The Bioscan 9(3): 959-963, 2014
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF CARBOFURAN ON THE FORMATION OF SOME BIOMOLECULES IN BRINJAL (*SOLANUM MELONGENA* L.) LEAF

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
Solanum melongena
Biochemical parameters
Leaf
Carbofuran

Received on :
18.02.2014

Accepted on :
10.07.2014

***Corresponding author**

ABSTRACT
The present work was carried out with the objective to study the effect of carbofuran on the quantitative formation of carbohydrate, total free amino acids, protein, total phenol and total chlorophyll contents in brinjal leaf as recorded on 1, 7, 14, 21, 28 and 35 days after application (DAA). The carbohydrate content decreased to the extent of 3.36, 4.53 and 4.60% respectively against control (T_1: 0), recommended (T_2: 33.0 kg ha^{-1}) and double of recommended (T_3: 66.0 kg ha^{-1}) doses of carbofuran formulation on the 35th DAA in comparison with 1st DAA. The total free amino acid content decreased to 42.82, 45.84 and 49.89% respectively against the above doses. In case of protein content also an overall decrease of 45.28, 43.10 and 45.61% with regards to 1st DAA was observed. The total phenol content, on the contrary, recorded an increase of 18.85, 21.77 and 5.35% respectively in case of above three doses. The total chlorophyll content again exhibited a decrease of 20.99, 22.73 and 36.05% respectively. So the formation of all the biomolecules under study except phenol in brinjal leaf was found to decrease with passage of time as an effect of application of carbofuran.

INTRODUCTION

Brinjal (*Solanum melongena* L.), a member of the family Solanaceae, is native of India and is one of the most popular vegetables cultivated and consumed in many countries of the world including India. It has a good nutritive value being rich in carbohydrate, protein, fibre, minerals and vitamins and low in fats and calories. It is a versatile crop adapted to different agro-climatic situations and can be grown throughout the year and so is attacked by a number of insect pests like shoot and fruit borer (*Leucinodes orbonalis* Guen.), hadda beetle (*Henosepilachna vigintioctopunctata* Fabr.), stem borer (*Euzophera particella* Rag.), whitefly (*Bemisia tabaci* Gennadius), brown leafhopper (*Cestius phycitis* Distant) and lace-wing bug (*Urentius sentis* Distant). Application of insecticides is, therefore, needed for their control.

Pesticides used on the crop plants can increase or decrease the quantitative formation of their various biomolecules and also can alter the physiological parameters of their growth. It was observed that the herbicide butachlor significantly reduced the carbohydrate level in the leaves of rice at panicle emergence stage and in the grains at maturity; the fungicide carbendazim caused a decreasing trend and the insecticide carbofuran, an increasing trend of the same (Bhattacharya *et al.*, 2001). Again carbendazim increased accumulation of phenolics when applied at half of the recommended dose and reduced the same at double dose in tobacco plants (Garcia *et al.*, 2001), whereas benlate and calixin rendered significant decrease in total protein and carbohydrate and increase in total phenol of wheat (Siddiqui and Ahmed, 2002). Cyanophos, an insecticide, caused increase in accumulation of total soluble sugar, remarkable drop in amino acid and peptide and increase in insoluble and total nitrogen content when applied on radish (El-Daly, 2008). The insecticide endosulfan applied individually as well as in combination with fungicide kitazin reduced protein content with increasing dose in brinjal (Sammaiah *et al.*, 2011). Different concentrations of carbaryl, a carbamate insecticide, reduced soluble sugar and free amino acid content of brinjal; whereas it increased the total protein and insoluble sugar content significantly (Goswami *et al.*, 2013). Four herbicides namely alachlor, butachlor, pendimethalin and pretilachlor applied at two doses with unweeded control in brinjal field soil one day after transplanting rendered increase in growth parameters and dry matter accumulation in the crop (Chnnappagoudar *et al.*, 2013).

The biochemical parameters in most of the works cited above were measured only once or twice after application of the pesticides. Therefore, specific trend in their formation during the course of growth of a crop could not be arrived at in the above studies. Keeping this gap of information in consideration, the present experiment was carried out to study the effect of carbofuran (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate), a systemic insecticide of carbamate group and used to control insect pests of crops including potato, brinjal, corn, soybean and others, on the quantitative formation of carbohydrate, total free amino acids, protein, total phenol and total chlorophyll contents in brinjal leaf recorded at different time intervals after its application.

MATERIALS AND METHODS

Cultivation of crop and sampling

The present study was carried out at the Instructional Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India under *Terai* agroclimatic zone situated between 25°57′N and 27°N latitude and 88°25′E longitude following randomized block design (RBD) with 3 treatments and 7 replications for each treatment. Brinjal (variety Muktakeshi) seedlings (4 weeks old) were transplanted in the prepared plots of size 3m x 3m at spacing of 90 cm x 60 cm. Fertilizers were applied as per recommended dose of 100:60:60 kg ha^{-1} of $N:P_2O_5:K_2O$ following standard practices. Three treatment doses of carbofuran (Furadan 3G) at the rates of control (T_1: 0), recommended (T_2: 33.0 kg ha^{-1}) and double of the recommended dose (T_3: 66.0 kg ha^{-1}) were applied once at the time of flowering. From each treated plot leaf samples were collected on 1, 7, 14, 21, 28 and 35 DAA of the pesticide. The samples were analyzed to quantify carbohydrate, total free amino acids, protein, total phenol and total chlorophyll using fresh leaves as soon as possible.

Physico-chemical analyses of soil

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 following methods were employed for physico-chemical analyses of the soil: soil pH by potentiometric method (Baruah and Barthakur, 1997) using pH meter; 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).

Biochemical analyses

Carbohydrate estimation

Carbohydrate was estimated by anthrone method (Sadasivam and Manickam, 2008). Briefly, 100 mg of leaf sample was hydrolysed with 5mL of 2.5 N HCl for 3 h and neutralized with sodium carbonate. It was then filtered and volume made up to 25 mL. From the filtrate 0.25 mL aliquot was taken and volume made up to 1 mL with distilled water. After adding 4 mL of anthrone reagent it was thoroughly mixed, heated for 8 min on water bath and cooled rapidly. The green-dark 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

Total free amino acids content was estimated as per the method of Misra *et al*. (1975). Briefly, 500 mg leaf sample was extracted twice with 5 mL of ethanol-water (4:1 v/v) followed by centrifugation at 10,000 rpm for 15 min. From the pooled supernatant, 0.1 mL was taken and 1 mL ninhydrin solution was added. After making up the volume to 2 mL with distilled water it was heated on a water bath for 20 min and then 5 mL of diluent solvent (water and n-propanol, 1:1 v/v) was added and mixed. After 15 minutes the intensity of the purple colour was read spectrophotometrically against reagent blank at 570 nm. 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 500 mg leaf sample with 10 mL sodium phosphate buffer (0.1 M, pH 7.0). The homogenate was centrifuged at 10,000 rpm for 20 min and supernatant was used for the assay. After addition of the recommended reagents the absorbance of blue colour was measured at 660 nm in a spectrophotometer against reagent blank and protein content was calculated by the standard graph of bovine serum albumin (BSA) and expressed as mg g^{-1} fresh weight of tissue.

Total phenol estimation

Total phenol was assayed by Folin–Ciocalteau method (Gopi *et al*., 2009). Briefly, 500 mg of fresh tissue was homogenized twice with 10mL and 5mL of 80% ethanol and centrifuged at

Table 1: Effect of carbofuran on carbohydrate content* (mg g^{-1}) in brinjal leaf

Treatment	Days after application					
	1	7	14	21	28	35
Control (T_1)	3.57 ± 0.72	3.42 ± 0.29(–4.20)#	3.33 ± 0.54(–6.72)	3.40 ± 0.19(–4.76)	3.52 ± 0.19(–1.40)	3.45 ± 0.16(–3.36)
33.0 kg ha^{-1} (T_2)	3.53 ± 0.31	3.47 ± 0.20(–1.70)	3.38 ± 0.23(–4.25)	3.43 ± 0.33(–2.83)	3.68 ± 0.28(+4.25)	3.37 ± 0.28(–4.53)
66.0 kg ha^{-1} (T_3)	3.48 ±0.20	3.35 ± 0.16(–3.74)	3.28 ± 0.12(–5.75)	3.35 ± 0.18(–3.74)	3.61 ± 0.23(+3.74)	3.32 ± 0.55(–4.60)
SEm(±)	0.186	0.095	0.140	0.092	0.067	0.154
CD at 5%	0.573	0.291	0.431	0.284	0.205	0.475

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

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

Treatment	Days after application					
	1	7	14	21	28	35
Control(T_1)	12.68 ± 0.49	8.25 ± 0.40(–34.94)#	11.12 ± 0.88(–12.30)	6.51 ± 0.33(–48.66)	6.53 ± 0.08(–48.50)	7.25 ± 0.18(–42.82)
33.0 kg ha^{-1} (T_2)	14.53 ± 0.98	8.84 ± 0.75(–39.16)	12.54 ± 0.44(–13.70)	8.57 ± 0.21(–41.02)	7.25 ± 0.36(–50.10)	7.87 ± 0.21(–45.84)
66.0 kg ha^{-1} (T_3)	13.85 ± 0.85	7.00 ± 0.45(–49.46)	11.82 ± 0.58(–14.66)	7.51 ± 0.57(–45.78)	6.25 ± 0.26(–54.87)	6.94 ± 0.40(–49.89)
SEm(±)	0.258	0.193	0.262	0.201	0.214	0.168
CD at 5%	0.794	0.594	0.808	0.620	0.659	0.516

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

10,000 rpm for 20 min. The supernatants were pooled and ethanol was evaporated to dryness. The residue was dissolved in 5mL distilled water and from it 0.2mL was taken and diluted to 3mL with distilled water. To it 0.5mL of Folin–Ciocalteau reagent was added and kept for 3 min. Next, 2mL of 20% sodium carbonate solution 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 determined 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

Total chlorophyll was estimated from the leaves by the method of Witham *et al.* (1971). Briefly, 1 g of finely cut fresh leaf was crushed in pre-chilled mortar and pestle with 20 mL 80% chilled acetone and centrifuged at 5,000 rpm for 5 min and the supernatant was collected. The process was repeated until the residue was colourless. Volume was made up to 100 mL with 80% chilled acetone. The absorbance of the solution was noted at 645 nm and 663 nm in a spectrophotometer against the 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 data obtained were subjected to statistical analyses by the ANOVA method (Gomez and Gomez, 1983). The computation and statistical analyses were done in Microsoft Excel 2007 and SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Physico-chemical analyses of soil

The physico-chemical properties of soil of the experimental field as analysed prior to start of the experiments showed that the soil was sandy loam in texture and acidic in nature with pH 5.62. The organic carbon as estimated was 0.87%, available nitrogen 163.71 kg ha^{-1}, available phosphorus 25.38 kg ha^{-1} and available potassium 112.35 kg ha^{-1}.

Carbohydrate content

As evidenced from the results (Table 1), the carbohydrate content in brinjal leaves due to recommended dose of applied carbofuran was non-significantly higher and the same at double of recommended dose was non-significantly lower than that of control dose. With passage of time the carbohydrate content initially decreased upto 14th DAA and then increased till 28th DAA followed by a decrease on 35th DAA in all three treatments. These results were in agreement with those of Bhattacharya *et al.* (2001) who observed decrease in carbohydrate content in rice due to butachlor and carbendazim and Ahmed *et al.* (2003) who recorded increase as well as decrease in carbohydrate level measured at 96h and one week after application of carbofuran, cypermethrin and Regent on maize.

Total free amino acid content

The results of total free amino acid content of brinjal leaves (Table 2) indicated a continuous decrease due to all three

Table 3: Effect of carbofuran on protein content* (mg g^{-1}) in brinjal leaf

Treatment	Days after application					
	1	7	14	21	28	35
Control (T_1)	0.53 ± 0.02	0.37 ± 0.02(–30.19)#	0.38 ±0.03(–28.30)	0.41 ±0.04(–22.64)	0.41 ±0.04(–22.64)	0.29 ±0.05(–45.28)
33.0 kg ha^{-1} (T_2)	0.58 ± 0.03	0.45 ± 0.02(–22.41)	0.42 ±0.03(–27.59)	0.56 ±0.08(–3.45)	0.54 ±0.04(–6.90)	0.33±0.03(–43.10)
66.0 kg ha^{-1} (T_3)	0.57 ± 0.03	0.42 ±0.02(–26.32)	0.40 ±0.02(–29.83)	0.49 ±0.03(–14.04)	0.49 ±0.03(–14.04)	0.31 ±0.02(–45.61)
SEm(±)	0.010	0.008	0.009	0.016	0.015	0.011
CD at 5%	0.031	0.024	0.027	0.049	0.045	0.032

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

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

Treatment	Days after application					
	1	7	14	21	28	35
Control(T_1)	9.55±0.63	10.25±0.23(+7.33)#	11.29 ± 0.74(+18.22)	11.93±0.75(+24.92)	9.83±0.62 (+2.93)	11.35±0.62(+18.85)
33.0 kg ha^{-1} (T_2)	9.92±0.56	10.89± 0.55(+9.78)	11.73 ± 0.67(+18.25)	12.96±0.89(+30.65)	10.23±0.89(+3.13)	12.08±0.62(+21.77)
66.0 kg ha^{-1} (T_3)	10.46±0.49	12.00±0.50(+14.63)	12.21 ± 0.52(+16.73)	11.35±0.55(+8.51)	9.25 ±0.58(-11.57)	11.02±0.63(+5.35)
SEm(±)	0.166	0.130	0.177	0.312	0.221	0.260
CD at 5%	0.510	0.399	0.547	0.960	0.682	0.800

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

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

Treatment	Days after application					
	1	7	14	21	28	35
Control(T_1)	0.81 ± 0.01	0.84 ± 0.02(+3.70)#	0.69 ± 0.03(–14.82)	0.80 ± 0.01(–1.24)	0.89 ± 0.02(+9.88)	0.64 ± 0.03(–20.99)
33.0 kg ha^{-1} (T_2)	0.88 ± 0.02	0.90 ± 0.02(+2.27)	0.72 ± 0.03(–18.18)	0.84 ± 0.04(–4.55)	0.98 ± 0.03(+11.36)	0.68 ± 0.03(–22.73)
66.0 kg ha^{-1} (T_3)	0.86 ± 0.02	0.87 ± 0.02(+1.16)	0.65 ± 0.02(–24.42)	0.78 ± 0.04(–9.30)	0.94 ± 0.06(+9.30)	0.55 ± 0.02(–36.05)
SEm(±)	0.016	0.014	0.010	0.021	0.029	0.029
CD at 5%	0.050	0.043	0.030	0.063	0.090	0.089

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

treatment doses of carbofuran except an increase on 14th DAA maintaining T_2 and T_3 doses significantly higher than control on most dates of analysis.

Earlier, Ahmed *et al*. (2003) observed decrease in total amino acid content of maize after one week due to first spray of different pesticides, and El-Daly (2008) recorded amino acid level of radish twelve days after each application of cyanophos and observed higher value at single dose than control and double doses. In the present study also total free amino acid content due to recommended dose was higher than control and double of recommended dose, thereby maintaining analogy with the earlier works. Furthermore, here the data were recorded till 35th DAA which showed gradual decrease with time in comparison with the first day of observation.

Protein content

The protein content as measured (Table 3) showed that due to both T_2 and T_3 doses it was significantly higher than that of control (T_1) on most of the sampling days. The protein level against all three treatments went on decreasing throughout the period of study keeping that of T_2 above T_3 and T_1. On 21st and 28th DAA an increase was observed in T_2 and T_3, still below that of 1st DAA.

In some earlier studies Siddiqui and Ahmed (2006) observed decrease in protein content of soybean with increase in concentration of five pesticides, while Ahmed *et al*. (2003) observed both increase and decrease in total soluble protein of maize measured up to one week after application of carbofuran. The present study showed a decrease in protein content with time, possibly due to inhibition of protein synthesis as suggested by Siddiqui and Ahmed (2006) and thus showed analogy with their works.

Total phenol content

The total phenol content of brinjal leaf as estimated (Table 4) showed that the level increased differently in the three applied doses. It increased till 21st DAA in case of all three doses maintaining its level a little higher than control due to T_2 and T_3 doses. After 21st DAA the phenol content declined and at the end it showed higher than that of first day of analysis.

High doses of pesticides treated on crops initiate a kind of abiotic or chemical stress and triggers formation of phenolic compounds (Siddiqui and Ahmed, 2006). Earlier, Siddiqui *et al*. (2001) observed elevation of root and shoot phenols in wheat by methyl thiophenate; Siddiqui and Arif-uz-Zaman (2004) recorded substantial increase of phenolic contents in root and shoot of maize and Gohar; and also Jaleel *et al*. (2008) found enhanced accumulation of total phenols in tubers of white yam. In the present study also the trend remained similar except the period of 21st to 28th DAA. So the results of the present study are found to be at par with the earlier findings.

Total chlorophyll content

The results of total chlorophyll content of brinjal leaf due to application of carbofuran are given in Table 5. A combination of increasing as well as decreasing trend in total chlorophyll content was observed during the course of study. Finally a percent decrease of 20.99, 22.73 and 36.05 respectively were recorded for the T_1, T_2 and T_3 doses with respect to the first DAA. The total chlorophyll content due to T_3 over T_1 was significant on majority of dates of analysis.

In some earlier studies, reduction in the chlorophyll content was observed by flumioxazin on grapevine (Saladin *et al*., 2003) and butachlor on rice (Bhattacharya *et al*., 2001). Here, an overall decrease in chlorophyll content was observed through the course of investigation which was in consonance with the earlier findings.

This study evaluated the quantitative formation of carbohydrate, total free amino acids, protein, total phenol and total chlorophyll content in brinjal leaves as measured on different days after application of carbofuran. The carbohydrate content, total free amino acid content and protein content in the brinjal leaves were found to decrease with passage of time. The level of total phenol increased with increasing treatment doses with respect to control. The total chlorophyll content was found to decrease at the end of study period with fluctuations in between. So the formation of the biomolecules under study in brinjal leaf as affected by carbofuran varied differently during the course of investigation.

ACKNOWLEDGEMENT

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

REFERENCES

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. *Intern. J. Agric. Biol.* **5(1):** 30-35.

Baruah, T. C. and Barthakur, H. P. 1997. A Textbook of Soil Analysis. *Vikas Publishing House Pvt. Ltd.,* New Delhi. p. 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 Res.* **21(1):** 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. *Res. J. Agric. Biol. Sci.* **4(3):** 210-218.

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. Agric. Food Chem.* **49(1):** 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*). *Acad. J. Pl. Sci.* **2(2):** 97-101.

Goswami, M. R., Banerjee, P., Swarnakar, S. and Mukhopadhyay, A. 2013. Carbaryl mediated biochemical alterations in Eggplant (*Solanum melongena* L.). *Intern. J. Res. Environ. Sci. Technol.* **3(2):** 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 Sci.* **26(4):** 298-307.

Lowry, O. H., Rosbrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193(1):** 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 Chem.* **52(6):** 844-848.

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

Saladin, G., Magne, C. and Clement, C. 2003. Impact of flumioxazin herbicide on growth and carbohydrate physiology in grapevine (*Vitis vinifera* L.). *Plant Cell Repor.* **21(8):** 821-827.

Sammaiah, D., Shekar, C. C., Prasad, V. R. and Reddy, K. J. 2011. Pesticide induced alterations in physiological responses in brinjal (*Solanum melongena* L.) *Intern. J. Pharma Bio Sci.* **2(1):** 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.). *Turk. J. Bot.* **26(3):** 127-130.

Siddiqui, Z. S. and Ahmed, S. 2006. Combined effects of pesticide on growth and nutritive composition of soybean plants (*Glycine max* L.). *Pak. J. Bot.* **38(3):** 721-733.

Siddiqui, Z. S. and Arif-uz-Zaman. 2004. Effects of benlate systemic fungicide on seed germination, seedling growth, biomass and phenolic contents in two cultivars of maize (*Zea mays* L.). *Pak. J. Bot.* **36(3):** 577-582.

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.) *Pak. J. Biol. Sci.* **4(10):** 1198-1200.

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 Sci.* **37(1):** 29-38.

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

APPLICATION FORM

NATIONAL ENVIRONMENTALISTS ASSOCIATION (N.E.A.)

To,
The Secretary,
National Environmentalists Association,
D-13, H.H.Colony,
Ranchi - 834 002, Jharkhand, India

Sir,
I wish to become an Annual / Life member and Fellow* of the association and will abide by the rules and regulations of the association

Name _____

Mailing Address _____

_____

Official Address _____

_____

E-mail _____Ph. No._____(R)_____(O)

Date of Birth _____ Mobile No. _____

Qualification _____

Field of specialization & research _____

Extension work (if done) _____

_____

Please find enclosed a D/D of Rs..... No. ..... Dated ..... as an *Annual / Life membership fee*.

*Attach **Bio-data and some recent publications along with the application form when applying for the Fellowship of the association.**

Correspondance for membership and/ or Fellowship should be done on the following address :

SECRETARY,
National Environmentalists Association,
D-13, H.H.Colony,
Ranchi - 834002
Jharkhand, India
E-mails : m_psinha@yahoo.com Cell : 9431360645
dr.mp.sinha@gmail.com Ph. : 0651-2244071