Journal of Crop and Weed, 15(1): 213-217 (2019)

Rapid and consistent QuEChERS based RP-HPLC methodology for the detection of various insecticides in cabbage and cauliflower

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Received : 14-08-2018 ; Revised : 26-11-2018 ; Accepted : 08-01-2019

ABSTRACT

A rapid and reliable reverse phase high performance chromatograph (RP-HPLC) method was developed for the instantaneous detection of insecticides residues of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and cypermethrin in cabbage and cauliflower. Modified QuEChERS method with the inclusion of primary secondary amine (PSA) sorbent and grafitized carbon black were used for extraction and clean-up process. The separation of insecticides was performed by C_{18} column with UV-VIS detector. The dual pump functions as isocratic flow of mobile phase of acetonitrile and water (90:10, v/v) with the flow rate of 0.5 mL min^{-1}. The peaks of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and Cypermethrin A, Cypermethrin B and Cypermethrin C in chromatograms observed at retention times of 2.66 min, 3.25 min, 3.54 min, 4.03min, 4.17 min, 8.63 and 9.94, 10.46 and 10.67 mins respectively. The average recoveries were found above 80 per cent for all the insecticides in both the vegetable samples when the samples were spiked at different levels of 0.05, 0.10, 0.25, 0.50 and 1.00 mg kg^{-1} levels.

***Keywords*:** Cabbage, cauliflower, cypermethrin and fipronil

The cole crops usually thrive best on cool climate. They are grown in the plains during the winter season whereas in hilly regions, it can be grown throughout the year. In India, cabbage and cauliflower were the only cole crops among Brassica vegetables, grown on commercial scale (Chatterjee, 1986) [1]. In North eastern states like Manipur, the cole crops are grown almost throughout the year and add a good contributor in the economy of vegetable growers. The sub-tropical monsoon type of climate found in Manipur has four different seasons namely the cold or winter season, the hot-dry season or spring-summer, the rainy season, and the retreating monsoon season. The state has an ambient temperature which is generally varies from a minimum range of 3.5 to 21°C and maximum of 22 to 32°C with the relative humidity ranging between 48 and 82 per cent. The state receives south-west monsoon in an annual rainfall of approximately 1436 mm in the plains. The agricultural economy of the country is contributed by the growing of different types of vegetable crops. To get the higher yields in different parts of the country, adoption of modern agricultural practices like use of high yielding varieties, heavy manuring and proper irrigation were practiced. Being succulent, the problems of insect pests attacking at different stages of crop growth are very high (Sachan and Gangwar, 1980). Fifty one insect pests have been reported to attack on cruficers (Lal, 1975). Among the insect pests, cabbage butterfly, diamond back moth, cabbage aphid are the most persistent in Meghalaya, Manipur, Mizoram and Sikkim. Different types of pesticides are using for increasing the crop production in north eastern states. The Manipur state consumed pesticide at the rate of 26.2 metric t $acre^{-1}$ in the year 2012 (Envis, 2015). At the same time, there is a rising public concern about the potential adverse effects of chemical pesticides on the human health, environment and biodiversity. Use of pesticide in crop production is a common incident, regular monitoring of residues levels of pesticides in food commodities is of vital importance to human being. The presence of residues of organochlorine, organophosphorus and synthetic pyrethroid insecticides on market samples of brinjal were found in Manipur (Singh *et al.*, 2010). The moderately toxic insecticides like cypermethrin, imidacloprid, profenofos, chlorpyrifos, propineb, dichlorvos were used in tomato crops (Shovarani *et al.*, 2015). It is well known that the pesticide application in crops represents a possible risk for the environment, farmers and consumers. Knowing the level of pesticides residues in vegetable crops especially cole crops is very essential as cabbage and cauliflower may consumed directly without much processing. There are no informations available on the nature and quantity of pharmacologically active compounds i.e. the active ingredients of different insecticides in cabbage and cauliflower which is necessary to ensure the safety of the consumers and the environment. Therefore, the method was developed for the estimation of various insecticides in cabbage and cauliflower.

The experiment was conducted at Indian Council of Agricultural Research-Research Complex for North Eastern Hill region Manipur Centre, Lamphelpat, India in January, 2018. The technical grade analytical standards of insecticides such as imidacloprid (99.9%), carbofuran (98.0%), chlorantraniliprole (99.20%), fipronil (97.5%), malathion (98.0%), chloropyriphos (98.0%) and

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cypermethrin (94.3%) and primary secondary amine (PSA) sorbent were obtained from sigma-aldrich, Kolkata. Chemicals such as sodium chloride, activated anhydrous magnesium sulphate ($MgSO_4$), anhydrous sodium sulfate and solvents like high-performance liquid chromatography (HPLC) grade acetonitrile, HPLC grade water were obtained from E. Merck (India) Ltd, Mumbai, India. All the solvents used in the experiment were of laboratory grade and were redistilled in all glass apparatus before experiment. The suitability of the solvents and other chemicals was ensured by running reagent blanks before real analysis.

The reverse-phase high-performance liquid chromatograph (series 200) was equipped with Brownlee analytical C18 column and a UV-VIS detector with dual pumps supplied by M/S Perkin Elmer, United States. The HPLC column, a Brownlee Analytical C18 column (150 mm column length, 4.6 mm inside diameter and 5 µm particle size) was also procured from M/S Perkin Elmer. For the control of instrument, data acquisition and processing, TC Nav software supplied from Perkin Elmer was also used. A good satisfactory separation of peak symmetry was obtained with an isocratic mobile phase comprising of acetonitrile : water (90 : 10, v/v) at a ûow rate of 0.5 ml min^{-1}. Quantiûcation was achieved with UV-VIS detection at 225 nm based on peak area with a retention factor of 10 min and injection volume of 20 µL.

A standard stock solution of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and Cypermethrin A, Cypermethrin B and Cypermethrin C (1 mg ml^{-1}) was prepared in HPLC grade acetonitrile. For the construction of a calibration curve (2.00, 5.00, 10.00, 15.00 and 20.00 ppm) the standard solutions required were prepared from stock solution by serial dilution with HPLC grade acetonitrile. All standard solutions were stored at 4°C before experiment.

Cabbage and cauliflower samples were used as substrates for standardization of the methodology proposed for estimation of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and cypermethrin. Cabbage and cauliflower treated to be control samples were collected a known source where there is no history of pesticides application. The cabbage and cauliflower samples were fortified at different levels, *i.e*., 0.05, 0.10, 0.25, 0.50 and 1.00 mg kg^{-1}. There were ûve replications for each treatment of both the samples.

The quick easy cheap effective rugged and safe (QuEChERS) method was modified by taking a representative sample of 15 g each of blended cabbage and cauliflower samples were weighed separately into 50 mL centrifuge tube. After that 30 ml of acetonitrile was poured into all the centrifuge tubes. The samples were vigorously shaken. Sodium chloride (10 g) was added to each sample and shaken dynamically by rotospin for around 5 min. The samples were centrifuged using a laboratory REMI centrifuge for 3 min at 6000 rpm. The top 15 mL organic layer from each of the 15 mL tube was decanted into another 50 mL centrifuge tube which was weighed with 10 g of activated sodium sulfate. It was again shaken using a rotospin for 2 min. The sample extract (6 mL) was transferred to a 15 ml centrifuge tube containing PSA sorbent (150 mg) and activated anhydrous magnesium sulfate (900 mg). The tube was tightly capped and vortexed for 30 s. The tubes were centrifuged for 3 min at 3000 rpm. The top extract (4 mL) was transferred into a test tube and concentrated to 2 mL with a rotary evaporator under 35°C for further quantification by HPLC.

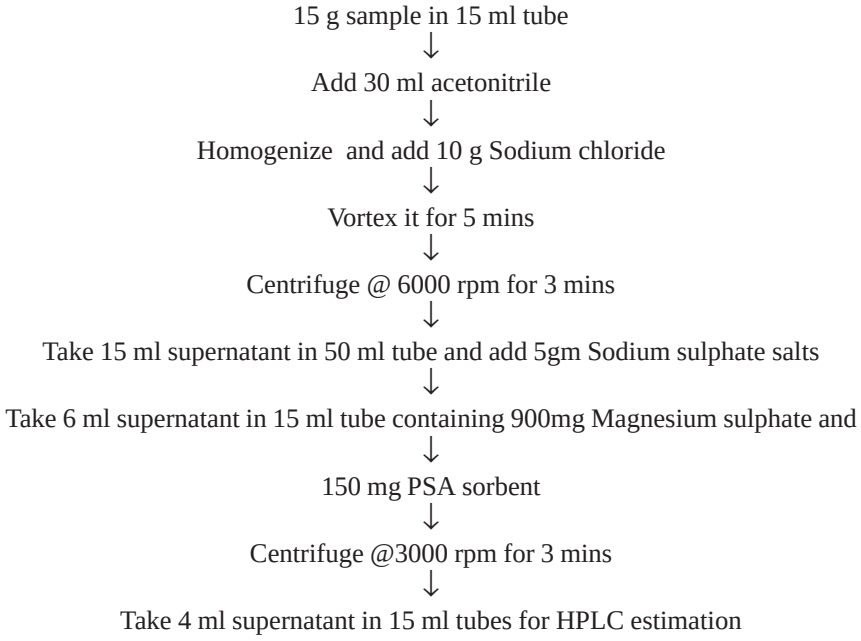

Table 1: Recovery (%) of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and cypermethrin A, cypermethrin B and cypermethrin C in cabbage (n=5, mean ± standard deviation)

Level of fortification (mg kg^{-1})	Imidacloprid	Carbofuran	Chlorantraniliprole	Fipronil	Malathion	Chloropyrifos	Cypermethrin A	Cypermethrin B	Cypermethrin C
0.05	80.24±0.45	90.96±2.56	87.70±2.00	82.10±3.97	80.24±1.89	90.33±2.67	85.51±1.72	92.26±1.98	92.19±1.71
0.10	96.62±0.53	88.76±3.89	82.45±2.67	91.19±1.06	80.55±1.76	85.17±1.65	85.92±3.40	84.05±2.02	80.56±2.00
0.25	90.67±0.90	82.34±1.38	81.10±3.05	81.18±0.97	85.10±2.63	85.78±1.67	90.56±2.22	90.07±3.51	85.53±2.06
0.50	96.10±2.92	85.10±1.79	83.32±1.98	86.64±2.08	88.90±3.89	86.45±0.87	80.56±2.14	91.46±2.10	92.88±2.45
1.00	96.21±2.08	89.90±1.80	85.45±3.03	97.56±3.00	86.56±2.04	92.34±0.95	86.60±0.88	84.40±1.34	81.80±1.70

Table 2: Recovery (%) of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and cypermethrin A, cypermethrin B and cypermethrin C in cauliflower (n=5, mean ± standard deviation)

Level of fortification (mg kg^{-1})	Imidacloprid	Carbofuran	Chlorantraniliprole	Fipronil	Malathion	Chloropyrifos	Cypermethrin A	Cypermethrin B	Cypermethrin C
0.05	90.64±2.78	89.90±3.29	88.60±2.67	80.54±0.43	81.12±3.78	93.27±0.65	89.90±0.91	90.45±0.76	92.30±2.56
0.10	98.80±1.65	89.62±2.09	87.07±1.65	90.08±2.28	97.56±0.96	88.14±0.43	82.14±0.77	85.45±0.68	83.65±2.90
0.25	80.36±1.56	84.48±2.41	82.02±4.00	86.64±1.00	82.26±1.86	87.72±2.08	96.65±2.43	94.55±2.77	96.10±0.58
0.50	98.80±0.35	87.42±1.66	82.21±1.97	87.76±1.98	86.60±4.04	86.08±2.55	90.00±0.18	94.44±1.65	92.25±0.91
1.00	92.20±3.08	87.77±0.38	82.12±2.00	98.80±2.35	82.29±1.63	86.62±1.91	84.42±1.09	85.52±2.89	90.18±2.74

Reversed-phase HPLC equipped with UV-VIS detector, was shown to be good for determination of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and cypermethrin because no need for derivatization step. Chromatographic separation in Brownlee analytical C18 columns provides good result. The detection at 225 nm provides suitable chromatograms for the quantiûcation of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and cypermethrin in real samples. Under the preferred conditions, imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and Cypermethrin A, Cypermethrin B and Cypermethrin C showed retention factors of 2.66 min, 3.27 min, 3.58 min, 4.03min, 4.17 min, 8.63 and 9.94, 10.46 and 10.67 min, respectively (Fig. 1). The recovery percentage of spiked samples of cabbage at different levels were found to be in the range of 80.24 to 96.62 % for imidacloprid, 82.34 to 90.96 % for carbofuran, 81.10 to 87.70 % for chlorantraniliprole, 81.18 to 97.56 % for fipronil, 80.24 to 88.90 % for malathion, 85.17 to 92.34 % chloropyriphos, 80.56 to 90.56 % for cypermethrin A, 84.05 to 92.26 % for cypermethrin B and 80.56 to 92.88 % cypermethrin C (Table 1). Similarly, in spiked samples of cauliflower, the recovery range were all above 80 % in all the levels (Table 2).

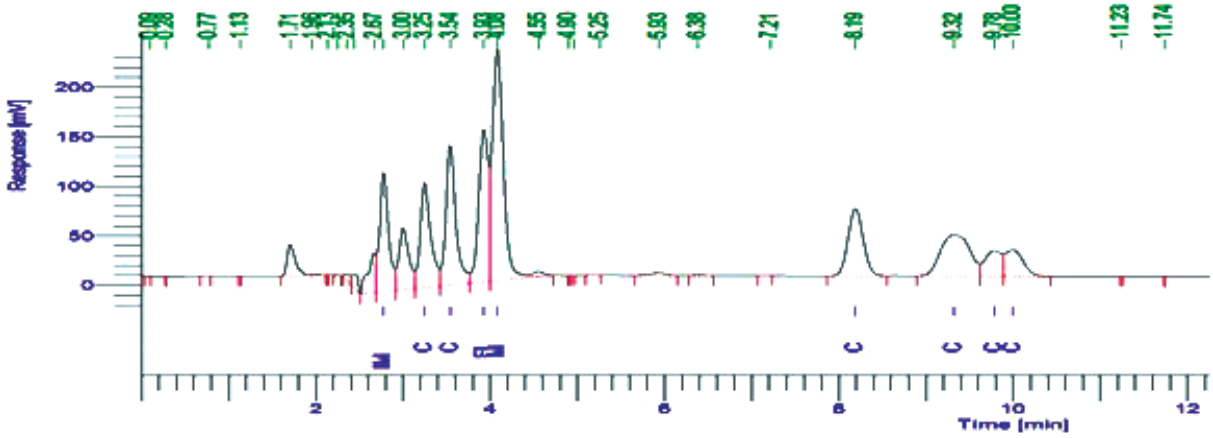

Fig. 1: HPLC chromatograms of standards of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and cypermethrin A, cypermethrin B and cypermethrin C in which retention times on x-axis and % deflection on y-axis.

A very fast and simple cost-effective reversed-phase HPLC method coupled with modified QuEChERS has been developed for the determination of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyriphos and cypermethrin. The consistent recoveries found in cabbage samples ranging from 80.24 to 97.56 per cent and in cauliflower, the it was ranged from 80.36 to 98.90 per cent for all the insecticides when both the samples were fortified at 0.05, 0.10, 0.25, 0.50 and 1.00 mg kg^{-1} levels. The present QuEChERS method is rather effective and provides the quickest, easy and cheap method as compared to other methods. In 2010, pesticide residues of chlorpyrifos and cypermethrin were found in Chinese cabbage and cauliflower (Szpyrka et al., 2011). A modified QuEChERS method was also developed for single stroke analysis of 86 multiclass pesticides including chloropyriphos and malathion in pineapple juice using Gas chromatography tandem mass spectrometry (GC-MS/MS) with the average recovery values within 70 - 120 % (Das *et al*., 2018). To evaluate multi-residual dynamics of the pesticides like chlorpyrifos, dimethoate, cyhalothrin, cypermethrin, fenvalerate, deltamethrin and chlorothalonil in the spring cabbage, *Brassica oleracea* L. var. *capitata*, a method was developed. The half-lives of the seven pesticides in the vegetable were 2.0, 1.6, 1.6, 2.3, 2.2, 1.5 and 1.8 days, respectively. If the cabbages were treated at normal recommended dosage of pesticides, and all pesticides were not exceeded the maximum residue limits (MRLs) according to the recommended pre-harvest interval (Zhang *et al*., 2007).

ACKNOWLEDGMENT

The authors are thankful to the Director, ICAR Research Complex for North Eastern Hill region, Manipur Centre, Lamphelpat, India, for providing the necessary research facilities.

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