Food Chemistry 131 (2012) 787–795

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Food safety evaluation of buprofezin, dimethoate and imidacloprid residues in pomegranate

Sagar C. Utture [a,b], Kaushik Banerjee [a,*], Sanjay S. Kolekar [b], Soma Dasgupta [a], Dasharath P. Oulkar [a], Sangram H. Patil [b], Sameer S. Wagh [a], Pandurang G. Adsule [a], Mansing A. Anuse [b]

[a] National Referral Laboratory, National Research Centre for Grapes, P.O. Manjri Farm, Pune 412 307, India
[b] Department of Agrochemicals and Pest Management, Shivaji University, Kolhapur 416 004, India

ARTICLE INFO

Article history:
Received 21 May 2011
Received in revised form 14 July 2011
Accepted 12 September 2011
Available online 19 September 2011

Keywords:
Pomegranate
Buprofezin
Dimethoate
Imidacloprid
Method validation
Residues
Dissipation
Pre-harvest interval
Dietary exposure
Processing by hot air drying

ABSTRACT

Food safety aspects of buprofezin, dimethoate and imidacloprid residues in pomegranate are reported. The residue analysis involved extraction of samples (15 g) with 10 ml ethyl acetate, cleanup by dispersive solid phase extraction with 25 mg primary secondary amine and 25 mg C_{18} sorbents and estimation by LC–MS/MS. The limit of quantification of each analyte was 0.001 mg kg^{-1} with recoveries within 76–109%. The residues of buprofezin and dimethoate were confined to outer rind, which degraded to below the maximum residue limit for the European Union (EU-MRL) after 10.5 and 31.5 days at standard dose and 32.0 and 44.0 days at double dose. Residues of imidacloprid penetrated into the albedo and membrane, although at less than the MRL in all samples even at double dose. The dietary exposure of buprofezin and imidacloprid was safe on all sampling days; whereas samples with dimethoate appeared safe after 15 and 30 days of field applications at standard and double doses, respectively.

1. Introduction

Pomegranate (*Punica granatum* L.) fruits are recognized for their high nutritional contents and medicinal properties (Aviram & Dornfeld, 2001; Aviram et al., 2000; Basu & Penugonda, 2009; Rosenblat, Hayek, & Aviram, 2006), which is responsible for their increasing demand in domestic and international markets. India is one of the major producers and exporters of fresh pomegranates. In addition to the fresh fruits, the processed products viz. juice and dried arils (local name: anardana) are also gaining popularity among consumers for their high antioxidant properties.

In India, pomegranate is mostly grown in peninsular region, where the cultivation suffers from frequent infestation of various insect pests like mealy bugs, and thrips (Ananda, Kotikal, & Balikai, 2009a) necessitating regular field applications of contact and systemic insecticides to prevent loss in economic yields. Once a pesticide is applied to a field, its residues might get accumulated on the surface of fruits. Residue deposits on fresh fruits located in the upper levels of the crop canopy could be at the highest concentrations immediately after foliar application; but for the fruits at lower canopy levels, residue deposits could even increase over time as a result of gravitational pull mediated transfer of some fractions from the fruits and foliage at upper to lower canopy levels. In case it is a systemic pesticide, some fraction of residue deposits get absorbed to the inside of fruit parts with time and may or may not remain stable in fresh matrix or while processing to juice and anardana depending on the physico-chemical properties and biochemical environment. Considering the consumption pattern, to assess the residue behavior of any plant protection products in pomegranate, the residue dynamics on fresh produce as well as the processed products are thus considered equally important.

The simultaneous challenges of managing insect pests through the application of insecticides and minimizing their residue accumulations in the produce at harvest could be addressed by applying the chemicals in a judicious manner so as to attain the necessary pest control with minimal field applications. However, the concentration of pesticide residues in fruits at below the maximum residue limits (MRL) could only be ensured if adequate waiting periods, estimated through GAP (good agricultural practices) based supervised experimentations, are maintained between last application and harvest (pre-harvest interval or PHI). From a review of the package of practices followed by the pomegranate farmers in India, the insecticides, namely buprofezin

* Corresponding author. Tel.: +91 2695 6091; fax: +91 2695 6099.
E-mail address: kbgrape@yahoo.com (K. Banerjee).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.09.044

[(Z)-2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one], dimethoate [*O,O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate, 2-dimethoxyphosphinothioylthio-*N*-methylacetamide] and imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] are identified as commonly used chemicals with satisfactory biological activities against sucking insect pests (Ananda, Kotikal, & Balikai, 2009b; Cabral, Garcia, & Soares, 2008). None of these chemicals however have recommended PHIs applicable for pomegranate under Indian agro-climatic conditions resulting in apprehensions of food safety issues associated with their usage for domestic marketing as well as export. To ensure food safety of the consumers, the European Union (EU) has set the MRLs of these chemicals in pomegranate at 0.05, 0.02 and 1.00 mg kg^{-1} for buprofezin, dimethoate and imidacloprid residues, respectively (http://ec.europa.eu/sanco_pesticides/public/index.cfm). A number of studies on the dissipation pattern and residue behavior of these chemicals in other crops have been reported earlier by several authors. Gupta, Sharma, and Shanker (2008) reported the dissipation of imidacloprid residues in tea following first order rate kinetics with PHI of 7 days and also studied the transfer of residues from 'made tea' to 'infusion' for a holistic risk assessment. In case of dimethoate, 3 days of waiting period was estimated to ensure degradation of its residues to below MRL following field applications at the rate of 120 g a.i. (gram active ingredient) per 100 l water (Khan, Farid, Asi, Shah, & Badshah, 2009). In another study (Oulkar et al., 2009), the degradation kinetics of buprofezin in grapes was reported to follow non-linear first + first order kinetics, where buprofezin residues dissipated to below the EU-MRL of 1 mg kg^{-1} after 31 days. This sort of information, however, is not available for these specific insecticides pertaining to pomegranate. The current endeavour thus aims to evaluate the food safety related to usage of these insecticides in pomegranates to ensure their safe usage by the farmers and minimize consumer risk.

To ensure precise and accurate residue analysis, the sample preparation and estimation methods were thoroughly validated with respect to different parts of pomegranate fruits (outer rind, albedo, membrane and arils) by considering the fact that, while arils are used for direct human consumption, other parts of pomegranate are generally used as cattle feed or as components of medicinal formulations. Furthermore, the fate of the residues of these insecticides on fresh fruits during processing to juice and anardana/dried arils was thoroughly investigated.

2. Materials and methods

2.1. Field experiment

Field experiments were conducted on a cultivated variety, "Bhagwa", at a farm located in Kalas (Latitude 18°9′58.74″N, Longitude 74°48′22.78″E), Indapur sub-district of Pune, as per the EU guidelines for crop field trials (Commission of the European Communities, 1997). The plant to plant and row to row distance was 8 ft and 10 ft, respectively. Dimethoate (formulation: Rogor 30% EC; Rallis India Ltd., Bangalore) and imidacloprid (formulation: Confidor 17.8% SL; Bayer Crop Science, Mumbai, India) were applied at the rate of 1.7 and 0.25 ml l^{-1} as standard dose (Ananda et al., 2009b) and 3.4 and 0.5 ml l^{-1} at double dose, respectively in separate plots. For buprofezin (formulation: Appalud 25% SC; Rallis India Ltd, Bangalore), sprays were conducted at 2 and 4 ml l^{-1} at standard and double doses, respectively, as per the farmers' practice. All these insecticides were applied twice in separate plots at intervals of 15 days after 45 and 60 days from the flowering stage (first and second spray, respectively) using Knapsack sprayers (hand operated sprayers). During spraying, the whole plant was sprayed for control of insects. An untreated control was simultaneously maintained during the study. Each treatment, including the untreated control, was replicated thrice. The crop was grown under drip irrigation. The Bhagwa variety used in this study takes around 120–140 days after the flowering stage for ripening.

Around 20 fruit samples (approximately 5 kg) were collected at random from each replicate of the treated and control plots (1 ha) separately at regular time interval 1 h after spraying on 0, 1, 3, 5, 7, 10, 15, 30, 45 and 60 days after the final foliar spray. The fruits hidden inside the canopy or those showing signs of infestation of insect pests, diseases or any physiological disorder were not considered during sampling. The samples were of different ripening stages on day wise basis. However, on each day of sampling, the fruits at a similar ripening stage were chosen. On the 60th day of sampling the fruits were ready for harvest. All the samples were transported to the laboratory at a controlled temperature (4 °C) and immediately stored at −18 °C until analysis to prevent any degradation losses of the residues. The atmospheric temperature in the field during the study period ranged between 18 and 38 °C with relative humidity ranging between 46% and 100% and no rainfall was recorded.

2.2. Juice and anardana preparation

Arils were separated from the fresh fruits harvested from the control plots and tested for confirmation of the absence of target analyte residues. The arils (1 kg) were then dipped into a solution of the pesticides (2 mg l^{-1}) considered in this study separately for 15 min, strained from the dipping solution and air dried for 15 min. A higher concentration of 2 mg l^{-1} was chosen so as to establish the efficacy of processing as a probable decontamination measure in highly contaminated samples. Anardana preparation was done as per the reported method (Parashar, Gupta, & Kumar, 2009). The brief method of anardana preparation included uniform distribution of arils on aluminum tray and drying it in a circulatory air-dryer at 60 ± 2 °C. The aril samples (20 g) were drawn at regular time intervals of 0 (15 min after treatment), 2, 4, 6, 8, 15, 24, 36 and 48 h and analyzed for the residue contents. The dry weights of the arils at respective time intervals were also recorded to account for the loss in water contents.

Pomegranate juice was prepared from the treated arils 15 min after treatment and analyzed immediately. The juice was obtained by crushing the arils in a food mill without damaging the seeds and the separated juice was collected. Extraction of residues from the arils and juice was done as per the method described in Section 2.4 and analysis was carried out as described in Section 2.5.

Samples of arils with incurred residues of individual chemicals from the field were processed in separate batches simultaneously to produce juice and anardana. The residue concentrations in fresh aril were compared to the residues in the corresponding processed products to account for the impact of processing in decontaminating residues.

2.3. Sample preparation for different fruit parts

The pomegranate fruits were separated into four parts, namely outer rind, albedo, membrane and arils. The samples of each of these components were separately crushed in a blender and extracted by the method described in Section 2.4 before analysis was carried out as described in Section 2.5.

2.4. Sample preparation

Whole fruit samples without washing or any kind of pretreatment were used for the residue analysis. The sub-sampling of the laboratory samples was as per the procedure validated in our