ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Suppl 3, 2013

ISSN - 0974-2441

Research Article

EFFECT OF IMIDACLOPRID INSECTICIDE RESIDUE ON BIOCHEMICAL PARAMETERS IN POTATOES AND ITS ESTIMATION BY HPLC

SHAILENDRA S. CHAUHAN*[1], SANJEEV AGRAWAL[2] AND ANJANA SRIVASTAVA[1]

[1]Department of Chemistry, [2]Department of Biochemistry, College of Basic Science and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India, E-mail: anj612003@yahoo.co.in

Received: 17 May 2013 , Revised and Accepted:14 June 2013

ABSTRACT

Pesticides are known to interfere with the biochemical processes of plants, lowering their food quality. The effects of imidacloprid insecticide (a very commonly used pesticide on vegetables) on some biochemical quality control parameters and some enzyme systems (CAT, PPO and POD) were studied. Estimation of imidacloprid residues in potatoes was done by HPLC. It was observed that insecticide treated potatoes have considerable amount of imidacloprid 0.35 mg/kg at the time of harvesting. Washing potatoes with tap water and boiling for 20min. decreased residue up to 33% and 80% respectively. It was also found that the imidacloprid insecticide treatment decreased the reducing sugar, total phenols, ortho-dihydroxy phenols and ascorbic acid contents of potatoes but increased the total protein content and enzyme (catalase, peroxidase and polyphenol oxidase) activity.

Keywords: Imidacloprid, potato, HPLC, biochemical parameters, enzyme activity

INTRODUCTION

Imidacloprid, [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylidene amine] is a systemic insecticide of neonicotenoid group, introduced by Bayer India Ltd. in 1993. It has a broad spectrum activity and low mammalian toxicity along with a unique property of excellent translaminar activity. Imidacloprid has high molecular mobility in the xylem of treated plants because of its high water solubility [1]. This insecticide is extensively used during vegetable and fruit production, to control pests. It is directly applied to the crops which lead to its persistence in the form of residues in vegetables and fruits at the time of harvest. The environmental and health problems and the risk involved in the use of chemicals, especially pesticides, in agriculture are very high [2], which not only leads to the chemical build up of pesticide residues in crops but also disrupts the biochemical parameters of plants. Potato is one of the most widely cultivated crops of India. The total phenolics, total ortho-dihydroxy phenols and ascorbic acid which act as non enzymatic antioxidants in potatoes and other crops are reported to vary because of the inability of the plants to uptake the essential micronutrients due to abiotic stress caused by pesticides. Similarly the activity of catalase, peroxidase and polyphenol oxidase (enzymatic oxidants) is likely to enhance by application of imidacloprid insecticide having amino group present in the insecticide molecule. The presence of certain functional groups such as –OH, $-NH_2$, –NHR, $-CONH_2$, –COOR and $-NR_3$, in the molecular structure of the pesticides hasten adsorption, especially on the soil humus and subsequently affect the plant growth through soil water plant relationship [3]. The inability of plants to take up the essential micro nutrients due to the presence of pesticide residues in soil create nutrient deficiency which is reflected in the abnormality in the different growth parameters. Pesticides which are commonly used for crop protection often reach to adjacent water systems from where they target the aquatic organisms like fish etc. Fish which are exposed to pesticides have a reduced lane of microbial population in their gut essentially needed for digestive processes [4].

It is therefore hypothesized that extensive and over use of systemic pesticides in agricultural land can exhibit negative effects on the growth of no targeted host plant as well as aquatic organisms when they enter into the water systems.

The present work was undertaken with a view to study the persistence of imidacloprid in potatoes after its application on a potato field and investigates the effects of the insecticide on some enzymes and biochemical quality control parameters in potato crop at harvest.

MATERIALS AND METHODS

Sampling of potato

Sowing of potato (variety- Kufri Bahar) crop was done in the month of October, 2011 in the experimental field of Crop Research Center (CRC) at G.B. Pant University of Agriculture and Technology, Pantnagar. Imidacloprid formulated as Confidor 200SL (manufacturer, Bayer Crop Science Inc.) was applied during planting and preplanting stage 3 to 4 times in the fields. Imidacloprid insecticides spray was also done at pre planting stage in soil. The insecticide treated and untreated potato samples were collected from the field in month of January, 2012. After sampling, the potato samples were stored in deep freeze at -20°C temperature.

Extraction and clean up of imidacloprid residue

Extraction and clean up experiment were conducted by following the method reported by Kadenezki [5]. Well homogenized 10 g vegetable samples were mixed with 4 g of anhydrous sodium sulfate, 0.1M NaCl solution and 20mL acetonitrile. The whole mixture was shaken on a mechanical shaker for 30 minutes and filtered. It was transferred onto a glass column, packed with 5 cm layer of anhydrous sodium sulfate and 2 g of activated charcoal for bleaching. The mixture was vacuum-filtered through a Whatman no. 6 filter paper. After filtering the solution was shifted into a 250mL round bottom flask and then, evaporated and concentrated on a rotary evaporator at 50ºC. The residue obtained was dissolved in 2mL acetonitrile and then filtered through a 0.45µM membrane filter. The collected elute was concentrated just to dryness, under a gentle stream of nitrogen gas and re-dissolved in acetonitrile making up the volume to 1mL for analyzing with HPLC. Decontamination of imidacloprid residue in potato samples was also done by washing and boiling methods, after which the residues were estimated as above.

Residue determination

Determination of imidacloprid residue was done on HPLC-UV (Dionex Ultimate 3000) equipped with an analytical column C-18 (250×4.6mm). The mobile phase was acetonitrile: water (60:40 v/v) at a flow rate was 0.5 mL/minute. Detection was done by UV detector at 270nm wavelength. Identification of insecticide residue

was accomplished by determining the retention time (t_R) which was 6.7 minutes and it was compared with known standards under the same conditions. The quantification of the insecticide was done on peak area basis.

Estimation of reducing sugar

The reducing sugar in potato was estimated by following the method given by Miller [6], using glucose as a standard. 100 µL extract of imidacloprid treated and untreated potato samples were taken in test tubes and mixed with DNS reagent (3 mL) in each tube. The mixture was incubated in boiling water bath for 5 minutes. After the color was developed, 1 mL of 40% Rochelle salt solution was added and mixed. The absorbance of the samples was measured at 540nm.

Quantitative estimation of protein

For estimation of protein content the experiment was conducted by the method given by Bradford [7] using Bovine serum albumin (BSA) as a standard. 40µL extract of imidacloprid treated and untreated potato samples were taken in test tubes. Phosphate buffer (pH 7) was added to make up the final volume to 300 µL. 3 mL of Bradford dye was added in each tube. The reaction mixture was incubated in water bath for 15 minutes in dark and absorbance was recorded at 595nm.

Total phenolic content (TPC)

The total phenolic content was determined by the Singleton and Rossi method [8]. 0.5mL of the sample extract was transferred into a test tube and mixed with 0.2 mL of 50% (v/v) Folin-Ciocalteu reagent. After 3 min., 0.5 mL of saturated Na_2CO_3 was added to the reaction mixture and the volume was made up to 10 mL by adding distilled water. The absorbance was read at 765 nm. The standard curve was prepared using various concentrations of Gallic acid and results were expressed as mg of gallic acid per 100 g of sample on dried weight (DW) basis.

Total *o*-dihydroxy phenols

Estimation of total *o*-dihydroxy phenols in the imidacloprid treated and untreated potato samples were done by the method of Arnow [9], using catechol as a standard. The potato extract (1 mL) was mixed with 0.5N HCL (1mL), Arnow's reagent (1 mL) and 1.0N NaOH (2mL). The absorbance of reaction mixture was recorded at 515nm. The amounts were expressed as mg per 100 g of sample on dry weight basis.

Ascorbic acid (Vitamin C) estimation

The analysis of ascorbic acid was carried out by the method of Niewiadomski [10]. The dye was prepared by dissolving 52 mg of 2, 6-dicholorophenolindophenol and 42 mg of sodium bicarbonate and the volume made up to 500 mL with distilled water. The burette was filled with the dye solution to zero mark. Ten mL of the working standard (10 mg ascorbic acid in 100 mL of 4% oxalic acid solution) was pipette out into a 100 mL conical flask and 10 mL of 4% oxalic acid was added. The mixture was titrated against the dye and the volume used was noted. The extract of imidacloprid insecticide of treated and untreated potato samples (10gm) in 4% oxalic acid was made up to a volume 20 mL and centrifuged. Ten mL of this supernatant was pipette out and after addition of 10 mL of 4% oxalic acid it was titrated against the dye. End point was observed by the appearance of pink color, which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid.

$$\boldsymbol{Vitamin\ C}\left(\frac{\boldsymbol{mg}}{\boldsymbol{100gm}}\right)=\frac{\boldsymbol{V_2\times 20mL}}{\boldsymbol{V_1\times weight\ of\ the\ sample\ (g)}}\times\boldsymbol{100}$$

Enzyme Assay

A valid enzyme assay was established where the initial rate of the enzyme-catalyzed reaction was proportional to the concentration of the enzyme extract.

Catalase (CAT) Assay

Catalase (EC 1.11.1.6) activity was measured with minor modifications in the method given by Beers and Sizer [11]. One gram imidacloprid treated and untreated (control) potato samples were homogenized in phosphate buffer (100mM, pH 7.0). The assay mixture consisted of 50µL of the enzyme extract, 100mM phosphate buffer (pH 7.0), and 0.1µM EDTA, and 20 mM H_2O_2 in a total volume of 1.5 mL. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm through a spectrophotometer (Genesys 10UV-Scanning, Thermo Scientific, USA). One international unit of enzyme activity was defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute change in the absorbance of 0.001/min.

Peroxidase (POD) Assay

Peroxidase (EC 1.11.1.17) activity was determined according to method of Tatiana [12] with minor modifications. The sample for enzyme assay was prepared by homogenizing potato in 0.05M phosphate buffer (pH 5.5). The reaction mixture contained 0.05M sodium phosphate buffer (pH 5.5), 2% H_2O_2 and 0.05M guaiacol and 0.1 mL enzyme extract in a final volume of 5 mL. The reaction was started by the addition of enzyme extract. The formation of tetraguaiacol, led to increase in absorbance which was recorded at 470nm by a spectrophotometer (Genesys 10UV-Scanning, Thermo Scientific, USA). One unit of peroxidase activity is defined as the amount of the enzyme that caused a change in absorbance of 0.001 per minute at 470nm.

Polyphenol oxidase (PPO) Assay

Polyphenol oxidase (PPO, EC 1.14.18.1) activity of imidacloprid treated and untreated potato samples were determined by the method of Jockusch [13]. The potato samples (treated and untreated) were peeled and crushed using mortar pestle. 1 g of crushed sample was mixed with 5 mL of 0.05M sodium phosphate buffer (pH 6.0) containing 5% (w/v) polyvinylpyrrolidone (PVP). The homogenate was filtered through a muslin cloth and the filtrate was centrifuged @10,000 rpm for 10 min at 4°C. 1 mL of 0.2 M sodium phosphate buffer, 1mL of 0.1M catechol and 1mL of supernatant of enzyme extract was transferred to a cuvette. The increase in absorbance at 400nm was recorded for 2 min at 10s intervals. One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001/min.

Statistical Analysis

Statistical analysis of the data was determined by the software Sigma Plot v11.

RESULTS AND DISCUSSION

Residue levels of imidacloprid in potato

The extractable residue of imidacloprid in potato samples was found to be 0.35 mg/kg at the time of harvest, which is near to the MRL value. The Codex MRL value for imidacloprid is 0.5 mg/kg for root and tuber vegetables. In none of the samples imidacloprid was found below detection limit (BDL < 0.05 mg/kg). Washing brought down the level of contamination by 33.33% and boiling reduced the residue level up to 80% (Table 1). The results obtained indicate that even after washing with water, the potatoes contained imidacloprid residues, which are likely to cause health hazards to consumers.

Table 1: Imidacloprid residue in unwashed, washed and boiled potato samples

Sample Name	Amount of imidacloprid residue (mg/kg)			MRL (mg/kg)
	Unwashed	Washed	Boiled	
Imidacloprid treated potato sample	0.35 ± 0.98*	0.24 ± 0.75*	0.07 ± 0.85*	0.5

*Values indicate Mean ± S.D.

Biochemical Studies

Pesticide residues may interfere with biochemical and physiological processes in plants retarding the growth and yield of the plant. They may also lower its food quality and prevent its use as food by affecting its quality characteristics [14]. Hence the possible effect of imidacloprid residue on some quality control parameters and activities of catalase, peroxidase, polyphenol oxidase were determined. The studies were done in triplicate and depicted in Tables 2 and 3.

Effect of imidacloprid residues on reducing sugar, protein, total phenols, o-hydroxy phenols and ascorbic acid content in potatoes

As depicted in Table 2, the content of reducing sugar, total phenols, o-hydroxy phenols and ascorbic acid in imidacloprid untreated (control) potato samples was higher as compared to insecticide treated potato samples. However the protein content increased in the imidacloprid treated potato samples.

The sugar content was 12.27% higher in imidacloprid treated potato samples. The obtained data was analyzed by applying t-test. There was a statistically significant difference between the mean of the sampled population and the hypothesized population mean ($p = 0.002$). The decrease in the reducing sugar content in imidacloprid contaminated tubers might be due to some enzymatic changes which are responsible for the conversion of starch to some reducing sugars.

It is well established that certain pesticides influence the chemical composition of the plants after they are applied. Profenofos insecticide residues also decreased the glucose content of potatoes [15]. The profenofos residue affects tomatoes and tomato products too, and it is reported that in the pesticide treated tomato samples the amount of glucose content was reduced by 4% as compared to the untreated tomato samples [16].

It was observed that the residue of imidacloprid insecticide in treated potato sample produced a remarkable decrease in the phenolic content level by 42.55% of the total phenolics in untreated potato sample. The decrease in the total phenolic content can be attributed to the decomposition of phenols which are responsible for the non enzymatic antioxidative properties. The obtained data was statistically analyzed by applying t-test. It was found that there was a statistically significant difference between the mean of the sampled population and the hypothesized population mean ($p = <0.001$). Hesam also reported a decrease in phenolic content in potatoes by application of pesticide from Iran [17].

There was also a high decrease in the total dihydroxy phenols in imidacloprid residue containing potato samples by 49.93% as compared to untreated samples. The data was analysed by applying t-test and here too there was a statistically significant difference between the mean of the sampled population and the hypothesized population mean ($p = 0.003$).

Similarly there was a decrease by 13.13% in the ascorbic acid content in pesticide treated potato samples as compared to the untreated ones. Here also there was a statistically significant difference between the mean of the sampled population and the hypothesized population mean ($p = 0.001$). Similar results have been reported for profenofos residues on the amount of ascorbic acid in tomato and tomato products. The ascorbic acid percentage was decreased by 20% in treated samples than in the untreated tomato samples [18].

The effect of imidacloprid residue on total protein of potato is also presented in Table 2. It was observed that the insecticide treated potato samples contained 18.26% higher amount of protein than the untreated potato sample. This may be due to the increase in nitrogen content because of the presence of amine group in imidacloprid. It is also reported that amine group containing insecticides induce efficient synthesis of amino acids leading to build up of proteins [19]. The obtained data was analyzed by one sample t-test at 95% confidence level and there was a statistically significant difference between the mean of the sampled population and the hypothesized population mean ($p = 0.003$).

Table 2: Effect of imidacloprid residues on some quality attributes of potatoes

Biochemical parameter	Untreated potato sample (Control) Mean ± S.D.	Imidacloprid treated potato sample Mean ± S.D.	± Percentage Change
Reducing sugar (g/100g)	5.62 ±3.45	4.93 ±3.49	-12.27%
Total protein (g/100g)	2.30 ±2.79	2.72 ±2.57	+18.26%
Phenolic content (mg/100g)	47.19 ±0.58	27.11 ±1.395	-42.55%
Ortho-dihydroxy phenols (mg/100g)	15.20 ±0.727	7.61 ±0.715	-49.93%
Ascorbic acid (mg/100g)	28.70 ±1.561	24.93 ±1.531	-13.13%

*Values indicate Mean ± S.D.

The organophosphorus insecticide malathion, at low concentration, has been shown to increase protein synthesis in plant [20]. Profenofos and primifos-methyl pesticide application significantly increased the total protein of potatoes and green pepper respectively [15, 18]. In profenofos residue contaminated tomato samples too, the amount of total protein content in the untreated tomato samples were 11-13% higher than in treated tomato samples [16].

Effect of imidacloprid residues on the activity of catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO)

Enzymes are important both commercially and industrially [21] and so to characterize the enzyme activity of any crop is essential. Peroxidase, a member of a large group of enzymes called the oxidoreductases, is also considered to have an empirical relationship to off-flavours and off-colours in raw and unblanched vegetables, although the reactions involved have not been conclusively identified [22].

The enzymatic antioxidants *viz.* catalases (CAT), peroxidase (POD) and polyphenol oxidase (PPO) were analyzed in imidacloprid treated and untreated potato samples. A valid enzyme assay was established where the initial rate of the enzyme-catalyzed reaction was proportional to the concentration of the enzyme extract.

The activity of imidacloprid on catalase (CAT), peroxidise (POD) and polyphenol oxidase (PPO) in treated and untreated potato samples are presented in Table 3. It was found that pesticide treated potato samples were rich in all the three activities as compared to untreated potato samples. After the application of imidacloprid application, catalase activity was enhanced by 17.94%, peroxidase enzyme activity by 22.26% and PPO activity by 10.09% respectively in the fully mature potato samples. The difference in the mean values of the two groups was found to be greater than would be expected by chance in all the three activities and there was statistically a significant difference between the input groups i.e. ($p = 0.004$), ($p = 0.025$) and ($p = 0.006$) for CAT, POD and PPA activities respectively.

Table 3: Effect of imidacloprid residues on the activity of catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO)

Enzyme	Untreated potato sample (Control) Mean ± S.D.	Imidacloprid treated potato sample Mean ± S.D.	± Percentage Change
Catalase (µMol/min.)	5.74±0.290	6.77 ±0.100	+17.94%
Peroxidase (U/100gm)	2.93 ±0.231	3.59 ±0.225	+22.26%
Polyphenol oxidase (µMol/min)	1.02 ±0.066	1.12 ±0.145	+10.09%

*Values indicate Mean ± S.D.

Ascorbic acid inhibits POD activity and addition of antioxidants has become increasingly popular as a means of increasing the shelf life of food. Ascorbic acid has been used as a peroxidase inhibitor [23]. During this study it has been observed that the ascorbic acid content was decreased and consequently the peroxidase activity increased in imidacloprid treated potato samples. This indicates that imidacloprid insecticide affected the shelf life of potato.

Various studies have been performed to evaluate the PPO enzyme activity in plant leaves, fruits, vegetables and plants under some stress conditions. Similarly quantitative estimation of biochemical contents of extracts of different parts of plants has been carried out to develop protocols for purification of extracts and then recommend their suitability for various purposes [24].

CONCLUSION

From the above study it can be concluded that the injudicious application of pesticides on vegetable and fruit crops results in lower yields and persistence of high levels of pesticide residues (above MRL) in the crops at the time of harvest. These residues produce disastrous effects on the crop quality by lowering or enhancing the biochemical parameters which make the crop unfit for consumption. More insight is needed to perform further studies related to phytotoxicity of insecticides so that the desired biochemical characteristics of the crop are retained and the residue levels are within the MRL values.

REFERENCES

1. Elbert A, Becker B, Hartwig J and Erdelen C. Imidacloprid a new systemic insecticide. Pflanzenschutz Nachricten Bayer, 1991; 44:113-136.
2. Lee Fook Choy LH and Seeneevassen S. Monitoring insecticide residues in vegetables and fruits at the market level. AMAS, Food and Agricultural Research Council, Reduit, Mauritius, 1998; pp. 95.
3. Misra SG and Mani D. Adverse effects of Pesticides. In: Agricultural pollution II. 1994; (Eds.):S.G. Misra and D. Mani Ashish Publisher, New Delhi.
4. Chairman K, Singh AJAR and Padmalatha C. Effect of pesticides on intestinal micro flora in the fish, *Mystus vittatus.* Asian Journal of Pharmaceutical and Clinical Research 2011; 4 (4):156-158.
5. Kadenezki L, Arpad Z, Gardi I, Ambrus A, Gyorfi L and Reese G. Column extraction of residues of several pesticides from fruits and vegetables; a simple multiresidue analysis method. Journal of AOAC International 1992; 75:53-61.
6. Miller GL. Use of dinitrosalicylic acid (DNS) reagent for determination of reducing sugar. Analytical Chemistry 1959; 31 (3):426-428.
7. Bradford MM. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry 1976; pp 248-254.
8. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic and phosphotungtic acid reagents, American Journal of Enology and Viticulture 1965; 16:144-158.
9. Arnow LE. Colorimetric determination of the components of 2, 4-dihydroxyphenyl alanine-tyrosine mixtures, Journal of Biology and Chemistry 1937; 118:531-53.
10. Niewiadomski H. Rape seed Chemistry and Technology. *Elsevier Science Publisher,* Amsterdam, Netherland, 1990; pp. 66-62.
11. Beers RF and Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, Journal of Biology Chemistry 1952; 195:133-140.
12. Tatiana Z, Yamashita K and Matsumoto H. Iron deficiency induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber root Plant Cell Physiology 1999; 40:273-280.
13. Jockusch H. The role of host genes, temperature and polyphenol oxidase in the necrotization of TMV infected tobacco tissue. Phytopathologische Zeischrift 1966; 55:185-192
14. Bartholomew ET, Stewart WS and Carman GE. Some physiological effects of insecticides on citrus fruits and leaves, The Botanical Gazette 1951; 112(4):501-511.
15. Habiba RA, Ali HM and Ismail SMM. Biochemical effects of Profenofos residues in potatoes. Journal of Agriculture Food Chemistry 1992; 40:1852-1855.
16. Ismail SMM, Ali HM and Habiba R. GC-ECD and GC-MS analysis of profenofos residues and the biochemical effects in tomatoes and tomato products, Journal of Agriculture Food Chemistry 1993; 41:610-615.
17. Hesam F, Balali GR and Tehrani RT. Evaluation of antioxidant activity of three common potato (*Solanum tuberosum)* cultivars in Iran. Avicenna Journal of Phytomedicine 2012; 2(2):79-85.
18. Radwan MA, Shiboob MH, Abu-Elamayem MM and Abdel-Aal A. Residues of primiphos-methyl and profenofos on green pepper and eggplant fruits and their effects on some quality properties Emirates Journal of Agriculture Science 2004; 16(1):32-42.
19. Bidwell RG, Barr RA and Steward FC. Protein synthesis and turnover in cultured tissue: Sources of carbon for synthesis and the fate of the protein breakdown products, Nature 1964; 203:367-373.
20. Rouchaud J, Moons C, Detroux L, Haquenne W, Seutin E. and Nys L. Quality of potatoes treated with selected insecticides and potato-halum killers. Journal of Horticulture Science 1986; 61(2):239-242.
21. Galante YM and Formantici C. Enzyme Applications in Detergency and in Manufacturing Industries. Current Organic Chemistry 2003; 7(13):1399.
22. Burnette FS. Peroxides and its relationship to food flavour and quality: a review. J. Food Science 1977; 42:1-5.
23. Shinkle JR and Jones RL. Inhibition of stem elongation in cucumis seedling by blue light requires calcium. Plant Physiology 1988; 86:960-966.
24. Pande S and Khetmalas M. Quantitative Estimation Of Biochemical Content Of Various Extracts Of Stevia Rebaudiana Leaves. Asian Journal of Pharmaceutical and Clinical Research 2012; 5 (1):115-117.