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Harvest Time Residues of Spinosad in Cardamom Capsules and Soil

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Studies were conducted to evaluate the harvest time residues of spinosad, composed of spinosyn A and D, an insecticidal derivative from fermentation of soil actenomycete species *Saccharopolyspora spinosa* from cardamom capsules and its cropped soil. Two sprays of spinosad 48 SC at 25 and 50 g a.i. ha^{-1} were given along with untreated check. Samples of green cardamom capsules and soil were collected at random at thirty days after last spray for analysis. The residues were estimated utilizing high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection for determining spinosad and its metabolites by Hitachi 6200 L Model. The results revealed that the harvest time residues of Spinosad 48 EC at 25 and 50 g a.i. ha^{-1} were below detectable level in green and cured cardamom capsules and soil samples.

Key words: Cardamom, residue, spinosyn, spinosad, HPLC.

Spinosad is a novel insect control agent derived by fermentation of the Actinomycete bacterium, *Saccharopolyspora spinosa.* The active ingredient is composed of two metabolites, spinosyn A and spinosyn D (Thompson *et al*., 1997). Spinosad controls many caterpillarpests in vines, pome fruit and vegetables (including tomatoe sand peppers), thrips in tomatoes, peppers and ornamental cultivation and dipterous leafminers in vegetables and ornamentals. Application rates vary between25 to 150 g of active ingredient per hectare (g a.i./ha) and 4.8 to 36 g of active ingredient per hectoliter (g a.i./hL) depending on the crop and target pest. High volume sprays in may lead to theoretical worse case application rates of 144,214 and 540 g a.i./ha. The mode of action of spinosad is completely novel, making it a useful resistance management tool. A novel mechanism of activity on the nicotinic acetylcholine receptors was identified as the primary cause of death (Salgado, 1997). Spinosad has additional effects on gamma amino butyric acid or GABA receptors, although it has not been shown that these effects contribute to insecticidal activity.

Cardamom, *Elettaria cardamomum* (L.) Maton the Queen of spices is indigenous to the Southern states of India. It is cultivated in Western Ghats (Kerala, Tamil Nadu and Karnataka) in an area of 73, 795 ha with a production of 12,540 MT (2005-6) and one of the important products fetching enormous foreign exchange (Stanley, 2007). India was the world's largest producer and exporter until it was taken over by Guatemala in the 18th century. One of the major constraints in the production of cardamom is the excessive damage by pests; the major ones being *Conogethes punctiferalis* Guenee and *Sciothrips cardamomi* Ramk in Tamil Nadu (Rao,1977). At present, these pests are kept under check with the help of synthetic insecticides. With the strict legislations enforced by the EPA, cardamom capsules with pesticide residues have a chance of being rejected by the hitherto importing countries, which in turn would have a major say in foreign revenues. The pesticide use pattern in the present day situations has led to resistance build-up by pests and pesticide residues, which demands newer and safer pesticides with different modes of action. Thus, there is a greater need to evaluate pesticides that would leave no or lesser residues in the commodity as well as in the environment. Spinosyns are the new group of crop protection agents highly effective against sucking pests and biting and chewing insects which act on receptor protein of insect nervous system. Spinosad provide excellent control of contemporary resistance pests. Extensive use of this novel compounds inevitably involves the risk of the residue problems in the produce. Keeping this in view, the present investigation was undertaken to determine the harvest time residues of spinosad 48 SC in cardamom capsules and its cropped soil.

Materials and Methods

Field experiment was conducted to determine the harvest time residues of spinosad (spinosyn A and spinosyn D) on Green gold variety cardamom during August 2012 in the farmer's holding at Kuppammal Patty near Thadiyankudisai, Kodaikanal Hills of Tamil Nadu. The experiments were conducted with three treatments viz., T1-

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Untreated control, T2 – Spinosad 48 SC @ 25 g a.i. ha-1 and T3 - Spinosad 48 SC @ 50 g a.i. ha^{-1}. The crop was maintained properly by adopting standard agronomic practices recommended by Tamil Nadu Agricultural University. The treatments were imposed when the pests crossed the economic threshold level (ETL). Two sprays were given with a pneumatic knapsack sprayer with a spray fluid volume of 500 l ha^{-1}.

Sampling and Extarction

Capsules

Matured and uniform sized cardamom capsules were collected at random on 30 days after second spray with the help of forceps for residue analysis. From each plot, 150 g of green capsules was collected and from this, a sub sample of 20 g green capsules in duplicate was taken for fresh sample analysis and transferred immediately into the sample container with acetonitrile. The remaining sample of 100 g was divided into two portions and was cured under conventional curing chamber at maximum temperature of 60-65°C maintained for 24 h and used as cured samples for residue analysis. The weights of the samples before and after curing were recorded from each plot to work out the residues on moisture free basis and curing loss.

The weighed sample of 25 g was soaked in acetonitrile (50 ml) overnight, homogenized and filtered through Buchner funnel. After repeated washing, the pooled acetonitrile extract was evaporated to near dryness.

Soil

The soil samples taken from treated plots were air dried. It was ground in a pestle and mortar and passed through 2 mm sieves. Five gram soil sample was taken in a 50 ml volumetric flask and mixed with 25 ml acetonitrile and kept for 48 hrs following sonication for 16 h in a sonicator. The solution was filtered and 1 ml aliquot was made up to 50 ml with acetonitrile mixture (650 ml acetonitrile + 350 ml HPLC water + 0.8 ml TEA). Twenty five microlitres of this sample was directly injected to HPLC (Hitachi L 6200 model) with a running time of 10 minutes.

Clean up

Liquid - liquid clean up

The aqueous remainder was treated with 50 ml of saturated sodium chloride and 150 ml of hexane (three 50 ml portions) in a 500 ml separating funnel. After shaking well, the lower aqueous phase was collected and 100 ml of hexane: ethyl acetate (98:2 V/V) was added and shaken well. Once again, the lower aqueous phase was collected and partitioned with three 50 ml portions of dichloromethane. The pooled dichloromethane extract was passed through anhydrous sodium sulphate. The extract was evaporated to near dryness and the aqueous remainder was dissolved in ethyl acetate.

Solid-liquid clean up

For column chromatography, 1.5 cm (id) x 50 cm (length) glass columns were used. Florisil® deactivated with 5 per cent water was used as an adsorbent at 4.5 g per sample. The drip tip of the chromatographic column was plugged with cotton wool. The Florisil® was slurried with 20 ml ethyl acetate and applied quantitatively into the column. This was sandwiched with two cm layers of anhydrous sodium sulphate. The column was prewashed with 20 ml ethyl acetate. The dry residue dissolved in small amount of ethyl acetate was poured on top of the column by means of a pipette and allowed to percolate. The active ingredient was eluted with 20 ml portions of acetonitrile (HPLC grade). The elutant was concentrated to near dryness, the residue dissolved in acetonitrile and fed into HPLC.

Preparation of standards

The stock solution of 1000 ppm was prepared by dissolving 101 mg of spinosad technical material (99.0% purity) in 100 ml of acetonitrile (HPLC grade). From this stock, intermediate stock solutions of 100 and 10 ppm were prepared. Using 10 ppm stock, working standards of 0.5, 1, 2, 3, 5 and 10 ppm were prepared in HPLC grade acetonitrile.

Recovery studies

Samples were fortified with working standards at 0.1, 0.5 and 1.0 ppm level to find out the recovery of spinosad.

Final quantification

End analysis was done with the aid of High Performance Liquid Chromatography (HPLC), Hitachi model L 6200 with the following operating parameters. The amount of residue was determined by comparing the sample response with the response of standard by using the formula.

Mobile phase: Acetonitrile & Water (35: 65 V/V HPLC grade)

Column: ODS 2

Flow rate: 1 ml min-1

Wave length: 270 nm

Quantity injected: 20 µl (fixed loop)

$$\text{Residues in ppm} = \frac{Hs}{Hstd} \times \frac{Wstd}{Ws} \times \frac{Vex}{Vs} \times \frac{As}{Astd}$$

where,

Hs - Peak height of the sample

Hstd - Peak height of the standard

Wstd - Weight of the standard injected in ng

Ws - Weight of the sample in g

Vex - Volume of the final extract in ml

Vs - Quantity of the sample injected in ìl

As - Attenuation of the sample

Astd - Attenuation of the standard

Results and Discussion

The mean recovery was 80.4 and 79.8 per cent for soil and green capsules of cardamom samples fortified at 0.1, 0.5 and 1.0 ppm level. Hence, the recovery factor was not used for working out the residues (Table1).

Table 1. Recovery of spinosad from capsules and soil samples of Cardamom

Matrix / Substrate	Amount fortified (ppm)	% recovered	Average % recovery
Soil	0.1	79.5	80.4
	0.5	81.2	
	1.0	80.5	
Green capsules	0.1	76.8	79.8
	0.5	81.7	
	1.0	80.9	

The minimum detection limit of the instrument was 0.5 ppm and the determinability level in the sample was 0.05 ìg g-1 considering the weight of the sample as 20 g and final volume of the extract as 2 ml. The harvest time residues of spinosad 48 SC at 25 and 50 g a.i. ha-1 as foliar spray were at below detectable level (BDL) in green and cured cardamom capsules and the soil as well (Table 2).

Table 2. Harvest time residues of spinosad 48 SC in cardamom capsules and soil

Treatment	Dose (g a.i. ha^{-1})	Residues in µg g^{-1} at harvest		
		Green capsules	Cured capsules	Soil
T1 - Untreated check		BDL	BDL	BDL
T2 - Spinosad 48SC	25	BDL	BDL	BDL
T3 - Spinosad 48SC	50	BDL	BDL	BDL

The interval between the last spray and sample picking was 30 days. Similar results were obtained by Stanley (2007), who reported that the residues of diafenthiuron dissipated to 0.08 and 0.16 per cent in green cardamom capsules at 15 DAT. Renuka (2001) and Rajabaskar (2003) reported a total loss of profenofos after 15 days of spray in both green and cured cardamom capsules when sprayed at 0.075 per cent. Imidacloprid residues in the cotton lint, seed and oil from first and second picking were at below detectable level (BDL) in the samples collected from the imidacloprid 200 SL treated plots, 12 and 15 days after the last spray in the first and second field trials, respectively (Preetha, 2008).

Vinothkumar et al., (2009) reported below detectable levels of imidacloprid 200 SL residues in green and cured capsules of cardamom. Since the picking of cardamom capsules was carried out at an interval of 30-35 days the residues of spinosad becomes zero or below detectable level. As harvest being the focal point for enforcement of residue tolerances, the suggested waiting periods of seven days is safe enough to contain the cardamom pests with spinosad without the problem of pesticide residues in harvestable produce. Gao (2007) reported that spinosad is unlikely to pose any health issues for higher animals including human not only in China but also in global community, if it is applied according to the good agriculture practices (GAPs) established by each countries. Recently, European Food Safety Authority (EFSA, 2012) concludes after conducting MRL analyses on celery, fennel, raspberries and blackberries that the intended use of spinosad on the crops under consideration will not result in a consumer exposure exceeding the toxicological reference value and therefore is unlikely to pose a public health concern.

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