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# Assessment of Iprovalicarb, a Systemic Fungicide in/on Cabbage (*Brassica oleracea var. capitata*)

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**Abstract** Iprovalicarb is a systemic fungicide and has high biological activity with particular efficacy against downy mildew. Iprovalicarb has an excellent toxicological and ecotoxicological profile. It has excellent fungicidal activity against *Plasmopara viticola*, *Peronospora vicia*, *Phytophthora* sp, *Alternaria* sp in grapes, potatoes, tomatoes, tobacco and vegetables. Iprovalicarb (Melody 50 W) was applied as a foliar spray on cabbage at the recommended dose, 300 g a.i $ha^{-1}$ and double the recommended dose, 600 g a.i $ha^{-1}$ at 50% head formation stage. Two application of each dose was made after an interval of 15 days. Residues of iprovalicarb in cabbage samples were analyzed by high performance liquid chromatography. It was detectable up to 15 days in both cabbage head and leaves at both the doses of application, after first and second applications. The dissipation model yielded rate constants 0.1157, 0.1121, 0.1170, 0.1114 $day^{-1}$ on cabbage heads after first and second applications at the recommended and double the recommended dose of application, respectively. The residual half-lives on cabbage heads and leaves were varied between 2.6–2.7 and 2.5–2.8 days, respectively. This suggested that dissipation was independent of initial doses and followed first order kinetics. The projected Theoretical Maximum Residue Contribution of iprovalicarb after first and second application was found to be lower than calculated Maximum Permissible Intake.

**Keywords** Iprovalicarb · Fungicide · Cabbage · Dissipation

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Iprovalicarb, {isopropyl-2-methyl-1-{[(RS)-1-p-tolyethyl] carbamoyl}-(S)-propyl carbamate (I, Fig. 1) belongs to a new class of antifungal compounds. The active substance constitutes of three units, isopropyloxycarbonyl, valine, the natural amino acid and 2-(4-methylphenyl) ethylamine. It is mixture of two diastereoisomers in 1:1 ratio. Its systemic action has protective, curative and eradicative effects against a large number of oomycetes. It has high biological activity with particular efficacy against downy mildew. Iprovalicarb has an excellent toxicological and ecotoxicological profile and is used at low application rates. It degrades rapidly and does not harm beneficial organisms. It has excellent fungicidal activity against *Plasmopara viticola*, *Peronospora vicia*, *Phytophthora* sp, *Alternaria* sp in grapes, potatoes, tomatoes, tobacco and vegetables (Dutzmann 1999; Jende et al. 1999).

The metabolic behavior of [phenyl-UL-14C] iprovalicarb was investigated in grapes under simulated field conditions (Babczinski and Thomas 1999). The parent compound metabolizes to a small degree in and on plant, and the active substance was the significant residual constituent. The cleavage of the amide bond between the L-valine and 2-(4-methylphenyl) ethylamine leads to formation of minute quantities of 2-(4-methylphenyl) ethylamine (0.001 μg $g^{-1}$). The rate of degradation on plants was, however, very low. The other metabolites detected in trace amounts were the aglycone Ω-hydroxy iprovalicarb, glucoside of Ω-hydroxy iprovalicarb, 3-hydroxy-improvalicarb, 3-hydroxy-iprovalicarb glucoside. Hennebole and Bornatsch (1997) and Hennebole (1997) investigated the degradation of iprovalicarb in the laboratory in four different soils. The active substance rapidly degraded or was firmly incorporated into the soil matrix. The active substance applied disappeared within 2–30 days (RL50) to half of the initial amount depending on the soil type. A series of

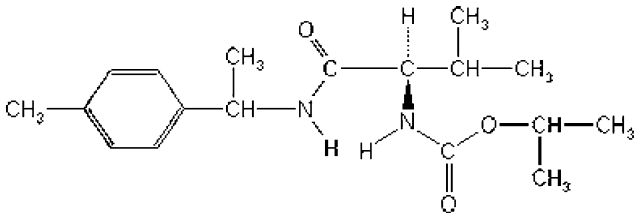

**Fig. 1** Iprovalicarb

degradation products were found in the experiment. One of these pathways led to the formation of 2-(4-methylphenyl) ethyl amine by cleavage of the molecule while another led to iprovalicarb-carboxylic acid and finally to tere-phthalic acid by oxidation of the methyl group on the aromatic ring. The main degradation product was $CO_2$ with up to 60 percent of the active substance used within 100 days.

The method of determination of residues of iprovalicarb documented in literature is by liquid chromatography—mass spectroscopy in the selected ion-recording mode (SIR) using an electrospray interface (Hennebole 1997; Brennecke 2000). There is not much information available on the method of determination of iprovalicarb residues in crops. This paper presents the behavior of iprovalicarb in/on cabbage (*Brassica oleracea var. capitata*).

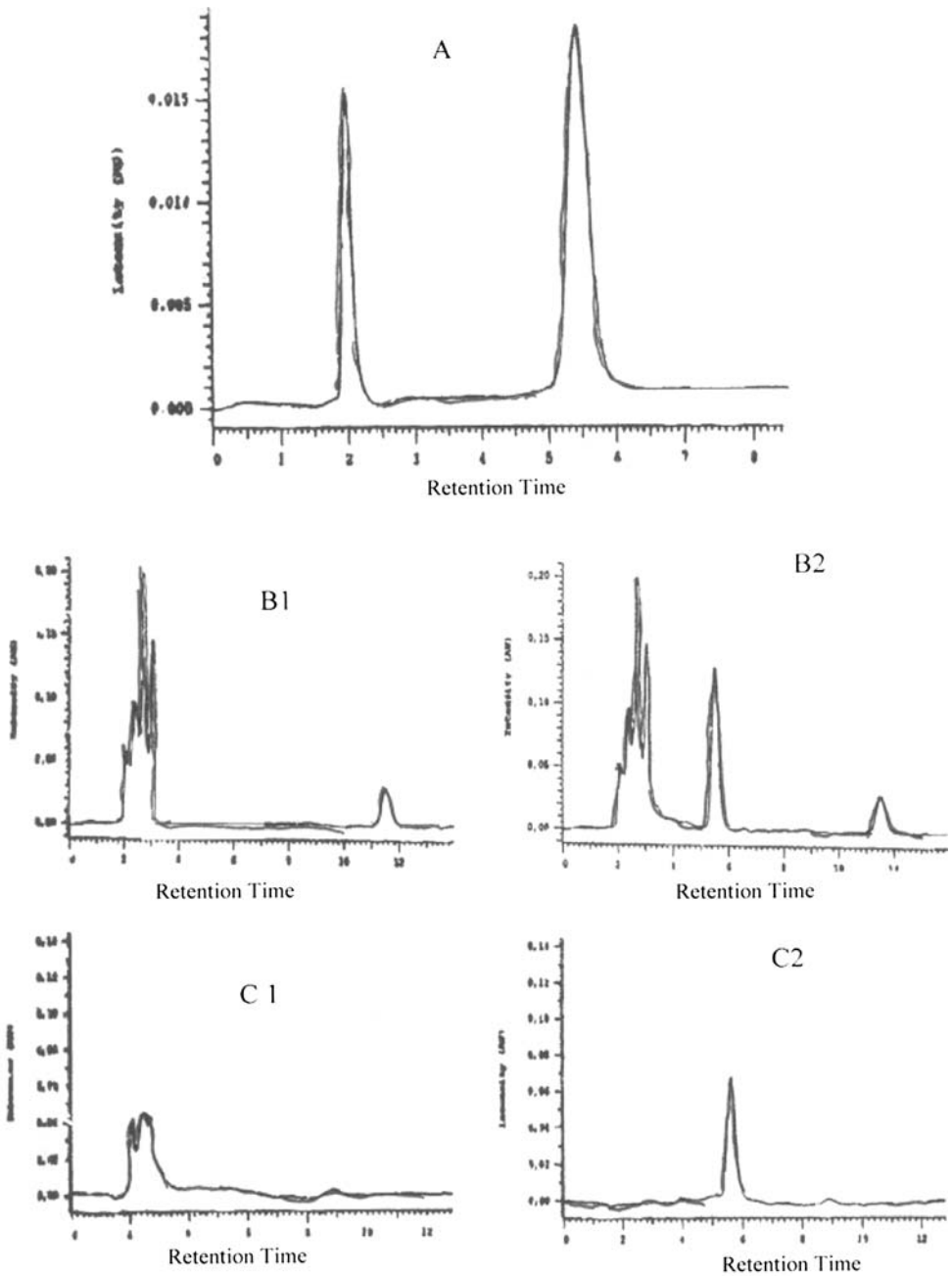


**Fig. 2** HPLC profile of iprovalicarb, **a** standard; **b1** from cabbage heads untreated; **b2** treated cabbage heads; **c1** cabbage leaves untreated; **c2** treated cabbage leaves

## Materials and Methods

Iprovalicarb technical (mp 163–165° and 98.7 per cent pure) was provided *gratis* by Bayer India Ltd (New Delhi, India). It was re-crystallized from methanol to obtain the analytical standard. NMR and IR spectroscopy confirmed the identity of the compound. Acetone, dichloromethane and hexane were obtained from S.D—Fine Chemicals Ltd, Mumbai, India. The solvents were distilled prior use, methanol (HPLC grade), neutral alumina—Brockmann grade-I HPLC system (Merck-Hitachi)—Consisting of a L-7100 (computer operated dual pump), a L-7400 (UV detector) and a L-7200 (Auto sampler), HPLC column (30 cm)—Lichrospher, RP-18 (5 μm) was used for analysis. Spectrophotometer—Varian, Series 634, UV–VIS double beam was used for determining the $\lambda_{max}$. A mixture of methanol–water (75:25, v/v) was used as the mobile phase, with a flow rate of 1.0 mL $min^{-1}$. The injection volume was 10 μL and the wavelength was set at 215 nm ($\lambda_{max,}$ determined by using spectrophotometer).

Field experiments were carried out in the field of the Division of Agronomy, Indian Agricultural Research Institute, New Delhi, during the Rabi season of 2000–2001. Cabbage (*Brassica oleracea var. capitata*), variety 'Golden Acre' was grown in the field to study the persistence of iprovalicarb. Nursery ($1 \times 1$ $m^2$) was solarized (using 1 mm polythene sheet in May–June) and was drenched (with captan) before sowing. Thirty days old seedlings were transplanted in the main plots ($3 \times 3$ $m^2$). Field sanitation and other agronomic practices were followed according to the recommended package of practices in the region. The fungicide was sprayed at recommended dose (300 g a.i $ha^{-1}$) and double the recommended dose (600 g a.i $ha^{-1}$) in 750 L water, using a knap-sack sprayer. A second application was made at 300 and 600 g a.i $ha^{-1}$, 15 days after the first application in the same season to validate the results. Three replicate for each field treatment was laid. A separate set of control experiment was laid, where no pesticide was applied. The average maximum and minimum temperature during the period of experiment were 28.04 and 12.09°C, respectively, with a mean of 20.06°C. The average relative humidity, sunshine hour and wind speed were 58.11%, 7.09 h and 1.40 km per hour, respectively. The total rainfall during the course of study was 12 mm.

A representative of about 2,000 g cabbage (including heads and leaves) were collected from each replicate of all the treatments on 0 (1 h after spraying), 1, 3, 5, 7, 10 and 15 days after spraying. Controls samples were also collected on the same day. During sampling, every precaution was taken so as to get a representative sample. Fifty grams of sub samples of both cabbage head and leaves were taken by repeated quartering. Fifty grams of representative sample of cabbage head and leaves taken separately, and were extracted with 250 mL (100 + 75 + 75) acetone in a homogenizer. The extract was filtered with suction. The combined extract was evaporated to aqueous concentrate (5 mL) using a rotary vacuum evaporator. The aqueous concentrate was transferred to a separatory funnel and 150 mL of sodium chloride solution (10%) was added to it. The aqueous portion was partitioned with dichloromethane ($3 \times 30$ mL). The combined organic layers were evaporated to dryness using a rotary vacuum evaporator and the residue obtained was dissolved in 5 mL 1:1 (v/v) acetone–

**Table 1** Concentration of iprovalicarb in/on cabbage head after first application

| Days | Treatment (g a.i $ha^{-1}$) | Residues (μg $g^{-1}$) | | | Average residues (μg $g^{-1}$) (SD) | Percent dissipation |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 300 | 3.07 | 3.07 | 3.09 | 3.08 (0.01) | – |
| | 600 | 6.79 | 6.25 | 6.35 | 6.46 (0.29) | – |
| 1 | 300 | 2.58 | 2.37 | 2.17 | 2.37 (0.21) | 23.05 |
| | 600 | 3.59 | 4.19 | 3.99 | 3.92 (0.31) | 39.32 |
| 3 | 300 | 1.64 | 1.40 | 1.16 | 1.40 (0.24) | 54.55 |
| | 600 | 3.04 | 2.90 | 3.19 | 3.04 (0.15) | 52.94 |
| 5 | 300 | 0.77 | 0.68 | 0.66 | 0.70 (0.06) | 77.25 |
| | 600 | 1.41 | 1.17 | 1.65 | 1.41 (0.24) | 78.17 |
| 7 | 300 | 0.40 | 0.39 | 0.41 | 0.40 (0.01) | 87.01 |
| | 600 | 0.85 | 1.07 | 0.60 | 0.84 (0.24) | 87.00 |
| 10 | 300 | 0.29 | 0.36 | 0.21 | 0.29 (0.08) | 90.58 |
| | 600 | 0.41 | 0.30 | 0.52 | 0.41 (0.11) | 93.65 |
| 15 | 300 | 0.05 | 0.06 | 0.05 | 0.05 (0.01) | 98.38 |
| | 600 | 0.12 | 0.17 | 0.11 | 0.13 (0.03) | 97.99 |

hexane mixture. The concentrate was subjected to clean up. A glass chromatographic column (1.5 i.d × 150 cm) was packed with neutral alumina (2 g) sandwiched between two layers of activated anhydrous sodium sulfate (2 g). The concentrate was transferred to the column, which was pre-washed with hexane (25 mL). The column was eluted with 150 mL 1:1 (v/v) acetone–hexane mixture. The eluate was concentrated to dryness using a rota-vapor and the residue was dissolved in 10 mL HPLC grade methanol before analysis.

## Results and Discussions

The percent iprovalicarb recovered from cabbage head and leaves was more than 70 per cent with a limit of quantitation (LOQ) of 0.1 μg $g^{-1}$ (Maity and Mukherjee 2004). The HPLC profile of the iprovalicarb standard, untreated cabbage heads and leaves and treated cabbage head and leaves is depicted in Fig. 2. The highest recovery was obtained from both from cabbage head and leaves when extracted with acetone, followed by liquid–liquid partitioning by dichloromethane and column clean up over neutral alumina as adsorbent and hexane–acetone used as the solvent, for and eluting solvent. The data on the persistence of iprovalicarb in/on cabbage heads after application of the fungicide at 300 and 600 g a.i $ha^{-1}$ are presented in Tables 1 and 2. It is evident from the data (Tables 1, 2; Fig. 3) that residues of iprovalicarb in/on cabbage head, decreased progressively with time. After the first spray at the recommended and double the recommended dose, the initial deposits on the cabbage heads (1 h after application) were 3.08 and 6.46 μg $g^{-1}$, respectively. The initial deposits after second spray were 3.75 and 7.20 μg $g^{-1}$ for normal and double dose of application, respectively. Residues in/on cabbage heads after the first application on day 1 day were 2.37 and

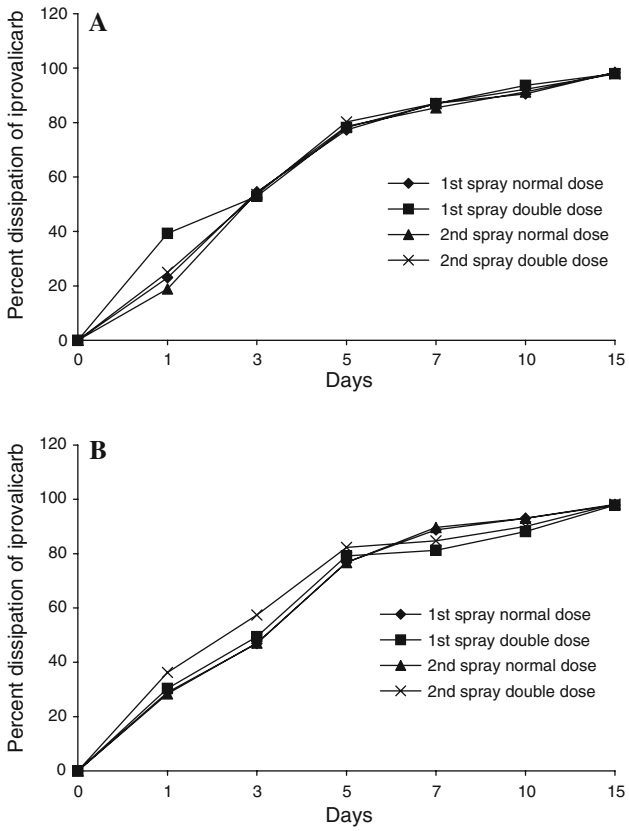


**Fig. 3** **a** Dissipation of iprovalicarb from cabbage head; **b** dissipation of iprovalicarb from cabbage leaves

**Table 2** Concentration of iprovalicarb in/on cabbage head after second application

| Days | Treatment (g a.i $ha^{-1}$) | Residues (μg $g^{-1}$) | | | Average residues (μg $g^{-1}$) (SD) | Percent dissipation |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 300 | 3.75 | 3.91 | 3.59 | 3.75 (0.16) | – |
| | 600 | 7.39 | 7.06 | 7.16 | 7.20 (0.17) | – |
| 1 | 300 | 3.18 | 2.98 | 2.96 | 3.04 (0.12) | 18.93 |
| | 600 | 5.55 | 5.33 | 5.32 | 5.40 (0.13) | 25.00 |
| 3 | 300 | 1.66 | 1.71 | 1.77 | 1.71 (0.06) | 54.31 |
| | 600 | 3.37 | 3.64 | 3.01 | 3.34 (0.32) | 53.61 |
| 5 | 300 | 0.86 | 0.80 | 0.74 | 0.80 (0.06) | 78.67 |
| | 600 | 1.49 | 1.42 | 1.35 | 1.42 (0.07) | 80.28 |
| 7 | 300 | 0.48 | 0.55 | 0.61 | 0.55 (0.07) | 85.42 |
| | 600 | 1.10 | 0.91 | 0.79 | 0.93 (0.16) | 87.08 |
| 10 | 300 | 0.33 | 0.36 | 0.29 | 0.33 (0.04) | 91.29 |
| | 600 | 0.56 | 0.52 | 0.60 | 0.56 (0.04) | 92.22 |
| 15 | 300 | 0.06 | 0.06 | 0.06 | 0.06 (0.00) | 98.40 |
| | 600 | 0.15 | 0.16 | 0.15 | 0.15 (0.01) | 97.92 |

3.92 μg $g^{-1}$, respectively, and 3.04 and 5.40 μg $g^{-1}$ after second spray, respectively, at the two applications rates. The residues of iprovalicarb were 21.75 and 28.27% higher on cabbage heads in the second spray as compared to the first spray on day-0 and day-1, respectively, for the recommended dose of application. Slightly higher initial residues after second spray could be attributed to the presence of residues left from the previous spray. The initial residues dissipated fast, till day-3 on cabbage heads, recording 53.61 per cent dissipation (Table 1; Fig. 4), it was followed by slow dissipation until day-15 of 97.9 per cent (Table 3). The data on persistence of the fungicide in/on cabbage leaves are presented in Tables 4 and 5. The initial deposits on leaves were 11.54 and 20.49 μg $g^{-1}$ for normal and double dose of application after first spray, respectively. The residues declined to 8.20 and 14.27 μg $g^{-1}$ on day-1 after application, recording a dissipation of 29.0 and 30.37% in the recommended and double dose of application. The day-0 residues for the second spray were 11.98 and 23.95 μg $g^{-1}$, respectively, for the normal and double dose of spray application. Comparisons of the amount of residues in/on cabbage heads and leaves on different sampling days of different treatments are given in Fig. 4. The figures indicate that the initial deposits on the leaf surface were much higher as compared to cabbage heads. Iprovalicarb deposit on leaves was about 3.75 and 3.17 times more than on cabbage heads at the normal and double dose of application, respectively, after the first spray on day-0. On an average cabbage leaves recorded 3.26 times higher residues on day-0 (Fig. 4) as compared to cabbage heads at both the application rates after the second spray.

This may be attributed to the fact that during the initial spray, leaves cover the cabbage head, which opens up gradually. This result in a reduction of pesticide residue recorded in/on cabbage head as compared to leaves. Data presented in Tables 1 and 2 and Fig. 3 indicates that iprovalicarb dissipated within 10 days in/on cabbage heads to about 90 to 93 per cent. A similar dissipation trend was observed in/on leaves also (Tables 3, 4; Fig. 4). The residues of iprovalicarb at normal and double dose of application dissipated at different rates during the initial stages after fungicide application. Initially, residues of double dose of application dissipated at faster rate as compared to the normal dose. Residues of iprovalicarb dissipated slowly from day-5 onwards. Similar observations were recorded at both the concentrations of applications. The percent remaining on cabbage heads after first spray was about 1.62 and 2.01 by day-15 which was

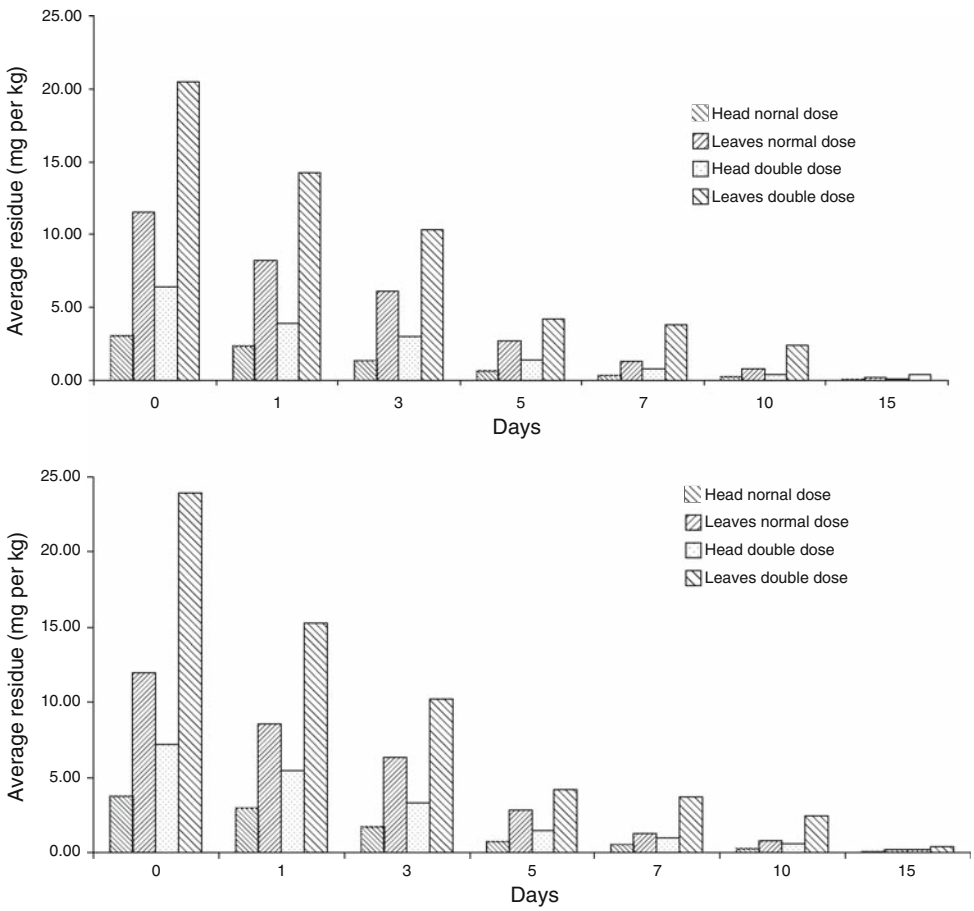


**Fig. 4** **a** Comparison of iprovalicarb in/on cabbage head and leaves (first application) **b** Comparison of iprovalicarb in/on cabbage head and leaves (second application)

**Table 3** Concentration of iprovalicarb in/on cabbage leaves after first application

| Days | Treatment (g a.i ha$^{-1}$) | Residues (μg g$^{-1}$) | | | Average residues (μg g$^{-1}$) (SD) | Percent dissipation |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 300 | 12.64 | 10.25 | 11.74 | 11.54 (1.21) | – |
| | 600 | 19.64 | 21.33 | 20.66 | 20.49 (0.94) | – |
| 1 | 300 | 7.43 | 8.49 | 8.67 | 8.20 (0.67) | 28.97 |
| | 600 | 14.70 | 14.54 | 13.56 | 14.27 (0.62) | 30.37 |
| 3 | 300 | 6.14 | 5.99 | 6.24 | 6.12 (0.13) | 46.94 |
| | 600 | 10.34 | 9.33 | 11.40 | 10.36 (1.04) | 49.46 |
| 5 | 300 | 2.86 | 2.98 | 2.18 | 2.67 (0.43) | 76.83 |
| | 600 | 4.58 | 3.37 | 4.84 | 4.26 (0.78) | 79.19 |
| 7 | 300 | 1.09 | 1.77 | 1.03 | 1.30 (0.41) | 88.76 |
| | 600 | 3.92 | 3.34 | 4.28 | 3.85 (0.47) | 81.23 |
| 10 | 300 | 0.97 | 0.87 | 0.58 | 0.81 (0.20) | 93.01 |
| | 600 | 2.43 | 2.33 | 2.53 | 2.43 (0.10) | 88.14 |
| 15 | 300 | 0.21 | 0.25 | 0.28 | 0.25 (0.04) | 97.86 |
| | 600 | 0.37 | 0.44 | 0.51 | 0.44 (0.07) | 97.85 |

**Table 4** Concentration of iprovalicarb in/on cabbage leaves after second application

| Days | Treatment (g a.i ha$^{-1}$) | Residues (μg g$^{-1}$) | | | Average residues (μg g$^{-1}$) (SD) | Percent dissipation |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | | |
| 0 | 300 | 13.49 | 10.41 | 12.05 | 11.98 (1.54) | – |
| | 600 | 25.56 | 22.62 | 23.67 | 23.95 (1.49) | – |
| 1 | 300 | 8.88 | 8.23 | 8.62 | 8.58 (0.33) | 28.38 |
| | 600 | 12.09 | 20.55 | 13.04 | 15.26 (4.60) | 36.28 |
| 3 | 300 | 7.09 | 6.20 | 5.74 | 6.34 (0.69) | 47.08 |
| | 600 | 10.70 | 9.52 | 10.42 | 10.21 (0.62) | 57.37 |
| 5 | 300 | 2.99 | 2.53 | 2.83 | 2.78 (0.23) | 76.79 |
| | 600 | 4.35 | 3.56 | 4.78 | 4.23 (0.62) | 82.34 |
| 7 | 300 | 1.35 | 1.21 | 1.16 | 1.24 (0.09) | 89.65 |
| | 600 | 3.59 | 3.55 | 3.83 | 3.66 (0.10) | 84.72 |
| 10 | 300 | 0.88 | 0.75 | 0.85 | 0.83 (0.07) | 93.07 |
| | 600 | 2.36 | 2.56 | 2.24 | 2.39 (0.16) | 90.02 |
| 15 | 300 | 0.25 | 0.23 | 0.18 | 0.22 (0.04) | 98.16 |
| | 600 | 0.44 | 0.44 | 0.42 | 0.43 (0.01) | 98.20 |

**Table 5** Theoretical dissipation models for iprovalicarb after foliar treatment

| Spray | Treatment (g a.i ha$^{-1}$) | Substrate | Regression equation | Half life (days) | Correlation coefficient ($r^2$) |
|---|---|---|---|---|---|
| First | 300 | Head | $Y = 3.4805 - 0.1157X$ | 2.60 | −0.987 |
| First | 600 | Head | $Y = 3.7549 - 0.1121X$ | 2.69 | −0.992 |
| First | 300 | Leaves | $Y = 4.0328 - 0.1127X$ | 2.67 | −0.985 |
| First | 600 | Leaves | $Y = 4.2898 - 0.1050X$ | 2.87 | −0.974 |
| Second | 300 | Head | $Y = 3.5752 - 0.1170X$ | 2.57 | −0.990 |
| Second | 600 | Head | $Y = .8182 - 0.1140X$ | 2.70 | −0.989 |
| Second | 300 | Leaves | $Y = 4.0588 - 0.1170X$ | 2.57 | −0.985 |
| Second | 600 | Leaves | $Y = 4.3197 - 0.1089X$ | 2.76 | −0.975 |

comparable with that observed in second spray recording 1.60 and 2.08% remaining, at the recommended and double the recommended dose of application (Tables 1, 2; Fig. 4). On cabbage leaves, a similar trend was observed in percent of iprovalicarb remaining (Fig. 4) at both the concentrations of spray by day-15.

The half-life (RL50) of iprovalicarb on cabbage heads and leaves was about 2.7 days at both the treatments and at both the spray application. Half-life of 2.60 and 2.69 days were recorded for single dose and double dose of application, respectively, on cabbage heads after first spray as compared to 2.57 and 2.70 days in second spray. On leaves the half-life (RL50) were 2.67 and 2.87 days for first spray and 2.57 and 2.76 days for second spray at the recommended and double dose of application, respectively (Table 5). The theoretical dissipation models for iprovalicarb through regression between time of application of the fungicide and corresponding residues in cabbage leaves and head yielded first order kinetics (Table 5). The dissipation models yielded rate constants—day of 0.1157, 0.1121, 0.1121 and 0.1127, after first application at the recommended and double the recommended dose in cabbage head and leaves, respectively. The corresponding to half-lives of 2.60, 2.69, 2.67 and 2.87 days suggests that the dissipation was independent of initial dose of iprovalicarb and confirmed first order kinetics. The theoretical concentration of residues at zero day, i.e., initial concentration were calculated as 3.02 and 5.68 μg $g^{-1}$ which do not differ substantially from the observed value of 3.08 and 6.46 μg $g^{-1}$ (Table 3). Therefore mathematical expressions described the observed persistence of iprovalicarb satisfactorily and hence can be used to predict residues at a particular time. The prescribed acceptable intake (ADI) of iprovalicarb is 0.013 mg $kg^{-1}$ body weight. The Maximum Permissible Intake (MPI) for a person weighing 55 kg was calculated and found to be 0.715 mg $person^{-1}$ $day^{-1}$. Scrutiny of the residue data revealed that maximum concentration was present in 0—old samples and accordingly Theoretical Maximum Residue Contribution (TMRC, daily consumption of food commodity residue in mg $kg^{-1}$ were calculated to be 0.123 and 0.150 mg $person^{-1}$ $day^{-1}$, respectively, for the recommended dose after first and second application, assuming that cabbage heads containing maximum residues were consumed as vegetable recommended (40 g) thus the projected TMRC from residue data was found to be lower than (Maximum Permissible Intake (MPI), 0.715 mg $person^{-1}$ $day^{-1}$), calculated from the toxicological data. Therefore two applications of iprovalicarb at both intervals could be taken to be safe from the risk to consumers and environmental contamination point of view.

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