Effects of Light Emitting Diodes (LEDs) On the Insect Predators Behavior against the Two Forms of *Tetranychus urticae* Koch

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ABSTRACT: Light emitting diodes (LEDs) with certain colors proved their role as an important agent in the biological control of *Tetranychus urticae*. Phototactic behavior of both *Stethorus punctillum* and *Scolothrips sexmaculatus* was strongly affected by LEDs to the two forms of *T.urticae* .The attraction incidence rate ratio (IRR) was estimated of the predators .The highest attraction IRR values of *S.punctillium* recorded in case of *T.urticae* green form exposed to White LED while it was detected with *S.sexmaculatus* preyed on red form exposed to blue LED. Concerning voracity, it was recorded 100 % in case of predation on the green and red forms of *T.urticae* exposed to white and blue LEDs by *Stethorus punctillum* and *Scolothrips sexmaculatus*, respectively. Moreover, the feeding preference and the predation efficiency of certain predators on the both forms of *Tetranychus urticae* adult males and females which exposed to LEDs were estimated depending on direct olfaction and Y-tube olfactory test. Results showed that there were significantly differences of insect predators' phototactic behavior which back mainly to colors of LEDs.

Keywords: Tetranychus urticae Culture, Stethorus punctillum and Scolothrips sexmaculatus Cultures.

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; Georghiou 1990).

Biological control, using natural enemies, is an alternative strategy to manage mites in agricultural systems. Natural enemies play a major role in the ecology of spider mites, including ladybird beetles (Coleoptera: Coccinellidae) (Obrycki and Kring 1998; Mori *et al*. 2005), which generally accept a large number of prey species and frequently show a preference for one species (Hodek 1973), and predatory mites (Acari: Phytoseiidae) (Gotoh *et al*. 2004; Friese and Gilstrap 1985). In addition, acarophagous thrips (Thysanoptera: Aeolothripidae, Thripidae) are important natural enemies, and have various degrees of specialization on various mites; however, many species of Scolothrips commonly known as acarophagous ladybird beetles, are predators of agricultural crop pests and significantly contribute to the control of spider mite pests (Lewis 1973; Gilstrap and Oatman 1976; Roy *et al*., 2003; Gotoh *et al*., 2004). The six-spotted thrips *Scolothrips sexmaculatus* Pergande is one of the important predators of spider mite. Its adult consumes about 1000- 3000 *T. urticae* during its lifetime (Hoddle, 2004).

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). The effects of lights may be directly or indirectly on pests. Directly, as 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).Indirectly,as the impact of LEDs on volatile infochemical that elicits a strong olfactory response of the predatory mite *Neoseiulus californicus*, an important natural enemy of the two-spotted spider mite *Tetranychus urticae* (Shimoda 2010).

So the new effective direction of both physical and biological control of pests is using LEDs with its wide spectrum colors. They are able to be used as a direct tool of control and traps of pests (Chu *et al*.2004) or as attractants of predators (Chu *et al*. 2003).Beside LEDs also able to direct olfaction to adjust movements of predators to their preys correctly (Shimoda and Honda 2013).

The aim of this study is mainly to compare the attraction incidence rate ratio (IRR), voracity, the feeding preference and the predation efficiency of adult females of *Stethorus punctillum* and *Scolothrips sexmaculatus* on the both forms of adult stage of *Tetranychus urticae*, exposed to two colors of light emitting diodes (LEDs).So the photo response of both predators will be compared depending on its interaction with received preys' infochemicals.

Materials and Methods

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.

Stethorus punctillum and *Scolothrips sexmaculatus* Cultures

The predators *S. punctillum* and *S. sexmaculatus* were explored from naturally infested castor oil bean plants. They were collected, brought to the laboratory and identified with the help of a stereo binocular microscope. After confirming their predation, 50 Adult females and males of each predator were kept under laboratory conditions in the big plastic cells (30*10*5 cm). Moistened pads of cotton with discs of castor oil leaves with both forms of *Tetranychus urticae* on them were as a preys of the certain predators. They were maintained for six months before testing their efficiency.

Light Emitting Diodes (LEDs) in Laboratory

The LED stimulator is designed around the Arduino prototyping microcontroller platform consisting of five components: a computer with chosen programming environment, the Arduino prototyping platform, the constant-current LED driver, the LED itself, and a power supply.

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 (Abd El-Wahab and Bursic 2014). 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. Table (1) showed the wavelength and voltage drop of the used two colors of LEDs.

Table (1) Wavelengths of LEDs

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)

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. In each experiment concerning estimation of attraction incidence rate ratio (IRR), voracity, direct olfaction, distribution and Y-tube olfaction, the target prays exposed to specific LED color for one hour till the experiment done.

Attraction Incidence Rate Ratios (IRR)

Direct count of each predator targeted its preferable prey of both forms of *T.urticae* was occurred. Various treatments were analyzed using the treatment incandescent light (control) as the reference category. The attraction incidence rate ratios (IRR) of each predator to preys with interaction other LED treatments colors beside the control were estimated according to Kleinschmidt *et al*.(2001) with modifications. The IRR for the treatments control values were depending on randomized preferable prey under performance relative to the control. Observed differences in the abundance of the predators and composition of *T.urticae* forms during experiments were analyzed independently of trapping and attracting period.

Voracity Assessment

Measurement of the voracity of adult females of predators to *T. urticae,* infested leaves, using a 3 cm diameter cut from the manipulated plants then served as the source of the volatiles for selection circular arena. Treatments depending mainly on exposure to LED's. Two LED lights, blue and white, with different wave lengths, were used. Every treatment was triple replicated beside control. The exposure was approx. for 1 hour (Raworth 2001).Every predator had a full choice to go to its preferable prey exposed to LEDs colors.

Olfactory Response

Direct Olfactory Experiments

Infested plants with spider mites, produce a unique blend of volatile compounds to which *Stethorus punctillum* and *Scolothrips sexmaculatus* respond more strongly than they do to uninfested plants (Sabelis and Van der Meer 1986; Dicke *et al.* 1990a, b) Assessment of the response of both predators' adult females to released biogenic amines from two forms of *Tetranychus urticae* as a prey, was done according (Nachappa *et al*.2009) with modifications as the following:

2 leaf discs, each (2.2 cm diameter) cut from fresh castor oil bean plants then served as the source of the phyto volatile. Two arenas were setup vertically side by side with a divider which is cardboard (60 cm htX55 cm w) between them to reduce air movement and possible drift of volatiles. In the central of one, there was a leaf disc with a *Tetranychus urticae* form, and on the other there was placed a leaf disc from an uninfested leaf disc as a control.

30 adult females of each predator were starved for 24 hours to join each test and then released at the edge of the arena. The numbers of the predators which found the central leaf disc (source of volatiles) in the arena in the first 10 mins were recorded. The high response line consisted of individuals that found the central leaf disc in the presence of the low volatile level in the vicinity (arena).The low response line consisted of individuals that did not find the leaf disc despite a high level of the volatiles in the arena. However, the low olfactory response individuals were actively moving around the arena.The low response line consisted of individuals that did not find the leaf disk despite a high level of volatiles in the arena. However, the low olfactory response individuals were actively moving around the arena and not stationary.

The mentioned experiment was separately done to study the olfaction response of predators to each form of *T.urticae* with both colors of LEDs under laboratory circumstances.

-Dispersal Response

30 adult females of predators were then introduced onto each leaf disc and allowed almost 30 mins acclimatization period, after the connection of 30X5 mm Parafilm Bridge to the infested discs with the prescence of *T.urticae* females. Predators that walked onto and out along the bridge were counted as dispersed under treatments of LEDs colors and control.

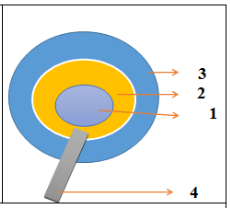

Figure (2) showed :

1. 30 adult females of each form of *T.urticae* removed after 24 hrs. on 2.2 D leaf plant
2. Water-saturated cotton wool.
3. 90 cm diameter plastic petri dish.
4. Parafilm bridge of the predator.

-Results were estimated as following:

High–Dispersal Line: dispersed from the leaf disc in the presence of 30 eggs (an abundant resource).

Moderate –Dispersal Line: dispersed from the leaf disc in the presence of 20 eggs

Low–Dispersal Line: Individuals remained on the leaf disc in the presence of only 10 eggs.

Y- Olfactory Experiments

Discs with spider mites were placed on the main opening arms of Y-tube in order to test olfaction response of females of both predators to males and females of their preys under LEDs.

2 leaf discs, each (2.2 cm diameter) cut from fresh castor oil bean plants then served as the source of the phyto volatile. The Y-tube olfaction depending on the determination of the highest predation, under two colors of LEDs, on females and males of the same form and then compare them with the control. The arrangements were done according (Nachappa *et al.*2009) with modifications as explained at direct olfaction test.

RESULTS

Detected data through the present research proved significantly the effect of Light Emitting Diodes (LEDs) on the attraction of main predators to certain preys.

Fig (2) showed the attraction incidence rate ratio (IRR) of the predator to the two forms of *T. urticae* exposed to LEDs colors. The highest IRR values recorded in case of green form exposed to White LED followed by green form under Blue LED,and then other treatments followed finally with the Red form in the control and under Blue LED.IRR of *S.punctillium* attracted to White LED and green form of *Tetranychus urticae* was highly significant than others.

While the difference occurred in the case of *S.sexmaculatus* .Fig (3) showed the attraction incidence rate ratio (IRR) of the predator to the two forms of *T. urticae* exposed to LEDs colors. The most IRR values recorded in case of red form exposed to blue LED followed by green form under White LED,and then other treatments followed finally with the control. IRR of *S.sexmaculatus* attracted to Blue LED and red form of *Tetranychus urticae* was highly significant than others.

Significant analysis of both mentioned treatments was done depending on Paired samples T-Test. Statically errors were estimated depending on fixed value 0.1, 5% and SE ±1. There’s a highly significant difference at P, 0.001 depending on paired samples test concerning the effect of LEDs on the behavior of the predators on both forms of *T.urticae*, with t=2.236, paired samples correlation=.478, Sig. (2-tailed) = .076**, with two correlations: Kendall's tau_b =.233* and Spearman's rho=.338* which showed the relation between LEDs and attraction IRR of the predators to their preys.

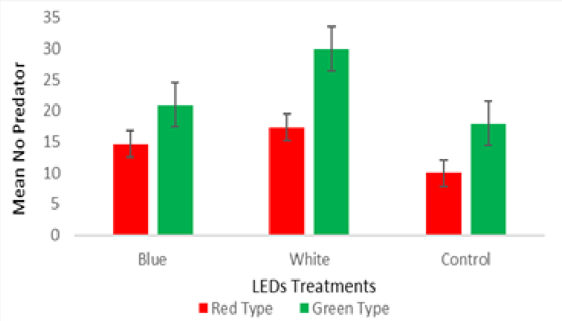

Fig.(2) Attraction Incidence Rate Ratios (IRR) of *Stethorus punctillum* to Two Forms of *Tetranychus urticae* Exposed to LEDs

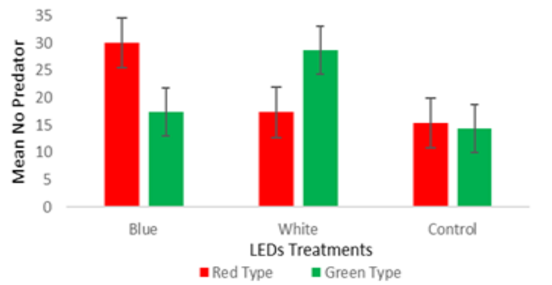

Fig.(3) Attraction Incidence Rate Ratios (IRR) of *Scolothrips sexmaculatus* to Two Forms of *Tetranychus urticae* Exposed to LEDs

Voracity of both predators to certain forms of *T.urticae* under Blue and White LEDs were estimated and showed at Table (2).Voracity values in all treatments incresed than control with percentages ranged from 100% till 23.26% with (S.*punctillum*+ *T.urticae*+White LED) and (*S.sexmaculatus* + Green *T.urticae* +Blue LED), resp.Among treatments there was highly significant difference at 99% depending on Kruskal Wallis Test (Chi-Square=30.321 and Asymp. Sig. = .001**).That was proved the significant difference mainly between S.*punctillum* and *S.sexmaculatus* under White and Blue LED with Green and Red *T.urticae*, resp. Highest Significant differences among groups'voracity showed by Kendall's tau_b(.004**) and Spearman's rho(.000**).

Table (2) Voracity of *Stethorus punctillum* Weise and The six spotted thrips, *Scolothrips sexmaculatus* Pergande to certain forms of *Tetranychus urticae* under two LEDs' colors

Tetranychus urticae	Predator	LEDs	% Voracity	% Increase than Control
Green Form	*Stethorus punctillum*	Blue	70	40
		White	100	100
	Scolothrips sexmaculatus	Blue	58.89	23.26
		White	97.78	37.21
	Stethorus punctillum	Control	57.78	
	Scolothrips sexmaculatus		47.78	
Red Form	*Stethorus punctillum*	Blue	48.89	41.94
		White	60	74.19
	Scolothrips sexmaculatus	Blue	100	96.65
		White	66.67	30.43
	Stethorus punctillum	Control	33.33	
	Scolothrips sexmaculatus		51.11	

Direct Olfactory and Dispersion Effect of LEDs on The Certain Predators of *Ttetranychus urticae* on Castor Bean Discs results recorded at Table (3) .Among treatments there was significant difference at 95% depending on Kruskal Wallis Test (Chi-Square=11.000 and Asymp.Sig.= .443*).So the significant olfactory response and disturbance were mainly showed between S.*punctillum* and *S.sexmaculatus* under White and Blue LED with Green and Red *T.urticae* ,resp.

To get the confirmed evidence that differences in data were mainly revised to the response of under LED, the Kolmogorov-Smirnov Z of predators recorded LED= .737 and Olfactory response=.619 but it was just significant in case of predators' response with .145**.

Table (3) Direct Olfactory and Dispersion Effect of LEDs on The Certain Predators of *Ttetranychus urticae* on Castor Bean Discs

Tetranychus urticae	Predator	LEDs	% Olfactory	Distribution Rate
Green Form	*Stethorus punctillum*	Blue	90	*High*
	Stethorus punctillum	White	100	*High*
	Scolothrips sexmaculatus	Blue	83.33	*Moderate*
	Scolothrips sexmaculatus	White	66.67	*Moderate*
	Stethorus punctillum	Control	66.67	*Moderate*
	Scolothrips sexmaculatus		40	*Low*
Red Form	*Stethorus punctillum*	Blue	83.33	*Moderate*
	Stethorus punctillum	White	80	*Moderate*
	Scolothrips sexmaculatus	Blue	96.67	*High*
	Scolothrips sexmaculatus	White	86.67	*Moderate*
	Stethorus punctillum	Control	63.33	*Moderate*
	Scolothrips sexmaculatus		50	*Low*

While Table (4) showed that there's a significant difference at 5% according non-parametric correlations (Kendall's =0.342), (Spearman's rho =0.348, R=1.30, SE=0.5064), among treatments in the comparable with the control in the experiments of Y-tube olfactory attraction .

In the same trend, Kruskal Wallis (Chi-Square)= 47.00* for predators and LEDs effect , Friedman Test (Chi-Square)=61.615** and Kendall's Coefficient of Concordance=.428*.Jonckheere-

Terpstra Test was used to show the significant difference of response of both predators to adult females and males of *Tetranychus urticae* exposed to LEDs .There was a highly significant difference of both predators firstly (.099)** with Observed J-T Statistic (644.000) and Std. Deviation of J-T Statistic (48.497).While the significant difference increased when the response was determined to adult females and males of the two forms of *T.urticae* with (.004)** depending on Observed J-T Statistic (401.000) and Std. Deviation of J-T Statistic (56.135).

Table (4) Y-Tube Olfactory Attraction Percentages of Certain Predators on *Ttetranychus urticae* Exposed to LEDs

Tetranychus urticae	Predators	LEDs	% Y-Tube Olfactory Attraction			
			With 30 Adult Females	Without 30 Adult Females	With 30 Adult Males	Without 30 Adult Males
			T.urticae			
Green Form	*Stethorus punctillum*	Blue	86.67	80	83.33	60
	Stethorus punctillum	White	100	86.67	96.67	83.33
	Scolothrips sexmaculatus	Blue	60	53.33	50	43.33
	Scolothrips sexmaculatus	White	46.67	33.33	40	30
	Stethorus punctillum	Control	13.33	0	16.67	3.33
	Scolothrips sexmaculatus		10	0	3.33	0
Red Form	*Stethorus punctillum*	Blue	66.67	56.67	53.33	30
	Stethorus punctillum	White	83.38	66.67	76.67	50
	Scolothrips sexmaculatus	Blue	100	86.67	90	80
	Scolothrips sexmaculatus	White	76.67	50	66.67	40
	Stethorus punctillum	Control	6.67	0	3.33	0
	Scolothrips sexmaculatus		16.67	0	13.33	6.67

DISCUSSION

Light Emitting Diodes could play an important role in controlling mites and insects effectively upon interaction directly or by indirectly by attracting pests' predators and parasitoids. As presented in our study, there's a significant difference between using biological control agents with LEDs and without.

Insect predators disappeared when the density of their prey became very low (Takahshi *et al.* 2001). That was occurred with the predator, *Scolothrips takahashii*, *Oligota kashmirica benefica* and *Stethorus japonicus* on *Tetranychus kanzawai* . Predator-traps with *T. kanzawai* infested bean plants attracted significantly more *S. takahashii* than traps with uninfested plants. However, LEDs showed through our study its ability to attract predators under any circumstances in Y-tube tests. That was because of the increase volatiles predominating and even concentrated them to attract predators.

Energy-efficient LEDs could be used for more environmentally friendly insect control tool (Kim *et al.*2012). They detected that the The Black Light 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.

Also LEDs could be used as an attractant to predators and parasitoids. Females of *Cotesia vestalis*, a parasitoid of diamondback moth (DBM), *Plutella xylostella* larvae, showed no significant preference for either green, yellow, orange, or red spotlighted areas over a control area with background fluorescent light (Uefune *et al.*2013).But after starved for 2 h, females preferred yellow and green light over the control area, but not orange or red light. *P. xylostella* larvae-induced cabbage-plant volatiles, which attract female wasps, to green versus yellow light.

Y-tube olfactometer was used to investigate the attraction of the predatory mite *Phytoseiulus persimilis* to volatiles from several host plants of its prey, spider mites in the genus *Tetranychus* (Takabayashi and Dicke 1992). Predators that were reared on spider mites (*Tetranychus urticae*) on Lima bean leaves did respond to volatiles from Lima bean leaves, while predators that had been reared on the same spider mite species but with cucumber as host plant did not respond to Lima bean leaf volatiles. Consequently, in our research we used fresh discs of castor bean plant as a constant source of phyto volatiles with a blend volatiles of the used forms of *Tetranychus urticae* .So we get a preferable form of the pray to each predator with the effect of LED colors.

About LEDs attraction of predators to uninfested castor bean leaves in Y olfactometer test, it can be explained on the allelopathy between both the plant and diode color. Also it is confirmed by the few number of predators and even null that reached the uninfested plant discs in control.The same results detected by Takabayashi *et al*. (2000) .They noticed olfactory response of the predatory mite *Phytoseiulus persimilis* to volatiles from *T. urticae*-infested tomato leaves in a Y-tube olfactometer when slightly infestation with the red strain, or moderately or heavily with the green strain. In contrast, neither leaves that were slightly infested with green-strain mites, nor leaves that were moderately or heavily infested with the red strain attracted the predators.

On the contrary, Tatemoto and Shimoda (2008) found that *Neoseiulus cucumeris* (a predatory mite) and the predatory insect *Orius strigicollis* showed a significant preference to onion thrips, *Thrips tabaci*, in a Y-tube olfactometer for volatiles from infested cucumber leaves without *T. tabaci* over clean air. However, they were not attracted to volatiles from uninfested cucumber leaves, artificially damaged cucumber leaves, or volatiles from *T. tabaci* plus their visible products collected from cucumber leaves. These results suggest that both predator species are capable of exploiting herbivore-induced volatiles from *T. tabaci*-infested cucumber leaves as a foraging cue. Neither predator was attracted to volatiles from uninfested spring onion leaves, infested spring onion leaves without *T. tabaci*, or volatiles from *T. tabaci* plus their visible products collected from spring onion leaves. Interestingly, they avoided volatiles from artificially damaged spring onion leaves.

The same case with *Phytoseiulus persimilis* (Choh *et al*. 2004) and *Scolothrips takahashii*, towards herbivore-induced plant volatiles emitted by Lima bean plants infested by two-spotted spider mites *Tetranychus urticae* (green form). The predatory insects showed a greater preference for Lima bean leaves infested by the two-spotted spider mites than for either clean air or uninfested bean leaves. They showed the same preference towards infested leaves from which all spider mites and their visible products had been removed. Neither the spider mites themselves nor their products attracted the predators (Shimoda *et al*. 1997).

The effect of volatiles related to feeding activity of nonprey caterpillars, *Spodoptera exigua*, on the olfactory response of the predatory mites *Phytoseiulus persimilis* was examined in a Y-tube olfactometer by Shimoda and Dicke (1999). At a low caterpillar density (20 caterpillars on 10 Lima bean leaves), the predators were significantly more attracted to volatiles from infested leaves on which the caterpillars and their products were present or from infested leaves from which the caterpillars and their products had been removed when compared to volatiles from uninfested leaves. The predators, however, significantly avoided odors from 20 caterpillars and their products (mainly feces) removed from bean leaves. In contrast, at a higher caterpillar density (100 caterpillars on 10 Lima bean leaves), the predators avoided volatiles from caterpillar-infested bean leaves. Volatiles from infested leaves from which the caterpillars and their products had been removed were not preferred over volatiles from uninfested leaves. Volatiles from feces collected from 100 caterpillars were strongly avoided by the predators, while the behavior of the predatory mites was not affected by volatiles from 100 caterpillars removed from a plant. The data show that carnivorous arthropods may avoid nonprofitable herbivores. This avoidance seems to result from an interference of volatiles from herbivore products with the attraction to herbivore-induced plant volatiles.

Allelopathy which was the responsible factor in all olfaction experiments could be explained as the volatile allelochemicals that originate from an interaction of the herbivore and its host plant

(Takabayashi *et al*. 1991). The composition of allelochemical blends emitted by herbivore-infested plants is known to be affected by both the herbivore and the plant. Moreover,blends emitted by apple leaves infested with spider mites of 2 different species, *T. urticae* and *P. ulmi*, differed less in composition (principally quantitative differences for some compounds) than blends emitted by leaves of two apple cultivars infested by the same spider-mite species,*T. urticae* (many quantitative and a few qualitative differences). Also the odors of *T. urticae* adults and their products might influence the attraction of *S. gilvifrons* females (Gencer *et al*.2009).Predators' attraction to red *Tetranychus urticae* was related mainly to cyanatelyase encoding gene that might be involved in feeding on cyanogenic plants (Grbic *et al*.2011).

CONCOLUSION

We could conclude all previous results briefly in the following chart:

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