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GC aided method for extraction and quantification of dithiocarbamate residues in cumin seeds

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Abstract

Cumin is an annual, herbaceous, high valued low volume crop of the family *Apiaceae*. Owing to its inherent flavour and aroma imparting biochemical constituents, there exists a greater demand from domestic as well as foreign markets as a food ingredient and as a raw material for cosmetic and pharmaceutical industries. Being predominantly grown as a rabi crop in major growing regions of the country, cumin is the crop of first choice among the farmers of arid and semi-arid parts of Rajasthan and Gujarat, the dominant cumin growing states of the country together sharing an area and production of ninety nine per cent. Despite of attractive income involved in farming, the crop is highly vulnerable to major dreadful diseases like *Alternaria Blight* provincially referred to as *kalia* and *Fusarium* wilt, apart from other pest and diseases like aphids and powdery mildew, respectively. Farmers with the fear of losing their crop and with an anticipation of harvesting blemish free produce, are indiscriminately spraying dithiocarbamate group of pesticides like mancozeb as a preventive and control measure against blight and wilt. Hence in the present study an easy and friendly analytical method to determine the residues of dithiocarbamate group of pesticides in the form of $CS_2$and its subsequent quantification in GC has been validated.

Key words: *Cumin, alternaria blight, fusarium wilt, powdery mildew, kalia, pesticides.*

Introduction

Since the dawn of civilization, humanity had two primary goals - obtaining enough food to survive and improving the standard of life. By then the most important task was to produce enough food to feed its population before it can devote resources to education, arts, technology or recreation. A basic fact of green revolution in India which converted a food deficient state to a food surplus state was the ability of controlling pests and diseases of high yielding varieties by providing effective plant protection umbrella with proper agronomic management. According to Ware and Whitcare (2014) about one third of the world's food crops are destroyed by pests during growth, harvesting and storage. Today, farmers regard pesticides as an essential tool to ensure crop production of good quality and quantity (Beena Kumari and Kathpal, 2010). Availability of advanced instrumentations like HPLC, GC, GC-MS, LC-MS and MS-MS detectors in the present era are helping in precisely tracing the type and quantity of pesticide residues with suitable extraction procedures.

Cumin (*Cuminum* cyminum L.) is an annual herbaceous high valued low volume seed spice grown predominantly as a rabi crop in Rajasthan and Gujarat states of India. The huge demand from western countries majorly European Union and USA is paving a greater opportunity for the country's foreign earnings and further expansion of the sector, it is estimated that 1,83,820 (Rs. In lakhs) valued cumin *i.e.*, 1,55,500 tonnes is being exported from India during the year 2014-15 (Spices Board Export Statistics). Owing to such a great potential involved in its trade, cumin is the crop of first choice among the farmers of arid and semi arid parts of Rajasthan and Gujarat, despite of its severe vulnerability to Alternaria Blight

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(*Alternaria burnsii*) and Fusarium wilt (*Fusarium oxysporum* f. sp. cumini) diseases. Though wilt is found to be infecting in seedling stages, occurrence of *Alternaria* blight is profound under specific favourable climatic conditions like moist humid conditions (Khare, *et.al*., 2014). According to Lakra (2005) the incidence of *Alternaria* blight increased with the increase in the duration of dewfall, leaf wetness, relative humidity, congenial temperature and number of rainy days. Mencozeb is the major pesticides used for controlling blight disease. Their residues in harvested seeds are creating hindrance in export of cumin to EU, USA and other developed countries. Hence, the present analytical experiment was carried out at AINP, PRL, AAU, Anand, Gujarat to validate Mancozeb assembly method to determine the residues of dithiocarbamate group of pesticides in cumin samples and subsequent quantification in GC.

Materials and methods

An analytical study under laboratory conditions was conducted to validate the method for extraction and quantification of dithiocarbamate residues in cumin seeds using GC, at Pesticide Residue Analysis Laboratory, Anand Agricultural University, Anand, Gujarat.

Cumin seed samples were collected from an organic source and used for fortification with definite concentrations of CS_2 and Mancozeb. Though, the sample thus collected is of organic origin, prior to fortification, it was pre examined to affirm the fact that, it is free from dithiocarbamate residues. After confirming its true organic origin, the cumin seeds were fortified by spiking with known concentrations of CS_2 and Mancozeb separately with three replications and then extracted using mancozeb assembly method (fig. 1). The extracted supernatant was then fed into GC to determine the recovery percentage.

Preparation of standards for fortification and analysis

Stock solution of CS_2: CS_2 of 99.97% purity was procured from Qualigen India Ltd. Exactly 1.318 g of CS_2 was weighed and later the final volume was made to 25 mL using iso-octane solution. The resultant concentration of CS_2 obtained was 52720 ppm.

Intermediate standard of mancozeb

Exactly 33.33mg of Mancozeb (75% purity) was dissolved in 1% EDTA solution and final volume was made up to 500 mL using Iso-octane. The resultant concentration as per 100 per cent purity is 66.6 ppm, since the purity of mancozeb used was 75 per cent, the final concentration obtained was 50 ppm.

Intermediate standards of CS_2

Through dilution, Intermediate standard of 5000 ppm of 25 mL CS_2 was prepared by dissolving 2.37 mL of 52720 ppm CS_2 in 22.63 mL of iso-octane solution and further dilution of 100 ppm was prepared by dissolving 0.5 mL of 5000 ppm CS_2 in 24.5 mL of iso-octane solution.

Working solutions of CS_2 and Mancozeb

Working concentrations of 10 ppm, 5 ppm, 2 ppm, 1 ppm, 0.5 ppm, 0.25 ppm, 0.1 ppm and 0.05 ppm was prepared from their respective intermediate standards by serial dilution.

Fortification with $CS_2$and Mancozeb standards

The organic cumin seed samples thus procured and examined were used for manual spiking (fortification) and subsequent extraction and quantification.

Apparatus

Three necked round bottom flask of 1000 mL volume, B-24 joint condenser, connecting tubes, two traps, glass tubes, air inlet glass tube and water suction pump (Fig. 1).

Fig. 1: Mancozeb Assembly

Reagents

Sodium Hydroxide (10%, w/v): NaOH pellets weighing 10 g were dissolved in 100 mL of distilled water to obtain 10% NaOH solution.

Stannous Chloride solution (40%, w/v): Arsenic free $SnCl_2.2H_2O$ weighing 40g was dissolved in 100 mL conc. HCL to obtain 40% stannous chloride solution

Procedure

Cumin control sample weighing 100 g was taken in a round bottom flask with three neck at the top. The sample was then fortified with CS_2 and mancozeb fungicide separately in different samples at a fortification level of 320 µL of 100 ppm concentration. To this mixture about 30 mL of 40 % stannous chloride and 30 mL of HCL was added through dropping funnel fixed on side neck of the round bottom flask. About 10 mL of NaOH and 10 mL of iso-octane (to trap the CS_2 gas from the evaporated gas) was taken in two individual absorption tubes (traps/improngers) attached distantly to the distillation apparatus and a water jet pump was connected to the last absorption tube containing iso-octane to facilitate the suction through the apparatus (approx. 300 mL min^{-1}) as shown in the above figure 1. The content in the flask was made to boil by igniting flame at the bottom of the flask using bunsen burner and water pump that was connected to suck the air from boiling mixture was switched on. The content was boiled for 60 minutes. After 60 min of boiling, the iso-Octane with the trapped CS_2 was taken in a 10 mL centrifuge tube and from this 2 mL of aliquot was taken in a vial for determining the recovery of mancozeb in terms of amount of CS_2 (1g mancozeb emits 0.557 mg of CS_2)by using GC-MS.

Quantitative analysis of CS_2

Dithiocarbamates was estimated by quantifying CS_2, the acidic degradation product of dithiocarbamate pesticides, by entrapping in iso-octane. The quantitative analysis of CS_2 was performed in 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 estimation. The initial temperature of column was fixed to 70° C and ramped at the constant pace of 25° C min^{-1} to 75° C and withheld for a duration of 2 min. and again ramped at a constant pace of 50° C min^{-1} to 250° C for a withhold duration of 5 min. The temperature of injector and detector was set to 75° C and 350° C, respectively. Nitrogen gas of purity 99.999% was used as carrier gas with a content flow of 1.8 mL min^{-1}. Combustion 1 (Air) flow, combustion H_2 flow and combustion 2 (Air) flow were 18, 14 and 7 mL min^{-1}, respectively. The multiplier voltage and trigger level was 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 CS_2 and Mancozeb standards was worked out and found to be R^2=0.998 (fig. 2 and fig 3, respectively).

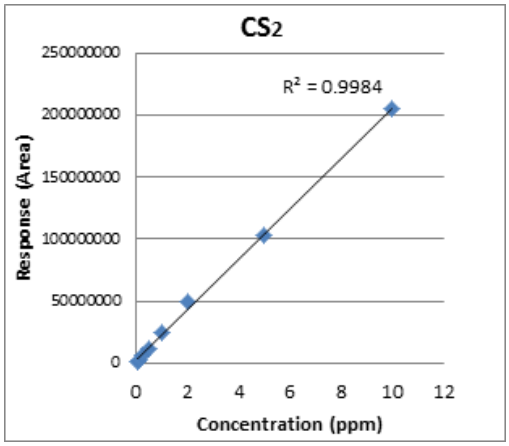

Fig. 2: Linearity curve of different concentrations of CS2

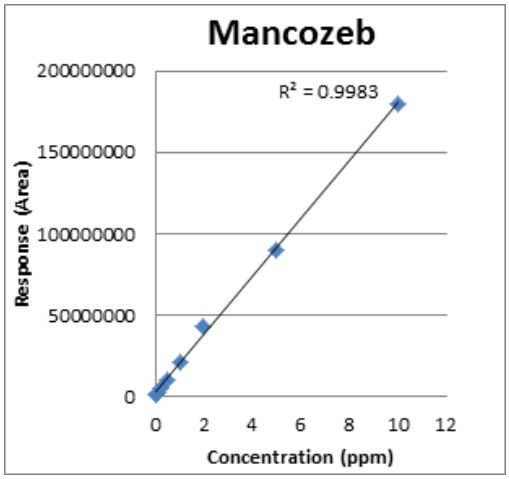

Fig. 3: Linearity curve of different concentrations of Mancozeb

Results and discussion

In the present analytical study it was found that, among all the fortified samples subjected for extraction and quantification of dithiocarbamate pesticide residues, an acceptable percentage of CS_2, the acid degraded product of dithiocarbamate pesticides was recovered from both the CS_2 and Mancozeb fortified samples. Since the recovery percentage thus obtained was within the acceptable range of 80 to 120 per cent, the method thus described above was considered as valid and was reliably used for extraction and GC aided quantification of dithiocarbamate group of pesticide residues in cumin seed samples.

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