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# Dissipation and Risk Assessment of Combi Product of Mancozeb and Carbendazim on Apple at Different Locations in North India

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In supervised field trials conducted jointly at five locations of North India to evaluate the dissipation pattern and risk assessment of combi product SAAF (carbendazim12%and mancozeb 63%) on apple fruits following foliar application of mixed formulation (carbendazim 120 and mancozeb 630 g ai $ha^{-1}$) fungicide at recommended and double the recommended (240 +1260 g a.i. $ha^{-1}$) doses, the residues of the two fungicides dissipated to reach below the detection limits at 15 and 25 days respectively. Average initial deposits ranged from 1.161 to 1.319 and 2.078 to 2.245 mg $kg^{-1}$for carbendazim and 3.074 to 3.434 and 5.430 to 6.073 mg $kg^{-1}$for mancozeb at the respective doses. No residues were not detected in mature fruits at all the five locations. Dissipation followed concentration dependent first order kinetics. The half life of mancozeb and carbendazim varied between 2.82-3.15 and 1.88-2.38 days, respectively. TMRC values were below the MPI in apple fruit and hence, the fungicide will not cause any adverse effect on consumption of fruits.

**Key words:** Carbendazim, mancozeb, persistence, half life, risk assessment, apple

Apple (*Malus domestica*) is the most important and fourth among the most widely produced temperate fruits in the world after orange, grapes and banana[1]. In India, it is commercially grown in Kashmir, hills of Uttar Pradesh and Himachal Pradesh. Apples are mostly consumed as fresh fruits though little production is processed to make jam, jellies, juices, canned slices and candies. India ranks fifth in apple production over the world[1,2].

Apple is attacked by a wide range of pests and diseases which at worst can reduce the crop to zero, and damage or even kill the tree. Among the diseases, apple scab, sooty blotch, premature leaf fall and Anthracnose canker are some of the major ones which can destroy the whole crop and eventually kill a tree[3]. To manage such diseases it becomes important that the crop should receive frequent application of a variety of fungicides viz., mancozeb, difenoconazole, carbendazim, thiophanate methyl, etc.[4], right from green tip stage to fruit harvest.

Apple alone consumes one third of total use of pesticides in agriculture/horticulture in Himachal Pradesh. As the fruits make the Indian diet more nutritive and are consumed fresh, they may carry the toxic levels of pesticide residues[5].Carbendazim and mancozeb are the most prominent fungicides used on apple throughout the world[6, 7]. Carbendazim (methyl benzimidazol-2-ylcarbamate), widely used in crop production, inhibits mitosis in fungi and is a broad-spectrum benzimidazole fungicide and a metabolite of benomyl with protective and curative action. Whereas, mancozeb, ethylene bis dithiocarbamate, a broad spectrum, non-systemic, contact fungicide with protective action, acts by disrupting lipid metabolism and has multi-site activity[8].Both fungicides are used to control plant diseases in cereals and fruits, including citrus, banana, strawberry, pineapple and pome.

In India, a combi formulation of carbendazim and mancozeb has been approved under Insecticide Act 1968 by the Registration Committee of Central Insecticide Board. It may become the choice of farmers to combat disease incidence. The persistence of the individual parent molecule has been evaluated and recommended on apple[5,9,10]. However, their behaviour as mixed formulation has been evaluated on mango in India[11] but not on apple so far. Therefore, keeping in view their joint/interactive role on plant

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factors, the present investigation was carried out to study the residual behaviour and risk assessment of the mixed formulation of carbendazim and mancozeb on apple fruits at different locations in North India.

## MATERIALS AND METHODS

### Chemicals and reagents

Reference analytical standards of carbendazim (purity 98.50 %) and mancozeb (purity 99.50 %), and the commercial formulation (SAAF; carbendazim 12 %+mancozeb 63 %) were supplied by United Phosphorus Ltd, Mumbai, India. All the solvents were of HPLC grade and chemicals of analytical grade, and were purchased from Merck (Mumbai, India). Carbon disulphide ($CS_2$), methanol, stannous chloride ($SnCl_2$), lead acetate, potassium hydroxide (KOH), sulphuric acid ($H_2SO_4$), hydrochloric acid (HCl), magnesium sulphate ($MgSO_4$), sodium chloride (NaCl), sodium sulphate ($Na_2SO_4$) were purchased from Merck and primary secondary amine(PSA) from Agilent Technologies.

### Preparation of standard solutions

A stock standard carbon disulphide ($CS_2$) solution containing 50 mg $CS_2$ in methanol (50mL) was prepared and working standard solutions were prepared by making suitable dilutions of stock solution in methanol. Stock solution (1000 mg $L^{-1}$) of carbendazim was prepared in HPLC grade acetonitrile by dissolving accurately weighed 25 mg of pure analyte into a 25 mL volumetric flask and making up the volume. Working standard solutions were prepared by serial dilution and used in sample spiking. All standard solutions were stored at -4°C and brought to room temperature before use.

### Field trials

**Lay out and sampling.** Field trials to study the dissipation pattern of SAAF were conducted at two locations of Himachal Pradesh (HP) viz., Matiana, Shimla (Location I) and Thanedhar, Shimla (Location II). However, harvest time residues of the fungicide were additionally investigated simultaneously in the apple and soil samples from three other locations of trials *viz*. Mashobra, Shimla (HP) (LocationIII), Kalpa, Kinnaur (HP) (Location IV) and one location of Jammu and Kashmir viz.,Youngora, Ganderbal (Location V). The combi formulation consisting of carbendazim 12 %+ mancozeb 63 % (SAAF 75 WP) was applied at the rate of 0.25% (X) and 0.50% (2X) by application of the recommended (120+630 g a.i. $ha^{-1}$) and double the recommended dose (240+1260 g a.i. $ha^{-1}$), respectively. Each application was sprayed two times at an interval of 10-15 days at fruiting stage by using a high volume power sprayer using 1000 L of spray fluid per hectare. A control plot of the same size was maintained and sprayed with water alone (size refers to 2 trees per replication). There were three replications per treatment. It was ensured that the test fungicides had not been used earlier in the experimental plots. The average minimum and maximum temperatures during the period of investigations ranged between 11.91-15.32°C and 22.03-29.35°C whereas the average relative humidity ranged between 61.70–77.63 % and the rainfall from 0.75–336 mm at the five locations.

Two kg of apple fruit samples were collected randomly from each treatment separately at 0 (2 h after application), 1, 3, 7, 10, 15, 20, 25 and 30 (at harvest) days after the second application of the fungicide mixture from both locations I and II. However, in locations III, IV and V, fruits were sampled at harvest i.e. at 34, 49 and 30 days, respectively post second application. These variations were due to different agroclimatic conditions. The soil samples (2kg) were collected at harvest from all the five locations. All the samples were transported to the laboratory and processed immediately.

**Mancozeb extraction and clean up.** The residues of mancozeb were analyzed as per the method of Dubey and Stan[12]. A 100g homogenized sample (fruit or dried, sieved soil sample) was taken separately (replication and treatment wise) in a three-necked round-bottom flask containing 200mL distilled water. The flask was refluxed on a heating mantle and when the content in flask started boiling, 20 mL of 40% $SnCl_2$ in HCl and 20 mL conc. HCl were added using dropping funnel. The contents were boiled for 40 minutes. The liberated carbon disulphide was passed through two traps. First trap contained 20 mL 30% lead acetate trihydrate solution and 2nd trap contained 15 mL conc. $H_2SO_4$. Finally carbon disulphide was trapped in 3rd trap (U-tube) containing 25 mL 0.5 M methanolic KOH. The content of U tube was transferred to 25 mL cylindrical tube. Absorbance of solution was measured at 302 nm against blank i.e 0.5 M methanolic KOH.

**Carbendazim extraction and clean up.** Apple fruits (2 kg) were homogenized in a Robot Coupe high volume homogenizer. Homogenized 15 g sample was taken in 50 mL centrifuge tube containing 30 mL acetonitrile and

homogenized at 15000 rpm for 2 min in high speed homogenizer. Three g sodium chloride was added to the tube, shaken at 50 rpm for 3 min with Rotospin and centrifuged at 3000 rpm for 3 minutes. Approximately 18 mL of upper organic layer was taken in 50 mL centrifuge tube containing 9 g anhydrous sodium sulphate to remove moisture. The supernatant (11 mL) was subjected to dispersive solid phase clean up (dSPE) by transferring it into 15 mL centrifuge containing 0.4 g primary secondary amine (PSA) sorbent and 1.15 g anhydrous magnesium sulphate. The contents were shaken for 5 minutes at 50 rpm in Rotospin mixer and then centrifuged at 3000 rpm for 3 minutes. The extract (6 mL) was transferred to the test tube and evaporated to dryness in the Turbovap concentrator at 45°C in the presence of air current. Residues were dissolved in 3 mL acetonitrile: water mixture (30:70 v/v), filtered through 13 mm nylon filter (0.45µm pore size) and 20 µL injected into HPLC.

**Soil.** Apple cropped soil (2kg) was drawn, sieved and 10 g of representative sample extracted in 20 mL acetonitrile. Four g magnesium sulphate and 1 g of sodium chloride were added into the tube, shaken at 50 rpm for 1 min in Rotaspin and centrifuged at 3000 rpm for 3 minutes. Approximately 10 mL of upper organic layer was subjected to dSPE with the addition of 1.5 g anhydrous magnesium sulphate and 0.25g PSA. The mixture was shaken for 1 min at 50 rpm in Rotaspin mixer and then centrifuged at 4000 rpm for 10 minutes. Supernatant (4 mL) was withdrawn and evaporated to dryness in the Turbovap concentrator at 45°C under nitrogen current. Residues were dissolved in 2 mL acetonitrile: water mixture (30:70 v/v), filtered through 13 mm nylon filter (0.45 µm pore size) and 20 µL injected into HPLC.

## Instrumentation

The quantitative analysis of mancozeb as $CS_2$ was performed spectrophotometrically using a UV–VIS spectrophotometer (Shimadzu 2008) at wavelength of 302 nm. Carbendazim residues were estimated by Liquid Chromatograph SHIMADZU LC-20AT equipped with DGU-$20A_5$ degasser, autoinjector SIL-20 AHT, SPD-M20A Photodiode Array Detector (PDA) connected with Waters µ BONDAPAK™ RP $C_{18}$ column (2.0 mm X 30 cm). The mobile phase used was acetonitrile: water (30:70), run in an isocratic mode at flow rate of 1 mL $min^{-1}$. Carbendazim was detected at 283 nm wavelength. Under these operating conditions, the carbendazim was eluted at a retention time of 7.0 min (Fig. 1).

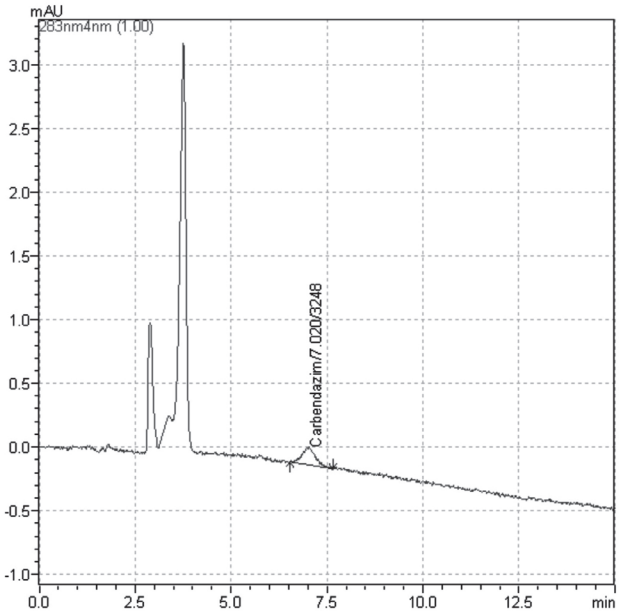


**Figure 1.** HPLC chromatogram showing carbendazim peak area and retention time (RT) for analytical standard of 0.05 mg $kg^{-1}$

## Recovery, limit of quantification and repeatability

The validity of the analytical methods for the determination of mancozeb[12]and carbendazim[13] residues, determined in the present investigation, was checked by spiking the untreated samples of apple fruit and soil with mancozeb at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 1.20 mg $kg^{-1}$, replicated thrice and carbendazim at 0.05, 0.10, 0.25, 0.50 and 1.00 mg $kg^{-1}$ fortification levels, replicated five times. The spiked samples were equilibrated and processed by adopting the standard procedure. The limit of quantification was established based on the recovery. The repeatability of the method was checked by calculating relative standard deviation (RSD) values.

# RESULTS AND DISCUSSION

## Recovery studies

The recovery of $CS_2$ at different levels viz., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 1.20 mg $kg^{-1}$ was 89.47, 84.21, 83.04, 85.96, 91.61, 85.71 and 99.85%, respectively in apple fruits. The relative standard deviation (RSD) for repeatability ranged from 0.65-3.25 % at 7 different levels of spiking. Similarly, the soil samples were fortified with mancozeb at different levels viz., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 1.20 mg $kg^{-1}$ and the recovery of $CS_2$ at the respective levels was 100.00, 95.32, 96.88, 98.39, 98.25, 99.22 and 98.83%. The

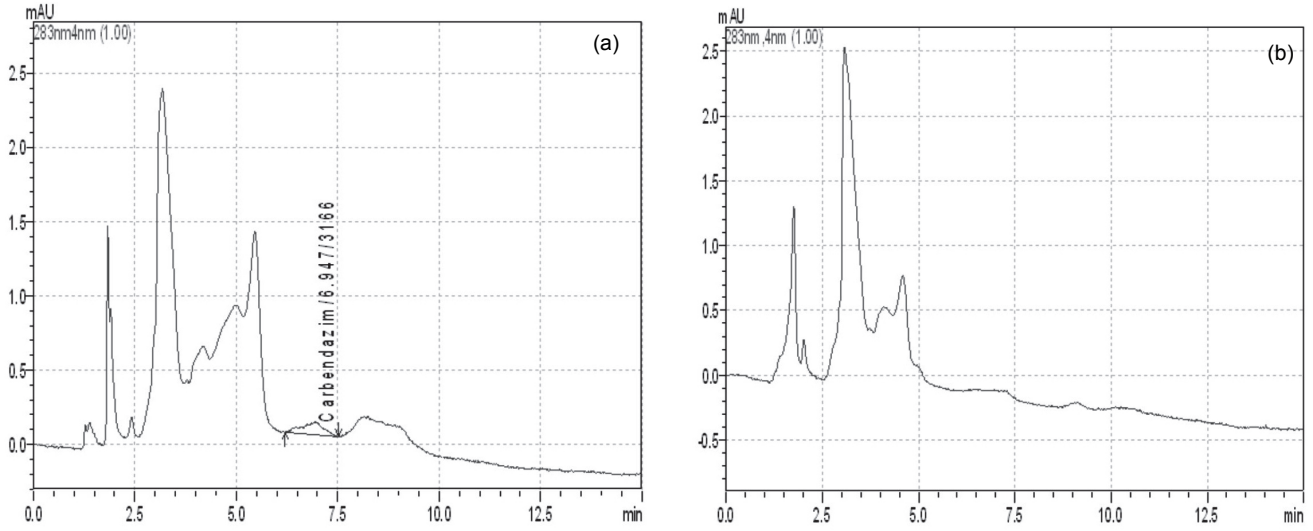


**Figure 2.** HPLC Chromatograms depicting carbendazim recovery in: a) spiked apple sample (0.05 mg $kg^{-1}$) b) apple blank sample

relative standard deviation (RSD) for repeatability ranged from 0.51-5.10 % at 7 different levels of spiking.

Homogenized apple fruits fortified with carbendazim at different levels viz., 0.05, 0.10, 0.25, 0.50 and 1.00 mg $kg^{-1}$ were analyzed by following the already described methodology. The recovery at the respective levels was 100.00, 106.00, 99.60, 100.00 and 100.20 per cent (Fig. 2). The recovery of carbendazim in apple cropped soil at different levels viz., 0.05, 0.10, 0.25, 0.50 and 1.00 mg $kg^{-1}$was 92.00, 87.00, 81.20, 102.20 and 103.60 per cent, respectively. The repeatability of method was checked by calculating RSD which ranged from 3.20-10.00 % in apple fruits and 1.33-7.65 % in soil at 5 levels of spiking.

The validation data showed linearity over the concentration range of mancozeb 0.1 to 1.20 mg $kg^{-1}$ with coefficient of correlation value of 0.9865 and of carbendazim 0.05 to 1.00 mg $kg^{-1}$ with coefficient of correlation value of 0.9974. The calibration curves and regression equations for both the fungicide are presented in Figure 3. The recovery percentage lied between 80 -120 % for the substrates. Hence, the method was adopted for residue dissipation study on carbendazim and mancozeb in apple fruit and soil samples. The limit of determination (LOQ) of the methods worked out to be 0.05 mg $kg^{-1}$ for both the fungicides i.e. mancozeb and carbendazim, and the limit of detection (LOD) 0.017 mg $kg^{-1}$.

## Persistence/dissipation of mancozeb on apple fruits in locations I and II

The initial deposits (2 h after application) of mancozeb expressed in terms of $CS_2$ on apple fruits due to the application of SAAF at single dose (@ 630 g.a.i. $ha^{-1}$) ranged from 3.074-3.434 and at double dose (1260 g a.i. $ha^{-1}$) from 5.430- 6.073 mg $kg^{-1}$, at both the locations. In location I

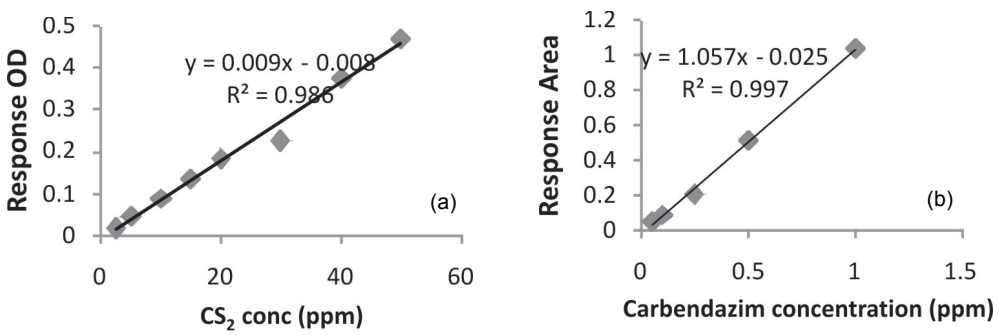


**Figure 3.** Calibration curves of a) $CS_2$, b) Carbendazim

**Table 1.** Persistence of mancozeb in /on apple fruits

| Interval (Days) | Mancozeb residues expressed as $CS_2$ in mg $kg^{-1}$ (n=3) | | | |
|---|---|---|---|---|
| | Location I | | Location II | |
| | X* | 2X** | X | 2X |
| 0 | 3.434+0.001 | 6.073+0.001 | 3.074+0.002 | 5.430+0.001 |
| 1 | 2.204+0.001 | 4.461+0.001 | 2.134+0.002 | 4.182+0.001 |
| 3 | 1.184+0.001 | 2.536+0.001 | 1.074+0.002 | 2.262+0.001 |
| 5 | 0.894+0.001 | 1.763+0.035 | 0.760+0.001 | 1.345+0.002 |
| 7 | 0.653+0.001 | 1.206+0.002 | 0.548+0.001 | 1.023+0.001 |
| 10 | 0.323+0.001 | 0.655+0.001 | 0.273+0.002 | 0.496+0.001 |
| 15 | 0.090+0.001 | 0.224+0.001 | 0.064+0.002 | 0.110+0.001 |
| 20 | BDL | 0.064+0.002 | BDL | 0.050+0.001 |
| 25 | - | BDL | - | BDL |
| Control | ND | ND | ND | ND |

ND = Not detected; BDL = Below limit of determination;
* Single dose; **Double dose; n = Replicatons

**Table 2.** Persistence of carbendazim in/on apple fruits

| Interval (Days) | Carbendazim residues (mg $kg^{-1}$) (n=3) | | | |
|---|---|---|---|---|
| | Location I | | Location II | |
| | X* | 2X** | X | 2X |
| 0 | 1.319+0.030 | 2.245+0.023 | 1.161+0.003 | 2.078+0.012 |
| 1 | 0.759+0.006 | 1.658+0.024 | 0.642+0.005 | 1.176+0.005 |
| 3 | 0.355+0.001 | 0.834+0.003 | 0.333+0.003 | 0.636+0.001 |
| 5 | 0.195+0.008 | 0.383+0.005 | 0.138+0.002 | 0.324+0.003 |
| 7 | 0.088+0.002 | 0.181+0.003 | 0.080+0.004 | 0.148+0.004 |
| 10 | BDL | 0.108+0.003 | BDL | 0.103+0.005 |
| 15 | - | BDL | - | BDL |
| Control | ND | ND | ND | ND |

ND = Not detected; BDL = Below limit of determination;
*Single dose; **Double dose; n = Replicatons

(Matiana), initial deposits at single dose dissipated to 2.204, 1.184, 0.894, 0.653, 0.323 and 0.090 mg $kg^{-1}$ after 1, 3, 5, 7, 10 and 15$^{th}$ day and reduced below the determination limit on 20$^{th}$ day of application. At double the recommended dose the initial deposits dissipated to 4.461, 2.536, 1.763, 1.206, 0.655, 0.224 and 0.064 mg $kg^{-1}$, and BDL after 1, 3, 5, 7, 10, 15 and 20, and 25$^{th}$ day of spraying, respectively (Table 1). Similarly, in location II (Thanedhar) the initial deposit, at the single recommended dose dissipated to 2.134, 1.074, 0.760, 0.548, 0.273 and 0.064 mg $kg^{-1}$ after 1, 3, 5, 7, 10 and 15$^{th}$ day and reduced below the determination limit on 20$^{th}$ day of application. However, when applied at double the recommended dose, the initial deposits dissipated to 4.182, 2.262, 1.345, 1.023, 0.496, 0.110 and 0.050 mg $kg^{-1}$ after 1, 3, 5, 7, 10, 15, and 20 days and then at 25$^{th}$ day of spraying, the residues were found below determination level (Table 1).The results are in contrast to the findings of Sharma and co-workers[14], who reported that mancozeb residues were non- detectable in tomato and brinjal within 10 days of application @ 1.9 and 3.8 kg a.i. $ha^{-1}$, respectively which in this study were detected till 20$^{th}$ day after application at double the recommended dose. However, the mancozeb residues reached below its MRL of 3 mg $kg^{-1}$ after 1 day at recommended dose and 3 days in double the recommended dose at both the locations.

## Persistence and dissipation of carbendazim on apple fruits in locations I and II

The initial deposits of carbendazim on apple fruits at location I and location II were 1.319 and 1.161 mg $kg^{-1}$ at the recommended dose (X) with the corresponding levels of 2.245 and 2.078 mg $kg^{-1}$ at double the recommended dose (2X), respectively (Table 2). More than 90 % of the initial deposits of this fungicide dissipated 7 days after field applications at both recommended and double the recommended doses at both the locations. The residues found at this stage were much lower than the MRL (5.0 mg $kg^{-1}$) prescribed by Food Safety and Standard Authority of India (FSSAI), India. The residues of carbendazim on apple fruits declined progressively with time in all the treatments and reached below determination level at 10 and 15 days after spraying of the recommended and double the recommended doses, respectively at both the locations. These results are in conformity with the results of Sharma and Nath[5] who reported that residues of carbendazim in apple persisted up to 10 days under the parallel agroclimatic zone conditions.

## Residues at harvest

Residues of both the fungicides were not detected in mature fruits (at harvest) at all the five locations. Soil samples collected after 30 days following the last spray application at recommended and double the recommended doses did not show the presence of carbendazim or mancozeb residues at the quantification limit of 0.05 mg $kg^{-1}$.

## Half-life and pre-harvest intervals of mancozeb and carbendazim

The dissipation kinetics equation, half-lives, and correlation coefficients ($R^2$) of the carbendazim and mancozeb residue dissipation were calculated from the experimental data and

**Table 3.** Dissipation kinetics of mancozeb and carbendazim on apple at two locations

| Fungicide | Dosages (g a.i. ha$^{-1}$) | Location | Regression equation | Correlation coefficient ($R^2$) | Half life (days) | PHI (days) |
|---|---|---|---|---|---|---|
| Mancozeb | 630 | I | Y=0.4652-0.0994x | 0.989 | 3.00 | 0.12 |
| | | II | Y=0.4398-0.1065x | 0.991 | 2.82 | 0.35 |
| | 1260 | I | Y=0.7454-0.0955x | 0.997 | 3.15 | 2.80 |
| | | II | Y=0.6999-0.1035x | 0.993 | 3.00 | 2.15 |
| Carbendazim | 120 | I | Y=0.0759-0.1622x | 0.994 | 1.91 | 3.80 |
| | | II | Y=0.0144-0.1643x | 0.990 | 1.88 | 4.16 |
| | 240 | I | Y=0.3282-0.1386x | 0.984 | 2.23 | 2.68 |
| | | II | Y=0.2236-0.1329x | 0.968 | 2.38 | 3.59 |

PHI= Pre-harvest interval

are summarized in Table 3.The data revealed that the dissipation of mancozeb and carbendazim in apple followed first order kinetics which is based on the first order rate equation: $C=C_o\ e^{-kt}$ and *t1/2= ln 2/k,* where *C*= (mg kg$^{-1}$) is the pesticide concentration at time *t,* $C_o$=apparent initial concentration(mg kg$^{-1}$), *k*=rate constant, and $t_{1/2}$ is the pesticide half-life. The half life values varied between 2.82–3.15 and 1.88–2.38 days, for mancozeb and carbendazim, respectively (Table 3).The present results are in cognisance to the finding of Sharma and Nath[5] and Sharma and co-workers[10] who reported the half-life values of mancozeb in the range of 3.9- 5.6 days and of carbendazim between 2.0-3.9 days on apple.

The results showed that the dissipation of carbendazim was faster than mancozeb. It may be due to the effect of environmental conditions. As per the MRL values of mancozeb (3 mg kg$^{-1}$) and carbendazim (5 mg kg$^{-1}$) in apple fixed by FSSAI, a pre-harvest interval of 3 and 4 days for mancozeb and carbendazim is suggested (Table 3).

### Risk assessment of mancozeb and carbendazim on apple fruits

Calculation of theoretical maximum residue contribution (TMRC) involves the assumptions that all pesticides legally allowed on a particular commodity will always be applied, that all residues are present at tolerance levels and that there is no post harvest effect on residue levels[15]. Therefore, to evaluate the risk assessment of SAAF, the TMRC was calculated by multiplying the maximum residue levels with average per capita daily consumption of 11 g of apple fruits in Indian context. These values were compared with the maximum permissible intake (MPI) of the fungicide. The prescribed acceptable daily intake (ADI) of carbendazim and mancozeb is 0.03 mg kg$^{-1}$ body weight day$^{-1}$ [16]. Multiplying the ADI with the average body weight of an adult taken as 60 kg, the MPI was found to be 1.80 mg person$^{-1}$ day$^{-1}$. TMRC values calculated from fruit samples collected on 0 day at recommended and double the recommended doses from both the locations ranged between 0.84-1.65 mg person$^{-1}$ day$^{-1}$ for mancozeb and 0.53- 1.02 mg person$^{-1}$ day$^{-1}$ for carbendazim which were less than the MPI in both the doses. Therefore, application of SAAF on apple appears safe from the consumer safety point of view.

## CONCLUSIONS

From the present findings it is concluded that the mancozeb residues dissipated slowly in apple as compared to carbendazim and were found below the limit of determination at 20 days after spraying of the mixed formulation which may be due to effect of environmental conditions. On the basis of dissipation data, half lives were calculated in the range of 1.88-2.38 and 2.82-3.15 days for carbendazim and mancozeb, respectively. TMRC values calculated from residue data generated from two locations were found to be below the MPI on apple fruit. Therefore, application of mixed formulation of carbendazim and mancozeb (SAAF) at the recommended dose on apple is safe for consumption of the fruits.

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