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RESEARCH **P**APER

Residues and dissipation of spinosad in pomegranate fruits

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Field and laboratory studies on residues and dissipation of spinosad in pomegranate fruits were conducted during 2010 at the Pesticide residue analysis laboratory, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, M.S. (India). The studies revealed that residues of spinosad persisted up to one day in arils, three days in whole fruits and five days in peel of pomegranate fruits at recommended and higher doses, respectively.

Key words : Pomegranate, Spinosad, 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. As a commercial crop, pomegranate is grown to a limited extent in selected locations in many states of India. The estimated area under pomegranate cultivation in India is about 124,926 ha presently with an export value of Rs 92 million *http://nhb.gov.in/statistics/area-production-statistics* (2011). 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, pomegranate is cultivated in the districts of Solapur, Nasik, Ahemadnagar, Pune, Sangli, Dhule, Latur, Usmanabad, Jalna, Parbhani, Aurangabad, Beed and Satara. The various varieties raised in Maharashtra are *Ganesh*, *Bhagwa*, *Mrudula* and *Arakta* (Maharashtra Pomegranate Growers Research Association, 2011). 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).

Pomegranate is attacked by several pests and diseases owing to which the quality of the pomegranate is hampered. 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. Of the 228 registered chemicals for use in Indian agriculture as per the Central Insecticides Board and Registration Committee (CIB and RC), 29 chemicals are recommended for use in pomegranates (Regulation of Export of Fresh Pomegranates to the European Union through Control of Pesticide Residues (2011). to control different insect pests and other diseases. 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.Considering the importance of food safety, the Government of India has made the certificate of residue analysis a mandatory prerequisite for issuance of a phyto-

sanitary certificate for export (APEDA, 2006). Pesticide residues are also becoming a major obstacle in reducing India's export to foreign market. Many multiresidue methods have been developed and applied for the determination of pesticide residues in fresh fruits and vegetables using gas chromatography with MS detection (Okihashi, *et al.*, 2007; Paya *et al.*, 2007; Walorczyk, 2008; Leandro *et al.*, 2005; Martinez *et al.*, 2006; Walorczyk and Gnusowski, 2006; Pihlstrom, 2007; Fernandez *et al.*, 2008 and Wong *et al.*, 2007). 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 spinosad 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 (56.25 g a.i ha^{-i}) and double the recommended dose (112.5 g a.i ha^{-i}) were evaluated for analysis of residues. The marketable quality fruits of pomegranate weighing one 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, 50 g representative samples were taken for extraction, cleanup and estimation as described under each compound. The analytical procedure followed for spinosad is as follows.

Extraction :

The analytical procedure outlined by Utting *et al.* (1998) and West and Turner (2000) was used for analysis of spinosad residues. The fruits were cut with a knife and arils were separated. Fifty g arils, peel and arils with peel (whole fruit) were blended separately with 100 ml acetonitrile:water (8:2). The sample was shaken at 500 rpm for 5 min and centrifuged at 3000 rpm for 5 min. Portion of the supernatant (75 ml) was then transferred to a bottle. After adding 40 ml water and 75 ml dichloromethane, the extract was shaken at 500 rpm for 5 min and centrifuged at 300 rpm for 5 min. Immediately, the upper aqueous layer was completely pipetted out and discarded. The lower organic layer was transferred to a 250 ml boiling flask and dried under vacuum at 40°C on rotary vacuum evaporator. The residue was taken in 20 ml hexane for cleanup.

Cleanup :

Silica gel column was wet packed with 10 ml of dichloromethane: methanol (75:25) followed by 10 ml of acetonitrile and 10 ml of hexane. The hexane extract (20 ml) was loaded on to the column and discarded eluate. Spinosad was eluted with 10 ml of dichloromethane: methanol (75:25) and transferred to 250 ml boiling flask. The sample eluent was immediately evaporated and evaporation process was closely monitored so that the sample could be removed from the evaporator immediately to prevent loss of residue. The residues were dissolved in 1 ml of acetonitrile, methanol and 2 per cent aqueous ammonium acetate (1:1:1). The sample vial containing residue was sonicated for 2 min and briefly centrifuged at 3000 rpm. Spinosad residue was transferred to another vial for estimation.

Estimation :

The determinations were made on the dual pump HPLC system. The mobile phase comprised of 30 per cent reservoir A and 70 per cent reservoir B (isocratic). Reservoir a contained methanol: water: acetonitrile: 2 per cent aqueous ammonium acetate (50:10:30:10) and reservoir B contained methanol: water: 2 per cent aqueous ammonium acetate (80:10:10). The flow rate was 1.2 ml min^{-1}.

HPLC parameters :

Name of the instrument: Liquid chromatograph (Model LC-20 AT, Schimadzu, Japan) equipped with PDA detector SPD-M20A and LC solution data software.

Liquid chromatographic conditions :

The chromatograms were generated on LC-MS and the parameters of LC are as under.

Mobile phase : Acetonitrile (42%) : Methanol (42%): 2 per cent ammonium acetate (16%)
Wave length : 250 nm
Flow rate : 0.4 ml/min
Column : RP C-18, Grace Vydac 150x3.2 mm i.d.
Injection volume : 10 µl
Retention time : Spinosyn A : 9.34 min
Retention time : Spinosyn D : 9.64 min.

Calculation :

Calibration curve was prepared for spinosad 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 co-efficient 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 :

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

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

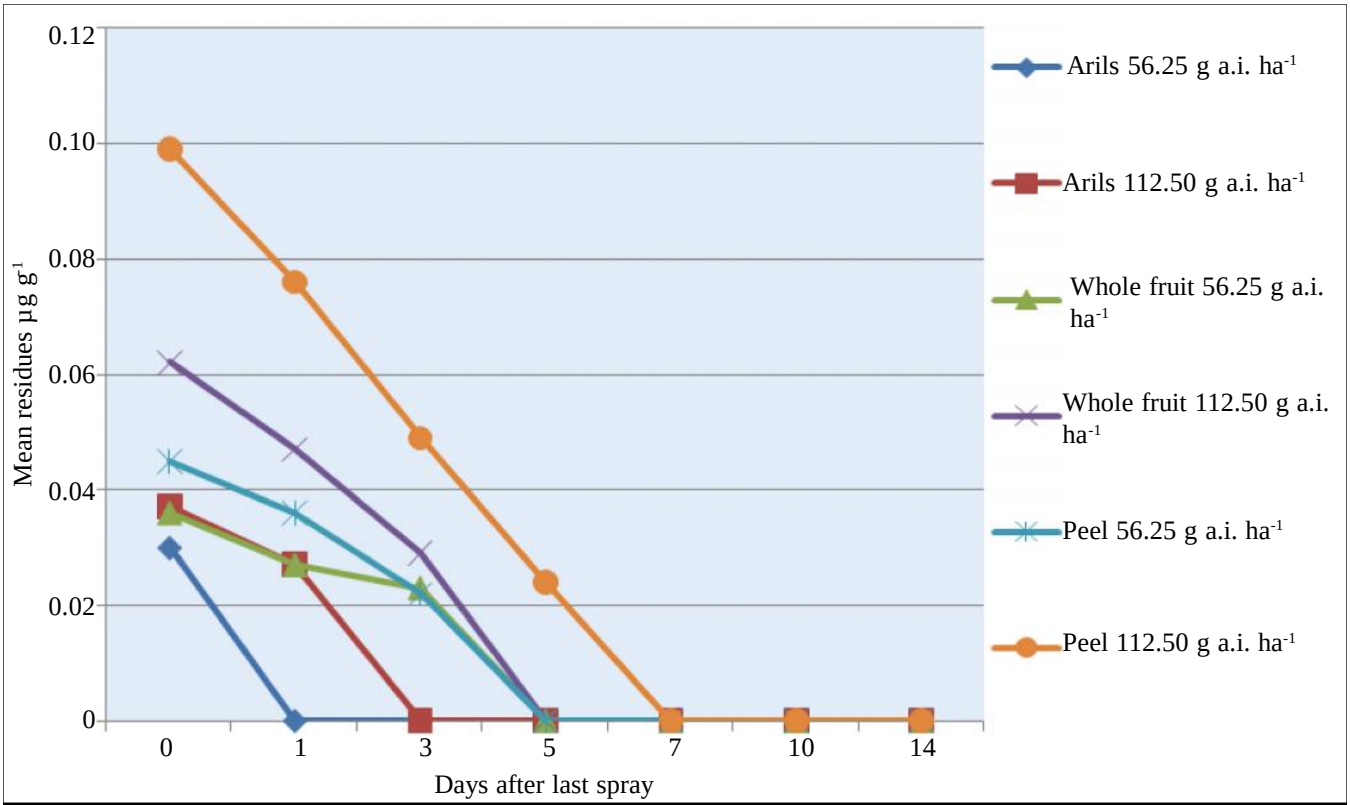
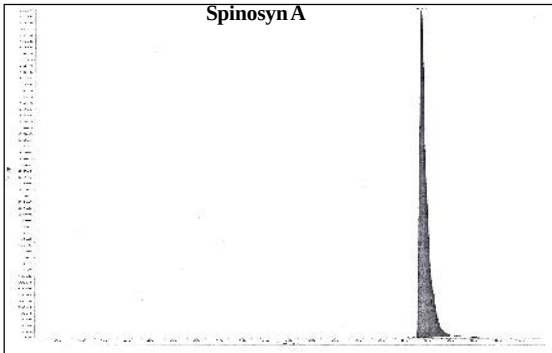


Fig. 1 : Residues of spinosad 45 SC (56.25 and 112.50 g a.i. ha^{-1}) in pomegranate fruits

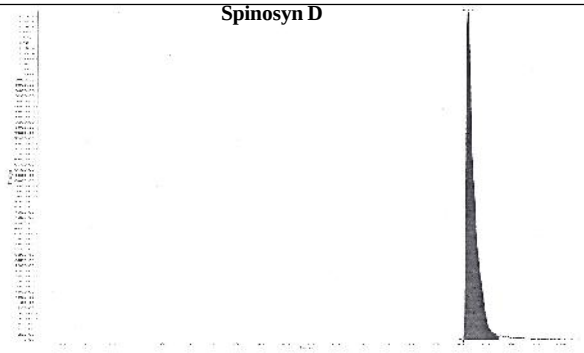
Table 1 : Residues of spinosad in pomegranate fruits

Days after spray	Spinosad residues (mg kg^{-1})					
	56.25 g a.i. ha^{-1}	112.5 g a.i. ha^{-1}	56.25 g a.i. ha^{-1}	112.5 g a.i. ha^{-1}	56.25 g a.i. ha^{-1}	112.5 g a.i. ha^{-1}
	Arils		Whole fruit		Peel	
0	0.030	0.037	0.036	0.062	0.045	0.099
1	BDL	0.027	0.027	0.047	0.036	0.076
3	BDL	BDL	0.023	0.029	0.022	0.049
5	BDL	BDL	BDL	BDL	BDL	0.024
7	BDL	BDL	BDL	BDL	BDL	BDL
10	BDL	BDL	BDL	BDL	BDL	BDL
14	BDL	BDL	BDL	BDL	BDL	BDL
RL_{50} (Days)		2.20	3.66	2.75	2.89	2.50
T_{BDL} (Days)		1.95	2.90	4.46	3.41	5.86
T_{MRL} (Days)		1.95	2.90	4.46	3.41	5.86

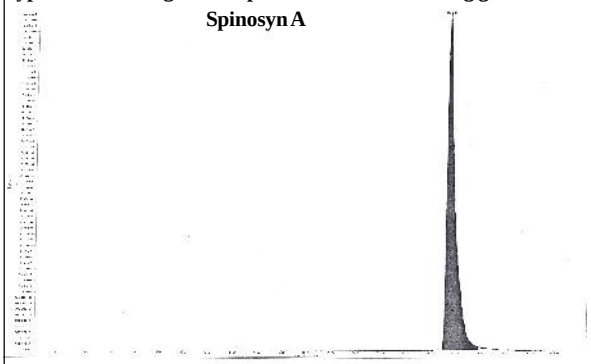
LOQ : 0.02 µg g^{-1}, BDL : Below detection limit, MRL : Maximum residue limit



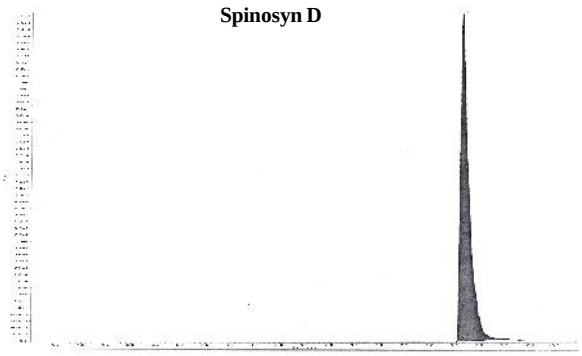
Typical chromatogram of spinosad standard 0.02 «g g^{-1}



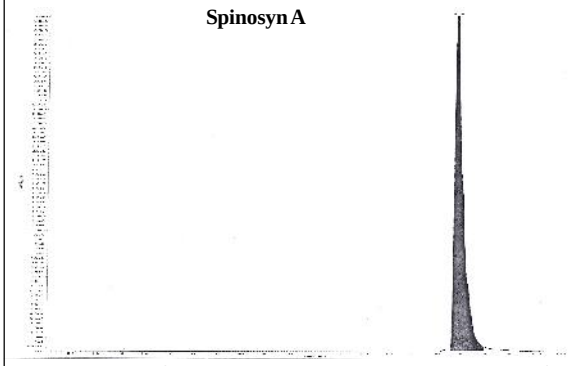
Typical chromatogram of spinosad standard 0.02 «g g^{-1}



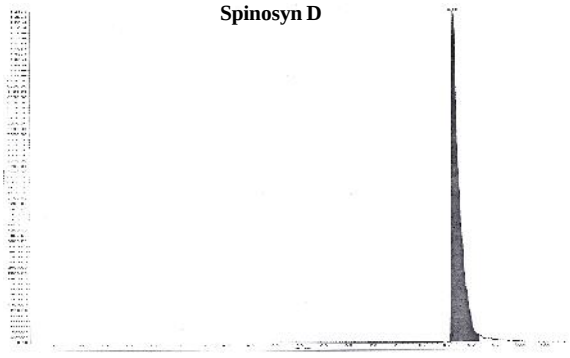
Chromatogram of pomegranate arils containing residues of spinosad standard 0 DAS



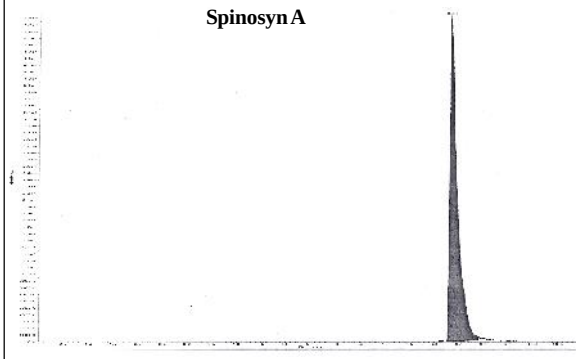
Chromatogram of pomegranate arils containing residues of spinosad standard 0 DAS



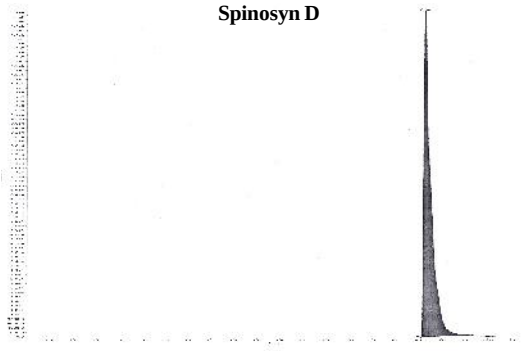
Chromatogram of pomegranate whole containing residues of spinosad standard 0 DAS



Chromatogram of pomegranate whole containing residues of spinosad standard 0 DAS

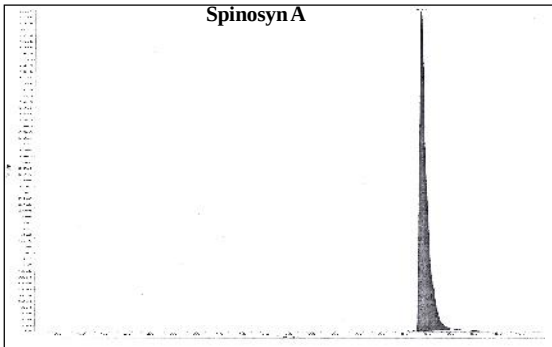


Chromatogram of pomegranate peel containing residues of spinosad standard 0 DAS

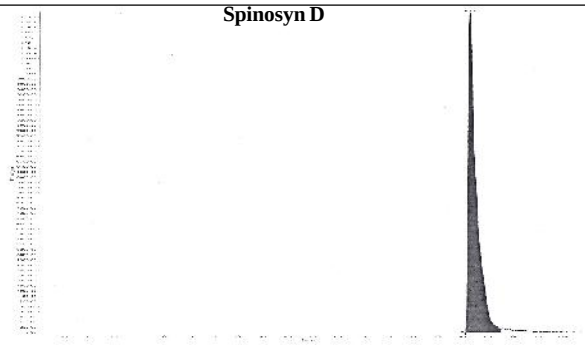


Chromatogram of pomegranate peel containing residues of spinosad standard 0 DAS

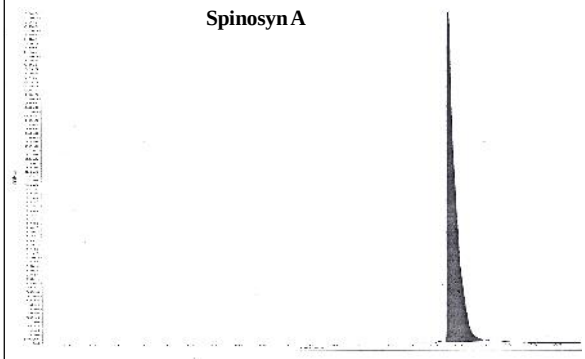
Fig. 2 : Typical Chromatogram of spinosad standard 0.02 µg g-1and its residues in pomegranate fruits at 0 DAS



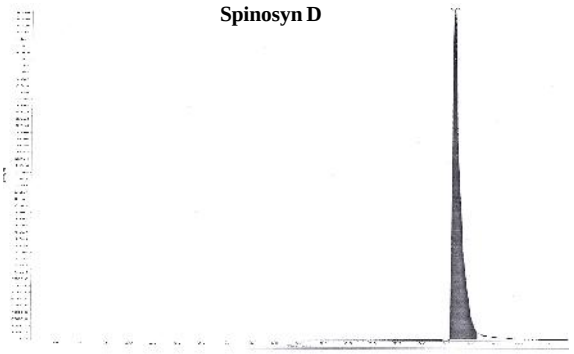
Typical chromatogram of spinosad standard 0.02 «g g^{-1}



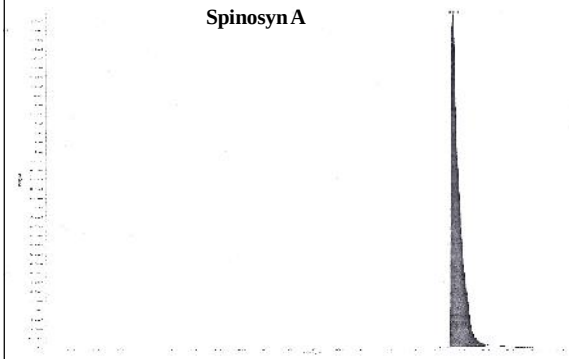
Typical chromatogram of spinosad standard 0.02 «g g^{-1}



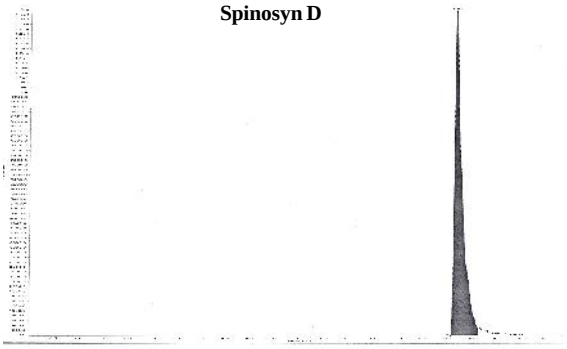
Chromatogram of pomegranate arils containing residues of spinosad standard 3 DAS



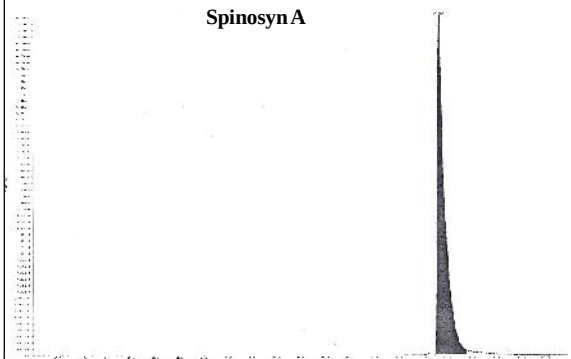
Chromatogram of pomegranate arils containing residues of spinosad standard 3 DAS



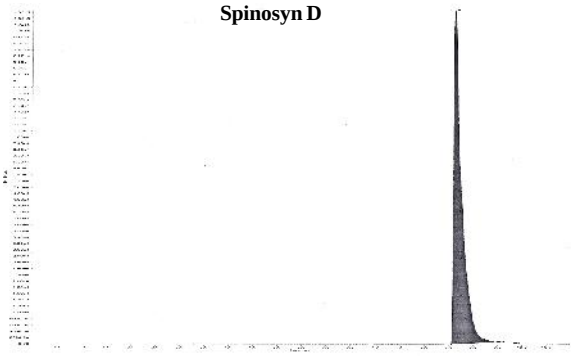
Chromatogram of pomegranate whole containing residues of spinosad standard 3 DAS



Chromatogram of pomegranate whole containing residues of spinosad standard 3 DAS



Chromatogram of pomegranate peel containing residues of spinosad standard 3 DAS



Chromatogram of pomegranate peel containing residues of spinosad standard 3 DAS

Fig. 3 : Typical chromatogram of spinosad standard 0.02 µg g^{-1} and its residues in pomegranate fruits at 3 DAS

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

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 spinosad in pomegranate fruits:

Arils :

Spinosad was estimated by reverse phase liquid chromatography at 250 nm wavelength with analytical parameters outlined. At recommended dose of spinosad 56.25 g a.i. ha^{-1} initial average deposit was 0.030 mg kg^{-1} which dropped down to below detection limit of 0.02 ppm on 1 DAS. The initial deposit of 0.037 mg kg^{-1} observed in the samples collected at 0 DAS (2 hrs) at double dose that fell to 0.027 mg kg^{-1}(1 DAS) and no residues were observed in the samples collected on 3 DAS.

Whole fruit :

Estimated residues on whole fruit samples at 0 days (2 hrs) interval indicated that higher application rate (112.5 g a.i. ha^{-1}) resulted in higher initial residues (Table 1 and Fig. 1-3). The initial residues of spinosad were 0.075 and 0.036 mg kg^{-1} at 56.25 and 112.5 g a.i. ha^{-1}. The spinosad residue levels at higher application rate fell with time from 0.075 to 0.036 mg kg^{-1} within 3 days with an estimated half-life of 3 days. No detectable residues were found in the samples brought 5 days after spray application. At recommended dose (56.25 g a.i. ha^{-1}) no residues were detected in the samples collected at 3 DAS.

Peel :

The concentration of spinosad at zero day in pomegranate peel was 0.099 mg kg^{-1} for higher dose and 0.045 mg kg^{-1} for normal dose. The initial deposit of 0.099 mg kg^{-1} dissipated to 0.024 mg kg^{-1} in 5 days with an estimated half-life of 2.47 days at 112.5 g a.i ha^{-1}. No residues were detected in the samples collected at 7 DAS. At recommended dose the initial deposit of 0.045 mg kg^{-1} took 5 days to reach below detection limit of 0.02 ppm. The estimated half-lives for both the doses were 2.89 and 2.50 days.

The initial residues of spinosad were 0.18 and 0.30 mg kg^{-1} at 15 and 30 g a.i. ha^{-1}, respectively on okra fruits (Kale, 2003). The initial deposit of spinosad 45 SC (96.4 g a.i. ha^{-1}) on cauliflower were 0.08 and 0.05 mg kg^{-1} at 0 and 1 DAS, respectively (Sable, 2005).

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