Bull Environ Contam Toxicol (2012) 89:1027–1031
DOI 10.1007/s00128-012-0785-6

Comparative Persistence of Thiacloprid in Bt-Transgenic Cabbage (*Brassica oleracea* cv. capitata) *vis-à-vis* Non-Transgenic Crop and its Decontamination

Debashis Dutta · Ram Niwas · Madhuban Gopal

Received: 4 August 2012 / Accepted: 7 August 2012 / Published online: 31 August 2012

Abstract Thiacloprid is a systemic neonicotinoid. The study hypothesized that difference may be seen in the rate of dissipation of thiacloprid when applied on non-transgenic and transgenic cabbage. Thiacloprid was estimated by HPLC. Half life of thiacloprid in transgenic as well as in normal cabbage ranged between 12.3–13.1 days in two doses of application. Under field condition, after 15 days, 59.2 % and 54.3 % dissipation was recorded at lower and higher rates of application in transgenic cabbage, where as the insecticide dissipated 57.5 % and 59.1 % for single dose and double dose application, respectively in non-transgenic cabbage. The study establishes that there is no significant difference in dissipation of a systemic pesticide in transgenic versus non-transgenic cabbage. Decontamination of thiacloprid contaminated cabbage was carried out by different chemical treatments. The application of 0.5 % $NaHCO_3$ (an edible alkali) may be recommended for decontamination. Thiacloprid residues in the day-3 field samples of cabbage could be reduced below Japanese MRL (1.0 mg kg^{-1}) by treating with 0.5 % $NaHCO_3$ solution for 1 h.

Keywords Transgenic · Isogenic · Cabbage · Thiacloprid · Dissipation · HPLC · Decontamination

Food products developed through biotechnology will be indistinguishable, at least on the market shelf, from conventional kinds. Ensuring the safety of these foods for people is an important concern for the producers of the new varieties, the regulatory and scientific agencies in the countries where these foods are grown, and for the governments of countries that import food grown elsewhere (Kaniewski et al. 2000). Safety assessment of transgenic crops in terms of allergen concentration is studied in recent past (Herman and Ladics 2011) but due to insertional mutagenesis, interaction with endogenous biochemical pathways, and gene modulation have all been said to bring change in metabolism. Thus the study of persistence of a systemic pesticide may be used as a criterion for safety evaluation for transgenic crop versus its isogenic line.

Cabbage is third largest vegetable grown with yield amounting to three million metric tons annually. One of the major problems in cabbage cultivation is insect pest, particularly diamond back moth (DBM) and aphid. Neonicotinoids are relatively new class of systemic insecticides with a distinct mode of action. Thiacloprid ([3-[(6-chloro-3-pyridinyl) methyl]-2-thiazolidinylidene] cyanamide) is a member of the group, which is having broad spectrum activity and particularly effective against aphids, whiteflies, jassid, thrips, leaf miner, beetles in various crops like okra, gram, mustard, cotton, citrus, etc. (Albuquerque et al. 1999; Li et al. 2000; Branco and Pontes 2001; Boselli and Vergnani 2001; Saimandir et al. 2009; Wang et al. 2011). Through the use of transgenic crop cultivation, the load of pesticide on this crop can be reduced in order to obtain higher yield of residue free cabbage. But the information on the residual behavior of pesticide on transgenic crop and its decontamination is scanty.

Wang et al. (2011) reported that thiacloprid dissipated rapidly with the half-life ($t_{1/2}$) 1.3–1.6 days in cabbage. Omirou et al. (2009) has reported the dissipation rate of the compound in greenhouse tomato with a $t_{1/2}$ of 1.9 days. In our earlier investigation, $t_{1/2}$ of the insecticide was found to be 11.1–11.6 days in eggplant (Saimandir et al. 2009).

D. Dutta · R. Niwas · M. Gopal (✉)
Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110012, India
e-mail: madhubangopal@gmail.com

Dubey et al. (2008) reported the $t_{1/2}$ 3.8–4.6 days and 3.3 days of thiacloprid in apple and tea respectively. Yu et al. (2007) reported the $t_{1/2}$ 0.7–1.2 days of thiacloprid in medicinal herbs.

From bio-safety point of view, the residual behavior of insecticide in transgenic cabbage is not available. So before releasing the Bt-transgenic cabbage in market, it is necessary to check whether there is any change in the persistence of pesticides residue in transgenic crop and explore the possibilities of their de-contamination, if residues are high.

Materials and Methods

The experiment was carried out in the farm of Indian Agricultural Research Institute, New Delhi, India. Good quality seeds of the two cultivars of cabbage (Golden acre and Transgenic Golden acre) were sown in the nursery bed during the month of November. In order to ensure that the seeds of transgenic crops used are possessing Bt-gene, PCR analysis and SDS-PAGE were carried out by using standard protocol (Dutta 2003). Seedlings of 5–6 cm height were transplanted in a segregated net house using safety protocol for transgenic crop and good agricultural practices were applied. At the curd formation stage, thiacloprid was applied at 72 g a.i. ha^{-1} (Normal dose) and 144 g a.i. ha^{-1} (Double dose). Samples of cabbage were taken randomly from treated and control plots at the interval 0, 1, 3, 7, 10 and 15 days in triplicate. Accurately weighed 25.30 mg of thiacloprid (analytical grade 99.7 %) was dissolved in HPLC grade acetonitrile in 25 mL volumetric flask. Volume was made up to the mark to obtain exactly 1,000 μg mL^{-1} solution. The stock solution was diluted with acetonitrile serially to obtain 0.5, 1, 1.5, 2.5, 5 and 10 μg mL^{-1} solutions, respectively and 10 μL aliquot of each solution was injected into HPLC and calibration curve was prepared by plotting concentration of thiacloprid in μg on X axis against average peak area on Y axis.

For recovery experiment cabbage was fortified with 0.4 and 4 μg g^{-1} thiacloprid. Enough amount of acetone was added in the flask to submerge the pieces of spiked cabbage head and left overnight. Control samples were treated only with the solvent. Each treatment was replicated thrice including control. Spiked cabbage sample (25 g) and acetone (50 mL) were added to the sample jar and then homogenized at a speed of 5,000 rpm for 5 min. The mixture was decanted and filtered through a Buchner funnel using Whatman No. 1 filter paper, the filter cake was washed twice, successively with 50 mL of acetone. The extracts were collected in a 250 mL volumetric flask, and acetone was added to make the volume up to 250 mL. A total of 50 mL of the mixture was transferred into a flask and concentrated on a rotary evaporator under reduced pressure with water bath temperature of 30°C. The solution remaining in the flask was then transferred onto a separatory funnel and 100 mL saline water was added to it and then partitioned with 50 mL of hexane. Organic layer was discarded off and the water was repartitioned thrice with dichloromethane (25 mL × 3). Dichloromethane was evaporated and the residue was dissolved in ethyl acetate and cyclohexane (1:1 v/v) before loading in a column packed with 5 g of anhydrous sodium sulfate followed by 6 g of Florisil and finally again with 5 g of sodium sulfate. The column was eluted with 100 mL ethyl acetate: cyclohexane (1:1 v/v) and the eluates were discarded. Finally the column was eluted with acetonitrile (40 mL) before injection in HPLC.

Reverse phase high performance liquid chromatography (Lachrom, HITACHI) (HPLC) was equipped with quaternary pump, UV detector connected with auto injector and computer for recording data. The stationary phase consisted of Lichrosphere RP-18 packed stainless steel column (250 mm × 5 mm id). Mobile phase used was acetonitrile: water (60: 40 v/v) with a flow rate of 0.5 mL min^{-1}. λmax for the analysis was 242 nm and injection volume was 10 μL using ambient column and sample temperature.

For decontamination experiment, the cabbage was fortified for 30 min with thiacloprid by dipping it in a thiacloprid solution of known strength. The solution of 0.5 % NaOH, 0.5 % $KMnO_4$, and 0.5 % $NaHCO_3$ was used separately for decontamination of thiacloprid in cabbage samples. One sample of cabbage was directly taken from the thiacloprid solution for determining thiacloprid content. Other replicates of the cabbage were kept in different containers, for 30 min and 1 h. After treating cabbage with different solutions, the remaining pesticide in cabbage was extracted, cleaned up and estimated again to calculate the effectiveness of the treatments. Solution of 0.5 % of $NaHCO_3$ was used to decontaminate the field samples of cabbage, collected on day 3 after application, by dipping them for 1 h.

Residue data were analysed and the degradation curve obtained using a simple linear regression equation. The dependent variable was thiacloprid concentration and independent variable was time (days/hour after treatment). The $t_{1/2}$ value (half life) was calculated from the degradation constant (first order rate kinetics) $t_{1/2} = \ln 2/b$ (where b is the slope of dissipation curve).

Results and Discussion

PCR analysis of transgenic plants showed positive results for the presence of Bt-gene (*cry*1Ab 1 kb and *cry*1B fragment 850 bp) in it (Fig. 1, 2). Protein in the transgenic cabbage was extracted and analysed through SDS-PAGE.

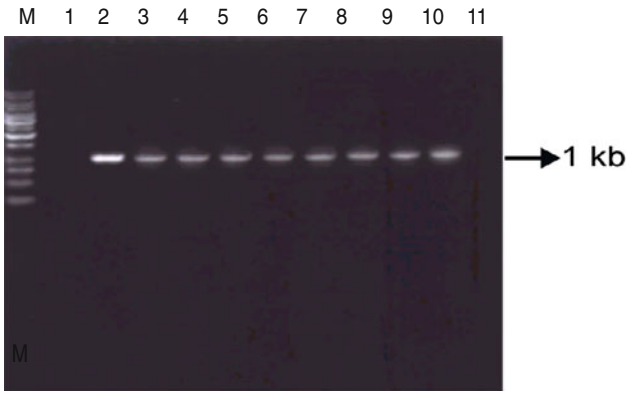

Fig. 1 PCR amplification of *cry*1Ab fragment, *Lane M* 1 kb DNA ladder. *Lane 1* Wild type cabbage plant negative for *cry* gene. *Lane 2* pBinBt6 positive for *cry*1Ab gene. *Lanes 3–10* Transformations positive for *cry*1Ab gene. *Lane 11* Reaction control without template DNA

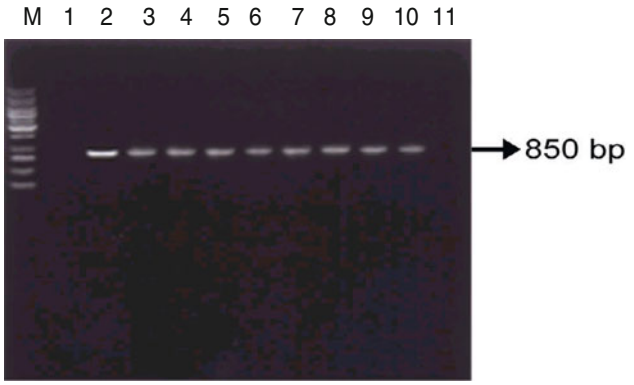

Fig. 2 PCR amplification of *cry*1B fragment, *Lane M* 1 kb DNA ladder. *Lane 1* Wild type cabbage plant negative for *cry* gene. *Lane 2* pBinBt6 positive for *cry*1B gene. *Lanes 3–10* Transformations positive for *cry*1B gene. *Lane 11* Reaction control without template DNA

Bt-fusion protein from transgenic cabbage plant gave a band at 135 kDa, which was absent in case of non-transgenic cabbage (Dutta 2003).

Trace analysis of thiacloprid in different matrices is currently performed by conventional reverse phase liquid chromatographic procedures using different detection strategies (Omirou et al. 2009; Kooner et al. 2010; Yu et al. 2007; Saimandir et al. 2009). In a recent study, UHPLC was used for the analysis of the compound with higher throughput (Tapparo et al. 2011).

Thiacloprid was estimated by RP-HPLC and retention time of thiacloprid was 6.8 min. The calibration graph of concentration versus peak area for thiacloprid followed the regression equation $y = 0.0136x + 1.87$. Linearity was recorded over the range of 0.2–10 μg mL^{-1} concentration of thiacloprid. The limit of detection of the instrument was found to be 0.02 μg g^{-1}.

From the data of recovery it was found that 86.4 %–97.1 % of treated thiacloprid was present in the recovery experiment (Table 1). Recoveries of thiacloprid in cabbage ranges between 86.2 %–108.8 %, when the fortified samples were extracted with acetonitrile followed by clean up by dispersive SPE (Wang et al. 2011).

Persistence of thiacloprid residues on cabbage heads of transgenic and isogenic line was studied after application of insecticide at 72 g a.i. ha^{-1} (Normal dose) and 144 g a.i. ha^{-1} (Double dose). Perusal of data indicated no significant changes in dissipation pattern of the compound in non-transgenic *vis-à-vis* transgenic cabbage. This implies that the dissipation of insecticide did not vary significantly across the matrix. After 15 days of application, thiacloprid dissipated by 59.3 % and 57.5 % in transgenic and non-transgenic cabbage, respectively. Similar trend was observed at double dose of application also. The dissipation curve of thiacloprid and data showed that the thiacloprid persisted for a relatively longer time in both lines of cabbage as compared to data reported in literature (Wang et al. 2011). This may be due to the systemic nature of the compound, which entered the plant system and degraded slowly. The fact may be further attributed to climatic conditions.

The initial concentrations of residues on transgenic and isogenic line were 2.041 and 2.060 μg g^{-1}, respectively for normal dose of thiacloprid (Table 2). The thiacloprid residues dissipated to 0.832 and 0.875 μg g^{-1} respectively after 15 days of thiacloprid application as normal dose. The dissipation equation of transgenic and non-transgenic cabbage were $C = 2.06e^{-0.056t}$ and $C = 2.04e^{-0.058t}$ (Table 1). At double dose of application the equations were $C = 3.16e^{-0.053t}$ and $C = 3.30e^{-0.058t}$ (where C is residues at time t in days).

The half life of thiacloprid residues was found to be 12.3 d and 12.4 d for transgenic and isogenic line of cabbage, respectively at normal dose of application. No difference in $t_{1/2}$ was observed at double dose also confirming 1st order

Table 1 Recoveries and degradation constants (b) and half-lives ($t_{1/2}$) of thiacloprid in non-transgenic and transgenic cabbage

Concentration level (mg kg^{-1})	Cabbage	Recovery (%)	RSD (%)	Slope (b)[a]	$t_{1/2}$ (Days)[a]
0.072	Non-transgenic	87.9	1.3	0.056	12.39
	Transgenic	90.5	2.3	0.056	12.34
0.144	Non-transgenic	86.4	1.8	0.058	12.04
	Transgenic	97.1	3.2	0.053	13.09

[a] Slope of the dissipation (b) line and half-lives of thiacloprid in non - transgenic and transgenic cabbage were determined on application of the pesticide at recommended dose and double the dose

Table 2 Dissipation of thiacloprid in transgenic versus non-transgenic cabbage at normal dose and double dose of application

Time (days)	Treatment (kg a.i. ha^{-1})	Transgenic cabbage		Non-transgenic cabbage	
		Residues (ppm) Mean ± SD	% Dissipation	Residues (ppm) Mean ± SD	% Dissipation
0	0.072	2.04 ± 0.201	0	2.06 ± 0.208	0
	0.144	3.16 ± 0.426	0	3.23 ± 0.337	0
1	0.072	1.77 ± 0.079	13.1	1.72 ± 0.099	16.6
	0.144	2.95 ± 0.325	6.7	2.82 ± 0.201	14.6
3	0.072	1.45 ± 0.078	18.7	1.61 ± 0.009	22.0
	0.144	2.57 ± 0.589	41.1	2.69 ± 0.265	18.4
7	0.072	1.20 ± 0.118	27.6	1.34 ± 0.129	35.0
	0.144	2.29 ± 0.083	47.5	2.09 ± 0.117	36.7
10	0.072	1.07 ± 0.176	47.5	1.00 ± 0.059	51.6
	0.144	1.70 ± 0.270	46.3	1.74 ± 0.174	47.3
15	0.072	0.83 ± 0.046	59.2	0.88 ± 0.058	57.5
	0.144	1.45 ± 0.309	54.3	1.35 ± 0.152	59.1

kinetics of the two dissipations. During the determination of thiacloprid in matrices, extra peaks were not found in HPLC implying that no extra metabolite was formed.

The recovery data of thiacloprid after treatment with different decontaminants are presented in Table 3. The decontamination study showed that simple washing with water was least effective for decontaminating the thiacloprid residue. By use of water, only 13.8 % of the initial concentration of the pesticide was decontaminated. When the pesticide contaminated cabbage was treated with 0.5 % $NaHCO_3$, and 0.5 % $KMnO_4$, only 39.1 % and 40.2 % of initial concentration respectively was decontaminated in 30 min of dipping in these solutions. When 0.5 % NaOH treatment was given to thiacloprid treated cabbage for 30 min, 61.8 % of initial concentration of pesticide was decontaminated that was nearly similar to the amount of decontamination (60.4 %) by 0.5 % $NaHCO_3$ treatment for 1 h. Among these treatments the decontamination of pesticides, was found to be maximum by the treatment with 0.5 % NaOH for 1 h which decontaminated 64.4 % of initial concentration of pesticide. This implies that the pesticide can be detoxified by alkali. Due to the toxic nature of NaOH, the application of 0.5 % $NaHCO_3$ (an edible alkali) may be recommended for decontamination. Thiacloprid residues in the day-3 field samples of cabbage could be reduced below Japanese MRL (1.0 mg kg^{-1}) by treating with 0.5 % $NaHCO_3$ solution for 1 h.

Table 3 Decontamination of thiacloprid contaminated cabbage by different chemical treatments

Treatments	Time (Min.)	Concentration (μg g^{-1})	% Decontamination
0.5 % NaOH	30	2.059	61.8
	60	1.918	64.4
0.5 % $NaHCO_3$	30	3.285	39.1
	60	2.138	60.4
0.5 % $KMnO_4$	30	3.433	36.4
	60	3.225	40.2
Water	30	4.647	13.8
	60	3.971	26.4

In contrast to this study, Zohair (2001) reported that acidic solutions in the form of, citric and ascorbic acid were more effective in decontamination of potato from organophosphorus and organochlorine pesticides than neutral or alkaline solutions. In our earlier investigation, eggplant could also be decontaminated by chemical method involving alkali (Saimandir et al. 2009). The study establishes that there is in no significant difference in dissipation of a systemic pesticide in transgenic versus non-transgenic cabbage.

Acknowledgments The authors wish to thank the Director, NRCPB Dr P. Anand Kumar for providing transgenic cabbage and encouragement for carrying out this interdisciplinary work. Contribution No. 1102.

References

Albuquerque FA, de Kaiser MA, Stulp M, Weber LF (1999) Control of thrips, *Frankliniella schultzei* (Tribon, 1920) (Thysanoptera:Thripidae), on cotton usingdifferent insecticides as sprays: anais II congresso brasileiro de algodao: o algodaono seculo XX, perspectivar para o seculo XXI, 5–10 Setembro. Ribeirao Preto, SP, Brasil, p 224–226

Boselli M, Vergnani S (2001) Efficacy of some insecticides for the control of first generation *Cydia pomonella* L. Inf Fitopatol 51(6):40–46

Branco MC, Pontes LA (2001) Efficiency of thiacloprid in controlling whiteflies. Hortic Bras 19(1):97–101

Dubey JK, Patyal SK, Sharma ID, Thakur M, Kumar A (2008) Residues of thiacloprid in/on apple and tea. Pestic Res J 20: 269–272

Dutta D (2003) Comparative persistence of pesticides in Bt-transgenic cabbage (*Brassica oleracea* L. var. capitita) cultivation. Ph. D. Thesis, Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110 012

Herman RA, Ladics GS (2011) Endogenous allergen upregulation: transgenic vs. traditionally bred crops. Food Chem Toxicol 49(10):2667–2669

Kaniewski W, Rogan G, Cline M (2000) Safety assessments for commercialization of transgenic crops and results of commercialization. Dev Plant Genet Breed 5:249–255

Kooner R, Sahoo SK, Singh B, Battu RS (2010) Dissipation kinetics of flubendiamide and thiacloprid on tomato (*Lycopersicon esculentum* Mill) and soil. Qual Assur Saf Crop Food 2(1):36–40

Li Z, Ye XX, Bao HY, Li K (2000) Study on safety assessment of some suitable insecticides applied in citrus of Zhejiang province. Acta Agric Zhejiang 12:390–392

Omirou M, Vryzas Z, Papadopoulou-Mourkidou E, Economou A (2009) Dissipation rates of iprodione and thiacloprid during tomato production in greenhouse. Food Chem 116:499–504

Saimandir J, Gopal M, Walia S (2009) Risk assessment of thiacloprid and its chemical decontamination on eggplant, *Solanum melongena* L. Pest Manage Sci 65:210–215

Tapparo A, Giorio C, Marzaro M, Marton D, Solda L, Girolami V (2011) Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds. J Environ Monit. doi:10.1039/c1em10085h

Wang C, Guan W, Zhang H (2011) Dissipation and residue of thiacloprid in cabbage and soil. Bull Environ Contam Toxicol 87:440–443

Yu Y, Wu J, Stahler M, Pestemer W (2007) Residual dynamics of thiacloprid in medical herbs marjoram, thyme, and camomile in soil. J Environ Sci 19:205–209

Zohair A (2001) Behaviour of some organophosphorus and organochlorine pesticides in potatoes during soaking in different solutions. Food Chem Toxicol 39(7):751–755