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PN Guru
All India Network Project on Pesticide Residues, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist: - Ahmednagar, Maharashtra, India

CS Patil
All India Network Project on Pesticide Residues, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist: - Ahmednagar, Maharashtra, India

Correspondence
PN Guru
All India Network Project on Pesticide Residues, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist: - Ahmednagar, Maharashtra, India

Dissipation studies of triazophos in/on polyhouse grown capsicum and cropped soil

PN Guru and CS Patil

Abstract
The dissipation pattern and to recommend the Pre Harvest Interval (PHI) of triazophos in/on capsicum was studied after application of insecticide at fruit initiation stage. Triazophos @ 500 and 1000 g a.i. ha-1 was applied twice at an interval of 10 days. The initial residues deposit was 3.17 and 6.13 mg kg^{-1} respectively, and it was dissipated according to first order kinetics at both the dosages to Below Quantification Limit (BQL) at 20th and 25th day, respectively @ 500 and 1000 g a.i. ha^{-1}. The half-life recorded was 2.95 and 2.63, respectively at recommended and double the recommended dose, respectively, whereas the cropped soil leaves with no residues. Considering the LOQ of 0.05 mg kg^{-1}, Pre-Harvest Interval (PHI) of twenty days can be suggested for triazophos for safe consumption of capsicum fruits grown under polyhouse.

Keywords: triazophos, dissipation, capsicum, half-life

Introduction
Now a day, the protected cultivation is getting preference over open field cultivation for off-season quality production of fruits and vegetables by extending availability of the quality produce, higher productivity and improved nutritional attributes of the poly house grown perishable produce [19]. In addition, the pesticide usage for the management of these polyhouse pests are common, while dissipation takes slow and longer period in poly house conditions than in open field conditions [23]. Among the different crops cultivated in polyhouse capsicum (*Capsicum annuum* L. var. grossum Sendt.) is one of the major crop which is having the B:C ratio of minimum 1:3.92 [25]. It is also called as shimla mirch, bell pepper, sweet pepper and green pepper. This crop is cultivated in most parts of the world, especially in temperate regions of Central and South America and European countries, tropical and subtropical regions of Asian continent mainly in India and China [28]. In India, capsicum is included under non-traditional category of vegetables and mainly cultivated during *rabi* and *kharif* seasons. Capsicum cultivation is in limited scale in Kerala, Telangana, Andhra Pradesh, Maharastra, West Bengal, Gujarat and Goa [2].
In India, it is cultivated in an area of 0.46 mha with production of 3.16mt [3]. Various biotic (pest and diseases), abiotic (rainfall, temperature, relative humidity and light intensity) and phonological factors (flower and fruit drop) limits the yield and fruit quality under open field conditions [9]. Among the biotic factors, insect pests reduces the quality of produce and even a small blemish on the fruit will drastically reduce its market value. Butani and Verma (1976) [4] reported over 20 insect species on chillies (*Capsicum* spp.) from India of which thrips, *Scirtothrips dorsalis* Hood, mite, *Polyphagotarsonemus latus* Banks are among the most damaging pests which cause greater loss in both open field and polyhouse [16, 21, 7]. In protected cultivation insecticide usage is one of the major management practices which farmers are depend on for the control of these severe damaging sucking pests. Since capsicum is consumed afresh, they may carry residues which warrant judicious use of pesticides in respect of persistence, dissipation, metabolism, movement and accumulation of residues [11]. There have been a number of scientific evidences which suggest that indiscriminate and impropriate use of pesticides has led to their residues in food chain and have exerted harmful effects on human beings as well as animals [12].
The organophosphate group of insecticides are mainly used in the polyhouse crops in western region of Maharashtra [8]. One among them is triazophos, [3-(o,o-diethyl)-1-phenyl thiophosphoryl-1,2,4-triazol] which is a broad spectrum insecticide and acraricide with some nematicidal properties [18]. Being a contact and stomach insecticide, it is used to control fruit

borer, aphids, leaf hopper, and cutworms on number of crops and vegetables including polyhouse crops [27]. Although, the molecules used by the farmers are non-recommended on the particular crop because of the improper knowledge they are applying these insecticides [15]. Therefore, recommendation of usage of an insecticide requires that it must not only provide an effective control of the pest, but at the same time its residues in the commodity should be toxicologically acceptable.

Keeping the above facts in view, the present study has aimed to study the persistence pattern, dissipation behavior of one of the majorly used non-recommended organophosphate chemical *i.e.,* triazophos in/on polyhouse grown capsicum fruits and in cropped soil by applying quick easy cheap effective rugged safe (QuEChERS) method for sample preparation followed by estimation of residues by gas chromatography with flame photometric detector. This data may be useful to recommend the safe waiting periods based on the Maximum Residue Limits (MRLs) calculated, as it helps in recommending risk mitigation protocols for food safety and also for recommendations of the triazophos for the management study against the major pests of the capsicum grown under polyhouse.

Material and Methods

Test chemicals

The Certified Reference Materials of triazophos were made available by Pesticide Residue Laboratory, AINP on Pesticide Residues, Mahatma Phule Krishi Vidyapeeth, Rahuri whereas the formulated products (Triazo) were made available by Modern Pesticide Testing Scheme, MPKV, Rahuri.

Field experiment

Field experiment for residue studies was conducted during *Kharif* -2016 at Hingoni (village), Nevasa (tehsil), Dist. Ahmednagar. Capsicum crop (Var. *bachata*) was raised by following recommended package of practices of the university except plant protection measures to avoid contamination. Overall two sprays were given at an interval of 10 days, initiating the first spray at fruit initiation stage (button size). According to residue studies protocol, prescribed by Central Insecticidal Board and Registration Committee (CIB & RC), two doses of triazophos (recommended dose @ 500 g a.i. ha^{-1} and double the recommended dose @ 1000 g a.i. ha^{-1}) were evaluated for residues.

Residue Analysis

Prior to pesticide application and field sample analysis, the residue analysis method was validated following the SANCO document (12495/2011).

Standard Preparation

An accurately weighed 10 mg of an individual analytical grade insecticide standard (CRM) was dissolved in 10 ml volumetric flask using suitable solvent to prepare the standard stock solution of 1000 mg kg^{-1}. Standard stock solution of each insecticide was further diluted to obtain immediate lower concentrations of 100 and 10 mg kg^{-1}. They were stored in a refrigerator at -20 ^{o}C. From intermediate standards, working standards of 1.00, 0.80, 0.50, 0.40, 0.25, 0.10 and 0.05 mg kg^{-1} were prepared by suitably diluting the stock solution in ethyl acetate and used as standard check in residue determination, linearity and recovery studies.

Method validation

Prior to analysis of samples, linearity of triazophos was established on GC-FPD. Accuracy and precision of the methods was determined by per cent mean recovery and per cent relative standard deviation [22].

Limit of Detection and Limit of Quantification

The limit of detection for triazophos was determined by considering a signal-to-noise ratio of three with reference to the background noise obtained for the blank sample. The limit of quantification determined as 3 times of LOD.

Linearity study

Seven linear concentrations as per international standards (0.05, 0.10, 0.25, 0.40, 0.50, 0.80 and 1.00 mg kg^{-1}) of each working standard were injected in triplicate [22]. Linearity curve was established with concentrations of the standard and corresponding peak area.

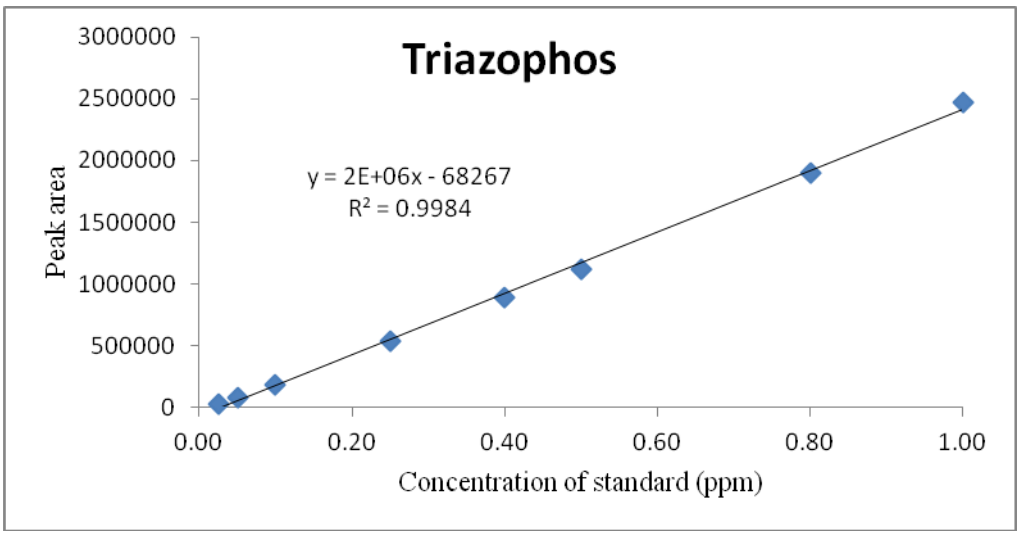

Fig 1: Linearity of triazophos standard

Recovery study

The analytical method for estimation of triazophos residues in/on capsicum fruits and soil has been validated by conducting recovery studies using control samples (or even standard matrix) of capsicum and soil [22]. Ten gram each of control sample of blended capsicum fruits and soil was taken

in 50 ml centrifuge (polypropylene) tubes in three replicates each were spiked with triazophos standards separately at the required fortification levels *i.e.,* LOQ, 5 LOQ and 10 LOQ, adding an appropriate volume of working standard of 10 mg kg^{-1}. This mixture was then shaken, in order to attain a proper homogeneity of pesticides in the samples and kept for 5min. The extraction and cleanup procedures were followed as described hereunder. Per cent recovery was calculated by using following formula.

$$\text{Per cent recovery} = \frac{\text{Quantity of pesticide recovered}}{\text{Quantity of pesticide added}} \times 100$$

Sampling - The medium marketable size capsicum fruit samples (1 kg) were collected at random from each replicate of the treated and control plots separately at regular time interval of 0 (2 hr after spraying), 1, 3, 5, 7, 10, 15, 20 and 25 days till BDL after the second spray, whereas, soil samples from each treatment plot were collected at final harvest. The collected samples (fruits and soil) were transferred to the laboratory within 6 hrs in an ice box. The samples were homogenized and kept at -20 °C in deep freezer until further analysis.

Extraction and clean up - The fruits and soil samples were processed for extraction and clean-up using QuEChERS method [1].

Fruit samples - The entire laboratory sample (1kg) was crushed/grinded thoroughly in a grinder and approximately 10 g homogenized sample was weighed in a 50 ml polypropylene tube and tube was kept in deep freezer for 10 min. Homogenised sample was extracted with 10 ml ethyl acetate in presence of 10 g anhydrous Na_2SO_4 and centrifuged at 3500 rpm for 5 min. Transfer 2 ml supernatant to the 15 ml tube containing 50 mg primary secondary amine (PSA). The content was vortexed for 30 sec and then centrifuged for 2 min at 2500 rpm. The supernatant was filtered through 0.2 micron filter and instrumental analysis was carried out [1]. Similarly, soil samples were also analysed according the structured method [1].

Determination

GC-FPD Parameter –

Analysis of triazophos was carried out by Gas Chromatography equipped with Flame Photometric Detector and automatic injection system (Shimadzu-GC-2010). GC solution software was used as the data analysis system. Typical GC conditions used for analysis are as below.

Column type	DB-1,30 m X 0.25 µm X 0.25 mm
Column temperature	170 °C.....3 min hold @ 6.5 °C min^{-1} 220 °C.....2min hold @ 10 °C min^{-1} 280 °C.....6min hold
Injector temperature	250 °C
Column temperature	170 °C
Detector temperature	300 °C
Injection volume	1µl
Column flow	0.99 ml min^{-1}
Hydrogen flow	90 ml min^{-1}
Air flow	120 ml min^{-1}
Retention time	16.58 min

Statistical Analysis

The simple statistical analysis like mean residues, standard deviation, regression equation, R^2 value and half life were calculated in Microsoft-excel programme.

Results and Discussion

Limit of Detection and Limit of Quantification

The limit of detection of the tested insecticides was 0.015 mg kg^{-1} resulted by considering a signal-to-noise ratio of compound with reference to the background noise (3:1) obtained for the blank sample. The limits of quantification determined as the lowest concentration in capsicum of a given compound giving a response that could be quantified with RSD lower than 20 per cent, was 0.05 mg kg^{-1} for all tested pesticides.

Linearity study

Seven linear concentrations (0.05, 0.10, 0.25, 0.40, 0.50, 0.80 and 1.00 mg kg-1) of working standard (triazophos) was injected in triplicate and the linearity lines were drawn. The response was linear over the range tested and an R^2 value was 0.998 as given in Fig.1. This indicated that the GC-FPD method is valid for residue determination of the tested insecticide in capsicum fruits and soil.

Recovery studies

Accuracy of the analytical method was determined by recovery studies. Mean recovery obtained from the studies reflected the accuracy of method. Precision of the method was reflected by the relative standard deviation. Recovery experiments were conducted on untreated capsicum fruits and soil, fortified with three concentrations of insecticide standard i.e., 0.05, 0.25 and 0.50 mg kg^{-1}. The mean of recovery of triazophos at the levels of 0.05, 0.25 and 0.50 mg kg^{-1} in/on capsicum fruits and soil (Table 1) were 94.74, 95.85, 96.15 per cent and 97.86, 89.01, 99.35 per cent, respectively.

Table 1: Recovery for triazophos in spiked capsicum fruit and soil samples (n=3) at different levels

Substrate	Level of fortification (mg kg^{-1})	Recovery (%)	SD	RSD (%)
Capsicum fruits	0.05	94.74	4.53	4.78
	0.25	95.85	6.92	7.22
	0.5	96.15	9.66	10.04
Soil	0.05	97.86	5.53	5.65
	0.25	89.01	9.12	10.25
	0.5	99.35	5.31	5.34

Note: SD- standard deviation, RSD- relative standard deviation

These results indicated that the analytical method employed for the extraction and clean up of capsicum fruits and soil samples was found accurate and precise as mean recovery percentages and RSD were within the limits prescribed by SANCO (2011) [22].

Dissipation of triazophos in/on capsicum fruits and soil

Dissipation of residues in plant depends on climatic conditions, type of application, dosage and intervals between application and time of harvest. The results revealed reduction in residue levels of these tested pesticides in capsicum fruits with time.

In case of triazophos 40EC @ 500 and 1000 gm a.i.ha^{-1}, initial residues were recorded as 3.17 and 6.13 mg kg^{-1}. The initial residues reached to below quantification limit (BQL) on 20^{th} and 25^{th} day at recommended and double the recommended dose, respectively. The half-life (RL_{50}) values of triazophos for capsicum were 2.95 and 2.63 days, for both

the dosages, respectively (Table 2). While, analysis of soil samples for the residues of triazophos was carried out on the 50th day after second application. No residues were detected in untreated samples. The residues of triazophos in soil at harvest was found to be below quantification limit (BQL) at both the doses.

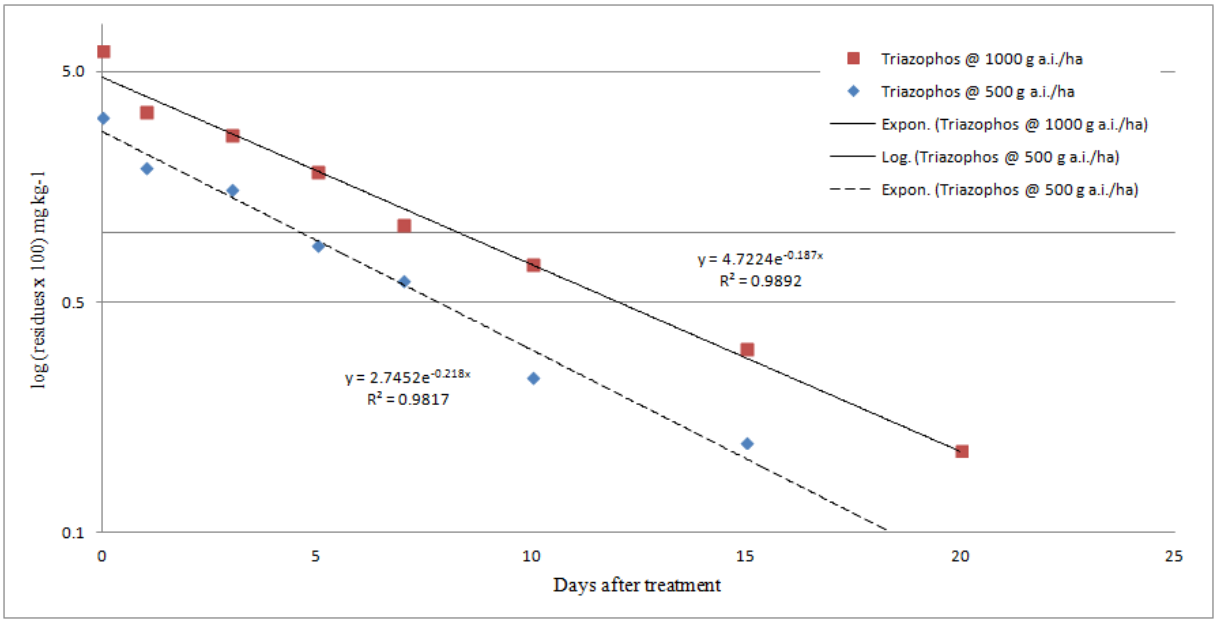

Fig 2: Semi-logarithm graph showing dissipation kinetics of triazophos residues in/on capsicum

Table 2: Residues of triazophos (mg kg-1) on capsicum fruits and soil at different time intervals

Interval between last spray and sampling (days)	Triazophos @ 500 g a.i. ha^{-1}					Triazophos @ 1000 g a.i. ha^{-1}				
	Replicates			Mean ± SD	% dissipation	Replicates			Mean ± SD	% dissipation
0 (2 hr)	3.29	2.92	3.29	3.17 ± 0.21	--	6.32	5.97	6.11	6.13 ± 0.18	-
1	1.93	1.91	1.98	1.94 ±0.04	38.74	3.42	3.35	3.25	3.34 ± 0.09	45.54
3	1.47	1.56	1.64	1.56 ±0.09	50.84	2.65	2.73	2.56	2.65 ± 0.09	56.85
5	0.91	0.89	0.87	0.89 ±0.02	71.89	1.73	1.89	1.92	1.85 ± 0.10	69.89
7	0.64	0.68	0.56	0.63 ±0.06	80.21	1.07	1.01	1.15	1.08 ± 0.07	82.45
10	0.25	0.24	0.22	0.24 ±0.02	92.53	0.75	0.78	0.65	0.73 ± 0.07	88.15
15	0.12	0.12	0.13	0.12 ±0.01	96.11	0.31	0.34	0.29	0.31 ± 0.03	94.89
20	BQL	BQL	BQL	--	--	0.11	0.09	0.14	0.11 ± 0.03	98.15
25	BQL	BQL	BQL	--	--	BQL	BQL	BQL	--	--
	RL50				2.95	RL50				2.64

ND-Not detected BQL-Below Quantification Limit of 0.05 mg kg^{-1}

These results obtained were found to be in agreement with those of [24] who studied the residues of triazophos on brinjal fruits. Following the application of triazophos on brinjal fruits at 500 and 1000 g a.i ha-1 respectively, triazophos dissipated about 94% after 6 days of last spray at both the dosages. Vijayalakshmi *et al.* (2000) [26] studied the persistence of triazophos on bhendi following its application at 350 and 700 g a.i ha-1. The initial deposits were found to be 0.68 and 1.39 mg kg-1 at low and high doses, respectively. The rate of dissipation was found to be rapid up to the 5th day and dissipated about 93%. Li *et al.* (2008) [14] studied the triazophos residues in wheat crop comprising of immature grain, leaves, stems, and soil in which crop was grown following the application at 900 g a.i.ha-1. 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. The residues dissipated to more than 95% after 30 days. Kumar *et al.* (2000) [13] reported the dissipation of triazophos (750 g a.i.ha-1) on chilli and found the initial deposit of 0.43 µg kg-1, to reach BDL by 15th day with respective half - life value of 10.03 and waiting period of 6.33 days, respectively. Diwan *et al.* (2015b) [5] studied the residues and persistence of triazophos (500 g a.i.ha-1) on tomato. Initial deposits (at 2 hours after spray) of 0.167 µg g-1, reached BDL on 10th day. Persistance of triazophos (500 and 1000 g a.i.ha-1) in okra fruits was reported by [17]. Initial deposits of 1.83, 3.09 µg kg-1 was observed in the fruits at both the doses, respectively. Rao *et al.* (2015) [20] reported initial deposits of 1.10 mg kg-1 triazophos was detected in fruit samples of tomato, dissipated to BDL by 7th day. The variation of results pertaining to the initial deposits (2.13 and 3.19 mg kg-1 in open and poly house conditions, respectively) of triazophos when sprayed thrice @ 1250 ml ha-1 and dissipated to BDL (10.00 and 20.00 days) of capsicum to chilli, tomato and okra may be due to due to variation in dosages of application, change in matrix and climatic conditions

The dissipation pattern of residues of triazophos from capsicum followed first order kinetics at both the dosages. Half-life value (T1/2) is usually defined as the time required for half of the given quantity of material to dissipate [6]. The T1/2 of triazophos was calculated using Hoskins' (1961) [10] formula. Half-life (T1/2) of triazophos on capsicum was observed to be 2.95 and 2.63 days, respectively, following the application of triazophos at 500 and 1000 g a.i.ha-1. 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 [24].

Conclusions

In polyhouse capsicum, dissipation half-life values for triazophos after two applications at recommended and double the recommended dosages were observed to be 2.95 and 2.64 days, respectively. At after 15th and 20th day respectively for recommended and double the recommended dose the residues found below quantification limit (BQL) i.e., the insecticide would not cause any adverse effects after consumption of capsicum fruit below this range and can also quote as maximum intake limit. A waiting period of 15 days may suggest to reduce the risk before consumption of capsicum and was consider to fix the pre harvest interval.

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