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Effects of Different Classes of Insecticides on Cotton Leaf Secondary Metabolites

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ABSTRACT

The influence of 5 insecticides: Karate, Sumi-alfa, Cypermethrin (pyrethroids), Avaunt (oxadizine) and Carbophos (organophosphate) on the nutritious soluble proteins, sugar quantity and the quantity of terpenoid aldehydes of cotton leaves on the plant defense mechanism against insects was studied. Field experiments were conducted on cotton plants before flowering. Treatment with pyrethroid insecticides changed plant leaf secondary metabolites significantly compared to control leaves treated with water. Colorimetrical analysis showed that Sumi-alfa, Cypermethrin and Avaunt increased the quantity of soluble proteins 5.8, 6.2 and 5.4 times (on the 10^{th}, 10^{th}, 16^{th} days), and the quantity of reducing sugars to 43.7%, 51.5% and 43.3% (on the 10^{th}, 7^{th}, 10^{th} days) over the control, respectively. An increase in the population of sucking insects such as aphids in the treated plants may be due to the more nutritious quality of the leaves compared to insect populations on the control plants. In addition, HPLC analysis of leaves showed the total concentration of defensive terpenoid aldehydes: gossypol, heliocides H1, H2, H3, H4 and their precursors hemigossypolone, and methylhemigossypolone were reduced in all insecticide-sprayed samples except for Carbophos. Avaunt and Karate decreased the total sum of these defensive compounds most of all. On the 10^{th} day of the treatment with Avaunt and on the 1^{st} day with Karate terpenoid aldehydes lowered 3.1 and 4.6 times, respectively. Sumi-alfa on the 4^{th} day and Cypermethrin on the 7^{th} day lowered terpenoids to 40% and 19%, the minimum level. In leaf samples taken on the 1^{st}, 4^{th} and 7^{th} days of the treatment with Carbophos, the quantity of defensive terpenoid aldehydes was 12, 33 and 19% higher than the control. This was followed by a slump in which their quantity was 38, 40 and 36% lower than the control. These results indicate that treatment with pyrethroid insecticides influences cotton plant secondary metabolites and makes the plant more attractive to pests.

Keywords: cotton plants, insecticides, HPLC, reducing sugars, soluble proteins, terpenoid aldehydes

INTRODUCTION

Cotton plants require a warm climate, sufficient light, and water supply; however, the plant is quite susceptible to different insect pests. These pests can cause serious losses in crop yield. The insects causing the highest losses are aphids (Ebert *et al.* 1997), bollworms (Mirmoayedi *et al.* 2010), budworms (Hedin *et al.* 1991), armyworms (McAuslane *et al.* 1997), whiteflies and stinkbugs (Torres *et al.* 2003), boll weevils (Hedin and McCarty 1990) and mealybugs (Nagrare *et al*. 2011). A significant part of the production cost is spent on the application of insecticides.

For the last two decades, interest in studying the effects of insecticides on plant biochemistry has increased. Some insecticides are known to increase aphid and mite number after treatment. A two-year study reported by Kerns and Gaylor (1993) on the influence of cypermethrin and sulprofos (used against *Heliothis virescens* and *Helicoverpa zea*) on aphid numbers showed that the number of aphids was much higher than in the non-treated control. The total amount of threonine and other essential amino acids of the treated plants were also higher than the control. Thus, it was concluded that insecticide spray leads to biochemical changes in plants that could indirectly cause an increase in the number of aphids.

The influence of bifenthrin, cyhalothrin, cypermethrin, and deltamethrin on aphid numbers was investigated by Leser (1994). This study showed that the number of aphids in cotton fields increased after treatment with those insecticides. Kidd *et al.* (1996) showed that aphid numbers increased in cotton fields after the use of cyhalothrin. These authors concluded that the decrease in aphids was not due to a decrease in natural predators. Ravindhran and Xavier (1997) found that aphid numbers in cotton fields treated with deltamethrin, cypermethrin and fenvalerate increased in that order. In the treated leaf samples the quantity of sugars increased but phenol compounds were less than in the control. Similar results were observed in cotton lines treated with cypermethrin. In addition, enzyme activities of peroxidase and polyphenoloxidase were higher, and phenylammonialyase activity was lower after treatment with pyrethroid.

A study by Parajulee and Slosser (2001) found that the number of aphids in fields treated with cyhalothrin was significantly higher than in non-treated fields. They attributed this to an indirect influence of the insecticide on reproductive performance of aphids.

However, Slosser *et al*. (2004) noted that total sugars of cyhalothrin-treated leaves within a week were statistically similar with the leaves of plants from untreated plots.

In work done by Kumar (2011), the effect of commonly used insecticides alone and in combination on resurgence of a mealybug population of cotton was studied. They showed a 15% resurgence in the mealybug population occurred due to spinosad and resurgence started after the 2^{nd} chemical spray; fields were treated on a weekly basis. The reason for resurgence was proposed to be due to biochemical changes in the plant or changes in insect reproduction physiology or other unknown ecological changes. Similar results have been observed in Uzbekistan cotton fields.

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Table 1 Treatment, chemical class and application rates.

Treatment	Class	Applications rates (l/ha)
Avaunt, Du Pont, Switzerland	Oxadiazine	0.4
Karate, Syngenta, United Kingdom	Pyrethroid	0.5
Sumi-alfa, Sumimoto Chemical, Japan	Pyrethroid	0.5
Cypermethrin, Changzhou Kangmei Chem, China	Pyrethroid	0.3
Carbophos, Aerosoyuz, Russia	Organophosphate	0.6

MATERIALS AND METHODS

Experimental design

A comparative analysis of five insecticides belonging to three different classes (pyrethroids, oxadiazines, and organophosphates; **Table 1**) was conducted. Cotton variety C6524 was employed in this study. 48 plants were ground for 8 weeks in lysimeters in spring, 2011. The experimental design was a randomized block with 4 replications. Two plants from each lysimeter were treated by five insecticides: Karate (Syngenta, United Kingdom), Sumi-alfa (Sumimoto Chemical, Japan), Cypermethrin (Changzhou Kangmei, China), Avaunt (Du Pont, Switzerland) and Carbophos (Aerosoyuz, Russia) were applied to cotton as treatment and water was used as the control (a total of 6 treatments). Three leaf samples from the each cotton plant were harvested after 1, 4, 7, 10, 13, 16 days after treatment.

Protein extraction and analysis

Lyophilized cotton leaves were ground with liquid nitrogen using a mortar and pestle. After grinding, the proteins of control and treated leaves were extracted with Tris-HCl buffer (0.5 M Tris-HCl pH 6.8, 20 mM EDTA, 2 mM PMSF, 1% Triton X-100, and 150 mM DTT) for 2 h with stirring. The mixture was filtered, and the protein was precipitated with ammonium sulfate. The residue was isolated by centrifugation (6000 rpm) and was desalted on Sephadex G-15 column. The quantity of proteins was determined according to Lowry (1951).

Extraction and analysis of reducing sugars

Control and treated cotton leaves were lyophilized and extracted with hot water (80-90°C) for 30 min. The extract was cooled under running tap water and the dissolved, high molecular compounds that precipitated were removed by filtration. An aliquot was removed and the amount of reducing sugars was determined according to Somogy-Nelson (1952).

Extraction and analysis of terpenoid aldehydes

Samples of lyophilized leaves were extracted for 1 h by shaking in a capped Erlenmeyer flask with hexane-ethyl acetate (3: 1) containing 10% HCl; the mixture was filtered through a fritted filter funnel. The residue was washed twice with the same solvent and the extracts were combined. The solvent was evaporated, the extract was concentrated, and then transferred onto Silica gel 60 (Fluka 60741). The gel was dried and washed with isopropanol: acetonitrile: water: ethyl acetate (35: 21: 39: 5) and transferred to a crimp-top vial. This product was prepared for HPLC analysis by evaporation with the residue dissolved in acetonitrile with a final concentration of 200-250 μg/ml of terpenoids. The samples were run on HPLC Agilent 1100, equipped with a column Discovery HS C18 (4.6 × 75 mm/3 μm) using a gradient of 0.1% phosphoric acid pH 2.5 and acetonitrile from 55-95% over a period of 15 min (n = 4, 0.7 ml/min, UV detection of eluate 272 nm). The terpenoid aldehydes standards were kindly provided by Dr. Robert Stipanovic (USDA/ARS, Southern Plains Agricultural research Center, College Station, Texas, USA).

RESULTS AND DISCUSSION

In Uzbekistan, the resurgence and increase of some secondary pests such as aphids and mites is often been observed after the treatment of cotton plants with insecticides with different chemistries. For example, a general practical use of the carbamate insecticide Sevin against cotton bollworm (*Helicoverpa armigera Hb*) led to an increase of mites (*Tetranychus urticae* Koch.) in cotton fields. Therefore, this insecticide began to be used in mixtures with acaricides. The same results were observed after treatment with the pyrethroid Decis.

In 2006-2007, after years of treatment with some insecticides: Atilla, Karate, Sumi-alfa, Phascord, Cypermethrin and others, a large increase in aphid (*Aphis gossypii* Glov.) populations were observed in cotton fields in the Baghdad district of Ferghana Region. The effect of insecticide treatments were measured by counting the number of aphids on 10 leaves/plot taken from the top, middle and bottom of the plant during the blooming period, with additional counts on the 5th, 10th, 15th, 20th day after the first sampling (1986-1987) and subsequently on the 3rd, 7th, 14th, 20th day after sampling (2006-2007). The number of aphids did not drop as was observed with the control plants. The number of aphids counted in the leaves was averaged and the efficiency was calculated (**Table 2**). We assumed that the increase in the cotton aphid population was associated with a change in the nutritional and defensive quality of cotton leaves after application of insecticides.

Soluble proteins

To investigate why the number of insect pests changed after insecticide applications, we conducted a biochemical investigation on cotton leave proteins, reducing sugars and terpenoid aldehydes, recognizing that proteins and reducing sugars are considered to be food for mites and aphis, terpenoid aldehydes have a defence property against insects.

Our results show that pyrethroids and Avaunt induced significant increases in the quantity of proteins starting with the fourth day after treatment (**Fig. 1**). Avaunt and Cypermethrin induced soluble proteins 5-6 times more than those found in the control 10-16 days after treatment. The effect of application of Carbophos on soluble proteins was not significantly different from the control (n = 3, SD was always < 6%).

In leaves taken 1, 4, 7 days after treatment, the quantity of soluble proteins was less than in the control. On subsequent days Carbophos induced a slight increase (n = 3, SD was always < 5%). It practically did not change the amount of soluble protein (**Fig. 1**).

Electrophoretic analysis

Protein extractions were also studied electrophoretically for samples collected on the 10th day after treatment. The soluble protein extractions showed that Carbophos- and Karate-treated samples were almost identical with the control. The quantity of proteins, which were expected to be 8, 35, and 55 kDa, were much larger in samples treated with Avaunt and Cypermethrin. We did not observe any *de-novo* synthesized protein in any of the samples (**Fig. 2**).

Reducing sugars

Carbophos did not influence the quantity of reducing sugars (n = 3, SD was always < 5%) whereas Karate lead to an increase on the 7th, 10th, and 13th days; Avaunt, Sumi-alfa, and Cypermethrin treatment lead to an increase in reducing sugars of 30-40% 4-10 days after treatment (**Fig. 3**). The results show that Avaunt, Sumi-alfa and Cypermethrin preparations stimulated an increase of proteins and sugars after treatment and this could be one of the probable causes of the attractiveness to cotton plants by sucking insects like aphids.

Table 2 Biological efficiency of some insecticides against aphid (*Aphis gossypii* Glov.) of cotton plant.

Insecticides	**Consumption norm l/ha**	**Average number of aphid per one damaged leaf**	**Efficiency (%) by days after treatment 1986-1987**			
			5	**10**	**15**	**20**
Karate 5% em.	0.5	88.3 ± 0.51	96.9 ± 0.45	97.2 ± 0.50	87.2 ± 0.39	73.5 ± 0.65
Talstar, 10% em.	0.6	79.5 ± 0.24	94.7 ± 5.54	97.6 ± 0.16	90.2 ± 0.26	75.2 ± 0.18
Decys, 10% em.	0.2	90.6 ± 0.26	86.2 ± 0.48	91.2 ± 0.56	80.7 ± 0.51	63.2 ± 0.57
Cypermethrin 25% em.	0.2	97.5 ± 0.16	91.7 ± 0.14	93.3 ± 0.24	78.3 ± 0.43	65.5 ± 0.16
Cypermethrin + Chlorpyrophos, 55% em. (etalon)	1	75.3 ± 0.14	100 ± 1.77	95.2 ± 0.36	96.2 ± 0.57	89.7 ± 0.16
Control (water)	-	89.0 ± 0.82	The amount of aphid naturally decreased 2-3 times			

Insecticides	**Efficiency (%) by days after treatment 2006-2007**			
	3	**7**	**14**	**20**
Karate 5% em.	65.2 ± 0.39	51.5 ± 0.50	15.2 ± 0.16	0
Talstar, 10% em.	71.8 ± 0.28	61.3 ± 0.14	24.4 ± 0.18	0
Decys, 10% em.	61.4 ± 0.16	50 ± 0.22	14.8 ± 0.08	0
Cypermethrin 25% em.	58.4 ± 0.08	44.4 ± 0.08	15.4 ± 0.16	0
Cypermethrin + Chlorpyrophos, 55% em. (etalon)	96 ± 0.16	94.5 ± 0.17	89.3 ± 0.36	73.8 ± 0.22
Control (water)	The amount of aphid naturally decreased 2-3 times			

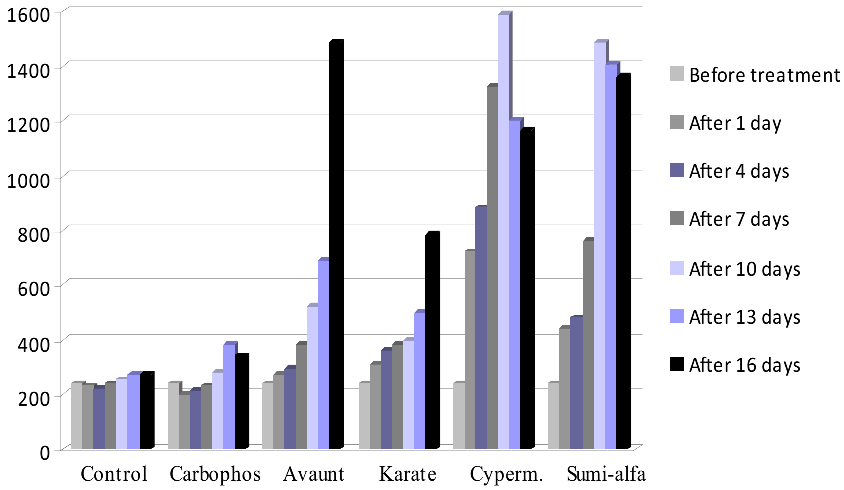

Fig. 1 The change dynamics of soluble proteins of cotton leaves after treatment with insecticides (µg/g). (n = 3, mean values are shown, SD was always less than 6%).

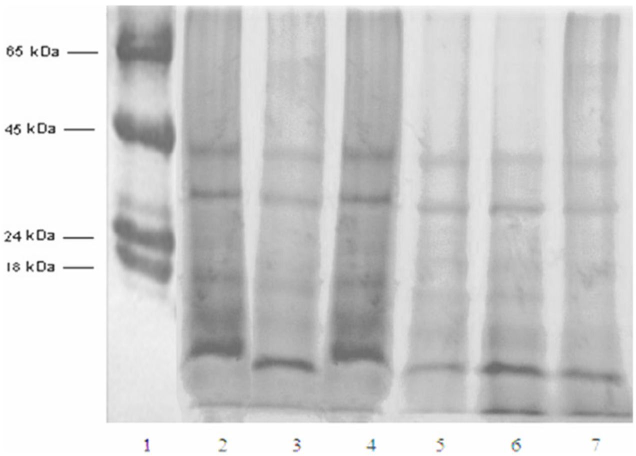

Fig. 2 Electrophoretical analysis of pattern total proteins from cotton leaves after treatment with insecticides. Lanes: 1 - marker proteins; 2 - Avaunt; 3 - Sumi-alfa; 4 - Cypermethrin; 5 - control; 6 - Carbophos; 7 - Karate. Obtained protein extracts were studied in SDS-PAGE gel as described by Laemmli (1970) 12% (w/v, pH 6.8) in the presence of 2-mercaptoethanol and stained with Coomassie G 250. Running time 3 h at a constant of 100 V.

Terpenoid aldehydes

Changes in the quantity of defensive compounds could also account for the resurgence of insect attack on cotton plants. The cotton plant has a unique group of terpenoids that include desoxyhemigossypol, hemigossypol, gossypol, hemigossypolone, and heliocides H_1, H_2, H_3 and H_4 (Bell *et al.* 1987; Stipanovic 1988; Hedin *et al.* 1992) that play a very important role in cotton plant protection against a wide range of pests. For instance, gossypol, hemigossypolone, and heliocides protect the plant from *Agrotis segetum* and *Heliothis armigera*. At the same time, cotton varieties with low gossypol content are damaged by insects, rodents and birds which commonly do not attack cotton (Stipanovic *et al.* 1999). Du *et al.* (2004) showed that aphids feeding on cotton cultivars with a high gossypol content showed significantly shorter adult longevity and lower fecundity than aphids feeding on plants with low and medium gossypol content.

We here report how the quantity of these individual compounds in leaves, which affect the defense mechanism of cotton plants, change after treatment with insecticides. As shown in **Table 3**, the concentration of terpenoid aldehydes in leaves treated with all insecticides, except for Carbophos, decreased. Only in the first samples treated with Carbophos was the total concentration higher than in the control; this was followed by a drop in concentration. Treatment with Karate gave the lowest concentration of total terpenoids. Except for the 4th day, samples treated with Cypermethrin, all the pyrethroids significantly reduced the terpenoid aldehydes concentration. These results provide evidence that another reason for the resurgence of some secondary pests on cotton could be that the defensive compounds of the plant are lower after treatment with insecticides and that the cotton plant is thus more susceptible to pests.

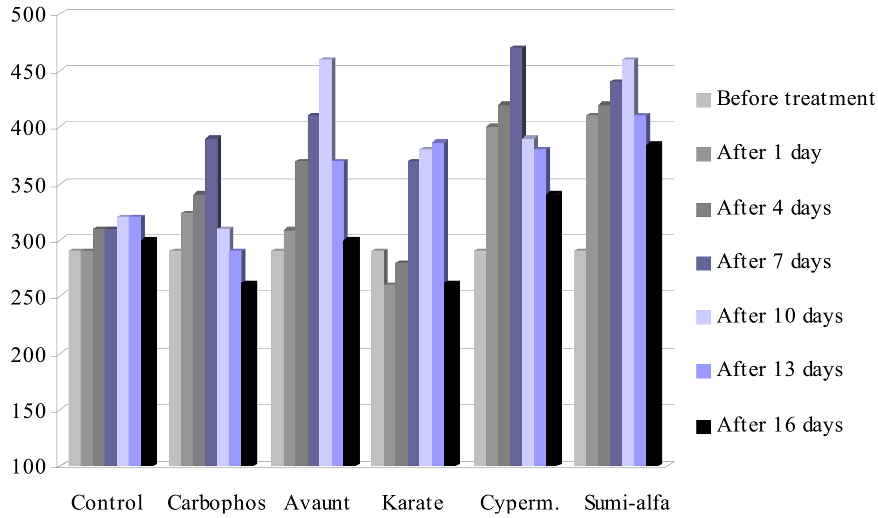

Fig. 3 The change dynamics of reducing sugars of cotton leaves treated with insecticides (µg/g). (*n* = 3, mean values are shown, SD was always < 6%).

Table 3 The changes in defensive terpenoid aldehydes after treatment with insecticides.

	Days	Control	Avaunt	Karate	Sumi-alfa	Cypermethrin	Carbophos
Hemigossypolone	1st day	10.1 ± 0.08	5.7 ± 0.18	0.21 ± 0.04	7.9 ± 0.14	8.1 ± 0.16	9.6 ± 0.32
	4th day	4.38 ± 0.03	6.0 ± 0.24	0.1 ± 0.02	2.2 ± 0.18	2.8 ± 0.09	6.7 ± 0.39
	7th day	5.2 ± 0.29	0.95 ± 0.05	3.4 ± 0.24	4.5 ± 0.16	2.4 ± 0.09	4.0 ± 0.24
	10th day	3.9 ± 0.18	0.63 ± 0.03	1.0 ± 0.18	3.5 ± 0.22	4.9 ± 0.04	1.8 ± 0.14
	13th day	5.2 ± 0.16	1.1 ± 0.18	5.4 ± 0.18	4.5 ± 0.26	5.2 ± 0.1	8.3 ± 0.1
	16th day	4.8 ± 0.18	0.52 ± 0.02	3.4 ± 0.14	6.3 ± 0.16	5.2 ± 0.17	1.6 ± 0.08
Methyl hemigossypolone	1st day	0.63 ± 0.01	1.8 ± 0.18	0.27 ± 0.04	0.60 ± 0.02	0.5 ± 0.02	0.55 ± 0.03
	4th day	0.408 ± 0.002	1.1 ± 0.18	0.3 ± 0.04	0.39 ± 0.01	0.84 ± 0.03	0.55 ± 0.03
	7th day	0.28 ± 0.02	1.1 ± 0.26	0.50 ± 0.02	0.44 ± 0.03	0.40 ± 0.01	0.55 ± 0.04
	10th day	0.63 ± 0.02	0.83 ± 0.04	0.45 ± 0.02	0.38 ± 0.01	0.46 ± 0.03	0.52 ± 0.02
	13th day	0.56 ± 0.03	1.3 ± 0.14	0.52 ± 0.02	0.52 ± 0.02	0.45 ± 0.02	0.48 ± 0.03
	16th day	0.50 ± 0.02	0.92 ± 0.02	0.60 ± 0.02	0.60 ± 0.02	0.70 ± 0.03	0.65 ± 0.02
Gossypol	1st day	0.56 ± 0.03	0.32 ± 0.02	0.12 ± 0.03	0.55 ± 0.02	0.20 ± 0.02	0.19 ± 0.02
	4th day	0.28 ± 0.02	0.45 ± 0.03	0.24 ± 0.03	0.14 ± 0.01	0.60 ± 0.02	0.36 ± 0.02
	7th day	0.46 ± 0.04	0.43 ± 0.02	0.2 ± 0.04	0.23 ± 0.02	0.24 ± 0.01	0.28 ± 0.02
	10th day	0.38 ± 0.01	0.30 ± 0.02	0.17 ± 0.02	0.22 ± 0.01	0.30 ± 0.02	0.30 ± 0.04
	13th day	0.32 ± 0.02	0.15 ± 0.04	0.50 ± 0.01	0.27 ± 0.01	0.26 ± 0.03	0.39 ± 0.05
	16th day	0.42 ± 0.02	0.16 ± 0.03	0.80 ± 0.04	0.37 ± 0.02	0.13 ± 0.01	0.24 ± 0.03
Heliocides H1-H4	1st day	117.06 ± 0.74	76.1 ± 0.34	27.35 ± 0.04	99.44 ± 0.05	110.6 ± 0.24	133.5 ± 0.32
	4th day	105.19 ± 0.03	93.8 ± 0.24	51.9 ± 0.18	62.8 ± 0.09	127.4 ± 0.56	139.5 ± 2.27
	7th day	89.11 ± 0.08	75.7 ± 0.81	81 ± 0.21	62.7 ± 0.03	74.3 ± 0.29	108.2 ± 0.34
	10th day	101.15 ± 0.03	32 ± 2.45	58.6 ± 0.34	82.9 ± 0.17	83.4 ± 0.29	63.4 ± 0.39
	13th day	102.22 ± 0.04	65.7 ± 0.36	67.7 ± 0.52	95.5 ± 0.44	71.9 ± 0.37	55.6 ± 0.26
	16th day	92.8 ± 0.22	52.7 ± 0.50	60.5 ± 0.44	86 ± 0.24	89.6 ± 0.48	48 ± 0.58
Σ terpenoid aldehydes	1st day	128.35 ± 0.04	83.92 ± 0.32	27.95 ± 0.04	108.49 ± 0.02	119.4 ± 0.24	143.84 ± 0.82
	4th day	110.26 ± 0.03	101.35 ± 0.17	52.54 ± 0.3	65.53 ± 0.05	131.64 ± 0.48	147.11 ± 0.08
	7th day	95.05 ± 0.04	78.18 ± 0.23	85.10 ± 0.14	67.87 ± 0.02	77.34 ± 0.07	113.03 ± 0.05
	10th day	106.06 ± 0.07	33.76 ± 0.03	60.22 ± 0.15	87 ± 0.39	89.06 ± 0.06	66.02 ± 0.08
	13th day	108.30 ± 0.13	68.25 ± 0.16	74.12 ± 0.1	100.79 ± 0.05	77.81 ± 0.11	64.77 ± 0.22
	16th day	98.52 ± 0.02	54.3 ± 0.24	65.30 ± 0.16	93.27 ± 0.05	95.63 ± 0.22	52.79 ± 0.23

CONCLUSION

Compared to Carbophos, pyrethroids induced nutritious compounds such as proteins and reducing sugars and lowered defensive compounds of the plant. Avaunt, which is an oxadiazine compound and not a pyrethroid but has a similar insecticidal mode of action, had the same effect as pyrethroids. Thus, pyrethroids affect the biochemistry of the cotton plant. This effect should be considered before introducing new pyrethroid insecticides.

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REFERENCES

Bell AA, Stipanovic RD, Elzen GW, Williams HJ (1987) Structural and genetic variation of natural pesticides in pigment glands of cotton (*Gossypium*). In: Waller GR (Ed) *Allelochemicals: Role in Agricultural and Forestry*, American Chemical Society, Washington D.C., USA, pp 477-490

Du L, Ge F, Zhu S, Parajulee MN (2004) Effect of cotton cultivar on development and reproduction of *Aphis gossypii* (Homoptera: Aphididae) and its predator *Propylaea japonica* (Coleoptera: Coccinellidae). *Journal of Economic Entomology* **4**, 1278-1283

Ebert TA, Cartwright B (1997) Biology and ecology of *Aphis gossypii* Glover (Homoptera: Aphididae). *Southwestern Entomologist* **22**, 116-153

Hedin PA, McCarty JC (1990) Possible roles of cotton bud sugars and terpenoids in oviposition by the boll weevil. *Journal of Chemical Ecology* **3**, 757-772

Hedin PA, Parrott WL, Jenkins JN (1991) Effects of cotton plant allelochemicals and nutrients on behavior and development of tobacco budworm. *Journal of Chemical Ecology* **17**, 1107-1121

Hedin PA, Parrott WL, Jenkins JN (1992) Relationships of glands, cotton square terpenoid aldehydes, and other allelochemicals to larvae growth of *Heliothis virescens* (Lepidoptera: Noctui). *Journal of Economic Entomology* **85**, 359-364

Kerns DL, Gaylor MJ (1993) Induction of cotton aphids outbreaks by insecticides in cotton. *Crop Protection* **12**, 387-393

Kidd PW, Rummel DR, Thorvilson HG (1996) Effect of cyhalothrin on aphid populations of the cotton aphid, *Aphis gossypii* Glover in the Texas High Plains. *Southwest Entomology* **21**, 293-301

Kumar R (2011) Insecticide induced resurgence of mealybug, *Phenacoccus solenopsis* Tinsley in cotton. In: *Proceedings of World Cotton Research Conference-5*, Mumbai, India, Kalyani Publisher, New Delhi, p 40

Laemmli VK (1970) Cleavage of structural proteins during the assembly of the head of bacteriphage T4. *Nature* **277**, 680-685

Leser JF (1994) Management of cotton aphids: Texas style. In: *Proceedings of the Beltwide Cotton Conferences*, 1994 Jan 4, San Diego, USA, pp 137-141

Lowry OH, Rosenbrough NJ, Farr AR, Randal RJ (1951) Protein measurements with folin-phenol reagent. *Journal of Biological Chemistry* **193**, 265-273

Madsen C, Claesson MH, Ropke C (1985) Immunotoxicity of the pyrethroid insecticides deltamethrin and α-cypermethrin. *Toxicology* **107**, 219-227

McAuslane HJ, Alborn HT, Toth JP (1997) Systemic induction of terpenoid aldehydes in cotton pigment glands by feeding of larval *Spodoptera exigua. Journal of Chemical Ecology* **12**, 2861-2879

Mirmoayedi A, Maniee M, Yaghutipoor A (2010) Control of cotton spiny bollworm, *Earias insulana* Boisduval, using three bio-insecticides, Bt, Spinosad and Neem-Azal. *Journal of Entomology* **7**, 89-94

Nagrare VS, Kranthi S, Kumar R, Jothi BD, Amutha M, Deshmukh AJ, Bisane KD, Kranthi KR (2011) *Compendium of Cotton Mealybugs*, Surya Offset, Ramdaspeth Nagpur, 1 p

Parajulee MN, Slosser JE (2001) Cotton aphid biology as affected by cyhalothrin (karate): Aphid or host plant modification? *Proceedings of the Beltwide Cotton Conferences*, Memphis, USA, pp 957-959

Ravindhran R, Xavier A (1997) Effect of pyrethroids on resurgence of aphids (*Aphis gossypii* G.) and alteration of plant metabolism in cotton. *Pesticide Research Journal* **9**, 79-85

Slosser JE, Parajulee MN, Hendrix DL, Henneberry TJ, Pinchak WE (2004) Cotton aphid (Homoptera: Aphididae) abundance in relation to cotton leaf sugars. *Environmental Entomology* **33**, 690-699

Somogy MJ (1952) Notes on sugar determination. *Journal of Biological Chemistry* **195**, 19-23

Stipanovic RD, Altman DW, Begin DL, Greenblatt GA, Benedict JH (1988) Terpenoid aldehydes in upland cotton: Analysis by aniline and HPLC methods. *Journal of Agricultural and Food Chemistry* **30**, 509-515

Stipanovic RD, Bell AA, Benedict CR (1999) Cotton pest resistance: The role of pigment gland constituents. In: Cutler HG, Cutler SJ (Eds) *Biologically Active Natural Products, Allelochemicals*, CRC Press, USA, pp 211-220

Torres JB, Silva-Torres CS, Barros R (2003) Relative effects of the insecticide thiamethoxam on the predator *Podisus nigrispinus* and the tobacco whitefly *Bemisia tabaci* in nectaried and nectariless cotton. *Pest Management Science* **59**, 315-323

Wing KD, Sacher M, Kagaya Y, Tsurubuchi Y, Mulderig L, Connair M, Schnee M (2000) Bioactivation and mode of action of the oxadiazine indoxacarb in insects. *Crop Protection* **19**, 537-545