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RP-HPLC Method for Determination of Organophosphate and Synthetic Pyrethroids Insecticides

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A B S T R A C T

A modified QuEChERS method for simultaneous detection of monocrotophos, deltamethrin, phosphamidon and dichlorovos insecticides in cabbage and cauliflower samples was developed. The detection was accomplished with the use of reverse phase high performance chromatograph (RP-HPLC) coupled with C_{18} column with UV-VIS detection system. The method employs the primary secondary amine (PSA) sorbent and activated charcoals were used for extraction and clean-up process. The quantification of monocrotophos and deltamethrin was done with an isocratic flow of 1.70 mL min^{-1} of mobile phase of acetonitrile and water (90:10, v/v) at 220 nm wavelength. The insecticides phosphamidon and dichlorovos was separated with an isocratic flow of 1.50 mL min^{-1} acetonitrile and water (70:30, v/v) at 210 nm wavelength. The peaks of monocrotophos and deltamethrin were depicted at retention times of 0.76 min and 2.85 min and the peaks of phosphamidon and dichlorovos was found at 1.10 min and 1.37 mins. The recovery percentage found at different levels of 0.05, 0.10, 0.25, 0.50 and 1.00 mg kg^{-1} levels were above 80 % for all the insecticides.

Introduction

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). 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%. 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). A total of 51 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 tones per acre 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 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 simultaneous estimation of monocrotophos, deltamethrin, phosphamidon and dichlorovos insecticides in cabbage and cauliflower.

Materials and Methods

The experiment was conducted at Indian Council of Agricultural Research-Research Complex for North Eastern Hill region Manipur Centre, Lamphelpat, India.

The technical grade analytical standards such as monocrotophos (99.8%), deltamethrin (99.90%), phosphamidon (94.80%) and dichlorovos (99.80%) and primary secondary amine (PSA) sorbent were procured from sigma-aldrich, Kolkatta (Fig. 1). 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 were of laboratory grade and were redistilled in all glass apparatuses before experiment. The suitability of the solvents and other chemicals was ensured by running reagent blanks before sample analysis.

The high-performance liquid chromatograph (series 200) was equipped with a reverse-phase, RP, C18 column and a UV-VIS detector, and 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 was 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 flow rate of 1.70 mL/min for method 1. In this, quantification of monocrotophos and deltamethrin was done with UV-VIS detection

at 225 nm based on peak area with a retention factor of 10 min and injection volume of 20 µL. Another method 2, the quantification of phosphamidon and dichlorvos was achieved with an isocratic mobile phase comprising of acetonitrile : water (70 : 30, v/v) at a flow rate of 1.50 mL/min with UV-VIS detection at 210 nm wavelength based on peak area with a retention factor of 10 min and injection volume of 20 µL.

A standard stock solution of monocrotophos, deltamethrin, phosphamidon and dichlorvos (1 mg/mL) was prepared in HPLC grade acetonitrile. For the construction of a calibration curve, the standard solutions 2.00 to 20.00 mg/mL required were prepared from stock solution by serial dilution with HPLC grade acetonitrile. Prior to experiment, all the standard solutions were stored at 4°C.

Cabbage and cauliflower samples were used as substrates for standardization of the methodology proposed for estimation of monocrotophos, deltamethrin, phosphamidon and dichlorvos insecticides. 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. There were five replications for each treatment of both the samples.

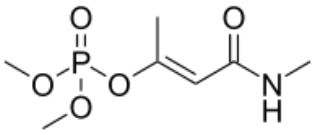
The quick easy cheap effective rugged and safe (QuEChERS) method was modified by taking a representative sample of 15 g each of blended cabbage and cauliflower samples were weighed separately into 50 mL centrifuge tube. After that 30 mL of acetonitrile was poured into all the centrifuge tubes. The samples 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.

Results and Discussion

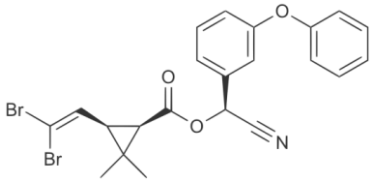
Reversed-phase HPLC equipped with UV-VIS detector, was shown to be good for determination of monocrotophos, deltamethrin, phosphamidon and dichlorvos because no need for derivatization step. Chromatographic separation in Brownlee analytical C18 columns provides good result. The detection at 225 nm and 210 nm provides suitable chromatograms for the method 1 i.e. monocrotophos and deltamethrin and method 2 i.e. phosphamidon and dichlorvos in real samples. Under the preferred conditions monocrotophos and deltamethrin showed retention times at 0.76 min and 2.85 min and the peaks of phosphamidon and dichlorovos was found at 1.10 min and 1.37 mins of retention factor (Fig. 2).

The recovery percentage of spiked samples of cabbage at different levels were found to be in the range of 82.28 to 97.75 % for monocrotophos, 85.15 to 93.16 % for deltamethrin, 80.38 to 95.38 % for phosphamidon and 83.23 to 98.26 % for dichlorvos (Table 1). Similarly, in spiked samples of cauliflower, the recovery ranges were all above 80 % in all the levels (Table 2).

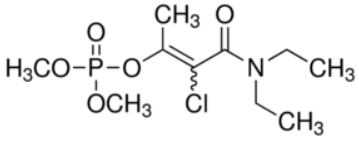
Fig.1 Chemical structure of (a) monocrotophos (b) deltamethrin (c) phosphamidon and (d) dichlorvos



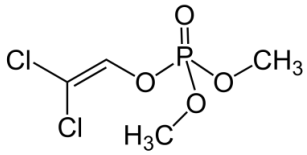
(a) Monocrotophos



(b) Deltamethrin



(c) Phosphamidon



(d) Dichlorvos

Fig.2 HPLC chromatograms of standards of monocrotophos and deltamethrin in which retention times on x-axis and % deflection on y-axis

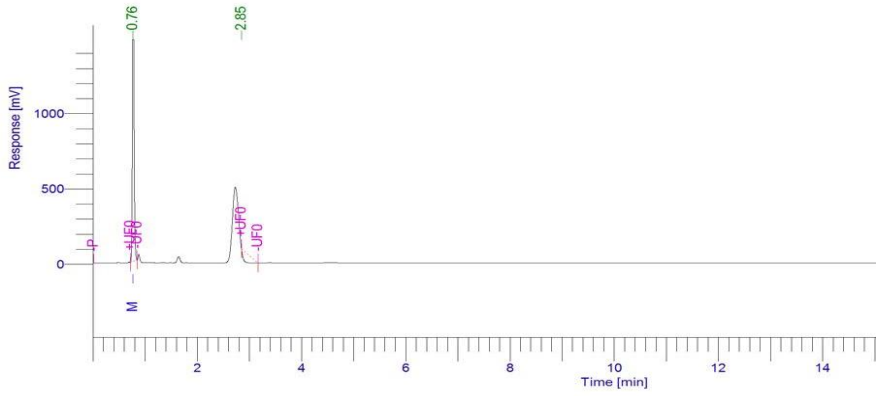


Fig.3 HPLC chromatograms of standards of phosphamidon and dichlorvos in which retention times on x-axis and % deflection on y-axis

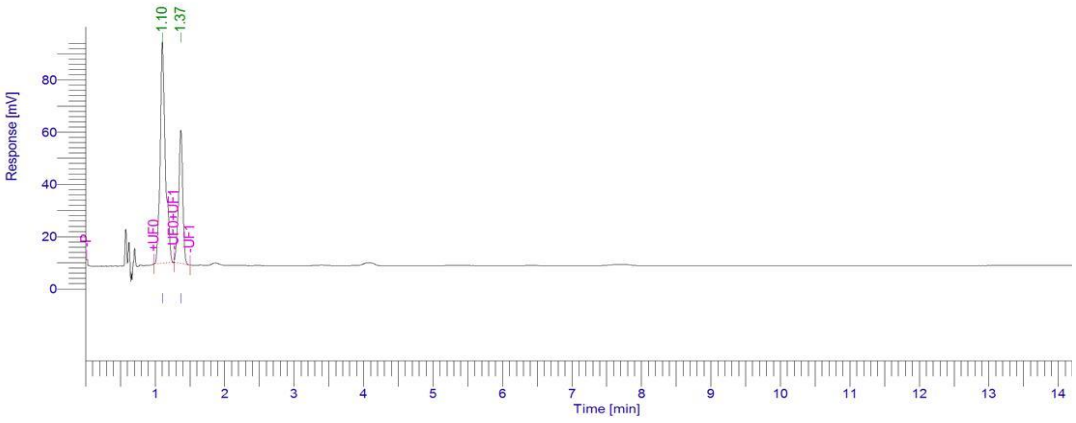


Table.1 Recovery (%) of monocrotophos, deltamethrin, phosphamidon and dichlorvos in cabbage (n=5)

Level of fortification (mg kg^{-1})	Monocrotophos	deltamethrin	phosphamidon	Dichlorvos
0.05	82.28±2.98	86.28±0.97	87.56±2.90	98.26±1.78
0.10	94.40±1.29	85.15±1.76	95.38±3.02	86.60±0.65
0.25	84.13±0.36	90.28±1.08	83.20±1.90	83.23±1.45
0.50	88.27±0.27	87.78±3.00	80.38±2.05	91.79±0.65
1.00	97.75±1.04	93.16±2.87	92.37±0.69	90.65±0.65

[a]Mean ± Standard deviation

Table.2 Recovery (%) of monocrotophos, deltamethrin, phosphamidon and dichlorvos in cauliflower (n=5)

Level of fortification (mg kg^{-1})	Monocrotophos	deltamethrin	phosphamidon	Dichlorvos
0.05	90.45±2.09	85.38±1.20	86.20±3.18	92.12±0.97
0.10	96.12±0.67	82.10±2.08	90.15±1.56	88.29±1.66
0.25	88.28±1.65	97.29±0.57	84.75±2.67	85.29±2.76
0.50	80.98±3.17	90.97±2.87	87.46±3.00	94.20±1.73
1.00	91.76±1.57	97.10±1.66	94.28±1.76	97.16±1.69

[a]Mean ± Standard deviation

A very fast and simple cost-effective reversed-phase HPLC method coupled with modified QuEChERS has been developed for the determination of monocrotophos, deltamethrin, phosphamidon and dichlorvos insecticides (Fig. 3). The consistent recoveries found in cabbage samples ranging from 80.38 to 98.26 % and in cauliflower, it was ranged from 80.98 to 97.29 % for all the insecticides when both the samples were spiked at 0.05, 0.10, 0.25, 0.50 and 1.00 mg/kg levels. The present QuEChERS method is rather effective and provides the quickest, easy and cheap method as compared to other methods.

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