Residual Fate and Safety Risk Assessment of Hexythiazox in Okra

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Abstract: A one season multi-location field study was conducted on okra under different agro-climatic conditionsof India where hexythiazox (Maiden 5.45 EC) was applied @ 25 ga.i./ha and 50 g a.i./ha along with untreated control. The okra fruit and soil samples were collected in different day intervals. The samples were extracted and cleaned up by modified QuEChERS method and quantified by GC-ECD. The LOD & LOQ of Hexythiazox was 0.01 ppm& 0.03 ppm respectively. The average recovery of hexythiazox was found in the range of 82-90% for Okra fruit and 86-90% for soil. Initial deposit of hexythiazox ranged between 0.19 - 0.45 ppm for recommended dose and 0.35 - 0.70 ppm for double dose. Hexythiazox dissipated with time following the first order reaction kinetics and the calculate half life value ranged between 1.43 - 2.01 days. No residue of hexythiazox was detected in soil. The Pre harvest interval of Hexythiazox in okra was calculated and found in the range of 2-5 days irrespective of dose and location.

Key words: Hexythiazox, Okra, Residue, Dissipation, Half life.

1. INTRODUCTION

Okra (Abelmoschus esculentus) is a vegetable under malvaceae family which is cultivated throughout India. From nutritional view point okra fruit is very rich in fats, carbohydrates, vitamins like A, B and minerals such as calcium, iron, magnesium and potassium [1]. India *ranks first in the world with 3.5 million tons (70% of total worlds production) of okra produced from over 0.35 million hectare of land [2].* However, okra production is highly affected by attack of wide range of insects and mites during its growth period resulting in yield loss [3]. Infestations by sucking insect pests and mites not only affect the crop but also hamper the crop health by transmitting

pathogenic diseases [4, 5]. Many insecticides have been used to encounter this problem but indiscriminate application of some insecticides has produced resistance in insects as well as environmental problems.

Hexythiazox ((4RS,5RS)-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide) is a non-systemic broad spectrum acaricide with contact and stomach action & good translaminar activity. It is applied at any growth stage of plant from budding to fruiting [6] and has ovicidal, larvicidal, and nymphicidal activity. It is widely used to control of red mites, tetranychid mite, and apple rust mite on apple, strawberry, cucumber, citrus, vegetables like- okra, soybean, vines, flowering plants (ex: rose) and cotton [7]. As Hexythiazox is a newly introduced pesticide in India, there is limited data concerning its dissipation behaviour in vegetables. So there is a need for determining the dissipation pattern of Hexythiazox in okra which could be effectively utilized for the fixation of maximum residue limits (MRL) in our country. In this regards the objective of the present study is to find out the residual fate, persistence behavior and safety risk assesment of Hexythiazox in okra under Indian climatic condition.
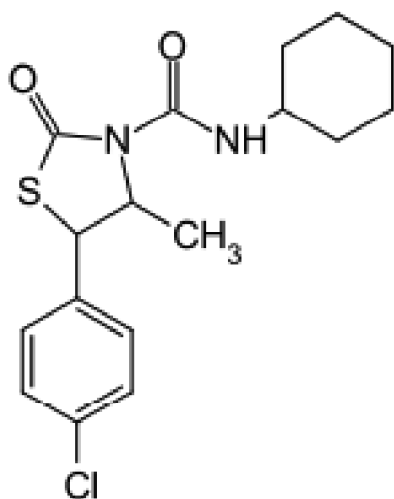

Figure 1: Structure of Hexythiazox

2. MATERIALS AND METHODS

Hexythiazox (Maiden 5.45% EC) was applied on okra at four different locations viz, (i) AB block Farm, C unit, B.C.K.V, Kalyani, Nadia, West Bengal. (ii) Rahuri, Maharashtra (iii) MPKV, Jabbalpur, Madhya Pradesh and (iv) G.B.Pant Univ. of Ag & Tech., Krishi Vigyan Kendra, Dhakarni, Dehradun, Uttarakhand during 2012-2013 seasons. Field spray was done at fruiting stage of okra in two different doses viz, recommended dose (T_1) i.e. 25 g a.i./ha and double the recommended dose (T_2) i.e. 50 g a.i./ha. A control plot was maintained as well where no pesticide was applied. Two treatment doses of the pesticide along with a control were replicated thrice in a randomized block design (RBD). Spraying was done with High volume Knapsack sprayer (Sampling Volume 500 L/ha) fitted with Hollow cone nozzle.

2.1. Sampling Information

Okra fruit samples (0.25 kg) were collected randomly from 8-10 places of each replicated plots at an interval of 0 (2 hr after application), 1, 3, 5, 7, 10 days after the application. Soil sample (1 kg) was collected at 10th day after the application of the chemical. A representative (100 g) sample of Okra fruit and soil were taken for analysis using quartering technique. The fruit sample was blended using blender (Bajaj). Soil samples were air dried and sieved through 80 mess sieve prior to extraction.

2.2. Preparation of Hexythiazox standard solution

Stock standard solution of 100 ppm Hexythiazox (Purity 99.90%, Supplied by M/S Biostadt India Ltd, Mumbai) was prepared by dissolving 10.01 mg Hexythiazox in 100 mL ethyl acetate. A seven point calibration curve consisting of 0.01, 0.02, 0.05, 0.1, 0.25, 0.5, and 1.0 ppm concentration levels was prepared from stock solution by serial dilution technique with ethyl acetate.

2.3. Extraction and cleanup of Okra fruit and soil sample

Extraction

An aliquot of ten gram (10 g) of the both Okra fruit and soil sample were taken in a 50 mL polypropelene centrifuge tube and 20 mL of ethyl acetate (HPLC grade, JT Baker) followed by 1.5 g anhydrous NaCl and 5 gm of activated Na_2SO_4 were added, the sample was again vortexed for 1 min and placed on a rotospin (Tarsons) for 30 minutes at 50 rpm. The sample was then centrifuged using Centrifuge (model: Avanti J-301, Beckman Coulter, Fullertron, CA) for 5 min at 8,000 rpm and 8 mL supernatant liquid was collected.

Clean-up

Out of 8 ml clear supernatant solution a representative 4 ml solution was transferred in a 15 mL polypropylene centrifuge tube containing 25 mg Primary secondary amine (PSA; Varian, Harbor City, CA; 40 mm particle size), 25 mg graphitized carbon black (GCB; United Chemical Technology) (for Okra fruit) and 150 mg Na_2SO_4 and the tube was subjected to vortex for 2 min. After that the extract was again centrifuged for 5 min at 8000 rpm. After centrifugation 2 mL clear solution was collected by micropipette (Eppendorf Research 1000) and filtered through 0.2 μm nylon membrane filter (0.2 μm ultipor nylon 6, 6 membrane filter, Pall Corporation) and analyzed by GC-ECD to quantify Hexythiazox content present in Okra fruit and soil.

2.4. Quantification of Hexythiazox residue by GC-ECD

Instrumental Parameters

Residues of Hexythiazox were determined on Agilent 6890N equipped with Electron capture detector and wide bore HP-5 column (30 m x 0.32 mm i.d. x 0.25 μm film thickness). Oven temperature

programming with GC parameters were as follows: Temperature (ºC): Oven: 140^0 C (1 min) $\rightarrow 10°min^{-1}$ $\rightarrow 240^0$ C (5min). Injection port: 275; detector: 300. Carrier gas (N_2) flow was 2 ml/min, Injection volume was 2 μL. Retention time (R_t) for Hexythiazox was 9.00 ± 0.2 min. LOQ for this method was 0.03 ppm. Chromatogram of hexythizox standard solution is presented in Fig. 3.

Estimation of Calibration curve

For the preparation of calibration curves, Hexythiazox standard was diluted with pure ethyl acetate in series (seven calibration point) from 0.01 to 1 ppm and injected in GC-ECD. A standard calibration curve of Hexythiazox was constructed by plotting analyte concentrations against corresponding area (Fig. 2). Good linear correlation between the peak area and the concentration assayed (0.01 to 1 ppm) with correlation coefficient 0.999 for Hexythiazox in all cases.
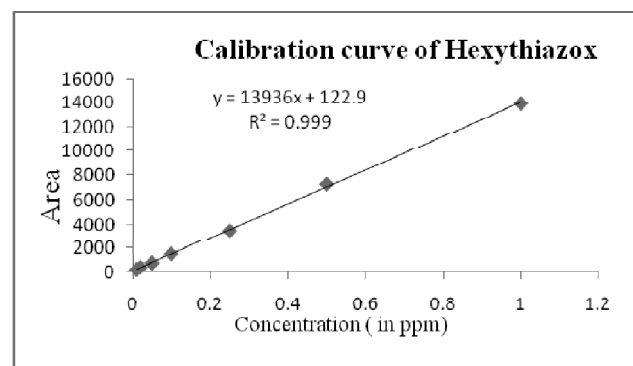

Figure 2: Calibration curve of analytical standard of Hexythiazox in GC-ECD
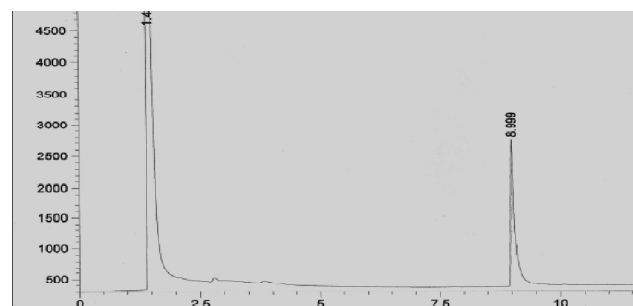

Figure 3: GC-ECD chromatogram of 1 ppm standard Hexythiazox solution in ethyl acetate

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RESULTS AND DISCUSSION

Results of recovery experiment in Okra fruit and soil

Recovery studies were carried out in order to establish the reliability of the analytical methods and to know the efficiency of extraction and clean up step for the present study by fortifying Okra fruit and soil samples separately with analytical standard of Hexythiazox at 0.05, 0.1 and 0.5 ppm level. It was observed that the mean percent recovery for Hexythiazox at three fortification levels was 89.43% for okra fruit and 86.55% for field soil. As the recovery percentage is more than 85% in both okra fruit and soil, the method can be adopted for the residue study of Hexythiazox in Okra fruit and soil sample. Zhang et al. studied the recoveries of Hexythiazox at three spiked concentration in three different matrix viz. mangos, tea, marrow and reported the average recoveries ranged from 83.6% to 116.9% which is in positive correlation with the present result [8].

Residue and Dissipation of Hexythiazox in okra fruit

The results obtained from the residue analysis of Hexythiazox in okra are presented in **Table 1.** The initial deposit of Hexythiazox in okra fruit was found in the range of 0.19 to 0.45 ppm for recommended dose (T_1) and 0.35 to 0.72 ppm for double (T_2) doses irrespective of location. Around 60% of initial residue was dissipated by 3rd day irrespective of dose and location. The residue of Hexythiazox was below the quantification level (0.03 ppm) at 5th day for both doses. The dissipation pattern of Hexythiazox in okra followed first order kinetics for all locations. First order kinetics has been extensively used to describe the degradation process of many chemicals [9]. The $T_{1/2}$ of hexythiazox was calculated using Hoskins formula [10]. The residual half life values were found to be in the range of 1.43 to 2.01 for recommended dose (T_1) and 1.63 to 2.08 days for double the recommended dose (T_2) respectively for all four locations. The soil sample was collected on 10th day after the application of the chemical. Hexythiazox residue was found below quantification level (0.03 ppm) in soil for all the four locations. Abd-Alrahman in a trial of Hexythiazox in bean-pods found that the initial deposit of Hexythiazox was 0.76 ppm and the half life value of Hexythiazox 2.70 days [6]. Thus the results of the current experiment qualify the previous findings.

The pre harvest interval (PHI) of Hexythiazox is the time required before the pesticide residue

Table 1
Residue and Dissipation of Hexythiazox (5.45 EC) in Okra

Trial Location	Dose	Residues in ppm					Regression Equation	Half Life ($T_{1/2}$)
		Day						
		0	*1*	*3*	*5*	*7*		*in Days*
Location I (Kalyani)	T_1	0.39±0.07	0.22±0.06	0.14±0.06	-	-	Y = 0.160x + 2.574	1.88
	T_2	0.61±0.03	0.39±0.03	0.20±0.03	-	-	Y = 0.153x + 2.77'	1.97
Location II (Rahuri)	T_1	0.45±0.03	0.29±0.02	0.13±0.02	-	-	Y = 0.175x + 2.644	1.72
	T_2	0.70±0.04	0.44±0.02	0.22±0.02	-	-	Y = 0.163x + 2.831	1.85
Location III (Jabbalpur)	T_1	0.20±0.02	0.13±0.01	0.06±0.01	-	-	Y = 0. 211x + 2.308	1.43
	T_2	0.36±0.04	0.23±0.03	0.10±0.01	-	-	Y = 0.185x + 2.554	1.63
Location IV (Dhakrani)	T_1	0.19±0.04	0.12±0.01	0.08±0.01	-	-	Y= 0.150x + 2.234	2.01
	T_2	0.35±0.05	0.19±0.02	0.12±0.01	-	-	Y = 0.145x + 2.494	2.08

reaches a level that is lower than the MRL's established. As there is no MRL data for Hexythiazox available in India, the pre harvest interval (PHI) of Hexythiazox in okra was calculated on the basis of MRL established by CODEX alimentary commission (MRL value of Hexythiazox in okra as 0.05 mg/kg) [11]. On the basis of CODEX MRL value the PHI of Hexythiazox in okra was found to be 5 day (Location I and II) and 3 day (Location III and IV)) for the recommended dose (T_1). Abd-Alrahman also found Pre Harvest Interval (PHI) value of 4 days for Hexythiazox in bean-pods which are in well agreement with the findings of the previous Study [6].

Safety risk assessment of Hexythiazox

Human health risk situation are a function of hazard and exposure to that hazard. If the hazard is small and fixed, then the risk will be proportional to the exposure, which can be reduced to low and occasional [12]. To assess the safety risk of Hexythiazox application in okra at recommended (25 g a.i/ha) and double dose (50 g a.i/ha), the values of maximum permissible intake (MPI) is compared with theoretical maximum residue contribution (TMRC). MPI is calculated as per the following formula- MPI = Acceptable daily intake (ADI) of Hexythiazox X average body wt of an Indian adult

Average body wt. of an Indian adult is considered to be 55 kg [13].

According to FAO JMPR 1991 report, The ADI of Hexythiazox is 0.03 mg/kg of b.w.

Whereas TMRC = Daily consumption of the food commodity X residue in ppm. It is expressed in mg of pesticide per day [14].

Considering the average daily per capita consumption of vegetables by an Indian active adult is 150 g [15], the TMRC value of Hexythiazox in okra was calculated and it is observed that the TMRC of Hexythiazox in okra was 0.02 (for Location I and II) and 0.01 (for Location III and IV) mg/person/ day at recommended dose at 3 days which were very lower than the MPI value (1.65 mg Hexythiazox/ day) which indicates that the schedule of plucking of okra at 5th day of application of Hexythiazox at the recommended dose is safe for human consumption under Indian scenario and the observation is in accordance with the WHO [16]. There is no MRL of Hexythiazox in vegetables under Indian condition; hence this study may become an important tool in the context of plant protection and safety evaluation.

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