The Bioscan 10(2): 539-543, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

VARIATION IN THE FORMATION OF SOME BIOMOLECULES IN CABBAGE (*BRASSICA OLERACEA* L. VAR *CAPITATA*) LEAF INDUCED BY ENDOSULFAN

MANZOOR A. ASHRAFI AND GOUTAM K. PANDIT*
Department of Biochemistry, Uttar Banga Krishi Viswavidyalaya,
Pundibari - 736 165, Cooch Behar, West Bengal, INDIA
e-mail: gkpandit@yahoo.co.in

KEYWORDS
Brassica oleracea var *capitata*
Biochemical parameters
Leaf
Endosulfan

Received on :
24.10.2014

Accepted on :
18.05.2015

***Corresponding author**

ABSTRACT

Synthetic pesticides, used to control the pests of various crops, have an effect in the quantitative formation of their biomolecules as well. The present experiment was carried out to study the effect of different doses (control, T_1: 0; recommended, T_2: 0.844 L ha^{-1} and double of recommended, T_3: 1.688 L ha^{-1}) of endosulfan on the formation of carbohydrate, total free amino acid, protein, total phenol and total chlorophyll contents of cabbage leaf on 1, 7, 14, 21, 28 and 35 days after application (DAA). In comparison with 1^{st} DAA, the carbohydrate content decreased to 6.20 and 5.29% for T_2 and T_3 doses respectively on 35^{th} DAA with no change in control. The total free amino acid content increased to the extent of 110.21, 92.87 and 99.79% and the protein content decreased to 57.37, 30.72 and 37.11% as recorded on 35^{th} DAA in case of T_1, T_2 and T_3 doses respectively. The total phenol level increased to 18.42, 27.65 and 35.33% for T_1, T_2 and T_3 doses respectively on the last DAA. The total chlorophyll content was also found to increase to 25.64, 23.81 and 27.50% on 35^{th} DAA in case of T_1, T_2 and T_3 doses respectively with a decrease in midway. So the trend of formation of the studied biomolecules as observed in cabbage leaf due to endosulfan varied differently.

INTRODUCTION

Pesticides, particularly the synthetic ones, are used to control various insect pests, diseases or weeds of the crops under cultivation. In addition, as revealed from survey of literature, they affect the quantitative formation of different biomolecules of the crop plants. The amounts of some biomolecules of the crops are found to be increased and those of some others are decreased as a result of pesticides applied. Significant reduction of carbohydrate level in rice leaves during panicle emergence and in the grains at maturity by the herbicide butachlor; a decreasing trend by the fungicide carbendazim and an increasing trend by the insecticide carbofuran were recorded by Bhattacharya *et al.* (2001). Increased accumulation of phenolics by the fungicide carbendazim applied at half of the recommended dose and reduction of the same at double dose in tobacco plants were recorded by Garcia *et al.* (2001), whereas Siddiqui and Ahmed (2002) observed significant decrease in total protein and carbohydrate and increase in total phenol of wheat by the fungicides benlate and calixin. The insecticide cyanophos rendered increase in accumulation of total soluble sugar as well as insoluble and total nitrogen level and remarkable decrease in amino acid and peptide when applied on radish (El-Daly, 2008). Endosulfan, when applied individually and in combination with fungicide kitazin with increasing dose in brinjal lowered its protein content (Sammaiah *et al.*, 2011), whereas different concentrations of carbaryl reduced soluble sugar and free amino acid content of the same crop and significantly increased its total protein and insoluble sugar content (Goswami *et al.*, 2013). Four herbicides applied one day after transplanting in the soil of brinjal field resulted in increase in growth parameters and dry matter accumulation in the crop (Chnnappagoudar *et al.*, 2013), whereas carbofuran applied on brinjal leaf caused decrease in the formation of carbohydrate, total free amino acid, protein, and total chlorophyll contents with an exception of increase in total phenol (Ashrafi and Pandit, 2014).

In most of the above works different biochemical parameters were quantified only once or twice after application of the pesticides. It was, therefore, difficult to get the specific trend in the formation of different biomolecules in crops as an effect of pesticides. Considering this gap of information, the present investigation was carried out to study the effect of endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepine-3-oxide), a highly persistent organochlorine insecticide, on the accumulation of carbohydrate, free amino acids, protein, total phenol and total chlorophyll contents of cabbage (*Brassica oleracea* L. var *capitata*) leaf at different time intervals after its application.

MATERIALS AND METHODS

Physico-chemical analyses of soil

The present experiment was carried out at the Instructional Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India situated under *Terai* agro-climatic

Table 1: Effect of endosulfan on carbohydrate content* (mg g^{-1}) in cabbage

Treatment	Days after application					
	1	7	14	21	28	35
Control (T_1)	3.52 ± 0.35	3.57 ± 0.16(+1.42)#	3.59 ± 0.24(+1.99)	3.55 ± 0.55(+0.85)	3.56 ± 0.24(+1.14)	3.51 ± 0.33(–0.28)
0.844 L ha^{-1} (T_2)	3.87 ± 0.43	3.71 ± 0.36(–4.13)	3.61 ± 0.36(–6.72)	3.62 ± 0.44(–6.46)	3.58 ± 0.35(–7.49)	3.63 ± 0.13(–6.20)
1.688 L ha^{-1} (T_3)	3.78 ± 0.38	3.60 ± 0.39(–4.76)	3.54 ± 0.31(–6.35)	3.51 ± 0.46(–7.14)	3.54 ± 0.34(–6.35)	3.58 ± 0.52(–5.29)
SEm(±)	0.110	0.107	0.124	0.177	0.137	0.132
CD at 5%	0.340	0.330	0.382	0.545	0.423	0.407

* Mean ± S.D. of 7 replicates. #Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

Table 2: Effect of endosulfan on total free amino acid content* (mg g^{-1}) in cabbage

Treatment	Days after application					
	1	7	14	21	28	35
Control (T_1)	4.31 ± 0.29	2.96 ± 0.08(–31.32)#	6.35 ± 0.08(+47.33)	4.65 ± 0.62(+7.89)*	11.75 ± 0.8(+172.62)	9.06 ± 0.80 (+110.21)
0.844 L ha^{-1} (T_2)	5.61 ± 1.02	3.93 ± 0.51(–29.95)	6.87 ± 0.09(+22.46)	5.89 ± 0.58(+4.99)	13.11 ± 0.85(+133.69)	10.82 ± 0.51(+92.87)
1.688 L ha^{-1} (T_3)	4.85 ± 0.40	3.78 ± 0.23(–22.06)	6.65 ± 0.37(+37.11)	5.21 ± 0.84(+7.42)	12.68 ± 0.59(+161.44)	9.69 ± 0.74(+99.79)
SEm(±)	0.229	0.124	0.293	0.188	0.299	0.250
CD at 5%	0.705	0.383	0.902	0.579	0.920	0.769

* Mean ± S.D. of 7 replicates. #Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

Table 3: Effect of endosulfan on protein content* (mg g^{-1}) in cabbage

Treatment	Days after application					
	1	7	14	21	28	35
Control (T_1)	0.72 ± 0.06	0.60 ± 0.03(–15.73)#	0.68 ± 0.04(–4.77)	0.32 ± 0.02(–54.78)	0.36 ± 0.05(–49.99)	0.31 ± 0.04(–57.37)
0.844 L ha^{-1} (T_2)	0.62 ± 0.03	0.55 ± 0.05(–10.39)	0.71 ± 0.04(+15.47)	0.46 ± 0.03(–26.11)	0.53 ± 0.03(–14.79)	0.43 ± 0.03(–30.72)
1.688 L ha^{-1} (T_3)	0.59 ± 0.05	0.51 ± 0.03 (–14.22)	0.74 ± 0.04 (+25.54)	0.39 ± 0.02 (–34.22)	0.44 ± 0.06(–25.30)	0.37 ± 0.03(–37.11)
SEm(±)	0.022	0.013	0.016	0.007	0.012	0.010
CD at 5%	0.066	0.041	0.050	0.022	0.037	0.032

* Mean ± S.D. of 7 replicates. #Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

Table 4: Effect of endosulfan on total phenol content* (mg g^{-1}) in cabbage

Treatment	Days after application					
	1	7	14	21	28	35
Control (T_1)	9.12 ± 0.76	11.01 ± 0.75(+20.72)#	11.90 ± 2.11(+30.48)	9.41 ± 0.55(+3.18)	11.17 ± 1.12(+22.48)	10.80 ± 0.95(+18.42)
0.844 L ha^{-1} (T_2)	9.26 ± 0.29	12.31 ± 1.79(+32.94)	12.18 ± 2.43(+31.53)	9.73 ± 1.69(+5.08)	11.56 ± 0.99(+24.84)	11.82 ± 0.79(+27.65)
1.688 L ha^{-1} (T_3)	9.54 ± 0.48	13.19 ± 1.35(+38.26)	12.58 ± 0.62(+31.87)	10.02 ± 0.62(+5.03)	12.81 ± 0.55(+34.28)	12.91 ± 0.98(+35.33)
SEm(±)	0.175	0.591	0.782	0.385	0.368	0.336
CD at 5%	0.539	1.822	2.410	1.187	1.134	1.035

* Mean ± S.D. of 7 replicates. #Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

region between 25°57′N and 27°N latitude and 88°25′E longitude. Composite soil samples were collected from 0-15 cm depth of the experimental field with the help of a soil auger before application of fertilizers and were dried under shade, pulverized and sieved through 0.2 mm sieve for soil analysis. The methods employed for the analyses were as follows: soil pH using pH meter by potentiometric method (Baruah and Barthakur, 1997); organic carbon by rapid titration method (Walkey and Black, 1934), available nitrogen by modified macro–Kjeldahl method (Jackson, 1967), available phosphorus by Bray's No. I method (Jackson, 1967) and available potassium by flame photometer method (Jackson, 1967).

Crop cultivation and sampling

Three weeks old cabbage seedlings (variety Golden Acre) were transplanted in the plots of size 3m x 3m at a spacing of 45 cm x 60 cm. Recommended dose of fertilizers were applied at the rate of 150:100:100 kg ha^{-1} of $N:P_2O_5:K_2O$ as per standard practices. Three treatment doses of endosulfan (Endocel 35% EC) at the rates of control (T_1: 0), recommended (T_2: 0.844 L ha^{-1}) and double of the recommended dose (T_3: 1.688 L ha^{-1}) were applied once at the time of head formation following randomised block design with 3 treatments and 7 replicates for each treatment. Leaf samples were collected from each plot on 1, 7, 14, 21, 28 and 35 DAA of the pesticide. All the collected samples were analyzed to estimate carbohydrate, free amino acids, protein, total phenol and total chlorophyll using fresh leaves as early as possible.

Biochemical analyses

Carbohydrate estimation

It was done by anthrone method as stated by Sadasivam and Manickam (2008). Briefly, leaf sample (100 mg) was hydrolysed with 2.5 N HCl (5 mL) for 3 h and neutralized with sodium carbonate. Then it was filtered and volume made up to 25 mL. From the filtrate aliquot (0.25 mL) was taken and volume made up to 1 mL with distilled water. Anthrone reagent (4 mL) was added to it, mixed thoroughly and was heated on water bath for 8 min and cooled rapidly. The intensity of the developed green colour was measured by spectrophotometer at 630 nm and carbohydrate content was calculated from the standard graph prepared by glucose and expressed as mg g^{-1} fresh weight of tissue.

Total free amino acids estimation

Table 5: Effect of endosulfan on total chlorophyll content* (mg g^{-1}) in cabbage

Treatment	Days after application 1	7	14	21	28	35
Control (T_1)	0.78 ± 0.02	0.81 ± 0.02(+3.85)#	0.79 ± 0.02(+1.28)	0.48 ± 0.03(–38.46)	0.81 ± 0.03(+3.85)	0.98 ± 0.02(+25.64)
0.844 L ha^{-1} (T_2)	0.84 ± 0.04	0.88 ± 0.03(+4.76)	0.87 ± 0.03(+3.57)	0.57 ± 0.03(–32.14)	0.89 ± 0.03(+5.95)	1.04 ± 0.16(+23.81)
1.688 L ha^{-1} (T_3)	0.80 ± 0.02	0.86 ± 0.01(+7.50)	0.82 ± 0.02(+2.50)	0.52 ± 0.05(–35.00)	0.87 ± 0.02(+8.75)	1.02 ± 0.07(+27.50)
SEm(±)	0.012	0.010	0.014	0.019	0.017	0.041
CD at 5%	0.038	0.032	0.042	0.057	0.052	0.128

* Mean ± S.D. of 7 replicates. #Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

This was estimated by the method of Misra *et al.* (1975) in which leaf sample (500 mg) was first extracted twice with ethanol-water (5mL, 4:1 v/v) followed by centrifugation at 10,000 rpm for 15 min. From the pooled supernatant, 0.1mL was taken and ninhydrin solution (1 mL) was added. After making up the volume to 2 mL with distilled water it was heated on a water bath for 20 min and diluent solvent (5 mL, water and n-propanol, 1:1 v/v) was added and mixed. The intensity of the purple colour developed after 15 min was read spectrophotometrically at 570 nm against reagent blank. The amount of total free amino acids was calculated from the standard graph of leucine and was expressed as mg g^{-1} fresh weight of tissue.

Protein estimation

Following the method of Lowry *et al.* (1951) protein was extracted from leaf (500 mg) sample with sodium phosphate buffer (10 mL, 0.1 M, pH 7.0). The homogenate was centrifuged at 10,000 rpm for 20 min and supernatant was used for the assay. The prescribed reagents were added and the absorbance of the blue colour formed therein was measured at 660 nm in a spectrophotometer against reagent blank, and protein content was quantified by the standard graph of bovine serum albumin (BSA) and expressed as mg g^{-1} fresh weight of tissue.

Total phenol estimation

The Folin–Ciocalteau method as described by Gopi *et al.* (2009) was taken up for this estimation. Briefly, fresh tissue (500 mg) was homogenized twice with 10 mL and 5 mL of 80% ethanol and centrifuged at 10,000 rpm for 20 min. Ethanol remaining in the pooled supernatants was evaporated to dryness and the residue was dissolved in 5 mL distilled water and from it 0.2 mL was taken and diluted to 3 mL with distilled water. To it Folin–Ciocalteau reagent (0.5 mL) was added and kept for 3 min. Next, 20% sodium carbonate solution (2 mL) was mixed to it and heated for 1 min. After cooling in ice–cold water the absorbance was measured at 650 nm in a spectrophotometer against reagent blank. Total phenol was estimated in gallic acid equivalent after comparing with the standard graph of gallic acid and expressed as mg g^{-1} fresh weight of tissue.

Total chlorophyll estimation

It was carried out by the method of Witham *et al.* (1971). Briefly, finely cut fresh leaf (1 g) was crushed in pre–chilled mortar and pestle with 80% chilled acetone (20 mL) and centrifuged at 5,000 rpm for 5 min and the supernatant was collected. The process was repeated until the residue was colourless. Finally volume was made up to 100 mL with 80% chilled acetone. The absorbance was recorded at 645 nm and 663 nm in a spectrophotometer against solvent blank. The amount of total chlorophyll was calculated using the given formula and results were expressed as mg g^{-1} fresh weight of tissue.

Statistical analysis

The statistical analyses of the data were done by the ANOVA method (Gomez and Gomez, 1983). The computation and statistical analyses were carried out in Microsoft Excel 2007 and SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Physico-chemical analyses of soil

The results of physico-chemical analyses of soil of the experimental field showed that texturally it was sandy loam soil in and acidic in nature with pH 5.62. The organic carbon was found to be 0.87% along with available nitrogen 163.71 kg ha^{-1}, available phosphorus 25.38 kg ha^{-1} and available potassium 112.35 kg ha^{-1}.

Carbohydrate content

From the results of carbohydrate content of cabbage as estimated on different days after application of endosulfan (Table 1) it was revealed that the carbohydrate level due to T_1 dose remained almost same till the last day of study with a little increase in between. Whereas in case of T_2 and T_3 doses a decrease of 6.20% and 5.29% respectively were recorded at the end of the study period.

Earlier, Osman *et al.* (1988) observed decrease in total and soluble carbohydrate after application of four herbicides on *Zea mays* grains and Bhattacharya *et al.* (2001) recorded an increasing trend in carbohydrate content in rice due to carbofuran; whereas Ahmed *et al.* (2003) observed increase as well as decrease in carbohydrates after application of three insecticides on two maize varieties. Thus the results of the present experiment are in conformity with the above findings.

Total free amino acid content

From the results (Table 2) it was observed that the total free amino acid content of cabbage leaf on different days after application of endosulfan increased with time showing a percent increase of 110.21, 92.87 and 99.79 respectively in case of T_1, T_2 and T_3 doses in comparison with the first DAA. The total free amino acid level of both the treatment doses remained higher than control all through the period of investigation, and the difference between their level due to T_1 and T_2 doses were significant and that between T_1 and T_3 were non-significant on most days of analysis.

In some earlier studies, Starratt and Lazarovits (1999) recorded increase in free amino acid levels due to herbicides trifluralin and acetochlor; Osman *et al.* (1988) and Ahmed *et al.* (2003) observed increase as well as decrease in some different amino acids due to different pesticides and El-Daly (2008) found remarkable drop in amino acid with high cyanophos concentrations. The present investigation also recorded increase in total free amino acid content with passage of time maintaining analogy with some of the earlier results.

Protein content

The protein content of cabbage leaf as measured on different days after application of endosulfan (Table 3) indicated an overall decline on the last day with regards to the initial day of analysis. The difference between T_1 and T_2 and also between T_1 and T_3 were significant during most of the period of study.

In most of the earlier studies the protein contents were measured only once after application of pesticides and varying trend in the results were observed. For example, Siddiqui and Ahmed (2006) observed decrease in protein content of soybean with increase in concentration of five pesticides; Osman *et al.* (2006) observed significant increase in protein content of cottonseed by metasystox, while Ahmed *et al.* (2003) observed increase as well as decrease in total soluble protein of maize measured at 96 h and one week after application of carbofuran. Here, excepting the rise on 14th DAA, there was a gradual decrease in protein content with time in all the treatments. This trend might be due to inhibitory effect of the insecticide on the synthesis of protein. So the results were similar to some of the earlier studies.

Total phenol content

The results of total phenol content of cabbage leaf on different days after application of endosulfan are summarised in Table 4. It was observed that the level of total phenol in cabbage leaves increased in case of both the treatment doses with respect to control.

In most of the earlier works (Siddiqui *et al.*, 2001; Jaleel *et al.*, 2008; Kaur *et al.*, 2011) the phenol level increased with application of pesticides. The trend in the present study also remained similar all through the period of investigation. In case of control treatment, the maximum increase was 30.48% on 14th DAA and for T_2 and T_3 doses it was 32.94% and 38.26% respectively on 7th DAA with respect to the first DAA. Pesticides applied on crops initiate a kind of chemical stress and triggers formation of phenolic compounds (Siddiqui and Ahmed, 2006). The increase in phenolics content as observed here might be due to this.

Total chlorophyll content

The results of total chlorophyll content of cabbage leaf on different days after application of endosulfan are given in Table 5. The results revealed that the level of total chlorophyll in cabbage increased at different rates with a decrease on 21st DAA in all the treatment doses. The difference between T_1 and T_2 doses was significant on most days of analysis and that between T_2 and T_3 doses were mostly non-significant.

Previously, an increase in the chlorophyll content was observed in *S. tuberosum* by deltamethrin (Fidalgo *et al.*, 1993) and in *H. esculentus* and *C. annuum* by Topsin (Ahmed and Siddiqui, 1995); whereas reduction by butachlor on rice (Bhattacharya *et al.*, 2001) was recorded. In the present study also increase of 25.64%, 23.81% and 27.50% in chlorophyll content were found on 35th DAA with respect to the initial amounts in T_1, T_2 and T_3 doses respectively excepting a sharp decrease on 21st DAA. So the results of the present study are comparable with the earlier findings.

The results of the present experiment revealed a minor decrease in carbohydrate content in case of two treatment doses of endosulfan keeping that of control treatment unaltered. The total free amino acid content of cabbage leaf increased with time in case of all three treatment doses in comparison with the first DAA whereas the protein content decreased gradually with elapse of time. This might be due to inhibition of protein synthesis due to pesticides. It was observed that the level of total phenol in cabbage leaves increased in case of both the treatment doses with respect to control and corroborated the earlier observations that chemical stress of pesticides augment yield of phenols. The total chlorophyll content was found to be increased at the end with a decrease on 21st DAA. So varying trend in the formation of the studied biomolecules was observed in cabbage due to endosulfan.

ACKNOWLEDGEMENTS

The financial assistance extended by Uttar Banga Krishi Viswavidyalaya to M. A. Ashrafi in the form of University Research Scholarship is duly acknowledged.

REFERENCES

Ahmed, S. and Siddiqui, Z. S. 1995. Effect of topsin fungicide (methyl thiophenate) on chlorophyll, protein and phenol contents of *Hibiscus esculentus* and *Capsicum annuum*. *Pakistan J. Botany*. **27:** 175-178.

Ahmed, S., Anzum, S., Naeem, M. and Ashraf, M. Y. 2003. Determination of efficacy of Cypermethrin, Regent and Carbofuran against *Chilo partellus* Swin. and biochemical changes following their application in maize plants. *International J. Agriculture and Biology*. **5:** 30-35.

Ashrafi, M. A. and Pandit, G. K. 2014. Effect of carbofuran on the formation of some biomolecules in brinjal (*Solanum melongena* L.) leaf. *The Bioscan*. **9(3):** 959-963.

Baruah, T. C. and Barthakur, H. P. 1997. A Textbook of Soil Analysis. *Vikas Publishing House Pvt. Ltd.,* New Delhi. **103**.

Bhattacharya, A., Pandit, G. K., Chakraborti, P. and Roy, D. 2001. Effect of carbofuran, butachlor and carbendazim on chlorophyll and carbohydrate contents of two summer rice (*Oryza sativa* L) cultivars at panicle emergence. *Crop Research*. **21:** 15-19.

Chnnappagoudar, B. B., Mane, S. S., Naganagoudar, Y. B. and Rathod, S. 2013. Influence of herbicides on morpho-physiological growth parameters in brinjal (*Solanum melongena* L.). *The Bioscan*. **8(3):** 1049-1052.

El-Daly, F. A. 2008. Biochemical influence of cyanophos insecticide on radish plant II. Effect on some metabolic aspects during the growth period. *Research J. Agriculture and Biological Sciences*. **4:** 210-218.

Fidalgo, F., Santos, I. and Salema, R. 1993. Effects of deltamethrin on field grown potato plants: biochemical and ultrastructural aspects. *Annals of Botany*. **72:** 263-267.

Garcia, P. C., Rivero, R. M., Lopez-Lefebre, L. R., Sanchez, E., Ruiz, J. M. and Romero, L. 2001. Direct action of the biocide carbendazim on phenolic metabolism in tobacco plants. *J. Agricultural and Food Chemistry*. **49:** 131-137.

Gomez, K. A. and Gomez, A. A. 1983. Statistical Procedures for Agricultural Research. *J. Wiley and Sons*, New York, pp. 20-30

Gopi, R., Jaleel, C. A., Divyanair, V., Azooz, M. M. and Panneerselvam, R. 2009. Effect of paclobutrazol and ABA on total phenol contents in different parts of holy basil (*Ocimum sanctum*). *Academic J. Plant Sciences*. **2:** 97-101.

Goswami, M. R., Banerjee, P., Swarnakar, S. and Mukhopadhyay, A. (2013). Carbaryl mediated biochemical alterations in Eggplant (*Solanum melongena* L.). *International J. Research in Environmental Science and Technology*. **3:** 51-57.

Jackson, M. L. 1967. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi. pp. 183, 347, 387-408.

Jaleel, C. A., Gopi, R. and Panneerselvam, R. 2008. Biochemical alterations in white yam *(Dioscorea rotundata* Poir.) under triazole fungicides: impacts on tuber quality. *Czech J. Food Science*. **26:** 298-307.

Kaur, N., Sohal, B. S. and Singh, K. 2011. Biochemical and physiological changes on *Bacillus thuringiensis* cotton after imidacloprid foliar spray. *Pesticide Biochemistry and Physiology*. **99:** 280-284.

Lowry, O. H., Rosbrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with folin phenol reagent. *J. Biological Chemistry* **193:** 265-275.

Misra, P. S., Mertz, E. T. and Glover, D. V. (1975). Studies on corn proteins. VIII. Free amino acid content of opaque-2 double mutants. *Cereal Chemistry*. **52:** 844-848.

Osman, G. A., Mustafa, A. I. and Abdelbagi, A. O. 2006. Effect of metasystox application on cottonseeds quality. *Pakistan J. Nutrition* **5:** 467-470.

Osman, R. O., Ahmed, F. A., Khalil, F. A. and Ali, M. S. 1988. Effect of some herbicides as plant growth regulators on the chemical composition of *Zea mays* grains. *Food Chemistry*. **28:** 167-176.

Sadasivam, S. and Manickam, A. 2008. Biochemical Methods. *New Age International (P) Ltd., Publishers,* New Delhi, pp. 7-8.

Sammaiah, D., Shekar, C. C., Prasad, V. R. and Reddy, K. J. 2011. Pesticide induced alterations in physiological responses in brinjal (*Solanum melongena* L.) *International J.Pharma and Bio Sciences* **2:** B374-383.

Siddiqui, Z. S. and Ahmed, S. 2002. Effects of systemic fungicides on protein, carbohydrate, amino acids and phenolic contents of susceptible (Mexipak) and resistant (Povan) varieties of wheat (*Triticum aestivum* L.) *Turkish J. Botany*. **26:** 127-130.

Siddiqui, Z. S. and Ahmed, S. 2006. Combined effects of pesticide on growth and nutritive composition of soybean plants (*Glycine max* L.). *Pakistan J. Botany*. **38:** 721-733.

Siddiqui, Z. S., Ahmed, S. and Arif-uz-Zaman. 2001. Effect of methyl thiophenate (systemic fungicide) on germination, seedling growth, biomass and phenolic content of resistant and susceptible varieties of wheat (*Triticum aestivum* L.) *Pakistan J. Biological Sciences*. **4:** 1198-1200.

Starratt, A. N. and Lazarovits, G. 1999. Herbicide-induced disease resistance and associated increases in free amino acid levels in melon plants. *Canadian J. Plant Pathology*. **21:** 33-36.

Walkey, A. and Black, I. A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*. **37:** 29-38.

Witham, F. H., Blaydes, D. F. and Devlin, R. M. 1971. Experiments in Plant Physiology. *Van Nostrand*, New York, p. 245.