PERSISTENCE OF HEXACONAZOLE AND TRIAZOPHOS RESIDUES ON GUAR

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Abstract: Application of pesticides has become common practice in modern agriculture initiated problems related to human health through pesticide residues. Hexaconazole (Fungicide) and Triazophos (Insecticide) are commonly used for control of fungal diseases and insect pest respectively in vegetable crops. In the present work, guar 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 50th and 65th days. The guar pods were analysed for the pesticidal residues after 10 days of their spraying with the pesticides (i.e. on 60th and 75th days of their growth). The Hexaconazole residue was recorded as below the maximum residual limit (MRL) at 0.05 to 0.20% after both sprays therefore, Hexaconazole can be recommended as safe pesticide to control diseases in guar at lower doses. The foliar sprays of Triazophos recorded its residue above the MRL. Hence, even single foliar dose of lower concentrations of Triazophos cannot be recommended for guar.

Keywords: Hexaconazole, Triazophos, Guar, Residue.

I. INTRODUCTION

Vegetables occupied important position in human regular diet, 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). Guar (Cyamopsis tetragonoloba (L.) Taub., family Leguminosae, sub family Fabaceae), has been grown in India since ancient time for its green pods, is used as vegetable, and grains as pulse and green plants as fodder. India is first rank producer of guar comprise 83% of world which followed by Pakistan and Afghanistan having 13%. The total global production is 3 million MT, India contributes 2.7 million MT from 5603 hectar having 485 Kg per hectare yield. In India, it is mainly growing in states like Rajasthan, Gujarat, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh, Orissa and Maharashtra. It got popularity due to its drought hardy nature (NRAA, 2014). But it is affected by leaf spot caused by Alternaria cucumerina var. cyamopsidis and also infested pest like Serpentine leaf miners (Liriomyza trifolii Burgress), Hairy caterpillars (Ascots imparat Walk., Spilosoma oblique Walk., Jassids (Empoasca fabae Harris, Empoasca kraemeri Ross & Moore ).

Now a days, pesticides are modern tool to control pests, diseases and weeds; mainly used for increase productivity off crop plants (Ahemad and Khan, 2010). Some pesticides are beneficial for plant growth even when used in lower concentrations (Kengar and Patil, 2016; Khan et al., 2000). Moreover, use of pesticides to protect vegetables has now become indispensable, their effectiveness depends upon properties. Non judicious use of pesticides caused irreparable damage to environment, soil and human health. Pesticides after application are known to persist on crop as residue and contaminate food grains, vegetables. 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 guar. In connection with this, the impact of foliar sprays of Hexaconazole 5% EC and Triazophos 40% EC on vegetable crop like guar has not yet been studied. Hence attempt has made to study the persistence of residues of Hexaconazole and Triazophos on guar pods.

II. MATERIALS AND METHODS

A) Experimental Design-
Experiments were conducted on guar 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 50th and 65th days. The plant pods were analysed for the pesticidal residues after 10 days of their spraying (i.e. on 60th and 75th days of their growth).

The experiments were conducted during January to March 2016. 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 °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.

B. Extraction for pesticide 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 pods 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 MgSO4 + 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 eppendorf tube and subjected to cleanup with 50 mg PSA + 150mg MgSO4. 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).

## III. RESULT AND DISCUSSION
The results obtained have been recorded in Table 1 for Hexaconazole and Triazophos.

1. **Hexaconazole:**
The results obtained for Hexaconazole have been recorded in Table 1. It is evident from the result that the amount of pesticidal residue recorded in guar pods after first sprayed with 0.10 to 0.30% Hexaconazole were 0.003, 0.007, 0.009 and 0.011. The residue at 0.30% concentration of Hexaconazole is 0.011 mg.Kg⁻¹ which remained above the MRL. However after second foliar spray of the Hexaconazole (on 65th day i.e. two sprays), the amount of residue recovered was relatively higher and that is above the MRL at the higher dose (0.20 and 0.30%). The values of pesticidal residues in the pods of guar was beyond the accepted limit only at the higher doses of pesticide i.e. 0.30% after first foliar sprays and that at 0.20 and 0.30% after second foliar spray (0.010 and 0.013 mg.Kg⁻¹ respectively). Thus Hexaconazole can be recommended as safe pesticide for guar vegetables but at lower doses only. In fact the growth of this guar was found promoted when the lower doses of pesticide (0.15% and below) were applied (Kengar and Patil, 2016a, 2016b).

2. **Triazophos:**
The amount of Triazophos residue in pods of the guar plants was recorded after first spray with 0.05 to 0.30% V/V concentrations are 0.014, 0.019, 0.025, 0.044 and 0.061 mg.Kg⁻¹ which remained above the MRL value. However after second spray of the Triazophos (on 65th day i.e. two sprays) the amount of residue recovered was relatively higher and above the MRL even at the lowest dose. The residues recorded after second foliar sprays at all concentrations are 0.021, 0.027, 0.031, 0.049 and 0.074 mg.Kg⁻¹ which remained above the MRL.

The insecticide Triazophos showed the residues in pods after both foliar sprays so even single foliar spray is not safe for guar.

Some evidences are there which correlated with this work. Kang *et al.* (2000) analyzed insecticide residues and observed that the levels were within the safe limits from market samples of cucumber and radish. The dissipation of Triazophos residue in okra fruits has been studied by Vijayalakshmi *et al.* (2000a) and they reported the presence of residues below the MRL level in the cooked samples. They also reported the presence of Quinolphos insecticide residues on paddy (Vijayalakshmi *et al.*, 2000b). Reddy *et al.* (2000) monitored residues of Monocrotophos, Chloropyriphos and Quinolphos, above MRL level in the market samples of grapes. Chinniah *et al.* (2000) also studied the dissipation of Dimethoate residues in chillies. Kaur *et al.* (2001) detected the presence of Methylparathion and Monocrotophos (Organophosphorus insecticide) residues below MRL from twenty market samples of muskmelon.
Pesticide residues or their toxicity 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 pods of guar 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 first and second sprays. However, Triazophos residue recorded above the MRL after all doses in both sprays, Therefore Hexaconazole persistent at higher concentration and triazophos even at lower concentrations. 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., 2013b and Mirani et al., 2012). Kangar and Patil (2017) recorded the Hexaconazole and Triazophos residue in spinach leaves, they reported their residues as below the MRL at only lower doses. The excessive pesticides residue enter in human and animal body accumulate 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 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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### Table 1: Analysis of Hexaconazole and Triazophos residues in the Guar pods.

| Pesticidal residue (mg.Kg⁻¹ fresh pods of guar) | First foliar spray | Second foliar spray |
|-----------------------------------------------|-------------------|-------------------|
| **Hexaconazole % (V/V)**                      |                   |                   |
| 0.05                                          | BLQ               | BLQ               |
| 0.10                                          | 0.003             | 0.005             |
| 0.15                                          | 0.007             | 0.009             |
| 0.20                                          | 0.009             | 0.010*            |
| 0.30                                          | 0.011*            | 0.013*            |
| **Triazophos % (V/V)**                        |                   |                   |
| 0.05                                          | 0.014*            | 0.021*            |
| 0.10                                          | 0.019*            | 0.027*            |
| 0.15                                          | 0.025*            | 0.021*            |
| 0.20                                          | 0.044*            | 0.049*            |
| 0.30                                          | 0.061*            | 0.074*            |

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