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Research Article

Persistence of hexaconazole and triazophos residues on spinach leaves

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Abstract

Application of pesticides as foliar spray has become common practice in modern agriculture. Hexaconazole and Triazophos are pesticides from triazole groups commonly used for control of fungal diseases and insect pest respectively in vegetable crops. In the present work, plants were sprayed with Hexaconazole and Triazophos at various concentrations (0.05, 0.10, 0.15, 0.20 and 0.30% V/V) at developmental stages of 10 th and 25 th days. The plant leaves were analysed for the pesticidal residues after 5 days of their spraying with the pesticides (i.e. on 15th and 30th days of their growth). The Hexaconazole residue was recorded as below the maximum residual limit (MRL) at 0.05 to 0.15% sprays therefore, Hexaconazole can be recommended as safe pesticide to control disease in spinach at lower doses. The first foliar spray of 0.05 and 0.10% Triazophos recorded its residue below the MRL. Hence, the single foliar dose of lower concentrations of Triazophos (0.05 and 0.10% V/V) can be recommended for spinach. In fact the growth of these spinach was found to promoted when the lower doses of pesticide (0.15% V/V and below) were applied.

INTRODUCTION

Vegetables are essential part of our human regular diet which supply nutrients and minerals for the good health and proper functioning of human body. Inclusion of vegetables in daily diet is very essential (Kengar *et al.*, 2014). Spinach (*Spinacea oleracea* L., family Chenopodiaceae) attain top of ranking in list of vegetables for nutrient richness (Schafer, 2011). But, it is commonly affected by fungal diseases i.e. leaf spot caused by *Alternaria spinaciae* Allesch & Noack and wilt caused by *Fusarium oxysporum f*.sp *spinaciae* (Sherb) Schl. and insect pest like leaf miners (Bhale, 2002; Rangaswami and Mahadevan, 2002; Halasi *et al*., 2008).

Now a days, pesticides are modern tool to farmers to control pests, diseases and weeds; used mainly for the purpose of increase productivity (Ahemad and Khan, 2010). Some of the pesticides are reported to beneficial for plant growth when used in lower concentrations (Khan *et al.*, 2000). Moreover, use of pesticides to protect vegetables is now become indispensable and their role, effectiveness depends upon properties. Indiscriminate use of these pesticides caused irreparable damage to environment, soil and human health. Pesticides after application are known to persist on crop, soil, water, air and contaminate food grains, vegetables, milk products, butter, ghee, meat etc. Their entry in to human body after consumption results in harmful effect on health and metabolism (Gnanasambanthan and Pillai, 2000). Pesticide residue in food and vegetable crops is a problem if persistence above the MRL at harvest. Even persistence at low level create problem as they can result in pest and disease resistance to pesticides being used.

Hexaconazole (fungicide) and Triazophos (insecticide) are broad spectrum systemic pesticides used to control leaf spot and leaf minors respectively in spinach. In connection with this, the impact of foliar sprays of Hexaconazole 5% EC and Triazophos 40% EC on vegetable crop like spinach has not yet been studied. Hence attempt had made to study the persistence of residues of Hexaconazole and Triazophos on spinach leaves.

MATERIALS AND METHODS

Experimental Design-

Experiments were conducted on spinach grown in the botanical garden of S.G.M college, Karad (Dist.: Satara, Maharashtra) located between 17° 15' - 18° 01' N latitude and 74° 12' - 74° 74' E longitude. The plants were raised from seeds in the soil in earthen pots and grown under normal environmental conditions with regular watering. The plants were sprayed with Hexaconazole and Triazophos at various concentrations (0.05, 0.10, 0.15, 0.20 and 0.3% V/V) at developmental stages of 10 and 25 days. The plant leaves were analysed for the pesticidal residues after 5 days of their spraying. (i.e. on 15^{th} and 30^{th}days of their growth).

The experiments were conducted during January to March 2014. Each treatment, including the untreated control, was replicated 3 times in randomized blocks. The meteorological conditions during the field experiment included the average maximum and minimum temperatures were 34.8 and 7.7 ^{0}C, with average relative humidity ranging between 47 and 75%. There was no rainfall during the study. The plants were grown under drip irrigation following a recommended package of practices.

Extraction for pesticidal residue:-

i) **Chemicals and reagents:-**

HPLC grade methanol was purchased from J.T. Baker (NJ, USA). Ethyl acetate dried and sodium sulfates anhydrous, glacial acetic acid AR, ammonium formate extra pure, diethylene glycol AR. were purchased from Thomas Baker, Mumbai, India. HPLC grade water, Bondesil-PSA (Primary secondary amine, 40μM) was purchased from Agilent technologies, Bangalore, India.

ii) **Apparatus:-**

Mixer and grinder, homogenizer (Heidolph 900, Germany), rough and precision balance (Vibra, Adair Dutt, Mumbai), vortex mixer (Geni 2T, Imperials Biomedicals, Mumbai, India), centrifuge (Kubota, Germany), micro-centrifuge (Microfuge Pico, Kendro, D-37520, Osterode, Germany).

iii) **Sample Preparation**:-

The samples for residue was analysed as per method given by Anastassiades *et al.* (2003). Approximately 100g of the spinach leaf samples taken separately were homogenized in mixer and grinder. A sample (10 g) was transferred into a 50 ml centrifuge tube followed by addition of 10 ml acetonitrile and 4 g $MgSO_4$ + 1g NaCl. The mixture was then homogenized at 15000 rpm for 2 min using a high speed homogenizer followed by centrifugation at 3000 rpm for 5 min. The 1 ml of the supernatant (acetonitrile extract) was transferred to a 2 ml eppendrof tube and subjected to cleanup with 50 mg PSA + 150mg $MgSO_4$. The tubes were then vortexed for 1 min and centrifuged at 5000 rpm for 5 min. A 0.5 ml of the cleaned extract was diluted with 0.5 ml water. The diluted extract was filtered through 0.2 µM 6.6 nylon filter paper and 10 µL of the extract was injected into LC-MS/MS.

iv) **Instrumentation**:-

The LC-MS/MS analysis was done with a Perkin Elmer HPLC linked to an API 2000 (ABS Sciex) mass spectrometer equipped with an electrospray ionization (ESI) probe. The HPLC separation was carried out using a C18 column viz. LiChroCART® (150 mm × 4.6 mm ID, 5 µM). The mobile phase was composed of (A) 5 mM ammonium formate in water: methanol (80:20) and (B) 5 mM ammonium formate in methanol: water (90:10). The gradient programme was, 0 – 1 min 15 % B, 1 to 3 min 15-98 % B, 3-10 min. 98 % B, 10-11 min. 98-15 % B and 11-17 min. 15 % B. The column oven temperature was maintained at 30 ˚C, and the flow rate was maintained at 0.9 ml min^{-1}. The injection volume was 20 µl and the resultant retention time for Hexaconazole 10.57 min. and Triazophos was 9.14 min successively.

The residues analysis experiment was performed in 'Analytical wing of Pesticidal Residue Testing Laboratory, Pune (Maharashtra).

RESULTS AND DISCUSSION

The results obtained have been recorded in Table 1 for Hexaconazole and Triazophos.

1. **Hexaconazole:**

An evident from the result that the amount of pesticidal residue recorded in spinach leaves of the plants first sprayed with 0.20 and 0.30% V/V concentration of Hexaconazole, are 0.012 and 0.014 $mg.Kg^{-1}$ which remained above the MRL. However after second spray of the pesticide (on 30^{th} day i.e.

two sprays), the amount of residue recovered relatively higher and that is above the MRL at these higher doses. Thus it appears that Hexaconazole can be recommended as safe pesticide to be used to control disease in spinach but at lower doses (0.05 to 0.15% V/V). In fact the growth of spinach was found to promoted along when the lower doses of pesticide (0.15% V/V and below) were applied (Kengar and Patil, 2016a, 2016b).

Table 1 : Analysis of Hexaconazole and Triazophos residues in the Spinach leaves

	Pesticidal residue ($mg.Kg^{-1}$ fresh leaves of spinach)	
	First foliar spray	Second foliar spray
Control	BLQ	BLQ
Hexaconazole % (V/V)		
0.05	BLQ	BLQ
0.10	0.005	0.006
0.15	0.009	0.009
0.20	0.012*	0.013*
0.30	0.014*	0.016*
Triazophos % (V/V)		
0.05	0.009	0.015*
0.10	0.010	0.017*
0.15	0.019*	0.021*
0.20	0.031*	0.039*
0.30	0.045*	0.051*

BLQ-Below the limit of Quantification * Values are above MRL (0.01)

Triazophos:

The amount of Triazophos residue in leaves of the spinach plants was recorded after first spray with 0.05 and 0.10% V/V concentration, are 0.009 and 0.010 $mg.Kg^{-1}$ which remained below the MRL. However after second spray of the Triazophos (on 30^{th} day i.e. two sprays) the amount of residue recovered was relatively higher and above the MRL even at the lowest dose. The doses beyond 0.15% V/V of first foliar sprays and all doses of secondary foliar sprays found residues above MRL in spinach. Thus single foliar dose of lower concentrations of Triazophos (0.05 and 0.10% V/V) can be recommended for spinach.

Some evidences are there which correlated with this work. Kang *et al.*(2000) analyzed insecticide residues in market samples of cucumber and radish and observed that the levels were within the safe limits. Dissipation of Triazophos (organophosphorus insecticide) in okra fruits has been studied by Vijayalakshimi *et al.* (2000a) and they reported the presence of residues in the cooked samples on the day of application were below the MRL level. They also reported the presence of Quinolphos insecticide residues in paddy (Vijayalakshimi *et al.*, 2000b). Reddy *et al.* (2000) monitored Monocrotophos, Chloropyriphos and Quinolphos residue above MRL in the market samples of grapes. Chinniah *et al.* (2000) studied the dissipation of Dimethoate residues in chillies. Kaur *et al.* (2001) determined twenty market samples of muskmelon for the quantification of Organophosphorus insecticide residues and detected the presence of Methylparathion and Monocrotophos residues below MRL.

Pesticide residues or their toxic metabolites certainly affect human health. WHO (1990) reported that, the developing countries population carry heavy pesticides in their bodies. The principle source of these residues is believed to be the diet which contains significant quantities of the persistent chemicals. Generally farmers do not observe the recommended doses and recommended waiting periods.

Therefore the persistence of pesticidal residue in vegetables is likely to be very high. As the vegetables are consumed raw also, the presence of residues may pose health hazards to the consumers. In order to know the extent of residual contamination and the magnitude of exposure to human being, there is a need of analysis and estimation of pesticide residues (Beena Kumari *et al.*, 2001). Although LC-MS/MS is a powerful tool for fast and selective analysis of residue in food. According to EU pesticide database the MRL for Hexaconazole and Triazophos is 0.01 mg.Kg^{-1} (EU pesticide databasehttp: //ec. europa. eu/sanco_pesticides /public/? event=commodity. resultat). Residues of Hexaconazole and Triazophos were determined in leaves of spinach after first and second foliar sprays. The results are discussed here.

Our results showed that the Hexaconazole residue recorded below MRL at the doses of 0.05 to 0.15% V/V after both sprays. However, Triazophos residue recorded below the MRL only after single dose of 0.05 and 0.10% V/V. This indicated that the higher concentrations of these pesticides persist the residue in spinach. The persistent nature of pesticides and their residues in vegetables has now became a global concern (Sheikh *et al.,* 2013a). Organophosphorous, Organochlorine and Nicotinoid pesticides, along with mixture of different pesticides in fruits and vegetables were also reported all over the world by many researchers (Sheikh *et al.*, 2013 and Mirani *et al.*, 2012). These excessive pesticides residue enter in human and animal body through the feed, fodder and water accumulate in fat and usually do not get converted to the water-soluble metabolites. Residues of pesticide accumulation in human adipose tissue, blood and milk of lactating women have been reported in several countries of the world (Kumar *et al.*, 2006). Persistence of pesticides are due to their wide spread occurrence, bioaccumulation and toxicity to human and animals. So for the betterment of the world and food safety the pesticides should be identified and compensate their doses with minimization of accumulation (Zheng *et al.*,2007, Mogle *et al*., 2013, Shabeer *et al*., 2015) is next stage of this study.

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