



Dynamics of difenoconazole and propiconazole residues on pomegranate over a period of two years under field conditions



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Renewed upto 16th October, 2016



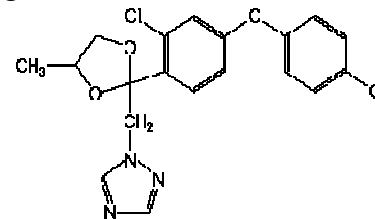
GENERAL INFORMATION



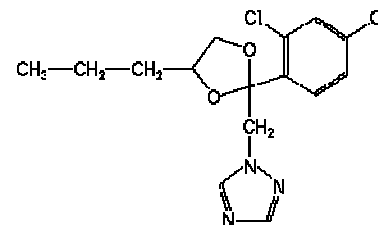
- ❖ Difenoconazole and propiconazole are broad spectrum fungicides.
- ❖ They are used for control of Wilt, leaf and fruit spot diseases of pomegranate.
- ❖ These diseases reduce the yield and marketability due to spotting of the fruits, can cause widespread destruction to pomegranate orchards.

❖ **Chemical structure:**

Difenoconazole - 1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole



Propiconazole- 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole





Experimental Field



Experimental Details



- ❖ A field study was undertaken as per good agricultural practices (GAP) to study the residue dynamics of difenoconazole and propiconazole on pomegranate .
- ❖ Spray application of the 2 fungicides was given to pomegranate crop (variety Baghwa) at the recommended and double dose of 125 and 250 g a.i./ha twice at 15 day intervals during August-October, 2012.
- ❖ The study was repeated during August-October, 2013.
- ❖ Residue analysis of pomegranate whole fruits and pulp (aril) was carried out after the second spray for a period of 1 month i.e., on 0, 1, 3, 5, 10, 15, 20, 25, 30, 35 and 40 days.
- ❖ Analysis of field soil was carried out after 30 days
- ❖ The environmental parameters such as temperature, humidity and rainfall was recorded.



RESIDUE ANALYSIS



- ❖ On every sampling day pomegranate fruits were collected from treated field.
- ❖ The whole fruits were cut into small pieces and homogenized.
- ❖ The fruits were washed under running water, peeled and the edible aril was homogenized.
- ❖ 15g of the whole fruit and aril was taken and sample preparation was carried out as per QuEChERS method.
- ❖ Soil samples were analyzed by QuEChERS method after adding water
- ❖ The final extract was analyzed by GC.
- ❖ Confirmatory studies were carried out by GC-MS.



QuEChERS Method



Approximately 2 kg pomegranate fruits were cut into small pieces.
Homogenized in a high volume Robot Coupe homogenizer



15 g sample was placed in a 50 mL Teflon tube.



15 mL of 1% acetic acid in HPLC grade acetonitrile was added to the tube.



6 g anhydrous magnesium sulphate, 1.5g of sodium acetate was added to the tube, mixed thoroughly by shaking and spinned for 2 min.



Centrifuged the tubes at 10,000 rpm for 10 min.



6 mL of the upper acetonitrile extract was placed in a centrifuge tube containing 50 mg primary secondary amine (PSA) sorbent and 150 mg anhydrous magnesium sulphate per mL of extract.



Shaken the tubes vigorously for 1 min and centrifuged at 10,000 rpm for 10 min.



3 ml acetonitrile extract was placed in a test tube and concentrated under nitrogen in a low volume concentrator and reconstituted in n-hexane: distilled acetone (9:1).



Analyzed by GC and confirmation by GC-MS.





Analytical method validation



- ❖ The analytical method was validated by studying the parameters such as recovery, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision, measurement uncertainty etc.
- ❖ The pomegranate used in the recovery study was grown in the experimental field of IIHR without the application of pesticides.
- ❖ Pomegranate whole fruit, aril and soil was spiked with difenoconazole and propiconazole at 0.05, 0.25 and 0.50 mg kg⁻¹ with five replications of each treatment.
- ❖ The precision of the analytical method was measured by the degree of repeatability (for five analyses) and expressed as the percent relative standard deviation.
- ❖ The linearity was determined in the concentration range of 0.01-1.0 µg/mL
- ❖ The measurement uncertainty (MU) of the method was calculated by considering all factors contributing to it. The uncertainties were determined individually and the expanded uncertainty was determined by using the coverage factor K=2, to give a confidence level of 95%.



Method validation results



- Recovery of difenoconazole and propiconazole from pomegranate whole fruit, aril and soil was in the range of 80.3-97.3% which was within the acceptable range of 70-120% (Method validation and quality control procedures for pesticide residue analysis in food and feed, SANCO/12495/2011).
- Matrix effect on recovery was nullified by calculating the recovery percent based on matrix matched standards.
- The calibration curve was linear in the range of 0.01-1.0 $\mu\text{g/mL}$ for both analytes with the correlation coefficient (R^2) > 0.99
- The LOQ of difenoconazole and propiconazole was 0.05 mg/kg.
- The precision expressed as relative standard deviation (RSD) remained within a range of 3.2-7.8%.
- The MU was in the range of 9.7-17.1%.



RESULTS

Residues of difenoconazole on pomegranate



Days after Treatment	Untreated control	Residues of difenoconazole recovered (mg/kg) \pm SD ^a			
		1 st year		2 nd year	
		Application at 125 g a.i./ha	Application at 250 g a.i./ha	Application at 125 g a.i./ha	Application at 250 g a.i./ha
0	ND	1.205 \pm 0.163	1.672 \pm 0.145	0.875 \pm 0.104	1.540 \pm 0.090
1	ND	1.012 \pm 0.105 (16.0)	1.464 \pm 0.172 (12.4)	0.700 \pm 0.033 (20.0)	1.236 \pm 0.110 (19.7)
5	ND	0.724 \pm 0.048 (40.0)	0.928 \pm 0.083 (44.5)	0.504 \pm 0.047 (42.4)	0.781 \pm 0.055 (49.3)
10	ND	0.348 \pm 0.017 (71.1)	0.460 \pm 0.018 (72.5)	0.346 \pm 0.026 (60.4)	0.484 \pm 0.030 (68.6)
15	ND	0.158 \pm 0.013 (86.9)	0.237 \pm 0.024 (85.8)	0.216 \pm 0.013 (75.3)	0.325 \pm 0.008 (78.9)
20	ND	0.102 \pm 0.006 (91.5)	0.174 \pm 0.006 (89.6)	0.173 \pm 0.016 (80.2)	0.204 \pm 0.017 (86.7)
25	ND	0.079 \pm 0.002 (93.4)	0.143 \pm 0.007 (91.5)	0.120 \pm 0.005 (86.3)	0.152 \pm 0.013 (90.1)
30	ND	0.050 \pm 0.001 (95.8)	0.096 \pm 0.008 (94.3)	0.058 \pm 0.001 (93.4)	0.112 \pm 0.006 (92.7)
35	ND	<LOQ	<LOQ	<LOQ	0.080 \pm 0.004 (94.8)

Values in the parenthesis is the dissipation % of residues



Dissipation of difenoconazole on pomegranate

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