

Residual fate and dissipation behaviour of Hexythiazox in brinjal

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ABSTRACT

A one season supervised multi location field trial of Hexythiazox on brinjal was conducted under Indian climatic condition. The Hexythiazox formulation (Maiden 5.45 EC) was applied @ 25 g a.i. ha⁻¹ and 50 g a.i ha⁻¹ along with untreated control. The samples were extracted and cleaned up using modified QuEChERS method and quantified by GC-ECD. The LOD & LOQ of Hexythiazox was 0.01 ug⁻¹g and 0.03 ug⁻¹g respectively. The average recovery of Hexythiazox was found in the range of 84-92 % for brinjal fruit and 86-90% for field soil. Dissipation of Hexythiazox followed first order kinetics and the half life values of Hexythiazox were in the range of 1.42 to 2.32 days. No residue was detected in field soil. The pre harvest interval of Hexythiazox in brinjal was calculated and found in the range of 3-5 days irrespective of dose and location.

Key words : Brinjal, GC-ECD, hexythiazox, half life, LOD, LOQ.

Brinjal (*Solanum melongena*) is a vegetable under solanaceae family which is cultivated throughout India. It is considered as one the most common food item in regular human diet (Banerjee, *et al.*, 2009) India accounts for about 8.7 million MTs of brinjal production with an area of 0.53 million hector area under cultivation. (Anon 2012-2013). Brinjal production is severely affected by attack of insects and mites. Many insecticides have been used to encounter this problem. 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 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 brinjal, okra, soybean, vines, flowering plants (ex: rose and cotton). (Mucinelli, 1987). As Hexythiazox is a newly introduced pesticide in India, there is limited data concerning the dissipation behaviour of Hexythiazox in vegetables. Thus the objective of the present study is to find out the residual fate and dissipation behavior of Hexythiazox in brinjal under Indian climatic condition.

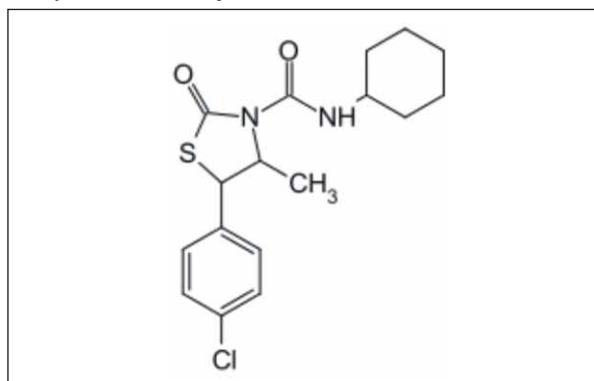


Fig. 1: Structure of Hexythiazox

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MATERIALS AND METHODS

Hexythiazox was applied on brinjal during 2012-2013 at four different locations viz, (i) AB block Farm, C unit, BCKV, 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. Hexythiazox (Maiden 5.45% EC) was applied in the field at fruiting stage of brinjal at two different doses viz, recommended dose (T₁) i.e. 25 g a.i ha⁻¹ and double the recommended dose (T₂) i.e. 50 g a.i. ha⁻¹ and a control where no pesticide was applied. Two treatment doses of the pesticide along with a control were replicated thrice in a randomized block design. Spraying was done with high volume knapsack sprayer (sampling volume 500 L ha⁻¹) fitted with hollow cone nozzle.

Brinjal fruit samples (1.00 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 of the acaricide. Soil sample (1 kg) was collected at 10th day after the application of the chemical. A representative (100 g) sample of Brinjal fruit and soil were taken for analysis using quartering technique. The fruit sample was blended using blender. Soil samples were air dried and sieved through 80 mesh sieve prior to extraction.

Stock standard solution of 100 ppm Hexythiazox (Purity 99.90%) was prepared by dissolving 10.01 mg Hexythiazox in 100 ml ethyl acetate. The working standard solutions of 0.01, 0.02, 0.05, 0.1, 0.25, 0.5, and 1.0 ug ml⁻¹ were prepared from stock solution by serial dilution technique with ethyl acetate.

An aliquot of ten gram (10 g) of the both brinjal fruit and soil sample were taken in a 50 ml fluorinated

ethylene polypropylene (FEP) centrifuge tube and 0.1 (N) NaOH solution 10 ml was added and subjected to vortex (Spinix) for 1 min. Then 20 ml of ethyl acetate (HPLC grade, JT Baker) followed by 1.5 g anhydrous NaCl and 5 g of activated Na_2SO_4 were added, the sample was again vortexed for 1 min and placed on a roto spin (Tarsons) for 30 min. at 50 rpm. The sample was then centrifuged using Centrifuge (model: Avanti J-301, Beckmen Coulter, Fullerton, CA) for 5 min at 8,000 rpm and 8 ml supernatant liquid was collected.

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) 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 (Eppendr of Research 1000) and filtered through 0.2 μm nylon membrane filter (0.2 μm ultipor N66 nylon 6, 6 membrane filter) and analyzed by GC-ECD to quantify Hexythiazox content present in brinjal fruit and soil.

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 ($^{\circ}\text{C}$): Oven: 140° (1 min) $\rightarrow 10^{\circ} \text{min}^{-1} \rightarrow 240^{\circ}$ (5min). Injection port: 275; detector: 300. Carrier gas (N_2) flow was 2 ml min^{-1} , Injection volume 2 μL . Retention time (R_t) for Hexythiazox was 9.00 ± 0.2 min. LOQ for this method was 0.03 ug g^{-1} . For the preparation of calibration curves, Hexythiazox standard was diluted with pure ethyl acetate in series (seven calibration point) from 0.01 to 1 ug g^{-1} and injected in GC-ECD. A standard calibration curve of Hexythiazox was constructed by plotting analyte concentrations against retention time of 9.00 ± 0.2 min (Fig. 2). Good linear correlation between the peak area and the concentration assayed (0.01 to 1 ug g^{-1}) with correlation coefficient 0.999 for Hexythiazox in all cases.

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 brinjal fruit and soil samples separately with analytical standard of Hexythiazox at 0.05, 0.1 and 0.5 ug g^{-1} level. It was found that the mean percent recovery for Hexythiazox at

three fortification levels was 87.33% for brinjal fruit and 88.07% for field soil respectively. Considering the recovery percentage is more than 85% in both brinjal fruit and soil, the method was adopted for the residue analysis of Hexythiazox in the above substrates.

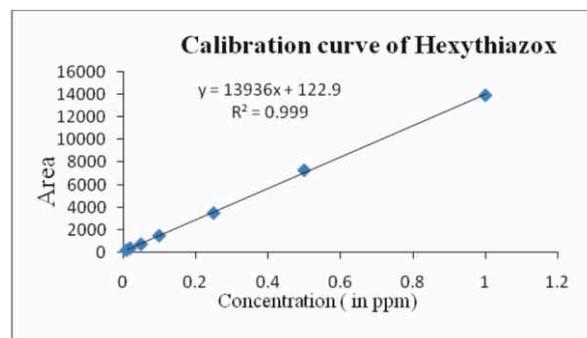


Fig. 2: Calibration curve of analytical standard of Hexythiazox in GC-ECD

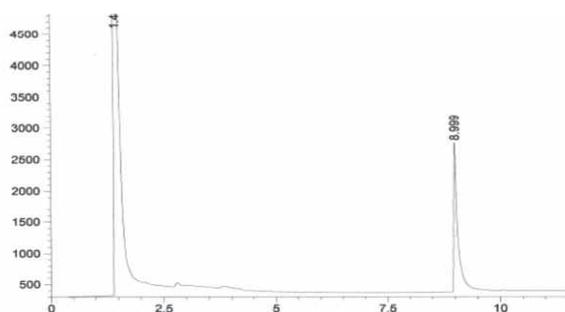


Fig. 3: GC-ECD chromatogram of 1 ppm standard Hexythiazox solution in ethyl acetate

RESULTS AND DISCUSSION

The results obtained from the residue analysis of Hexythiazox in brinjal are presented in table 1. The initial deposit of Hexythiazox in brinjal fruit were found in the range of 0.23 to 1.18 ug mL^{-1} for recommended dose (T_1) and 0.42 to 2.30 ug g^{-1} 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 ug g^{-1}) at 7th day for both doses. The dissipation pattern of Hexythiazox in brinjal followed first order kinetics for all locations. The $T_{1/2}$ of Hexythiazox was calculated using Hoskins formula (Hoskins, 1961). The residual half life values were found to be in the range of 1.42 to 2.13 for recommended dose (T_1) and 1.84 to 2.47 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

Table 1: Residue and dissipation of Hexythiazox (5.45 EC) in brinjal ($\mu\text{g g}^{-1}$)

Location	Dose	Days after application					Regression Equation	Half Life ($T_{1/2}$) in Days
		0	1	3	5	7		
(Kalyani)	T ₁	0.24±0.03	0.16±0.02	0.09±0.01	-	-	Y = 0.141x + 2.368	2.13
	T ₂	0.38±0.03	0.26±0.03	0.16±0.03	0.09±0.01	-	Y = 0.122x + 2.559	2.47
(Rahuri)	T ₁	0.26±0.04	0.19±0.01	0.08±0.01	-	-	Y = 0.182x + 2.432	1.65
	T ₂	0.42±0.05	0.28±0.03	0.18±0.02	0.08±0.01	-	Y = 0.130x + 2.605	2.32
(Jabalpur)	T ₁	1.18±0.07	0.70±0.03	0.27±0.01	-	-	Y = 0.164x + 3.373	1.42
	T ₂	2.3±0.13	1.55±0.05	0.90±0.06	0.33±0.04	-	Y = 0.212x + 3.067	1.84
(Dhakrani)	T ₁	0.23±0.02	0.15±0.02	0.08±0.01	-	-	Y = 0.145x + 2.350	2.08
	T ₂	0.53±0.03	0.38±0.03	0.19±0.02	0.10±0.02	-	Y = 0.142x + 2.471	2.12

*T₁ = 25 g a.i ha⁻¹, T₂ = 50 g a.i ha⁻¹

quantification level ($0.03 \mu\text{g mL}^{-1}$) in soil for all the four locations. Abd-Alrahman SH (2012) in a trial of Hexythiazox in bean-pods found that the initial deposit of Hexythiazox was $0.76 \mu\text{g g}^{-1}$ and the half life value of Hexythiazox 2.70 days. 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 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 brinjal was calculated on the basis of MRL established by CODEX alimentary commission (MRL value of Hexythiazox in brinjal as 0.1 mg kg^{-1}) (Anon, 2012). On the basis of CODEX MRL value the PHI of Hexythiazox in brinjal was found to be 3 day (Kalyani and Dhakrani), 2 day (Rahuri) and 5 day (Jabalpur) for the recommended dose (T₁). Abd-Alrahman, SH (2012) 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.

On the basis of the above study, it might be concluded that Hexythiazox may be safely recommended for use in brinjal and is quite safe for human consumption if the crop is harvested at least 5 days after treatment.

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