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Absorption, Translocation, and Accumulation of Carbendazim in Opium Poppy (*Papaver somniferum* L.)

R. Banerji, B. S. Dixit, S. P. Singh, S. C. Verma

National Botanical Research Institute, Lucknow-226001, India

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Papaver somniferum L. is cultivated in European countries for edible seeds and seed oil, while in India, Bulgaria and Yugoslavia, it is a dual purpose crop cultivated both for seeds and opium. Since the crop is susceptible to fungal diseases (Hussain and Sharma 1983), it is usually treated with Bavistin 50 WP (Carbendazim, methyl benzimidazol-2-yl carbamate). We (1993) have recently estimated the levels of carbendazim in leaves and fruits of opium poppy to study the residue levels when a recommended dose of the fungicide was used. In continuation of the previous work a study was undertaken to investigate the absorption, translocation and accumulation of carbendazim in various tissues when sprayed with different doses of the fungicide on opium poppy plant during gowth.

MATERIALS AND METHODS

The crop (*P. somniferum* L.) was raised over 0.2 ha on sandy loam soil at NBRI, Lucknow situated at 26-52'N lat. and 80°56'E long. and at an altitude of 161.5 m above the sea level. Agronomical practices recommended were strictly followed while raising the crop. The crop experienced winter season from mid November to the end of February followed by transitional period in March.The mean maximum temperature fluctuated between 23.30°C to 34.03°C while the mean minimum was between 6.72°C to 14.65° C. The relative humidity ranged between 56% to 90% while the rainfall recorded over the study period was about 100 mm. The pH value of the soil was 7.8 and the organic carbon content in the soil was 0.3425% (Walkey, 1953).

Foliar sprays of three different concentrations (0.125, 0.25 and 0.50%) of fungicide were given to the leaves only, of alternate plant rows, by bottle sprayers at fortnightly intervals. Three sprays were given instead of four recommended for maintaining a healthy crop. Precautions were taken to avoid spraying on the whole plant.The leaves selected for the treatment had an average area 37.31 cm^2 at

Correspondence to: R. Banerji

the time of first spray,128.75 cm^2 at 2nd spray and 168.3 cm^2 during third spray. The total number of leaves exposed were same in all the concentrations for each spray.

First leaf sample was collected after 24 h of spray while subsequent sampling was done after 14 days, just before the next spray and 24 h thereafter. For latex, samples were collected after the lancing. The mature seeds were, however, collected separately for the control and each of the concentrations. Three replicates of each sample were taken.

The residue of carbendazim was extracted from the leaves, stem, root, capsule, capsule husk, latex (opium), mature seeds as well as from the soil. Sample (50 g) in each case, except leaves (100 g) and latex (1.0 g), was cut into small pieces and mixed thoroughly. This was extracted in 100 ml distilled chloroform for one hour.The residues were further extracted by taking 50 ml of the solvent as described. The extracts were combined, filtered and concentrated to a known volume (25 ml) in a rotary vacuum evaporator and extracted with 1M sulphuric acid (10 ml). The acidic phase from each sample was neutralized separately with 0.1N sodium hydroxide (pH 7.8-8.2). Each neutralized solution was extracted with ethyl acetate (3 x 25 ml). The combined ethyl acetate layer was washed with distilled water, dried over sodium sulphate and concentrated by rotary vacuum evaporator. The residue was redissolved in methanol (1 ml, HPLC grade).

The soil samples were collected from 4 sampling sites where the highest concentration (0.5%) of fungicide was used, air dried, ground and sieved through a 2 mm mesh screen before use. Finally these specimens were combined and an aliquot (50 g) was processed for carbendazim residue as described above.

For the analysis of carbendazim residue, a 20 µl aliquot of each sample was injected into the HPLC system (Gynkotek, Germany, model 300 C) with Shimadzu SPD-6A UV detector connected to a chromatopak C-R3A data processor. The column was packed with Nucleosil C-18 (4.5 x 250 mm) and the mobile phase was methanol at 0.5 ml/min using UV detection at 281 nm.

Before an unknown sample was analysed the response factor of carbendazim (BASF, India) standard was determined from the chromatogram of 0.1-10 ppm solutions. Recovery studies with fortified samples gave percent recovery values 87 ± 0.02.

RESULTS AND DISCUSSION

Under the prevailing agro-climatic conditions of Uttar Pradesh (Lucknow) where the opium poppy crop was grown in sandy loam soil during the winter 1991-92, carbendazim residues resulting from

Table 1. Residue level of carbendazim on various parts of *P. somniferum*

Sampling time (Days)	Residue (µg/g)* C-1 L	C-1 S	C-1 R	C-2 L	C-2 S	C-2 R	C-3 L	C-3 S	C-3 R
0 (Control)	0	0	0	0	0	0	0	0	0
1 (Ist spray)	1.10	0.44	ND	2.23	0.89	ND	4.64	1.85	ND
14	0.66	0.26	ND	1.34	0.53	ND	2.78	1.11	ND
15 (IInd spray)	1.87	0.75	ND	3.82	1.53	ND	7.28	2.91	ND
28	1.03	0.41	ND	2.10	0.84	ND	4.01	1.60	ND
29 (IIIrd spray)	1.72	0.69	ND	3.49	1.39	ND	6.58	2.63	ND
42	0.78	0.31	ND	1.58	0.63	Tr	2.96	1.18	0.03
57	0.40	0.16	ND	0.79	0.31	ND	1.47	0.59	Tr
72	0.28	0.11	ND	0.54	0.21	ND	1.01	0.40	ND
84 (At harvest)	0.16	0.06	ND	0.31	0.12	ND	0.57	0.23	ND

* Average of three replicates
Concentrations used, C-1 = 0.125%, C-2 = 0.25%, C-3 = 0.5% , L = leaves, S = stem, R = root
ND = <0.001 µg/g, Tr = < 0.01 µg/g

Table 2. Residue level of carbendazim in capsule of *P. somniferum*

Sampling time* (Days)	Residue (µg/g)** C-1	C-2	C-3
Capsule			
29	0.32	0.59	1.23
44	0.18	0.32	0.67
56 (At harvest)	0.11	0.20	0.41
Capsule husk	0.10	0.17	0.35
Mature seed	ND	ND	ND
Latex (Opium)	ND	ND	ND

* After final spray
** Average of three replicate
ND = <0.001 µg/g.

0.125%, 0.25% and 0.50% doses, each applied in three sprays at fortnightly intervals on various parts of the crop are presented in Table 1 and 2. The data reveal that the carbendazim residue in different tissues of the plant was more or less in accordance with the concentration of the fungicide sprayed.

With the absorption of carbendazim in the leaves of the plant, the initial levels of residue after first spray for all the three concentrations were observed to be 1.10, 2.23 and 4.64 µg/g respectively. An increase of 2.8 times in the absorption of carbendazim was observed after the second spray for the recommended and half the recommended doses only while 2.6 times for double the recommended dose. But an over all increase of 1.6 times in the absorption was noticed for all the doses of the fungicide after third spray. The increase in the quantum of the fungicide in the plant over the same period can be attributed to growth of the plant (Table 3). Further, it was observed that the concentration of the fungicide in leaves was maximum after second spray in all the concentration. However, this value declined in the third spray. The fungicide was translocated to different parts between first and second spray and second and third spray. The level of carbendazim in leaves, stems, and roots of opium poppy showed marked decrease after third spray. By the end of our study i.e. after 80 days, low levels of carbendazim residue were observed. At the time of harvest, the residue levels of carbendazim was 0.16 µg/g in the leaves and 0.06 µg/g in the stem at the lowest concentration (0.125%). However, 0.31 and 0.12 µg/g residue was detected in leaves and stem respectively at the traditionally used concentration (0.25%) which was almost double the residue observed for lower concentration.The residue level was about 1.8 times higher in the leaves and stem at the time of harvest where the concentration was double (0.5%). But in all the cases carbendazim failed to reach the root zone.The trace amounts of carbendazim found in the roots after the final spray may be due to the absorption of the fungicide through soil. However,the carbendazim residue was below detectable limits in the edible commodities i.e. latex and seeds.

It was observed that after the first spray, carbendazim residue reduced about 40% in a fortnight i.e. just before the second spray for all the concentrations but before the third spray by 45% , while after the third and final spray by about 55% within a fortnight. This suggests the greater translocation capacity of plant at advanced stages.

It is evident from Table 1 that 76.7% of the carbendazim residue dissipated within 29 days after 3rd and final spray for the lowest concentration of fungicide used, while 84.5% residue dissipated within 44 days of the final spray for the recommended dose bringing the residue below guideline level values of various crops which ranged from 0.10-10 µg/g (WHO/FAO Report, 1978). For double the

Table 3 Growth behaviour of *P. somniferum*

Sampling time* (Days)	Av.height (cm)	Av. Weight (g)	Leaves/ plant	Average Area
60	2.2	1.1	7	37.3
74	8.0	6.3	9	128.7
88	33.7	16.5	12	160.2
105	80.0	30.2	14	168.0
117	85.6	32.3	14	168.5

* From the date of planting.

Table 4. Yield of capsule and seeds of *P. somniferum*

Treatment	Capsules/plant	Yield*(wt of seeds/capsule) g
Control	0.90	1.56
C-1	2.41	2.09
C-2	2.25	2.21
C-3	2.31	2.03
GS**	2.24	2.61

* Data based on three replicates of 10 plant each
** GS = General spray, data based on 50 plants

Table 5. Residue level of carbendazim in soil sample

Sampling time (Days)	Residue (µg/g)*
0 (Control)	0.0
1	0.25
14	0.18
15	0.40
28	0.28
29	0.52
42	0.36
57	0.24
72	0.15
84**	0.09

* Average of three replicates
** At harvest time of Opium poppy

recommended dose, 91.3% residue dissipated till the time of harvest. However, for all the concentration, about 91% residue of the fungicide dissipated by the harvest time bringing the level of carbendazim below the guideline level values as mentioned above.

The data presented in the tables indicate that the lowest concentration of carbendazim is effective in controlling the fungal diseases of the crop which is supported by yield data in regard to capsules and seeds. The number of capsules per plant was found to be 2.4 for the whole plant spray which was highest among the treated plants. However, in the untreated plants the number was 0.9 (Table 4). A marginal increase in yield of seed per capsule was noticed in traditionally used concentration of carbendazim. Further, it was observed that the foliar application was at par with the whole plant spray. The lower dose of the fungicide is, therefore, recommended as compared to the traditionally used one.

Besides, studying carbendazim residue in different parts of opium poppy, the residue was also monitered in the soil but only for the highest concentrations (0.5%) of the fungicide and presented in Table 5. The low level of the residue found in the soil might be due to spilling of the fungicide during the spray.

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