IJCBS RESEARCH PAPER VOL. 1 [ISSUE 3] JUNE, 2014 ISSN:- 2349–2724

The Relation Between Vertimec Resistance in The Two-Spotted Spider Mite, *Tetarnychus urticae* and Climate Changes in Egypt

[1]Rania Ahmed Abd El-Wahab
Plant Protection Research Institute,
Agriculture Research Center,
Egypt
Email: *rania-proline@hotmail.com*

[2]Thair Mahmood Taha
Biology Dept.,Faculty of Girls Eduction,
Kufa University,
Iraq
Email: *thairtaha_2008@yahoo.com*

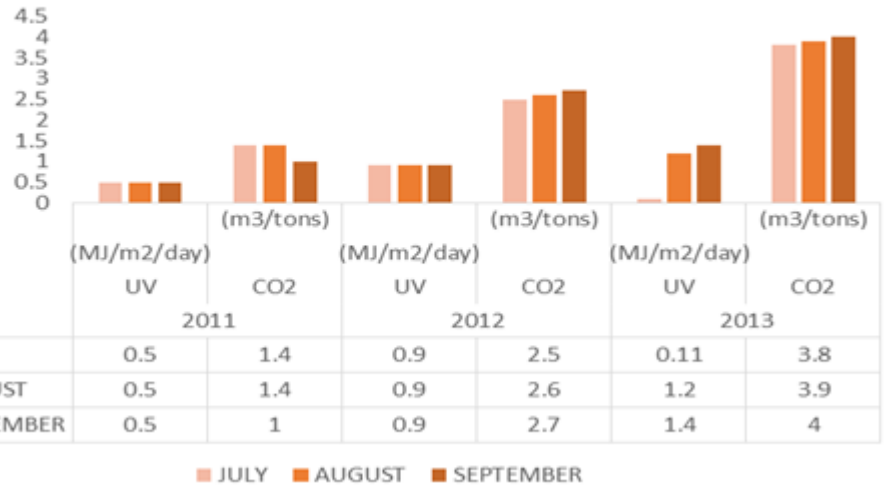
ABSTRACT: Pesticides resistance in *Tetranychus urticae* is a phenomenon which caused by many reasons. One of their causes is the exposure of highly levels of UV and CO2, which could play an important role to get a resistant field strain. The Vertimec ,a pronounced miticide , LC_{50} of the laboratory resistant strain which maintained under selection pressure till F40 and the resistant field strain in comparable with susceptible strain were 2099.38,200.01 and 50.822 μLL^{-1} ,resp. Field studies through 2011,2012 and 2013 showed that initial kill percentages of *T.urticae* infested cotton were 86.08,62.74 and 40.32 % for the same arrangement, and the residual effect percentages were 94.62,67.11 and 52.90 %, resp. Moreover, the elevated esterases and mixed function oxidases (MFO) in both the laboratory and the 2013 field resistant strains mainly proved the effect of increased radiation of UV on the highly resistance levels recorded for both strains.

Keywords: Pesticide resistance, Cotton, UV, CO2, *Tetranychus, Vertimec*, Esterases, Mixed function oxidases (MFO)

1. INTRODUCTION

Pesticide resistance is a vital topic especially in general pest as the two spotted spider mite, *Tetranychus urticae*. So it's important to take most effective element of climate changes in mind when the resistance is the problem which farmers should give a hand to it. Figure (1) showed the levels of both UV and CO2 in Egypt during cotton cultivation of this study through three years 2011, 2012 and 2013.

Figure (1): Levels of UV and CO2 emissions determination by Egyptian Meteorological Organization From 2011-2013



	2011		2012		2013	
	(MJ/m2/day) UV	(m3/tons) CO2	(MJ/m2/day) UV	(m3/tons) CO2	(MJ/m2/day) UV	(m3/tons) CO2
JULY	0.5	1.4	0.9	2.5	0.11	3.8
AUGUST	0.5	1.4	0.9	2.6	1.2	3.9
SEPTEMBER	0.5	1	0.9	2.7	1.4	4

© Virtu and Foi

Hence, global warming with increased CO2 can be expected to certainly affect the chemical-defense-signaling system in plants and that will render them more susceptible insect pest attack The increased number of generations per year and frequent population outbreaks of potential insect pests necessitate continual applications of high amount of insecticides and that will make the insects to develop resistance against these chemicals (**Petzoldt and Seaman, 2007**).

Further, the increased voltinism with prolongation of lifespan in insects under high CO2 and temperature will stabilize such insecticide resistant insect varieties in the population, which will cause greater damage to plants even under extensive insecticide control measures. Additionally, some classes of pesticides have been shown to be less effective in controlling insects at higher temperatures (**Musser & Shelton 2005**).

Entomologists predict additional generations of important pest insects in temperate climates as a result of increased temperatures, probably necessitating more insecticide applications to maintain populations below economic damage thresholds. A basic rule of thumb for avoiding the development of insecticide resistance is to apply insecticides with a particular mode of action less frequently (**Shelton et al 2001**). With more insecticide applications required, the probability of applying a given mode of action insecticide more times in a season will increase, thus increasing the probability of insects developing resistance to insecticides.

In addition , elevated CO2 increases the carbon to nitrogen (C:N) ratio and reduces the N content in the tissue of most plant species, elevated CO2 is expected to alter plant synthesis of phenolics, terpenes, and other secondary metabolites (**Bezemer et al.2002,Sun et al.2010**) .Such changes in C:N and in the content of secondary metabolites will alter the nutritional quality and palatability of host plants for herbivores and could therefore affect the performance of herbivorous insects (**Couture et al.2010**).

The elevated resistance in *Tetranychus urticae* of acaricides such as Vertimec could be defined and explained depending on many causes. Some of them related to the internal mode of action of the pesticide. Others are directed by environmental factors and mainly climate changes under egyptian circumstances. So this study will take place to explain resistance depending on both directions on the same time.

MATERIALS AND METHODS

Tested pesticide: VERTIMEC

Trade names: Abba, Affirm, Agri-Mek, Avid, Dynamec, Vertimec and Zephyr.

Common names: Avermectin B1 and MK-936.

Tested mite: Two Spotted Spider Mite, *Tetranychus urticae*

Maintenance of the mite and the Assessment of acaricidal activity were done according to Dittrich (1962). Mortality percentages were determined and corrected by using **Abott's formula (1925**) and they were stastically analyzed according to **Finney (1971)** to estimate LC50, LC90 and slope values.All were done for susceptible, resistant and field strains. Rearing of pest colonies under pesticides selection pressure were done for laboratory resistant strain according to **Abd El-Wahab (2010),** by using leaf –dip technique **(Dittrich 1962).**

Field study: was done at Aga district in 2011, 2012 and 2013 in the area about 4200 m2. All data concerning IK, Residual effect and general effect were compared depending on the values of UV and CO2 which affected on the strength of Vertimec to do its action.

- **Biochemical Studies**
- **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

© Virtu and Foi

stopped and colour 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.

.Statistical Analysis: A split-split plot design was used to analyze the univariate responses of the measured variables. Effects were considered significant if $P<0.05$. The effect of block and the interactive effects of block and other factors were not significant ($P>0.45$), and the effect of block and its interaction with other factors are not presented so as to simplify the presentation. Least significant difference (LSD) tests were used to separate means when ANOVAs were significant.

RESULTS

Effect of Vertimec selection pressure on the susceptibility of *T.urticae* adult females. The data of laboratory tests in Table (1) presented building up of Vertimec resistance in the laboratory strain of mite during 40 generations. The results indicated that LC_{50} values gradually increased from 50.822 μLL^{-1} in parents' generation to 218.763 μLL^{-1} of F4, while in F10, that value was suddenly increased to 825.794 μLL^{-1}, then the susceptibility to Vertimec decreased with continuous selection,thus increasing LC_{50} of F40 to 2099.38 μLL^{-1}. Hence, in the case of $LC_{90's}$, (Table 1), which gradually increased from144.38 μLL^{-1} for parents' generation to 523.894 μLL^{-1} in F2, while in F6, that value was increased to 2014.333 μLL^{-1}, then the susceptibility to Vertimec sharply decreased with continuous selection,thus increasing the LC_{90} for F40 to 7192.297 μLL^{-1}. Depending on toxicity index , the F40 was affected as parents as 02.42 % and 02.00 % at LC_{50} and LC_{90}, resp. Concerning slope values for Vertimec resistant generations, it varied from 04.20 , 03.14 and 02.79 to 05.86 to parents' generation,F2,F4 and F40, resp.

Table (1) Effect of Vertimec selection pressure on the susceptibility of *T.urticae* adult females under laboratory conditions.

G.	LC_{50} (μLL^{-1})			LC_{90} (μLL^{-1})			Slope	Toxicity index	
	Main	U.L.	L.L.	Main	U.L.	L.L		LC_{50}	LC_{90}
S.	50.822	111.30	23.206	144.38	316.192	65.613	4.20	100	100
F2	70.416	147.874	33.531	523.894	1100.178	249.473	3.14	70.17	27.56
F4	218.763	437.526	109.382	998.145	1996.29	499.073	2.79	23.23	14.46
F6	599.431	1192.868	301.222	2014.333	4008.523	1012.228	3.01	8.48	7.17
F8	714.379	1500.196	324.718	3520.141	7392.296	1676.258	3.24	7.11	4.10
F10	825.794	2064.485	330.318	4199.375	10498.438	1679.75	3.57	6.15	3.44
F20	1032.119	2745.437	384.631	5210.413	13859.699	1958.802	4.23	4.92	2.77
F30	1571.245	4415.199	559.162	6031.157	16947.551	2146.319	5.09	3.23	2.39
F40	2099.38	5941.245	741.830	7192.297	20354.201	2541.448	5.86	2.42	2.00

Related to the susceptible strain (S), the resistant ratios (RR) to Vertimec for *T.urticae* resistant generations, showed at Table (2). RR's at Vertimec $LC_{50's}$ were ranged from 1.386- folds to 41.308- folds, of 2nd. and 40th. generations, resp. According to Hayashi scale (1983), RR of 40th resistant generation to Vertimec LC_{50} can be ranked as moderate resistance.

RR's at Vertimec $LC_{90's}$ were generally higher than those to $LC_{50's,}$ they were ranged from

© Virtu and Foi

03.629- folds to 49.815- folds, of 2nd. and 40th. generations,resp. According to Hayashi scale (1983), RR of 40th resistant generation to Vertimec LC_{90} can be ranked as moderate resistance. (Table 2). RR's of Vertimec slopes also were estimated and ranged from 00.748-folds to 01.395-folds, for 2nd. and 40th. generations,resp.

Table (2) Resistance ratios of Vertimec resistant adult females of *T.urticae* till 40th generation.

G.	LC_{50} μLL^{-1}			LC_{90} μLL^{-1}			RR Slope
	RR50	RR50 U.L.	RR50 L.L	RR90	RR90 U.L	RR90 L.L	
S.	--------	-------	------	-------	-------	-------	------
F2	1.386	1.329	1.44	3.629	3.479	3.802	0.748
F4	4.304	3.931	4.714	6.913	6.314	7.606	0.664
F6	11.795	10.718	12.98	13.952	12.677	15.427	0.717
F8	14.056	13.479	13.993	24.381	23.379	25.548	0.771
F10	16.249	18.549	14.234	29.086	33.203	25.601	0.85
F20	20.309	24.67	16.575	36.088	43.833	29.854	1.007
F30	30.917	39.67	24.956	41.773	53.599	32.712	1.212
F40	41.308	53.38	31.967	49.815	64.373	38.734	1.395

Moreover, the toxicity of Vertimec was compared field and resistant strains with the susceptible strain. As shown in Table (3), LC_{50} values were 2000.01, 2099.38 and 50.822 μLL^{-1}, resp., which showed that LC_{50} of cotton field strain exposured to highly levels of CO2 and UV recorded so close value to that of 40th generation of the laboratory resistant strain. The same situation was in the case of $LC_{90's}$ which recorded 7142.433,7192.297 and 144.38 μLL^{-1},resp. Relative to the laboratory strain (S),the resistant ratios (RR) to Vertimec for *T.urticae* laboratory resistant strain and field strain,showed at Table (3). RR's at Vertimec $LC_{50's}$ were 41.38- folds and 39.353- folds, resp. According to Hayashi scale (1983), RR of both strains were showed resistance to Vertimec LC_{50} and it could be ranked as moderate resistance.

Table (3) Vertimec toxicity against adult females of *T.urticae* (Field, Resistant strain and susceptible strains)

Tetranychus urticae Strains	Vertimec				Slope Values		LC_{90}/LC_{50} Ratio
	LC_{50} (μLL^{-1})		LC_{90} (μLL^{-1})				
	Main	RR50	Main	RR90	Main	RR Slope	
Field	2000.01 b	39.353	7142.433 b	49.47	3.27 a	0.78	3.571
Resistant	2099.38 b	41.308	7192.297 b	49.815	5.086 a	1.211	3.426
Susceptible	50.822 a	------	144.38 a	-----	4.20 a	-----	2.841

Cotton Field treatments

Results of cotton field treatments with Vertimec (40 ml/100 L) were showed at Table (4). Initial kill (IK) results were 86.8, 62.74 and 4.32 % for treatments in 2011,2012 and 2013, resp. Percentages of residual effect of Vertimec treatments decreased from 2011 to 2013 which showed that the biocide decomposed rapidly by UV while the percent of CO2 increased in the same time. In the same trend % general reduction educed from 90.35 to 46.61%.

© Virtu and Foi

Table (4) Effect of Vertimec on the reduction percentage of *T. urticae* infesting cotton under field conditions with UV and CO2 changes

Year of Treatment	Rate of Application	I.K. After 3 Days	% Reduction After Days				% Residual Effect	General Reduction
			7	10	14	21		
2011	40 ml/100L	86.08 a	95.23	96.98	98.75	87.51	94.62	90.35a
2012		62.74b	70.43	79.95	63.92	54.14	67.11	64.93b
2013		40.32c	53.95	60.75	50.05	46.83	52.90	46.61c

Biochemical Studies

Resistance to pesticides was conferred by genes controlling penetration, detoxification and sensitivity of the target protein (**Brown, 1990**), however, linkage relationships among these genes was not defined in most agriculture pests, especially mites. Concerning metabolic resistance, this was the potential of pests to expel poisonous pesticides from their body through chemically driven deterioration especially in conjugation with UV increase and CO2. The main enzymes related to resistance mechanism: Nonspecific esterases and mixed function oxidase MFO, (**Brogdon & McAllister, 1998**).

***T.urticae* Esterases Activity**

Data in Table (5) referred to the changes of the rate of α-NA hydrolysis by LC50's of Vertimec laboratory resistant strain, beside two field strains tested in 2011 and 2013 and the susceptible strain. The data generally revealed that Vertimec (abamectin) caused increasing in α-NA hydrolysis in the tested strains in comparable with the susceptible strain.From these data seems that the highest level of α-NA hydrolysis (2.08) µg/mite/minute of the laboratory resistant strain, followed by field strain 2013 (2.01) µg/mite/minute,then field strain 2011 (0.81) µg/mite/minute and finally the susceptible strain (0.77) µg/mite/minute.Esterases activity of tested populations of Vertimec $LC_{50's}$ towards α-NA was much higher than that of (S) in nearly 2.70, 2.61 and 1.05 folds, in the same previous order with comparison with susceptible strain.

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

Data in Table (5) referred to the changes of the rate of PNA hydrolysis by LC50's of Vertimec laboratory resistant strain, beside two field strains tested in 2011 and 2013 and the susceptible strain. The data generally revealed that Vertimec (abamectin) caused increasing in PNA hydrolysis in the tested strains in comparable with the susceptible strain. From these data seems that the highest level of PNA hydrolysis (47.95) µg/mite/minute of the laboratory resistant strain, followed by field strain 2013 (45.98) µg/mite/minute, then field strain 2011 (11.28) µg/mite/minute and finally the susceptible strain (11.26) µg/mite/minute. Mixed function oxidases activity of tested populations of Vertimec $LC_{50's}$ towards PNA was much higher than that of (S) in nearly 4.26, 4.08 and 1.00 folds, in the same previous order with comparison with susceptible strain.

© Virtu and Foi

Table (5) Rate of α –NA & PNA hydrolysis (2.5 x 10^{-4} M) by esterases and oxidases, resp., of (F), (s) and (r) strains homogenates of *T.urticae*.

Tested pesticides	Substrate hydrolysis	Strains			
		F		S	R
		2011	2013		
Vertimec	α –NA	0.81	2.01b	0.77a	2.08 b
	1*EST. A	1.05 a	2.61 b	----------	2.70 b
	PNA	11.28a	45.98b	11.26 a	47.95b
	2*Oxidases A.	1.00 a	4.08 b	----------	4.26b

Values are expressed as the means ±SE. Mean

1* EST.A = Esterases Activity = Hydrolysis of α –NA in R Strain / Hydrolysis of α –NA in S Strain

2* Oxidases. A = Oxidases Activity = Hydrolysis of PNA in R Strain / Hydrolysis of PNA in S Strain

DISCUSSION

It could be detected that increased atmospheric carbon dioxide affects plant photosynthesis and chemistry (Cure and Aycock 1986, Kimball 1986), thereby influencing plant tissue nutritive quantity and quality for arthropods.CO2 enrichment effect studies showed different results winged between suppression of whiteflies on tomato (Tripp *et al*.1992) and a noticeable increase of *Tetranychus urticae* populations on white clover (Heagle *et al.*1994 and 2002).

Whereas there were none significantly effects combined with the increase of CO2 also noticed with thrips on milkweed (Hughes and Bazzaz 1997) and both thrips (Butler 1985), and whiteflies (Butler *et al*.1986) on cotton host. On the other hand, many studies have evaluated the relationship between plant resistance and tolerance to herbivores (Bailey and Schweitzer 2010, Muola *et al*.2010), but little information is available regarding how the relationship between tolerance and resistance is affected by an abiotic stress such as global CO2 enrichment. Some studies suggested that elevated CO2 decreased tomato plant resistance against *H. armigera* by suppressing the critical defensive signal molecule jasmonic acid (JA) and JA-Pathway-related defensive enzymes as the most important defense hormone involved in resistance against chewing insects. Tomato plants grown under elevated CO2 are less tolerant to *H. armigera* than plants grown under ambient CO2 (Guo *et al*.2012).Phenotypic plasticity is a principal means by which plants cope with biotic or abiotic stress (Valladares *et al*.2007), and the decreased resistance and tolerance to herbivores under elevated CO2 suggests that elevated CO2 reduces the phenotypic plasticity of plant response to herbivorous insect attack.

Concerning UV radiations, the most point which all studies agree with that is related to the inhibition of *T. urticae* on the undersides of leaves which possibly used as a filter to avoid the deleterious effects of UV-B. (Barcelo 2008, Ohtsuka K. and Osakabe 2009, Suzuki *et al*. 2009, 2014). Chosen sheltered areas because of low UV transmission through leaves as the accumulation of compounds that act as selective sunscreens (e.g., phenolics). Through presented study, there was a noticeable adults females with orange body color in case of exposed to highly UV and CO2.That could be explained by the accumulation of carotenoids, a scavenger for

© Virtu and Foi

UV-induced reactive oxygen species. It was the same occurred in case of diapausing females which may overcome the deleterious effects of UV-B during winter in the absence of leaves by emigrating to UV-free environments and by accumulating carotenoids. (Suzuki *et al*. 2009).Mentioned diapausing females, low mortality was observed even at high doses of UV radiation, but more than half escaped even at low doses. Moreover,the lethal effects of solar UV radiation may also affect the population dynamics of spider mites, and habitat (resource) limitation may increase the probability of interspecific interactions, such as competition and predation. In turn, the occurrence of these interactions in sheltered areas may be associated with observed increases in herbivory under conditions of solar UV-B-attenuation. (Ohtsuka and Osakabe, 2009). In the same line, photoreactivation in *T. urticae* eggs and larvae efficacy was determined by the cumulative irradiance of visible light (VIS) after exposure to UVB radiation.(Murata and Oosakabe,2014).The possibility that the timing of photoreactivation occurs related mainly with phase-specific UVB vulnerability and outbreak symptoms due to UVB-induced DNA damage.

In the same trend UV radiations could be used to control the eggs of stored grain pests,*Tribolium castaneum* (Herbst), *T. confusum* (Duval) (Coleoptera: Tenebrionidae) and *Cadra cautella* (Walker) (Lepidoptera; Pyralidae. Exposure time increase to UV-rays caused a gradual decrease in eggs hatchability. No hatching occurred after 24 minutes of exposure in 2 and 3 day-old eggs of *T. confusum. C. cautella* eggs were less sensitive to UV-rays than were *T. castaneum* and *T. confusum* eggs. All the exposure periods significantly reduced the eclosion of adults in all the experimental insects. No adults emerged when 3 day-old eggs of *T. castaneum* were irradiated for 16 or 24 minutes, or from 2 and 3 day-old eggs *T. confusum* irradiated for 16 or 24 minutes. The explanation of that phenomenon was admitted by Seidel et al. 1940.They detected that the higher sensitivity of the older eggs to UV-rays than the younger eggs, depending on insect physiology, that during early embryonic organization injury to the peripheral parts of the eggs by UV-exposure did not impede the viability of the activation centre. As development proceeds the embryonic regions became more specialized, and different organ fields can no longer replace each other. Thus, damaging of the surface tissue of the eggs can be fatal at the advanced stages of development by non-penetrating radiations like UV-rays. (Faruki *et al*.2007).

Similar reduction in adult eclosion was reported by Hasan *et al.*1998 working with UV-irradiated pupae of *E. sorbillans*. The present findings are also similar to the findings of Beard (1972), who reported that adult emergence was progressively decreased by higher doses when late stage larvae of *P. interpunctella* were irradiated with UV-rays.

Then if both UV and CO2 interacted with pesticide resistance, it could be said that both played an important role in pesticide formation especially in tiny pests with little number of chromosomes which are capable to be multiple resistant effectively. Even with use with synergists to neutralize resistance in case of metabolic resistance, synergists loss their activity and being unstable under UV light (Savinelli 2014).

So the resistance elevation occurred in the case of exposed *T.urticae* to UV and CO2 could be explained by two ways, firstly depending on escaping to remaining sheltering with low amount of UV transmission on the lower leaf surfaces, then the oviposition and other physiological indexes would be affected slightly. Secondly, explained by the elevation of reactive oxygen scavengers (ROS) production as a stress of Ultraviolet (UV) radiation which is able to eliminate reactive oxygen species. Most known elements of ROS in *Tetranychus urticae* are melatonin and arylalkylamine N-acetyltransferase (NAT).(Suzuki *et al.*2008).Moreover, both environmental factors play an important role to decrease the concentration of sprayed pesticide under field conditions which lead to exposure of under lethal concentrations and contribute in resistance formation gradually.

© Virtu and Foi

References

1. Abd El-Wahab,R.A.(2010) Toxicity and Subtle Effects Of Pesticides On Piercing-Sucking Pests.pp.238. Lambert Academic Publishing GmbH & Co.KG. ISBN 978-3-8433-7596-2.

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

3. Bailey JK and Schweitzer JA. (2010) .The role of plant resistance and tolerance to herbivory in mediating the effects of introduced herbivores. Biol Invasions 12: 337–351.

4. Barcelo J. A.(2008) Photo-effects of visible and ultraviolet radiation on the two-spotted spider mite,*Tetranychus urticae*.Photochem.Photobiol.33 (5): 703-706.

5. Beard RL. (1972) Lethal action of UV-irradiation on insects. J.Econ. Entomol.65: 650 654.

6. Bezemer TM, Jones TH, Newington JE (2000) Effects of carbon dioxide and nitrogen fertilization on phenolic content in *Poa annua* L. Biochem Syst Ecol 28: 839–846.

7. Brogdon, W. G. and McAllister, J. C. (1998) Insecticide resistance and vector control. Centers for Disease" Control and Prevention, Atlanta, Georgia, USA. Vol. 4: 605-613.

8. Brown ,T.M.(1990) Biochemical and genetic mechanisms of insecticide resistance ,in managing resistance to agrochemicals", ed by Green , M.B.; Moberg,W.K. and LeBaron, H.American Chemical Soc. Books, Washington, DC.pp.456.

9. Butler, G. D. (1985) Populations of several insects on cotton in open-top carbon dioxide experiment chambers. Southwestern Entomologist. 10: 264-267.

10. Butler, G. D., Kimball B. A., and Mauney J. R.. (1986) Populations of *Bemisia tabaci* (Homoptera:Aleyrodidae) on cotton grown in open-top field chambers enriched with CO2. Environ. Entomol. 15: 61-63.

11. Couture JJ, Servi JS, Lindroth RL (2010) Increased nitrogen availability influences predator -prey interactions by altering host-plant quality. Chemoecology 20: 277–284.

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

13. Faruki SI, Das DR., Khan,AR., Khatun MM. (2007) Effects of ultraviolet (254 nm) irradiation on egg hatching and adult emergence of the flour beetles, *Tribolium castaneum* (Herbst), *T. confusum* (Duval) (Coleoptera: Tenebrionidae) and the almond moth, *Cadra cautella* (Walker) .J.Insect Physiol.7(36):1536-2442.

14. Finney,S.J.(1971)"Probit analysis .A statistical treatment of the sigmoid response curve."7th Ed. Campridge Univ.Press,Cambridge,England.

15. Guo H, Sun Y, Ren Q, Zhu-Salzman K and Kang L (2012) Elevated CO2 Reduces the Resistance and Tolerance of Tomato Plants to *Helicoverpa armigera* by Suppressing the JA Signaling Pathway. PLoS ONE 7(7): e41426. doi:10.1371/journal.pone.0041426.

16. Hasan M, Jahan MS, Khan AR. (1998) Effect of UV-radiation on the Uzi-fly, Exorista sorbillans Widemann, an endoparasitoid of the silkworm, *Bombyx mori* L. Insect Science & its Application 18: 87-91.

© Virtu and Foi

17. Hayashi,A.(1983).History,present status and management of insecticide resistance .Pest Reistance to Pesticides Soft Sciences,Tokyo,pp.31-53.

18. Heagle,A.S., Brandenburg R.L., Burns J.C. ,and Miller J.E.(1994) Ozone and carbon dioxide effects on spider mites in white clover and peanut. J. Environ. Qual. 23: 1168 - 1176.

19. Heagle,A.S., Burns J.C., Fisher D.S., and Miller J.E.(2002) Effects of carbon dioxide enrichment on leaf chemistry and reproduction by two spotted spider mites on white clover. Environ. Entomol. 31: 594-601.

20. Hughes, L., and Bazzaz F. A.. (1997) Effect of elevated CO2 on interactions between the western ßower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) and the common milkweed, Asclepias syriaca. Oecologia (Berl.). 109: 286-290.

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., Vol. 60,pp. 1001-1006.

22. Kimball 8A (1986) CQ2 stimulation of growth and yield under environmental restraints. In: Carbon Dioxide Enrichment of GreenhouseC rops. II. Physiology, Yield and Economics (eds Enoch, HZ, Kimball, 8A), pp. 53-68. CRC Press, Boca Raton, FL.

23. Muola A, Mutikainen P, Laukkanen L, Lilley M, Leimu R (2010) Genetic variation in herbivore resistance and tolerance: the role of plant life-history stage and type of damage. J Evol Biol 23: 2185–2196.

24. Murata Y. and Oosakabe M. (2014) Factors affecting photoreactivation in UVB-irradiated herbivorous spider mite (*Tetranychus urticae*).Experiment.Appl. Acarol. 63 (2) : 253-265.

25. Musser, F. P and Shelton A. M.. (2005) The influence of post-exposure temperature on the toxicity of insecticides to *Ostrinia nubilalis* (Lepidoptera:Crambidae). Pest Manag Sci. 61:508- 510.

26. Ohtsuka K. and Osakabe M.)2009)Deleterious effect of UV-B radiation on herbivorous spider mites: They can avoid it by remaining on lower leaf surfaces. Environ. Entomol.38 (3) : 920-929.

27. Petzoldt, C. and Seaman, A. (2007) Climate Change Effects on Insects and Pathogens. Fact Sheet. http://www.climateandfarming.org/clr - cc.php.

28. Savinelli C (2014) Insecticide Resistance: Causes and Action. http://pesticidestewardship. org/resistance / Insecticide/Pages/ InsecticideResistance.aspx.

29. Seidel F, Bock E, Krause G.(1940) Die organisation des insekteneis. Naturwissenschaften 28: 433-446.

30. Shelton, A.M., Wilsey W.R., and Soderlund D.M. (2001) Classification of insecticides and acaricides for resistance management. Dept. of Entomology, NYSAES, Geneva, NY 14456. 315-787-2352. http://www.nysaes. cornell. edu/ent /faculty/Shelton /pdf / res_mgmt.pdf.

31. Sun YC, Cao HF, Yin J, Kang L, Ge F (2010) Elevated CO2 changes the interactions between nematode and tomato genotypes differing in the JA pathway. Plant Cell Environ 33: 729–739.

32. Suzuki T, Watanabe M, Taked M. (2009) UV tolerance in the two-spotted spider

© Virtu and Foi

mite, *Tetranychus urticae*.J Insect Physiol. 55(7):649-54.

33. Suzuki T., Takashima T., Izawa N., Watanabe M., Takeda M. (2008) UV radiation elevates arylalkylamine N-acetyltransferase (NAT) activity and melatonin content in the two-spotted spider mite, *Tetranychus urticae*.J.Insect Physiol.54 (7):1168-1174.

34. 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.

35. Tripp, K. E., Kroen W. K., Peet M. M., and Willits D. H.(1992) Fewer white flies found on CO2 enriched green- house tomatoes with high C: N ratios. Hort. Sci. 27:1079-1080.

36. Valladares F, Gianoli E, Gomez JM (2007) Ecological limits to plant phenotypic plasticity. New Phytol 176: 749–763.

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

© Virtu and Foi