Direct Effects of Light Emitting Diodes (LEDs) on The Two-Spotted Spider Mite, *Tetranychus urticae*

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**Abstract.** Light emitting diodes (LEDs) with two certain colors, white and blue, proved successfully its direct effects on the two forms of *Tetranychus urticae*. LEDs, Vertimec (the most pronounced compound against mites), and joined treatments were compared in the two ways under laboratory conditions: mortality percentages and translaminar effect. Gained results clarify that the interaction of Vertimec and LEDs always recorded the highest direct effect, 100% mortality, against adult females of both forms even by determination of mortality percentages or translaminar effects followed by others. Besides, LEDs had sufficient translaminar effect more than Vertimec, significantly. White LEDs were more effective against the green form in all treatments while Blue LEDs were more effective against the red form. The specific activity of Mono amine oxidases was determined in all treatments and compared with the control. All treatments caused inhibition of the enzyme but White LEDs and Blue LEDs joined with Vertimec LC50 decreased the enzyme activity of the green type of *T. urticae* (0.09 and 0.13 mOD min-1 mg-1 proteins, resp.) more than the red type (0.1 and 0.17 mOD min-1 mg-1 proteins, resp.). In conclusion, there was a close positive relation among accumulation of biogenic amines and ratio of mortality which was affected mainly by exposure to LEDs colors in comparison with the control.

**Keywords:** Diodes, *Tetranychus*, Vertimec, Translaminar, mortality, monoamine oxidases (MAO)

1. **INTRODUCTION**

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a widespread agricultural pest, causing severe damage on a variety of greenhouse and field crops (Cranham, 1985). Spider mites are difficult to control with pesticides (Nahar et al., 2005) due to inaccessibility of lower leaf surfaces, short life cycle, high reproductive capacity, and ability to develop resistance to miticides (Cranham and Helle, 1985; Georgiou 1990).

The means of controlling mites mainly is the used of commercially available miticides that either have contact or translaminar activity (Cloyd et al., 2008). Pesticides with contact activity include fenpyroximate, hexythiazox and bifenthrate which provide minimal residual activity once spray, residues have dried. However, other compounds with translaminar properties include but not limited to abamectin, chlorfenapyr and etoxazole, which can penetrate the leaf cuticle and the active ingredient resides within the leaf tissue including the spongy mesophyll and palisade parenchyma cells, providing a reservoir of active ingredient. This allows for extended residual activity against certain foliar-feeding insects and mites which normally feed on leaf undersides, even after spray residues have dried. Translaminar -penetration of a foliar applied pesticide from the adaxial cuticular surface of the leaf, through the epidermis layer and distributing into the mesophyll on the abaxial side (Wise, 2014).

The white LED is clean and efficient but also more attractive to flying invertebrate (Zielinski, 2014), and the procedure of attracting specific predator to each pery depending on the different wavelengths through LEDs were published by mine in many research paper (Abd EL-Wahab and Abouhatab, 2014; Abd EL-Wahab et al., 2014) and as a new trend able to reduce metabolic Vertimec resistance in mites (Abd EL-Wahab and Bursic, 2014). Moreover, Hori et al. (2014) found that blue-light irradiation by a common LED can kill insect pests of various orders and that highly lethal blue-light wavelengths are species-specific in insects.

So the main target of the present paper was about examination of lethal effect of Light Emitting Diodes (LEDs) on both types of *Tetranychus urticae* adult females and the efficiency of interaction between LEDs and Vertimec.

2. **MATERIALS AND METHODS**

2.1. *Tetranychus urticae* culture

The green and red forms *Tetranychus urticae* were collected from naturally infested cowpea (*Vigna unguiculata*) and strawberry (*Fragaria ananassa*) plants respectively. Then they were reared under
laboratory conditions on discs of castor oil bean plants according to Abd El-Wahab (2010) for six months before treatments.

2.2. Tested Compound and its experiment

The compound: Vertimec (Abamectin 18 g L⁻¹). The experiment: Colonies of the spider mite, *T. urticae* were reared under laboratory conditions (25±2°C, and 60±5%RH) to evaluate the activity of Vertimec against *T. urticae* adult females. The leaf-dip technique described by Dittrich (1962) was used as following: Several concentrations of Vertimec (18 g L⁻¹) were prepared. Castor oil leaf discs (2 cm diameter) were dipped in each concentration for 10 seconds, and left to dry. Discs were placed onto cotton wool pads in Petri-dishes (9 cm diameter). 10 adult females of *T. urticae* were transferred to the treated castor oil leaf-discs, to each replicate, by using camel hair brush with the aid of stereomicroscope. All of treatments were left under laboratory conditions . Each treatment was replicated three times. In addition, control discs were exposed to fluorescent only. Observations were taken after 24h. Mortality percentages were determined and corrected by using Abott's formula (1925).

2.4. *Tetranychus urticae* exposure to Light Emitting Diodes (LEDs) treatments

The two forms of *Tetranychus urticae* 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. 10 adult females of *T. urticae* were transferred to the treated castor oil leaf-discs, to each replicate, by using camel hair brush with the aid of stereomicroscope. All of treatments were left under laboratory conditions . Each treatment was replicated three times. In addition, control discs were exposed to fluorescent only. Observations were taken after 24h. Mortality percentages were determined and corrected by using Abott’s formula (1925).

2.5. Conjugated experiments

Light Emitting Diodes (LEDs) treatments with the two main colors, White and Blue controlled by Arduino were conjugated with Vertimec LC50. Observations were taken after 24h. Mortality percentages were determined and corrected by using Abott's formula (1925).

2.6. Translaminar experiments

Each experiment was done as described before but the difference was about treated just one side of the leaf discs and the mites were put on the other untreated side. Observations were taken after 24h and mortality percentages were determined.

2.7 Monoamine Oxidase Inhibition

MAO-A is a flavin adenine dinucleotide (FAD) containing enzyme which is tightly anchored to the mitochondrial outer membrane. MAO-A inhibition potencies treatments were determined in the homogenates of each treatment. The rate of the MAO-catalyzed oxidation of Kynuramine was measured according to Aiyegoro and Van Dyk (2011). 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 4-hydroxyquinoline in the incubation mixtures was determined by comparing the fluorescence emitted by the samples to that of known

<table>
<thead>
<tr>
<th>Light Color</th>
<th>Wavelength [nm]</th>
<th>Voltage drop [AV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>450 &lt; λ &lt; 500</td>
<td>2.48 &lt; n &lt; 3.7</td>
</tr>
<tr>
<td>White</td>
<td>Broad spectrum</td>
<td>ΔV = 3.5</td>
</tr>
</tbody>
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Table 1: Wavelengths of LEDs
amounts of authentic 4-hydroxyquinoline at excitation (310 nm) and emission (400 nm) wavelengths. All enzymatic reactions were carried out to a final volume of 500 µL in potassium phosphate buffer and contained kynuramine as substrate, MAO-A (0.0075 mg/mL) and various concentrations of the test inhibitor (treatment). Stock solutions of the test inhibitors were prepared. 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).

2.8. Data Analysis

SPSS (V.16) was used to show differences among treatments and test significance among groups through experiments in the present research.

3. RESULTS

Direct effects of light emitting diodes were appeared in the two ways under laboratory conditions:

3.1. Mortality Percentages

Figure (1) showed that both White and Blue LEDs always caused 100% mortality against green and red types of *T. urticae*, respectively. While, White LEDs caused 90% against the red type and Blue LEDs caused 96.67 against the green type. All joined treatments of LEDs and Vertimec LC50 caused 100% mortality against both types. Pearson Correlation (Sig. (2-tailed <1) at 1% was highly significant at 1% between Direct and translaminar effects of treatments.

3.3. Effects on Mono Amine Oxidases (MAO)

Effects of Light Emitting Diodes (LEDs) and Vertimec and the action of both treatments together on Mono Amine Oxidases (MAO) of *T. urticae* adult Females had highly significantly differences in comparison with control (Figure 3). T Paired Samples Test (*t*=5.291**, Kendall's W Coefficient of concordance=.694** and Chi-Square =8.333** were calculated to confirm results. Moreover, with Two-stage Least Squares Analysis (Multiple R=.271, R² =.074), there was a significant effect of LEDs on MAO at (*t*=3.709 at 1%) while Vertimec efficiently affected with (*t*=.891 at 5%).

4. DISCUSSION

LEDs have been commercially available since the 1960s, but they came only in a limited range of colors. Without a blue LED, there was no way to generate white light. Normile (2014) mentioned that trio of Japanese researchers in 2014 captured the Nobel Prize in physics for their invention of blue light-emitting diode (LED), which initiated a revolution in artificial lighting. That will lead to more specifically control of all agricultural pests and I expected that LEDs will be an important part of IPM in a few years. IPM also promoted the search and utilization of natural active principles (insecticides, repellents, attractants, etc.) which could be helpful in dealing with insect pests of crops and forest plantations. The concept of “Green Pesticides” is also one that is gaining a lot of attention presently. It refers to all types of nature-oriented and beneficial pest control materials, which can contribute to reduce the pest population and increase food production. They are safe and ecofriendly. They are more compatible with the environmental components than synthetic pesticides (Isman and Machial, 2006). Depending on the present paper, it could be considered that LEDs are an important and new tool of green pesticides which are totally specific on target pests and have no bad hazards.
Some insecticide/miticides have translaminar, or local, systemic activity. These materials penetrate leaf tissues and form a reservoir of active ingredient within the leaf. This provides residual activity against certain foliar-feeding insects and mites. Insecticides/miticides with translaminar properties include abamectin (Avid), pyriproxyfen (Distance), chlorfenapyr (Pylon), spinosad (Conserve), and acephate (Orthene). In general, these types of materials are active against spider mites and/or leafminers. Because the active ingredient can move through plant tissues (that is, leaves), thorough spray coverage is less critical when using these materials to control spider mites, which normally feed on leaf undersides.

LEDs are identified to cause direct control of both tested pests as both Vertimec (as its translaminar effect) and also close to Formamidines (as its the mode of action). But LEDs are considered as physical...
treatments which are so safe and without any bad side effects on plants, water, non-target organisms such as bees, predators (Abd El-Wahab et al., 2014) and parasitoids. Besides, 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 mites became quiescent and died and that was the same which occurred by LEDs treatments also.

Energy-efficient LEDs could be used for more environmentally friendly insect control as explained by Kim et al. (2012). Photo-response of the tobacco whitefly to light-emitting diodes of four different wavelengths and various intensities in an LED-equipped Y-maze chamber were compared with the response to black light (BL). 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%). In the same way, Pawson and Bader (2014) hypothesized that white LEDs would be more attractive and thus have greater ecological impacts than high-pressure sodium vapor lamps (HPS) due to the peak UV-green-blue visual sensitivity of nocturnal invertebrates. Results supported this hypothesis; on average LED light traps captured 48% more insects than were captured with light traps fitted with HPS lamps, and this effect was dependent on air temperature (significant light × air temperature interaction).

In the agreement with results of the present research, Hori et al. (2014) investigated the lethal effects of visible light on insects by using light-emitting diodes (LEDs). The toxic effects of ultraviolet (UV) light, particularly shortwave (i.e., UVB and UVC) light, on organisms are well known. However, the effects of irradiation with visible light remain unclear, although shorter wavelengths are known to be more lethal. Irradiation with visible light is not thought to cause mortality in complex animals including insects. Here, however, they found that irradiation with short-wavelength visible (blue) light killed eggs, larvae, pupae, and adults of Drosophila melanogaster. Blue light was also lethal to mosquitoes and flour beetles, but the effective wavelength at which mortality occurred differed among the insect species. Their findings suggest that highly toxic wavelengths of visible light are species-specific in insects, and that shorter wavelengths are not always more toxic. For some animals, such as insects, blue light is more harmful than UV light.
Concerning the inhibition of monoamine oxidase (MAO) by LEDs in mites, which also could be inhibited by formamidines (Atkinson et al., 1974) and that was not the sole mechanism in formamidine poisoning.

In the same trend, Kaufman and Sloley (1996) assessed biogenic amine catabolism in haemolymph and saliva of *Amblyomma hebraeum* Koch. As a result, Dopamine (DA) and 5-hydroxytryptamine (5-HT) were rapidly converted to dihydroxy phenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) respectively, indicating that monoamine oxidase (MAO) constituted a major catabolic pathway for biogenic amines in this species. Deprenyl was about 44-72 times more potent an inhibitor of MAO than clorgyline when either DA or 5-HT was offered as substrate, suggesting that this MAO was of the MAOB type. Conversion of DA to DOPAC was also detected in several tissues incubated with DA in vitro; in descending order of MAO activity (pmol mg⁻¹ h⁻¹ at about 18 degrees C) tissues tested were: skeletal muscle (approximately 100), Malpighian tubule (approximately 50), ovary (approximately 45), salivary gland (approximately 20), and haemolymph (approximately 4-5). So inhibition of MAO would lead to accumulation of biogenic amines with their effects.

To conclude, there was a positive relation in the presence of LEDs among accumulation of biogenic amines with the increased inhibition of MAO activity, and mortality ratios in mites in comparison with the control. Mentioned relation increased with joined action if Vertimec and LEDs affected. This paper recommended using LEDs as a new trend in controlling *T. urticae* effectively and the efficiency increased when use Vertimec LC50.

REFERENCES


The Fifth International Conference Coordinators of Arab Union for Sustainable Development & Environment AUSDE: "Future of Water, Energy, Climate and Food Nexus in the Arab Countries"