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Spectrophotometric Determination of Fungicides from Treated Seeds of Wheat

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Abstract

Spectrophotometry was found to be suitable for quick and accurate quantitative estimation of carbendazim, carboxin and thiram from treated seeds of wheat. The simplicity of technique has made it an indispensable tool for chemical manufacturer, formulation chemist and commercial producer of seed treatment equipments. Higher amount of fungicides was detected in slurry treatment than dry seed treatment, which indicates increase in loading of fungicides in slurry treatment as compared to dry seed treatment method at all doses. The recovery of fungicides varied from 30.00—46.00% in dry method and 70.00—89.26% in slurry method. Fungicides were quantitatively estimated after 12 months of storage of treated seeds. Persistence of fungicide decreased with increase in storage period. The per cent recovery of fungicides was low at seed treatment with 1.0 and 1.5 g/kg as compared to 2.0 and 2.5 g/kg dose during storage.

Key words : Spectrophotometry, Quantitative estimation, Seed treatment, Storage, Fungicides.

The use of fungicides as seed treatment is the most widely followed disease control measure used in all crops globally. Wheat seeds are routinely treated by seed producing agencies with a broad spectrum (captan, thiram) and systemic fungicides like carbendazim, carboxin and tebuconazole. The efficacy of seed treatment for the management of seed and soil borne diseases may depend on method of seed treatment, dose of fungicide, quantity of fungicide received by the seed, coverage of fungicide over the seed and degradation of fungicide during storage. Estimation of fungicides from treated seeds is being done in a regular manner in some countries like Denmark and United State of America. However, it appears that at the moment chemically treated seeds are not subjected to quantitative and qualitative estimation by any seed corporation in India. Techniques like bioassay, thin layer chromatography (TLC) and spectrophotometry have been found to be useful for estimation of the fungicides, but information on estimation of chemicals used for seed treatments are lacking globally. Amongst the available methods, the spectrophotometry has proved to be the most sensitive and rapid method for estimation of fungicides. This technique has successfully been used for the estimation of carbendazim and carboxin in wheat, carboxin and oxycarboxin in pearl millet, captan in sorghum, thiram in wheat, sorghum, pea and maize (1—4). The present experiment was carried out in 2003-04 at GB Pant University of Agriculture and Technology, Pantnagar aimed for quantitative estimation of carbendazim, carboxin, thiram and persistence of fungicide during storage from dry and slurry treated seeds by spectrophotometry.

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Methods

Spectrophotometry as described for the estimation of carbendazim (1), carboxin (3) and thiram (4) was followed during the present studies with slight modifications. The standard curve for carbendazim, carboxin and thiram was prepared by using ethyl acetate (for carbendazim) and chloroform (for carboxin and thiram) as a solvent, respectively. Ten mg active ingredient of each fungicide namely carbendazim, carboxin and thiram was dissolved separately in 100 ml of their respective solvents to prepare 100 ppm

Table 1. Quantity of fungicides detected by spectrophotometry in seeds treated by dry and slurry methods.

Fungicide/ Method of seed treatment	Dosages (g/kg)	Expected amount (µg/g)	Detection µg/g	Detection Per cent
		Carbendazim		
Dry	1.0	1000	356.25	35.62
	1.5	1500	618.75	41.25
	2.0	2000	800.00	40.00
	2.5	2500	1150.00	46.00
Mean			731.25	40.72
Slurry	1.0	1000	788.00	78.80
	1.5	1500	1206.25	80.41
	2.0	2000	1662.50	83.12
	2.5	2500	2231.50	89.26
Mean			1472.06	82.90
		Carboxin		
Dry	1.0	1000	312.50	31.25
	1.5	1500	450.00	30.00
	2.0	2000	675.00	33.75
	2.5	2500	981.25	39.25
Mean			604.69	33.56
Slurry	1.0	1000	700.00	70.00
	1.5	1500	1075.00	71.66
	2.0	2000	1592.50	79.62
	2.5	2500	2050.00	82.00
Mean			1354.38	75.82
		Thiram		
Dry	1.0	1000	340.00	34.00
	1.5	1500	525.00	35.00
	2.0	2000	750.00	37.50
	2.5	2500	1025.00	41.00
Mean			660.00	36.88
Slurry	1.0	1000	768.75	76.87
	1.5	1500	1137.50	75.83
	2.0	2000	1612.50	80.62
	2.5	2500	2100.00	84.00
Mean			1404.69	79.33

(100 µg/ml) solution. Standard solution of carbendazim, carboxin and thiram at 0.1, 0.2, 0.4, 0.8, 1.0 and 3.2 ml were added to 10 ml graduated test tubes. These solutions were diluted to 10 ml with ethyl acetate and chloroform, respectively and mixed properly. The resultant solutions then contained 1, 2, 4, 8, 16 and 32µg carbendazim, carboxin and thiram per ml respectively. The absorbance of developed color complex was measured in beckman DU 640 B spectrophotometer at 281, 300 and 284 nm against the respective fungicide and solvent system. The absorbance of all the concentration of each fungicide was plotted separately to prepare standard curve of each fungicide. The standard curves were used to estimate the respective fungicide of unknown concentration.

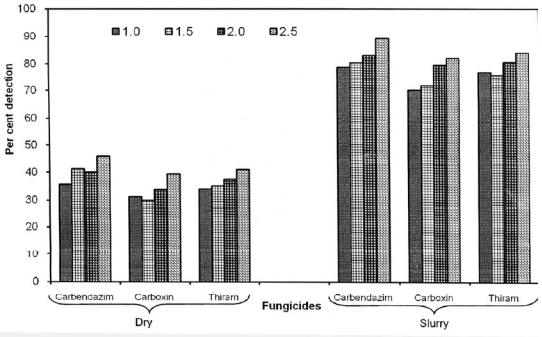

Figure 1. Amount of different doses (g/kg) of carbendazim, carboxin and thiram detected by spectrophotometry from immediately treated seeds.

The seeds of wheat variety Sonalica were treated with fungicides at the rate of 1.0, 1.5, 2.0 and 2.5 g/kg seed. The treatment was done by shaking the seed in a 500 ml volumetric flask for 5 minutes and flask mouth was covered by butter paper. In slurry seed treatment, 5.0 ml water was added to per kg seed. Treated seeds were stored in butter paper bags at room temperature for 12 months. Two grams sample of seeds treated separately with carbendazim, carboxin and thiram were shaken with 10 ml ethyl acetate and chloroform, respectively, in a 50 ml conical flask for 15 minute. Extraction process was repeated twice. The extract was pooled and the volume was adjusted to 25 ml by adding ethyl acetate and chloroform respectively. One ml of each fungicide was further diluted to 10 ml by adding their respective solvents and spectrophotometric measurements were taken as described for standard curve. The same procedure was used for untreated and stored seeds also. The absorbance of the extract of untreated seeds was subtracted from the absorbance of the extract of treated seeds and the concentrations of carbendazim, carboxin and thiram were determined from the calibration graph i. e. standard curve. The quantity of the fungicide was determined with the help of following formula (4) :

$$\text{Fungicide content of seed } (\mu g/g) = \frac{C \times 25 \times \text{dilution}}{\text{Weight of seeds (g)}}$$

Results and Discussion

All the three fungicides were successfully estimated by spectrophotometry method. On theoretical basis it is expected that seed treatment at 1.0, 1.5, 2.0 and 2.5 g/kg will results 1000, 1500, 2000, 2500µg fungicide respectively, from per gram of seed, respectively (Table 1). The amount of fungicide detected by spectrophotometry was lower as compared to expected ones. The seed washed with solvents revealed zone of inhibition on testing by bioassay. Thus it is indicated that not all fungicide is removed by washing seeds with solvents. The results revealed that a higher amount of fungicide was detected in slurry seed treatment as compared to dry seed treatment (Fig.1). The average amount of carbendazim, carboxin and thiram detected in slurry seed treatment was 1,472.06, 1,354.38, 1,404.69 µg/g while in dry seed treatment was 731.25, 604.69, 660.00 µg/g, respectively. The results showed that recovery of fungicides varied from 30.00 to 46.00% in dry method and 70.00 to 89.26% in slurry method. Amongst the fungicides average detection of carbendazim was highest i. e. 40.72 (dry method) and 82.90 (slurry method) followed by thiram and carboxin with 36.88, 79.33 and 33.56, 75.82%, respectively, in dry and slurry method of treatment (Table 1). It is inferred from the results that slurry treatment is better than dry seed treatment. In slurry treatment a higher amount of fungicide is loaded on seed by sticking as compared to dry seed treatment. In dry seed treatment, the fungicide from the surface of seed may be partially removed during treatment and handling processes. The seed treatment with wettable powder, emulsifiable concentrate and slurry has been found to be better than dry treatments earlier by number of workers (1, 5, 6).

The per cent recovery of fungicides after 12 month of storage ranged from 17.50 to 27.69 and 37.52 to 56.00% in dry and slurry treated seeds, respectively. Carbendazim was found to be most stable fungicide and showed better persistence compared to thiram and carboxin with 22.71and 47.15 % recovery after 12 month of storage followed by thiram (21.49 and 45.96%) and carboxin (19.96 and 44.87%), respectively, in dry and slurry method. The loss of fungicides during storage was higher in 1.0 and 1.5 g/kg dosages than 2.0 and 2.5 g/kg dosages and comparatively higher amount of fungicide retained at higher dose (at 2.5 g/kg) than lower dosages (1.0 and 1.5 g/kg) (Table 2, Fig. 2). These results showed better persistence of carbendazim on seed during storage. The quantity of fungicides on seed decreased gradually with the storage period. The information on persis-

Table 2. Quantity of fungicides detected after 12 months of storage by spectrophotometry in seeds treated by dry and slurry methods.

Fungicide/ Method of seed treatment	Dosages (g/kg)	Expected amount (µg/g)	Detection µg/g	Per cent
		Carbendazim		
Dry	1.0	1000	181.68	18.16
	1.5	1500	326.70	21.78
	2.0	2000	464.00	23.20
	2.5	2500	692.30	27.69
Mean			416.17	22.71
Slurry	1.0	1000	398.00	39.80
	1.5	1500	637.50	42.50
	2.0	2000	1005.81	50.29
	2.5	2500	1400.00	56.00
Mean			860.33	47.15
		Carboxin		
Dry	1.0	1000	175.00	17.50
	1.5	1500	270.00	18.00
	2.0	2000	407.43	20.37
	2.5	2500	625.00	25.00
Mean			365.42	19.96
Slurry	1.0	1000	375.20	37.52
	1.5	1500	593.00	39.58
	2.0	2000	987.50	49.37
	2.5	2500	1325.00	53.00
Mean			820.18	44.87
		Thiram		
Dry	1.0	1000	187.40	18.74
	1.5	1500	294.00	19.60
	2.0	2000	442.50	22.12
	2.5	2500	637.55	25.50
Mean			390.36	21.49
Slurry	1.0	1000	403.60	40.36
	1.5	1500	613.11	40.80
	2.0	2000	983.62	49.18
	2.5	2500	1337.70	53.50
Mean			834.51	45.96

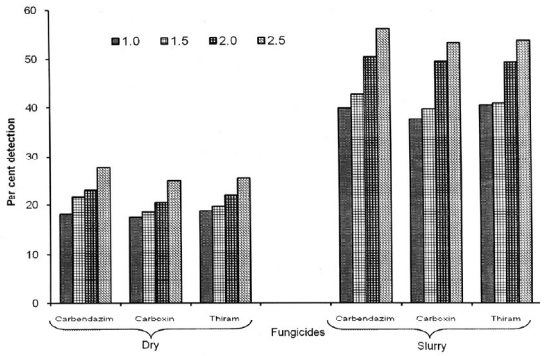

Figure 2. Amount of different doses (g/kg) of carbendazim, carboxin and thiram detected spectrophotometry after 12 months storage of treated seeds.

tence of fungicides on seeds is important in determining the longevity of effective seed treatment in storage. Further, the fungicide itself may be degraded during storage and may become ineffective after a certain period of storage (7).

These findings are supported by several workers as they observed 27.00 to 33.00% and 70.00 to 80.00% recovery of thiram from dry and slurry treated seeds of pea, respectively (4). Earlier, spectrophotometry has been used for the estimation of carbendazim, carboxin, captan and thiram from freshly treated and stored seeds of different crops (3, 5— 8). Thus, spectrophotometry appears to be more sensitive than other methods like TLC and bioassay, which are indirect methods of detection, less sophisticated, time consuming and not enough sensitive to detect small differences in the quantity of fungicide on seed. This technique can be used to measure the amount fungicides retained on seed surface after treatment and persistence of fungicides during storage. It is suitable for chemical companies producing various seed treatment formulations of different fungicides and those concerned with production of seed treatment equipments.

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