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Dissipation pattern and effect of household processing on reduction of Indoxacarb residues in tomato fruits (*Lycopersicon esculentum* Mill.)

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Abstract

In the present research dissipation behaviour of indoxacarb 4.5% SC was investigated in tomato fruits sprayed at two doses i.e. 37.13 and 74.26 g a.i. ha⁻¹. Tomato fruit samples were collected periodically on 0 (1h after application), 1, 3, 5, 7, 10, and 15 days after application. Tomato samples were fortified at 0.01 to 0.25 mg kg⁻¹ levels and recovered 84.50 to 90.58 percent average residues. The initial deposits were found to be 0.63 and 0.82 mg kg⁻¹ at single and double doses, respectively. Dissipation of indoxacarb fitted well to first order kinetics with half-life values ranging from 2.37 to 2.48 days at respective doses. Residues decreased substantially during household processing among which peeling was found most effective which resulted 87-89% reduction. LOD and LOQ were calculated to be 0.005 and 0.01 mg kg⁻¹.

Keywords: Indoxacarb, dissipation, half-life period, tomato fruits, processing, GLC

1. Introduction

In Indian agriculture, tomato crop plays an important role and India ranks fourth in the worldwide production of tomato and it is the second largest produced vegetable after potato in India [10]. The estimated area production of tomato for India is about 350000 hectares and 5300000 tons. As it is a short duration crop, and gives high yield, it is important from an economic point of view [16, 26].

Considering yield, crop is badly affected by pests which always attack on it into different life cycle stages like nursery, foliage, fruit formation stage etc. So it is a challenge for farmers to protect their crop from pests like jassids, aphids, tobacco caterpillar, flea beetles, leaf miner, spider mites and fruit borer [21, 26]. Hence, in order to combat the insect pest problem, lots of pesticides are used by the farmers which are available in market. Newer crop protection agents (CPA) like imidacloprid, indoxacarb, spinosad along with conventional molecules like fenvalerate, cypermethrin, deltamethrin are applied to protect the crop from pest infestation [21]. Exorbitant use of pesticide applications for the control of tomato pests has led to the development of resistance in target pest species. Therefore tomato crops have become very much dependent on intensive use of pesticides when grown in open fields. To overcome these problems many efforts have been made to develop selective, safe and non-hazardous compounds, thus broadening the spectrum, even for economy of applications also.

Indoxacarb, is such type of pesticide which is used to overcome such type of problems and chemically known as {Methyl 7-Chloro-2,5-dihydro-2-[(methoxy-carbonyl)[4-(trifluoromethoxy) phenyl] amino] carbonyl] indeno[1,2-e] [1,3,4] oxadiazine-4a (3H)-carboxylate}. It is a non-systemic, synthetic organophosphate replacement insecticide with broad spectrum activity. This new pesticide was developed by E. I. du Pont de Nemours and Company and was registered as Avaunt [15]. It is a highly effective new insecticide having a low side effect on non-target insects [14] and allows most predators and immature wasp parasites to survive [9, 23]. Although, many references citing the efficiency of this insecticide and its safety to beneficial organisms [1, 6] is available, but no work has been carried out to evaluate the persistence of indoxacarb residues in tomato fruits especially in Haryana state. Besides this, information on reduction of residues of indoxacarb in tomato fruits by kitchen processes like unwashed, washing, boiling, peeling is also lacking. Thus, keeping this in view, the present investigation was undertaken to study the dissipation of indoxacarb residues and effect of different decontamination processes on reduction of residues in/on tomato (*Lycopersicon esculentum* Mill.).

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2. Materials and Methods

2.1 Field experiment

Present experiment was conducted at the Research Farm of Department of Entomology, CCS HAU, Hisar in a 4m x 4m, randomized block design (RBD) plots as per good agricultural practice (GAP). Two doses of indoxacarb were used, for single dose 43.31 g a.i. ha⁻¹ and double dose 86.62 g a.i. ha⁻¹ and applied in the field on the tomato crop (variety HS-86) at 50% fruiting stage in the month of May, 2015 with the help of Knapsack sprayer, where one plot was left untreated and used for the sampling of fruits as control in each treatment. Tomato samples (1kg) were collected randomly and separately from the control and treated plots of each treatment at 0 (1 h), 1, 3, 5, 7, 10 and 15 days after the application of the pesticide. These samples were subjected to household processing like washing, washing followed by boiling/cooking and peeling as explained below. Residues from the processed samples were extracted, cleaned and analyzed by gas liquid chromatography (GLC). Residue status was compared with control.

2.2 Extraction and Clean-up

Collected tomato fruits samples were chopped into small pieces and after quartering, a representative sample was macerated in Warring blender to make a fine paste. 20 g representative tomato sample was taken in a flask and then 100 ml of acetone was added. Flasks were shaken for one hour on the mechanical shaker. Extract was filtered through Buchner funnel. Than filtered extract was transferred to 1L separatory funnel, 600 ml of brine solution (10% NaCl) was added and then partitioned thrice with dichloromethane and hexane (75, 50, 25 ml) by vigorous shaking for 5 min to remove the non-emulsifying impurities. Each time, organic phase was collected and passed through the 2-3 cm pad of anhydrous sodium sulphate and pooled together. The pooled organic layer was concentrated to 5 ml on Rotary Vacuum Flask Evaporator.

For clean-up of extract, adsorbent mixture i.e. silica + activated charcoal (5 + 0.2 w/w) was used as per method of [7] and mixed uniformly. Preparation of column: A cotton plug was put at the bottom glass column (90 cm x 22 mm i.d.) over which 1 cm layer of anhydrous sodium sulphate was laid.

After this adsorbent mixture was transferred in to column and ensure the compact packing of the column. 1cm layer of anhydrous sodium sulphate was added over the adsorbent mixture. The column was pre wetted with 40 ml hexane. The extract was transferred quantitatively to the column and eluted with 100 ml solution of hexane: acetone (9:1 v/v). Then the eluate was concentrated to near dryness on Rotary Vacuum Flask Evaporator. The final volume was made to 5 ml in hexane for GC analysis.

2.3 Instrumentation

The residues of indoxacarb were estimated using gas liquid chromatography (Shimadzu GC-2010) equipped with a fused silica capillary column, Restek -1(30 m x 0.32 mm i.d. x 0.25 µm film thickness) of 5% diphenyl + 95% dimethyl polysiloxane and electron capture detector (ECD). The operating GC parameters were: injector temperature: 280°C, detector temperature: 300°C and oven temperature programme was 150 (5min) increased at 8°C min⁻¹ up to 190 (2min) further increased at 15°C min⁻¹ up to 280° (5 min) with a split ratio 1:10. Total gas flow rate was 60 ml min⁻¹. Retention time (R_i) for indoxacarb was 8.058 minute.

2.4 Limit of detection and quantification

For indoxacarb half-scale deflection was obtained for 0.5 µg ml⁻¹, which could be easily identified from the baseline as 0.1 µg ml⁻¹ of the compound produced 10% deflection, which can be measured. When 1 µL (5 mg) of the sample was injected then did not produce any background interference. Thus, the limit of quantification (LOQ) was found to be 0.01 mg kg⁻¹ and the limit of detection (LOD) observed to be 0.005 mg kg⁻¹ for tomato fruits.

2.5 Recovery

Representative 20 g of chopped tomato fruits were taken in 250 ml Erlenmeyer flasks and were fortified @ 0.01 to 0.25 mg kg⁻¹ with standard pesticides. As evident from data given in Table 1, average recoveries from samples of tomato fruits fortified at 0.01, 0.10 and 0.25 mg kg⁻¹ levels were 84.50, 88.27 and 90.58 percent for indoxacarb.

Table 1: Percent recovery of indoxacarb from tomato fruits

Fortification Levels (mg kg ⁻¹)	Indoxacarb Average Recovery* (%) ± SD
0.01	84.50 ± 2.01
0.10	88.27 ± 3.32
0.25	90.58 ± 5.55

*Average of three replicates

3. Results and Discussion

3.1 Persistence and Dissipation kinetics of Indoxacarb

Experimental data obtained from tomato fruits at two treatments i.e. 37.13 g a.i. ha⁻¹ and 74.26 g a.i. ha⁻¹ for indoxacarb is given in Table 2. Data revealed that initial residues of 0.63 mg kg⁻¹ from T₁ at 0 day (1h after spray) dissipated to 0.31, 0.22, 0.16, 0.06 and 0.01 mg kg⁻¹ in 1, 3, 5, 7 and 10 days after treatment, respectively, thereby recording 50.79, 65.07, 74.60, 90.47 and 98.41 percent dissipation in this period while at T₂, initial residues 0.82 mg kg⁻¹ were

dissipated to 0.54, 0.30, 0.14, 0.10, 0.05 and 0.01 mg kg⁻¹ with percent dissipation of 34.14, 63.41, 82.92, 87.80, 93.90 and 98.78, respectively. Residues of indoxacarb were found below MRL value of 0.05 mg kg⁻¹ on 10th and 15th day at both doses. Residues half-life values were 2.37 days and 2.48 days at T₁ and T₂ treatments. The values of correlation coefficient for degradation kinetics in tomato fruits were 0.941 and 0.977 for single and double dose respectively, given in Table 2.

Table 2: Residues (mg kg⁻¹) of Indoxacarb in tomato fruits

Days	Indoxacarb residues (mg kg ⁻¹)			
	T ₁ (37.13 g a.i. ha ⁻¹)		T ₂ (74.26 g a.i. ha ⁻¹)	
	Average residues* ± SD	% Dissipation	Average residues* ± SD	% Dissipation
0	0.63 ± 0.04	-	0.82 ± 0.03	-
1	0.31 ± 0.02	50.79	0.54 ± 0.05	34.14
3	0.22 ± 0.03	65.07	0.30 ± 0.03	63.41
5	0.16 ± 0.03	74.60	0.14 ± 0.03	82.92
7	0.06 ± 0.04	90.47	0.10 ± 0.02	87.80
10	0.01 ± 0.004	98.41	0.05 ± 0.01	93.90
15	BDL	100.00	0.01 ± 0.003	98.78
	Correlation coefficient R ² = 0.941 Regression equation y = -0.127x + 1.731 t _{1/2} = 2.37 days		Correlation coefficient R ² = 0.977 Regression equation y = -0.121x + 1.849 t _{1/2} = 2.48 days	

For regression equation [residues (mg kg⁻¹)×10³] is taken

*Average residues±SD of three replicates; below detectable level (BDL) < 0.01 mg kg⁻¹

Maximum Residue Limit (MRL), 0.05 mg kg⁻¹

Initial deposits of indoxacarb on eggplant fruits were found to be 2.60 to 3.64 mg kg⁻¹ and 2.63 to 3.68 mg kg⁻¹ from 75 and 150 g a.i. ha⁻¹ treatments from first and second year field trials [18]. The reported concentration reached non-detectable (< 0.02 mg kg⁻¹) after 15 - 20 days. Residues dissipated with half-life of 3.0 - 3.8 days. It was observed that dissipation of indoxacarb enantiomers in cabbage follow first-order kinetics [24]. The enantiomeric concentration of (-)-indoxacarb and (+)-indoxacarb increased from 5.08 to 12.03 mg/kg in the first 2 days because of the dominant absorption process and then declined to 0.1 and 0.06 mg/kg in 30 days for the Beijing cabbage. The half-lives of two enantiomers in cabbage ranged from 2.8 to 4.6 days. It was reported that average initial deposits of 0.23 and 0.45 mg kg⁻¹ of indoxacarb on cauliflower following the last application dissipated more than 78 and 68 percent after 3 days at single and double doses. Residues dissipated below its LOQ of 0.01 mg kg⁻¹ after 7 days with half-life periods of 1.12 and 1.31 days, respectively [25]. Initial deposits of indoxacarb were found to be 0.11 and 0.21 mg kg⁻¹ on brinjal @ 70 and 140 g a.i. ha⁻¹ [22]. It was reported that residues of indoxacarb persisted up to 10 days with half-life of 0.58-1.02 days [8]. Persistence of indoxacarb residues in tomato at foliar application of indoxacarb at 0.5 and 1.0 ml/l was observed. The residues of the insecticides dissipated fast below detectable limits within 7-10 days after their last application with half-lives of 1.1 to

1.5 days [19]. The results of indoxacarb were in agreement with those of above findings as our experiment also show that dissipation of indoxacarb follow first-order kinetics and maximum percent dissipation was observed on 7th and 10th day of application.

3.2 Effect of household processing in tomato fruits

The processing methods played a major role in reducing the residues from the vegetable and render them suitable for human consumption by decreasing the residues within prescribed limits of MRLs [20]. In this investigation when the samples were subjected to washing with simple tap water than residues of indoxacarb tend to decrease with increase in time duration. Residues data presented in Table 3 and Fig.1 revealed that at single dose residues levels of 0.63, 0.31, 0.22, 0.16, 0.06 and 0.01 mg kg⁻¹ was observed on 0 (1h after spray), 1, 3, 5, 7 and 10 days after treatment and reduced to 0.29, 0.18, 0.15 and 0.14 mg kg⁻¹ resulting in 53.96, 41.93, 31.81 and 12.50 percent reduction due to washing. Where in case of double dose, data presented in Table 4 and Fig. 2 revealed that the initial residues of 0.82, 0.54, 0.30, 0.14, 0.10, 0.05 and 0.01 mg kg⁻¹ were reduced to 0.37, 0.31, 0.20 and 0.12 mg kg⁻¹, respectively due to washing and thus showed 54.87, 42.59, 33.33 and 14.28 percent loss at corresponding time intervals and reached BDL on 7th day of application.

Table 3: Processing data on reduction of Indoxacarb residues at single dose (T₁)

Days after treatment	Indoxacarb residues (mg kg ⁻¹)			
	T ₁ (37.13 g a.i. ha ⁻¹)			
	Average residues* ± SD (mg kg ⁻¹)	Washing	Washing +Boiling	Peeling
0	0.63 ± 0.04	0.29 ± 0.02 (53.96)	0.18 ± 0.03 (71.42)	0.08 ± 0.02 (87.30)
1	0.31 ± 0.02	0.18 ± 0.03 (41.93)	0.08 ± 0.03 (74.19)	0.07 ± 0.03 (77.41)
3	0.22 ± 0.03	0.15 ± 0.03 (31.81)	0.05 ± 0.02 (77.27)	0.07 ± 0.03 (68.18)
5	0.16 ± 0.03	0.14 ± 0.02 (12.50)	0.03 ± 0.01 (81.25)	0.09 ± 0.02 (43.75)
7	0.06 ± 0.04	BDL	BDL	0.04 ± 0.01 (33.33)
10	0.01 ± 0.004	-	-	BDL
15	BDL	-	-	-

*Average residues ± SD of three replicates

Figures in parenthesis is percent reduction of residues

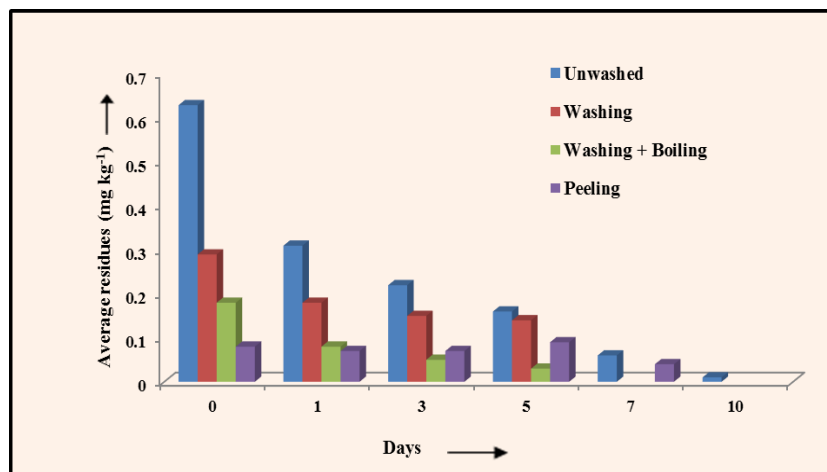


Fig 1: Effect of processing on reduction of residues of indoxacarb at single dose

However, when the sample were subjected to washing + boiling, initial residue level reduced to 0.18, 0.08, 0.05 and 0.03 mg kg⁻¹ resulting in 71.42, 74.19, 72.27 and 81.25 percent reduction, respectively. While in case of double percent loss of indoxacarb residues was found to be 73.17, 75.92, 80.00 and 85.71 at corresponding time intervals. After 7th day residues reached below detectable level of 0.01 mg kg⁻¹. Tomato fruits were washed and then peeled off with the

help of the knife than results show that the average initial residues of 0.63 mg kg⁻¹ in single dose reduced to 0.08 mg kg⁻¹ showing 87.30 percent reduction and 0.82 mg kg⁻¹ reduced to 0.09 due to peeling showing 89.02 percent reduction at double dose. After that percent reduction decreased with increase of time duration and reached BDL on the 10th day of application.

Table 4: Processing on reduction of Indoxacarb residues at double dose (T₂)

Days after treatment	Indoxacarb residues (mg kg ⁻¹)			
	T ₂ (74.26 g a.i. ha ⁻¹)			
	Average residues* ± SD (mg kg ⁻¹)	Washing	Washing + Boiling	Peeling
0	0.82 ± 0.03	0.37 ± 0.02 (54.87)	0.22 ± 0.04 (73.17)	0.09 ± 0.01 (89.02)
1	0.54 ± 0.05	0.31 ± 0.04 (42.59)	0.13 ± 0.02 (75.92)	0.11 ± 0.02 (79.62)
3	0.30 ± 0.03	0.20 ± 0.02 (33.33)	0.06 ± 0.03 (80.00)	0.09 ± 0.02 (70.00)
5	0.14 ± 0.03	0.12 ± 0.02 (14.28)	0.02 ± 0.02 (85.71)	0.07 ± 0.01 (50.00)
7	0.10 ± 0.02	BDL	BDL	0.06 ± 0.04 (40.00)
10	0.05 ± 0.01	-	-	BDL
15	0.01 ± 0.003	-	-	-

*Average residues±SD of three replicates

Figures in parenthesis is percent reduction of residues

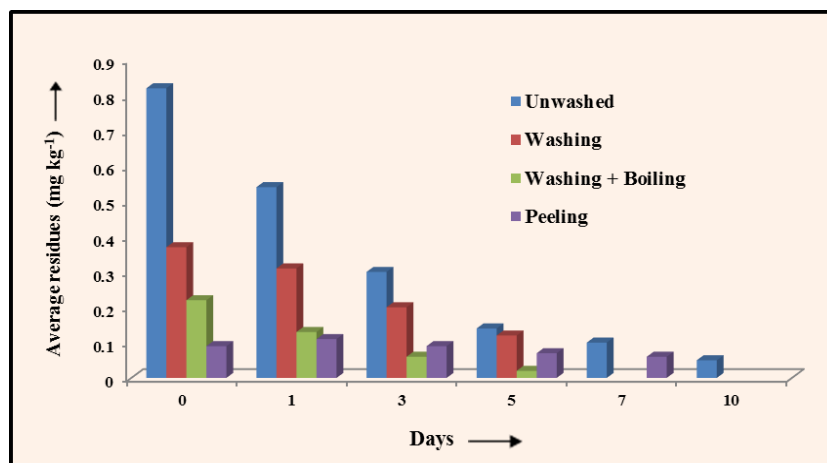


Fig 2: Effect of processing on reduction of residues of indoxacarb at double dose

Residues of indoxacarb decreased substantially during household processing among which peeling was found most effective which resulted 87 to 89% reduction. Research studies were carried out by [5] and determined maximum reduction in concentration of indoxacarb residues up to 79.244% by following traditional procedure i.e. washing with tap water. Washing with boiled water has significantly reduced indoxacarb residues to 95.5886%. The group, which was washed with boiled haridra water, has significantly reduced 96.631% of indoxacarb residues. Results indicated that ozonated water eliminated the indoxacarb residues from the detected levels of 2.0, 1.0 and 0.0 ppm at low and high doses, respectively, in potato samples [13]. Peeling by hot water at 95 °C for 2 min eliminated about concentration of 0.797, 0.628, 0.916, 0.468 and 1.069 ppm and caused the reduction of the previous pesticides in prepared tomato fruits by 67.27, 68.43, 67.69, 75.22 and 63.43%, respectively [17]. Boiling process reduced the residues by 32 - 100 percent in case of OC, SP, OP and carbamates in three vegetables viz. brinjal, cauliflower and okra. Peeling off potatoes reduced 91-98 percent chlorpyrifos residues from an initial concentration of 3.8 ppm in individual tubers 10 days of post application. The amount of pesticide residues removed by peeling was 70 percent for pyridaben and 100 percent for pyrifenox and tralomethrin. The initial diazinon residue level (0.822 ppm) on cucumber was decreased by 67.3 percent by peeling [2, 3, 11 and 12]. Initial procymidone residues level (0.86 ppm) on tomatoes was decreased 77 percent by peeling procedure [4]. The present results were in agreement with above findings which show maximum reduction of residues in peeled samples.

4. Conclusion

From the research it was concluded that residues of indoxacarb were found below MRL value of 0.005 mg kg⁻¹ on 10th day at both single and double dose, respectively. Residues of indoxacarb decreased substantially during household processing among which peeling was found most effective which resulted 80 - 89% reduction of pesticide residues.

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