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CUMIN SEED OIL AS AN ANTIFUNGAL PRESERVATIEE OF FOOD GRAINS IN STORAGE

BY

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The antifungal activity of cumin seed oil was studied in plate culture as well as on stored grains of pearl-millet. The test oil showed fungitoxicity *invitro* against *Aspergillus flavus* and *Aspergillus glaucus*, the predominant storage fungi in grains. The oil also controlled the grain infection and subsequent deterioration completely when treated at the level of 25 μl/g of grains. The ED_{50} was found to be at 20 μl/g.

INTRODUCTION

The post-harvest preservation of food grains from fungal deterioration remains a primary challenge to be resolved. The use of antifungal volatile compounds and other synthetic fungicides as grain preservatives, though attracted a lot of attention, has never been fully satisfactory (Jones, 1970 ; Christensen, 1973 ; Nelson *et al*, 1973 ; Hearting and Drury, 1974 ; Frankenfeld *et al.*, 1975 ; Nandi and Fries, 1976; Nandi, 1978 a&b; Ghosh *et al*, 1982). Moreover, the indiscriminate application of these synthetic preservatives may pollute the environment and thus poses a serious health threat. In addition, the development of undesirable resistant fungi cannot be totally ruled out. Hence, a hectic research is certainly called for to exploit the antifungal efficacy of natural phytochemicals to controls the field as well as storage fungi in grains. In recent years, inhibition of fungal growth by several essential oils of aromatic plants has been extensively studied (Nigam and Rao, 1977 ; Gulati and Suri, 1980 ; Kurita *et al.*, 1979, 1981 ; Kurita and Shigerukoike,1982 ; Forstrenter and Burkhard 1985; Karapinar,1985), However, reports on their grain preservative efficacy is still very meagre.

The present paper describes the fungitoxicity and grain preservative efficacy of natural cumin seed oil.

MATERIALS AND METHODS

Test Organisms

Aspergillus flavus and *Aspergillus glaucus*, which infected the pearlmillet grain in storage, was isolated from the grains and used in the present study.

Test chemicals

Essential oil of cumin seed (*Cumin cyminum*), procured from S. H. Kelker and Co,, Pvt. Ltd., Bombay, was tested for antifungal activity and grain preservative efficacy.

Assay af Cumin seed oil for fungitoxicity to plate culture

Sterilized plates of salt malt agar (10% NaCl in 2% Difco-malt agar) and malt agar were inoculated with single loopfuls of test fungi, Sterilized paperdiscs (10 mm. diam.) soaked in cumin seed oil were kept on top of the inocula. In control set, water was used instead of the essential oil. The petriplates were incubated at 30°±1°C for 7 days. The diameter of the fungal colonies were measured and the fungitoxic activity of the test oil calculated by the formula,

$$F = E/C \times 100$$

Where F is the percentage inhibition of fungal growth and E represents the diameter of the fungal colony in the presence of the test oil, and C is the colony diameter in control set.

Effect of the oil on spore germination in vitro

Cumin seed oil, diluted in 70% ethanol, was added to the solid salt malt agar and medium (5 ml medium/plate) at the concentration of 10%, 15%, 20% and 25% respectively. A measured volume of spore suspension (1.0×10^4 spores/ml) of *Aspergillus flavus* and *Aspergillus glaucus* (taken earlier from 10 days old slant culture) was sprayed on the medium. In control set, an equal volume of ethanol in place of the test oil, was used. After incubation in a moist chamber at 30°±1°C for 10 h, agar discs were aseptically punched out with a sterilized cork borer and placed on slides under cover slips. The percentage of spore germination was recorded by microscopic count and compared with the control.

Grain preservative efficacy of the oil and ED_{50}

Grains of pearl millet (*Pennisitum typhoideum*) used in this experiment, were collected locally after harvest. Grain moisture was elevated to 15% following Lutey and Christensen (1963) by adding requisite quantity of sterile distilled water to the grains in tightly stoppered bottles and storing them for 3 days at 4°C with occassional shaking to facilitate uniform distribution of moisture. Grains (1000 g) were then kept in closed polythene bags and sprayed with cumin seed oil at measured volume of 10, 15, 20 and 25 μl/g of grains. Replicate sets together with control (untreated) were kept as such with periodic shaking for 3 days after which the polythene bags were made perforated for a easy escape

of the excess volatile oil. Grains were then stored under normal conditions for 180 days.

The preservative efficacy of the test oil was recorded by evaluating the grain deterioration after 30, 90 and 180 days of storage. The ED_{50} (effective dose for controlling infection in 50% of the seeds) was also determined.

The percentage of grain infection was estimated by placing the grains (surface sterilized with 2% sodium hypochlorite) both on malt agar and salt malt agar at pH 6.5 in petriplates (40 grains per plate) and incubating at 30°±1°C for 10 days.

The extent of fungal growth in the grains was measured in terms of chitin content of the invading mycelia following Nandi (1978a).

The total grain carbohydrate was estimaied following Viles and Silverman (1949) and McCready *et al.* (1959).

RESULTS

Fungitoxicity of cumin seed oil

The cumin seed oil arrested the growth *A. flavus* and *A. glaucus* in plate culture showing a 100% fungitoxic activity. No growth inhibition was, however, recorded in control.

Invitro inhibition of spore germination

The test oil suppressed the spore germination completely at 25% concentration. The inhibition by the oil at 20% concentration was also considerably high. (Table 1).

Table 1. *Percentage germination of Aspergillus spores treated with cumin seed oil (Each value represents the arithmatic mean of five replicates)*

Fungi	% spore germination Concentration (%)				
	0	10	15	20	25
Aspergillus flavus	82	72	48	15	0
Aspergillus glaucus	84	71	52	17	0

Preservation of grains by the oil

The control grains deteriorated progressively with storage showing a 100% fungal infection. Grain infection was controlled completely when treated with the test oil at 25μl/g. (Table 2).

The fungal chitin in control grains increased considerably over initial value (220μg/g of grains) with the period of storage. Treatment of oil (25μl/g)

inhibited the fungal growth completely showing no apparent increase of chitin content. (Table 2).

Table 2. *Fungal deterioration of the control and the oil-treated grains during storage (Each value represents the arithmatic mean of five replicates)*

Storage period (days)	Infected grains (%)		Increase in chitin content (%)		Decrease in total carbohydrate (%)	
	Control	Treated	Control	Treated	Control	Treated
30	70	0	13.63	N.D.	2.0	—
90	100	0	45.90	N.D.	7.2	—
130	100	0	174.54	N.D.	20.5	—

N.D. : Not detected

— : Value did not vary significantly from the initial.

In control grains, the total carbohydrate was also on the decline during storage. Treated grains (25μl/g), however, showed no evident of reduction. (Table 2).

ED_{50}

The test oil at 25μl/g was optimum for 100% growth inhibition. However, ED_{50} was found to be at 20μl/g.

DISCUSSION

From the results it is evident that the cumin seed oil is antifungal in nature and can be used as an effective grain preservative against fungal deterioration. Moreover, the *invitro* experiment shows that the test oil controls the infection at the spore germination level in the life cycle of the test fungi. In addition, the oil also checked the mycelial growth as is revealed by the unchanged chitin content in the treated grains during storage. The fungitoxic efficacy of the test oil is confirmed further by estimating the total carbohydrate which shows no decline over the initial value in the treated grains. However, control grains shows profuse fungal growth with a concomitent increase in their chitin content and a significant loss in total carbohydrates.

It is interesting to note that a single spray of the oil at 25μl/g not only killed the seed-borne fungi, but also preserved the grains from reinfection throughout the test period. This indicates the durability of the antifungal action of the test oil. The mechanism by which cumin oil inhibits growth, however, remains to be seen. It was earlier reported that the constituents of volatile oils interfere with the selective permeability of fungal cell wall (Gracza and Szasz, 1968). Kurita *et al* (1979, 1981), however, observed that the antifungal activity of the essential oils was related to the molecular orbital energies of the component aldehydes, i e. the lower the energy, the higher is the antifungal activity. It was

further suggested that the aldebydes possibly form charge transfer complexes with electron donors in the fungal cells.

Thus, the treatment of grains with cumin oil will reduce the fungal population and the possibility of mycotoxin contamination during storage. Moreover, the text oil, being of food-grade quality, will never be toxic to men and animals.

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