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Residue Levels of Carbendazim in Opium Poppy (*Papaver somniferum*)

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The Opium poppy (*Papaver somniferum* L. Papaveraceae) has been grown as a medicinal plant for thousands of years mainly for opium alkaloids, which are frequently used as analgesic, antitussive, and antispasmodic agents in modern medicine. It is cultivated in European countries as a source of edible seeds and seed oil. In India, Bulgaria and Yogoslavia, it is a dual purpose crop grown for both seeds and opium (Hussain and Sharma 1983; Singh et al. 1991).

Carbendazim, methyl benzimidazol-2-ylcarbamate, is a broad spectrum systemic fungicide that has been reported to effectively control most fungal phytopathogens. Four to six sprays are recommended as a prophylactic measure for maintaining a healthy crop. Cline et al. (1981), Bhattacharya et al. (1989) and White and Kilgore (1972) have analysed its residues in various fruits; however, no such work is reported on opium poppy.

MATERIALS AND METHODS

An opium poppy crop (variety BR-87) was raised on 0.20 ha of land of sandy-loam texture at the Institute during 1990-91 (Nov.-April, Temp. 35-40°). Plants were grown in a row using small beds (30 x 10 cm) for easy culture. The first irrigation was done after 15 days of sowing; light irrigation was done at regular intervals of 15 days until the start of lancing.

Four sprays of the recommended dose of 0.25% carbendazim (Bavistin 50% WP, BASF, India) was sprayed at 2-wk intervals. The first leaf sample was collected after 6 hr of spray which was taken as the minimum time required for the absorption of the fungicide. The subsequent sampling was done at regular weekly intervals. For latex, samples were collected after lancing. The mature seeds were, however, collected randomly at the time of harvesting. The oil was extracted from mature powdered seeds (40 mesh) in a Soxhlet extractor with n-hexane (6 hr) by the method of Singh et al. (1990).

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For the extraction of the carbendazim residue, the leaf (100 g), capsules (50 g), seeds (50 g), and latex (1.0 g) samples (in triplicate) were macerated with chloroform (100 mL) and left overnight. After centrifugation the supernatant was concentrated to 25 mL and extracted with 1 M H_2SO_4 (10 mL). The 1 M H_2SO_4 extract from each sample was neutralized separately with 0.1 N NaOH (pH 7.8-8.2). Each neutralized solution was extracted with ethyl acetate (3 x 15 mL). The combined ethyl acetate layer was washed with distilled water and dried with anhydrous Na_2SO_4. The ethyl acetate was removed by distillation. The residue thus obtained was dissolved in methanol (5 mL). The absorbance of the solution was recorded at λmax 300 nm using a Bosch and Lomb Spectronic 2000 spectrophotometer against methanol as a blank (White and Kilgore 1972). Further analysis was performed with HPLC (Gynkotek, Germany model 300 C) using methanol as a mobile phase at 0.5 mL/min, Nucleosil 5C-PMSC$_{18}$ (250 x 4.5 mm) column and UV detector at 281 nm. The residue levels (µg/g) were calculated with the help of a standard curve of carbendazim (5, 10, 20, 100, 200 µg/mL). A 20 µl aliquot of each of the samples was injected into the HPLC system.

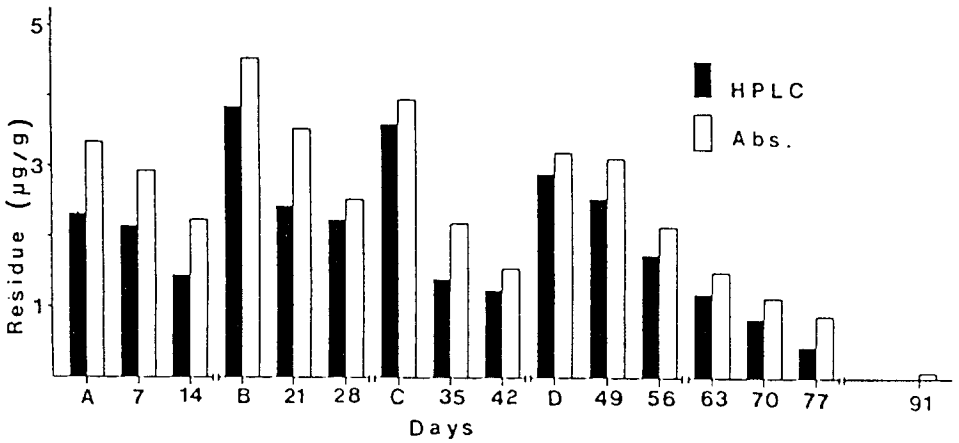
RESULTS AND DISCUSSION

The residue levels as determined by the spectroscopic method were always slightly higher than those determined by the HPLC method (Fig. 1). It is evident from Table 1 that the residue of carbendazim was recorded as 2.25 µg/g after 6 hr of the first spray. With the absorption of carbendazim in different part of the plant, it was reduced to 2.07 µg/g after 7 days and to 1.35 µg/g after 14 days, i.e., just before the 2nd spray. After the 2nd spray, an increase of 2.50 µg/g residue was recorded which subsequently lowered down to 2.15 µg/g before the 3rd spray. This shows an increase of 1.88 times in absorption of fungicide in the plant over the same period of plant development. This absorption further increased to 2.35 µg/g within the same period after 3rd spray. However the absorption after the 4th spray was only 1.2 µg/g in a fortnight. At this stage there was initiation of flowering in the poppy crop; therefore, the reduction of absorption was recorded.

Table 1. Residue of carbendazim in opium poppy leaves at different time interval after four sprayings.

	Residue, µg/g*			
Sampling time (days)	1st spray (19.12.90)	2nd spray (02.01.91)	3rd spray (16.01.91)	4th spray (30.01.91)
0.25	2.25 (3.32)**	3.85 (4.50)	3.50 (3.97)	2.87 (3.18)
7	2.07 (2.95)	2.35 (3.48)	1.32 (2.08)	2.45 (3.06)
14	1.35 (2.17)	2.15 (2.47)	1.15 (1.47)	1.67 (2.10)

*Mean values of three replicate samples
**Data from HPLC, spectrophotometric data in parenthesis.



A= 1st spray; B= 2nd spray; C= 3rd spray; D= 4th spray

Figure 1. Residue levels in leaves at different time intervals of four sprays as determined by HPLC and spectroscopic methods.

Table 2. Cumulative residue of carbendazim in opium poppy after the fourth and final spray.

Sampling time (days)	Plant part	Residue, µg/g	
		HPLC	Spectroscopy
21	Leaf	1.17	1.46
28	Capsule	2.32	2.63
35	Leaf	0.77	1.02
	Capsule	1.17	1.75
49	Leaf	0.37	0.89
	Capsule	0.58	0.76
63	Leaf	ND	Tr
	Capsule	0.28	0.38
	Seeds	0.07	0.15
	Latex	ND	Tr
	Mature seeds	ND	Tr
	Seeds	ND	Tr

ND= < 0.001 µg/g; Tr= < 0.01 µg/g

The absorption of fungicide in opium leaves during the first 6 hr after spraying shows an interesting trend. It was 2.25 µg/g after 1st spray followed by 2.50, 1.35 µg/g and after 4th spray it was 1.72 µg/g. It is evident from Table 2 that 73% of the residue dissipated within 35 days while after 63 days of the 4th and final spray it was below the detectable levels. The carbendazim levels in the total capsules harvested at the mid season were 2.32 µg/g (70 days) and 1.17 µg/g (77 days) however, at the harvest time the residue found in the capsule hull (0.28 µg/g) and seeds (0.07 µg/g) were well within the tolerance limit (WHO/FAO, 0.5 to 1.0 ppm). Further, the quantities of residue in the mature seeds, seed oil and latex (Table 2) were below the detectable levels.

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