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Research Article

Cotton Leaf Urease and PAL activities, Disulfide Bonds under Treatment with Insecticides

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Abstract

Cotton plant is susceptible to different pests and deceases and is necessarily treated with pesticides. Insecticides are used most of all pesticides in agriculture worldwide. These chemicals are efficiently used; however they often cause the plant to be more susceptible to pests and deceases. The reason of this phenomenon is explained with the fact that defensive compounds in plant tissues lower. Herein we report the results on the effects of insecticides relating to three classes: carbophos (organophosphate), lannate (carbamate) and sumi-alfa (pyrethroid) on cotton leaf urease, phenylalanine ammonia-lyase activities and disulfide bonds amounts. Insecticides were sprayed with the concentration recommended by the producer against cotton pests. Field experiments were conducted on the cotton plant at the prebloom stage and leaf samples were taken on the 10th and 13th days of the treatment. Colorimetric analysis showed that urease and PAL activities in all samples treated with insecticides were lower than the control. Carbophos more strongly affected on the enzymes activities. Carbophos and lannate increased the amount of disulfide bonds, whereas pyrethroid sumi-alfa decreased.

INTRODUCTION

In Uzbekistan cotton fields a number secondary plant pests; cotton aphids and mites often increase after plants are treated with insecticides against cotton bollworms. Many researches, give evidence that the reason of this phenomenon is connected with observations that, natural enemies of secondary pests perish as the plant is treated with insecticides and their resurgence takes a part faster. On the other hand, nutritious compounds increases and lowering process in defensive compounds are suggested to be another reason of this problem. In our former investigations we studied that, essential amino acids and reducing sugars increase in cotton leaves after treatment with insecticides especially with pyrethroids [1]. Besides our results showed that the PR proteins; chitinase and peroxidase and polyphenoloxidase activities in plant leaves lowered [2]. Herein we report the effects of insecticides relating to three different classes on urease and phenylalanine ammonia-lyase enzymes and disulfide bonds determining the plan defense.

Urease (urea amidohydrolase EC 3.3.1.5) catalyzes the hydrolysis of urea, a major nitrogenous waste product of biological actions, to form ammonia and carbon dioxide and the process spontaneously continues [3] and is a nickel-dependent metalloenzyme, the activity of which is assayed by measuring the quantity of ammonia production. Ureases are widespread in plants, fungi and bacteria. In plants ureases from different parts were identified and studied. Better characterized plant urease was isolated from jack bean Canavalia ensiformis [4]. Besides urease from mulberry (*Morus alba*) leaves was isolated and well characterized [5]. Plant ureases possess insecticidal properties independent of its ureolytic activity; as first described for canatoxin [6] and later for jack bean urease and soybean seed-specific urease [7,8]. Becker-Ritt [9] demonstrated that (Glycine max) embryo-specific soybean urease, jackbean (Canavalia ensiformis) major urease and a recombinant *H. pylori* urease impair growth of selected phytopathogenic fungi at sub-micromolar concentrations. Scanning electron microscopy of urease-treated fungi suggests plasmolysis and cell wall injuries. They assumed ureases contribute to the plant defense against predators and phytopathogens independently of ammonia release from urea. Urease is produced in bacteria, fungi, yeast,

and plants where it catalyzes the urea degradation to provide these organisms with a source of nitrogen for growth [10].

Similar leaf-tip necrosis were observed after the fertilization with urea resulting the accumulation of toxic amounts of urea rather than the toxic amount of ammonia as a result of urease action, since the addition of urea acted as urease inhibitor and increased leaf-tip necrosis [11]. The reason of this phenomenon could be connected with nickel shortage. Gerendas [12] demonstrated the importance of nickel for urease activity by the observation of urea-grown nickel-deprived rice (*Oriza sativa*) plants showing reduced growth and accumulating large amounts of urea due to reduced urease activity. Besides urease-negative mutant plants and nickel deprived wild type plants have the same phenotype, since they accumulate urea and exhibit necrotic leaf tips, apparently due to urea "burn" [13].

Phenylalanine ammonia-lyase (PAL) catalyzes nonoxidative deamination of L-phenylalanine forming trans-cinnamic acid and ammonium ion. Accumulation of phenylpropanoid compounds under stress conditions is considered to be the result of increased PAL activity [14]. Phenylalanine ammonia-lyase (EC 4.3.1.24, PAL) has been reported to change during stress conditions. Researches on different plants species showed increased PAL activity with the biotic and abiotic stresses [15]. Jeannette Vera showed a linear correlation between the increase of PAL activity and decrease of necrotic lesions in tobacco and in tobacco mosaic virus capsid protein transcript level. The induction PAL activity in response to stressful conditions has been considered to be defensive mechanisms of plants against stress [16].

The results obtained on antifungal activity of PAL enzyme, testified by Bhattacharyya and Ward [17] in susceptible soybean hypocotyls, indicated the rapidly increased level of PAL beginning 2 h after inoculation with *Phytophthora megasperma*. The authors also demonstrated small increases in PAL activity caused by wounding. Laporte [18] concluded, PAL and lypooxigenase, as the first enzymes of the phenylpropanoid and octadecanoid pathways, are key enzymes that regulate both metabolic pathways and their activation leads to the synthesis of secondary metabolites with antiviral, antibacterial and/or antifungal activities Another class of compounds of protein nature involved in plant defence is antimicrobial peptides (AMPs), the structures of which stabilized through formation of 2–6 **disulfide** bridges. The activities of plant AMPs are primarily directed against fungal and bacterial microorganisms, but certain members of a class can be directed against other targets, including herbivorous insects. Structural features common to plant AMPs are disulfide bridges and secondary structures like α-helices and β-sheets [19].

Harrison and Sternberg suggested that disulfide bonds stabilize the protein's folded state by restricting the protein's conformation, reducing the entropy of the unfolded state [20]. Meanwhile, disulfide bonds increase the enthalpy of the folded state of protein molecule stabilizing local interactions [21]. Hogg concluded disulfide bonds, besides the above mentioned functions, increase the protein's half-life by enhancing protein protection against proteases by maintaining the integrity of protein structure against local unfolding events [22]. Disulfide bonds act together with sulfhydryl groups. Under moderate oxidative stress conditions, oxidation of cystein residues lead to reversible formation of mixed disulfides between protein thiol groups and low-molecular-mass thiols (S-thiolation), especially with glutation (S-glutathionylation). Protein S-glutathionylation can directly regulate protein function (redox regulation) and also might have a role in protection from irreversible (terminal) oxidation [23].

MATERIALS AND METHODS

Experimental design

Field experiments were conducted on cotton variety S 26, growing at the pre-bloom stage in the cotton fields of the Institute for Plant Protection (Ministry of Agriculture and Water Resources of Uzbekistan, Tashkent Region, Kibray District, Salar Township). Insecticides relating to three different classes: carbophos (organophosphate), lannate (carbamates) and sumi-alfa (pyrethroid) were sprayed in concentrations recommended by the producers against cotton pests and leaf samples were taken on the 10^{th} and 13^{th} days of the treatment. Control leaves were treated with water. The solutions were sprayed once in the early morning at 6:00 to 6:30AM on 5 July 2012**.** Young and old leaves were taken from the upper, middle, and lower parts of the plant on the10^{th} and 13^{th} days after treatment. They were averaged and lyophilized (Table 1).

Protein extractions for urease and PAL activities

Proteins were extracted with 50 mM phosphate buffer pH 7.5, 10 mM PMSF, 50 mM NaCl, 1 mM EDTA, 1 M DTT and 1.5 mM PVP in homogenizer and solution was mixed in mixer (Vortex CL 001). The supernatant was freed from the residue by centrifugation 12000r/min for 30 minutes at 4^{o}C. In order to release DTT protein crude extract was dialyzed.

Determination of urease activity

Urease activity was determined by the phenol-nitroprussid method; Reagent A: 5 g phenol and 25 g sodium nitroprussid dissolved in 500 ml water. Reagent B: 2.5 g sodium hydroxide and 4.2 ml sodium hypochlorite (by 5% solution of free chlorine) in 500 ml solution. 1 ml of protein solution was taken to a tube and 600 µl 10 mM carbamide was added. The solution kept in thermostat 37^{o}C for 20 minutes. Then 20 µl reagent A was added, thoroughly mixed and 5 ml reagent B added. The solution was kept at 37 ^{o}C for 20 minutes. Blank solutions were included. The optical density of formed blue colour was read at 625 nm. Activity unit was counted as µmol/mg protein per min.

Determination of PAL activity

PAL activity was determined in 1 mL of reaction mixture containing 100 mM phosphate buffer, pH 7.5, 13 mM phenylalanine

Table 1: Treatment chemical class and application rates.

Treatment	Class	Application rate l/ha
Control		Water
Carbophos, Aerosoyuz, Russia	Organophosphate	0,6
Lannate, Du-Pont, France	Carbamate	0,25
Sumi-alfa, Sumimoto chemical, Japan	Pyrethroid	0,5

and protein extract at 40˚C. The increase in absorbance, caused by cinnamic acid accumulation, was monitored at 290 nm. PAL activity was calculated using the extinction coefficient of cinnamic acid (e = 17.4 mM-1 cm-1) [24].

Protein extractions for disulfide bond determination

Lyophilized cotton leaves were ground with liquid nitrogen using a mortar and pestle. After grinding, the proteins 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 two hours with stirring. The mixture was filtered and supernatant proteins were precipitated with cold absolute acetone and centrifuged 30 min (8 000 r/min, at 4^0C). The residue was dissolved in water and freeze-dried. The quantity of soluble proteins was determined according to Lowry et al. [19]. For calibration, albumin bovine (from bovine serum; Sigma A7030) in appropriate amounts was weighed and dissolved in Na_2HPO_4 buffer pH 7 to provide concentrations of 10-100 µg/ml.

Disulfide bonds and sulfhydryl groups were determined using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) according to [25]. To a 3-mL aliquot of the protein solution in the standard buffer 0.03 mL of Ellman's reagent solution (4 mg of DTNB/mL of standard buffer) was added. After the solution was rapidly mixed and allowed to stand at room temperature for 15 min, absorbance was read at 412 nm. The standard buffer blanks were included. A molar extinction coefficient of $1.36x10^4$ M-l cm-l was used for calculating micromoles of SH/gram of protein [26].

RESULTS

Our former results obtained on the effects of insecticides on soluble proteins of cotton plant leaf showed that, the highest quantities of proteins were on the 10th and 13th day of the treatment [1]. Therefore control and treated leaf samples were taken on the corresponding days.

Urease activity on the 10th day of treatment was higher in all samples; in control and treated samples, exceptionally insignificant differences in samples treated with lannate. Treatment with insecticides decreased the enzyme activity. The effects of carbophos on urease were more than other insecticides that the activity was 30 and 23% lower than the untreated control. 22% and 5% lower, than control, urease activity was determined in samples treated with carbamate lannate. Urease activity in samples treated pyrethroid sumi-alfa was 11% and 20% lower than the control on the corresponding 10th and 13th days after the treatment (Figure 1). It is suggested, depending on the day the leaves taken, PAL activity much differed in control, lannate and sumi-alfa samples, that on the 10th day of the treatment nearly twice lower enzyme activity was determined. Sumi-alfa decreased PAL more than carbophos and lannate that 2.5 and 3 times lower activity, than the control, was calculated. On the corresponding 10th and 13th days of the treatment PAL activity after carbamate lannate spray was 1.75 and 2 times lower than in the leaves treated with water. 30% lower than the control enzyme activity determined on the 10th day of carbamate treatment, and it still decreased on the 13th day differing from others (Figure 2).

Carbophos caused the disulfide bonds and sulfhydryl groups, one of the defense mechanisms of plants against pests, to increase, that their quantity was 40% and 9% higher than control samples. Lannate increased the quantity to 35% over the control on the 10th day of the treatment and not significant differences were observed on the 13th day. After the treatment with pyrethroid sumi-alfa, the amount of disulfide bonds and sulfhydryl groups was 27% and 40% less than the control which gives evidence that sumi-alfa showed more side-effects on the defensive compounds of plant tissues (Figure 3).

DISCUSSION

Ureases were determined and studied in cotton plant seeds and leaves; there is no information on cotton plant urease activity under the influence of pesticides. The first purified cotton urease was studied by Menegassi [26] in *Gossypium hirsutum* seeds. The

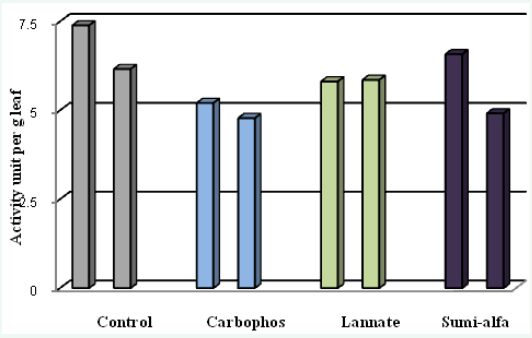

Figure 1 Urease activity changes after treatment (n=4, RSD≤5%).

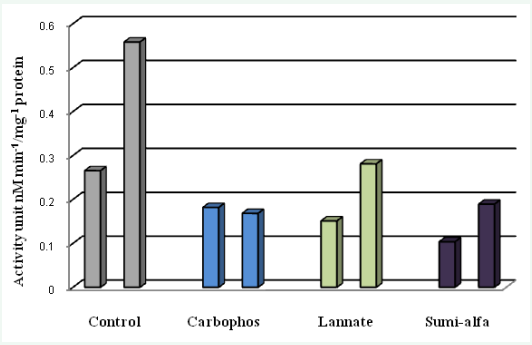

Figure 2 PAL activity changes after treatment (n=4, RSD≤5%).

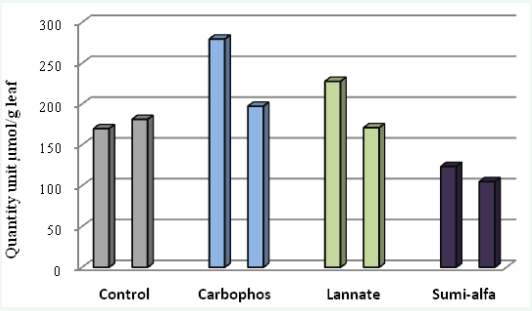

Figure 3 Disulfide bonds quantity changes after treatment with insecticides (n=4, RSD≤5%).

98.3 kDa enzyme had low ureolytic activity but displayed potent antifungal properties at sub-micromolar concentrations against different phytopathogenic fungi.

Jafaar [28] demonstrated the increase in PAL activity which could be related to reduction in nitrogen content in *Labisia pumila* plant exposed to high CO_2 levels. Zucker [29] demonstrated that fresh potato tuber tissues have no PAL activity. But the enzyme activity was found in extracts of tuber disks maintained in a moistened condition for 16 to 24 hours previous to extraction. The appearance of enzyme activity was suggested to be stimulated by exposure of the tissue to white light during culture, a condition which stimulates protein synthesis in the tissue. Analogues of purines and pyrinmidines inhibited the appearance of enzyme activity. Quiujua demonstrated induced PAL activity in cotton seedlings, after aphid infestation and mechanical wounding. They indicated that PAL activity was greatly induced by mechanical wounding and aphid infestation in cotton seedlings [30]. Results taken on the effects of *Xanthomonas Campestris* pv. *malvacearum* on PR proteins activities of cotton plant showed that, PAL activity was enhanced and it reached a maximum at 24h after challenge inoculation with *Xcm* (*Xanthomonas campestris* pv *malvacearum*) declined drastically after 48 h and remained constant at 96 h [31]. In our investigations PAL activity decreased in all treated samples. Ravindhran and Xavier Anne demonstrated the lower than control PAL activity in leaves treated with pyrethroid insecticides: deltamethrin, cypermethrin and fenvalerate which correspond to our results [32].

Disulfide bonds quantity changes correspond with our former results on other PR protein changes of cotton leaves under the influences of the same applied insecticides that, sumi decreased the enzymic activity of peroxidase, polyphenoloxidase, β-1,3-glucanase and chitinase, whereas carbophos and lannate increased polyphenoloxidase and chitinase acitivities on the same 10^{th} and 13^{th} days of the treatment [2].

Total soluble proteins quantity changed differently; carbophos lowered the quantity to 45 and 30% and after lannate their quantity were 20% less and 10% more, than untreated control, on the corresponding days of the treatment. Sumi-alfa increased total soluble proteins amounts till 60% and 40% over the control, whereas defensive proteins decreased under its spray (Table 2). These data conform to that that PR proteins make up 5-10% of all proteins [33].

Carbophos is not very often used in cotton defense against cotton bollworm and other *Heliothis* species, although it has acaricide property that the probability to cause the outbreaks of secondary pests as mites and aphids, after insecticide application, is not high as expected by lannate and sumi-alfa. Lannate increased the enzyme activity of peroxidase, polyphenoloxidase and chitinase [2], and by the effects on PR enzymes it can be evaluated the best among these threes.

Table 2: Total soluble proteins in cotton leaves treated with insecticides (n=4, M±m).

Samples treated	Control	Carbophos	Lannate	Sumi-alfa
The 10^{th} day	11,30±1,33	6,36±0,29	9,07±0,45	17,90±0,75
The 13^{th} day	10,07±0,18	7,11±0,411	11,24±0,41	14,09±0,34

CONCLUSION

Sumi-alfa a pyrethroid insecticide is very efficiently used in cotton defense against *Heliothis* species however; it causes the activities of many of the PR proteins including urease and PAL, and disulfide bonds quantity to decrease. We suggest sumi-alfa should better be used with acaricides and aphicides; otherwise their probable side-effects have to be taken into consideration. Carbophos, an organophosphate insecticide increased the quantity of disulfide bonds; however it decreased the urease activity and caused the PAL activity to be three times lower than the control. The treatment with carbamate lannate slight differences in urease activity and disulfide bonds quantity were determined on the 13^{th} day after the spray, which may support its application. Before the spray of pyrethroid sumi-alfa, its probable effects on biological control agents have to be thoroughly considered, that the treatment might cause some sucking and biting pests to increase as a result a phytoimmunity decrease and decreased number beneficial insects.

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REFERENCES

1. Asrorov A, Sultanova E, Veshkurova O, Uzbekov V, Sattarov N, Khodjayev Sh, et al. Effects of different classes insecticides on cotton leaf secondary metabolites. The Asian and Aust J Plant Sci and Biotechnol, Special Issue: 2013; 43-47.
2. Asrorov A, Sultanova E, Veshkurova O, Sattarov N, Khodjayev Sh, Salikhov Sh. Effects of different classes of insecticides on the activity of hydrolases in cotton plant leaves. American-Eurasian J Agric & Environ Sci. 2013; 3: 296-300. (a)
3. Andrews RK, Blakeley RL, Zerner B. Urea and urease. Adv Inorg Biochem. 1984; 6: 245-283.
4. Balasubramanian A, Ponnuraj K. Crystal structure of the first plant urease from jack bean: 83 years of journey from its first crystal to molecular structure. J Mol Biol. 2010; 400: 274-283.
5. Hirayama C, Sugimura M, Saito H, Nakamura M. Purification and properties of urease from the leaf of mulberry, Morus alba. Phytochemistry. 2000; 53: 325-330.
6. Carlini CR, Oliveira AE, Azambuja P, Xaviera-Filho J, Wells MA. Biological effects of canatoxin in different insect models: evidence for a proteolytic activation of the toxin by insect cathepsinlike enzymes. J Econ Entomol. 1997; 90: 340-348.
7. Follmer C, Wassermann GE, Carlini CR. Separation of jack bean (Canavalia ensiformis) urease isoforms by immobilized metal affinity chromatography and characterization of insecticidal properties unrelated to ureolytic activity. Plant Sci. 2004; 167: 241-246. (a)
8. Follmer C, Real-Guerra R, Wasserman GE, Olivera-Severo D, Carlini CR. Jackbean, soybean and Bacillus pasteurii ureases: biological effects unrelated to ureolytic activity. Eur J Biochem. 2004; 271: 1357-1363.
9. Becker-Ritt AB, Martinelli AH, Mitidieri S, Feder V, Wassermann GE, Santi L, Vainstein MH. Antifungal activity of plant and bacterial ureases. Toxicon. 2007; 50: 971-983.

10. Mobley HL, Hausinger RP. Microbial ureases: significance, regulation, and molecular characterization. Microbiol Rev. 1989; 53: 85-108.

11. Krogmeier MJ, McCarty GW, Bremner JM. Phytotoxicity of foliar-applied urea. Proc Natl Acad Sci U S A. 1989; 86: 8189-8191.

12. Gerendas J, Zhu Z, Sattelmacher B. Influence of N and Ni supply on nitrogen metabolism and urease activity in rice (Oryza sativa L). J Exp Bot. 1998; 49: 1545-1554.

13. Polacco JC, Holland MA. Roles of urease in plant cells. Jeon KW, Jarvik J editors. In: International Review of Cytology: Academic Press. San Diego. 1993; 65-103.

14. Maldonado R, Goni O, Escribano MI, Merodio C. Regulation of phenylalanine ammonia-lyase enzyme in Annona fruit: Kinetic characteristics and inhibitory effect of ammonia. Journal of Food Biochemistry. 2007; 31:161-178.

15. Hammerschmidt R. Induced disease resistance: how do induced plants stop pathogens? Physiol Mol Plant Pathol. 1999; 55: 77-84.

16. Martinez-Tellez MA, Lafuente MT. Effects of high temperature conditioning on ethylene, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase activities in Flavedo of chilled "Fortune" Mandarin fruit. J Plant Physiol. 1997; 150: 674-678.

17. Bhattacharyya MK, Ward EWB. Phenylalanine ammonia-lyase activity in soybean hypocotyls and leaves following infection with Phytophthora megasperma f.sp. glycinea. Can J Bot. 1998; 66: 18-23.

18. Laporte D, Vera J, Chandia NP, Zuniga EA, Matsuhiro B, Moenne A. Structurally unrelated algal oligosaccharides differentially stimulate growth and defense against tobacco mosaic virus in tobacco plants. J Appl Phycol. 2007; 19: 79-88.

19. Stotz HU, Waller F, Wang K. Innate immunity in plants: The role of antimicrobial peptides. Hiemstra PS, Zaat SAJ editors. In: Antimicrobial peptides and innate immunity. Springer Basel. 2013; 29-51.

20. Harrison PM, Sternberg MJ. Analysis and classification of disulphide connectivity in proteins. The entropic effect of cross-linkage. J Mol Biol. 1994; 244: 448-463.

21. Wedemeyer WJ, Welker E, Narayan M, Scheraga HA. Disulfide bonds and protein folding. Biochemistry. 2000; 39: 4207-4216.

22. Hogg PJ. Disulfide bonds as switches for protein function. Trends Biochem Sci. 2003; 28: 210-214.

23. Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev. 2004; 84: 1381-1478.

24. Vera J, Castro J, González A, Barrientos H, Matsuhiro B, Arce P, et al. Long-term protection against tobacco mosaic virus induced by the marine alga oligo-sulphated-galactan Poly-Ga in tobacco plants. Mol Plant Pathol. 2011; 12: 437-447.

25. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82: 70-77.

26. Shimada K, Cheftel JC. Determination of sulfhydryl groups and disulfide bonds in heat-induced gels of soy protein isolate. J Agric Food Chem. 1988; 36: 147-153.

27. Menegassi A, Wassermann GE, Olivera-Severo D, Becker-Ritt AB, Martinelli AHS, Feder V, et al. Urease from cotton (Gossypium hirsutum) seeds: Isolation, physicochemical characterization, and antifungal properties of the protein. J Agric Food Chem. 2008; 56: 4399-4405.

28. Jaafar HZE, Ibrahim MH, Karimi E. Phenolics and flavonoids compounds, phenylanine ammonia lyase and antioxidant activity responses to elevated CO2 in Labisia pumila (Myrisinaceae). Molecules. 2012; 17: 6331-6347.

29. Zucker M. Induction of Phenylalanine Deaminase by Light and its Relation to Chlorogenic Acid Synthesis in Potato Tuber Tissue. Plant Physiol. 1965; 40: 779-784.

30. Quiujua Q, Shi Xueyana, Liang Peia, Gao Xiwu. Induction of Phenylalanine ammonia-lyase and lipoxtgenase in cotton seedlings by mechanical wounding and aphid infestation. Progress in Natural Science. 2005; 15: 419-423.

31. Raghavendra VB, Lokesh Siddalingaiah, Nagesh K Sugunachar, Chandra Nayak, Niranjana S. Ramachandrappa, et al. Induction of Systemic Resistance by biocontrol agents against bacterial blight of cotton caused by Xanthomonas campestris pv. Malvacearum. eSci J. Plant Pathol. 2013; 02: 59-69.

32. Ravindhran R, Xavier Anne. Effect of Pyrethroids on Resurgence of Aphids (Aphis gossypii G) and alteration of Plant Metabolism in Cotton Pesticide. Research Journal. 1997; 9: 79-85.

33. van Loon LC. Pathogenesis-related proteins. Plant Mol Biol. 1985; 4: 111-116.

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