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# Estimation of Indoxacarb Residues by QuEChERS Technique and Its Degradation Pattern in Cabbage

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**Abstract** Indoxacarb residues were estimated by employing standardized QuEChERS technique in cabbage following three applications of Avant$^{R}$ 14.8 EC @ 52.2 and 104.4 g a.i. ha$^{-1}$. The average recoveries of indoxacarb on cabbage for fortification levels of 0.01, 0.05 and 0.1 mg kg$^{-1}$ were observed to be 83.93, 89.86 and 95.40%, respectively, with relative standard deviation of 1.21, 1.53 and 2.23. The method was also validated with respect to parameters of linearity, precision and limit of quantification (LOQ). The LOQ for cabbage was found to be 0.01 mg kg$^{-1}$. The average initial deposits of indoxacarb on cabbage were observed to be 0.18 and 0.39 mg kg$^{-1}$, respectively, at single and double the application rate. These indoxacarb residues dissipated below its LOQ of 0.01 mg kg$^{-1}$after 7 and 10 days, respectively, at single and double dosages. Half-life of indoxacarb was observed to be 2.88 and 1.92 days, respectively, at recommended and double the recommended dosages.

**Keywords** Cabbage · Indoxacarb · QuEChERS · Degradation

Cabbage (*Brassica oleracea*) is one of the most popular winter vegetable grown in India. In India, it is cultivated in 265.4 thousand hectare with the total production of 5,887.8 thousand tonnes (Anonymous 2009a) where as in Punjab, average production is 73.23 thousand tonnes in 3.34 thousand hectare of land cultivated (Anonymous 2008). It is the fourth most widely grown vegetable crop of India and the country ranks third in cabbage production in the world. Cabbage is used in a variety of dishes for its naturally spicy flavor. The so called "cabbage head" is widely consumed raw, cooked, or preserved in a great variety of dishes. It is the principal ingredient in coleslaw. The pest incidence in cabbage is generally more during February to September, though it is noticed throughout the year. This crop suffer from the ravages of pests such as cabbage borer (*Hellula undalis*), leaf webber (*Crocidolomia binotalis*), diamond back moth (*Plutella xylostella*), cut worm (Lepidoptera: Noctuidae), cabbage aphids (*Brevicoryne brassicae*) and cabbage flea beetle (*P. cruciferae*), resulting in severe loss of quality and production (Regupathy et al. 1985; Patel et al. 1999).

Indoxacarb, (S)-methyl 7-chloro-2, 5-dihydro-2- [[(methoxycarbonyl) [4 -(trifluoromethoxy) phenyl] amino]carbonyl] indeno[1,2-e][1,3,4] oxadiazine-4a(3H)-carboxylate ($C_{22}H_{17}$ $ClF_3$ $N_3O_7$) registered in California, January 2001, is a new oxadiazine insecticide produced by DuPont and marketed as Steward™, Avaunt™ and Technical Indoxacarb™. The indoxacarb racemate contains two enantiomers (*S*: *R*), designated DPX-KN128 and DPX-KN127, but only the *S* enantiomer has insecticidal activity (Mc Cann et al. 2001). Indoxacarb is considered a reduced risk pesticide with low mammalian toxicity and a benign profile for avian and aquatic toxicity as compared to that of conventional insecticides. It is also the first commercialized insecticides that acts by blocking the sodium channel in insect neurons (Lahm et al. 2000) and was designated a reduced risk product by Environment Protection Agency (Anonymous 1998). It is a broad spectrum, highly effective, nonsystemic, synthetic organophosphate replacement insecticide recently registered for the use on vegetables to control lepidopteran

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pests and selected insect pests with sucking mouthparts (Harder et al. 1997; Anonymous 1998; Wing et al. 1998; Liu et al. 2002). It has been used for controlling insect pests of cotton, fruits and vegetables in USA (Allen et al. 1999; Liu and Sparks 1999), Australia (Holloway and Forrester, 1998), France (Olszak and Pluciennik 1999), Italy (Bassi et al. 2000) and many other countries (Pluschkellm et al. 1998).

In India, indoxacarb has been used to control of leaf folder, fruit borer in chillies (Singh et al. 2005a) and also tomato fruit borer (Singh et al. 2005b). Indoxacarb has been found to be very effective for the control of diamond back moth Lepidoptera: *Plutellidae* (Gill et al. 2008; Murthy et al. 2006) and cabbage looper Lepidoptera: Noctuidae (Liu et al. 2002). As these crops invariably retain some residues of the insecticides, the potential health hazards posed by these consumable commodities depend on the quantity of pesticide residues present in them.

Therefore the present investigation was carried out with the objective to study the dissipation pattern and residue levels of Indoxacarb (Avant 14.8 EC) in cabbage as well as soil under the sub tropical conditions.

## Materials and Methods

Indoxacarb reference standard (≥99.9% purity) was purchased from Dr. Ehrestorfer Augusburg, Germany. Acetonitrile was of HPLC grade and acetone was of GR grade and purchased from Merck (Mumbai, India). Before use these solvents were redistilled in all glass apparatus and suitability of solvents was ensured by running reagent blank along with actual analysis. Sodium chloride (ACS reagent grade ≥99.9%, sodium sulfate anhydrous (AR grade) were obtained from sd fine chemicals, Mumbai, India. Analytical-grade $MgSO_4$ was purchased from Merck, India. $MgSO_4$ was activated by heating at 400°C for 4 h in muffle furnace, cooled and kept in a desiccators' before use. Graphatised carbon and primary secondary amine (PSA) (40 mm, 100 GM) were obtained from Sigma Aldrich and Varian, Mumbai, India, respectively.

The stock solution containing 1,000 μg $mL^{-1}$ of analyte was prepared using acetone as solvent. The standard solutions used for fortification of the matrices and instrument calibration purposes were prepared by serial dilution. All standards solutions were stored at 4°C before use. Standard calibration curve of indoxacarb was constructed by plotting analyte concentrations versus peak area (Fig. 1).

Cabbage (var. hybrid *Meera*) was raised during November 2009 – March 2010 according to recommended agronomic practices at Entomological Research Farm, Punjab Agricultural University, Ludhiana, India using

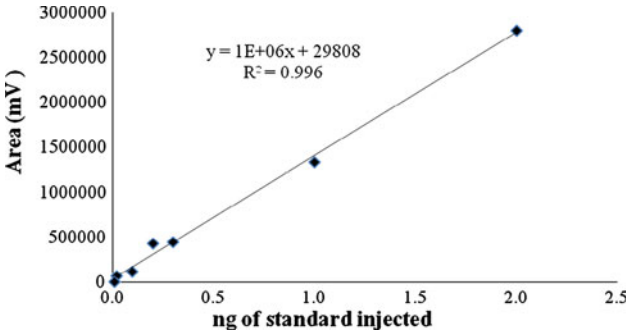


**Fig. 1** Linearity curve of Indoxacarb standard on GC

randomized block design (RBD) (Anonymous 2008). The first application of Indoxacarb (Avant 14.8EC) @ 52.2 and 104.4 g a.i. $ha^{-1}$ was made at head formation stage using Aspee Knapsack sprayer equipped with hollow cone nozzle. Subsequently the second and third application was made at 10 days interval. Each treatment was replicated thrice and size of each plot was 50 $m^2$. In control plots only water was sprayed.

About 5–6 marketable size cabbage heads were collected from each treated and control plots separately and brought to laboratory at 0 (2 h), 1, 3, 5, 7, 10 and 15 days after the last application of indoxacarb. Samples were extracted and cleaned up immediately after sampling. This paper describes a simple and effective extraction procedure using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method to determine indoxacarb in cabbage and soil samples. The cabbage samples were cut into small pieces, mixed in a blender and a representative 15 g sample of cabbage was taken into a 50 mL centrifuge tube, capped and stored overnight under refrigerated conditions. Next day, 30 mL of acetonitrile was dispensed into each tube. The samples were well shaken and then homogenized @ 15,000 rpm for 2–3 min using a homogenizer (Heidolph). Added 5–10 g sodium chloride and shook the tubes vigorously first by hand and then by rotospin for 5 min. The samples were centrifuged using a laboratory centrifuge for 3 min @ 2,500 rpm. Decanted the upper 15 mL layer into another 50 mL centrifuge tube containing 10 g of activated sodium sulfate and again shook the contents using a rotospin for 2–3 min so as to remove even small traces of moisture. Transferred 6 mL of supernatant into 15 mL centrifuge vial containing 150 mg sorbent PSA and 900 mg $MgSO_4$ and 50 mg graphitized carbon. The samples were again vortexed for 1 min and then centrifuged for 1 min at 2,500 rpm. Transferred an aliquot of 4 mL into a 50 mL round bottom flask and carefully concentrated to near-dryness and again added about 20 mL distilled acetone, concentrated using rotary vacuum evaporator at <30°C to completely remove

**Table 1** Recovery studies of indoxacarb on cabbage and soil at various fortification levels

| Substrates | Level of fortification (mg $kg^{-1}$) | Recovery (%)[a] | RSD (%)[b] |
|---|---|---|---|
| Cabbage | 0.10 | 95.40 | 1.21 |
| | 0.05 | 89.86 | 1.53 |
| | 0.01 | 83.93 | 2.23 |
| Soil | 0.50 | 96.94 | 1.54 |
| | 0.05 | 92.53 | 0.89 |
| | 0.01 | 86.67 | 1.46 |

[a] Each value is mean of six replicate determinations

[b] Relative standard deviation

acetonitrile. Repeated the process and final volume was reconstituted to about 2 mL using distilled acetone. While proceeding for soil samples 10 mL of distilled water is added in 15 g of soil sample. Rest of method is same as for cabbage samples.

Perkin gas liquid chromatograph (GLC) equipped with electron capture detector (ECD) was used for residue analysis. A capillary column Elite 35 (50 m × 0.53 mm i.d, 1.5 μm film thickness) with spilt ratio 1:10 was used for the estimation of indoxacarb residues. The carrier gas flow was 2 mL $min^{-1}$ of high purity nitrogen. Injector, detector and oven were held at 300, 310 and 290°C, respectively. Under these operating conditions, the retention time of indoxacarb was 5.78 min. Residues were

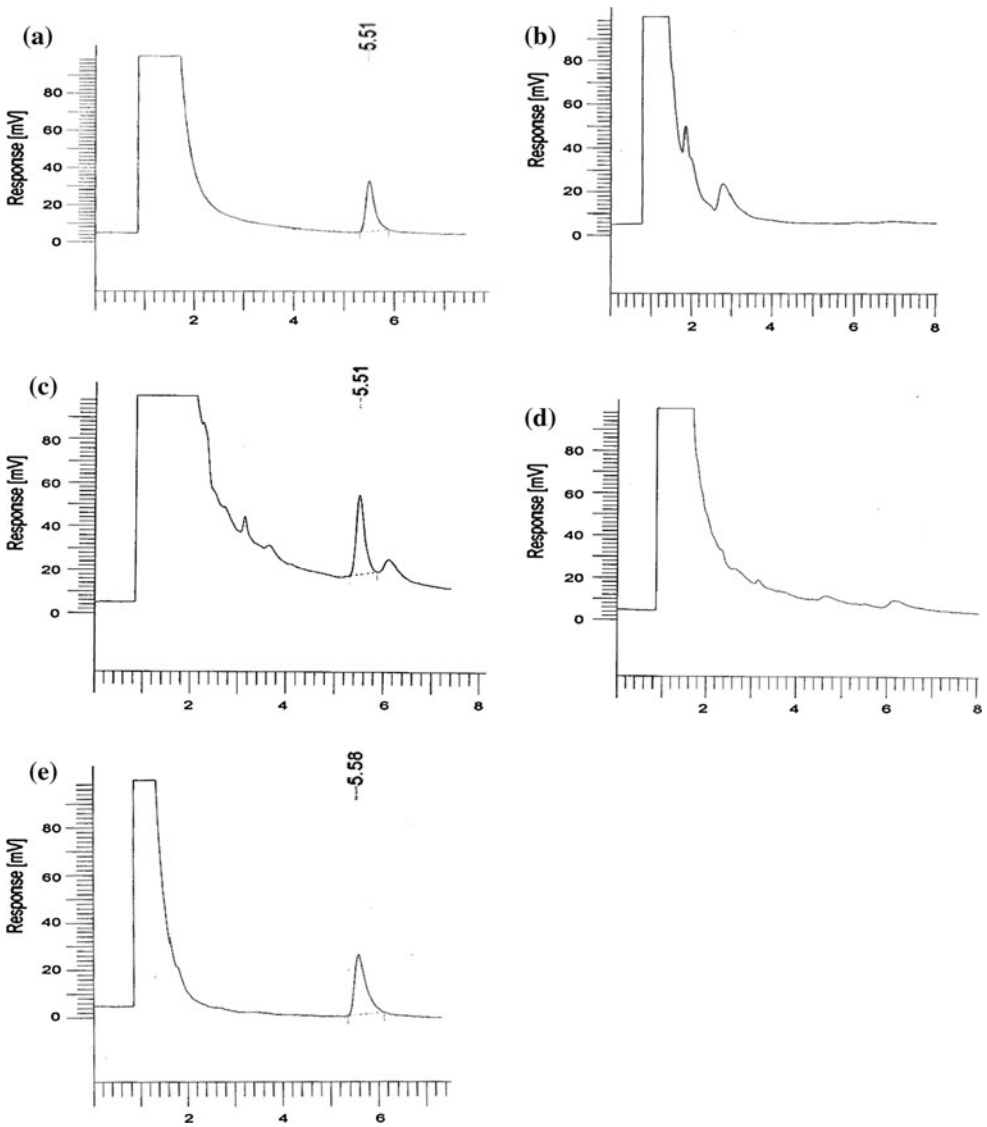


**Fig. 2** GC Chromatograms **a** Indoxacarb 0.02 ng standard, **b** Untreated cabbage sample, **c** Spiked cabbage sample, **d** Untreated soil sample, **e** Spiked soil sample

estimated by comparison of peak height/peak area of standards with that of the unknown or spiked samples run under identical conditions.

The confirmation of indoxacarb was done by GC coupled with mass detector (Fisons MD-800, quadrupole mass detector) equipped with capillary column (GCMS-QP 2010 plus, Shimadzu, Rtx-5 Sil MS). Helium was used as a carrier gas with flow rate of 1 $mL^{-1}$. The injector temperature was maintained at 285°C and oven was in temperature programming from 200°C (4 min hold) to 280°C at the rate 10°C $min^{-1}$ (10 min hold). Injection volume was 1μL in split less mode. The samples were injected and ionized using electron ionization (EI) mode. The compounds were identified both in total scan and SIM mode based on m/z ratio. The mass spectra of standard indoxacarb showed the most abundant ions at *m/z* 249, 293, 496, 529 and base peak at 529. These values of mass ions were compared with the cabbage samples spiked with indoxacarb and cabbage samples collected from treated plots for confirmation of indoxacarb residues.

In order to estimate the efficiency of the method, a recovery experiment was conducted by fortifying untreated sample (cabbage and soil) with analytical grade indoxacarb standard at the rate of 0.1, 0.05, and 0.01 mg $kg^{-1}$ as per the methodology described above (Table 1, Fig. 2). Satisfactory results were achieved at three spiking levels with recoveries ranges between 83.93% and 97.0%.

Half-scale deflection was obtained for 0.05 ng indoxacarb. A total of 15 g cabbage sample was extracted, cleaned up using QuEChERS method. An aliquot of 1 μL (equivalent to 1 mg of sample) when injected did not produce any background interference at the retention time of standard indoxacarb. Thus, limit of quantification (LOQ) was found to 0.01 mg $kg^{-1}$ and limit of detection (LOD) being 0.003 mg $kg^{-1}$.

The precision of this analytical method was determined by repeatability and reproducibility studies and expressed by and RSD values. Variation in batch recovery and repeatability (RSD) of spiked indoxacarb in cabbage samples at the levels of 0.01, 0.05 and 0.10 mg $kg^{-1}$ are summarized in Table 1. The reproducibility of this analytical method was determined by analyzing spiked samples under various test conditions (different analysts and different days). The RSD was measured by comparing the SD values of the recoveries from spiked samples analyzed the same day. The RSD values, determined by three analysts at spiking level of 0.1 mg $kg^{-1}$ were within 15%.

## Results and Discussion

The residual data at different day's intervals, dissipation pattern percentage, and half-life values in cabbage heads for indoxacarb has been presented in Table 2. Following the three applications of Avant 14.8EC @ 52.2 g a.i. $ha^{-1}$ resulted in the initial deposits of 0.18 mg $kg^{-1}$ in cabbage. The same formulation when applied @ 104.4 g a.i. $ha^{-1}$, the initial residues of indoxacarb detected was 0.39 mg $kg^{-1}$. These deposits dissipated to 0.04 and 0.11 mg $kg^{-1}$ after 3 day of their application, thereby, showing a loss of about 78 and 72%, respectively at single and double dosages. The residues of indoxacarb on cabbage reached below the LOQ of 0.01 mg $kg^{-1}$ in 7 days at recommended and 10th day for double the recommended dosage, respectively. In the untreated control samples the residues of indoxacarb were remained below LOQ of 0.01 mg $kg^{-1}$. Soil samples collected after 15 days did not reveal the presence of indoxacarb residue at the LOQ level of 0.01 mg $kg^{-1}$.

Half-life values calculated from the best fit lines of the log of residual concentration vs time period, suggested first order reaction kinetics with respect to dissipation of residues of indoxacarb (Fig. 3). Half-life ($T_{1/2}$) of indoxacarb calculated as per Hoskins (1961) was observed to be 2.88 and 1.92 days, respectively when applied @ 52.2 and 104.4 g a.i. $ha^{-1}$.

The results are in agreement with Sinha et al. (2010) who studied the dissipation behavior of indoxacarb (Avant 14.5 SC) on brinjal and reported initial deposits of 0.11 and 0.209 μg $g^{-1}$ following three applications at the rate of 70 and 140 g a.i. $ha^{-1}$. The residues dissipated with half-life of 1.6–2.3 days. Gupta et al. (2009) reported the initial

**Table 2** Residues of indoxacarb (mg $kg^{-1}$) found in cabbage and soil at different times after the application of Avant 14.8 EC @ 52.2 and 104.4 g a.i. $ha^{-1}$

| Days after application | $T_1^a$ Mean ± SD (mg $kg^{-1}$) | $T_2^b$ Mean ± SD (mg $kg^{-1}$) |
|---|---|---|
| Before application | BDL | BDL |
| 0 | 0.18 ± 0.01 | 0.39 ± 0.008 |
| 1 | 0.13 ± 0.003 (27.8)[c] | 0.26 ± 0.02 (33.3)[c] |
| 3 | 0.04 ± 0.004 (77.7)[c] | 0.11 ± 0.005 (71.8)[c] |
| 5 | 0.01 ± 0.002 (94.4)[c] | 0.03 ± 0.002 (92.3)[c] |
| 7 | BDL (100)[d] | 0.01 ± 0.002 (97.4)[c] |
| 10 | – | BDL (100) |
| 15 | – | – |
| Soil samples after 15 days | – | – |
| $T_{1/2}^e$ | 2.88 | 1.92 |

[a] $T_1$ Avant 14.8 EC @ 52.2 g a.i.$ha^{-1}$

[b] $T_2$ Avant 14.8 EC @ 104.4 g a.i.$ha^{-1}$

[c] () Per cent dissipation after spraying

[d] *BDL* Below determination limit of 0.01 mg $kg^{-1}$

[e] $T_{1/2}$ = Half-life time

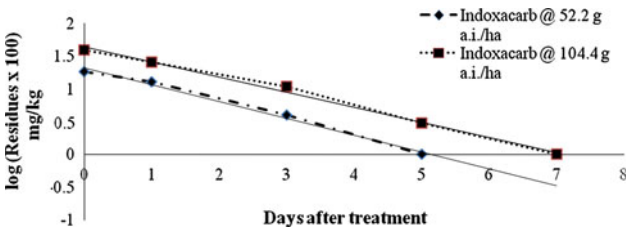


**Fig. 3** Semi-logarithm graph showing dissipation kinetics of indoxacarb on cabbage. Regression equation $y = -0.299x + 1.639$ (single dose) and $y = -0.256x + 1.319$ (double dose)

deposits of 0.26 and 0.67 μg $g^{-1}$, respectively, when indoxacarb was applied at 70 and 140 g a.i. $ha^{-1}$ on okra. Thereafter, analysis of samples collected 10 days after application did not reveal the presence of indoxacarb at 0.01 mg $kg^{-1}$.

The maximum residue limit (MRL) of indoxacarb on cabbage has been prescribed as 3.0 mg $kg^{-1}$ (Anonymous 2009b). As per the Prevention of Food Adulteration Act 1954, maximum residue limit for indoxacarb is 0.2 mg $kg^{-1}$. Following third application @ 52.2 g a.i. $ha^{-1}$, the initial deposits of indoxacarb on cabbage were found to be below the MRL. Therefore, the present investigations suggest a waiting period of one day for the safe consumption of cabbage.

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