Light Emitting Diodes (LEDs) Reduce Vertimec, Resistance in Tetranychus urticae (Koch)

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ABSTRACT: The system based on light emitting diodes (LEDs) controlled by Arduino microcontroller could play an important role in the steadily reduction of Vertimec resistance in adult females of *Tetranychus urticae*. The resistance ratio of vertimec resistant strain gained after 30 selected generations (VERT30), recorded 34.81-, 36.84-, 39.76- and 72.30- Folds, resp., of Vertimec, Chlorpyrifos, Fenoxyprimate and Propargite. White LEDs decreased multiple resistances more than Blue LEDs in (VERT30). The inheritance mode of Vertimec resistance was incompletely dominance (1>DD>0) but it was so close to Zero value after treatments. Decreased activity of both Esterases and Oxidases was sharply occurred with diode which means revision of resistance. Also, reactive oxygen scavengers (ROS) increased gradually by exposure to White and Blue LEDs which contributing in the reduction of resistance with increased ratios ranged from 11.49- to 6.11- Folds. Resulted data were discussed and explained in details.

Keywords: Tetranychus, Resistance, LEDs, Arduino, Vertimec , Fenoxyprimate, Chlorpyrifos, Propargite, ROS.

INTRODUCTION

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 [EEG, 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 on-time (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*

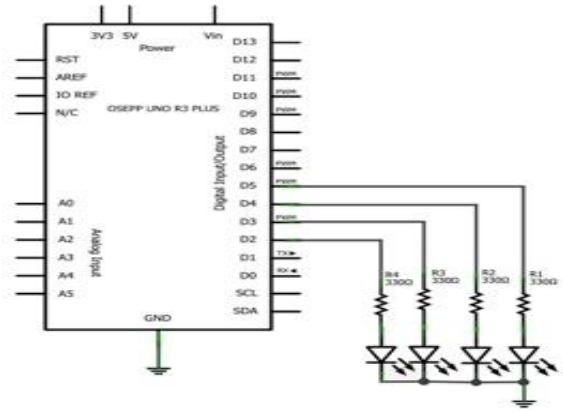
vivo multispectral imaging system for rodents (Sun et al.,2010),teaching violin bowing by providing an interface for combining motion sensing and vibrotactile feedback (Van Der Linden et al.,2011), wearable computing (Isoyama et al.,2011), and in cross-disciplinary teaching of biology and computer science (Grasel et al.,2010).

So the conjugation between arduino and LEDs in one system was used to test its ability to play a role as a tool to break pests' control and specifically mites such as *Tetranychus urticae.* Moreover, their contribution in the breakage of pesticide resistance as which will be showed in details and explained.

Materials and Methods

Light Emitting Diodes

Each lighting treatment was conducted in separately controlled chambers, to be free from spectral interference among treatments. Totally used 8 LEDs (Light Emitting Diodes), (4+4), to provide an 18 h light/6 h dark photoperiod at the duration of exposure. Treatments were done under two different light colors with broad-spectrum-white LED (BSWL, 420-680 nm) and blue LED (460 nm), while control was under normal fluorescent light. Light quality and quantity were estimated using a Testo545 light meter (Testo, Germany). Tow colors, white and blue were used and controlled by Arduino Uno .C++ language was used in the programming to On/Off lights automatically.



Schematic Fig. (1) Shows 330 ohm resistor in series to each LED device. In use digital pin 2, 3, 4 and 5

Maintenance of *Tetranychus urticae* strains:

1-Susceptible strain

Colonies of the spider mite, *T.urticae* were reared under laboratory conditions (25±2˚C,and 60±5%RH)at Plant Protection Research Institute for several years without exposure to any pollutions or pesticides .

2-Resistant strain

Original colony of the spider mite, *T.urticae* was established from mites collected from castor oil plants without exposing to pesticides. It was 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 dipped in water only. Observations were taken after 24h. Mortality percentages were determined and corrected by using Abott's formula (1925).Pooled data were subjected to probit analysis (POLO PC) (LeOra software, 1994). LC50 values of the selection strain were compred to those of susceptible strain (GSS). Females of the original strain were selected for Vertimec for 30 generations. Selection pressure used a modified form of the method developed by Yang *et al*.(2002). 1000 adult females of this colony started this selection. Every two generations, the LC50, LC90 and slope were evaluated. New LC50 was applied as subsequent selection pressure. Mortality percentages were recorded.The next selection transferred to untreated leaves .LC50 values of the selection strain were compared to those of the susceptible strain. The resistance ratio (RR) was calculated according to the following formula, RR=LC50

value of the resistant strain / LC50 value of the GSS strain.

Multiple resistance of other pesticides to Vertimec selected strain

Multiple resistance of Vertimec , Chlorpyrifos , Fenoxyprimate and Propargit was evaluated using GSS and VERT30 strains of *T.urticae*. The same bioassay method used for all pesticides was done in this case. Mortality percentages were determined for both GSS and VERT30 strains. Data were subjected to probit analysis (POLO PC) (LeOra software, 1994). LC50 values of the selection strain were compared to those of susceptible strain (GSS). The resistance ratio (RR) was calculated according to the following formula, RR=LC50 value of the resistant strain / LC50 value of the GSS strain.

Exposure to Light Emitting Diodes (LEDs)

Resistant strains 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 . Each resistant strain maintained after the 30 generations for 10 days during larval and nymphal durations. After that, resulted adult females were collected and the resistance ratio calculated for each resistant strain.

Crossing Experiments

To estimate the dominance of resistance, the GSS and the Vertimec resistant strain VERT30 were reciprocally crossed to produce hybrid F1 females by placing 30 females, emerged newly from deutonymphal stage, of one strain and 30 adult males of the other strain on the upper side of a primary castor bean leaf disc, which was then placed on wet cotton pad in petri dish. Directly after molting, the diploid females were fertilized by the haploid males, and 1 day later they began to lay eggs. Both males and females were removed after 5 days. If mating was successful, the haploid-diploid mating system resulted in F1 females. Resulting F1 females were then transferred to leaf discs and the bioassay experiment was started when F1 females reached the adult stage. The bioassay method was the same as described previously in the toxicity test. The experiment was conducted and replicated triple. Then the experiment has done again under LEDs with both its tested colors, White and Blue, respectively.

The degree of dominance (D) of the resistance strain trait in the F1 females from both reciprocal crosses was estimated using the next formula (Stone, 1968).

D= (2 X2-X1-X3) / (X1-X3)

X1=The log of LC50 of the resistant strain.

X2=The log of LC50 of the F1 females.

X3=The log of LC50 of the susceptible strain.

Biochemical Studies

Esterases and oxidases were determined and compared in all *T.urticae* strains all over its resistant generations to used pesticides at LC50's.

Preparation of *T.urticae* samples for analysis

0.1gm of each stored sample was weighed and then homogenized in a cold porcelain mortar containing 1 ml of distilled water. Each sample was then centrifuged 12000 r.p.m. for 5 min. and the supernatant (fluid) was transferred to an Eppendorf tube. The supernatant was kept frozen at -2˚C until analyzed.

Estimation of esterases activity

EST activity was measured using α –Naphthyl Acetate (α-NA) by the method of Van Asperen(1962) with slight modifications .The reaction mixture contained 450 µl of potassium phosphate buffer (4mM,PH 6.8) and 50 µl of enzyme solution (from 0.01 gm of each stored sample) was incubated at 37˚ C for 15 min after addition of 0.5ml of α-NA in ethanol(from 2 mg of α-NA dissolved in 10 ml) .The reaction was stopped and color developed by adding 0.5ml of dye solution (10g litre¯1 diazoblue B salt+50g litre¯1 sodium lauryl sulfate) 2:5 by volume for 20 min.The absorbance was read at 600nm for α-NA by a Gilford 260PS spectrophotometer.

Estimation of oxidases assay

MFO activity was measured using p-nitroanisole-O-demethyl (PNA) by the method

of Kim *et al.*(2004). The reaction mixture contained 50 µl of miocrosomal preparation (5-50 protein equivalents) , 50 of NADPH – generating system (Magnesium chloride 12 mM , NADPH 2.7m M,NADP 8.1mM, glucose -6-phosphate 240Mm,glucose-6-phosphate dehydrogenase 25 units ml^{-1}, 390 µl of potassium buffer (0.1M,PH7.4)and 10 µl of PNA in ethanol (0.05mM).The reaction was run at 37° C for 3min.Absorbance was measured at 400nm by a Gilford 260PS spectrophotometer .The concentration of P-nitrophenol generated was determined from a standard curve.

Synergism Test

PiperonylButoxide (PBO-Technical product from Sigma, Milwaukee, WI) and S,S,S tributylphosphorotrithioate (DEF- Technical product from ChemServices West Chester, PA) were used to inhibit detoxification mechanisms by mixed-function oxidases (MFO) and esterases (EST) ,respectively, by adding 10 µl of synergist to the mite homogenates in a final concentration of 10^{-7} M and was incubated for 5min. at 27°C.Synergistic ratio (SR) was calculated as following formula:

SR=LC50 of Vertimec without synergist/ LC50 of Vertimec with synergist

Antioxidant of enzyme activities

POD activity was assayed according to the method of Hemeda and Klein (1990). A total of 100 mL of reaction mixture containing 10 mL of 1% guaiacol (v/v), 10 mL of 0.3% H2O2 and 80 mL of 50 mM phosphate buffer (pH 6.6). Enzyme extract (75 µL) was added to reaction mixture in a final volume of 3 mL. The increase in absorbance due to oxidation of guaiacol (extinction coefficient 26.6 mM/cm) was monitored at 470 nm. Enzyme activity was expressed as unit's min/mg protein.

APX activity was measured by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mM/cm). The 3 mL reaction mixture contained 50mM phosphate buffer (pH 7.0), 0.1 mM H2O2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The change in absorbance was monitored at 290 nm and enzyme activity was expressed as unit's min/mg protein (Nakano and Asada 1981).

SOD activity was measured by the photochemical method as described by Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm in the presence of riboflavin and light. The reaction mixture contained 45 mM potassium phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 13 mM methionine, 0.17 mM NBT in ethanol, 0.007 mM riboflavin and enzyme aliquot. Blanks were kept in the dark and the others were illuminated for 15 min. One unit of SOD is the amount of extract that gives 50% inhibition to the rate of NBT reduction.

Exposure to Light Emitting Diodes (LEDs)

Both strains GSS and VERT30 of *Tetranychus urticae* adult females 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 . The released larvae from the 29th generation till adult stage were developed under LEDs to get the 30th generation for 10 days during larval and nymphal durations. After that, resulted adult females were collected and all required toxicological estimations and the resistance ratio were calculated for the resistant strain.Also exposure was occurred during the tests of toxicity, multiple resistance and cross resistance in case of GSS and VERT30.

Statistical Analysis

XLSTAT's statistical analysis software (V.2011) was used to show significant differences among collected data.

Results

Selection Pressure

VERT30 after selection recorded 1502.88 µl/l with increase by 34.81 folds in comparison with GSS strain (43.17 µl/l). Highly slope value of GSS showed computability in the sensitivity to Vertimec .Table (1).

Table (1) Test of multiple resistances to Vertimec resistant (VERT 30) and susceptible strains of *Tetranychus urticae*

Pesticide	Strain	LC50 (ML-LL)	LC90 (ML-LL)	Slope	RR
Vertimec	GSS	43.17 (30.84-60.44)	302.33 (215.95-423.26)	2.01	----
	VERT30	1502.88 (834.93-2705.18)	9888.13 (5493.41-17798.63)	1.91	34.81
Chlorpyrifos	GSS	49.91 (40.91-60.89)	489.04 (400.85-596.63)	0.35	----
	VERT30	1838.52 (1193.84-2831.32)	10201.28(6624.21-15709.97)	0.82	36.84
Fenoxyprimate	GSS	54.62 (31.39-95.04)	892.73 (513.06-1553.35)	0.98	----
	VERT30	2171.94 (1085.97-4343.88)	11509.51(5754.48-23134.12)	0.20	39.76
Propargite	GSS	73.57 (70.74-76.51)	1301.38 (1249.32-1353.44)	1.24	----
	VERT30	3319.22 (2289.12-4812.87)	14800.65(10207.34-21460.94)	0.71	72.30

Multiple Resistances

The toxicity of certain pesticides to the resistant Vertimec (VERT30) and the susceptible (GSS) strains of *T.urticae* was examined using leaf dip technique. The activities of Chlorpyrifos, Fenoxyprimate and Propargite against both strains of *T.urticae* were shown in Table (1).The VERT30 strain showed multiple resistances to all previous tested pesticides. Resistance ratios recorded 34.81, 36.84, 39.76 and 72.30 for Vertimec, and multiple resistance against Chlorpyrifos, Fenoxyprimate and Propargite, respectively, under White LED exposure.

Effect of Light Emitting Diodes (LEDs) on Multiple Resistances

Light Emitting Diodes (LEDs) showed their effects on the multiple resistances in Table (2 and 3). LC50s reduced gradually but seemed that the effect of White LED was higher than Blue LED. Resistance ratios recorded 25.62, 18.84, 30.12 and 35.64 for Vertimec, and multiple resistance against Chlorpyrifos, Fenoxyprimate and Propargite, respectively, under White LED exposure. While they recorded 29.60, 20.26, 33.29 and 29.32 for the same arrangement resp., under Blue LED.

Table (2) Test of multiple resistances to Vertimec resistant (VERT 30) and susceptible strains of *Tetranychus urticae* after exposure to White LEDs

Pesticide	Strain	LC50 (ML-LL)	LC90 (ML-LL)	Slope	RR
Vertimec	GSS	10.66(8.08-14.07)	78.27(59.30-103.32)	1.96	----
	VERT30	273.15(137.26-543.57)	1102.33(553.93-2193.64)	2.05	25.62
Chlorpyrifos	GSS	18.85(12.57-28.28)	220.62 (147.08-330.93)	1.73	----
	VERT30	351.44(276.72-446.33)	1722.03(1355.93-2186.98)	1.34	18.64
Fenoxyprimate	GSS	20.92(13.95-31.38)	300.41(200.27-450.62)1.5	1.23	----
	VERT30	630.16(428.68-926.34)	1013.57(689.50-1489.95)	1.58	30.12
Propargite	GSS	23.03(20.56-25.79)	390.01(348.22-436.81)	1.47	----
	VERT30	820.71(509.76-1321.34)	12003.08(7455.33-19324.96)	1.83	35.64

So multiple resistance occurred when the use of Vertimec joined with Chlorpyrifos, Fenoxyprimate and Propargite, respectively.But, this linkage could be breaked significantly by the exposure to mainly White LEDs, followed by Blue LEDs,rep.

Table (3) Test of multiple resistances to Vertimec resistant (VERT 30) and susceptible strains of *Tetranychus urticae* after exposure to Blue LEDs

Pesticide	Strain	LC50 (ML-LL)	LC90 (ML-LL)	Slope	RR
Vertimec	GSS	13.19(7.95-21.90)	101.53(61.16-168.54)	1.22	----
	VERT30	390.42(379.05-402.13)	1801.28(1748.82-1855.32)	1.55	29.60
Chlorpyrifos	GSS	25.77(16.63-39.94)	422.53(272.60-654.92)	1.70	----
	VERT30	522.08(474.62-574.29)	1922.76(1747.96-2115.04)	1.78	20.26
Fenoxyprimate	GSS	30.04(28.88-31.24)	341.27(328.14-354.92)	1.33	----
	VERT30	1000.12(990.22-1010.12)	7111.89(7040.77-7183.01)	1.41	33.29
Propargite	GSS	35.72(34.02-37.51)	401.01(381.91-421.06)	1.09	----
	VERT30	1047.37(824.70-1330.16)	12999.10(10235.51-16508.86)	1.23	29.32

Inheritance mode of Vertimec resistance

In an attempt to estimate the dominance of the resistance, individuals of the susceptible (GSS) and resistant Vertimec (VERT30) strains reciprocally crossed to produce hybrid F1 females.

The response of F1 females showed that the resistance trait before exposure to LEDs was inherited from both the maternal and parental line and was incompletely dominant (Table 4).The dominance value was found to be 0.66 and 0.24 in the VERT30♀X GSS♂ crossed F1and GSS♀X VERT30♂ crossed F1,resp.

Effect of Light Emitting Diodes (LEDs) on the inheritance mode of Vertimec resistance

Noticed reduction in the dominance of the resistance which related strongly with the detected low LC50s in the response of the susceptible (GSS) and resistant Vertimec (VERT30) strains reciprocally crossed to produce hybrid F1 females exposed to LEDs (Table 4). White LEDs minimized the dominance sharply till to be close to zero.

Table (4) Inheritance mode of Vertimec resistance

Strain	LC50	RR	Slope±SE	D
GSS	13.19 (9.84-17.67)	------	2.18±1.02	-
VERT30	390.42(205.48-741.80)	29.60	1.99±0.38	-
F1(VERT30♀X GSS♂)	235.08(159.85-345.71)	16.73	1.23±0.11	0.66
F1(GSS♀X VERT30♂)	220.65(200.60-242.72)	17.82	1.29±0.02	0.42
Exposure to White LED				
F1(VERT30♀X GSS♂)	31.01(25.84-37.21)	2.35	1.53±0.18	0.26
F1(GSS♀X VERT30♂)	22.55(22.10-23.01)	1.71	1.77±0.10	0.01
Exposure to Blue LED				
F1(VERT30♀X GSS♂)	48.64 (31.58-74.91)	3.69	1.41±0.21	0.56
F1(GSS♀X VERT30♂)	30.19(24.54-37.13)	2.29	1.49±0.35	0.13

F1 females showed that the resistance trait was not dependent on parents and was incompletely dominant (Table 4).The dominance value was found to be 0.26 and 0.01 in the VERT30♀X GSS♂ crossed F1and GSS♀X VERT30♂ crossed F1, resp.

In the same trend, but with semi-higher dominance ratios under Blue LEDs , F1 females showed that the same incompletely dominant (Table 4).The dominance values were found to be 0.56 and 0.13 in the VERT30♀X GSS♂ crossed F1and GSS♀X VERT30♂ crossed F1, resp.

Biochemical assays

***T.urticae* Esterases Activity:**

Data in Table (5) referred to the changes of the rate of α-NA hydrolysis by susceptible and VERT30 strains of *T.urticae* adult females' homogenates. The data generally revealed that all treatments caused significant increased difference in α-NA hydrolysis in the VERT30 in comparable with the susceptible strain. Vert 30 recorded the highest level of α-NA hydrolysis (7.44)µg/mite/minute,with 2.75- folds more than GSS.While Vert 30 +Blue LED and Vert 30 +White LED recorded (7.29) and (6.31) µg/mite/minute resp., with 2.70- and 2.33- fold increased than GSS.

Table (5) Rate of Esterases and Oxidases Activity of (S) and (R) Strains of *T.urticae* homogenates, with and without Synergists

***T.urticae* Strain**	**Enzyme**	**Specific Activity mOD min-1 mg-1 proteins (±SE)**	**Synergist (±SE)**	**[1]R/S**	**[2]Synergism Ratio**
S	Esterase	2.71±0.10	3.55±0.43		0.76c
S+ WHITE		3.08±1.04	4.92±0.52		0.63a
S+ BLUE		4.73±0.31	7.21±0.62		0.65b
R		7.44±0.81	11.00±0.93	2.75c	0.68b
R+ WHITE		6.31±0.09	8.14±0.90	2.33a	0.78d
R+ BLUE		7.29±0.54	10.88±0.34	2.70b	0.67c
S	Oxidases	12.40±1.22	18.98±0.45		0.65b
S+ WHITE		13.33±1.03	21.01±0.72		0.63a
S+ BLUE		13.87±0.42	21.03±1.32		0.66b
R		34.84±0.45	42.22±1.09	2.81c	0.83d
R+ WHITE		25.99±1.03	37.57±1.88	2.10a	0.69c
R+ BLUE		29.52±0.41	42.17±0.11	2.38b	0.70b

[1]R/S = Resistance Ratio = LC50 of the (Tested) resistant strain / LC50 of the susceptible strain.
[2]Synergism Ratio Enzyme+Synergist =Potentiation of synergist to the tested pesticide.

Synergistic effects showed the less effect of esterases in Vertimec resistance formation. However there was a significant observed role of LEDs exposure to reduce resistance. Synergistic ratios were arranged decently from VERT30, Vert 30 +Blue LED and Vert 30 +White LED with 2.75- , 2.70- and 2.33- fold, resp., than GSS.

***T.urticae* Mixed function Oxidases Activity:**

The oxidases activity in VERT30 showed significantly higher increase than GSS strain ($P < 0.05$). Table (6)showed that PNA hydrolysis by VERT30 2.81- fold in comparable with GSS .Followed by Vert 30 +Blue LED and Vert 30 +White LED which recorded (29.52) and (25.99) µg/mite/ minute resp., with 2.38- and 2.10- fold more than GSS.

Synergistic effects were studied to prove the main role of oxidases of resistance formation and then the significant effect of LEDs exposure to reduce it efficiently. Synergistic ratios were arranged decently from VERT30, Vert 30 +Blue LED and Vert 30 +White LED with 2.81- , 2.38- and 2.10- fold, resp., than GSS.

T.urticae Reactive Oxygen Scavengers (ROS) Ratio

Reactive Oxygen Scavengers (ROS) in VERT30 showed significantly higher increase than GSS strain ($P < 0.05$). Table (6) and Fig.(2) showed that Ascorbate Peroxidase (APX), Peroxidase (POD) and Superoxide dismutase (SOD) in VERT30 is higher than GSS with 5.99-, 6.02- and 4.71-folds, resp. The most efficient light treatment recorded with light LED. It was higher than GSS with 9.87-, 8.34- and 11.94-folds, resp. Resistance reduction occurred with specific relation with increased reactive oxygen scavengers (ROS) ratio after exposure of VERT30 to White and Blue LEDs.

The activity of free radical scavenging enzymes viz., APX, POD and SOD showed inverse relationship with Vertimec resistance. Also, it is stated that LEDs and specifically White LED able to increase antioxidants with reduction of free radicals in Verimec resistant adult females.

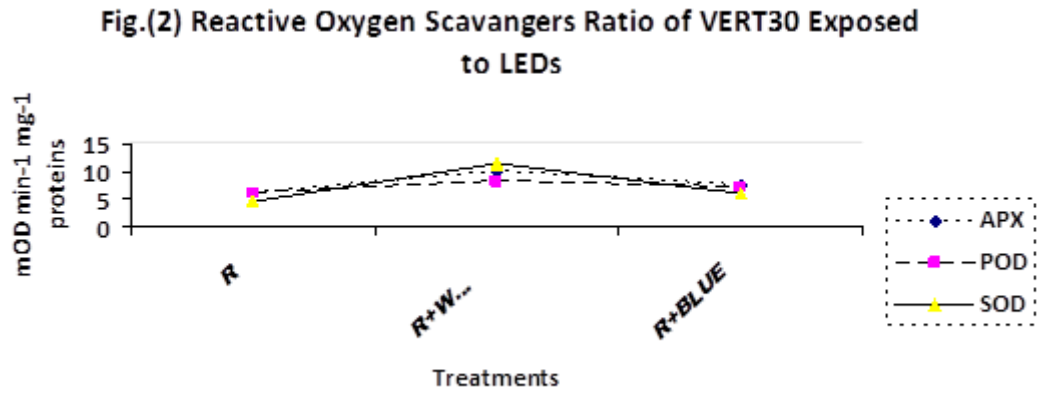
Table (6) Reactive Oxygen Scavengers (ROS) Ratio Before and After Exposure To LEDs

T. urticae **Strain**	**[1]ROS (Reactive Oxygen Scavengers)**			**[2]Increased Ratio**		
	APX	**POD**	**SOD**	**APX**	**POD**	**SOD**
S	5.44±0.02a	7.32±0.11a	4.26±0.14a	NE		
S+WHITE	10.11±0.20b	15.24±0.04	11.88±0.09b			
S+BLUE	7.14±0.31a	10.46±0.19a	8.18±0.12a			
R	32.64±0.35c	44.09±2.00c	20.06±0.22c	5.99	6.02	4.71
R+WHITE	53.71±1.01e	72.01±1.94e	48.93±0.92e	9.87	8.34	11.49
R+BLUE	40.20±0.97d	53.12±1.18d	26.04±0.15d	7.39	7.26	6.11

[1]ROS (Reactive Oxygen Scavengers) APX-Ascorbate peroxidase, POD-Peroxidase and SOD-Superoxide dismutase.

Values are expressed as the means ±SE. Mean

[2] Increased Ratio = LC50 of the tested strain / LC50 of the susceptible strain



DISCUSSION

Light Emitting Diodes proved its effectiveness against the most of agricultural pests with good effects on exposed plants such as tomato, cucumber (Kim *et al*.2013) and strawberry (Samuolienė *et al*. 2010). In higher plants, blue-light is mainly perceived by cryptochromes and phototropins, which subsequently orchestrates phototropism, chloroplast relocation, stomatal opening, rapid inhibition of hypocotyl elongation and leaf expansion. Blue-light signaling is also known to mediate the plant responses to biotic stresses, but relevant mechanisms are largely unknown. Here, we demonstrated that blue LED (Light Emitting Diode)-driven inhibition of gray mold disease was highly correlated with the increases in cellular protectants like proline, antioxidants and ROS (Reactive Oxygen Species) scavenger activities. The activities of various ROS (Reactive Oxygen Species) scavenging enzymes were also slightly increased under the blue-LED lighted conditions. Finally, blue-LED significantly suppressed symptom development of tomato infected by gray mold. Combined results suggest that blue LED light inhibits the development of gray mold disease, which can be mechanistically explained by the enhanced proline accumulation and antioxidative processes at least in partial.

Pests and mainly insects' responses to light are substantially influenced by a variety of factors, including light intensity and wavelength, combinations of wavelengths, time of exposure, direction of light source, and the contrast of light source intensity and color to that of ambient light. In addition, the impact of light on insect behavior varies both qualitatively and quantitatively depending on the light source (light bulb or light-emitting diode [LED]) and material (light-reflecting plate) (Antignus 2000; Honda 2011; Johansen *et al*. 2011; Matteson *et al*. 1992 and Nissinen *et al*. 2008). In the same trend insects which are able to see ultraviolet (UV) radiation could be controlled by the same tool while future development and use light-emitting diodes is anticipated for promoting integrated pest management more safely (Shimoda and Honda 2013). The effect of Red LED on Sporulation of *Neozygites floridana* on *Tetranychus urticae* host was tested by Holthe (2012). He found that the sporulation *N. floridana* was prolonged and had more efficiency as a biological control tool.

Diodes can be used effectively also against the gray mold disease infested tomato by *Botrytis cinerea* (Kim *et. al.*2013). After twenty one days of exposure to various wavelengths of LED lights, blue-LED treated tomato displayed significant increases in proline accumulation in the leaves and stems, whereas red- and green-LED treated tomato exhibited the lower proline contents. Similarly, the blue-LED treatment increased the amount of polyphenolic compounds in tomatoes, compared to other wavelength of LED lights. The activities of various ROS (Reactive Oxygen Species) scavenging enzymes were also slightly increased under the blue-LED lighted conditions which demonstrated that blue LED (Light Emitting Diode)-driven inhibition of gray mold disease was highly correlated with the increases in cellular protectants like proline, antioxidants and ROS (Reactive Oxygen Species) scavenger activities.Cowan and Gries (2009) tested the hypothesis that moths of *Plodia interpunctella* use wavelengths of visible blue/violet light as orientation cures that trigger phototactic responses. In a four choice lab experiment, blue light was more effective than green, orange, or red light. In subsequent experiments that tested LEDs emitting peak wavelengths in the blue/violet light range, 405 nm was significantly more effective than 435-, 450-, or 470-. Nakamoto and Takushi (2002) confirmed the positive phototactic response of the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) to yellow, green, and blue rays of a LED (light emitting diode).Also mounted trips under field conditions with diodes captured significantly more weevils than that without the LED source in the field.

Diodes can be joined its properties to control Vertimec resistance in mites specially with the known information concerning the small number of chromosomes (n = 3) in *T.urticae*, which increased the possibilities of multiple-resistance development (Helle & Bolland 1967) .

The selection with an acaricide (e.g. abamectin) may select populations resistant to another group of acaricides (e.g. pyrethroids), if the genes responsible for resistance to these two groups were located in the same chromosome (Omoto,1995). Abamectin resistance in *T. urticae* was also reported by several authors (Campos et al. 1996, Beers et al. 1998). Stumpf&Nauen (2002), investigating enzymes involved in abamectin resistance in the two-spotted spider mite, observed that resistant strains (NL-00 and COL-00) presented several fold higher MFO (cytochrome P450-dependent monooxygenase) activity than the susceptible strain GSS. Abamectin resistance in strain NL-00 was strongly synergized by PBO (piperonyl butoxide) and DEM (diethyl maleate), suggesting that MFO and GST (glutathione S-transferases) might be involved in abamectin resistance. These results were in the same trend with ours, which vertimec resistant strains recorded higher oxidases activity than all resistant strains of other treatments. It could be said clearly that metabolic resistance of vertimec was depending on oxidases activity in the treated mite. Also it is obviously in the present study that even in case of VERT 30 exposed to White LED , the adult females recorded the lower level of esterases and oxidases in comparable with others under blue light beside that all mites stopped feeding and moving.(unpublished data).In the same trend, White LED showed higher reactive oxygen scavengers ratio which caused mortality of all individuals have resistance genes in comparable with its behavior with susceptible mites under the same light color.That caused the appearing of new susceptible generation of Vertimec and put a difference tool to stop resistance easily and fastly .Mechanism used in this research could be used under greenhouses conditions specially its depending mainly on Arduino UNO which can contacted with solar panel systems or batteries and then it'll be able to do many tasks in the same time.

The most helpful information depending on the lowest emission of CO2 is contributed in the explanation of results. Under usual maintenance of *T.urticae* under field or laboratory conditions, there are highly levels of CO2 resulted from many forms of activities around minute refuge of mites. Specially ascending radiation ratio from the sun in field or fluorescent lamps in the laboratory or in incubator. LEDs contains many small semiconductor units; each emits light when a voltage is applied. Besides, LEDs are advantaged with their highly capability and easily replaceable bulb with no wasted energy at all. Certainly, such as light source is LED, power consumption is just 20% of traditional incandescent; working life is 10 times of traditional save-power light, safe and stable. Also with comparable with other sorts of lights such as traditional incandescent, you can find that wasted energy is over 95% and the electric current heats the bulb's tungsten filament until it glows. The same with compact fluorescent (CFL), which excited gas in a CFL tube emits UV photons that coax the bulb's coating to emit visible light. The wasted energy recorded 35%. Finally, our findings in this paper can be in the same side explanation with Suzuki *et al.*(2014) . They found that the photolyases activated by white LED may reduce UV-B–induced DNA damage in spider mite eggs of *Tetranychus okinawanus* .So any changes could be happened and led to get resistance case not allowed.

CONCLUSION

Certain wave lengths of LEDs have two mechanisms:

1-As a resistance breakage tool which able to increase the Reactive Oxygen Scavengers (ROS) effectively as shown in this research paper.

2-As a physical control as anti-feedant factor and in biological control can contribute also with certain wave lengths by attracting specific predators (unpublished data yet).

Different light quality did show a profound effect on the breakage of Vertimec resistance and assimilate distributed of resistant mites. LED lighting devices that take up little space and have low energy consumption are likely to enable pest control in places where conventional light sources are impractical. At the same time, our studies are being carried out to investigate the wavelengths that are effective against mites but have little effect on the occurred plants. This research area aims to develop a new pest control technology that is

fully compatible with cultivation technology and integrated pest management (IPM).

REFERENCES

1. Abott, W.S. (1925) A method of computing effectiveness of an insecticides. J. Econ. Entomol. 18:265-267.

2. Albeanu D.F., Soucy E., Sato T.F., Meister M. and Murthy V.N. (2008) LED arrays as cost effective and efficient light sources for widefield microscopy. PLoS ONE. 3 (5):2146-2148.

3. Antignus Y (2000) Manipulation of wavelength-dependent behavior of insects: an IPM tool to impede insects and restrict epidemics of insect-borne viruses. Virus Res 71:213–220.

4. Beauchamp C, Fridovich I. Superoxide dismutase improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971; 44:276–287.

5. Beers, E.H., Riedl, H. and Dunley, J.E. (1998) Resistance to abamectin and reversion to susceptibility to fenbutatin oxide in spider mite (Acari: Tetranychidae) populations in the Pacific Northwest. J. Econ. Entomol. 91: 352-360.

6. Campos, F., Krupa, D.A. and Dybas ,R.A. (1996) Susceptibility of populations of twospotted spider mites (Acari: Tetranychidae) from Florida, Holland, and the Canary Islands to abamectin and characterization of abamectin resistance. J. Econ. Entomol. 89: 594-601.

7. Cowan T & Gries G. (2009) Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella*. Entomol. Experiment. Applicata.131: 148- 158.

8. Da Silva Pinto M.A., de Souza J.K.S., Baron J. and Tierra-Criollo C.J. (2011) A low-cost: portable, micro-controlled device for multi-channel LED visual stimulation.J Neurosci Methods. 197 (1) : 82–91.

9. Demontis G.C., Sbrana A., Gargini C. and Cervetto L. (2005) a simple and inexpensive light source for research in visual neuroscience.J Neurosci Methods. 146 (1): 13–21.

10. Dittrich,V.(1962) A comparative study of toxicological test methods on a population of the two-spotted spider mite, *T.urticae*. J. Econ. Entomol. 55: 633-648.

11. Fadda A. and Falsini B. (1997) Precision LED-based stimulator for focal electroretinography. Med Biol Eng Comput. 35 (4): 441–444.

12. Grasel J., Vonnegut W. and Dodds Z. (2010) Bitwise biology: cross disciplinary physical computing a top the Arduino. 2010 AAAI spring symposium series.

13. Helle, W. and Bolland, H.R. (1967) Karyotypes and sex determination in spider mites (Tetranychidae). Geneti. 38: 43-53.

14. Hemeda HM, Klein BP.(1999) Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. J Food Sci. 55:184–185.

15. Hogg C. (2006) Stimulus devices, calibration, and measurement of light.JR Heckenlively, GB Arden (Eds.), Principles and practice of clinical electrophysiology of vision (2nd ed.), The MIT Press, Cambridge.

16. Holthe, M. P. (2012) Effect of Red Light on Sporulation of *Neozygites floridana* on Tetranychus *urticae* host. M. Sc. Thesis, Agroecol. Dept., Plant Environ. Sci., Norwegian Univ. Life Sci. pp43.

17. Honda K (2011) Reactions to light in insects and practical applications. J Soc Biomech. 35:233–236.

18. Isoyama N., Terada T., Akita J. and Tsukamoto M. (2011) A method to control LED blinking for position detection of devices on conductive clothes. Proceedings of the 9th international conference on advances in mobile computing and multimedia. MoMM, ACM, New York, NY, USA, pp. 123–130.

19. Johansen N.S. , Pinto D.M., Nissinen A.I. and Shipp L. (2011) In the light of new greenhouse technologies: 2. Direct effects of artificial lighting on arthropods and integrated pest management in greenhouse crops. Ann Appl Biol. 159:1–27.

20. Kim K. , Kook H., Jang Y. , Lee W. , Kamala-Kannan S., Chae J. and Lee K. (2013) The Effect of Blue-light-emitting Diodes on Antioxidant Properties and Resistance to *Botrytis cinerea* in Tomato. J Plant Pathol Microb. 4(9): 203-207. http://dx.doi.org/10.4172/2157-7471.1000203

21. Kim, Y.J., Lee, S.H. ; Lee ,S.W. and Ahn,Y. (2004). Fenpyroximate resistance in *Tetranychus urticae* (Acari: Tetranychidae): cross-resistance and biochemical resistance mechanisms. Pest Manag.Sci., 60: 1001-1006.

22. LeOra Software.1994.POLO-PC.A User's Guide to Probit or Logit Analysis LeOra Software, 28 p., Berkeley, CA.

23. Matteson N., Terry I., Ascolichristensen A., Gilbert C. (1992) Spectral efficiency of the western flower thrips, *Frankliniella occidentalis*. J Insect Physiol 38:453–459.

24. Nakamoto Y. and Takushi J. (2002) A Newly Developed LED (Light Emitting Diode) Trap for the West Indian Sweet Potato Weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae). (Fruit-fly Eradication Project Office, Okinawa Prefectural Government, Naha, Okinawa 902–0072, Japan). Japan. J. Appl. Entomol. Zool. 46: 145–151.

25. Nakano Y and Asada K. (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22:867–880.

26. Narra P. and Zinger D.S. (2004) An effective LED dimming approach.IEEE industry applications conference, 2004, 39th IAS annual meeting, vol. 3. IEEE: 1671–167.

27. Nissinen A, Kristoffersen L, Anderbrant O (2008) Physiological state of female and light intensity affect the host-plant selection of carrot psyllid, *Trioza apicalis* (Hemiptera: Triozidae). Eur J Entomol. 105:227–232.

28. Nygaard, R.W. and Frumkes, T.E. (1982) LEDs: convenient: inexpensive sources for visual experimentation. Vision Res, 22 (4) : 435–440.

29. Pokorny J. , Smithson H.E. and Quinlan J. (2004) Photostimulator allowing independent control of rods and the three cone types .Vis Neurosci, 21 (03) , pp. 263–267.

30. Rogers B., Shih Y.I., Garza B.D.L., Harrison J.M., Roby J. and Duong T.Q. (2012) A low cost color visual stimulator for MRI.J Neurosci Methods, 204 (2) : 379–382.

31. Rumyantsev S.L., Shur M.S., Bilenko Y., Kosterin P.V. and Salzberg B.M. (2004) Low frequency noise and long-term stability of noncoherent light sources. J Appl Phys. 96 (2) (2004), pp. 966–969.

32. Salzberg B., Kosterin P., Muschol M., Obaid A., Rumyantsev S. and Bilenko Y. (2005) An ultra-stable non-coherent light source for optical measurements in neuroscience and cell physiology. J Neurosci Methods. 141 (1): 165–169.

33. Samuolienė G.,Urbonavičiūtė A., Šabajeviene G. and Duchovskis P. (2010) The effect of red and blue light component on the growth and development on frigo strawberries.Zamdirbyste-Agricult.97(2): 99-104.

34. Scholfield C. and Murdock, M. (1978) Pulse-modulated light source for psychometric and vision experiments. J Neurosci. Methods. 19 (3): 203–207.

35. Schubert E.F. and Kim J.K. (2005) Solid-state light sources getting smart. Science. 308 (5726): 1274–1278.

36. Shimoda M. and Honda K. (2013) Insect reactions to light and its applications to pest management.Appl. Entomol. Zool. 48:413–421.

37. Stone,B.F.(1968) Formula for determining degree of dominance in cases of monofactorial inheritance of resistance to chemicals.Bull.World Health Org.38:325-326.

38. Stumpf, N. and Nauen R. (2002) Biochemical markers linked to abamectin resistance in *Tetranychus urticae* (Acari-Tetranychidae). Pestic. Biochem. Physiol., 72: 111-121.

39. Sun R., Bouchard M.B. and Hillman E.M.C. (2010) SPLASSH: open source software for camera-based high-speed: multispectral in vivo optical image acquisition. Biomed Opt Express. 1 (2): 385–397.

40. Suzuki, T., Yoshioka Y., Tsarsitalidou O., Ohno S., Ohyama K., Kitashima Y., Gotoh T., Takeda M. and Koveos D. S. (2014) An LED-based UV-B irradiation system for tiny organisms: system description and demonstration experiment to determine the hatchability of eggs from four Tetranychus spider mite species from Okinawa. J. Insect Physiol. 62 (1): 1-10.

41. Svilainis L. (2009) LED PWM dimming linearity investigation.Displays, 29 (3) :243–249.

42. Svilainis L. (2012) Comparison of the EMI performance of LED PWM dimming techniques for LED video display application. J Disp Technol, 8 (3) :162–165.

43. Van Asperen,K. (1962) A study of house fly esterase by means of a sensitive colorimetric method. J. Insect Physiol. (8):401-408.

44. Van der Linden J., Schoonderwaldt E., Bird J. and Johnson R. (2011) MusicJacket – Combining motion capture and vibrotactile feedback to teach violin bowing. IEEE Transactions on Instrumentation and Measurement, 60 (1): 104–113.

45. Watanabe, T., Mori N. and Nakamura F. (1992) A new superbright LED stimulator: photodiode-feedback design for linearizing and stabilizing emitted light.Vision Res, 32 (5) : 953–961.

46. Wilson J.D., Adams A.J., Murphy P., Eswaran H. and Preissl H. (2009) Design of a light stimulator for fetal and neonatal magnetoencephalography. Physiol Meas, 30 (1):1-10.

47. Yang,X.,L.L. Buschmann,K.Y. Zhu and D.C. Margolies.2002.Susceptibility and detoxifying enzyme activity in two spider mite species (Acari : Tetranychidae) after selection with three secticides.J.Econ.Entomol.95:399-406.