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Dissipation of fluopyram and tebuconazole residues in/on pomegranate and soil in western maharashtra

Abstract

Field and laboratory experiments were conducted to study the residues and dissipation of Fluopyram, a succinate dehydrogenase inhibitor (SDHI) and tebuconazole, demethylation inhibitor (DMI) used for the control of powdery mildew and Anthracnose diseases in grape. Residues of fluopyram dissipated with a half life of 4.04 and 5.18days, at recommended and double dose, respectively. For tebuconazole, the half life values recorded were 4.75 and 5.42days, respectively. The residues reached below quantification limit (BQL) on 10th and 15th day, in both the fungicides at recommended and double the recommended dose, respectively, which suggests a Pre-Harvest Interval (PHI) of 7.76 and 9.91days for fluopyram and tebuconazole, respectively when applied at 75g a.i./ha and 150g a.i./ha.

Keywords: fluopyram, tebuconazole, residues, persistence, QuEChERS

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Introduction

Pomegranate (*Punica granatum*) also called as "fruit of paradise" is one of the major fruit crops grown in tropical and subtropical regions of the world. The pomegranate fruit is known for its cool, refreshing juice and valued for its medicinal properties. In India, it is cultivated over an area of about 19689 ha with a production of 230644 MT.[1] Maharashtra is the leading producer of pomegranate followed by Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu. Insect pests and diseases are the major constraints in the pomegranate cultivation. Shoot and fruit borer (*Deudorix isocrates*), thrips (*Rhiphiphorothrips cruentatus*) and aphids (*Aphis punicae*) are the major insects which cause severe damage to pomegranate Of late, this crop is under threat due to number of serious diseases such as bacterial blight (*Xanthomonas axonopodis* pv. punicae), wilt (*Ceratocystis fimbriata)*, anthracnose (*Colletotrichum gloeosporioides*) and leaf spot and fruit rot (*Alternaria alternata*, *Cercospora sp*., *Pseudocercospora sp*., *Drechslera sp*. and *Sphaceloma sp*. etc). Farmers rely heavily on synthetic pesticides for the control of insect pests and diseases in pomegranate.

However there are no insecticides and fungicides with label claim for use in pomegranate except cyantraniliprole 10.26% OD, quinalphos 25% EC, difenoconazole 25% EC, kitazin 48% EC, propineb 70% WP and metiram 55% + pyraclostrobin 5% WG. (http://cibrc.nic.in/mup.htm). Sometimes pesticides are applied even during fruiting stage. Indiscriminate use of pesticides has resulted in the accumulation of pesticide residues in the primary agricultural products as well as soil.[2] Luna Experience is a combination of fluopyram and tebuconazole and offers two different modes of action. Fluopyram, a pyridylethylamide broad spectrum fungicide belongs to a new chemical class. It is succinate dehydrogenase inhibitor (SDHI) and breaks the respiration chain in the mitochondria of the fungus cell by blocking its energy production. Tebuconazole is a demethylation inhibitor (DMI). It interferes in the process of building structure of fungal cell wall. Finally, it inhibits the reproduction and further growth of fungus.

Fluopyram is a new fungicide and there is no data on its dissipation in pomegranate. Hence, studies were undertaken to validate the method for residue analysis of fluopyram and tebuconazole on liquid chromatography and mass spectrometry (LCMS) to determine the dissipation pattern of combination product, fluopyram and tebuconazole in pomegranate in western Maharashtra. The degradation or dissipation of insecticide is influenced by climatic conditions, type of application, plant species, dosage interval between application and time of harvest.[3] Hence it necessary to determine the dissipation pattern of these two fungicides by following Good Agricultural Practices (GAP). Keeping this in view an attempt was made to conduct studies on the persistence of fluopyram, its metabolite and tebuconazole in/on pomegranate

Material and methods

Chemicals and reagents

Analytical grade fluopyram (99.60%), its metabolite fluopyram benzamide (99.40) and tebuconazole (95.60) as well as commercial formulation.ie. Luna Experience 400 SC was provided by Bayer CropScience Ltd, Mumbai. The solvents and sorbents used in extraction and analysis were distilled and checked for impurities prior to use.

Field experiment

Residues and dissipation of fluopyram, its metabolite and tebuconazole in/on pomegranate and in soil was studied by conducting supervised field experiment during 2015 at the research farm of Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar. During the period of investigation, the maximum and minimum temperature was 32.9 and 23.14per cent. Humidity was 60.36per cent. The experiment was conducted in RBD with three replications. Luna Experience 400 SC was used at 75g a.i./ha (X dose),150g a.i./ha (2X dose) along with untreated control. Two sprays of fluopyram 200 + tebuconazole 200 (400 SC) were sprayed at an interval of 10days initiating first spray at fruit setting stage. Water was sprayed in the control plot. Samples were collected periodically at 0, 1, 3, 5, 7, 10, 15

and 20th day after second spray. Third spray was given 15days before harvest and mature fruits and soil samples were collected at harvest. About 1kg immature fruits, 1kg mature fruits and 1kg of soil were collected from each treated plot. Collected samples were transported by keeping in dry ice and analysed at AINP on Pesticide Residues, PJTSAU, Hyderabad for the residues of fluopyram, its metabolite and tebuconazole by modified QuECHERS method.[4]

Residue analysis

Standard preparation: An accurately weighed 10mg of an individual standard was dissolved in 10ml volumetric flask using suitable solvent to prepare the standard stock solution of 1000mg kg^{-1}. Standard stock solution of each insecticide was serially diluted to obtain intermediate lower concentration of 100mg kg^{-1}. They were stored in a refrigerator at -40°C. From the intermediate standards, working standards were prepared by suitably diluting the stock solution in n-hexane and used as standard check in analysis, linearity and recovery studies.

Method validation

Prior to analysis of samples, linearity of these fungicides was established on LCMS. Accuracy and precision of the method was determined by per cent mean recovery and per cent relative standard deviation. Linearity was studied by injecting standard solution of fungicides under study at five linear concentrations i.e. 0.05, 0.10, 0.25, 0.50 and 1.00µg g^{-1} in triplicate. The linearity curve was established with concentration of the standard and corresponding peak area. Recovery study was conducted in different matrices i.e. whole pomegranate fruit, edible arils, juice and cropped soil in order to establish the reliability of the method of analysis. For this purpose, pomegranate samples and soil from control plots were used. The samples were spiked with three different concentrations viz. 0.05 (LOQ), 0.25 (5×LOQ) and 0.5 (10×LOQ)mg/kg. The extraction and clean up were performed as described hereunder. Per cent recovery was calculated by using following formula.

$$\text{Percent recovery} = \frac{\text{Quantity of pesticide recovered}}{\text{Quantity of pesticide added}} \times 100$$

Extraction and clean up

Pomegranate fruits, edible aril and juice: The pomegranate immature fruits, edible aril and juice samples were analyzed for fluopyram, its metabolite - fluopyram benzamide and tebuconazole residues following the AOAC official method 2007.01 (QuEChERS) after validation of the method at the laboratory. The pomegranate fruits, edible aril and juice samples were homogenized separately with robot coupe blixer and homogenized 15±0.1g sample was taken in 50ml centrifuge tube. 30±0.1ml acetonitrile was added to sample tube. The sample was homogenized at 14000-15000rpm for 2-3min using Heidolph silent crusher. 3±0.1g sodium chloride was added to sample, mixed thoroughly by shaking gently followed by centrifugation at 2500-3000rpm for 3min to separate the organic layer. The top organic layer of about 16ml was taken into 50ml centrifuge tube and added with 9±0.1g anhydrous sodium sulphate to remove the moisture content. 8ml of extract was taken in to 15ml tube containing 0.4±0.01g PSA sorbent (for dispersive solid phase d-SPE cleanup) and 1.2±0.01g anhydrous magnesium sulphate. The sample tube was vortexed for 30sec then followed by centrifugation at 2500-3000rpm for 5min. The extract of about 1ml (0.5g sample) was taken for analysis on LC-MS/MS under standard operational conditions.

Soil: The soil samples were analyzed following the QuEChERS method after validation of the method at the laboratory. The soil samples were dried at room temperature under shade, ground, passed through 2mm sieve and a representative 10g sample was taken in to 50ml centrifuge tube. 20ml acetonitrile was added to sample tube and shaken vigorously for 2min. The samples were then added with 4±0.1 g magnesium sulphate and 1±0.1 g sodium chloride and centrifuged at 2500-3000rpm for 5min to separate the organic layer. The top organic layer of about 10ml was taken into 15ml centrifuge tube and added with 250±0.1mg PSA sorbent and 1.5±0.01g magnesium sulphate and sonicated for 1min then centrifuged at 2500-3000rpm for 10min. The extract of about 1ml (0.5g sample) was taken for analysis on LCMS/MS under standard operational conditions (Table 1 & Table 2).

Table 1 LC-MS/MS parameters for fluopyram and tebuconazole

LC-MS/MS	**SHIMADZU LCMS/MS - 8040.**
Detector	Mass Spectrophotometer
Column	Kinetex, 2.6µ, C18 Column, 100 x 3.0.
Column oven temperature	40°C
Retention time	Tebuconazole - 5.2 min
	Fluopyram - 5.4 min
Nebulizing gas	Nitrogen
Nebulizing gas flow	2.0 litres/min
Pump mode/ flow	Gradient / 0.4 ml/ min
Solvents	A: Ammonium Formate in Water (10Mm)
	B: Ammonium Formate in Acetonitrile (10Mm)
LC programme	Time solvent Conc.
	0.01 B Conc. 50%
	1.00 B Conc. 80%
	4.00 B Conc. 50%
	8.00 B Conc. 50%
Total Time Programme	8 min

Table 2 LC-MS/MS parameters for fluopyram metabolite - fluopyram benzamide

LC-MS/MS	**Shimadzu LCMS/MS - 8040.**
Detector	Mass Spectrophotometer
Column	Kinetex, 2.6µ, C18 Column, 100 x 3.0.
Column oven temperature	40°C
Retention time	Fluopyram Benzamide - 1.5 min
Nebulizing gas	Nitrogen
Nebulizing gas flow	2.0 litres/min
Pump mode/ flow	Gradient / 0.4 ml/ min
LC programme	A: Ammonium Formate in Water (10Mm) - 40%
	B: Ammonium Formate in Methanol (10Mm) - 60%

Citation: Patil CS, Vemuri S, Deore BV, et al. Dissipation of fluopyram and tebuconazole residues in/on pomegranate and soil in western maharashtra. *J Nutr Health Food Eng.* 2018;8(5):351–356. DOI: 10.15406/jnhfe.2018.08.00294

Table Continued...

LC-MS/MS	**Shimadzu LCMS/MS - 8040.**
LC programme	Time solvent Conc.
	0.01 B Conc. 50%
	2.00 B Conc. 80%
	3.00 B Conc. 50%
	4.00 B Conc. 50%
Total Time Programme	4 min

Results and discussion

Method validation

The detector response to the neat standards of the fungicides was studied by injecting five linear concentrations of these fungicides. The graph was plotted with detector response against respective concentrations and linearity line was drawn. The response of the instrument was linear over the range tested and R^2 value was 0.99 for all the fungicides under study (Figure 1-6). These results indicated that the LC-MS analysis is a valid method for residue determination of the tested fungicides in pomegranate fruits. Accuracy of the analytical method was determined by recovery studies. The per cent recovery was within acceptable range of 70-120 per cent prescribed by SANCO (2011) and mentioned in Table 3.

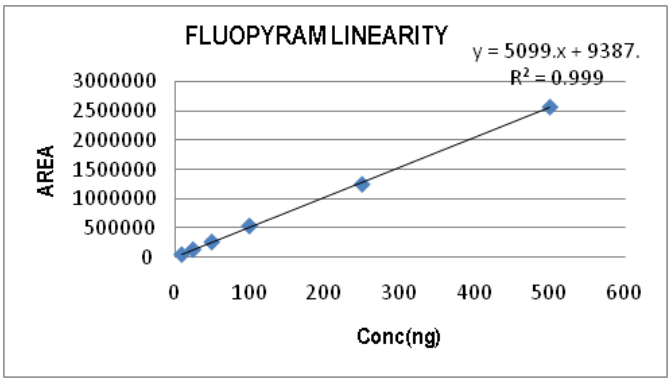

Figure 1 Linearity of fluopyram.

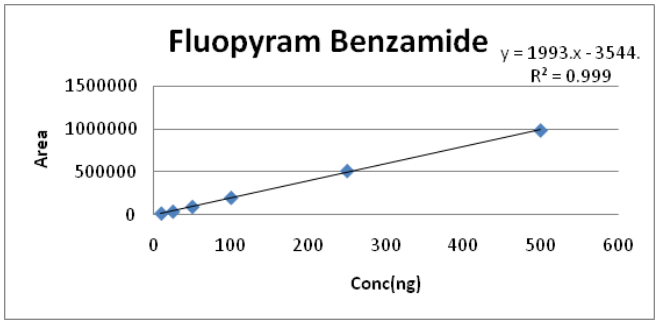

Figure 2 Linearity of fluopyram benzamide.

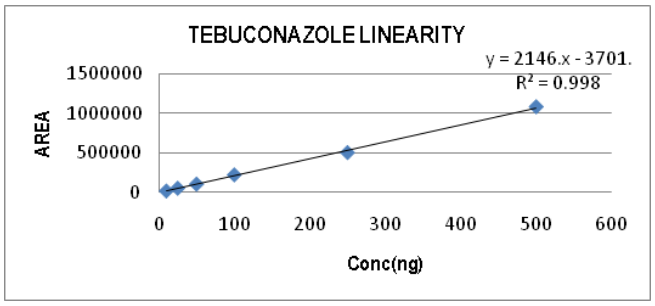

Figure 3 Linearity of tebuconazole.

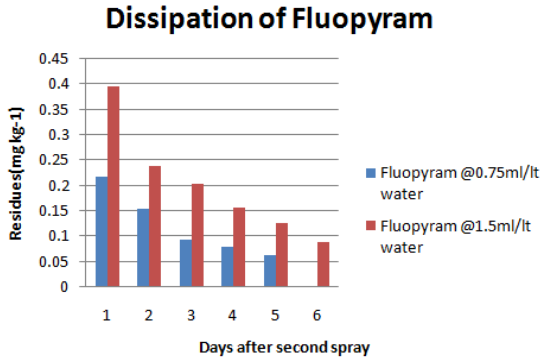

Figure 4 Dissipation pattern of fluopyram in/on pomegranate.

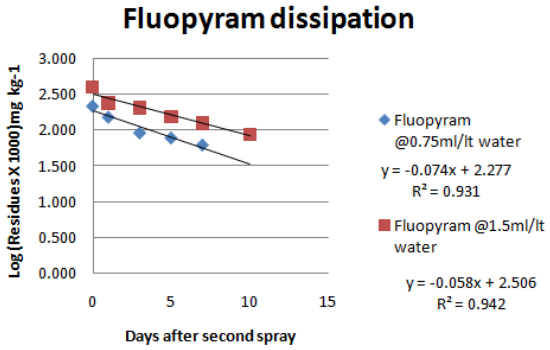

Figure 5 Semi logarithmic graph showing dissipation kinetics of Fluopyram in pomegranate.

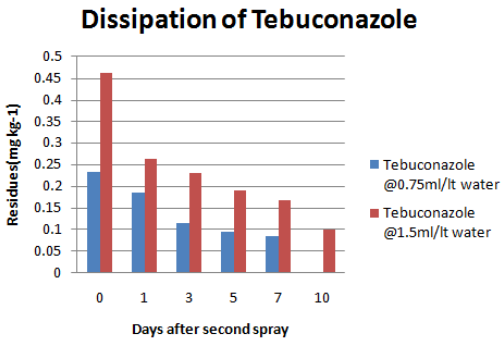

Figure 6 Dissipation pattern of tebuconazole in/on pomegranate.

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Table 3 Recoveries of fluopyram, fluopyram benzamide and tebuconazole at various fortification levels in different matrices

Matrix	Fortification level	Recovery (%)		
		Fluopyram	Fluopyram Benzamide	Tebuconazole
Immature fruits	0.05 mg/kg	106.66(3.095)	88.0(3.770)	114.0(0.949)
	0.25 mg/kg	83.46(2.635)	87.73(0.652)	95.46(2.457)
	0.50 mg/kg	102.53(3.095)	102.0(4.507)	117.2(1.628)
Juice	0.05 mg/kg	102.0(2.156)	88.0(2.099)	112.60(4.416)
	0.25 mg/kg	85.6(2.662)	96.26(2.325)	94.80(1.977)
	0.50 mg/kg	99.53(3.796)	107.13(0.882)	115.00(0.822)
Soil	0.05 mg/kg	103.33(1.631)	96.0(5.137)	114.0(2.247)
	0.25 mg/kg	84.53(1.719)	96.4(2.131)	102.8(13.129)
	0.50 mg/kg	106.13(3.247)	105.86(0.372)	117.13(1.593)

Dissipation of insecticides

Dissipation pattern of fluopyram and tebuconazole are presented in Table 4 and Figure 7. In immature pomegranate, initial residues of fluopyram were 0.219 and 0.395mg/kg^{1} in recommended and double dose, respectively 2h after the second application. Dissipation followed a linear trend with gradual degradation. The residue reached 0.063 and 0.089mg/kg^{1} on 7th and 10th day, respectively on recommended and double dose. In mature fruits of pomegranate and juice, the residues of fluopyram were below quantitation limit (BQL) of 0.05mg/kg^{1}. The residues of fluopyram were also estimated in oil at harvest which was below quantitation limit of 0.05mg/kg in both the doses. As regards fluopyram benzamide, samples of immature fruits, mature fruits, juice and soil did not record any residues in recommended and double dose. They were below Quantitation limit of 0.05mg kg^{1}.

Table 4 Dissipation of fluopyram and tebuconazole in pomegranate fruits, edible aril, juice and soil

Days after 2nd Application	Residues of Fluopyram (mg/kg)					
	Fluopyram		Fluopyram benzamide		Tebuconazole	
	Recommended Dose (0.75 g/L)	Double Recommended Dose (1.5 g/L)	Recommended Dose (0.75 g/L)	Recommended Dose (1.5 g/L)	Recommended Dose	Recommended Dose
	Mean	Mean	Mean	Mean	Mean	Mean
Pomegranate fruits	SD(±)	SD(±)	SD(±)	SD(±)	SD(±)	SD(±)
0 Day	0.219 (0.046)	0.395 (0.085)	BQL	BQL	0.234 (0.048)	0.465 (0.079)
1 Day	0.154 (0.039)	0.238 (0.062)	BQL	BQL	0.186 (0.050)	0.266 (0.071)
3 Days	0.093 (0.037)	0.205 (0.006)	BQL	BQL	0.117 (0.037)	0.232 (0.011)
5 Days	0.079 (0.002)	0.157 (0.035)	BQL	BQL	0.097 (0.015)	0.191 (0.042)
7 Days	0.063 (0.002)	0.126 (0.018)	BQL	BQL	0.085 (0.003)	0.168 (0.003)
10 Days	BQL	0.089 (0.005)	BQL	BQL	BQL	0.102 (0.018)
15 Days	BQL	BQL	BQL	BQL	BQL	BQL

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Days after 2nd Application	Residues of Fluopyram (mg/kg)					
Edible aril at harvest	BQL	BQL	BQL	BQL	BQL	BQL
Juice at harvest	BQL	BQL	BQL	BQL	BQL	BQL
Soil at harvest	BQL	BQL	BQL	BQL	BQL	BQL
Regression Equation	y = -0.0745x + 2.277	y = -0.058x + 2.5069			y = -0.0634x + 2.3276	y = -0.0555x + 2.5693
Regression coefficient	R2 = 0.931	R2 = 0.942			R2 = 0.9348	R2 = 0.9125
Half life (Days)	4.04	5.18			4.75	5.42
PHI (Days)	7.76	13.9			9.91	15.68

LOQ: Fluopyram-0.05 mg/kg, fluopyram benzamide-0.05mg/kg Tebuconazole-0.05mg/kg figures in parenthesis are ± SD values.

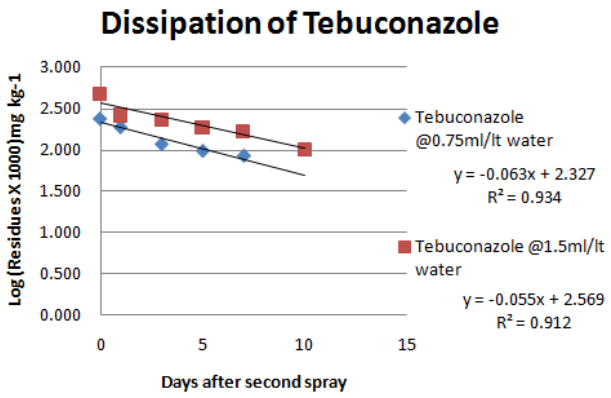

Figure 7 Semi logarithm graph showing dissipation kinetics of tebuconazole in/on pomegranate.

The dissipation of tebuconazole also followed similar pattern of degradation. Initial residue level of 0.234 and 0.465mg/kg[1] were detected in immature fruits of pomegranate. The residues gradually degraded and reached 0.085 and 0.102mg/kg[1] in recommended and double dose, respectively on 7th and 10th day after second application. However, the residues were below quantitation limit of 0.05mg/kg[1] in mature pomegranate fruits and juice and also soil at harvest. In the present study, both fluopyram and tebuconazole showed first order kinetics for dissipation and followed linear degradation pattern. Half life was calculated from dissipation pattern curves of first order kinetics. The results in respect of dissipation of fluopyram cannot be compared due to lack of literature. The dissipation of residues of fluopyram and tebuconazole (Luna Experience 400SC) was studied in chilli.[5] onion[6] and watermelon[7] and tebuconazole alone applied in/on onion,[8,9] mango,[10] ginseng,[11] chilli[12], tomato[13] and apple[14].

According to Patel et al.,[6] half life of fluopyram was 8.85 and 9.12days, respectively in recommended and double dose. Dong & Hu [15] showed half life of 6.48days for fluopyram in watermelon. In chilli, Saha (2016)[5] found a half life of 1.161 and 1.241days for single (100g a.i./ha) and double (200g a.i./ha) application rate. For tebuconazole, half life of 6.69 and 7.72days was reported in onion[6] and 0.866 and 1.083 days in case of chilli[5] at the single and double dose, respectively. In other studies, the reported half life was 1.7 days Mohopatra et al.[8] and 6days in onion Mohopatra et al.[9] and mango[10] 5.87 and 6.93 days in watermelon[15] 4.49 days in ginseng,[11] 1day in chilli[12] and 0.9days in tomato (Sing and Sing, 2014). However, half life of tebuconazole ranged between 19.38 and 25.99days and 19.84 and 28.86days at the application rate of 200 and 400g ai/ha in apple.[14] A PHI of 21days was recorded for tebuconazole on onion by CIB & RC of India Mohopatra et al.[16] suggested a PHI of 16days and 35days for tebuconazole at 187.5 and 375g a.i./ha in immature onion bulb with leaves. From the present study, the pre harvest interval (PHI) of 7.76 and 9.91days for fluopyram and tebuconazole can be considered safe for harvesting residue free pomegranate at application rate of 75 and 150g a.i./ha.[17]

Acknowledgements

None.

Conflict of interest

None.

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