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RESEARCH PAPER

Residues and dissipation of imidacloprid in pomegranate fruits

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Field and laboratory studies on residues and dissipation of imidacloprid in pomegranate fruits were conducted during 2010 at the Pesticide Residue analysis laboratory, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, M.S. (India). The studies revealed that residues of imidacloprid persisted up to 3 days and 5 days in arils, 5 days and 7 days in whole fruits and 7 days and 10 days in peel of pomegranate fruits at recommended and higher doses, respectively.

Key words : Pomegranate, Imidacloprid, Residues

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INTRODUCTION

Pomegranate (*Punica granatum*) is a native of Iran and one of the favorite table fruits of tropical and subtropical regions. India ranks first in area (0.12 million ha) and production (9.0 million tonnes) of pomegranate followed by Iran with an area of 0.065 million ha and production 8.00 million tonnes. Pomegranate is an important tropical fruit crop extensively cultivated in India in an area of 124,926 ha with an export value of INR 92 million (http://*www.pomegranateindia*,2010). The incidence of fungal diseases, e.g. wilt caused by *Fusarium oxysporum*, *Rhizoctonia solani*, *Ceratocystis fimbriata*; leaf and fruit spots caused by *Cercospora punicae*, *Colletotrichum gloeosporioides*, *Alternaria alternata*, and fruit rot caused by *Rhizopus* sp. and *Colletotrichum* spp. (ttp://*www.nrcpomegranate.org/research.htm*, 2010), is one of the major causes of economic loss in pomegranate production, which necessitates regular application of fungicides (ttp://*www.nrcpomegranate.org,* 2010) to secure desired yield and fruit quality for domestic sales as well as export. In India, Maharashtra ranks first (0.096 million ha) contributing 70 per cent of the total area under pomegranate followed by Karnataka (0.013 million ha) and Andha Pradesh (0.0051 million ha). Again average productivity of pomegranate in Maharashtra is very less *i.e* only 6.2 t ha^{-1}. In Maharashtra, Nashik district has an area of 0.0354 million ha followed by Solapur 0.0310 million ha, Ahmednagar 0.00639 million ha and Sangli 0.00630 million ha. However the productivity of this crop in India is only 7.4 t ha^{-1} which is significantly lower than other pomegranate growing countries like Spain (18.5 t ha^{-1}), USA (18.3 t ha^{-1}) and Iran (9.23 t ha^{-1}) (Anonymous, 2008).

The insect pests and diseases play significant role in reducing the productivity of this crop. The pomegranate crop is susceptible to some disorders *viz.,* internal breakdown of arils and sun scald. Sucking pests like mealy bugs, thrips, etc. are the major obstacles during pomegranate cultivation (Ananda *et al*., 2009a and b). The pomegranate crop (*Punica granatum* L.) suffers from the attack of several insect and non-insect pests. Eighty six species of insect pests infesting pomegranate have been reported from various parts of the world (Zirpe, 1966). Thrips, *Scirtothrips dorsalis* (H) contributes major losses in pomegranate cultivation, both qualitatively and quantitatively. In order to protect the crop from pest problems; farmers are spraying a number of chemical pesticides on this crop. The disadvantages of pesticides as known as 4R (Resistance, resurgence, risk and residue) are well known. Since this fruit is mostly accepted as a table purpose fresh fruit, pesticide residues in this crop are of very much concern. Literature survey reflects non-

availability of residue dissipation data for either of these fungicides in pomegranate as per the good agricultural practices, which creates apprehension of accumulation of their residues at levels above the maximum residue limits (MRLs) at the stage of harvest (Ananda *et al.*, 2009a), resulting in food safety issues. Pesticide residues are also becoming a major obstacle in reducing India's export to foreign market. Pomegranate peel extract has more potential as a health supplement rich in natural antioxidants (Duhan *et al.*, 2010). The export scenario shows that India contributes only 5 per cent of International market while Spain is most dominant with 80 per cent share. Keeping in view the above facts and figures the present study to study residues and dissipation of imidacloprid in pomegranate fruits were carried out in the field experiment conducted in *Ambia* bahar (*Summer* season) of 2010. Pesticide residues were analyzed in peel, arils and whole fruits separately, collected periodically after the third spray to decide the safety of treatments to consumers.

RESEARCH METHODOLOGY

The field experiments on bioefficacy of newer insecticides against thrips, *Scirtothrips dorsalis* (H) of pomegranate were conducted during the *Ambia* bahar (*Summer*) and *Mrig* bahar (*Kharif*) seasons of 2010 on a five year old orchard of '*Bhagva*' variety at the Research project on Arid zone fruits, Horticulture farm, Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar. Over all three sprays were given at an interval of 15 days, initiating first spray at the time of fruit setting. According to residue studies protocol prescribed by Central Insecticidal Board (CIB) two doses recommended (27 g a.i ha^{-i}) and double the recommended dose (27 g a.i ha^{-i}) were evaluated for analysis of residues. The marketable quality fruits of pomegranate weighing 1 kg were collected separately from each plot and packed properly in labeled polythene bags with rubber band and shifted to laboratory. Samples were collected at an interval of 0 (~ 2 hr), 1, 3, 5, 7, 10, 14 and 21 days after last spray for residue analysis. From composite samples by quartering method after cutting, 50g representative samples were taken for extraction, cleanup and estimation as described under each compound. The analytical procedure followed for imidacloprid is as follows.

Extraction :

Weighed 100 g of analytical sample in a 500 ml glass bottle. Added 250 ml acetonitrile and homogenized with the high speed blender for about 3 minutes. Added 10 to 15 g Celite 545 filter aid and filtered through Buchner funnel using filter paper. Blender jar and funnel were washed with 100 ml acetonitrile, filtered and combined the acetonitrile in 1000 ml round bottom flask. The acetonitrile was evaporated by means of a rotary vaccum evaporator and 50 ml saturated sodium chloride solution was added to aqueous remainder and transferred extract into a 500 ml seperatory funnel. Rinsed the flask with 100 ml hexane and added n-hexane to separatory funnel. After vigorous shaking, drained off the lower aqueous phase into the 1000 ml n-hexane and repeated extraction. The hexane was discarded. The extract was taken into a 500 ml seperatory funnel. The container was washed with 100 ml of hexane/ethyl acetate mixture (98:2) or (v/v) and was shaken. The aqueous phase was drained into a 250 ml seperatory funnel and discarded the organic phase. The aqueous phase was shaken with 3×100 ml dichloromethane and the dichloromethane was collected in a 500 ml seperatory funnel, washed with 50 ml of 0.01 molar aqueous potassium carbonate solutions. The aqueous phase was drained and discarded. Dichloromethane was dried over anhydrous sodium sulfate and sodium sulfate was washed with 50 ml of dichloromethane. Dichloromethane was evaporated just to dryness.

Clean up :

The sintered glass column was rinsed with 10 ml ethyl acetate. The column was slurry-packed by taking 4.5 g Florosil deactivated with 5 per cent water in 20 ml ethyl acetate and applied quantitatively into the column. This was covered with a 0.5 cm layer of anhydrous sodium sulfate. The solvent was allowed to drain down to the sodium sulfate layer. The residue was dissolved in a small amount of ethyl acetate. The solution was applied on top of the column by means of a pipette and allowed to percolate. The column was rinsed with 20 ml acetonitrile. The eluate was concentrated just to dryness and the residue was dissolved in 1.0 ml acetonitrile (analytical grade).

Estimation :

The purified sample solution was injected into a high performance liquid chromatograph after the standard solution. Liquid chromatograph (Model LC-10 AT, Schimadzu, Japan) equipped with UV/VIS (SPD 10 A)

Calculation :

Calibration curve was prepared for imidacloprid by plotting the concentration of the calibration standards on X-axis and resulting peak height or area on Y-axis. Using regression analysis, the equation for the calibration curve was determined with respect to the X-axis. The concentration (C) of the analyte in the final solution was calculated from the measured peak height or area response (PR) and the least square coefficient for the slope (m) and Y-axis intercept (b) as follows.

$$C = \frac{(PR - b)}{m}$$

The concentration (ug g^{-1}) of the analyte in the sample

was calculated from the concentration (C) in final volume (V), the weight (W) of the sample that was extracted, and the aliquot factor (AF) using the following equation :

$$\mu g\ g\text{-}1 \text{ or } ug\ g\text{-}1 = \frac{(C \times AF \times V)}{W}$$

The aliquot factor was calculated from the appropriate extraction and aliquot volumes for each sample type :

$$AF = \frac{\text{Total extraction volume}}{\text{Aliquot volume}}$$

The calibration of standards at various ng/ml levels showed good correlation between the concentration (X) and peak-height (Y) with the co-efficient of determination (r^2) averaging 0.955 or above.

RESEARCH FINDINGS AND ANALYSIS

The findings of the present study as well as relevant discussion have been presented under the following heads :

Residues and dissipation of imidacloprid in pomegranate fruits :

Arils :

Samples at zero days after spray (2 hrs) indicated that the higher application rate resulted in higher initial deposit (Table 1 and Fig. 1). The initial deposits were 0.097 and 0.167 µg g^{-1} in 27 and 54 g a.i.ha^{-1} imidacloprid treated fruits, respectively. At recommended dose of 27 g a.i.ha^{-1} the initial deposits dissipated from 0.097 µg g^{-1} to 0.050 µg g^{-1} at 1 DAS indicating half life of 1.05 days. Imidacloprid level at higher rate fell with time from 0.197 to 0.051 µg g^{-1} within 3 days with an estimated half life of 1.76 days at 54 g a.i.ha^{-1}. No detectable residues were found in the samples brought after 3 and 5 DAS at recommended and higher doses, respetively.

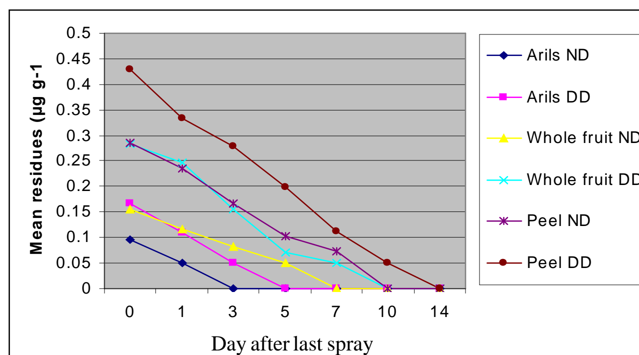


Fig. 1: Residues of imidacloprid 17.8 SL (27 and 54 ga.i. ha^{-1}) in pomegranate fruits (Summer 2010)

Whole fruit :

The mean initial deposits of imidacloprid at zero time were 0.156 and 0.284 µg g^{-1} for 27 and 54 g a.i.ha^{-1} field application, respectively (Table 1). The residues dissipated rapidly up to 5 days for 27 g a.i.ha^{-1} and 7 days for 54 g a.i.ha^{-1}. The residue half-life under field condition ranged from 2.64 to 3.16 days.

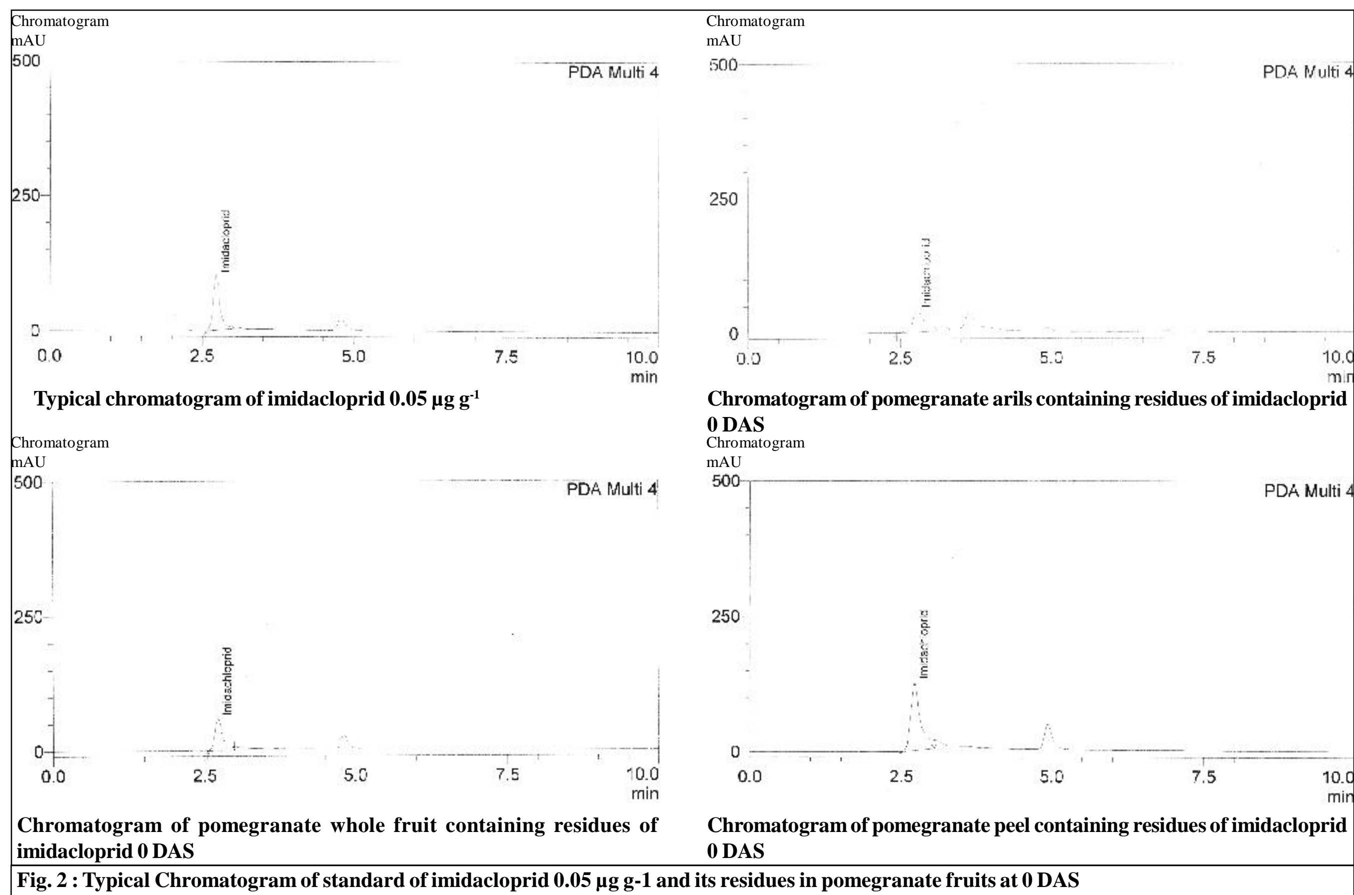
Peel :

The immediate post application (2 hr) initial deposits on pomegranate peel were 0.286 and 0.429 µg g^{-1} at 27 and 54 g a.i. ha^{-1}, respectively at field rates (Table 1). The initial deposits of 0.286 µg g^{-1} at recommended dose dissipated to 0.235 µg g^{-1} (1 DAS), 0.166 µg g^{-1} (3 DAS), 0.102 µg g^{-1} (5 DAS) and 0.073 (7 DAS). These values were exceeding the MRL of 0.05 µg g^{-1} which is specified for other crops. However, at higher dose of 54 g a.i. ha^{-1} imidacloprid dissipated from 0.429 to 0.051 in a period of 10 days. The residues of imidacloprid were not detected after 10 days in both the doses.

Table 1: Residues of imidacloprid (27 and 54 g a.i. ha^{-1}) in pomegranate fruits (*Summer* 2010)

Days after spray	Residue level µg g^{-1}					
	Arils		Whole fruit		Peel	
	ND	DD	ND	DD	ND	DD
0	0.097	0.167	0.156	0.284	0.286	0.429
1	0.050	0.110	0.116	0.246	0.235	0.334
3	BDL	0.051	0.083	0.156	0.166	0.278
5	BDL	BDL	0.050	0.071	0.102	0.199
7	BDL	BDL	BDL	0.051	0.073	0.112
10	BDL	BDL	BDL	BDL	BDL	0.051
14	BDL	BDL	BDL	BDL	BDL	BDL
RL50 (Days)	1.05	1.76	3.16	2.64	3.49	3.34
T_{MRL} (Days)	1.00	3.00	5.08	6.88	8.82	10.73
T_{BDL} (Days)	1.00	3.00	5.08	6.88	8.82	10.73

LOD : 0.05 µg g^{-1} ND: Normal dose (27 g a.i. ha^{-1}) DD: Double dose (54 g a.i. ha^{-1})



Typical chromatogram of imidacloprid 0.05 µg g-1

Chromatogram of pomegranate arils containing residues of imidacloprid 0 DAS

Chromatogram of pomegranate whole fruit containing residues of imidacloprid 0 DAS

Chromatogram of pomegranate peel containing residues of imidacloprid 0 DAS

Fig. 2 : Typical Chromatogram of standard of imidacloprid 0.05 µg g-1 and its residues in pomegranate fruits at 0 DAS

The residue half-life under field condition ranged between 3.34 and 3.49 days. The time required for initial deposit to degrade below detectable limit ranged between 8.82 to 10.73 days.

Arora *et al.* (2000) studied that residue of imidacloprid (0.008 and 0.016 % a.i.) in fruits of kinnow mandarin. The initial deposits at both the dosage on the rind were 2.40 and 3.90 mg kg^{-1}, respectively and corresponding values in the pulp were 0.03 and 0.04 mg kg^{-1}, respectively. Gupta *et al.* (2005) studied persistence of imidacloprid seed dressing (3 and 6 g a.i kg^{-1}seed) and foliar application (20 and 40 g a.i. ha^{-1}) on chickpea and found that the residues of imidacloprid persisted beyond 3 days but no residues were detected on 5th day. Battu *et al.* (2007) found that acephate and imidacloprid when applied on cotton using ready-mix SP formulation of acephate 50 per cent + imidacloprid 1.8 per cent (LancerGold) at acephate 500 and 1000 and imidacloprid 18 and 36 g a.i. ha^{-1}. Imidacloprid residues reached below detectable level of 0.02 mg kg^{-1} after 5 and 7 days of its application at lower and higher rates of application.

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