

Decontamination of Mancozeb from Cumin and Fennel

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An adoptable technique for decontamination of mancozeb from cumin and fennel is reported. Spiked samples when subjected to rubbing with muslin cloth, heat treatment, exposure to ultra violet (UV) radiation and washing with sodium hypochlorite revealed decontamination of mancozeb in the range of 28.95 to > 97.85% from cumin and 15.86 to > 93.10% from fennel. Physical and various heat treatments recorded lower while chemical and UV-irradiation recorded higher decontamination in both the commodities. Exposure of mancozeb contaminated cumin and fennel to UV radiation for 45 minutes was the best decontamination treatment from the point of view of practical adoption.

Key words: Dithiocarbamate, mancozeb, decontamination, UV radiation, spices, cumin, fennel

Spices are used to improve the flavour, acceptability and the appetizing property of food. These are important for human health and have not only played a crucial role in Indian cuisine but also generate a lot of foreign exchange. India, the “land of spices” is the world’s largest producer, consumer and exporter of spices¹. India grows about 52 spices, prominent among them that occupy a sizeable area and have entered in national and international trade, are black pepper, cardamom, ginger, fennel, chilli, clove, coriander, cumin, *ajwain* and *suwa* (dillseed). Cultivation of spices in India was spread over 5.93 million ha of land with 3.04 million tones of production annually in the year 2010-11. Export was only around 8.86% of the total spices production in India².

Seed spices namely, cumin, coriander and fennel are cultivated mainly in the states of Gujarat and Rajasthan. Area devoted to cumin and fennel cultivation was 311755 and 42833 ha while production was 221906 and 76128 tons in the year 2009-10, respectively in Gujarat state³. A sizeable quantity of the produce is exported every year to countries like Japan, USA, Malaysia, Singapore, UAE, Saudi Arabia, South Africa etc. earning valuable foreign exchange.

Being a potential source of foreign exchange, assurance of quality of spices as per the strict norms set by the importing nations needs to be maintained which has

been often neglected by the growers in India. Detection of pesticide residues in exported agricultural produce is one of the major benchmarks of quality which comes under the umbrella of SPS (Sanitary and Phytosanitary) measures.

Decontamination of pesticide residues is relevant nowadays considering the fact that arable land is shrinking rapidly and pressure of providing food to ever-increasing population is quite high. In such circumstances, the question quality versus quantity of produce becomes more significant as the agricultural produce cannot be refused merely on detection of pesticide. Development of decontamination techniques for pesticide residues in the crops, especially the high value crops such as seed spices is an immediate need of time. Seed spices especially cumin and fennel are susceptible to fungal diseases where mancozeb, a dithiocarbamate fungicide is commonly used by the farmers. Dithiocarbamates (DTCs) are important organosulfur compounds, which act as inhibitors of metal dependant and sulphhydryl enzymes and have a serious consequence on biological systems. There are reports that more than 90% samples of seed spices collected from market places were contaminated with dithiocarbamate pesticides^{4,5}. Although, uses of DTC (Drug Testing Compliance) cannot be altered but their residues can be minimized by adopting various decontamination processes. Keeping this point under consideration, an experiment was conducted to evaluate the effect of different decontamination treatments on mancozeb residues on cumin and fennel.

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MATERIALS AND METHODS

Experimental

A laboratory experiment was conducted to study the decontamination of mancozeb from cumin and fennel at Anand Agricultural University, Anand (Gujarat). Cumin and fennel were collected from the farmers and analysed for the probable presence of mancozeb. Contaminated and fortified cumin and fennel samples were taken for decontamination experiment. Mancozeb formulation (Dithane M-45) as aqueous solution was used. For fortification, samples of cumin (1 kg) each were treated with 166.67 ml of 120 $\mu\text{g ml}^{-1}$ mancozeb solutions with the help of baby sprayer of 1l capacity to get 20 $\mu\text{g g}^{-1}$ level of fortification while fennel samples were fortified at the rate of 5.0 $\mu\text{g g}^{-1}$ by applying 41.68 ml of 120 $\mu\text{g ml}^{-1}$ mancozeb solution. After fortification, samples were left for drying under ambient conditions for two days. The dried cumin and fennel samples were subjected to decontamination treatments. The experiment was conducted in duplicate. The details of the treatments are shown in Table 1 and effect of different decontamination treatments along with unspiked control sample is depicted in Table 2.

The heat treatment was given in hot air oven (Model NV 858/859) manufactured by Nova Instruments Private Limited, Ahmedabad, Gujarat. Prior to treatment, the oven was set at 60 and 90 °C for 1 h for homogeneous heating. Then the samples were subjected to heat treatment (T_2 to T_9). Samples were preserved after heat treatments in aluminum foil separately, till their extraction.

Table 1. Treatment details

S. no.	Notation	Treatment details
1.	T_0	Absolute control
2.	T_{00}	Treated with mancozeb formulation
3.	T_1	Rubbing with muslin cloth for five minutes
4.	T_2	Heat treatment (60°C for 2.0 min)
5.	T_3	Heat treatment (90°C for 2.0 min)
6.	T_4	Heat treatment (60°C for 4.0 min)
7.	T_5	Heat treatment (90°C for 4.0 min)
8.	T_6	Exposure to UV radiation for 30 min
9.	T_7	Exposure to UV radiation for 45 min
10.	T_8	Dipping in starch solution followed by rubbing with muslin cloth
11.	T_9	Washing with 5% sodium hypochlorite solution

UV irradiation was given to samples with the help of 6-W self-ballasted long-wave lamp (365 nm) with input voltage 220 V, 60 Hz which was housed in a steel cabinet, 18 × 15 × 12 inches. The UV source was fitted at a height of 20 cm above the bottom. The lamp was illuminated for 2 h prior to UV treatment of the samples. Treated samples of cumin and fennel were irradiated for 30 and 45 minutes respectively.

Decontamination of mancozeb treated samples of cumin and fennel with starch was carried out by dissolving commercially available starch in 25 ml water. The fortified samples were immersed for 10 minutes in starch solution and then taken out and spread over aluminum foil and dried at ambient temperature. After drying, samples were rubbed with muslin cloth to detach the starch from the samples and analysed for mancozeb residues.

Samples were also treated with 5% sodium hypochlorite and air dried before analysis. In another treatment, fortified samples were simply rubbed with muslin cloth for 5 minutes and then analysed for the residues of mancozeb.

Preparation of CS_2 standard for the GLC analysis

Stock Solution

Purity of the CS_2 , procured from Qualigen India Ltd was 99.97%. Exactly 0.1003 g CS_2 was weighed, dissolved in toluene and volume made to 100 ml. The resultant concentration of CS_2 standard was 1000 $\mu\text{g g}^{-1}$.

Intermediate standard and working standards

Stock solution was serially diluted to 10, 20, 40, 100 and 200 times to prepare intermediate (100 $\mu\text{g g}^{-1}$) and working standards (50, 25, 10 and 5 $\mu\text{g g}^{-1}$) with toluene.

Extraction of mancozeb

Apparatus

Three necked round bottom flask (500 ml), small condenser with B-24 joint, two traps, glass tubes, air inlet glass tube and a water suction pump.

Reagents

Sodium hydroxide (10%, w/v)

NaOH pellets (10 g) were dissolved in distilled water and final volume was made up to 100 ml.

Table 2. Decontamination of mancozeb from cumin and fennel

S. no.	Treatment	Degradation of mancozeb					
		Cumin			Fennel		
		Mean of CS ₂ detected* (µg g ⁻¹)	Degraded amount (µg g ⁻¹)**	Decontamination (%)	Mean of CS ₂ detected* (µg g ⁻¹)	Degraded amount (µg g ⁻¹)**	Decontamination (%)
1	T ₀	0.00	-	-	-	-	-
2	T ₀₀	18.67	0.00	0.00	4.35	0.00	0.00
3	T ₁	6.88	11.79	63.14	1.65	2.73	62.75
4	T ₂	13.26	5.41	28.95	3.69	0.69	15.86
5	T ₃	10.64	8.03	43.02	1.66	2.69	61.83
6	T ₄	8.21	10.46	56.03	2.65	1.70	39.08
7	T ₅	6.74	11.93	63.87	0.68	3.67	84.36
8	T ₆	8.27	10.4	55.68	0.45	3.90	89.65
9	T ₇	1.76	16.92	90.60	BDL	>4.05	>93.10
10	T ₈	BDL	>18.27	>97.85	BDL	>4.05	>93.10
11	T ₉	0.55	18.12	97.05	BDL	>4.05	>93.10
			C.D. at 5%	11.62		C.D. at 5%	9.02

BDL = Blow Determination Limit < 0.42 for cumin and 0.30 µg g⁻¹ for fennel*Mean of two replicates ** Degraded amount = (Mean of replicates of T₀₀ - Mean of replicates of each of the other treatments)**Stannous chloride solution (40%, w/v)**

Arsenic free SnCl₂·2H₂O (40 g) was dissolved in conc. HCl and diluted to 100 ml.

Procedure

A representative mancozeb treated sample (50 g) was taken in 500 ml capacity three necked round bottom flask. Mixture of distilled water (300 ml), conc. HCl (30 ml) and stannous chloride solution (30 ml) was added to it with the help of a dropping funnel. Initially, two absorption tubes (traps/impingers) were attached to the distillation apparatus (Leibig condenser). First tube was filled with NaOH (10 ml) solution. The second absorption tube was filled with 5 ml toluene. A water jet pump was connected to the last absorption tube so that constant rate of air flow through the apparatus was indicated on a flow meter (approx. 300 ml min⁻¹). The content of the flask was heated to boiling. Boiling was continued for 60 minutes. At the end of this period, water supply of water jet pump was stopped and the second absorption tube disconnected. Wash content of the tube was transferred to a 25 ml measuring cylinder and the final volume made up to 15 ml with toluene. The sample was subjected to gas chromatographic analysis.

Efficiency of CS₂ trapping assembly

Prior to the analysis of treated samples, a dummy sample (water fortified with 50 µg g⁻¹ of CS₂) was run in triplicate

and CS₂ trapping efficiency of the assembly was determined by trapping it in toluene using the procedure mentioned above. The quantitative analysis of CS₂ entrapped in toluene was performed on Varian GC 450 equipped with Pulse Flame Photometric Detector (PFPD). The result obtained in the study revealed that 42.2 + 1.66 µg g⁻¹ of CS₂ was detected which is 84.4 + 3.32% of the spiked level.

Recovery of mancozeb from cumin and fennel

An experiment was carried out to determine the recovery of mancozeb from cumin and fennel samples. Each sample was spiked with mancozeb at 1.0 and 5.0 µg g⁻¹ level. Recovery study was performed with 7 replications along with a control and reagent blank. The mean recovery of mancozeb recorded in cumin was in the range of 80.82-83.93% at either dose while per cent Relative Standard Deviation (RSD) was in the range of 3.79-4.49. LOD and LOQ were 0.14 and 0.42 µg g⁻¹ for cumin. The mean data obtained from recovery of mancozeb from fennel was in the range of 88.61-90.99% at respective spiking level while per cent RSD was in the range of 4.66-5.08. LOD and LOQ were 0.10 and 0.30 µg g⁻¹ for fennel. The method applied for the extraction of mancozeb from cumin and fennel was found accurate and precise as described by SANCO (2012)⁶.

Quantitative analysis of CS₂

Mancozeb was estimated by CS₂, its acidic degradation product entrapped in toluene. The quantitative analysis of

CS₂ was performed on Varian GC 450 equipped with PFPD detector. The DB-1 capillary column (30 m x 0.25 mm x 0.25 µm film thickness) was used for analysis. The column was initially held at 70°C and then ramped at the rate of 25 °C min⁻¹ to 75°C and held for 2.0 min and ramped again at the rate of 50.0°C min⁻¹ to 250°C at which it was held for 5.0 minutes. The temperature of injector was set at 75°C while that of detector at 330°C. Nitrogen (purity 99.999%) was used as carrier gas in constant flow mode while flow rate was set at 1.8 ml min⁻¹. Combustion 1 (Air) Flow, Combustion (H₂) Flow and Combustion 2 (Air) Flow were 18, 14 and 7 ml min⁻¹, respectively. The multiplier voltage and trigger level were set to 660 V and 200 mV, respectively. Gate delay and gate width were 4 and 10 min. Sample was injected with autosampler (CP-8400) in the split mode and split ratio was 1:5 while injection volume was 1.0 µl. Prior to analysis, linearity of CS₂ estimation was worked out and found in the range of 5.0 -100 µg ml⁻¹.

RESULTS AND DISCUSSION

The results of the decontamination study on mancozeb in cumin and fennel samples are reported in Table 2. Overall, the heat treatment (T₉), UV-radiation treatments (T₆ and T₇) and chemical decontamination treatments (T₈ and T₉), were found effective in the decontamination of mancozeb from spiked cumin and fennel samples.

Decontamination from cumin

Decontamination of mancozeb from cumin was observed in the range of 28.95 - > 97.85%. Mancozeb residues (as CS₂ µg g⁻¹) in cumin samples subjected to physical decontamination treatments: T₁ (rubbing with muslin cloth for five minutes) and various heat treatments (T₂ to T₉) were in the range of 6.88 - 13.26 CS₂ µg g⁻¹ which indicates that mancozeb was decontaminated in the range of 28.95 - 63.87%. Among physical decontamination treatments, T₂ (heat treatment, 60 °C for 2.0 min) recorded the minimum decontamination i.e. 28.95% while it was maximum (63.87%) in treatment T₅ (heat treatment, 90 °C for 4.0 min) for cumin.

Generally, higher decontamination was observed in chemical decontamination treatments as compared to physical decontamination treatments. Decontamination of mancozeb with chemical treatments was in the range of 97.05 → 97.85%. Chemical treatment T₈ (dipping in starch solution followed by rubbing with muslin cloth) showed the

highest decontamination value (> 97.25%) followed by T₉ (washing with 5% sodium hypochlorite solution) which recorded 97.05% mancozeb decontamination.

Among the irradiation treatments, T₇ (exposure to UV radiation for 45 min) gave higher decontamination of mancozeb (90.60%) as compared to treatment T₆ (exposure to UV radiation for 30 min) which recorded only 55.68% decontamination of mancozeb from cumin samples.

Cumin treatments T₇, T₈ and T₉ though statistically at par, T₇ was better from the point of view of its practical applicability. Application of starch initiated the growth of fungi while foul odour of sodium hypochlorite reduced the acceptability and economical value of cumin seeds.

Decontamination from fennel

Mancozeb decontamination from fennel samples with various treatments ranged from 15.86 → 93.10%. Treatment T₂ recorded the minimum decontamination i.e. 15.86% among all treatments while maximum decontamination (> 93%) was observed in treatments T₇, T₈ and T₉. The treatments T₁, T₃, T₄ and T₅ recorded decontamination of mancozeb from fennel in the range of 39.08-84.36%.

The treatments T₆, T₇, T₈ and T₉ recorded maximum decontamination of mancozeb from fennel i.e. 89.65- >93.10% and were found statistically at par. However, the treatment T₇ is preferable to other treatments because of its practical advantages.

Chemical and photolytic degradation may be potential reasons for degradation of mancozeb from cumin and fennel samples. The quantum energy available at longer wavelengths (above 400 nm) is insufficient to break most kinds of chemical bonds commonly found in pesticides⁷. The ultraviolet region is the most important portion of the sunlight spectrum because pesticides generally absorb sunlight most readily at this wavelength⁸. It is believed that absorption of light causes breakage of the carbonyl C-S bond producing two radicals. These can combine with protons from the solvent to give formamide and mercaptan. Formamide is further degraded by ultraviolet radiation (UVR) to dialkylamine by the elimination of carbon monoxide. Collision of two mercaptan radicals would lead to the formation of a disulfide. Because the sulphur-sulphur bond is quite susceptible to photolysis, continued exposure to UV radiation would result in separate mercaptan radicals, and the possible reformation of the disulfide. Changes in the availability of protons could influence the concentrations

of mercaptan and disulfide formed. Considerably high degradation of mancozeb with UV radiation in both commodities is in confirmation of above mentioned justification where UV radiation is found suitable for removing the residues of mancozeb from various matrices.

Chlorine, chlorine dioxide, ozone, and hydrogen peroxyacetic acid (HPA) treatments were found effective for the degradation of mancozeb in apples at neutral pH and ambient temperature. Mancozeb residues were decreased by 56-99% with chlorine and 36-87% with chlorine dioxide treatments⁹. Cumin and fennel recorded >90% degradation of mancozeb when treated with 5% sodium hypochlorite for the decontamination of mancozeb. Similar results were obtained for the decontamination of mancozeb in fresh apples with the use chlorinated decontaminants¹⁰.

In general chemical treatments gave higher decontamination as compared to physical treatments in cumin and fennel. But these treatments reduce the acceptability and economical value of cumin and fennel. Therefore, on the basis of present investigation, exposure to UV radiation for 45 minutes has been found better decontamination treatment from the point of view of practical adoption.

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