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Persistence and Safety Evaluation of Alphamethrin on Mustard (*Brassica campestris* Linn.)

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Mustard is the second most important oilseed crop after groundnut in area and production (Yadav et al. 1988) and considered to be one of the major source of edible oil in India. It is attacked by as many as 38 insect pests at various stages of plant growth (Bakhetia 1987) which limit in its profitable cultivation.

Many insecticides belonging to organochlorines (Bhatia and Sekhon 1989), organophosphates (Anonymous 1984 and Mature et al. 1987) and pyrethroids (Prasad et al. 1983) have been used to control insect pests but many of them were associated with undesirable traits such as high persistence, deposition in oil and development of resistance (Lal 1996). Alphamethrin, 1R *cis*, αS and

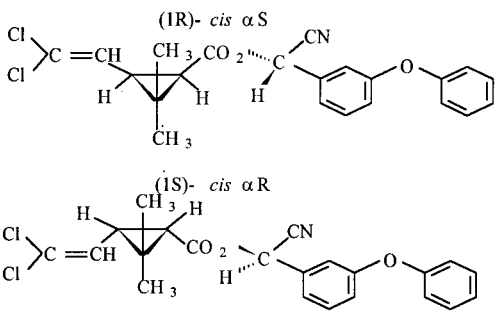

Fig. 1. Chemical structures of alphamethrin

1S *cis*, αR enantiomer pair of α-cyano-3-phenoxybenzyl-3-(2, 2-dichlorovinyl)-2, 2-dimethyl cyclopropanecarboxylate (Fig. 1), an environmentally safe pyrethroid insecticide, having broad spectrum of activity has recently been registered in India and it has been recommended for control of wide range of insect pests. Its persistence and residues has been evaluated only on few crops in Indian conditions viz., tea (Barooah and Borthakur 1994), cotton (Tamilselvan 1995) and cabbage (Pandit et al. 1996). Since, it is used at flowering and pod formation stage, the residues may remain in the crop including grain and may pose hazards to consumers. The reports on the persistence and safety evaluation of alphamethrin in mustard crop are not available and therefore studies were carried out on its persistence and safety evaluation on this crop. Besides, the theoretical dissipation models were developed and predicted data thus obtained

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was compared with observed data (true data). In the absence of MRL values and waiting period of this insecticide and based on ADI value, an effort was made to establish its TMRC and MPI values and also waiting period is suggested.

MATERIALS AND METHODS

Mustard crop variety Pusa Kalyani was raised in the fields of Indian Agricultural Research Institute, New Delhi during rabi season. Alphamethrin (diluted from Alphaguard 10 EC, Garda Chemicals, Mumbai, India) was sprayed at the rate of 40 and 80 g ai ha^{-1} (700 l of spray fluid ha^{-1}) at the pod formation stage. The control plots were sprayed with water at the rate of 700 l ha^{-1}. Samples of foliage including pods (herbage) were drawn from each treatment and replicate on 0 day (after 1 hr of insecticide spray) and 1, 3, 7, 15 and 28 days after spray application. Samples of matured grains were collected at harvest. After harvest of the crop soil samples from 0-10 cm depth were also collected to determine the residues of alphamethrin.

The analytical procedure was optimised by conducting recovery experiments. Representative sample of herbage 200 g was drawn and chopped and a sub sample of 25 g was taken. The samples were extracted with acetone-hexane (1:1, v/v) three times (100+2 x 50 ml) using a mixer grinder. To the pooled extract 150 ml of 5% aqueous sodium chloride solution was added in a separatory funnel and shaken for two minutes. The aqueous layer was discarded. The hexane layer was dried over anhydrous sodium sulfate and concentrated to 1-2 ml in a rotary flash evaporator. The concentrated extracts were cleaned up by passing through 10 g of alumina (neutral), prewashed with 50 ml of hexane, in a glass column (450 x 15 mm). The elutions were done with 50 ml of the hexane-acetone (9:1, v/v). The eluates were made up to 50 ml in a volumetric flask and were quantified.

Samples of matured grains (15 g) were crushed and extracted with 150 ml of acetone-hexane (1:1, v/v) in a Soxhlet apparatus for 6 hrs. Solvent was evaporated to dryness and 50 ml of hexane was added. Hexane layer was extracted with acetonitrile (3 x 50 ml). The acetonitrile extract was combined and evaporated to dryness and the residues were dissolved in 1-2 ml of hexane. The extracts were then cleaned up as described for herbage and subjected for quantification of alphamethrin.

A single step method was used for extraction and clean up of alphamethrin from soil samples. Soil samples (25 g) were air dried, ground and screened through 2 mm sieve and then 0.5 g activated charcoal (Darco-G-60) was mixed. The mixture was packed in a glass column (450 x 15 mm) containing 4 cm layer of anhydrous sodium sulfate and eluted with 100 ml hexane-acetone (9:1, v/v). The eluates were concentrated to 50 ml and subjected for quantification without further clean up.

The residues were determined by using Hewlett Packard 5890 series II, gas-

liquid chromatograph equipped with ^{63}Ni-ECD. Conditions for analysis of alphamethrin were, column, megabore (10 m x 0.53 mm i.d.) coated with HP-1 (methyl silicon gum), film thickness, 2.65 µm; oven temperature, 260°C; injection temperature, 300°C; detector temperature, 300°C; carrier gas (N_2), 20 ml min^{-1}; injection volume, 3 µl; retention time, 3.26 min.

During the period of experiment the minimum and maximum temperature varied between 0.8-17.2 and 13.0-31.6°C, respectively and mean relative humidity ranged from 51 to 95 per cent. Total rainfall was 154.9 mm. The dissipation rate of alphamethrin residues was worked out by regression analysis between time after application and corresponding residues (Hoskins 1961) using microstat software on computer.

RESULTS AND DISCUSSION

The recoveries of alphamethrin from fortified samples of brassica herbage and grains and soil using various extracting solvents and adsorbents are reported in Table 1.

Table 1. Recoveries of alphamethrin from fortified brassica herbage and grains and soil

Extraction method/solvent	Adsorbent used	Fortification (mg kg^{-1})	Per cent recovery* Herbage	Grains	Soil
Cold extraction/ Hexane-acetone (9:1, v/v)	Alumina (neutral)	1.0	82.00	-	-
		2.0	80.50	-	-
	Alumina (neutral) +5% charcoal	1.0	75.00	-	-
		2.0	77.50	-	-
	Silica gel	1.0	61.67	-	-
		2.0	60.83	-	-
	2% charcoal mixed in sample	1.0	-	-	88.00
		2.0	-	-	91.00
Cold extraction/ Hexane-acetone (1:1, v/v)	Alumina (neutral)	1.0	96.00	77.00	-
		2.0	96.20	74.00	-
	Florisil	1.0	78.00	-	-
		2.0	79.00	-	-
	Silica gel	1.0	63.00	-	-
		2.0	61.80	-	-
Hot extraction**/ Hexane-acetone (1:1, v/v)	Alumina (neutral)	1.0	-	99.33	-
		2.0	-	96.83	-

* Average of three replicates, ** Soxhlet method

Hexane-acetone (1:1, v/v) was found optimum in extracting alphamethrin from different matrices. Therefore, this mixture of solvents was used for the extraction of the insecticide from herbage and matured grains. In case of herbage, alumina (neutral) gave 96.00 to 96.20 per cent recovery. Florisil was found less effective and silica gel the least.

Extraction by Soxhlet method and cleanup through alumina (neutral) was found best for matured grains and recoveries were 96.83 to 99.33 per cent. Fairly good recoveries (88-91%) from fortified soil samples were attained by single step extraction and cleanup method using 2 per cent charcoal (Darco-G-60) mixed with soil and eluted with hexane-acetone (9:1, v/v).

The data on alphamethrin residues and its dissipation in/on brassica crop are presented in Table 2.

Table 2. Alphamethrin residues in/on brassica herbage

Days	Treatment (g ai ha^{-1})	Residues* (mg kg^{-1})	Dissipation (%)
0	40	7.60±0.62	-
	80	13.53±0.49	-
1	40	5.27±0.25	30.66
	80	8.66±0.28	35.85
3	40	2.59±0.20	64.61
	80	4.21±0.39	68.69
7	40	1.18±0.07	84.74
	80	1.52±0.65	88.76
15	40	0.55±0.05	92.76
	80	0.88±0.06	93.50
28	40	0.17±0.02	97.76
	80	0.29±0.02	97.86

* Average of three replicates

The initial deposits in/on brassica herbage were 7.60 and 13.53 mg kg^{-1} from 40 and 80 g ai ha^{-1} treatments, respectively. The observed high initial deposits may be due to the hairy, waxy and dense foliage of brassica and high lipophilicity of alphamethrin. The residues declined exponentially with the time lapse and reached to 0.17 and 0.29 mg kg^{-1} in 28 days from both the rates of application. There was no significant difference in dissipation between the two rates of application, suggesting that dissipation was independent of dose of insecticide and followed first order kinetics for dissipation.

The theoretical models representing dissipation during the period of 0-28, 0-7 and 7-28 days and their corresponding half-lives, correlation coefficients and initial deposits are listed in Table 3.

The dissipation model (0-28 days) yielded dissipation rate constant of 0.0559 and 0.0556 day^{-1} with correlation coefficient of -0.9659 and -0.9465 at lower and higher rates of application. The equation yielded half-lives of 5.38 and 5.45 days from both the rates of application which indicate that half-lives are independent of rate of insecticide application. However, predicted initial deposits on the basis of theoretical dissipation model were 4.77 and 7.59 mg kg^{-1} which are substantially different than the observed values of 7.60±0.62 and 13.53±0.49 mg kg^{-1} (Table 1) which indicate that this dissipation model behaves differently than the true data.

Table 3. Statistical data on regression analysis

Treatment (g ai ha^{-1})	Observation period (days)	Dissipation phase	Dissipation model	Correlation coefficient (r)	Initial deposit (mg kg^{-1})	Half-lives (days)
40	0-28	-	Y= 0.6792-0.0559x	-0.9659	4.77	5.38
	0-7	Fast	Y= 0.8355-0.1140x	-0.9872	6.85	2.65
	7-28	Slow	Y= 0.3475-0.400x	-0.9999	2.22	7.52
80	0-28	-	Y= 0.8804-0.0556x	-0.9465	7.59	5.45
	0-7	Fast	Y= 1.0844-0.1329x	-0.9932	12.15	2.26
	7-28	Slow	Y= 0.4386-0.0346x	-0.9948	2.74	8.70

Experimental data for residues of alphamethrin revealed that following 7th day after application, the residues of alphamethrin at various interval of time showed gentle slopes for dissipation. It showed a two phase profile of dissipation behaviour of alphamethrin in/on brassica plants. The residues dissipated more quickly during initial days after application, presumably, due to loose association of insecticide residues with plant foliage and consequently quick loss of insecticide by natural elements. The dissipation rate became slower at later stages. This could be due to the phenomenon that the association of insecticide molecules and brassica herbage became stronger in the adsorbed form with time resulting in less and less alphamethrin being subjected to loss by weathering.

The predicted initial concentrations from fast dissipation phase (0-7 days) were 6.85 and 12.15 mg kg^{-1} (Table 3) which were approximately equal to the observed values of 7.60±0.62 and 13.53±0.49 mg kg^{-1} (Table 1). Hence dissipation rate expressions described the observed data satisfactorily. According to the bi-phasic dissipation models, the residues dissipated quickly with half-lives 2.65 and 2.26 days for faster dissipation phase (0-7 days) from both the rates of application. The dissipation rate during later period (7-28 days) became much slower with dissipation half-lives of 7.52 and 8.70 days for both the application rates.

To evaluate the risk to the consumer, as brassica leaves are used as vegetable (saag) in India, the Theoretical Maximum Residue Contribution (TMRC) through

the consumption of brassica leaves treated @ 40 and 80 g ai ha^{-1} can be compared with Maximum Permissible Intake (MPI). The prescribed Acceptable Daily Intake (ADI) value of alphamethrin is 0.05 mg kg^{-1} body weight. Multiplying ADI with the weight of average Indian, 55 kg, the MPI is obtained. The TMRC is calculated by multiplying the maximum amount of residue detected on herbage with the amount of vegetable consumed $person^{-1}$ day^{-1}. Assuming the worst case that only brassica leaves (40 g) are consumed as leafy vegetable, calculated TMRC even on zero day (0.336 and 0.565 mg $person^{-1}$ day^{-1}) were lower than MPI (2.75 mg $person^{-1}$ day^{-1}) calculated from toxicological data. Therefore spray application of alphamethrin at both the levels could be taken safe from crop protection and environmental contamination point of view. On the basis of the above discussion, the suggested waiting period for alphamethrin is one day.

The recommended balanced diet for Indians is given in Table 4.

Table 4. Balanced diet for an Indian

Food	Diet (g day^{-1})
Cereal	670
Pulses	62
Leafy vegetables	40
Other vegetables	80
Root and tubers	80
Milk	250
Oil and fats	65
Sugar and jaggary	55

The terminal residues of alphamethrin in grain samples collected at harvest were non detectable resulting from 40 and 80 g ai ha^{-1} application.

Alphamethrin residues were not detected in the field soil samples collected after harvest of the crop.

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