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FUNGITOXIC PROPERTIES OF THE ESSENTIAL OILS (ANETHUM & CUMINUM OIL) AGAINST *Alternaria alternata*

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ABSTRACT

The minimum inhibitory concentrations (MIC) of the Anethumand Cuminum oils were found to be 500 ppm against the test fungus and these oils exhibited fungistatic nature on their MIC(s). However the oils exhibited fungicidal nature at above their MIC(s). The effect of increased inoculum density of the test fingus on the fungitoxicity of the Anethum and Cuminum oils was studied and the oils inhibited the fungal growth of the treatment set containing 16 fungal discs indicating their capacity to withstand high inoculum density. Moreover, the oils remained absolutely effective up to 240 days of their storage, the maximum period taken into considertion against test fungus. Effect of temperature on fungitoxicity of Anethum and Cuminumoils were studied by exposing the oils at different temperatures *viz*. 50^0, 70^0 and 90^0 C for three hours and testing of fungitoxicity against test fungus at their respective MIC(s) by the usual poisoned food technique. The fungitoxic potentiality of the oils remained unaltered showing thermostable nature of toxicity. The fungitoxic potency of Anethum and Cuminum oils was compared with some prevalent synthetic fungicides and these were found to be more efficacious than some prevalent fungicides *viz.* Capton, Dhanuka M-45, Agrozim, Bavistin, Benlate, Emison and Dithane M-45. The fungitoxic spectrum of the oils were studied against 24 storage fungi at 500 and 1000 ppm concentration and these exhibited a broad fungitoxic spectrum. The volatile activity of the oils was studied for estimating the amount of oil vaporizing on fixed temperature in known time. The study reveals that Cuminum oil exhibited better evaporation in comparision to that of Anethum oil.

KEYWORDS: MIC, Fungus, Fungitoxicity, Cuminum Oil

The side-effects of synthetic fungicides means that alternative strategies need to be developed for reducing losses due to post-harvest decay that are perceived as safe by the public and pose negligible risk to human health and environment (Sbragia, 1975; Cook & Baker, 1983; Suryanarayan, 1978; Wilson & Wisniewski, 1989;

Thus, replacement of synthetic fungicides by natural products (particularly of plant origin), which are non-toxic and specific in their action, is gaining considerable attention. Because of their biodegradable nature they do not cause serious alternations in ecological systems of the nature. There has been a renewed interest in botanical pesticides because of several distinct advantages: (1) Plant origin pesticides are much safer than conventionally used synthetic pesticides. Pesticides plants have been in nature as its component for millions of years without any ill or adverse effect on ecosystem. (2) Plant based pesticides will be renewed in nature and would be cheaper. (3) Some plants have more than one chemical as active principle responsible for their biological properties. The research and development cost of biopesticides from discovery to marketing is much less compared to chemical pesticides (Cariton, 1988; Woodhead *et al.,* 1990).

The plant world comprises a rich storehouse of biochemicals that could be tapped for use as pesticides. The toxic constituents present in plants represent the secondary metabolites and have only insignificant role in primary physiological process in plants that synthesizes them (Cooper and Johnson, 1984). This is an interesting approach in exploiting the plant products in enhancing the shelf life of stored fruits and vegetables from their microbial deterioration without altering the taste and quality of the treated commodities. Some essential oils have been reported as synergists in enhancing the insecticidal activity of pyrethrum. Combination of *Blumea* sp. andpyrethrum have been compared to piperonyl butoxide and pronounced variation in synergistic coefficient is recorded (Saxena and Koul, 1982). The pesticidal plant receiving global attention for the last two decades is the wonder tree of Indian origin Neem (*Azadirachta indica*). The aqueous neem leaf extracts have shown inhibition to DNA polymerase enzyme of hepatitis B virus. Multinational firms from Japan, Germany and U.K. are trying to extract an enzyme from neem, which inhibits division of AIDS infected cells (Dev Kumar & Sukhdev, 1993).

The essential (volatile) oils produced by different plant genera are in many cases biologically active, endowed with allelopathic antioxidant and bioregulatory properties (Elakovich, 1988; Deans *et al.* 1990, Caccioni and Guizzardi, 1994). The volatility, ephemeral nature and biodegradability of flavour compounds of angiosperms will be specially advantageous if they are developed as pesticides (French,

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1985). Therefore it is important to test the fungitoxicity of essential oils.

MATERIALS AND METHODS

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration at which the oils showed absolute fungitoxicity was determined by the usual poisoned food technique. Different concentrations of the oils (250, 500, 750 and 1000 ppm) were prepared by dissolving the requisite amount in 0.5ml acetone and then mixing with 9.5 ml potato dextrose agar medium separately. The medium of control set contained requisite amount of sterilized water and 0.5 ml acetone in place of the oils. As usual the plates were inoculated aseptically with the assay disc of the test fungus *Alternaria alternata* and incubated for six days at 25 ± 2^0C. The observation was recorded on the seventhday and percentage mycelial inhibition was calculated. Results are presented in Table 1 and Fig.1a, b.

Nature of Toxicity

Nature of toxicity (fungistatic/fungicidal) of the oils against the test fungus was determined following Garber and Houston (1959) and Thompson (1989).

Requisite amounts of the oils were dissolved in 0.5 ml acetone and then mixed with 9.5 ml potato dextrose agar medium to get final concentration of 250, 500, 750 and 1000 ppm. Sterilized water was used in control sets in place of the oils. The plates were inoculated aseptically with fungal disc (4mm diameter taken from the periphery of a seven day old culture of the test fungi) and were incubated for six days. On seventh day the incubated discs were taken out from the plates, washed with sterilized water and reinoculated aseptically to plates containing fresh potato dextrose agar medium. The revivals of the discs were observed and the percentage mycelial inhibition with respect to control was calculated on the seventh day and results are presented in Table-1.

Table 1: Minimum inhibitory concentrations and nature of fungitoxicity of oils of *Anethum graveolens* and *Cuminum cyminum*

Concentration in ppm	Percent Mycelial Inhibition of test fungus ± SD	
	Anethum Oil	Cuminum Oil
250	52.43±6.93	53.82±2.59
500	100*	100*
750	100**	100**
1000	100**	100**

Where, * is Denotes Fungistatic nature, ** is Denotes Fungicidal nature

Effect of Increased Inoculum on Toxicity of the Oils

The effects of increased inoculum density of test fungus *A. alternata* on fungitoxicity of the oils were studied following Mishra(1992) at MIC(s) of oils.

Requisite quantity of Anethumand Cuminum oils were dissolved in 0.5ml acetone and was mixed to 9.5 ml Potato dextrose broth medium separately to make the MIC(s) of oils i.e.500 ppm. Five sets thus prepared from each oil were inoculated separately by the assay discs (4mm) of the test fungi in geometrical progression of two, i.e., 1, 2, 4, 8 and 16. For control, sterilized water dissolved in acetone was mixed to the liquid medium. All flasks were incubated for five days at 25 ± 2^0C. Observation was recorded on the sixth day. (Table- 2)

Table 2: Effects of increased inoculum density of *Alternaria alternata* on the antifungal activity of the Anethum and Cuminum oils

Number of inoculated discs	Control	Mycelial growth of the Test Fungus Treatment at MIS (s)	
		A. graveolens Oil	*C. cyminum* Oil
1	+	-	-
2	+	-	-
4	+	-	-
8	+	-	-
16	+	-	-

Where, – = Indicates no growth of fungus, + = Indicates growth of fungus

Effect of Storage and Temperature on Fungitoxicity of the Oils

Anethum and Cuminumoils were stored in air tight specimen tubes separately at room temperature and their activity against the test fungus *A. alternata* was tested at regular interval of one month at their respective minimum inhibitory concentrations following poisoned food technique. The observation were recorded and percentage mycelial inhibition was calculated (Table-3).

Table 3: Fungitoxicity of the oils stored for different periods at their MIC(s)

Period of storage of the oils (in days)	Percent mycelial growth of the test fungus *Alternaria alternate*	
	Anethum Oil	Cuminum Oil
30	100	100
60	100	100
90	100	100
120	100	100
150	100	100
180	100	100
210	100	100
240	100	100

To study the effects of temperature on toxicity of Anethumand Cuminumoils, 2 lots of oils each containing 2 ml of the oils separately were taken for the study. First lot of each oils were kept in Borosil glass specimen tubes air tight with their caps (close) and second lot of the oils were also kept in similar specimen tubes without caps (open) and were treated at different temperatures *viz.* 50, 70 and 90^{0}C) for three hours. The oils of each lots were cooled to room temperature and tested for fungitoxicity by the usual poisoned food technique at their minimum inhibitory concentration. The data are presented in Table-4

Table 4: Fungitoxicity of the oils exposed to different temperature on their MIC(s)

Oil incubated at temp. ^{0}C	**Percent mycelial growth of test fungus *A. alternate***			
	Anethum oil		**Cuminum oil**	
	Open	**Close**	**Open**	**Close**
50	100	100	100	100
70	100	100	100	100
90	100	100	100	100

Comparison of Effectiveness of the Oils with some Prevalent Synthetic Fungicides

The comparative efficacy of the Anethum and Cuminum oils with some available standard synthetic fungicides *viz.* Agrozim, Bavistin. Emison, Capton, Dithane M-45, Benlate and Dhanuka M-45 were studied against the test fungus *A. alternata* by the usual poisoned food technique. The fungicides and oils in requisite amounts were dissolved separately in 0.5 ml acetone and mixed with 9.5ml at potato dextrose agar medium to obtain different concentrations. For control, requisite amount sterilized water dissolved in 0.5 ml acetone was used in place of fungicides/oils. The plates were inoculated aseptically with the assay disc and incubated for five days at 25±2^{o}C. The observation was recorded on the sixth day and percentage mycelial inhibition was calculated. The data are presented in Table 5 & Fig 2.

Table 5: Comparative efficacy of synthetic fungicides and the oils of Anethum and Cuminum against test fungus

Fungicides / Oils (in ppm)	Minimum inhibitory concentration against A. alternate
Anethum oil	500
Cuminum oil	500
Capton	4500
Dhanuka M-45	4500
Agrozim	5000
Bavistin	5000
Benlate	5000
Emison	5000
Dithane M-45	5500

RESULTS

(I) It is evident from Table 1 that *Anethum* and *Cuminum* oils exhibited absolute toxicity upto 500 ppm against test fungus. Therefore, the minimum inhibitory concentrations of *Anethum* and *Cuminum* oils were assigned to be 500 ppm.

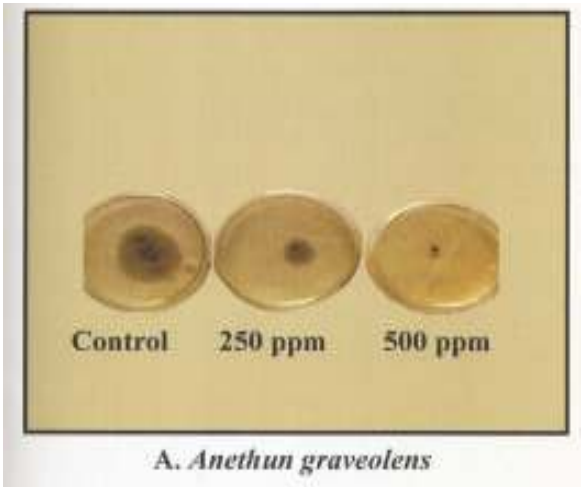
(II) Table 1 shows that *Anethum* and *Cuminum* oils were fungistatic against the test fungus on their respective MIC(s). However the oils become fungicidal at above concentration

(III) The results of the Tables 2 show that the oils inhibited the mycelial growth of the treatment set containing even 16 fungal discs, indicating their capacity to withstand high inoculum density.

(IV) The data represented in Table 3 shows that fungitoxicity of Anethum and Cuminum oils remained unaltered up to 240 days of its storage against test fungus *A. alterata* the maximum time taken into consideration showing the long self life of oils.

(V) Table 4 predicts that the oils remained effective even up to 90°C the maximum temperature taken into consideration showing the thermostable nature of their fungitoxicity

(VI) The data presented in Table 5 & Fig. 2 shows that the Anethum and Cuminum oil were 11 times superior to Dithane M-45, 10 times to Agrozim, Bavistin, Benlate, Emison and 9 times to Capton and Dhanuka M-45. Moreover Citrus oils were 5 times superior to Celphas & Sulphex, 3.5 times to Dithane M-45, 3 times to Agrozim, Bavistin Emison & Benlate and 2.5 times to Dhanuka M-45.



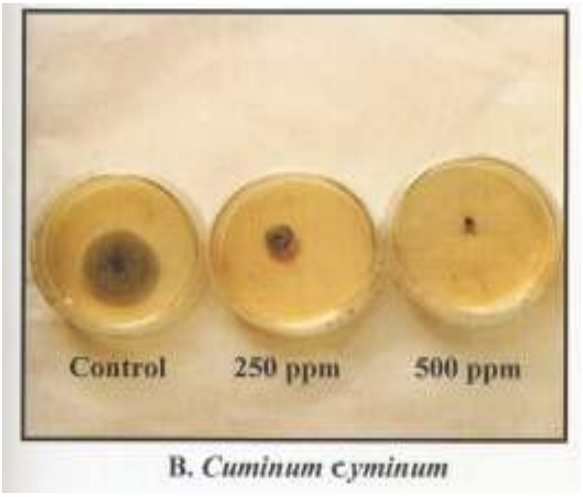


Figure 1: MIC Experiment showing variation in growth of *A.alternata*

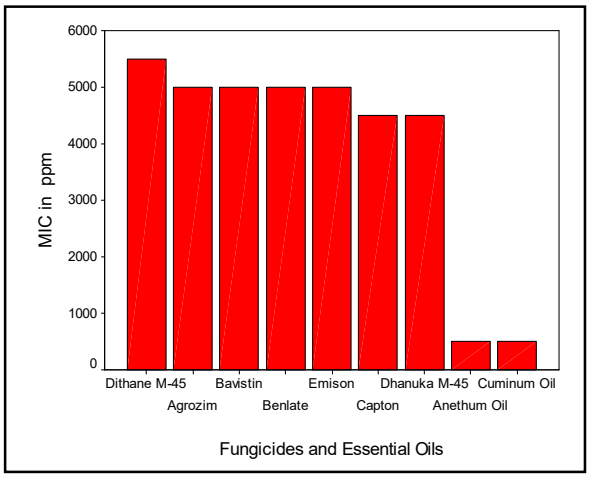


Figure 2: Comparative efficacy of synthetic fungicides and the oils of Anethum and Cuminum agains test fungus

DISCUSSION

There are two principal factors which make plant products more susceptible to spoiling: the high water content in fruit which allows pathogen attack (Harvey, 1978) and the wounds present on the plant organs during storage, often as a result of harvesting and transportation, which give microorganism easy access.

When permitted, synthetic fungicides are the primary means to control postharvest diseases. However, several reasons, such as the public's growing concern for the human health conditions and the environmental pollution associated with pesticide usage on orchards (Wilson and Wisniewski, 1994), the development of

fungicide-resistant strains of postharvest pathogens (Romano *et al.*, 1983; Spotts and Cervantes,1986) and the lack of continued approval of some of the most effective fungicides (Gullino and Kuijpers, 1994) have motivated the search for alternative approaches.

Therefore in the present study it was thought desirable to find out the potentiality of some higher plant products (essential oils) in control of postharvest Alternaria rot of Tomato during storage and transportation. However different test fungi of stored fruit commodities *viz. Aspergillus flavus* (Mishra & Dubey, 1994); *A. niger* (Pandey, 2003); *Penicillium expansum* and *P. digitatum* (Agrawal, 2003) have been taken by various workers during screening of essential oils. In the present study *Alternaria alternata* was selected as test fungi since it causes severe postharvest rotting of tomato during storage and produces mycotoxins, which are hazardous to the health of animal and man. (Mary *et al.,* 1987; Agrawal *et al.,* 2001).

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