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Dissipation Kinetics of Spinosad on Cauliflower (*Brassica oleracea* var. *botrytis*. L.) Under Subtropical Conditions of Punjab, India

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Abstract Residues of spinosad were estimated in cauliflower curds using high performance liquid chromatography (HPLC) and confirmed by high performance thin layer chromatography (HPTLC). Following three application of spinosad (Success 2.5 SC) at 15 and 30 g a.i. ha^{-1}, the average initial deposits of spinosad were observed to be 0.57 and 1.34 mg kg^{-1}, respectively. These residues dissipated below the limit of quantification (LOQ) of 0.02 mg kg^{-1} after 10 days at both the dosages. The half-life values ($T_{1/2}$) of spinosad were worked out to be 1.20 and 1.58 days, respectively, at recommended and double the recommended dosages. Thus, a waiting period of 6 days is suggested for the safe consumption of spinosad treated cauliflower.

Keywords Spinosad · Residues · Dissipation · Waiting period

Cauliflower (*Brassica oleracea* var. *botrytis*. L.) is one of the important cruciferous vegetable crops of India. It is widely cultivated throughout the sub-tropical parts of north India. The crop is subjected to high degree of instability in production mainly because of the losses caused by insect pests, of which diamond-back moth [*Plutella xylostella* (Linnaeus)] is the most serious. Recently, spinosad has been found to be very effective for the control of diamond-back moth (Gill et al. 2008).

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Spinosad is a biologically derived insecticide that consists of two active compounds, spinosyns A and D, produced by fermentation culture of an actinomycete isolated (*Saccharopolyspora spinosa* Mertz & Yao) from soil. Structurally, these compounds are macrolides and contain a unique tetracyclic ring system to which two different sugars are attached (Kirst et al. 1992). It possesses both contact and stomach poison activity against insects belonging to order Coleoptera, Diptera, Hymenoptera, Isoptera, Lepidoptera, Siphonoptera and Thysanoptera, but has little or no activity against sucking insects, predatory insects and mites (Elzen et al. 1998). However, considerable concern is being expressed over the magnitude of pest control chemicals left in food stuffs following their use on crops. It is important to ensure that the level of harvest time residues of pesticides on foodstuffs do not pose any hazard to consumers and are acceptable in domestic as well as in international trade. Therefore, the present studies were undertaken to know the persistence of spinosad on cauliflower under subtropical conditions of Punjab, India.

Materials and Methods

Cauliflower (var. Giant Snowball) was raised during August–October 2007 at Entomological Research Farm, Punjab Agricultural University, Ludhiana following recommended agronomic practices (Anonymous 2007). The first application of spinosad (Success 2.5 SC) at 15 and 30 g a.i. ha^{-1} was made at curd formation stage followed by another two applications at 10 days interval. About 1 kg curds of marketable size were collected randomly before and 0, 1, 3, 5, 7, 10 and 15 days after the third application of the insecticide.

A representative 50 g sample of chopped and macerated cauliflower curds was dipped for overnight into 100 mL methanol in an erlenmayer flask. The extract was filtered through glass wool plugged in filtering funnel into one litre separatory funnel. The residual material was rinsed with methanol and transferred to the same separatory funnel. The contents of separatory funnel were diluted with 600 mL brine solution and partitioned thrice into 100, 75 and 75 mL dichloromethane and once into 100 mL hexane. The combined organic layers were drained into 500 mL beaker through 1.5″ layer of anhydrous sodium sulphate supported on a pre-washed glasswool in a funnel. The combined extract was concentrated to about 5 mL under rotary *vacuum* evaporator.

The extracts thus obtained were cleaned up by column chromatography using silica gel (60–120 mesh) as an adsorbent. Before use, the silica gel was activated at 110°C for 2 h. The extract was mixed with a mixture of silica gel (10–12 g), anhydrous sodium sulphate (10 g) and 2 g activated charcoal. A glass column (60 cm × 1.5 cm i.d.) containing 40 ml dichloromethane supported on a cotton plug was prepared. Sample slurry was prepared using dichloromethane and transferred to the column. The glass beaker containing extract was rinsed with methanol and the contents were transferred to the column. The column was allowed to stand for 90 min. Then the dichloromethane present in the column was allowed to elute drop wise. When about 5 mL dichloromethane remained on the surface of the adsorbent, the extract was eluted with 150 mL of freshly prepared solvent mixture of dichloromethane: hexane (1:1, v/v). The eluate was concentrated to near dryness in a rotary evaporator under *vacuum* and transferred to 5 mL of methanol for further analysis.

The residues were determined by using high performance liquid chromatography (Shimadzu model DGU-2045) equipped with C_{18} column with photo diode array (PDA) detector. The instrument was set at wavelength 274 nm, mobile phase methanol with pump flow at 1 mL min^{-1}. Residues of spinosad were quantified by comparison of peak height/peak area of standards with that of unknown or spiked samples run under identical conditions. Under these operating conditions the retention time of spinosad was found to be 5.67 min.

The control samples of cauliflower curds were spiked at 0.2, 0.5 and 1.0 mg kg^{-1}, respectively, and processed by following the methodology as described above. The average recovery values from the fortified samples were found to be more than 80 % cent (Table 1).

The confirmation of spinosad residues was carried out by high performance thin layer chromatography (HPTLC). The experiment was carried out at 254 nm wavelength using methanol as a mobile phase. The Rf value of spinosad was observed to be 0.48.

Table 1 Recovery of spinosad from cauliflower curds

Substrate	Level of fortification (mg/kg^{-1})	Recovery (%) (mean ± SD)[a]
Cauliflower curds	0.2	83.3 ± 2.9
	0.5	86.7 ± 3.1
	1.0	86.3 ± 3.1

[a] Each value is mean ± SD of three replicate determinations

Results and Discussion

The overall results of the analysis of cauliflower curds following 3rd application of spinosad at 15 and 30 g a.i. ha^{-1} are presented in Table 2. The mean initial deposits of spinosad were 0.57 and 1.34 mg kg^{-1} on the curds following 3rd application of spinosad at minimum effective and double the effective dosages, respectively. These deposits dissipated to 0.14 and 0.27 mg kg^{-1} after 3 days, respectively, thereby showing a loss of about 75 and 80 per cent following application of spinosad at 15 and 30 g a.i. ha^{-1}. These residues reached below the detectable limit of 0.02 mg kg^{-1} in 7 and 10 days, respectively thereby showing 100 % cent loss following application of spinosad at both the dosages (Fig. 1).

Half-life ($T_{1/2}$) of spinosad calculated as per Hoskins 1961 was observed to be 1.20 and 1.58 days, respectively when applied at 15 and 30 g a.i. ha^{-1}. Half-scale deflection was obtained for 20 ng spinosad and limit of quantification (LOQ) was found to be 0.02 mg kg^{-1}.

The results are in agreement with those of Sharma et al. (2007) who reported an initial deposits of 2.66 and 3.89 mg kg^{-1} following application of spinosad at 17.5 and 35.0 g a.i. ha^{-1}. Sharma et al. (2007) also reported that spinosad residues persisted up to 7 and 10 days, respectively following its application at lower and higher dosages. The half lives for both spinosyns had been reported to vary with rate i.e. lower persistence at the lower application rates (Tomkins et al. 1999). Spinosad was quickly converted to degradation products by sunlight on leaf surfaces. Half lives for spinosad had been reported to vary from 1.6 to 16 days depending on the amount of sunlight received. The possible routes of spinosad dissipation and transformation in the environment included photodegradation and biotransformation on plant surfaces, abiotic hydrolysis, aqueous photolysis, photodegradation on soil and biotransformation via soil microorganisms (Saunders and Bret 1997). Singh and Mukherjee (1993) also observed fast dissipation of monocrotophos on leaves and curds of cauliflower following its application at 350 and 700 g a.i. ha^{-1}, the residues dissipated below the detection limit of 0.01 in 7 days.

The maximum residue limit (MRL) of 0.02 mg kg^{-1} has been prescribed for spinosad on cauliflower (Sharma

Table 2 Residues of spinosad (mg kg^{-1}) in the curds of cauliflower

Days after application	15 g a.i. ha^{-1}			30 g a.i. ha^{-1}		
	Replicates	Mean ± SD	% Dissipation	Replicates	Mean ± SD	% Dissipation
Before application	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
0	0.51	0.57 ± 0.06	–	1.36	1.34 ± 0.13	–
	0.57			1.45		
	0.63			1.20		
1	0.33	0.31 ± 0.04	45.6	0.67	0.72 ± 0.06	46.3
	0.34			0.71		
	0.27			0.78		
3	0.14	0.14 ± 0.01	75.4	0.25	0.27 ± 0.02	79.9
	0.15			0.28		
	0.13			0.27		
5	0.03	0.03 ± 0.01	94.7	0.15	0.15 ± 0.04	88.8
	0.02			0.12		
	0.03			0.19		
7	BDL	BDL	100.0	0.05	0.06 ± 0.01	95.5
	BDL			0.07		
	BDL			0.06		
10	BDL	BDL	100.0	BDL	BDL	100.0
	BDL			BDL		
	BDL			BDL		
15	BDL	BDL	100.0	BDL	BDL	100.0
	BDL			BDL		
	BDL			BDL		
$T_{1/2}$	1.20			1.58		
T_{MRL}	5.80			7.26		

BDL, below detectable limit (<0.02 mg kg^{-1})

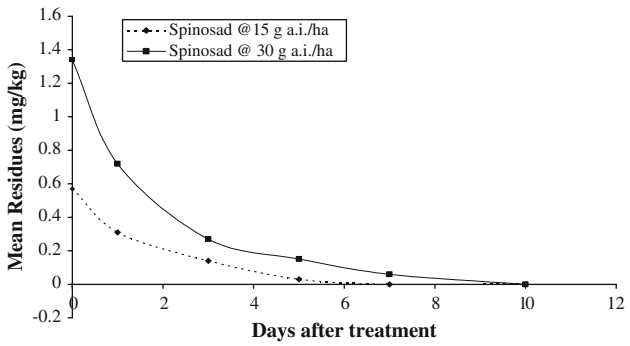

Fig. 1 Dissipation of spinosad residues on cauliflower

2007). Following application at 15 g a.i. ha^{-1}, the residues of spinosad in/on cauliflower were found to be below the MRL after 6 days application. These results can be further substantiated with the help of dissipation parameters calculated in the present investigations. It was found that residues reached below the MRL on 5.80 and 7.26 days (T_{MRL}) at single and double dose, respectively. These studies, therefore suggest that the use of spinosad at the minimum effective dosages does not seem to pose any hazards to the consumers if a waiting period of 6 days is observed.

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