DETERMINATION OF PESTICIDE RESIDUES IN BRINJAL FRUIT AT SUPERVISED TRIAL

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ABSTRACT

Supervised field trial of brinjal was conducted during May, 2006 to determine Chlorpyriphos, Acephate, Dichlorovos, Carbofuran and Imidachloprid residues by adopting HPTLC method. These insecticides were sprayed directly on the brinjal crop and data were recorded after 0, 3 and 7th day after application. The samples were treated with organic solvent ethyl acetate and cleaned-up by activated charcoal. All the samples were found contaminated with pesticide residues except Imidachloprid. After 0 day the quantity of pesticide residues was maximum followed by 3rd day that contained lesser amount of insecticide residue and after 7th day the quantity of these residues were negligible in the brinjal fruit which led to conclusion, that the brinjal fruit was suitable for the consumption of public after three days with out posing any hazard to human health.

Key Words: Pesticides, residues, brinjal fruit, HPTLC

INTRODUCTION

Many types of vegetables are grown in Pakistan on 225.4 thousand hectares with a production of 2879.9 thousand tones (Anonymous, 2004). Brinjal (*Solanum melongena* L.) is an important vegetable and has a serious insect pest problem in the field. The main pests that attack plants are Brinjal Fruit Borer, Stem borer, Spider Mite, Aphid, Jassid, Whitefly and Roots-cutworm. For the control of these insect pests, the farmers are used different types of insecticides. The farmer community uses the major insecticides such as Acephate, Chlorpyriphos, Dichlorovos, Carbofuran and Imidachloprid. Residues of DDT, DDE, chlorinated hydrocarbons and other pesticides have been found in different vegetables included brinjal, Salwa *et al*. (1999), Tahir *et al*. (2001), (Dogheim *et al*. (2002) Mukherjee (2003).

International deal in foodstuff and agricultural commodities is governed by agreement of the World Trade Organization (WTO). The overall intention of the WTO agreement is to consent countries to formulate legitimate measures to defend the life and vigor of their consumers. Present study is an exertion toward the WTO scenario to find out the insecticides residues in brinjal fruit by the application of above cited insecticides.

MATERIAL AND METHODS

Assortment of Samples: The samples were collected from randomly chosen brinjal plants. After collection of samples, about 1kg sample was separated, sealed in polythene bags and stored at -4^{o}C for residues analysis of carbofuran, acephate, chlorpyriphos, dichlorovos, and imidachloprid. The extraction, clean-up and analytical work was followed by Asi. *et.al*. (2002).

Extraction and Clean-up of Brinjal Samples : These brinjal samples were chopped and mix systematically. A sub-sample of 50g was taken out from the above said sample. The sample was blended for 2-3 minutes in 20ml of ethyl acetate solution. Thereafter, 20g anhydrous sodium sulphate was added into it so that sodium sulphate absorbed the remaining water present within the blended medium. The solvent layer was decanted, filtered with Whatman flute filter paper (42). Then this extract was cleaned-up by activated charcoal. This charcoal was activated at 105 ^{o}C temperature for about four hours. The cleaning material was transferred in the round bottom flask and reduced the volume upto 1 ml using Rotary evaporator (Buchi 011, Switzerland). The final volume was evaporated in water bath (40^{o}C) with nitrogen stream for dryness and reconstituted in for analysis on HPTLC plates.

High Performance Thin Layer Chromatographic Analysis (HPTLC): Pre-coated silica gel 60 glass plate (each measuring 20 x 20 cm with 0.25 mm layer thickness) was activated at 105^{o}C for 30 minutes in oven (0-250 ^{o}C, Memmert, Germany). The plate was spotted with sample along with marker compounds and developed in pre-saturated tank of ethyl acetate. After developed the plate, the extra solvent was evaporated in fume hood, and again developed the plate in other pre-saturated tank with bromine vapors (8g $KMnO_4$ + 10mL

HBr) for 30 seconds. The excess bromine was removed in fume hood for about 45 minutes and the plate was sprayed with solution of acetylcholine esterase enzyme. Blue spots with white background were appeared on silica gel plates. The distance traveled by solvent and eluted compounds was noted. R_f values of each pesticide was calculated and compared with the marker compound. The concentration of each pesticide was calculated by comparing the average spot diameter.

HPTLC Residual Analysis method: Brinjal samples were extracted by ethyl acetate and analyzed by HPTLC with enzyme inhibition horse blood serum method (acetylcholine esterase enzyme) which was very sensitive for the detection of insecticide residues. The extract was spotted on silica gel plate, which was developed in mobile phase (ethyl acetate) and spot visibility was determined after spraying with acetylcholine esterase enzyme and tris-buffer solution. R_f value and average spot diameter was measured and imidachloprid, carbofuran, acephate, dichlorovos and chlorpyriphos residue concentration in ppm was calculated comparing the standard reagent spot diameter Asi. *et.al*., (2002).

RESULTS AND DISCUSSION

The result of the present study regarding residues of imidachloprid, carbofuran, acephate, dichlorovos and chlorpyriphos analyzed after 0 day, 3 days and 7th day after spraying are given in the Table 1.

Results of the present study indicated that higher concentration of carbofuran, acephate; dichlorovos and chlorpyriphos residues were detected in all the samples of brinjal after three days of application of these aforesaid insecticides but imidachloprid residues were detected below MRLs. It was further revealed that there was negligible change in carbofuran, acephate, dichlorovos and chlorpyriphos residues in brinjal samples but minute change in residues occur after 7th day. The change in residues after 0 to 7^{th} day might be due to mismanagement or photo-degradation of pesticide residues in environment. When chlorpyriphos was exposed to sunlight, it undergo hydrolysis in the presence of water to liberate 3, 5, 6-trichloro-2-pyridinol, which undergo further decomposition to diols and triols and ultimately cleavage of the ring to fragmentary products (Smith, 1968). Relative flow value (R_f) of HPTLC method were not affected with time period shown in table 1, which were in agreement with the previously reported work of IAEA in which different HPTLC methods were validated for marker and selected compounds (Asi, *et al*., 2000; Qian and Liu 2002 and Asi, *et al*., 2002). Kadenczki *et al*. (1992) methods with a little change was followed because it was faster, less laborious, friendly to environment and less expensive. The ethyl acetate was used in this study for the extraction of pesticide residues from vegetables. Similarly the methodology involving the use of the HPTLC for the estimation of pesticide residues in food grains, vegetables, fruits, water and soil had been used by workers like Sundararajan and Chawala (1983), Ohlin (1986), Dekok and Hiemstra (1992), Pang-Goufang *et al*. (1996), Pasha *et al*. (1996) and Takatsuki *et al*. (1999).

The results showed that at 0 day (three hours after application of pesticide) just after spraying the quantity of insecticide residues was maximum followed by 3rd days that contain lesser amount of insecticide residue and after 7th day the quantity of residues of carbofuran, imidachloprid, chlorpyriphos, dichlorovos and acephate were negligible in the brinjal fruit. This led to conclusion that brinjal fruit is suitable for the consumption of public after three days of spraying with out posing any hazard to human health as MRLs adopted by Central Committee for Food Standards (CCFS) under the Ministry of Health & Family Welfare India. They recommend residue for fruits and vegetables range from 0.01-0.50 mg/kg for Chlorpyriphos and for Carbofuran 0.05-0.10 mg/kg (Chandurkar and Bhatnagar 2002).

Table 1: Quantitative analysis of residues in Brinjal fruit (ppm)

Insecticide	Insects	Insecticide residues in Brinjal fruit (ppm)*			
		Sampling Periods			
		0 day	3 days	7 days	R_f value
Carbofuran	Fruit & Stem Borer	0.084	0.039	0.026	0.05
Chlorpyriphos	Fruit/Stem Borer/Sucking Insects	0.095	0.049	0.035	0.05
Imidachloprid	Sucking Insects	0.097	0.051	0.039	0.230
Dichlorovos	Fruit/Stem Borer/Sucking Insects	0.070	0.049	0.034	0.05
Acephate	Fruit & Stem Borer	0.071	0.046	0.028	0.05

*Values are mean of 5 samples from supervised trail.

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