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Determination of Triazophos residues in/on Chilli fruits using Gas Chromatography-tandem Mass Spectrometry

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ABSTRACT: In the present study, triazophos dissipation was observed on chilli, the residues of triazophos on human beings were assessed, and a waiting period was suggested for safety reasons for consumers. After applying triazophos (Truzo 40 EC) at 500 g a.i.ha^{-1}, we found that initially 1.19 mg/kg of triazophos was accumulated on average. With the recommended dosage, the residues dissipated below the limit of quantification (LOQ, 0.05 mg/kg) in 10 days. The dissipation of triazophos in chilli followed first-order kinetics. Based on the calculations, the half-life is 2.18 days for recommended dosage. A waiting period of 12 days is suggested for the consumption of chilli sprayed with triazophos at the recommended dosages.

Keywords: Triazophos, dissipation, QuEChERS, waiting period, half-life.

INTRODUCTION

Indian chilli (*Capsicum annum* Linn.), commonly termed 'miracle spice is well acknowledged for the two most valuable characteristics it possesses in terms of trade names, pigment and scorching levels (Ahlawat *et al*., 2019) and its peppery flavour is attributed to capsicin in the fruit's skin and septa. It has therapeutic use and may be utilized in chutneys and pickles (Sahoo *et al*., 2009). India is the leading producer, trader, and exporter of chilli but its production is intermittent. In India chilli is farmed in 3,09,000 hectares of land, producing 3592 MT (Pattanayak, 2017). Chilli is susceptible to a wide range of bacterial, fungal, and viral diseases, which distort plant parts and cause a substantial quantitative and qualitative loss in productivity and the overall drop in chilli fruit production is up to 34% (Varghese *et al*., 2011).

Consumers throughout the world demand low residue levels of pesticides in agricultural goods. Therefore, residue levels must be monitored and controlled to ensure safe food production. This requires careful consideration of the use of pesticides and the application of integrated pest management strategies (Ahmed and Mohammed 2014). Therefore, considering the growing concern of the world about environmental pollution by pesticides, it becomes logical to investigate the state and residue levels of pesticides in the environment (Qin and Hu 2022). Moreover, the detection of these chemicals also reduces the potential risk of contamination of food items, soil, and water sources (Dong *et al*., 2018). Triazophos is a polyfunctional organophosphate compound (3-(o,o-diethyl)-1-phenyl thiophosphoryl-1,2,4-triazole), which is a broad-spectrum insecticide and acaricide (Fig. 1) with nematicidal properties (Rani *et al.*, 2001). In addition to being a contact and stomach pesticide, it controls fruit borer, aphids, leafhoppers, and cutworms (Vijayalakshmi and Kuttalam 2000). Its translaminar properties allow triazophos to penetrate deeply into plant tissues despite it being non-systemic (Yang *et al*., 2019). Using insecticides to combat insect pests has undoubtedly improved food production and quality (Jawale and Gogate 2018); however, a variety of scientific studies have indicated that the use of pesticides indiscriminately and inappropriately has resulted in their residues in the food chain and has adversely affected humans and animals (Ahmad *et al*., 2014). Thus, in order to recommend using an insecticide, the product must not only control the pest effectively, but its residues in the commodity must also be acceptable. Therefore, analysis of residual amounts of pesticides in raw agricultural crops and processed foods is a leading preventive measure of public health.

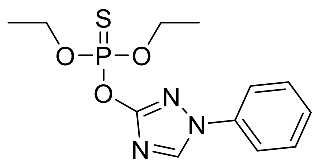

Fig. 1. Chemical structure of Triazophos.

This study aims to investigate the persistence pattern, dissipation behavior, and risk assessment of triazophos residues on chilli by applying a quick, easy, cheap, effective, rugged safe (QuEChERS) method to sample preparation before estimating residues using gas chromatography based on the above facts.

MATERIAL METHOD

A. Chemicals and Reagents

The certified reference materials of Triazophos with a purity of 99.8% were acquired from Sigma Aldrich, Pvt, Limited. All the analytical organic solvents and reagents such as acetonitrile, acetone, sodium chloride, magnesium sulphate, and anhydrous sodium sulphate, were purchased from Merck (Darmstadt, Germany). Primary secondary amine (PSA) was supplied by Agilent Technologies Private Limited, Bangalore, India. Each of the chemicals used for the analysis was first subjected to glass distillation and then ran as a reagent blank.

B. Field Application and Sampling

The "Kanshi Anmol" variety of Chilli (*Capsicum annum Linn*) was raised following recommended agronomic practices at the Research Farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar (29.14°N, 75.70°E). Truzo 40 EC formulation was sprayed only once at the time of 50% fruiting stage with a dosage of 500 g.a.i. ha^{-1}on selected experimental plots with the knapsack sprayer. Additionally, one of the experimental fields was left untreated to serve as a control. The samples in triplicate were collected randomly at 0 (2 h), 1, 3, 5, 7, 10, and 15 days after application (DAA). Samples were transported to the laboratory for residue analysis.

C. Sample Preparation

Chilli samples were processed using the QuEChERS method proposed by Sharma, (2007). A representative sample of 15 g macerated chilli fruits was combined with 30 mL acetonitrile and homogenized using a low-volume homogenizer (Heidolph) for 3-4 minutes at 14,000 rpm. To separate the water (chilli) and acetonitrile phases of the aforementioned representative sample, 3.3 g of sodium chloride (NaCl) is added to the extract and vortexed for 2 minutes. Following the 3 min centrifugation of the extract at 2500 rpm, the upper 18 mL acetonitrile layer was deposited over sodium sulphate to eliminate any remaining moisture traces. The dispersive solid phase extraction (DSPE) technique was used for the clean up of the extract with primary secondary amine (PSA) 0.4 g and 1.15 g magnesium sulphate ($MgSO_4$) as adsorbent. Then, the extract was recomposed to a volume of 3 mL in n-hexane and filtered through a 0.2-micron filter before GC–MS/MS analysis.

D. GC-MS/MS Analysis

Pesticide analytes in samples were determined by GC-MS/MS (Shimadzu GC-MS TQ 8040) equipped with a capillary column (SH-Rxi-Sil MS column of 0.25 µm thick film having 30 m length and 0.25 mm internal diameter) using helium gas as the carrier gas at a constant flow rate of 1.5 mL min^{-1}. Samples were injected (1 µl) with an autosampler (20iAOC) in splitless injection mode. The temperature of the injection port was 250°C and programming of the oven temperature was done to optimize the working conditions. The oven temperature programming began at 80°C and remained at this temperature for 2 min, then start to increase up to 180 °C at 20 °C/min ramp rate and attain the temperature of 300 °C, at the rate of 5 °C/min and remains for 10 min. Pesticide residues could be confirmed and quantified by using GC-MS/MS in Multiple Reaction Monitoring (MRM) with an ESI(+) source of ionization throughout a scanning mass range of 40-1000 m/z, Peaks in the total ion chromatogram of the sample recorded in MRM mode were detected based on their particular retention time (R_T) and their characteristic ion peaks in the mass chromatogram. The analysis was carried out in a completely air-conditioned laboratory with a temperature of less than 22°C and a relative humidity of less than 60%.

E. Dissipation Studies

The data on the residues over days were analysed using first-order kinetics with the equation (1) as follows:

$$C_t = C_0 e^{-K_1 t} \quad (1)$$

Where C_0 represents the initial concentration (mg kg^{-1}); C_t concentration of the pesticide residue (mg kg^{-1}) at time t (in days), and K_1 denotes the rate constant (day^{-1}). A regression coefficient (R^2) was used to depict the link between residue data and time by plotting the log[residues (mg kg^{-1}) x 10^3] on the y-axis and days after application on the x-axis. The half-life ($t_{1/2}$) of residues was calculated according to Hoskin's formula (Hoskins, 1961). Along with half-life, pre-harvest interval (PHI) was also calculated using equation **(2)** (Mukherjee *et al.*, 2021) depicted below:

$$\text{PHI} = \frac{\text{log of initial deposit} - \text{log of MRL}}{\text{Slope of regression line}} \quad (2)$$

Where, MRL is the maximum residue limit (mg kg^{-1}) of Triazophos in chilli.

F. Data Analysis

Data are represented as mean ± S.D (Standard deviation). For each parameter involved in the dissipation and decontamination processes, analysis of variance (ANOVA) was performed to analyze the interactions among different treatments, and days after the application. Differences in means were determined to be statistically significant at a p-value of 0.05. The software Origin Pro 9.0 (Origin Lab Corporation, Northampton, MA, USA) was used to create all of the figures.

RESULTS AND DISCUSSION

Recovery experiments at different levels, i.e., 0.05 and 0.10 mg kg^{-1}, were conducted to establish the validity and reliability of the analytical method used in this study. As described above, control samples of hot pepper were fortified with different insecticides at the above-stated levels and analyzed. Across all insecticides, all hot pepper percent recoveries were higher than 80% (Table 1). There was a limit of quantification (LOQ) of 0.05 mg/kg for hot pepper fruits and a limit of detection (LOD) is 0.01 mg/kg.

The average initial deposits of triazophos on chilli were found to be 1.19 mg/kg for applications of Triazophos 40 EC at 500 g a.i.ha^{-1}. The residues were observed to be 0.92 mg/kg after 1 day showing a percent dissipation of about 23.17 % at the recommended dosage (Fig. 3). About 93 % of residues of triazophos dissipated after 7 days of spray (Table 2). Residues were found to be below the determination limit of 0.05 mg kg^{-1} at 10 days for recommended dosages. On all the days, there was a significant reduction in the residues of triazophos (p=0.05).

The results obtained were consistent with those obtained by Singh and Kapoor (1998) when examined the residues of triazophos on brinjal fruits. The residual effects of triazophos on brinjal fruits caused by the application of it at 500 and 1000 g a.i ha^{-1} day^{-1} were approximately 94% after 6 days of last spraying at both dosages. Using 350 and 700 g a.i ha^{-1} application, (Vijayalakshmi and Kuttalam, 2000), examined the persistence of triazophos in bhendi. Initially, the deposits were 0.68 and 1.39 mg/kg at low and high doses, respectively. A rapid dissipation rate was observed up to the fifth day, with nearly 93% dissipation. An experiment conducted by Kumar *et al*., (2000) examined the persistence of triazophos in chilli after 700 g a.i ha^{-1} of triazophos were applied per hectare per annum. The initial deposits were found to be 0.43 mg/kg at the recommended dosage. By 15 days, the residues had dissipated to more than 70%. However, residues continued to persist after 15 days. After applying 900 g a.i ha^{-1} of triazophos to wheat crops comprising immature grain, leaves, stems, and soil in which it was grown, Li *et al*. (2008) investigated the residues from this application. The initial deposits of triazophos on plant parts were found to be 32.44 and 19.41 mg kg^{-1} for the crops grown in Shandong and Beijing, China, respectively. After 30 days, the residues dissipated to over 95 percent.

A. Half-life and PHI values of triazophos on chilli samples

The correlation coefficients were found to be 0.961 at the recommended dosage (Fig. 2). The dissipation pattern of residues of triazophos from capsicum followed first order kinetics. Half-life value ($T_{1/2}$) is usually defined as the time required for half of the given quantity of material to dissipate (Gunther and Blinn, 1955).

Table 1: Recovery for Triazophos in spiked chilli samples at different levels.

Substrates	Level of fortification (mg/kg)	% Recovery* (Mean ± S.D)
Chilli	0.05	80.12 ± 2.26
	0.01	81.20 ± 1.23

*Mean ± S.D of three replicates

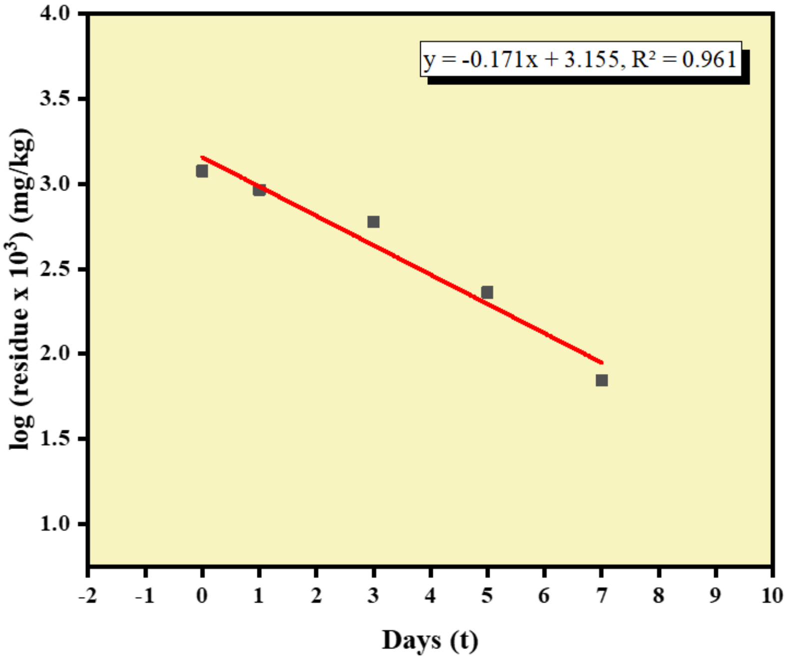

Fig. 2. Plot of log[residues (mg/g) × 10^3] of Triazophos in Chilli fruits v/s days.

Table 2: Residues of Traizophos (mg/kg) in chilli fruits after the application of T_1 dose.

Days after the treatment	Dose (T_1= 500 g a.i.ha^{-1})				
	R_1	R_2	R_3	Average residues*±SD (mg/kg)	% Dissipation
0 (2h)	1.115	1.250	1.220	1.19 ± 0.015	-
1	0.910	0.918	0.925	0.92 ± 0.013	22.69
3	0.598	0.612	0.605	0.60 ± 0.017	49.58
5	0.222	0.231	0.225	0.23 ± 0.011	80.67
7	0.71	0.075	0.069	0.07 ± 0.002	94.12
10	<LOQ	<LOQ	<LOQ	-	-
15	<LOQ	<LOQ	<LOQ	-	-

LOQ = 0.05 mg/kg LOD = 0.01 mg/kg

*Average residues of three replicates

SD = Standard Deviation

The $T_{1/2}$ of triazophos was calculated using Hoskins, (1961) formula. Half-life ($T_{1/2}$) of triazophos on chilli was observed to be 2.18 days, following the application of triazophos at 500 g a.i.ha^{-1} (Table 3). Half-life values of triazophos on brinjal fruits (Kharif) were 1.51 and 1.42 days following the application of triazophos at 500 and 1000 g a.i.ha^{-1}, respectively (Singh *et al*., 2015). The pre-harvesting value calculated by equation (2), was 12.12 days. This suggests that consumers should wait for picking chilli after the Triazophos.

Table 3: Dissipation parameters of Fenpropathrin residues in chilli fruits.

Dissipation parameters	Dose (T_1= 500 g a.i.ha^{-1})
Regression equation	y = -0.171x + 3.155
R^2	0.961
K_1	0.317
C_0	1.19
$t_{1/2}$	2.18

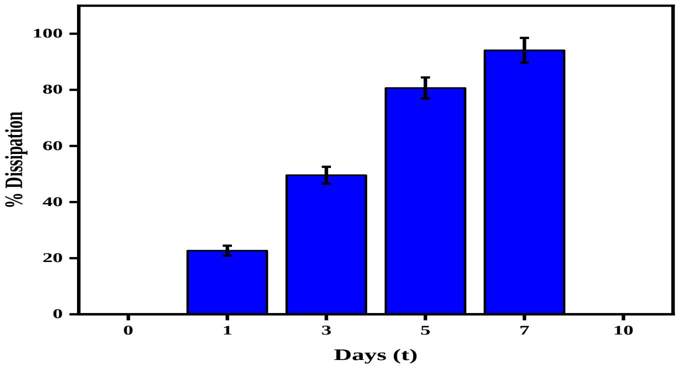

Fig. 3. Chart showing the dissipation pattern of Triazophos in chilli at T_1 dose.

CONCLUSION

Half-life values for triazophos on chilli at the recommended dosage were observed to be 2.18 days. A waiting period of 12 days is suggested to reduce the risk before consumption of chilli. The residues of triazophos were found to be eliminated after 15 days. Therefore, it is recommended to wait for 15 days before harvesting and consuming chilli after the application of triazophos.

FUTURE SCOPE

The future of research on the dissipation of Triazophos in the environment holds significant importance in ensuring the health and sustainability of ecosystems, as well as human well-being. Triazophos are widely used to control pests in agriculture, forestry, and public health, but their widespread application has raised concerns about their impact on the environment and potential risks to human health. Therefore, research in this field aims to understand the fate, behavior, and impacts of triazophos in the environment, and develops strategies to mitigate their adverse effects.

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Conflict of Interest. The authors state that they have no known competing financial interests or personal ties that might seem to have influenced the research reported in this paper.

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