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Persistence and Effectiveness of Iprodione Against *Alternaria blight* in Mustard

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Diseases cause considerable loss in terms of yield and quality of oil seed crops. Use of fungicides is effective and economically viable option in order to control fungal pathogens, which cause plant diseases. This is especially true in case of rapeseed mustard, which is adversely affected by *Alternaria blight*, and use of fungicide is the only practical method to control losses, as a suitable resistant donor is not yet available in order to evolve a resistant variety (Shiv Puri et al., 1988). Since fungicides are bioactive xenobiotics, their excessive and indiscriminate use can pose problems for the safety of the consumers. Residues of pesticides have indeed been observed in mustard seed (Mukherjee and Gopal, 1994; Mukherjee and Gopal, 1998; Mukherjee and Gopal, 2000) specially when the pesticides were applied near the maturity period and a minimum waiting period was not allowed before harvesting the crop.

The earlier fungicides belonging to the benzimidazole group were not as effective in controlling *Botrytis cinerea* infestation due to increasing resistance. They are now being substituted by dicarboximide group of fungicide (Pommer and Lorenz, 1995). Iprodione [3-(3,5-dichlorophenyl)-N–isopropyl-2,4-dioxoimidazoline-1-carboxamide [I] belongs to this group and this contact fungicide inhibits the germination of spore and fungal mycelium. It is effective against *Botrytis cinerea*, *Sclerotinia*, *Alternaria*, *Monilia*, *Rhizoctonia solani*, *Phoma*, *Pellicularia sasakii* (Soper and Cox, 1977; Bompeix et al., 1979; Cox et al., 1981; Adas Kaveg and Organia, 1994; Patil et al., 1995; Smith et al., 1995). Iprodione has been found to be very effective against pests of vegetables especially for the control of *Botrytis cinerea* and pathogens of fruits (Sadlo, 2000; Cabras and Angioni, 2000). Three year field trials were carried out to evaluate the bioefficacy of iprodione (Rovral 50 WDP) for controlling *Alternaria blight* and its effectiveness was compared with that of commonly used fungicide mancozeb.

Determination of pesticide residues is necessary to establish the waiting period and to propose the MRL (maximum residue limit) of the pesticide in oilseed crop. There is no literature available on the quantification of the terminal residues of iprodione in mustard crop. Its residues were therefore estimated in leaves and pods periodically and in grains at the time of harvest, along with bioefficacy experiment to evaluate the suitability of the fungicide.

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MATERIALS AND METHODS

Investigations were carried out for three years (1998-2000) during the Rabi season at the farm of Indian Agricultural Research Institute, New Delhi. Mustard (*Brassica juncea,* variety Pusa Bold) was sown in October 1998, 1999 and 2000 for the bioefficacy trial in split plot design with 4 replicates.

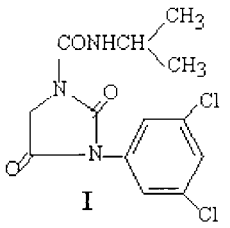
Iprodione (Rovral 50 WP) formulation was applied @ 500 g a.i. ha^{-1} once at the time of early pod stage as recommended. Recommended growing practices were adopted. The crop was grown in an area of 144 m^2 (12 m x 12 m) divided into 12 equal sized quadrates. Similarly in four subplots (3 m x 4m) mancozeb was applied and 4 control plots were not subjected to any fungicidal treatment. The maximum and minimum temperatures during the crop season were 28.2°C and 12.0°C, respectively with relative humidity of 32%. The average sunshine hours recorded was 8.53. There was no rainfall during the period of study.

Five plants from each quadrate were randomly chosen and marked before *Alternaria* infestation, thereby selecting same number of plants every year. The intensity of *Alternaria blight* on both leaf and pod were recorded in 5 plants tagged in each subplot. The mean of percentage of *Alternaria blight* was calculated on both leaf and pod. The yield of mustard seed was also recorded in control as well as in mancozeb and iprodione treated plots in quintal per hectare after harvest each year.

The residue trial experiment was carried out in the year 1999-2000. Periodic samples of leaves and green pods were collected on 0 (one hour after application), 1, 5, 10, 15 days after spray application and grains were collected at harvest. Samples were collected from control plots also, in which no pesticide was sprayed.

M/s Rhone Poulenc, India provided analytical standard (a.i. 92.4%) of iprodione and its formulation Rovral 50WDP. Mustard leaves, green pods and seeds were fortified in triplicate at two concentrations, 0.1 and 1 mg kg^{-1} level for recovery experiments. The samples were extracted, partitioned and cleaned up of co-extractives as given below.

A representative sample of mustard leaves (25 g) and pods (25 g) were extracted with acetone three times (3 x 30 mL) using a Waring blender. The combined organic solvent was concentrated under reduced pressure. The concentrate was transferred to a separatory funnel, saline water (2% W/V, 150 mL) was added and the pesticide was partitioned into dichloromethane (3 x 50 mL) by liquid-liquid extraction. The extract was further cleaned of co-extractives by passing through a glass column packed with sodium sulfate (2 g), neutral alumina (6 g) and sodium sulfate (2 g). The pesticide was eluted with a mixture of hexane -acetone (1:1 V/V, 100mL). The solvent was rotary evaporated under vacuum. Hexane (10mL) was added to the flask and it was again evaporated under vacuum so as to remove



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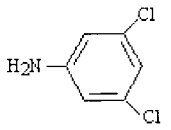

II

Figure. 1. Iprodione (I) and its degradative product 3,5-dichloroaniline (II)

traces of dichloromethane. The concentrate was finally made up in 10mL hexane for estimation.

Harvest time mustard grains (20 g) and pod covers (10 g) were extracted with a mixture of hexane-acetone (1:1, V/V) in a Soxhlet extractor for 6 hours. The solvent was concentrated and the clean up for pod covers was as carried out given above. The clean-up procedure followed for grain samples was as documented by Mukherjee and Gopal (1998). Summarily the extract was concentrated to about 15 mL, and then partitioned into acetonitrile (3 x 50 mL). Saline water (2% W/V, 150 mL) was added to it and the pesticide was further partitioned into hexane (3 x 30 mL). The hexane portion was concentrated to 10 mL before analysis by glc.

In order to prepare major degradative product 3,5-dinitroaniline (II), iprodione (I) was refluxed with aqueous potassium hydroxide (10 %) for 2 h. The reaction was monitored on TLC (Figure 1). When the starting material was used up on TLC, the reaction was worked up by neutralizing the reaction mixture with dilute hydrochloric acid (1 N) and extracting the solution with ethyl acetate. The product II, 3,5-dichloroaniline, was obtained as oil. The identity of the compound was confirmed by IR (ν cm-1 3200), and PMR CDC13(δ) (7.63, dd, J=6HZ, Ar-H), 8.91 (m, Ar-H).

The residues of iprodione were estimated using Hewlett Packard 5890 series II gas liquid chromatograph fitted with an electron capture detector. The megabore column (5 m x 0.53 mm i.d. x 2.65 μm film thickness) used for the analysis was coated with HP1 (methyl silicone gum). The operating temperature conditions were column maintained at 1900C, injector port at 2000C and detector at 2750C. The carrier gas, nitrogen flow was maintained at 32 ml min -1. Iprodione eluted at the retention time of 5.1 min and 3,5-dichloroaniline at 2.35 min. There were no interfering peaks in the region when the control samples were injected.

RESULTS AND DISCUSSION

Three-year bioefficacy trials were carried out and percentage of *Alternaria blight* was recorded in both leaves and pods following application of iprodione and mancozeb. The latter was used as standard for comparison along with the results of the control plot. The percentage incidence of *Alternaria blight* was less on

using both the fungicides as compared to control (Table 1). Iprodione was found to be more effective than the conventional fungicide mancozeb in all the three years of bioefficacy trial as mean incidence of *Alternaria blight* was less in both leaf as well as in pods. Increase in yield of mustard seed in iprodione treated plots ranged from 24-59 percent as compared to that realized from control plots. The information generated proves the effectiveness of the fungicide for usage in rapeseed mustard.

Table 1. Mean incidence of *Alternaria blight* (%) in leaves and pods and yield (q/ha) in field trials

Fungicide	1998-1999			1999-2000			2000-2001		
	Leaf	Pod	Yield	Leaf	Pod	Yield	Leaf	Pod	Yield
Iprodione	10.0	14.0	13.36	10.4	6.66	12.96	19.5	17.7	9.21
Mancozeb	22.8	19.16	11.06	23.4	17.0	10.74	38.4	27.3	10.84
Control	43.8	35.0	8.38	40.0	44.1	8.40	65.3	53.4	7.37
CD 5%	2.2	2.27	1.57	2.46	2.36	1.17	2.45	1.6	1.35

The average recovery of iprodione from mustard leaves and grain was 98 percent at 0.1 and 1 mg kg-1 level of spiking. The identity of the fungicide was confirmed by GC-MS m/e 335.

Iprodione has been analyzed by GLC using NPD detector (Loper and Riba, 1999). Since iprodione contains two chlorine atoms and carbonyl functions, it was envisaged that the compound could be analyzed more efficiently using an electron capture detector. The limit of detection was indeed lowered to 0.01 mg kg-1. The residues of iprodione recorded on mustard leaves and pods at different interval of time and in seed and pod cover at the time of harvest are presented in Table 2. The fungicide was found to dissipate on leaves following first order kinetics. Its residues were 15.68 mg kg-1 in mustard leaves for the recommended dose of spray on 0 day (1 hour after the application) and it the dissipated to 1.59 mg kg-1 in 15 days. The degradation product 3,5–dinitroaniline was quantified only in the harvest samples. The metabolite was however not detected in the harvest grains and pod covers. Residues of iprodione have been estimated on fruits like pears and other greenhouse crops (Loper and Riba, 1999; Brouwer et al., 1997).

The residues of mancozeb were detected in cabbage up to 30 days after application and in tomatoes and eggplant up to 7 days (Kumar and Kumar, 1996). The half-life of mancozeb on different crops has been documented by Brouwer et al. (1997) and is about 3 days.

Although mancozeb is cheaper than iprodione (Kumar and Kumar, 1996). The latter is being preferred as it is more effective and gave higher yield during trials conducted in first two years. The residues of iprodione were detected is pod cover as well as seeds. Its average value was 0.039 and 0.012 mg kg-1, respectively. The residues of the fungicide, iprodione in edible commodity was however less

than the MRL (0.5 mg kg-1) recommended by Codex Alimentarius Commission (1996) proving the safety of the schedule.

Table 2. Persistence of residues of iprodione on mustard leaves and pods

Days of Sampling	Average Residues in Leaves * (mg kg^{-1})	Average Residues in Pods * (mg kg^{-1})
0	15.68	13.23
1	8.83	7.47
5	6.03	5.08
7	3.57	2.16
10	1.59	1.02
Harvest time Pod cover		0.039
Harvest time grains		0.012
Regression Equation	Y =3.10 - 0.059 X	Y =3.03 - 0.069 X
R^2	0.9640	0.9788
Half life RL_{50} (days)	6.02	5.00

*Average of three replicates

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REFERENCES

Adas Kaveg JE, Organia JM (1994) Penetration of iprodione into mesccarp fruit tissue and suppression of gray mold and brown rot of sweet cherries. Plant Dis. 78: 293-296.

Bompeix G, Saindrenan P, Mourichon X, Chalandon A (1979) Control of various storage fungi of apples and peaches by post harvest dipping in iprodione or Aluminium tri (ethyl phosphonate). Proc. Brighton Crop Protection Conference Pest Dis Brighton, Proc. Brighton 1: 87-94.

Brouwer DH, Haan M de, Leenheers L H, Vreede SAF (1997) Half -lives of pesticides on Greenhouse Crops. Bull Environ Contam Toxicol 58: 976-984.

Cabras P, Angioni A (2000) Pesticide residues in grapes, wine and their processing products. J Agric Food Chem 48: 967-973.

FAO / WHO (1996) Codex Alimentarius Commission. Codex Committee on Pesticide Residues, 28th Session, 15-20 April 1996. Cx/PR96/5 Part A1-88. The Hague, The Netherlands, 1996

Cox TW, Swash D, Paviot J (1981) The control of Alternaria brassicae and Sclerotinia sclerotiorum on oil seed Rape with iprodione. Proc. Brighton Crop Protection Conference - Pests Dis Brighton 2:513-520.

Datta A, Gopal M (1999) Safety evaluation of the fungicide iprodione on cauliflower (*Brassica oleracea* var. botrytis L.). Bull Environ Contam Toxicol 62: 496-501.

Datta A, Prasad S, Singh R, Gopal M (2001) Persistence of iprodione residues on cruciferous crops. Ann Plant Protec Sci 9: 169-170.

Kumar A, Kumar A (1996) Efficacy of different fungicides against Alternaria blight, white rust and staghead infection of mustard. Plant Dis Res 11: 174-177.

Loper M I, Riba M (1999) Residue levels of ethoxyquin, imazalil and iprodione in pears under cold storage conditions. J Assoc Anal Chem Int 82: 1229-1239.

Mukherjee I, Gopal M (1994). Methodology for the estimation of chlorothalonil and its metabolite in mustard crop by gas liquid chromatography. Fresenius J Anal Chem 351: 590-591.

Mukherjee I, Gopal M (1998) Persistence behavoiur of organochlorine and organophosphorus pesticides on mustard crop. Indian J Plant Protec 26: 138-144.

Mukherjee I, Gopal M (2000) Environmental behaviour and translocation of imidacloprid in eggplant, cabbage and mustard. Pest Manage Sci 56: 932-936.

Patil PV, Hiremath PC, Hegde RK (1995). In vitro evaluation of different fungicides against *Alternative temuissima* (Kunze Fr.) w shire, incidence of leaf blight of groundnut. Current-Research University of Agricultural Sciences Bangalore 19:123-124.

Pommer EH, Lorenz G (1995). Dicorboximide fungicides. In H. Lyx (Ed.) Modern selective fungicide. Gustav Fisher Verlag, Jena, Germany. 99-118pp. and *Sclerotinia sclerotiorum* on oil seed Rape with iprodione. Proc Brighton Crop Protection Conference - Pests Dis Brighton 2: 513-520.

Sadlo S (2000) Disappearance of iprodione deposits on peppers, eggplant and tomato. J Plant Protec Res 40: 173-178.

Sharma ID, Nath A , Dubey JK (1994) Persistence of mancozeb (Dithane-45) in some vegetables and efficacy of decontaminations process. J Food Sci Technology -Mysore, 31: 215-218.

Shiv Puri A, Siradhana BS, Bansal RK (1988) Management of *Alternaria blight* of mustard with fungicides. Indian Phytopathol 41:644-646.

Smith FD, Phipps DM, Stipes RJ, Brenneman TB (1995) Significance of insensitivity of Sclerotinia minor to iprodione in control of *Sclerotinia blight* of peanut. Plant Dis 79: 517-523.

Soper D, Cox TW (1977) The use of iprodione for the control of Botrytis in glasshouse tomatoes and lettuce. Proc Brighton Crop Protection Conference - Pests Dis Brighton 2: 315-325.