Studies on Residues of Flufenzin and Fenpyroximate on Brinjal

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SUMMARY

After application (twice) of flufenzin @ 100 and 200 g a.i./ha and fenpyrozimate @ 25and 50 g a.i./ha, residue level in brinjal inpested from red spider mite dissipated within 7 and 10 days and 3 and 5 days, respectively. The levels were reduced in the range of 43 to 48 and 46 to 55.6% due to washing. Combined effect of both washing and cooking, further reduced the levels in the range of 70 to 75% and 67 to 70% in fenpyrozimate and flufenzin.

Key words : Brinjal, Residues, Flufenzin and fenpyrozimate, Red spider mite, *Tetranychus* sp.

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Red spider mites (*Tetranychus* sp.) have now become serious menace for the cultivation of vegetables. Intensive year-round cultivation often leads to increased flare-ups necessitating more rigid pest control. Chemical control mostly constitutes use of broad-spectrum insecticides/acaricides, which poses deleterious impact on biodiversity resulting into reduction of overall population of natural enemies and other beneficial biological species. Repeated use of such chemicals and/or increase in their dosages have unnecessarily led to severe problems such as additional insecticide cost, elimination of beneficial fauna, unintended toxic residues and more importantly, the ability of pests to adapt to high pesticides use environment. In turn, field management has now become a more difficult task.

In recent years, the emphasis is being laid on low risk acaricide active at low dose and is less harmful to non-targeted species. New generation acaricides with novel chemistries are being developed and claimed to be selective against target mite species with little or no effect on beneficials / environment. Two such new molecules *viz.*, Fenpyroximate 5 EC (5%) and Flufenzin 200 SC (20%) were selected for the studies. Fenpyroximate is a contact poison belonging to pyrazole group and acts as mitochondrial electron transport- inhibitor. Flufenzin is a mite growth regulator (new tetrazine analogue) having both contact and translaminar action. Contemplating their use on vegetables, especially on brinjal, the data on magnitude of residues of these compounds after application is of immense importance from the consumers' safety point of view.

MATERIALS AND METHODS

Acaricides *viz.*, fenpyroximate (@ 25 and 50 g a.i./ha) and flufenzin (@ 100 and 200ga.i/ha) were evaluated after second spray under the field conditions for studying their dissipation pattern and the residue levels in/on brinjal fruits, both processed and unprocessed. Pesticide residues were analyzed in edible quality fruits collected periodically after the last spray to decide the safety of treatments to consumers. Fruit samples from each plot were collected at an interval of 0(1h), 1, 3, 5, 7 and 10 days after the last treatment application and subjected to analysis by extraction, cleanup and estimation. The reference standard of parent compound and method employed for residue analysis was obtained from the respective manufactures. Revised–phase high performance liquid chromatography (HPLC) with ultraviolet detection at specified wavelength was used to determine residues of both the acaricides. Percentage recovery experiments validated the suitability of the analytical method. The linearity of the detector was determined using calibration standards. Peak identification was made by comparing the characteristic retention times of peaks in the standard chromatograms and sample chromatograms. Standards were injected at the beginning and at the end of the analysis of asset of samples to evaluate stability of chromatography system and the standards. All the analyses were carried out in duplicate and the data were analyzed statistically to interpret the results.

Fenpyroximate:

Residues were extracted as per the

protocol supplied by M/S Excel Industries Ltd, Mumbai. The sample fruits were chopped into pieces and mixed thoroughly from which a representative 50 g sample was taken. It was homogenized in a tissue homogenizer with acetone. The sample extract was vacuum-filtered through Buchner funnel, using Celite filter aid. The filter cake was then re-extracted with 50 ml acetone. The blender jar and filter bed were rinsed with 50 ml additional acetone for complete extraction. The combined extract was concentrated to about 20 ml on rotary vacuum evaporator at 40^{0}C. After transferring to 500 ml separator funnel, the extract was diluted with 200 ml of 10% aqueous sodium chloride solution. The solution was partitioned with 3 x 100 ml of dichloromethane by vigorous shaking for two minutes and the lower organic phase (dichloromethane) was collected after separation of the phases. It was filtered and dried over anhydrous sodium sulphate. Combined dichloromethane was concentrated to near dryness. Concentrated dichloromethane extract (approximately 3 ml) was subjected to cleanup by column chromatography using Florisil as adsorbent. The analyte was eluted with 100 ml acetonitrile into 500 ml round-bottomed flask and reduced to dryness on rotary vacuum evaporator at 40^{0}C. The residue was reconstituted in 1 ml of HPLC-grade acetonitrile for injecting into HPLC system.

Residues were determined on Shimadzu High Performance Liquid Chromatograph (HPLC) equipped with LCD-10 AT pump and SPD-10A UV-VIS detector connected to Shimadzu ^{a}-6 RA data integrator. The column was RP-18 (25 cm length x 4.6mm i.d.) and the mobile phase was 20% water in acetonitrile isocratic at a flow rate of 1.2 ml/minute. The UV detector was operated at 235 nm. The injected volume was 20 µl. The residue levels expressed as mg/kg. Data on dissipation of residues on fruits were analyzed by the method suggested by Hoskins (1961) to work out the RL_{50} (residue half-life in days) and T_{BDL} (days required for the residue to reach below detectable limit).

Flufenzin:

Residues were extracted as per the protocol supplied by M/S Rotam India, Mumbai. The sample fruits were chopped into pieces and mixed thoroughly. The fruit sample weighing 50 g was blended with 10-12 ml water in tissue homogenizer. Ten gram homogenate was made into free flowing powder in mortar by adding enough filter aid. The free flowing powder was transferred into glass column (300 x 18 mm i.d.). The column was tapped gently, each time the powder added for uniform loading. The mortar and pestle were rinsed with 20 ml extracting solvent, hexane: dichloromethane (7:3) and the solvent mixture was poured onto the column. The flow rate was adjusted to 5 ml/minute. The analyte was eluted with 100 ml extracting solvent. The solvent was collected in round-bottomed flask and the extract was concentrated to 1 ml on rotary vacuum evaporator. Three ml of hexane was added to round bottom flask and the extract was transferred to test tube. The extract was then concentrated after adding 1 ml methanol. The final extract, after adjusting to 1 ml, was sonicated and filtered through 0.45 ìm membrane filter for injection on HPLC.

Residues were determined on Shimadzu High Performance Liquid Chromatograph (HPLC) with SPD-10A UV-VIS detector at 270 nm wave length. Chromatograms were plotted on Shimadzu ∈-R6A data integrator. The column was RP-18 (250 x 4.6 mm i.d.) and the mobile phase was methanol plus water at 75:25 ratio at a flow rate of 1 ml/minute. The volume injected was 20 µl. Residue levels were expressed as mg/kg. Data on dissipation of residues on fruits were analyzed by the method suggested by Hoskins (1961) to work out RL_{50} and T_{BDL}

Recovery percentage for fenpyroximate and flufenzin from fruits was determined by fortifying flufenzin standard at 2 and 5 µg/g of sample. After few minutes, samples were analyzed following the analytical procedure prescribed earlier. Per cent recoveries were calculated as difference between the amount of fenpyroximate/ flufenzin added in the fortified sample and the amount recovered. In order to evaluate the effect of simple home-processing on reduction of residues, fruit samples harvested at zero (1h) were subjected to washing of fruits with tap water and washing followed by cooking. Samples were extracted and analyzed. Average percentage recoveries in flufenzin and fenpyroximate were 90 and 87.70%, respectively which, were within commonly acceptable range (80 to 110%). The limit of detection was 0.05 mg/kg for both the analyses.

RESULTS AND DISCUSSION

Mean residues at different sampling days and residue levels prior to and after the home-processing are depicted in Table 1 and 2, respectively.

Flufenzin:

The retention time for the compound was 4.80 minutes. In control samples, no peaks were detected in the chromatograms. In highest application rate (double the recommended dose) of 200 g a.i./ha, the concentration of flufenzin at zero day (≈ 1h) was 0.37 mg/kg. A decrease in the level of total residue was observed with elapsed

Table 1: Dissipation of the acaricide residues on brinjal fruits

Days after spray	Mean residues (mg/kg)			
	Flufenzin (g a.i./ha)		Fenpyroximate (g a.i./ha)	
	100	200	25	50
0	0.20	0.37	0.10	0.18
1	0.16	0.32	0.06	0.12
3	0.10	0.22	BDL	0.07
5	0.05	0.13	BDL	BDL
7	BDL	0.06	BDL	BDL
10	BDL	BDL	BDL	BDL
RL_{50} (days)	2.52	2.72	1.36	2.25
T_{BDL} (days)	5.20	8.28	1.36	4.03
Regr. Equn.	Y = 2.3196 – 0.1193 x	Y = 2.6163 – 0.1109 x	Y = 2.0 – 0.2219 x	Y = 2.2384 – 0.1339 x

(BDL: Below detectable limit)

Table 2: Effect of processing of fruits on decline of acaricide residues

Particulars	Mean residues (mg/kg)			
	Flufenzin (g a.i./ha)		Fenpyroximate (g a.i./ha)	
	100	200	25	50
Residues at 0-DAS (Pre-washing)	0.200	0.370	0.100	0.180
Residues at 0-DAS (Post-washing)	0.104	0.210	0.054	0.080
Residues decline after washing (%)	48.00	43.24	46.00	55.56
Residues after washing & cooking at 0-DAS	0.060	0.120	0.030	0.045
Residues decline after washing and cooking (%)	70.00	67.57	70.00	75.00

DAS: Days after (second) spray

time. Residue level of 0.06 mg/kg detected at 7th days was dissipated to below detectable level within 8 days post-application. The initial residue of 0.20 mg/kg resulted from the lowest dose (100 g a.i./ha) was further dissipated with the time to a level of 0.05 mg/kg on 5th day and below this detectable level within 6 days. Under field conditions, the half-life (RL_{50}) was estimated to be 2.52 and 2.72 days for the recommended and double the recommended dose, respectively. The days required for the residue to reach below the detectable limit (T_{BDL}) for the lowest and highest dose were 5.20 and 8.28 days, respectively (Table 1).

Fenpyroximate:

Retention time for the compound was 8.55 minutes. The immediate post-application (zero time) residue level on fruits was 0.18 mg/kg for double the recommended dose (50 g a.i./ha). The level of residue decreased with time, to a level of 0.07 mg/kg within 3 days, which was further decreased below the detectable limit within 4 days. Initial residue level of 0.10 mg/kg resulted from the lowest rate of application (25 g a.i./ha) was detected to be 0.06 mg/kg at 1 day while on 3rd day, the residue was not detected in the samples. Under filed conditions, half-life (RL_{50}) was estimated to be 1.36 and 2.25 days for the lowest and the highest dose, respectively. The days required for the residue to reach below the detectable limit (T_{BDL}) for the recommended and double the recommended dose were 1.36 and 4.03 days, respectively (Table 1).

The fruit samples harvested immediately (zero time, 1h) after the second spray were subjected to two home-processes, thorough washing of fruits with tap water and washing followed by cooking. Data depicted in Table 2 indicate that in flufezin, initial residue level was 0.37 mg/kg in the highest dose (200 g a. i./ha) and 0.20 mg/kg in the lowest dose (100 g a. i./ha). Post-washing residues were estimated to be 0.21 mg/kg in the highest dose while 0.104 mg/kg in the lowest dose, indicating 43.2 and 48% reduction. The residue level after the processes *viz.*, washing and cooking was 0.12 mg/kg in the highest dose and 0.06 mg/kg in the lowest dose, which resulted in 67.6 and 70% reduction in residues, respectively. Pre-washing fenpyroximate residue level was 0.18 mg/kg in the highest dose (50 g a.i./ha) while 0.10 mg/kg in the lowest dose (25 g a.i./ha). The residues level after washing was 0.08 mg/kg in the highest dose and 0.054 mg/kg in the lowest dose, which showed 55.6 and 46% removal of residues by washing alone, respectively. Due to both washing and cooking processes, the decline in residues were 70% and 75% in the recommended and double the recommended dose of fenpyroximate, respectively.

In flufenzin at 100 and 200 g a.i./ha dosages, the initial residue level was 0.20 and 0.37 mg/kg, which fell down below the limit of detection on 7th and 9th day thereafter, respectively. In the studies conducted in apple and grape by Pap *et al.* (1994), flufenzin when applied at 60 g a.i./ha, its residues were detectable up to 61 days. Under the present studies, it was dissipated within 7 days when applied at 100 g a.i./ha. Present finding is in contradiction probably due to brinjal plant pattern wherein fruits are more covered by foliage in turn exposed less to spray droplets and also due to higher tropical temperature. The half-lives (RL_{50}) were estimated to be 2.52 and 2.72 days for the higher and lower dosages, respectively

Initial levels of fenpyroximate residues were estimated to be 0.10 and 0.18 mg/kg at 25 and 50 g a.i./

ha dosages, respectively which, fell down rapidly below the limit of detection on 3rd and 5th day after the application. Estimated half-lives were 1.36 days for the recommended and 2.25 days for double the recommended dose. In field studies carried out in green chilies by Pentareddy (2003), residues dissipated below the detectable limit of 0.05 mg/kg at 3.6 and 5.92 days of application of fenpyroximate at 25 and 50 g a.i./ha, respectively. Under the present studies in brinjal fruits, it was dissipated within 2 and 4 days for the same dosages. FAO panel has advocated its use on various crops as a safer acaricide (Anonymous, 1996). Studies on dissipation of residues under the field conditions revealed that flufenzin and fenpyroximate at the recommended rate of 100 and 25 g a.i./ha, dissipated at faster rate and reached the limit of detection within 7 and 3 days, respectively.

Simple processes such as washing, peeling and cooking are helpful in removing the residues from the fruits and vegetables. Residue level due to the process of washing was reduced in the range of 43 to 48% in flufenzin and 46 to 55.6% in fenpyroximate. The combining effect of washing and cooking further reduced the levels in the range of 67.6 to 70.0% in flufenzin and 70 to 75% in fenpyroximate. Thus, it is clear that routine process of washing as well as cooking before consumption certainly helps in minimizing the pesticide load on vegetable-fruits. Earlier studies on other pesticides also exhibited similar results in respect of various other vegetables (Agnihotri, 1999).

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