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Dissipation analysis of Difenaconazole (25 EC) on apple under temperate conditions of Kashmir

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ABSTRACT: In present study, the dissipation pattern and persistence of foliar application of difenaconazole (25 EC) on leaves and fruits of apple were investigated at 300 g ai ha^{-1} and 600 g ai ha^{-1}. The samples were obtained at different time intervals *viz.*, 0, 1, 3, 5, 7, 10, 15, 20, 25, and 30 days after treatment. The cost-effective, efficient, and robust QuEChERS method coupled with a gas chromatography mass spectrometery was used to analyze the residual behaviour of difenaconazole. The residual concentration in leaves and fruits dropped below the detection limit on 30th day at 300 g ai ha^{-1} and 25th and 30th day at 600 g ai ha^{-1} during 2021. The mean recovery rates in leaves and fruits ranged from 84.80 to 101.6 per cent and the relative standard deviation was 1.69 to 3.97 per cent. The half-life of difenaconazole in leaves and fruits were 9.9 to 10.19 days and 5.21 to7.3 days at single and double dose, respectively. In terms of pre-harvest intervals (PHI), the time required for residues to decrease to permissible levels (maximum residue limits) ranged from 6.8 to 11.75 days for the single dose and from 3.65 to 8.54 days for the double dose.

Keywords: Difenaconazole, Dissipation, Residue, Half-life, QuEChERS, Recovery.

INTRODUCTION

The apple, scientifically known as (*Malus* × *domestica*. Borkh) stands as one of the most economically important temperate fruit and ranks fourth among the most extensively cultivated fruits globally, following bananas, oranges, and grape Originating over 4000 years ago in the Middle East, the apple's popularity has reached every corner of the Earth. Apples are rich in flavonoids, polyphenols, vitamins and minerals, along with a wealth of beneficial phytochemicals. However, in the realm of apple production, the use of pesticides has become a common place. Pesticides serve as a cornerstone in the management of pests and the mitigation of diseases in fruit cultivation however, growers have long faced accusations of excessive use, which can have detrimental impacts on food safety, the ecosystem, and human well-being Cai *et al*. (2021). The pesticides noted by Sauphanor *et al*. (2009) Simon *et al.* (2011), Mladenova *et al*. (2009), and Pennel (2006), serve the critical purpose of safeguarding apple crops against the pathogens and pests that threatens during various stages of production, storage, and transport (Ticha *et al*., 2008). Pesticides empower growers to increase their harvest yields from each tree and in turn, enhance the quality and longevity of the fruits. Yet, the tan extensive array of potential risks to human health, ranging from immediate effects like as headaches and nausea to enduring consequences such as cancer, harm to reproductive functions, and interference with the endocrine system. (Baldi *et al*., 2006; Carreno *et al*., 2007; Jankuloska *et al*., 2017 and Benbrook, 2021). Among the fungicides, difenaconazole is a broad-spectrum which pertains to14 α-demethylation inhibition (DMIs). Difenaconazole exhibits a remarkable capacity to inhibit the synthesis of ergosterol in fungi, as elucidated by Yun *et al*. (2012). Its unique attribute lies in its exceptional absorption by plant roots, enabling seamless distribution to various plant tissues through the xylem, a key vascular tissue (Zheng *et al*., 2020). The utilisation of difenaconazole in agriculture has been rising faster than that of other fungicides due to its remarkable efficacy against plant diseases. This versatile fungicide finds application through methods like spraying, seed coating, drenching of infected plant roots, and direct application, making it an indispensable tool in safeguarding of our apple orchards. This fungicide exhibits protective and therapeutic effects and also possess lower toxicity to non-target organisms (Dong *et al*., 2013). In the context of current study our main focus was to analyse the dissipation pattern and fate of difenaconazole residues in the leaves and fruits of apple.

MATERIAL AND METHODS

The research experiment was conducted at apple orchard block of Division of Fruit Science within the Faculty of Horticulture at SKUAST-K Shalimar, during the year 2021. The research followed a randomized

experimental framework, incorporating three replicates for each treatment. A planting configuration with a spacing of 1.5 meters between individual plants and 3.0 meters between rows was consistently maintained. The fungicide difenaconazole (25 EC) was sprayed at two concentrations i.e., 0.03 per cent and 0.06 per cent on active ingredient basis during the phenological stage of apple FD II. About one kg of samples (leaves and fruits) treated with the desired concentrations were collected at 2 hours post application followed by 01, 03, 07, 10, 15, 20, 25 and 30 days and control trees were sprayed with water only. Sample collection was done in poly bags and brought to the laboratory for future use. The composite sample of one kg from three replications was taken in blender which was chopped and grinded for about 2-3 minutes. About 15g of homogenised samples were taken in a 50 ml centrifuge tube in which 30 ml of acetonitrile (leaves) and ethyl acetate (fruits) were added. Further, 3g± 0.1 g of NaCl was added to the centrifuge tube which was centrifuged at 2500–3000 rpm for 3 minutes to separate the organic layer. Approximately 18ml of upper organic layer was taken in separate centrifuge tubes in which 9g ± 0.1 g of Na_2SO_4 was added. The purification process of the acetonitrile extract involves a method known as dispersive solid-phase extraction (DSPE). In this procedure, an 11 mL portion of acetonitrile is placed into a test tube, and to this, 1.15 grams of magnesium sulfate ($MgSO_4$) and 0.4 grams of primary secondary amine (PSA) are added. Subsequently, the mixture is agitated using a vortex for approximately 30 seconds, followed by centrifugation at 3000 rotations per minute (rpm) for 5 minutes. The resulting 6 mL of acetonitrile aliquot is then subjected to evaporation to achieve dryness, accomplished using a low-volume evaporator set at 35°C. Finally, the volume is adjusted to its final state by reconstituting it with 3 mL of n-hexane. This solution is subsequently filtered through a PTFE syringe filter with a pore size of 0.2 micrometers and subsequently analyzed using gas chromatography-mass spectrometry (GC-MS).

RESULTS AND DISCUSSION

In order to establish the trustworthiness and efficiency, a recovery process was carried out to validate the extraction and purification process. The leaves and fruits were fortified with diverse analytical standard solutions containing of difenaconazole. The recovery has dual purpose of verifying the methods accuracy and assessing the performance of our sample preparation. The fortification of control samples was done at three different concentrations of 0.25, 0.50, 0.75 µg g^{-1} and the processing of samples was done by the method as mentioned above. The average recovery ranged from 84.80 to 101.60 per cent. From Table 1 and Table 2, the three recovery levels were within the range of 70 to 120 per cent (SANCO/1257/2014). The relative standard deviations were less than 20 per cent for all the concentrations.

The dissipation behaviour of difenaconazole on apple is summarized in Table 3 and Table 4, that shows the maximum initial concentrations of 0.83±0.03 µg g^{-1} and 1.32±0.02 µg g^{-1} at zero day for single and double dose, respectively.

Table 1: Recovery of Difenaconazole from apple leaves.

Amount fortified (µg g^{-1})	Amount recovered (µg g^{-1})*	Average recovery (%) ± SD	% relative standard Deviation (RSD)
0.25	0.254	101.6 ± 1.721	1.69
0.50	0.490	98.00 ± 2.321	2.36
0.75	0.666	88.80 ± 1.762	1.98

*average of three replications.

Table 2. Recovery of Difenaconazole from apple fruits.

Amount fortified (µg g^{-1})	Amount recovered (µg g^{-1})*	Average recovery (%) ± SD	% relative standard Deviation (RSD)
0.25	0.212	84.80 ± 2.112	2.49
0.50	0.456	91.20 ± 3.621	3.97
0.75	0.732	97.60 ± 1.751	1.79

*average of three replications

Residues on 10th day declined to 0.47 ±0.03 and 0.68±0.01 µg g^{-1} with the dissipation rate of 51.86 and 61.89 per cent in single and double dose, respectively. There was a steep decline in the residues and by the 25th day the residues degraded to 0.10±0.06 and 0.20± 0.09µg g^{-1} with dissipation rate of 87.84 and 84.69 per cent in single and double dose, respectively. After 30th day the final residue reached below the detection limit at both doses. The half-life values were 9.9 days for single dose and 10.19 days for double dose. The waiting period of 6.8 days and 11.75 days were recorded in single and double dose respectively. Similar results were found in case of fruits (Table 4) in which the maximum initial concentration was 0.73±0.01 and 0.98±0.03 µg g^{-1} at zero day. The residues on 10th day dissipated to 0.21±0.04 and 0.48 µg g^{-1} with the dissipation rate of 70.95 and 50.76 per cent in single and double doses, respectively. Thereafter, on 25th day the residues reached below detection limit for both single and double doses, respectively. The half-life values were found to be 5.21 and 7.53 days and pre-harvest interval of 3.65 and 8.54 days at recommended and double the recommended doses, respectively. The findings closely resembled those observed in other research studies (Xu *et al*., 2019). Specifically, the degradation half-lives of difenaconazole were found to be in the range of 6.0 to 11.5 days in pepper fruits according to Wu et al. in 2018, 6.3 to 10.2 days in apples as reported by Guo *et al.* in 2010, 6.6 to 7.8 days in cabbages as indicated by Wang *et al*. in 2008, 2.15 to 2.32 days in chili fruits as documented by Mukhopadhyay *et al.* in 2011, and 4 to 11.5 days and 4.6 to 6.7 days for apples as per the research conducted by Sharma and Nath in 1992.

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Table 3. Dissipation pattern of Difenaconazole 25 EC on leaves of apple cv. Gala Redlum under HD orchard system.

Interval (Days)	Leaf							
	Residues* (µg g^{-1}) ±SD (x)	Residues* (µg g^{-1}) ±SD (2x)	$T_{½}$(Days)		T_{tol}(Days)		r^2	
			(x)	(2x)	(x)	(2x)	(x)	(2x)
0	0.83±0.03	1.32±0.02	9.9	10.19	6.8	11.75	0.90	0.99
1	0.71±0.04 (14.07)	1.13±0.01 (14.24)						
3	0.66±0.05 (19.97)	0.93±0.06 (29.46)						
5	0.53±0.06 (35.13)	0.77±0.03 (40.10)						
7	0.47±0.03 (42.47)	0.68±0.01 (48.10)						
10	0.40±0.07 (51.86)	0.50±0.07 (61.89)						
15	0.36±0.03 (56.55)	0.43±0.08 (66.74)						
20	0.25±0.04 (68.95)	0.33±0.08 (75.00)						
25	0.10±0.06 (87.84)	0.20±0.09 (84.69)						
30	<BDL	< BDL						

*= Mean of three replicates in parts per million (ppm), figures with in the parenthesis are dissipation per cent, BDL= Below Detection Limit

Table 4: Dissipation pattern of Difenaconazole 25 EC on fruits of apple cv. Gala Redlum under HD orchard system.

Interval (Days)	Fruit							
	Residues* (µg g^{-1}) ±SD (x)	Residues* (µg g^{-1}) ±SD (2x)	$T_{½}$(Days)		T_{tol}(Days)		r^2	
			(x)	(2x)	(x)	(2x)	(x)	(2x)
0	**0.73** 0.73±0.01 **±0.01**	0.98±0.03	5.21	7.53	3.65	8.54	0.96	0.92
1	0.68±0.09 (6.71)	0.90±0.04 (8.62)						
3	0.59±0.08 (19.17)	0.84±0.05 (14.21)						
5	0.47±0.07 (35.20)	0.76±0.06 (22.74)						
7	0.37±0.06 (49.04)	0.60±0.03 (38.98)						
10	0.21±0.04 (70.95)	0.48±0.06 (50.76)						
15	0.10±0.05 (85.89)	0.36±0.05 (62.94)						
20	<BDL	0.13±0.06 (86.80)						
25	**0.73±0.01**	<BDL						

2.15 to 2.32 days in chili fruits as documented by Mukhopadhyay *et al.* (2011) and 4 to 11.5 days and 4.6 to 6.7 days for apples as per the research conducted by Sharma and Nath in 1992. Data obtained harmonized with Ruilan *et al.* (2010), whereas Wang *et al.* (2008) found that the half-life of difenoconazole in Chinese cabbage were 6.6 days in 2005 and 7.8 days in 2006. Data obtained harmonized with Ruilan *et al.* (2010), whereas Wang *et al.* (2008) found that the half-life of difenoconazole in Chinese cabbage were 6.6 days in 2005 and 7.8 days in 2006. Data obtained harmonized with Ruilan *et al.* (2010), whereas Wang *et al.* (2008) found that the half-life of difenoconazole in Chinese cabbage were 6.6 days in 2005 and 7.8 days in 2006.

CONCLUSION

From the present investigation it was clearly depicted that the dissipation behaviour of difenaconazole showed the different dissipation rates on leaves and fruits of apple. In the context of leaf samples, the observed half-life and the corresponding waiting period were 9.9 and 10.19 days at the recommended dosage, and 6.8 and 11.75 days at double the recommended dosage. As for fruit samples, the half-life and associated waiting period were 5.21 and 7.53 days at the recommended dose, and 3.65 and 8.54 days at double the recommended dose. It's worth noting that the reduction of residues in both dosages adhered to the principles of first-order kinetics for degradation.

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