THE INDIRECT ROLE OF SOLAR ENERGY IN CONTROLLING COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOSID) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT: Light Emitting Diodes (LEDs), which showed a direct effect on pests, were powered by solar energy to play an indirect role in controlling *Spodoptera littoralis*. Laboratory studies were conducted to determine the mortality and both of antifeedant and growth inhibitory effects of light-emitting diodes (LEDs) against 1st, 2nd, 3rd and 4th larval stages of *S.littoralis.* White LEDs were more effective against all larval stages more than Blue LEDs even mortality ratios proved that.White LEDs caused 100 % mortality of both 1st and 2nd stages, and recorded 96.67% and 90% of 3rd and 4th larval stages. While, Blue LED caused mortality with 96.67, 90, 86.67 and 80 % against the same mentioned larval stages resp. LEDs have an antifeedant effect against *S. littoralis* larve. Mean weights of exposed larvae to White and Blue LEDs were lower than control with significant difference at the end of each stage. Besides, malformation was noticed in all stages with different percentages decreased gradually from 1st to 4th larval instars. Monoamine oxidase (MAO) was determined in *S.littoralis* homogenates and LEDs showed its effect as an effective inhibitor of the enzyme activity significantly. It could be concluded that, a close relation between biogenic amines affected by LEDs which contributed as an antifeedant till mortality. Light-emitting diodes (LEDs) showed their effective importance in the promising pest management tools which could be used easily with certain effects on the exposed pest.

Keywords: Diodes, mortality, *Spodoptera*, Antifeedant, malformation, monoamine oxidase (MAO)

INTRODUCTION

Spodoptera littoralis (Bosid) (Lepidoptera: Noctuidae) is an extremely serious pest. The larvae can defoliate many economically important crops cutting across over 40 families, such crop includes cotton and tomatoes (EPPO 2008).The use of insecticides in agriculture field causes biological imbalance (Yadav 2010), and some new eco-friendly formulations pesticides became the target (Bulmer *et al*. 2009, Zhang and Xiao-zhen 2010, Cloyd and Bethke 2011).So the main trend nowadays is upon using new environmental safely methods to control *S.littoralis* .

Light-emitting diodes (LEDs) have become more and more common as a low-cost and flexible way to provide light stimuli in vision research (Nygaard and Frumkes 1982,Scholfield and Murdock 1978,Watanabe *et al*.1992, Pokorny *et al*. 2004, Demontis *et al*.,2005, Da Silva Pinto *et al*. 2011 and Rogers *et al*.2012).LEDs offer easier and more versatile control of light characteristics (Schubert and Kim 2005) compared to traditional light sources such as xenon, mercury, metal halide and halogen lamps which often require various auxiliary devices in experimental settings such as a set of filters for spectral tuning and shutters to control exposure duration. LEDs are close to being monochromatic light sources (half-bandwidths of 20–30 nm) and they provide roughly linear light output (Svilainis 2009) in response to pulse-width modulation (Narra and Zinger 2004) control signal over an extended dynamic range. The response time of LED chips it is very fast (in the order of nanoseconds).The whole system response of the LED driver and the LED chip together is slower, but still sufficient for most purposes, the response time being in the order of microseconds (Albeanu *et al*., 2008).

An additional advantage of LEDs is their characteristic low electromagnetic interference (EMI) emission (Svilainis 2012), as compared to cathode ray tube (CRT) monitors that can cause significant electromagnetic interference to electrical cables (Hogg 2006), making them especially suitable to be used with electrophysiological techniques such as electroretinography (Fadda and Falsini 1997), magnetoencephalography (Wilson *et al*. 2009), and electroencephalography (Da Silva Pinto *et al*. 2011) in which the physiological potentials are typically very weak and are prone for artifacts. LEDs are typically controlled with a discrete on–off PWM signal, in which the ontime (duty cycle) is in theory linearly proportional to the light output (Rumyantsev *et al*., 2004 and Salzberg *et al*., 2005).

Arduino [http://arduino.cc/], is an easy-to-use hardware and software and it has been employed in scientific applications .They were such as LED light control of an open-source in vivo multispectral imaging system for rodents (Sun *et al*.,2010),teaching violin bowing by providing an interface for combining motion sensing and vibrotactile feedback (Van Der Linden *et al*.,2011), wearable computing (Isoyama *et al*.,2011), and in cross-disciplinary teaching of biology and computer science (Grasel *et al*.,2010).

LEDs could be contributed strongly in pest control as attracting light traps as a photo-response of the tobacco whitefly to light-emitting diodes of four different wavelengths and various intensities in an LED-equipped Y-maze chamber and compared with the response to black light (BL), which is typically used in commercial traps (Kim *et al*. 2012).The BL showed the highest attraction rate (90.3%) to *Bemisia tabaci*, followed by a similarly strong attraction to the blue LED (89.0%), the yellow LED (87.7%), the green LED (85.3%), and the red LED (84.3%). These results suggest that energy-efficient LEDs could be used for more environmentally friendly insect control.

The aim of this work is to reduce the using of pesticides to control *S.littoralis* by the conjugation between solar energy and LEDs in one arduino system which used to test its ability to play a role as an indirect control tool of insects. The mentioned system could be applied in greenhouses and any such places if it is being with successful constructed solar energy system.

MATERIAS AND METHODS

Spodoptera littoralis Culture

Spodoptera littoralis was cultured on leaves of the castor oil plant (*Ricinus communis* L.) in Plant Protection Research Institute, Agriculture Research Center, Mansoura Branch, Egypt. Larvae were kept at 25±1 °C, 70% RH and 12L: 12D of photoperiod. The second instar larval stage of the insect was used in the insecticidal bioassay.

- Solar Panel with Light Emitting Diodes (LEDs) System

10Watt monocrystalline solar panel - 14" X 12" , 20-foot/18-gauge cables , 12V/3aH 20-hour battery,solar charge controller to protect battery from overcharge / overdischarge and Light Emitting Diodes (LEDs) were used to make the full required system.

LEDs with two colors, powered by solar energy were used effectively. LEDs provided 12 h light/12 h dark photoperiod at the duration of exposure.Treatments were done under two different light colors with broad- spectrum-white LED and blue LED ,Table (1) showed the wavelength and voltage drop of the used two colors of LEDs, wheile control was under normal fluorescent light (Abd El-Wahab and Bursic 2014). Light quality and quantity were estimated using a Testo545 light meter (Testo, Germany). Tow colors were used and controlled by Arduino Uno C++ language was used in the programming to On/Off lights automatically. Six units of each color were used and placed, as one unit for each replicate.

Table (1) Wavelengths of

Light Color	Wavelength [nm]	Voltage drop [ΔV]
Blue	$450 < \lambda < 500$	$2.48 < \Delta V < 3.7$
White	Broad spectrum	$\Delta V = 3.5$

-Exposure to Light Emitting Diodes (LEDs)

S.littoralis 1st,2nd, 3rd and 4th larval stages ,which were reared on discs of castor oil plant leaves, were exposed to Light Emitting Diodes (LEDs) with the two main colors, White and Blue controlled by Arduino and powered by solar energy .Each treatment had three replicates with 30 larvae/treatment . In each experiment, estimation effect of LEDs on exposed larvae such as mortality (Percentages of larvae affected by LEDs), antifeedant (Depending on the mean weight of larvae before and after treatment compared with control), and malformation (Percentages of deformed larvae affected by LEDs).

-Monoamine oxidase (MAO-A) Determination

MAO-A is flavin adenine dinucleotide (FAD) containing enzymes which are tightly anchored to the mitochondrial outer membrane. To determine the MAO-A inhibition potencies of LEDs, the extent by which LEDs treatments able to reduce the rate of the MAO-catalyzed oxidation of Kynuramine, were measured. Kynuramine is non-fluorescent until undergoing MAO-catalyzed oxidative deamination and subsequent ring closure to yield 4-hydroxyquinoline, a fluorescent metabolite. The concentrations of the MAO-generated 4hydroxyquinoline in the incubation mixtures was determined by comparing the fluorescence emitted by the samples to that of known amounts of authentic 4-hydroxyquinoline at excitation (310 nm) and emission (400 nm) wavelengths. Microsomes prepared from insect cells expressing recombinant human MAO-A and MAOB (5 mg/mL) obtained from Sigma–Aldrich and were pre-aliquoted and stored at -70°C. All enzymatic reactions were carried out to a final volume of 500 µL in potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl, 20.2 mM) and contained kynuramine as substrate, MAO-A or MAO-B (0.0075 mg/mL) and various concentrations of the test inhibitor (LEDs colors) (0–10.0 mg/ml). Stock solutions of the test inhibitors were prepared in DMSO and added to the reactions to yield a final concentration of 4% (v/v) DMSO. The reactions were carried out for 20 min at 37°C and were terminated with the addition of 200 µL NaOH (2 N). After the addition of distilled water (1200 µL) to each reaction, the reactions were centrifuged for 10 min at 16000 × g. To determine the concentrations of the MAO-generated 4-hydroxyquinoline in the reactions, the fluorescence of the supernatant at an excitation wavelength of 310 nm and an emission wavelength of 400 nm were measured (Novaroli, et al. 2005). Quantitative estimations of 4 hydroxyquinoline were made with the aid of a linear calibration curve ranging from 0.047 to 1.56 µM of the reference standard dissolved in potassium phosphate buffer (100 mM, pH 7.4). Each calibration standard was prepared to a final volume of 500 µL in potassium phosphate buffer (100 mM, pH 7.4) and contained 4% DMSO. To each standard was also added 200 µL NaOH (2 N) and 1200 µL distilled water. IC50 values were determined according to Strydom *et al.* (2010) and Cheng and Prusoff (1973).

- Data Analysis

SPSS (V.16) was used to show differences among treatments with LEDs. Paired samples test, Kendall's Wa Coefficient, Chi-Square and One-Sample Kolmogorov-Smirnov Test were used to test significance among groups through experiments in the present research.

RESULTS AND DISCUSSION

1-LEDs direct effect on *S. littoralis*

Gained results were analyzed depending on the concerned relation test statics between LED colors and their impact that caused mortality of each exposed larval stage of *Spodoptera littoralis* in comparable with control Table (2).

White LEDs caused 100 % mortality of both 1st and 2nd stages while mortality percentages recorded 96.67% and 90% of 3rd and 4th larval stages, respectively. Therefore, Blue LED caused in general mortality also but lower than that occurred in case with White LED. Blue LEDs showed mortality with 96.67, 90, 86.67 and 80 % against 1st,2nd,3rd and 4th larval stages of *S.littoralis*, resp. There was a noticed significant difference between treatments in the same larval stage (Paired Samples Correlations=-.074**) and increased when comparison was among treated stages (Paired Samples Correlations=-.898**).

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Table (2) Effect of Light Emitting Diodes (LEDs) on larval stages of *Spodoptera littoralis*

Larval Stages	LEDs Colors	% Mortality	Weight (10 Larvae) gram	% Malformation
1st	White	100	0.0003	70
	Blue	96.67	0.0005	63.33
2nd	White	100	0.0002	50
	Blue	90	0.0006	43.33
3rd	White	96.67	0.0004	43.33
	Blue	86.67	0.0035	33.33
4th	White	90	0.0037	27.27
	Blue	80	0.0075	23.33
1st	CONTROL		0.0011	
2nd			0.0023	
3rd			0.0064	
4th			0.0116	

2-LEDs as Insect Growth Regulators (IGRs) against *S. littoralis*

Revealed data were analyzed depending on the concerned relation test statics between LED colors and their effect as insect growth regulators (IGRs) on each exposed larval stage of *Spodoptera littoralis* in comparable with control Table (2). Insects larvae were malformed mainly during ecdysis after exposing to LEDs.White LEDs caused malformation with 70, 50, 43.33 and 27.27 % of 1st, 2nd, 3rd and 4th larval stages, while Blue LEDs caused 63.33, 43.33, 33.33 and 23.33 % against the same mentioned arrangement. resp. There was no a significant difference between treatments in the same larval stage but it was noticed when comparison was among treated stages at 5% (Kendall's Wa Coefficient of Concordance =0.111, Chi-Square=1.333).

3- LEDs as Antifeedant Assessment against *S. littoralis*

Table (2) showed the effect of LEDs and antifeedant against *S. littoralis* .It was assessed through weight of tested larval stages before and after treatments in comparison with control. Revealed data were analyzed depending on the concerned relation test statics between LED colors and their effect as antifeedant on each exposed larval stage of *Spodoptera littoralis* in comparable with control with significant difference at 1% among larval stages affected by treatments (Kendall's Wa Coefficient of Concordance =0.917, Chi-Square=11).Mean weight of larvae exposed to White and Blue LEDs were lower than control with highly significant difference at 1% among treatments (Kendall's Wa Coefficient of Concordance =1, Chi-Square=12).Reaction of both significant antifeedant effect of LEDs treatments on tested larval stages showed clearly by (Kendall's Wa Coefficient of Concordance =0.775, Chi-Square=18.591).

-Effect of Light Emitting Diodes (LEDs) on monoamine oxidase of *Spodoptera littoralis*

Monoamino oxidase was inhibited effectively by LEDs especially White color (Fig. 1). The specific activity of monoamino oxidase in the control showed significantly higher increase than that in treatments ($P < 0.05$). Fig. (1) Showed that White LEDs inhibited MAO with 1.24, 2.85, 6.45 and 7.59 and Blue LED caused 1.62, 3.90, 7.33 and 8.04 µg/larva/ minute in comparable with control (3.67, 5.29, 9.24 and 10.33 µg/larva/ minute) of tested larval stages. Partial correlation among treatments showed highly significance (2-tailed).

Through gained results, it could be mentioned that there was a close relation between biogenic amines affected treatments and contributed in the stop feeding of tested pest and kept them as so till death occurred.So antifeedant directly to mortality as a response of LEDs.

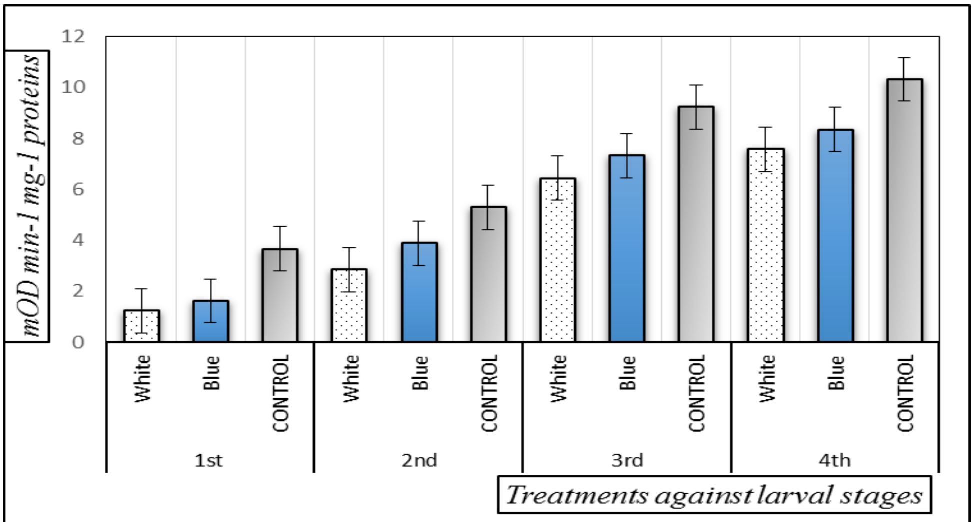

Fig. (1) Light Emitting Diodes (LEDs) Effect on Monoamine Oxidase (MAO) of *Spodoptera littoralis* Larvae

One-Sample Kolmogorov-Smirnov Test showed highly significance (2.192** at 1%) among LEDs treatments especially with White LED and less significant difference (1.878* at 5%) was among tested larval stages of *S.littoralis*.Moreover, Kendall's Coefficient of Concordance (0.339**) showed the highest significant differences of LEDs treatments on larval stages of *S.littoralis*.

DISCUSSION

Sustainable crop production is a way of growing or raising food in an ecologically and ethically responsible manner. This includes adhering to agricultural and food production practices that do not harm the environment, and able to use renewable energy resources such as solar energy.In addition, sustainable crop production practices can lead to higher yields over time, with less need for expensive and environmentally damaging inputs.

The application of renewable energy technologies can be assumed to have a positive impact on agricultural output and can improve pest control methods such as operating LEDs which proved their effects on *S.littoralis* larvae.

Solar energy used before in invention as solar insect killer and catcher which included a casing, a solar plate, a power supply, a control switch, a timer, a circuit board and a plurality of light-emitting elements to emit the lights having various wavelengths. But that system used to kill or catch the insects and prepared with a water-collecting basin outdoors. When the casing emits the lights having various wavelengths according to the preset timings, many kinds of insects can be attracted by the lights and then fly into the water-collecting basin. As a result, the insects cannot escape from the water collecting basin or water collecting pit and be captured therein (Lin 2007).The main difference in the present research is mainly on the effect of LEDs on *S.littoralis* larvae which were not used as attractants but as a control instrument powered by solar energy.

The mode of action of LEDs against various insects and mites is close to that by Formamidine and the poisoning symptoms of both are distinctly different from other pesticides. Their proposed action is the inhibition of the enzyme monoamine oxidase, which is responsible for degrading the neurotransmitters norepinephrine and serotonin (Ware and Whitacre 2004). This results in the accumulation of these compounds, which are known as biogenic amines. Affected insects become quiescent and die and that was the same which occurred by LEDs also.

LEDs affected insects successfully with highly attractiveness of their predators (Abd El-Wahab *et al*. 2014).LEDs are totally safe and can attract such predators to each prey in addition to the direct effects on pests (Abd El-Wahab and Abouhatab, 2014),which are so close to biogenic amines-based-pesticides (neonicotinoids and formamidines) but not with their disruptive effects on biogenic amine signaling causing olfactory dysfunction in honeybees (Farooqui 2013) .

Biogenic amines have a wide variety of functions in both the central and peripheral nervous systems of insects. They can act as neurotransmitters, neuromodulators and even circulating neurohormones. Although our knowledge about the pharmacology of the receptors that mediate the actions of biogenic amines in insects is increasing, at present there is only one known example of a pesticide that activates biogenic amine receptors. The knowledge of the mode of action of insect biogenic amine receptors is mediated through second messenger systems. However, Evans (1985) mentioned that no pesticides are known to bring about their actions by directly interfering with second messenger systems in insects ,but now and after our research, it can be said that LEDs which are ecofriendly are able to do so action without any bad hazards of pesticides.

Biogenic amines could be revealed potential new target sites for the development of future pesticides such as targeted in the present paper by LEDs and also as mentioned by Fuchs *et al.* (2014).They recently proved that biogenic amines affected mosquito fertility. Even egg melanisation was regulated by adrenergic signalling, whose disruption caused premature melanisation specifically through the action of tyramine. The strong cumulative negative effect was on mosquito locomotion and survival. Dopaminergic and serotonergic antagonists such as amitriptyline and citalopram recapitulated this effect.

CONCLUSION

LEDs played an important role to control *Spodoptera littoralis* as:

- Antifeedants. Depending on the condensed biogenic amines.
- IGRs .Insects larvae were malformed mainly during ecdysis.
- Biocides: Because larvae were killed biologically without any chemicals.

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