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Effect of Imidacloprid on Accumulation of Some Biomolecules in Cabbage (*Brassica oleracea* var *capitata* L.) Leaf

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ABSTRACT

Synthetic pesticides, in addition to controlling pests, affect the quantitative formation of different biomolecules in crop plants and thereby alter their growth and yield. It was, therefore, thought worthwhile to investigate the accumulation of carbohydrates, total free amino acids, total protein, total phenol and total chlorophyll contents in cabbage leaf on 1, 7, 14, 21, 28 and 35 days after application of imidacloprid. The results revealed that carbohydrate content showed non-significant fluctuations. The total free amino acid content in cabbage leaves increased with passage of time excepting some decrease in between, whereas the protein content decreased gradually. The total phenol content also exhibited an increasing trend with time. The total chlorophyll content of cabbage on different days was inconsistent although significant increase in case of recommended dose of imidacloprid over control was observed on most of the days of study. The results suggested that the formation of different biomolecules in cabbage due to imidacloprid varied differently during the course of study.

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INTRODUCTION

Cabbage (*Brassica oleracea* L. var *capitata*) is one of the most popular green cruciferous vegetables cultivated and consumed in many countries of the world including India. It is rich in carbohydrate, protein, minerals and vitamins like A, B_1, B_2 and C. One of the major constraints that happens during its cultivation is the infestation of insect pests like cut worm, *Spodoptera litura* (F.), diamond-back moth, *Plutella xylostella* (L.), cabbage butterfly, *Pieris brassicae* (L.), cabbage aphids, *Brevicoryne brassicae* L. and *Lipaphis erysimi*, cabbage leaf webber, *Crocidolomia bionotalis* (Zell), head borer, *Hellula undalis* (F.), painted bug, *Bagrada cruciferarum* (Kirk.) and flea beetle, *Phyllotreta cruciferae* (Goeze) (Kumar *et al.* 2021).

Synthetic pesticides used to control various insect pests, diseases or weeds additionally affect the quantitative formation of different biomolecules. It was observed earlier that the herbicide butachlor caused significant reduction in carbohydrate content in the leaves of two varieties of rice during panicle emergence and in the grains at maturity, and the fungicide carbendazim resulted a decreasing trend and the insecticide carbofuran, an increasing trend of the same (Bhattacharya *et al.* 2001). The contents of sugar, soluble amino acid and soluble protein after

application of chlorpyrifos were measured on 5, 10 and 20 days after application (DAA) which showed lower concentration of chlorpyrifos exerted activity as stimulant and higher concentrations (0.6 and 1.5 mM) caused decrease in soluble sugar and protein content and increase in soluble amino acid (Parween *et al.* 2011) whereas insecticide endosulfan applied individually and in combination with fungicide kitazin on brinjal reduced protein content with increasing dose (Sammaiah *et al.* 2011). The carbamate insecticide carbaryl applied at different concentrations on brinjal reduced its soluble sugar and free amino acid content and significantly increased total protein and insoluble sugar content (Goswami *et al.* 2013). In two earlier studies by the present authors, 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); and endosulfan applied on cabbage leaf decreased the carbohydrate and protein content and caused increase in total free amino acid, total phenol and total chlorophyll content (Ashrafi and Pandit 2015).

In most of these works quantification of biochemical parameters were studied only once or twice or thrice after application of the pesticides. So exact trend in the formation of different biomolecules during a course of time could not be ascertained in the above studies. Considering this limitation, the present experiment was carried out to study the effect of imidacloprid (N-[1-[(6-chloro-3-pyridyl) methyl]-4,5-dihydro-imidazol-2-yl] nitramide), a systemic neonicotinoid class of insecticide widely used on cereals including rice and maize, on potato and other vegetables, fruits, cotton and also for seed treatment, on the accumulation of total of carbohydrate, free amino acids, protein, phenol and chlorophyll contents of cabbage head at different time intervals after its application.

MATERIALS AND METHODS

Chemicals and instruments

The chemicals used in the present experiment were of Sigma Aldrich Inc, USA, Merck Specialities Pvt Ltd, Mumbai, India and SRL Pvt Ltd, Mumbai, India. Water used was double distilled. Of the instruments, centrifuge used was R–8C laboratory centrifuge of Remi Motors Ltd, Mumbai, India; the ultracentrifuge was Sorvall RC–90 of Thermo Fisher Scientific, USA and UV–VIS spectrophotometer was Lambda 25 of Perkin Elmer (India) Pvt Ltd, Mumbai. Flame photometer (Model no. 128) of Systronics, Ahmedabad (India), Macro–Kjeldahl (Model no. Distyl–EM) of Pelican Equipments, Chennai (India) and digital pH meter 335 of Systronics (India) Ltd were used for soil analysis.

Physico-chemical analyses of soil

The composite soil samples were collected from 0–15 cm depth of the experimental field with the help of a soil auger prior to application of fertilizers and were dried under shade, pulverized and sieved through 0.2 mm sieve for physico–chemical analyses. Soil pH was determined by potentiometric method (Baruah and Barthakur 1997) using pH meter. Organic carbon was estimated by rapid titration method (Walkley and Black 1934). The modified macro–Kjeldahl method was employed to determine the available nitrogen in the soil (Jackson 1967). Available phosphorus and available potassium content were determined by Bray's No. I method (Jackson 1967) and flame photometer method (Jackson 1967) respectively.

Cultivation of crop and sampling

The present experiment was carried out at the Instructional Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal (India) following Randomized Block Design (RBD) with 3 treatments and 3 replications for each treatment. Three weeks old cabbage seedlings (variety Golden Acre) were transplanted in the plots of size 3 m × 3 m keeping a spacing of 45 cm × 60 cm. Application of fertilizers were done as per recommended dose of 150 : 100 : 100 kg ha^{-1} of $N : P_2O_5 : K_2O$ following standard practices. Three treatment doses of imidacloprid (trade name A-One 17.8% SL) at the rates of control (T_1: 0), recommended (T_2: 0.075 L ha^{-1}) and double of the recommended dose (T_3 : 0.150 L ha^{-1}) were applied once at the time of head formation. Leaf samples were collected from each plot on 1, 7, 14, 21,

Table 1. Effect of imidacloprid on carbohydrate content* (mg g^{-1}) in cabbage. * Mean ± SD of 7 replicates. # Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

Treatment	Days after application (DAA) 1	7	14	21	28	35
Control (T_1)	4.67 ± 0.75	4.57 ± 0.49 (–2.14)#	4.65 ± 0.56 (–0.43)	4.52 ± 0.52 (–3.21)	4.62 ± 0.58 (–1.07)	4.66 ± 0.68 (–0.21)
0.075 L ha^{-1} (T_2)	4.68 ± 0.57	4.58 ± 0.56 (–2.14)	4.68 ± 0.65 (0.00)	4.56 ± 0.59 (–2.56)	4.65 ± 0.57 (–0.64)	4.67 ± 0.55 (–0.21)
0.150 L ha^{-1} (T_3)	4.69 ± 0.67	4.60 ± 0.52 (–1.92)	4.70 ± 0.66 (+0.21)	4.54 ± 0.49 (–3.20)	4.61 ± 0.49 (–1.71)	4.65 ± 0.64 (–0.85)
SEm(±)	0.274	0.201	0.202	0.267	0.246	0.241
CD at 5%	0.846	0.619	0.623	0.822	0.758	0.743

28 and 35 DAA of the insecticide. All the samples were analyzed to quantify total of carbohydrate, free amino acids, protein, phenol and chlorophyll using fresh leaves immediately after collection.

Carbohydrate estimation

Carbohydrate was quantified by anthrone method as described by Sadasivam and Manickam (2008). Briefly, 100 mg of finely cut leaf sample was hydrolyzed with 5 mL of 2.5 N HCl on a water bath for 3 h and cooled to room temperature and neutralized with solid sodium carbonate. It was then filtered through Whatman No. 1 filter paper 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 on vortex, heated for 8 min on water bath and cooled rapidly. The intensity of greenish color developed was measured by UV–VIS 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.

Free amino acids estimation

Total free amino acids content was estimated following the method of Misra *et al.* (1975). Briefly, 500 mg of the leaf sample was ground into fine mesh in a mortar and pestle and extracted two times with 5 mL of ethanol-water (4:1 v/v) followed by centrifugation at 10,000 rpm for 15 min. From the pooled supernatant (25 mL) 0.1 mL was taken in a boiling tube 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 (mixture of water and n-propanol, 1:1 v/v) was added and mixed on vortex. After 15 minutes the intensity of the purple color was read against a reagent blank in a UV–VIS spectrophotometer 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.

Table 2. Effect of imidacloprid on total free amino acid content* (mg g^{-1}) in cabbage. * Mean ± SD of 7 replicates. # Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

Treatment	Days after application (DAA) 1	7	14	21	28	35
Control (T_1)	6.15 ± 0.49	5.24 ± 0.95 (–14.80)#	9.25 ± 1.65 (+50.41)	5.56 ± 0.39 (–9.59)	15.68 ± 1.75 (+154.96)	12.45 ± 0.99 (+102.44)
0.075 L ha^{-1} (T_2)	9.95 ± 1.11	6.21 ± 0.87 (–37.59)	10.12 ± 1.02 (+1.71)	7.25 ± 0.27 (–27.14)	17.65 ± 0.87 (+77.39)	15.21 ± 0.59 (+52.86)
0.150 L ha^{-1} (T_3)	7.28 ± 1.10	5.15 ± 0.94 (–29.26)	10.78 ± 0.90 (+48.08)	6.50 ± 0.77 (–10.71)	16.79 ± 0.67 (+130.63)	13.54 ± 0.89 (+85.99)
SEm(±)	0.4101	0.208	0.317	0.272	0.278	0.133
CD at 5%	1.2637	0.642	0.976	0.839	0.857	0.409

Table 3. Effect of imidacloprid on protein content* (mg g^{-1}) in cabbage. * Mean ± SD of 7 replicates. # Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

Treatment	Days after application (DAA)					
	1	7	14	21	28	35
Control (T_1)	0.60 ± 0.04	0.43 ± 0.03 (–28.33)#	0.41 ± 0.06 (–31.67)	0.29 ± 0.05 (–51.67)	0.19 ± 0.04 (–68.33)	0.13 ± 0.03 (–78.33)
0.075 L ha^{-1} (T_2)	0.73 ± 0.03	0.43 ± 0.05 (–41.10)	0.43 ± 0.06 (–41.10)	0.35 ± 0.04 (–52.06)	0.21 ± 0.03 (–71.23)	0.13 ± 0.03 (–82.19)
0.150 L ha^{-1} (T_3)	0.52 ± 0.03	0.41 ± 0.06 (–21.15)	0.38 ± 0.03 (–26.92)	0.33 ± 0.06 (–36.54)	0.23 ± 0.03 (–55.77)	0.20 ± 0.08 (–61.54)
SEm(±)	0.011	0.020	0.020	0.015	0.009	0.015
CD at 5%	0.032	0.062	0.061	0.047	0.028	0.046

Protein estimation

Protein was extracted from 500 mg leaf sample with 10 mL sodium phosphate buffer (0.1 M, pH 7.0) following the method of Lowry *et al.* (1951). The homogenate was centrifuged for 20 min at 10,000 rpm and the supernatant was employed for the analysis. After addition of the specified reagents the absorbance of the blue color was measured in a UV–VIS spectrophotometer at 660 nm against a reagent blank, and amount of protein was calculated by the standard graph prepared using bovine serum albumin (BSA) and expressed as mg g^{-1} fresh weight of tissue.

Total phenol estimation

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

Total chlorophyll estimation

Total chlorophyll in the leaves of cabbage was estimated as per the method of Witham *et al.* (1971). Briefly, 1 g of fresh leaf was crushed with 20 mL 80% chilled acetone in a pre–chilled mortar and pestle and centrifuged for 5 min at 5,000 rpm and the supernatant was collected. The process was repeated until the residue was colorless. Volume was made up to 100 mL with 80% chilled acetone. The absorbance of the solution was recorded at 645 nm and 663 nm in a UV–VIS 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 analyzed prior to start of the experiments revealed that the soil was sandy loam in texture and acidic in nature having a pH of 5.62. The organic carbon as estimated was 0.87%, available nitrogen

Table 4. Effect of imidacloprid on total phenol content* (mg g^{-1}) in cabbage. * Mean ± SD of 7 replicates. # Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

Treatment	Days after application (DAA)					
	1	7	14	21	28	35
Control (T_1)	9.25 ± 0.44	11.05 ± 0.52 (+19.46)#	12.27 ± 1.16 (+32.65)	10.74 ± 0.84 (+16.11)	11.66 ± 0.65 (+26.05)	12.60 ± 0.96 (+36.22)
0.075 L ha^{-1} (T_2)	9.39 ± 1.01	11.27 ± 0.51 (+20.02)	13.37 ± 2.21 (+42.39)	12.38 ± 0.79 (+31.84)	12.57 ± 0.79 (+33.87)	13.26 ± 0.70 (+41.21)
0.150 L ha^{-1} (T_3)	9.44 ± 0.48	12.20 ± 0.93 (+29.24)	13.93 ± 1.42 (+47.56)	12.90 ± 0.96 (+36.65)	13.98 ± 0.66 (+48.09)	14.65 ± 0.73 (+55.19)
SEm(±)	0.239	0.290	1.232	0.221	0.237	0.300
CD at 5%	0.735	0.894	3.796	0.680	0.731	0.923

163.71 kg ha^{-1}, available phosphorus 25.38 kg ha^{-1} and available potassium 112.35 kg ha^{-1}.

Carbohydrate content

The results of carbohydrate content of cabbage leaf on different days after application of imidacloprid were presented in Table 1. The carbohydrate content due to all three treatments went on decline on 7th DAA and then increased closely to the extent of 1st DAA on 14th DAA. Again it declined on 21st DAA showing a decrease of 3.21, 2.56 and 3.20% in T_1, T_2 and T_3 doses respectively. After that the level increased and attained a value close to that of 1st DAA on the last day of study.

Earlier, the sugar content in the leaves of *Vigna radiata* was found to decrease in a dose dependent manner in comparison with control after chlorpyrifos treatment at 5, 10 and 20 DAA (Parween *et al.* 2011). In a previous study by the present authors also a decrease in the formation of carbohydrate was noticed in cabbage after application of endosulfan (Ashrafi and Pandit 2015). The results of the present study also exhibited non-significant fluctuations in the amount of carbohydrate measured at different time intervals after application of imidacloprid. So the results of the present investigation are in agreement with some of the earlier studies.

Total free amino acid content

The results (Table 2) revealed that the total free amino acid content of cabbage leaf after application of imidacloprid increased gradually with passage of time with the exception of decrease on 7th and 21st DAA in case of all three treatments. The values of both T_2 and T_3 doses were significantly higher than T_1 on most of the days of analysis.

A significant increase in soluble free amino acid content in the leaves of *Vigna radiata* with a maximum of 30.76% due to chlorpyrifos treatment (Parween *et al.* 2011); increase as well as decrease in different amino acids due to different pesticides in maize (Ahmed *et al.* 2003) were found in some earlier studies. As revealed from these studies, different pesticides affect differently on the amino acid content of various crops. In the present investigation the total free amino acid content increased with time showing a similar trend with some of the earlier studies in all three treatments excepting some decreases as shown in Table 2, thereby indicating accumulation of free amino acids due to imidacloprid in cabbage.

Protein content

As evidenced from Table 3, the protein content of cabbage on different days after application of imidacloprid declined in all three doses T_1, T_2 and T_3 during the entire course of study showing a decrease of 78.33, 82.19 and 61.54% respectively with respect to 1st DAA. Significant difference in values were observed between T_1 and T_2 on 1st, 21st and 28th DAA and that between T_1 and T_3 on 1st, 21st, 28th and 35th DAA.

In their works Parween *et al.* (2011) recorded significant increase in total soluble protein content in the leaves of *Vigna radiata* at 10 DAA at low dosage of chlorpyrifos, while at higher dose it decreased on

Table 5. Effect of imidacloprid on total chlorophyll content* (mg g^{-1}) in cabbage. * Mean ± SD of 7 replicates. # Figures in parentheses indicate percent increase (+) / decrease (–) with respect to 1st DAA.

Treatment	Days after application (DAA)					
	1	7	14	21	28	35
Control (T_1)	0.80 ± 0.04	0.78 ± 0.01 (–2.50)#	0.98 ± 0.05 (+22.50)	0.75 ± 0.03 (–6.25)	0.78 ± 0.05 (–2.50)	1.42 ± 0.05 (+77.50)
0.075 L ha^{-1} (T_2)	0.88 ± 0.04	0.86 ± 0.02 (–2.27)	1.05 ± 0.05 (+19.32)	0.83 ± 0.04 (–5.68)	0.84 ± 0.05 (–4.55)	1.28 ± 0.03 (+45.45)
0.150 L ha^{-1} (T_3)	0.84 ± 0.02	0.81 ± 0.03 (–3.57)	1.02 ± 0.02 (+21.43)	0.80 ± 0.03 (–4.76)	0.81 ± 0.03 (–3.57)	1.21 ± 0.05 (+44.05)
SEm(±)	0.023	0.014	0.026	0.018	0.020	0.021
CD at 5%	0.070	0.043	0.081	0.054	0.061	0.064

5, 10 and 20 DAA and Ashrafi and Pandit (2015) found decrease in protein content of cabbage leaf when endosulfan was applied. Here, the results of the present study also showed very much similarity with the earlier findings. Therefore, all the results including those of the previous studies indicate that pesticides have a lowering effect in the protein yield of plants.

Total phenol content

From the results (Table 4) it was observed that the level of total phenol in cabbage leaves increased in both the treatment doses (T_2 and T_3) with respect to control (T_1) after application of imidacloprid. Significant increase in case of T_3 over T_1 was observed on most days except 1st and 14th DAA.

In most of the earlier studies (Kaur *et al.* 2011; Ashrafi and Pandit 2014) the phenol level increased with application of pesticides. The present investigation also showed the trend to be similar all throughout the period of investigation excepting 1st DAA. The percent increase in comparison with the 1st DAA was highest in case of T_3 treatment dose (55.19%) and lowest (36.22%) in control dose. So the results of the present experiment are in well agreement with the earlier studies.

Total chlorophyll content

The results of total chlorophyll content of cabbage leaf on different days after application of imidacloprid (Table 5) showed that after a little decrease on 7th DAA it increased on 14th DAA in case of all three treatment doses. Again it declined on 21st DAA followed by a sharp increase on 35th DAA. Significant increase in case of treatment dose T_2 over T_1 was observed on most days except 14th DAA.

In some earlier studies increase as well as decrease in chlorophyll content of different crops was observed. Chagas *et al.* (2008) observed significant reduction in chlorophyll content on application of paraquat after 48 h exposure at concentrations higher than 2 mM whereas Ashrafi and Pandit (2015) recorded increase in chlorophyll content in cabbage leaf due to endosulfan. In the present study increase in total chlorophyll content to the extent of 22.50, 19.32 and 21.43% on 14th DAA and 77.50, 45.45 and 44.05% on 35th DAA were recorded with respect to the initial amounts due to T_1, T_2 and T_3 doses respectively. But there was a decreasing trend in between. So the total chlorophyll content of cabbage on different days after application of imidacloprid was found to be inconsistent. However, normal dose maintained higher level of total chlorophyll than double and control doses all through the period of investigation.

CONCLUSION

This study evaluated the variations in carbohydrate, total free amino acids, protein, total phenol and total chlorophyll content of cabbage leaves on different days after application of imidacloprid. The results showed non-significant fluctuations in carbohydrate level measured at different time intervals. The total free amino acid content increased excepting some decrease in between, whereas the protein content decreased with passage of time. This might be due

to inhibition of protein synthesis as an effect of pesticides. The level of total phenol increased with increasing treatment doses with respect to control corroborating the well established fact that chemical stress of pesticides augment yield of phenols. The total chlorophyll content was found to be fluctuating and was similar to some earlier studies. So varying trends in the formation of different biomolecules was noticed in cabbage due to imidacloprid. Thus it may be concluded that some pesticides may alter the accumulation of different biomolecules and thereby may be responsible for altered growth and development and also the yield of horticultural crops.

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REFERENCES

Ahmed S, Anzum S, Naeem M, Ashraf MY (2003) Determination of efficacy of cypermethrin, Regent and carbofuran against *Chilo partellus* Swin. and biochemical changes following their application in maize plants. *Intl J Agric Biol* 5 (1) : 30—35.

Ashrafi MA, Pandit GK (2014) Effect of carbofuran on the formation of some biomolecules in brinjal (*Solanum melongena* L.) leaf. *The Bioscan* 9 (3) : 959—963.

Ashrafi MA, Pandit GK (2015) Variation in the formation of some biomolecules in cabbage (*Brassica oleracea* L var *capitata*) leaf induced by endosulfan. *The Bioscan* 10 (2) : 539—543.

Baruah TC, Barthakur HP (1997) A Textbook of Soil Analysis. Vikas Publishing House Pvt Ltd, New Delhi, pp 103—107.

Bhattacharya A, Pandit GK, Chakraborti P, 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.

Chagas RM, Silveira JAG, Ribeiro RV, Vitorello VA, Carrer H (2008) Photochemical damage and comparative performance of superoxide dismutase and ascorbate peroxidase in sugarcane leaves exposed to paraquat-induced oxidative stress. *Pestic Biochem Physiol* 90 (3) : 181—188.

Gomez KA, Gomez AA (1983) Statistical Procedures for Agricultural Research. John Wiley and Sons, NY.

Gopi R, Jaleel CA, Divyanair V, Azooz MM, 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 MR, Banerjee P, Swarnakar S, Mukhopadhyay A (2013) Carbaryl mediated biochemical alterations in eggplant (*Solanum melongena* L.). *Intl J Res Environ Sci Technol* 3 (1) : 51—57.

Jackson ML (1967) Soil Chemical Analysis. Prentice Hall of India Pvt Ltd, New Delhi, pp. 183— 347 and 387—408.

Kaur N, Sohal BS, Singh K (2011) Biochemical and physiological changes on *Bacillus thuringiensis* cotton after imidacloprid foliar spray. *Pestic Biochem Physiol* 99 (3) : 280—284.

Kumar A, Monobrullah M, Kishor DR, Kumar R (2021) Documentation of the major insect pest of cabbage and their associated natural enemies in Bihar, North-eastern India. *Curr J Appl Sci Tech* 40 (25): 58—66.

Lowry OH, Rosbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. *J Biol Chem* 193 : 265—275.

Misra PS, Mertz ET, Glover DV (1975) Studies on corn proteins. VIII. Free amino acid content of opaque-2 double mutants. *Cereal Chem* 52 : 844—848.

Parween T, Jan S, Mahmooduzzafar, Fatma T (2011) Alteration in nitrogen metabolism and plant growth during different developmental stages of green gram (*Vigna radiata* L.) in response to chlorpyrifos. *Acta Physiol Pl* 33 : 2321—2328.

Sadasivam S, Manickam A (2008) Biochemical Methods. New Age Intl. (P) Ltd., New Delhi, pp. 8-9, 107 : 108—109.

Sammaiah D, Shekar CC, Prasad VR, Reddy KJ (2011) Pesticide induced alterations in physiological responses in brinjal (*Solanum melongena* L.). *Int J Pharmac Biol Sci* 2 (1) : B374—383.

Walkley A, Black IA (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 FH, Blaydes DF, Devlin RM (1971) Experiments in Plant Physiology. Van Nostrand, NY, pp 245.