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Safety Evaluation of the Fungicide Iprodione on Cauliflower (*Brassica oleracea* var. *oleracea* L.)

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Alternaria blight is an important disease of cruciferous crops. Cauliflower (*Brassica oleracea* var *oleracea*) which is one of the most important vegetable crops in India, is attacked by *two Alternaria* species, *Alternaria brassicae* and *Alternaria brassicicola* (Duhan and Suhag, 1989). Mainly two fungicides are reported for the control of alternaria - captafol and mancozeb (Chakrabarty, 1993). Captafol has been banned in India for foliar spray and chronic toxicity test with ethylenebisdithiocarbamate shows goiteriogenic, oncogenic and teratogenic effects in experimental mammals (Matolcsy *et al.,* 1988, Rose et *al.,* 1980). Iprodione [3 - (3,5-dichlorophenyl) - N - isopropyl - 2,4 dioxoimidazolidine - 1 - carboxamide] - an environmentally safe fungicide having broad spectrum of activity has been recently registered in India. Though it is recommended for controlling disease in cauliflower crops, its MRL is not established due to lack of residue data and hence waiting period cannot be recommended (FAO/WHO, 1996). A detailed study has been conducted on the persistence of iprodione in/on cauliflower.

Iprodione has been found effective against *Alternaria* species attacking mustard crop. *In vitro* bioactivity test was conducted to evaluate the activity of iprodione against local strain of *two* species of the *Alternaria* affecting cauliflower. A new infrared spectroscopic method for the estimation of active ingredient of iprodione in commercial formulation was also developed.

MATERIALS AND METHODS

Cauliflower crop variety Pusa Snowball 1 was raised in the fields of IARI, New Delhi during Nov-Dec., 1997 following RBD. Rovral® 50 WP formulation of iprodione was sprayed twice (10th and 25th February, 1996) at two doses, 500 g a.i./ha and 1000 g a.i./ha starting from the curd initiation stage. Curds were collected for both the treatments on 0 (1 hour after spraying), 1, 3, 5, 7 and 10 days after spraying.

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The leaves were collected on 0, 1, 3, 5, 7 and 10 days after first spraying only. Control samples were collected from untreated plots. The analytical procedure was optimized on the basis of recovery experiments. A representative sample 25 g of cauliflower curd was drawn by quartering and then extracted with acetone (3 x 50 mL) in a waring blendor. After filtration, the filtrate was concentrated under reduced pressure in a rotary vacuum evaporator and partitioned with dichloromethane (3 x 30 mL) in the presence of 100 mL water saturated with NaCI. The dichloromethane layer was dried with sodium sulfate and the extract was concentrated before column chromatographic cleanup. The concentrated extract was cleaned up by passing it through Florisil® (4 g) in a glass column. The extract was transferred into column with 30 mL dichloromethane which was discarded. Final elution was done with 80 mL dichloromethane - ethylacetate (95: 5) solvent mixture. The eluate was collected and completely evaporated. 10 mL hexane was added which was evaporated subsequently to remove residual dichloromethane. Finally the volume was made up to 10ml with toluene. For the leaf sample the same method was followed except that instead of Florisil®, magnesia: celite: charcoal (1:2:2) mixture (5 g) was taken as adsorbent.

Residues were determined by using Hewlett Packerd 5890A-gas liquid chromatograph equipped with Ni 63 - ECD. GLC condition for the analysis of iprodione was - column: megabore (5 m x 0.53 mm i.d.), coated with HP1 (methyl silicone gum), film thickness 2.65 µm; oven temperature: 190°C; injector temperature: 200°C; detector temperature: 275°C; injector volume: 1 µL; retention time: 4.9 min.

During the experiment, mean temperature, average relative humidity, average sunshine hour, average wind speed and total rainfall were 16.83°C, 53.9%, 7.41 hr, 4.36 km/hr and 0 mm, respectively. The rate of dissipation of iprodione residues was worked out by determining half-life values (RL_{50} values using statistical methods (Hoskins, 1961).

Infrared spectrophotometer (Model Impact 400) was used for the estimation of active ingredient in the formulation. A standard curve was first prepared by plotting area and height of the N-H peak vs. concentration (10,000 to 50,000 ppm). The N-H stretching peak of 3355 cm-' was chosen for the analytical work. Rovral® 50 WP formulation was extracted with dichloromethane by stirring with Teflon coated magnetic stirrer for 15 min. The extract was then separated from residual matter by centrifugation and filtration. Finally the height and area of N-H peak of the extract was recorded and plotted in the graph.

In vitro bioefficacy by poison food technique in a potato-dextrose-agar medium was done against local strains of *Alternaria brassicicola,*

Alternaria brassicae and *Sclerotium rolfsii* following the method already reported (Nene and Thapliyal, 1979).

RESULTS AND DISCUSSION

Acetone is a good solvent for iprodione, so it was chosen for the extraction of the fungicide from the curd and leaf. Dichloromethane was found to be the most suitable solvent for liquid - liquid partitioning as compared to hexane and chloroform.

In the case of curd, Florisil® gave higher recovery (85.71%) than alumina (66.2%) or silica gel (47.64%) in column cleanup. Magnesia: Celite: Charcoal (1: 2: 2) mixture was found suitable for cleanup of leaf samples which gave a colorless extract and 82.28% recovery. For the cleanup of extract of leaves, Florisil® was unable to remove the green colour completely. As the fungicide is polar, dichloromethane-ethylacetate mixture (95: 5) was used as eluent. Ethyl acetate was used to increase the polarity otherwise more amount of dichloromethane was required to complete the elution of the fungicide.

Three columns megabore (5m, HP-1), capillary (25m, OV - 1), glass (2m long, OV - 17) were tried but the best result was found when a short megabore column was used. It gave higher sensitivity and better resolution. Use of short column for the analysis of iprodione was also reported by Lacroix *et al.* (1980). The residue data of iprodione on cauliflower curd is shown in Table 1.

Table 1. Iprodione residues in/on cauliflower curd and leaves

Sampling Day	Treatment kg a.i./ha	Iprodione residues (μg/g) curd 1st spray	curd 2nd spray	leaves 1st spray
0	0.5	2.42	2.67	3.71
	1.0	4.22	4.48	7.14
1	0.5	2.20(0.91)	2.35(11.98)	3.25(12.40)
	1.0	3.73(11.61)	3.91(12.72)	6.10(14.57)
3	0.5	1.50(38.02)	1.43(46.44)	2.08(43.94)
	1.0	2.31(45.26)	2.61(41.74)	3.40(52.38)
5	0.5	0.87(64.05)	0.99(62.92)	1.12(69.81)
	1.0	1.44(65.88)	1.50(66.52)	2.42(66.11)
7	0.5	0.67(72.31)	0.72(73.03)	0.76(79.51)
	1.0	1.13(73.22)	1.02(77.23)	1.64(77.03)
10	0.5	0.35(85.54)	0.35(86.89)	0.35(90.57)
	1.0	0.49(88.39)	0.50(88.84)	0.63(91.18)

Figures in parentheses indicate percentage dissipation.

In case of 1st spray initial residues were 2.42 µg/g and 4.22 µg/g for single and double dose respectively. Within 10 days the fungicide dissipated to 0.35 µg/g and 0.49 µg/g. Thus more than 86% iprodione dissipated within 10 days. For 2nd spray a similar trend was obtained.

Initial leaf residues were 3.71 µg/g and 7.14 µg/g, respectively and after 10 days 0.35 µg/g and 0.63 µg/g. More than 90% of iprodione residues dissipated within 10 days. Higher amount of residues were detected on leaves as compared to curds because leaves are more exposed and have a greater surface to mass ratio. Dissipation was relatively faster in leaves than curds.

Iprodione dissipated uniformly at both rates of application on curds and leaves showing a first order rate kinetics. The half-life of iprodione was calculated to be 3.25 to 3.50 days during the first application and 3.10 to 3.43 days during second application at 0.5 and 1.00 kg a.i./ha rates of application. In the case of leaves RL_{50} values were 2.88-2.93 days at two rates of application. Dethe ***et*** a/. in 1992 have reported half-life of 4.03 - 4.33 days for iprodione on tomato fruits. So it can be concluded that pesticide does not persist in the environment for a longer period in the tropical and subtropical climates of India.

Table 2. Statistical data on RL_{50}

Treatment (kg a.i./ha)	Regression Equation	RL_{50}	R^2
	Curd, 1st spray		
0.5	Y = 0.4075 - 0.0859X	3.50	0.9932
1.0	Y = 0.6452 - 0.0926X	3.25	0.9914
	Curd, 2nd spray		
0.5	Y = 0.4385 - 0.0877X	3.43	0.9957
1.0	Y = 0.6775 - 0.0970X	3.10	0.9968
	Leaf, 1st spray		
0.5	Y = 0.5911 - 0.1047X	2.88	0.9958
1.0	Y = 0.8736 - 0.1028X	2.93	0.9893

The ADI of iprodione is 0.06 mg/kg body weight (FAO/WHO, 1996) Multiplying ADI with the weight of average Indian 55kg, Maximum Permissible Intake (MPI) is obtained. Multiplying maximum amount of residue detected on cauliflower curds with the amount of vegetable consumed per day per person (Gopal and Mukherjee, 1995) Theoretical Maximum Residue Contribution (TMRC) is obtained. TMRC thus calculated (1.035 mg/person/day) from toxicological data was found to be lower than Maximum Permissible Intake (3.30 mg/person) calculated from

residue data. Therefore, the application of iprodione could be taken to be safe from the crop protection and environmental contamination point of view.

So far no MRL (Maximum Residue Limit) for iprodione on cauliflower has been assigned by FAO although iprodione has been used for the control of the plant diseases of cauliflower. These data along with other international data can be used for the establishment of MRL for iprodione on cauliflower. From the published residue data (FAO/WHO, 1995) TMRC has been calculated which is shown in Table 3.

Table 3. TMRC of iprodione on cauliflower in different countries

Country	Application		Maximum	TMRC
Year	Form	kg a.i./ha	residue (mg/kg)	
Canada,1991	Rovral WP 50	0.75	4.55	1.297
Canada,1990		0.75	2.3	0.655
France, 1986	Kidan	1.25	0.16	0.046
France, 1987		0.75	0.05	0.014
France, 1991	Rovral Aqua Flo	0.75	0.02	0.006
India, 1997	Rovral 50 WP	0.5	2.77	0.789
India, 1997	Rovral 50 WP	1.0	4.58	1.305

Maximum residue of 4.58 mg/kg was found in the Indian trial. This can be rounded up to 5 mg/kg and proposed as MRL of iprodione in cauliflower.

For analytical estimation by IR spectroscopy, N-H peak was chosen because the area and height of the peak varied linearly with the iprodione concentration ranging from 10,000-50,000 ppm. Rovral® 50WP was found to contain 49% iprodione by IR spectroscopy which was confirmed by GLC analysis (Datta, 1997). The IR method has been found to be accurate, precise and rapid when a number of samples are to be analyzed.

From the *in vitro* bioefficacy experiment iprodione has been found to be very effective against all the fungi tested. Its Ed_{50} was found to be 1.34, 1.82 and 5.16 µg/mL against *Alternaria brassicicola, Alternaria brassicae* and *Sclerotium rolfsii,* respectively.

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