h durations respectively but for UV the attraction increases according to time duration *i.e.* 57.78%, 58.89%, 60% and 65.56% respectively for 2,6,12

Table 1: Attraction rate of *C. chinensis* (in percentage) under various lighting conditions (n=90 individuals tested in each bioassay).

		Duration of light				
Colour (nm)	Intensity	2h (A)	6h (B)	12h (C)	24h (D)	$F_{3,8}$ value A×B×C×D
Red	25 lux (a)	53.33% (48#)	57.78% (52)	61.11% (55)	65.56%*(59)	14.44 (*P*=0.001)
	50 lux (b)	47.78% (43)	54.44% (49)	56.67% (51)	61.11% (55)	16.667 (*P*=0.001)
	100 lux (c)	45.56% (41)	50% (45)	53.33% (48)	57.78% (52)	10.833 (*P*=0.003)
	$F_{2,6}$value a×b×c	7.800 (*P*=0.021)	7.400 (*P*=0.024)	5.256 (*P*=0.047)	12.33 (*P*=0.007)	
Green	25 lux (a_2)	63.33% (57)	66.67% (60)	70% (63)	74.44% (67)	7.300 (*P*=0.011)
	50 lux (b_2)	66.67% (61)	72.22% (65)	73.33% (66)	77.78%*(70)	4.833 (*P*=0.033)
	100 lux (c_2)	62.22% (56)	67.78% (61)	67.78% (61)	68.89% (62)	7.333 (*P*=0.011)
	F value a_2×b_2×c_2	1.857 (*P*=0.236)	1.909 (*P*=0.228)	2.714 (*P*=0.145)	16.333 (*P*=0.004)	
Blue	25 lux (a_3)	61.11% (55)	62.22% (56)	70% (63)	74.44%*(67)	21.944 (*P*<0.0001)
	50 lux (b_3)	55.56% (50)	56.67% (51)	62.22% (56)	67.78% (61)	8.556 (*P*=0.007)
	100 lux (c_3)	57.78% (52)	61.11% (55)	66.67% (60)	70% (63)	12.167 (*P*=0.002)
	F value a_3×b_3×c_3	6.333 (*P*=0.033)	4.200 (*P*=.072)	2.846 (*P*=0.135)	5.600 (*P*=.042)	
UV	-	57.78% (52)	58.89% (53)	60% (54)	65.56%*(59)	
IR	-	50% (45)	51.11% (46)	52.22% (47)	52.22%*(47)	

#In the bracket the digits designate the actual number of insects showed positive response.

*Marks beside the number denote the optimum condition of attraction for each light intensity and duration.



Numbers in brackets are the number of insects that did not respond to either treatment (N = 90 in each bioassay).

Fig 2: Behavioral responses of *C. chinensis* individual towards Blue vs Green; Red vs Blue; Red vs Green colour light [two choice test].

and 24 h time duration (Table 1). So, 24 h UV light projection would be optimum.

Two-choice test

After the deduction of optimum condition for each light a series of two-choice test were conducted to reconfirm the efficiency of each light in the presence of other. Two-choice test between optimum condition of green light and red light, attraction showed that green light (82.22%; $\chi 2$ = 37.37 df = 1, *p*<0.00002) was more efficient than red light (17.78%). Comparison between blue and red colour light again blue light (70%; $\chi 2$ = 14.4, df = 1, *p*<0.0001) produces more attraction than red light (30%). Between green and blue light still green light produces little more attraction (61.11% $\chi 2$ = 4.44, df = 1, *p* = 0.035) than blue light (38.89%).From the test it confirms that green light at 50lux and 24 h duration provide the most effective attraction among green, red and blue. However the attraction of blue light was next to green light at a lower intensity (25 lux) at 24 h duration (Fig 2).

Phototactic behavior is well known fact in insect world especially in lepidopteran and coleopteran insects (Sambaraju *et al*., 2008; Kim *et al*., 2012; Song *et al*., 2016) but this light loving behavior depends on different factors like wavelengths, intensity and light exposure time (Sambaraju and Phillips, 2008). Under suitable wavelength (*e.g.* red, green, blue *etc*), exposure times and luminance intensities of light have been proved to be attractive for stored-product insect species like weevil and moth (Nakamoto and Kuba, 2004; Kühnle and Müller, 2011; Cho and Lee, 2012; Jeon *et al.,* 2012; Kim and Lee, 2014 ; Park *et al.,* 2015; Park and Lee, 2016; Song *et al*., 2016). Our results also indicate that the phototactic behavioral responses of the *C. chinensis* adults depend on the specific wavelength, luminance and light exposure time. Sensitivity of weevil to shorter wavelengths is also documented by Nakamoto and Kuba (2004) and Hausmann *et al.* (2004) for *Euscepes postfasciatus* and *Anthonomus pomorum* weevil respectively. In this experiment *C. chinensis* insects also showed highest attraction towards green light (50 lux and 24 h duration) followed by blue light (25 lux and 24 h duration). Red light and UV light showed same response but 1.2 times lesser than green light which is according to the phototactic response of the *Sitophilus oryzae* by Jeon *et al.* (2012). Insect activities were lowest in IR it may be due to less presence of IR receptor in this insect or heat generated by IR that can be correlated with experiments on phototactic response of *Sitophilus oryzae* observed by Jeon *et al.* (2012) and response of *Aulacophora foveicolis* under IR spectrum by Mukherjee and Makal (2021). To identify the relationship between insect behavior and light wavelengths investigation on the responses of pest insect towards the LED sources have been conducted worldwide with a specific goal to identify the effective attractant and/or repellent wavelengths for target insect pests (Hironaka and Hariyama, 2009; Endo *et al.,* 2022). In addition a research on attracting natural enemies of insect pests (parasitoids) by the help of using specific wavelengths of light is also being investigated (Shimoda and Honda, 2013).

CONCLUSION

Chemical based insecticides (*e.g.* pyrethroids, organophosphates *etc*) are the most popular and cost effective way to control those stored product pest outbreaks but application of those insecticide had a greater risk for environment as well as human health. Therefore it is necessary to develop new ways which might be included in integrated pest management (IPM) schemes for those pest. Results of this study indicate the phototactic response of insects depends on specific wavelength, luminance and exposure time of light. The result of this study suggests that light traps equipped with green (520 nm) LEDs at intensity of 50 lux can be used as better control strategy for this pulse weevils. However, further research is needed to evaluate the efficacy of these light sources in a broad range and to investigate if olfactory stimulants (seed volatiles *etc*) can produce synergistic effects.

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Conflict of interest

Authors have declared that no conflict of interests exist.

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