International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(1) January -2016

EFFECT OF IMIDACLOPRID ON THE ACTIVITIES OF SOME ENZYMES OF CABBAGE (BRASSICA OLERACEA L. VAR. CAPITATA) LEAF

Manzoor A. Ashrafi and Goutam K. Pandit

THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 1, pp. 8232-8235, January, 2016

International Journal of Recent Scientific Research

RESEARCH ARTICLE

EFFECT OF IMIDACLOPRID ON THE ACTIVITIES OF SOME ENZYMES OF CABBAGE (*BRASSICA OLERACEA* L. VAR. *CAPITATA*) LEAF

Manzoor A. Ashrafi and Goutam K. Pandit*

Department of Biochemistry, Uttar Banga Krishi Viswavidyalaya, Pundibari-736165, Cooch Behar, West Bengal, India

ARTICLE INFO

Article History:

Received 05th October, 2015
Received in revised form 08th November, 2015
Accepted 10th December, 2015
Published online 28st January, 2016

Key words:

cabbage, imidacloprid, enzyme activity, catalase, peroxidase, polyphenol oxidase

ABSTRACT

The variation in the activity of the three antioxidant enzymes catalase, peroxidase and polyphenol oxidase in cabbage (*Brassica oleracea* L. var. *capitata*) leaf induced by foliar application of imidacloprid was evaluated. Studies carried out upto 35 days after application (DAA) of the insecticide indicated that there was a continuous increase in the activity of catalase with increasing dose and also with elapse of time. In case of peroxidase the activity was found to remain higher at normal and double of normal doses over control throughout the duration of study and the difference with control reached maximum on 7th DAA. Polyphenol oxidase activity at double dose showed to remain higher than that of control and normal doses all through the period of investigation, the difference being maximum on 14th and 28th DAA.

INTRODUCTION

Cabbage *(Brassica oleracea* var. *capitata)*, one of the most popular and oldest cultivated winter vegetables in India, is rich in carbohydrate, protein, minerals and vitamins like A, B_1, B_2 and C. The cultivated types of cabbage differ in size, shape, texture and colour of head. It grows well under varied soil conditions and in a relatively cool moist climate. The crop is affected by a number of insect pests and fungal diseases and so demands application of different pesticides for its protection and also for getting desired yield.

While controlling the pest infestation, these pesticides enter into the plant system and affect the physiological and biochemical activities of the plants (Jood *et al*., 1995 and Kaur *et al*., 2011). Various studies have revealed that the quantitative formation of different biomolecules and activity of some enzymes of the crop plants are affected due to application of pesticides (Bhattacharya *et al*., 2001; Saladin *et al*., 2003 and Kaur *et al*., 2011). It was observed that profenofos while applied foliarly increased the activity of polyphenol oxidase and acid phosphatase along with adversely affecting peroxidase activity (Habiba *et al*., 1992). Ribulose bisphosphate carboxylase activity of *Solanum tuberosum* exhibited a higher value due to deltamethrin than control (Fidalgo *et al*., 1993). Trichloroacetate showed reduction in germination and biomass production of oat, chinese cabbage and lettuce significantly, whereas it increased activities of enzymes superoxide dimutase, catalase, peroxidase and glutathione reductase significantly at its lowest concentration (Radetski *et al*., 2000).

Imidacloprid (N-[1-[(6-chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl] nitramide), a systemic insecticide of neonicotinoids family, is widely used as soil and foliar applied one on cereals including rice and maize, potato, vegetables, fruits, cotton etc and also for seed treatment (Daraghmeh *et al*., 2007). In the present study the effect of imidacloprid on the activity of three enzymes namely catalase, peroxidase and polyphenol oxidase in cabbage leaf during a course of time after its application are being reported.

MATERIALS AND METHODS

The field study of the present experiment was conducted at the Instructional Farm and the laboratory study at Department of

Corresponding author:* **Goutam K. Pandit
Department of Biochemistry, Uttar Banga Krishi Viswavidyalaya, Pundibari-736165, Cooch Behar, West Bengal, India

Biochemistry of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal (India). The experimental design was randomized block design (RBD) with 3 treatments and 7 replications for each treatment. Imidacloprid was applied only once during the course of study.

Chemicals and instruments

The chemicals and reagents used in the study were of Sigma Aldrich Inc, USA; Merck Specialities Pvt. Ltd, Mumbai, India and SRL Pvt. Ltd, Mumbai, India. Water used was double distilled. The ultracentrifuge used was Sorvall RC–90 of ThermoFisher Scientific, USA and centrifuge was R–8C Laboratory Centrifuge of Remi Motors Ltd, Mumbai, India; the UV–VIS spectrophotometer was Lambda 25 of Perkin Elmer India Pvt. Ltd, Mumbai, India.

Field study: cultivation and pesticide application

Three weeks old cabbage (variety Golden Acre) seedlings were collected locally and transplanted in the prepared plots of size 3m x 3m at a spacing of 45 cm x 60 cm. Recommended dose of 150:100:100 kg ha^{-1} of $N:P_2O_5:K_2O$ fertilizers were applied in the form of urea, single super phosphate and muriate of potash respectively to each plot following standard practices. Three treatment doses of imidacloprid (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.

Sampling

Leaf samples were collected from each replicate plot of all the treatments on 1, 7, 14, 21, 28 and 35 DAA of the pesticide. All the samples were collected from the field and brought to the laboratory under cold condition (around 4°C) and analyzed to determine the activity of catalase, peroxidase and polyphenol oxidase using fresh leaves as soon as possible.

Laboratory study: enzyme assay

The enzyme catalase was assayed by the method of Sadasivam and Manickam (1996) with some modifications. Briefly, 0.2 g of fresh cabbage leaf was crushed with 2 mL of M/150 phosphate buffer (pH 7.0) and centrifuged at 4°C at 10,000 rpm for 30 minutes and the supernatant was used as enzyme source. In a cuvette 2.3 mL of above phosphate buffer, 0.2 mL of enzyme extract and 0.5 mL of 0.2 M H_2O_2 were taken. The rate of change of its absorbance was recorded at 240 nm at 30 seconds interval for 5 minutes in a UV–VIS spectrophotometer. The catalase activity was expressed as "ΔA_{240} min^{-1} g^{-1} fresh weight", where ΔA_{240} represents the change in absorbance at 240 nm.

The method of Sadasivam and Manickam (1996) was employed for extraction of the enzyme peroxidase. Briefly, 0.2 g of fresh cabbage leaves were crushed with 2 mL of 0.1M phosphate buffer (pH 7.0), centrifuged at 10,000 rpm at 4°C for 30 minutes and the supernatant was used as enzyme source. The activity of peroxidase was assayed by the method of Neog *et al*. (2004). The assay mixture containing 2.5 mL of 0.1 M phosphate buffer (pH 6.5), 0.2 mL of enzyme extract and 0.1 mL of *o*-dianisidine (1 mg mL^{-1} in methanol) was incubated at 28°C in a water bath for 2 minutes. The reaction was started by adding 0.2 mL of 0.2 M H_2O_2. Rate of change in absorbance was recorded at 430 nm at 30 seconds interval for 5 minutes in a UV–VIS spectrophotometer. The enzyme activity was expressed as "ΔA_{430} min^{-1} g^{-1} fresh weight", where ΔA_{430} represents the change in absorbance at 430 nm.

The assay of the enzyme polyphenol oxidase was done by the method of Mayer *et al*. (1965). Briefly, 0.2 g of leaf was finely cut and crushed with 2 mL phosphate buffer (0.1 M, pH 6.6). The homogenate was centrifuged at 10,000 rpm for 30 minutes and the supernatant was used as enzyme source. The enzyme activity was assayed using pyrogallol as substrate. The assay mixture was prepared by taking 100 µL enzyme extract, 2.4 mL of the above phosphate buffer and 0.5 mL 0.05 M pyrogallol and the decrease in the rate of absorbance was recorded at 495 nm at 30 seconds interval for 5 minutes in a UV–VIS spectrophotometer. The enzyme activity was expressed as "ΔA_{495} min^{-1} g^{-1} fresh weight", where ΔA_{495} represents the change in absorbance at 495 nm.

Statistical analyses

The data obtained from the laboratory experiments were subjected to statistical analyses by the ANOVA method and the computation and statistical analyses were done in Microsoft Excel-2007 and SPSS software version 17.

RESULTS AND DISCUSSION

Catalase activity

The results of catalase (ΔA_{240} min^{-1} g^{-1}) activity of cabbage leaf on different days after application of imidacloprid are presented in Table 1 and Figure 1 (A). On the 1st DAA the T_1, T_2 and T_3 treatments recorded catalase activity in terms of change in absorbance (ΔA_{240}) to be 0.32 ± 0.07, 0.38 ± 0.10 and 0.39 ± 0.07 min^{-1} g^{-1} respectively. The enzymatic activity increased on the 7th DAA to an extent of 34.38%, 36.84% and 41.03% for the treatment doses T_1, T_2 and T_3 respectively. It is noteworthy that the enzyme activity of the two treatments (T_2 and T_3) remained higher than the control (T_1) dose all through the period of study. Catalase activity of T_3 dose was significantly higher than T_1 during most part of the investigation. Maximum increase in activity was observed on 21st DAA to 75.00, 55.26 and 58.97 percent respectively for T_1, T_2 and T_3 doses.

Among the very few number of works done earlier it was observed that significant increase in activity of catalase took place in oat, chinese cabbage and lettuce at lowest concentration of trichloroacetate (TCA) applied (Radetski *et al*., 2000). An enhancement in the activity in bean and onion, inhibition in okra and non-consistency in guar by methyl parathion took place (Shirashyad *et al*., 2009).

Table 1 Effect of imidacloprid on catalase activity* (ΔA_{240} min^{-1} g^{-1}) in cabbage

Treatment	Days after application (DAA)					
	1	7	14	21	28	35
Control (T_1)	0.32 ± 0.07	0.43 ± 0.05 (+34.38)#	0.41 ± 0.10 (+28.13)	0.56 ± 0.04 (+75.00)	0.49 ± 0.06 (+53.13)	0.53 ± 0.21 (+65.63)
0.075 L ha^{-1} (T_2)	0.38 ± 0.10	0.52 ± 0.09 (+36.84)	0.43 ± 0.10 (+13.16)	0.59 ± 0.07 (+55.26)	0.51 ± 0.09 (+34.21)	0.59 ± 0.05 (+55.26)
0.150 L ha^{-1} (T_3)	0.39 ± 0.07	0.55 ± 0.10 (+41.03)	0.47 ± 0.11 (+20.51)	0.62 ± 0.05 (+58.97)	0.56 ± 0.07 (+43.59)	0.62 ± 0.05 (+58.97)
SEm(±)	0.016	0.019	0.015	0.015	0.023	0.047
CD at 5%	0.049	0.058	0.045	0.046	0.072	0.144

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

Table 2 Effect of imidacloprid on peroxidase activity* (ΔA_{430} min^{-1} g^{-1}) in cabbage

Treatment	Days after application (DAA)					
	1	7	14	21	28	35
Control (T_1)	12.66 ± 1.72	14.63 ± 1.29 (+15.56)#	12.85 ± 1.74 (+1.50)	14.77 ± 2.63 (+16.67)	16.42 ± 2.26 (+29.70)	15.73 ± 1.27 (+24.25)
0.075 L ha^{-1} (T_2)	13.24 ± 1.11	17.09 ± 1.07 (+29.08)	13.15 ± 2.84 (–0.68)	15.40 ± 2.93 (+16.31)	16.93 ± 2.57 (+27.87)	16.90 ± 2.91 (+27.64)
0.150 L ha^{-1} (T_3)	14.27 ± 2.37	17.97 ± 2.43 (+25.93)	14.45 ± 2.12 (+1.26)	15.87 ± 1.43 (+11.21)	17.54 ± 3.27 (+22.92)	18.59 ± 2.38 (+30.27)
SEm(±)	0.447	0.660	0.340	0.396	0.343	0.968
CD at 5%	1.377	2.034	1.047	1.221	1.057	2.982

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

Table 3 Effect of imidacloprid on polyphenol oxidase activity* (ΔA_{495} min^{-1} g^{-1}) in cabbage

Treatment	Days after application (DAA)					
	1	7	14	21	28	35
Control (T_1)	0.31 ± 0.06	0.44 ± 0.06 (+41.94)#	0.48 ± 0.08 (+54.84)	0.46 ± 0.07 (+48.39)	0.45 ± 0.11 (+45.16)	0.52 ± 0.11 (+67.74)
0.075 L ha^{-1} (T_2)	0.34 ± 0.07	0.46 ± 0.04 (+35.29)	0.55 ± 0.09 (+61.76)	0.48 ± 0.07 (+41.18)	0.51 ± 0.11 (+50.00)	0.54 ± 0.10 (+58.82)
0.150 L ha^{-1} (T_3)	0.37 ± 0.09	0.49 ± 0.08 (+32.43)	0.57 ± 0.07 (+54.05)	0.51 ± 0.08 (+37.84)	0.58 ± 0.07 (+56.76)	0.57 ± 0.11 (+54.05)
SEm(±)	0.009	0.019	0.020	0.023	0.024	0.037
CD at 5%	0.029	0.057	0.063	0.071	0.073	0.113

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

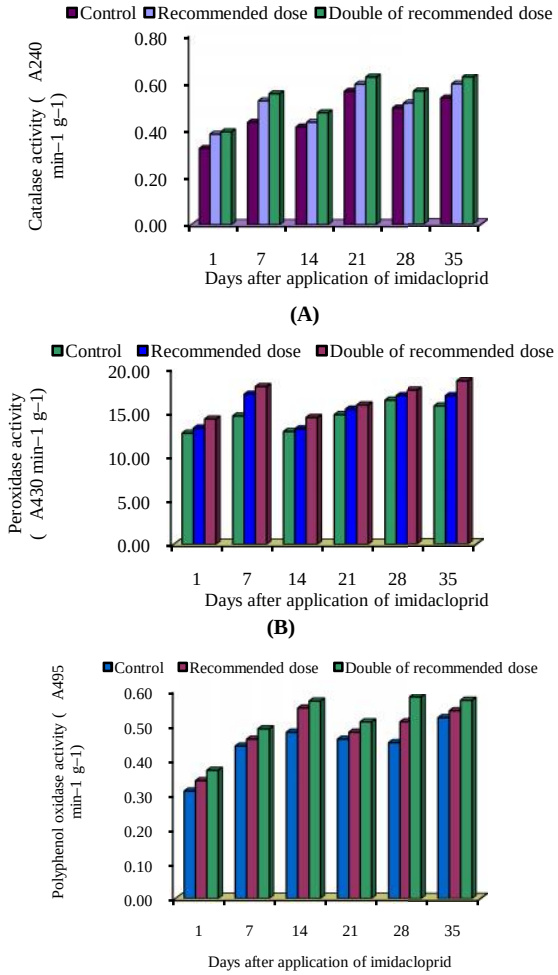

Figure 1 Change in activity of (A) catalase, (B) peroxidase and (C) polyphenol oxidase enzymes of cabbage due to imidacloprid

In the present investigation also increase in activity of catalase due to application of imidacloprid was observed all throughout the period of study. Double dose showed higher activity than normal as well as control. Therefore, it may be mentioned that the results of the present investigation followed similar trend with some of the earlier findings.

Peroxidase activity

The results of peroxidase (ΔA_{430} min^{-1} g^{-1}) activity of cabbage leaf on different days after application of imidacloprid are given in Table 2 and Figure 1 (B). On the 1st DAA, the T_1, T_2 and T_3 treatments recorded peroxidase activity in terms of change in absorbance (ΔA_{430}) to be 12.66 ± 1.72, 13.24 ± 1.11 and 14.27 ± 2.37 min^{-1} g^{-1} respectively. The activity of T_3 dose remained higher than T_1 and T_2 doses all through the period of study (T_3 remaining significantly higher over T_1 on most days of analysis) and the difference with control reached maximum on 7th DAA.

In some earlier studies, higher activity of peroxidase on chilli by hexaconazole (Mareeswari *et al*., 2003); highly significant stimulation in its activity with increasing dose of chlorpyrifos and malathion in tomato and brinjal (Nasrabadi *et al*., 2011) and an increase in peroxidase activity of B.T. cotton by imidacloprid (Kaur *et al*., 2011) were observed. Here also higher activity of the enzyme was recorded by the two treatments T_2 and T_3 over control throughout the duration of study. So it can be said that the peroxidase activity gets increased with increasing the concentration of imidacloprid which is supportive of the earlier results.

Polyphenol oxidase activity

The results of polyphenol oxidase (ΔA_{495} min^{-1} g^{-1}) activity of cabbage leaf on different days after application of imidacloprid are given in Table 3 and Figure 1 (C). On the 1st DAA the T_1, T_2 and T_3 treatments recorded polyphenol oxidase activity in terms of change in absorbance (ΔA_{495}) to be 0.31 ± 0.06, 0.34 ± 0.07 and 0.37 ± 0.09 min^{-1} g^{-1} respectively. The activity of the dose T_3 remained higher than that of T_1 and T_2 doses all through the period of investigation (T_3 remaining significantly higher over T_1 on most of the days of analysis), the difference being maximum on 14th and 28th DAA.

Among works done earlier Mareeswari *et al*. (2003) observed increase in activity of polyphenol oxidase by hexaconazole on chilli and Nasrabadi *et al.* (2011) also observed highly significant stimulation in the activity of the enzyme with increasing dose of chlorpyrifos and malathion in tomato and brinjal. The present study also revealed higher activity of the enzyme at normal and double doses over control all along the course of investigation.

CONCLUSION

In most of the earlier investigations the activity of different enzymes were measured on a single day after application of a pesticide. But in the present experiment the activities of three enzymes namely catalase, peroxidase and polyphenol oxidase were quantified at different time intervals upto 35 DAA. So the results of the present study show a trend in the rate of activity of the enzymes under consideration during the course of study. Furthermore, the activity of the three enzymes remained higher all through the period of study at higher dose of the insecticide than the lower ones. So it can be concluded that with increasing xenobiotic stress the activity of the above antioxidant enzymes are stimulated in order to protect the plants from the deleterious effects of the free radicals or reactive oxygen species (ROS) generated during the stress.

Acknowledgements

The authors are indebted to Uttar Banga Krishi Viswavidyalaya for financial assistance for carrying out the programme.

References

1. 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: 15-19.
2. Daraghmeh, A., Shraim, A., Abulhaj, S., Sansour, R. and Ng, J.C. 2007. Imidacloprid residues in fruits, vegetables and water samples from Palestine. *Environ. Geochem. Health* 29: 45–50.
3. Fidalgo, F., Santos, I. and Salema, R. 1993. Effects of deltamethrin on field grown potato plants: biochemical and ultrastructural aspects. *Annals Bot.* 72: 263-267.
4. Habiba, R.A., Ali, H.M. and Ismail, S.M. 1992. Biochemical effects of profenofos residues in potatoes. *J. Agric. Food Chem.* 40: 1852-1855.
5. Jood, S., Kapoor, A.C. and Singh, R. 1995. Polyphenol and phytic acid contents of cereal grains as affected by insect infestation. *J. Agric. Food Chem.* 43: 435-438.
6. Kaur, N., Sohal, B.S. and Singh, K. 2011. Biochemical and physiological changes on *Bacillus thuringiensis* cotton after imidacloprid foliar spray. *Pestic. Biochem. Physiol.* 99: 280-284.
7. Mareeswari, P., Prakasam, V., Samiyappan, R., Kamalakannan, A. and Rajinimala, N. 2003. In: *Annu. Meet. Symp. Rec. Dev. Diag. Mgt. Pl. Dis. Meet Global Challenges*. Dept. Pl. Pathol., Univ. Agric. Sci., Dharwad, pp. 66.
8. Mayer, A. M., Harel, E. and Shaul, R. B. 1965. Assay of catechol oxidase: A critical comparison of methods. *Phytochem.* 5: 783–789.
9. Nasrabadi, M., Ghayal, N. and Dhumal, K. N. 2011. Effect of chloropyrifos and malathion on antioxidant enzymes in tomato and brinjal. *Intern. J. Pharma and Bio Sci.* 2 (B): 202-209.
10. Neog, B., Yadav, R.N.S. and Singh, I.D. 2004. Peroxidase, polyphenol oxidase and acid phosphatase activities in the stigma-style tissue of *Camellia sinensis* (L) O. Kuntze following compatible and incompatible pollination. *J. Indian Inst. Sci.* 84: 47-52.
11. Radetski, C.M., Cotelle, S. and Ferard, J.F. 2000. Classical and biochemical endpoints in the evaluation of phytotoxic effects caused by the herbicide trichloroacetate. *Environ. Experi. Bot.* 44: 221-229.
12. Sadasivam, S. and Manickam, A. 1996. *Biochemical Methods.* New Age International (P) Ltd., New Delhi and TNAU, Coimbatore, pp. 107-109.
13. Saladin, G., Magne, C. and Clement, C. 2003. Effects of flumioxazin herbicide on carbon nutrition of grapevine (*Vitis vinifera* L.). *J. Agric. Food Chem.* 51: 4017-4022.
14. Shirashyad, V. S., Kanade, M. B. and Karadge, B. A. 2009. Effect of organophosphorus pesticide on the enzyme catalase in some vegetable crops. *Bioinfolet.* 6: 347–348.

****

How to cite this article:

Manzoor A. Ashrafi and Goutam K. Pandit.2016, Effect of Imidacloprid on the Activities of some Enzymes of Cabbage (Brassica Oleracea l. Var. Capitata) Leaf. *Int J Recent Sci Res.* 7(1), pp. 8232-8235.

TSSN 0976-3031