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Multi Residue Evaluation Protocol for Coriander (*Coriandrum sativum*) Seeds

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Abstract

Along with the means for optimum crop production, the management of persistent pesticide residues as a result of application of pesticides in the produce is also a major challenge being faced now a days due to stringent quality control norms implemented by various agencies. To attend this issue the first step was to design / modify and validate a protocol that is quick, easy, cheap, effective, rugged and safe (QuEChERS). In the present study a modified multi residue QuEChERS method for estimation of pesticide residue in coriander samples from field trials at NRCSS, Tabiji, Ajmer was developed and validated for commonly used pesticides incorporated as carbamates, neonecotinides, pyrethroids and some broad spectrum insecticide groups. The results were verified and the MRL values for most of the insecticides were found to be at par within the prescribed safety limits.

Keywords: QuEChERS, coriander, maximum residue levels, GC MS-MS, pesticides and insecticides

Introduction

Coriander (*Coriandrum sativum* L.) is one of the main export commodities after cumin from India. It is cultivated as a *annual* crop during winter in arid and semi-arid parts of Rajasthan, Gujarat and other parts of the country. The crop faces severe management issues from fungal diseases such as wilt, blight, powdery mildew, damping off and insect infestation by aphids and thrips in moderate to severe form. A broad array of fungicides and insecticides are used to control these diseases by the growers and often these control agents are used injudiciously. Being an export commodity the importers are very much conscious about the residue levels of various insecticides and pesticides and a stringent rider has been imposed in terms of maximum residue levels by the various controlling agencies such as the European Union Quality control Agency and *Codex Alimentarious*. An attempt has been made to derive and standardise a Gas Chromatography Mass Spectrometer- Mass Spectrometer (GC MS-MS) supported by tandem LC MS-MS multi residue methodology for simultaneous evaluation of insecticides belonging to organophosphates, carbamates, neonecotinides, pyrethroids and some broad spectrum insecticides used in the coriander cultivation experimental trials at the ICAR- National Research Centre on Seed Spices (NRCSS), Tabiji, Ajmer (Mastovasta *et al*. 2010).

Materials and methods

Experimental set up

An experiment was designed for testing some insecticide molecules mentioned below towards their control effiacy against sucking pests and borer pest population at the different crop stages. A spray schedule with set of insecticides was applied on coriander (Var ACr-1). The crop was sown at the experimental field of ICAR-NRCSS, Tabiji, Ajmer on 30th October, 2014. (Table 1). Two sprays of the insecticides were carried out at 15 days interval depending upon the onset of insect arrival on the commodity *i.e.* coriander. The quantity of insecticide residues present in the harvested produce was estimated as per the methodology mentioned below in detail and the validity of the protocol was also ascertained.

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Table 1. Treatment schedule for spraying insecticides (15 days interval)

S.No	Treatment for sucking pests
1.	Carbosulfan @ 0.03%
2.	Dimethoate@0.04%
3.	Carbofuran @ 1.0kg ai ha^{-1}
4.	Thiacloprid @ 0.24 %
	Treatment for borer pests
5.	Imidacloprid @ 0.005 %
6.	Triazophos @0.05 %
7.	Chlorantranilipole @ 20si, 20 gm ai ha^{-1}
8.	Deltamethrin @ 0.003 %
9.	Carbosulfan @ 1.0 kg ai ha^{-1} ATS
10.	Phorate @ 1.0 kg ai ha^{-1} ATS
11.	Clothianidin 0.5 kg ai ha^{-1} ATS
12.	Carbofuran @ 1.0 kg ai ha^{-1} ATS

Pesticides and solvents

The analytical grade (>99 %) pesticides namely carbosulfan, dimethoate, carbofuran, thiacloprid, imidacloprid, triazophos, chlorantranilipole, deltamethrin, phorate and clothianidin were procured from M/S Sigma Aldrich. Solvents were purified by glass distillation. The glasswares were pre washed with chromic acid solution and rinsed with acetone in order to avoid contamination (Banerjee *et al*, .2007 and Anastassiades *et al*., 2003),.

Preparation of standard solutions

Stock solutions (1000 ppm) for each pesticide were prepared in acetone and 5 ml of individual stock solution (1000 ppm) was diluted in 100 ml volumetric flask with distilled hexane to obtain 50 µg ml^{-1} pesticide mixture stock solutions. Working standard solutions of 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005, and 0.001 µg/ml were prepared by serial dilutions with hexane.

Standardization of GC MS-MS

Gas chromatograph (Thermo TSQ 8000 Evo) equipped with Triple quad mass spectrometer as detector was used for the analysis. Oven temperature program with an incremental rate of 10°C min^{-1}, temperature of 65-320 (°C), hold time - 1.5 -5 (min) was used. The injection parameters with splitless, injection mode split flow -10 ml min^{-1}, flow rate -1 ml min^{-1}, sample volume-2.5 µl, ion source temperature – 280(°C) and MS transfer line temperature of 310 (°C) was used.

Sample history

Coriander samples harvested from the experimental field trials conducted at the ICAR-National Research Centre on Seed Spices (NRCSS), Tabiji, Ajmer were processed for the analysis of pesticide residue status.

Extraction technique

Two gram of powdered seeds was soaked for 30 minutes in 8 ml of chilled distilled water in 50 ml capacity centrifuge tube. After completion of soaking time, 10 ml acetonitrile + 4 mg magnesium sulphate + 1 gm sodium chloride was added and the sample mixture was vortexed for 120 seconds. This mixture was then centrifuged at 5000 rpm for 5 minutes. Four milli litre of the supernatant from this stage was subjected to clean up operations so as to remove the unwanted impurities and moisture by treating with 25 mg PSA + 150 mg magnesium sulphate + 100 mg C-18 bulk adsorbent. The contents were centrifuged in micro centrifuge tubes at 3500 rpm (Utture *et al*., 2012). About 2.0 ml of the aliquot (equivalent to 0.6 g sample) was taken in 15 ml test tube and evaporated to dryness in Turbovap concentrator. Later a final volume of 1.0 ml was made by adding the mixture of methanol: water (1:1, v/v) + 0.1% acetic acid in water so as to buffer the pH. One ml of this supernatant was used for analysis of the targeted pesticides in the coriander samples with GC MS-MS at APEDA laboratory of ICAR-NRC on Grapes, Pune (Steinwandter, 1985 and Philip, 2003).

Recovery and limit of quantification (LOQ)

The fortified coriander samples (10.0, 1.0 and 0.1 µg/L of each pesticide) in triplicate were processed through standardized extraction and cleanup procedure and the extract was analyzed for recovered pesticide residues. LOQ, the minimum amount of pesticide residue that can be quantified by the method was estimated. The recovery factor

for the various pesticides standards analysed was 85 to 108 %.

Standardization of analytical method

Gas chromatographic conditions, extraction and cleanup procedures were standardized to suit to multi-pesticide residue analysis of 10 pesticides in coriander seeds. The parameters of GC MS-MS for analysis were standardized for proper resolution and sensitivity of selected pesticides in the produce. The oven temperature programming as reported above was found to be suitable for analysis. The linearity range of different pesticides was between 0.001-10.00 µg ml^{-1}.

Table 2. Maximum residue level (ppm) of the insecticides in coriander samples after harvest

Sample label	Pesticide compound	Concentration (mg kg^{-1})	MRL values (mg kg^{-1}) (As per Prevention of Food Adulteration Act & Rules)
1	Carbosulfan	0.23	0.01
2	Dimethoate	0.17	0.05
3	Carbofuran	0.01	0.10
4	Thiacloprid	0.01	0.01
5	Imidacloprid	0.01	0.05
6	Triazophos	0.01	0.05
7	Chlorantranilipole	0.01	0.05
8	Deltamethrin	0.03	0.20
9	Phorate	0.02	0.05
10	Clothianidin	0.01	0.02

Results and discussion

After the analysis, results were interpreted in terms of extent of retention of various pesticides as residues in coriander samples after harvest in terms of minimum residue levels in the produce after harvest within the prescribed MRLs for coriander. The pesticides were applied in different combinations to test and evaluate their effectiveness towards various diseases and insects (sucking pests and borers) in coriander so as to derive an effective combination for further use. The residues were analysed easily and perfectly. The analytical results have been presented in the Table 2.

The results indicate that the pesticide residues in the results for the insecticides Carbosulfan and Dimethoate were slightly high above the permissible limits (Sharma, 2013). The doses for these insecticides needs to be corrected in the trials for the next year. The application of most of these insecticides for the control of sucking and borer pests in coriander crop gave desirable results, hence the use of these pest control agents can be promoted to the crop growers with the schedule followed at the trial site for their benefit after procedural registration.

Conclusion

The multi residue evaluation protocol for coriander *(coriandrum sativum* L.*)* seeds designed and explained in this article was found to be well fitting for the purpose of estimating the pesticide residues in coriander seeds. The experimental results towards the MRL values were also within the permissible limits except few (Carbosulfan and Dimethoate) which needs to be corrected in the future experiments.

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