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CONTROL OF FOOD PATHOGENIC FUNGI BY OIL DERIVED FROM SEEDS OF INDIAN SPICE PLANT, *Foeniculum vulgare*

Priyanka Chaudhary[1] and Padma Singh[2]

[1,2]*Dept. of Microbiology, Kanya Gurukul Campus, GKV, Haridwar-249407*
E-mail: [1]priyanka.chaudhary817@gmail.com, [2]dr.padmasingh06@gmail.com

Abstract—*Food borne diseases of microbial origin are major international health problem associated to food safety and are important cause of death in the developing countries. Food safety and conservation has been characterized for nutritious and microbiologically stable foods and it can be achieved by controlling the growth of spoiling and pathogenic food related micro-organisms.Due to residual toxicity associated with chemical preservatives, spices and essential oils are used by the food industry as natural agents for extending the shelf life of foods. The present study was done to evaluate the antifungal potency of oil derived from F.vulgare seeds by Food Poisoned Technique (FPT) and Agar Well Diffusion Assay against Aspergillus flavus and A.niger. In FPT, F.vulgare essential oil in pure as well as diluted form showed 100% Inhibition against both the fungi, A.flavus and A.niger. Similar results were observed in Agar Well Diffusion Assay against both the fungal species, with maximum zone of inhibition against A.niger (17-12mm) having MIC value of 128µg/ml. The result provided us a strong basis for the development and utilization of F.vulgare as both pharmaceuticals and dietry supplement*

Keywords: *Aspergillus flavus, Aspergillus niger, FPT, Agar Well Diffusion Assay, MIC.*

1. INTRODUCTION

Food borne diseases are a severe health problem in the world, even in well developed nations. The consumption of food contaminated with food borne micro-organisms can pose a serious threat to human health. The existence of micro-organisms causes spoilage and results in reduction of the quality and quantity of processed foods. Some biologically active compounds isolated from spices and herbs have been in use for the inhibition of growth of pathogenic micro-organisms because of the resistance that micro-organisms have built against antibiotics [1].

Herbal spices, being promising source of phenolics, flavonoids, anthocyanins and carotenoids are usually used to impart flavor and enhance the shelf life of dishes and processed food products. Essential oils, extracts and bioactive constituents of several spices and herbs are well known to exert antioxidant and antimicrobial activities.

Fennel (*Foeniculum vulgare*) is a biennial medicinal and aromatic plant belonging to the family Apiaceae. It is a highly aromatic and flavorful herb with culinary and medicinal uses. Dried fennel seed is an aromatic, anise, flavored spice, brown or green in colour when fresh, slowly turning a dull grey as the seed ages. Traditionally, fennel seeds are used as anti-inflammatory, analgesic, carminative, diuretic and antispasmodic agents. Recently, there has been considerable interest in the antioxidant potential and antimicrobial activities of fennel seed extracts and essential oil.

The purpose of the present study was to evaluate the antifungal activity of essential oil derived from seeds of *F.vulgare.*

2. MATERIALS AND METHODS

2.1. Collection of spice material

Mature and dried fennel seeds were collected from the local market of Hardwar (Uttarakhand), India. The spice material was taken to the laboratory and was authenticated by reffering taxonomic literature and herbarium available in University Library.

2.2. Preparation of essential oil [3].

For the preparation of essential oil, the seeds were washed and dried in hot air oven at 40^0C. The dried seeds were powdered and subjected to conventional steam distillation using Clevenger apparatus for 24-48 hrs in which water is heated to produce steam that carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The essential oil normally float on the top of the hydrosol was separate and dried over anhydrous Na_2SO_4. The essential oil obtained was stored and for antifungal activity, it was used in two forms: pure (100mg/ml) and diluted (10mg/ml).

2.3. Antifungal Assay

The assessment of fungitoxicity was done by Poisoned Food Technique [4-5] and Agar well diffusion Technique [6] against *Aspergillus flavus* and *A.niger* isolated from various spice mixes.

2.3.1. Poisoned Food Technique

A volume of 0.5 ml of essential oil (100 mg/ ml) was aseptically poured into the petriplate followed by the addition of 9.5 ml of melted PDA and was swirled gently to achieve thorough mixing of the contents. After the solidification of the media, seven day old fungal culture disk of 6mm diameter was aseptically inoculated at the center of the petriplate. Sterile DMSO was used as negative control while Sodium propionate (5 mg/ml) was served as positive control. All the plates were incubated at 25 ± 2^0C and radial growth of colony was measured on the 7th day of incubation.

2.3.2 Agar well diffusion method

1 ml of standardized inoculum (10^6spores/ml) was poured into sterilized petriplates followed by pouring of sterilized Sabrourd Dextrose Agar medium. A well of 8mm in diameter was made with a sterile cork borer aseptically in the middle of the plate. 100 µl of essential oil having 100mg/ml (pure) and 10mg/ml (diluted) of concentration was then introduced into the bored agar well and incubated at 25 ± 2^0C for 5-7 days. Sterile DMSO was used as negative control while Sodium propionate (5 mg/ml) was served as positive control. The zone of inhibition was measured and expressed in millimeters [6].The diameter of inhibition zone <9mm was considered as inactive; 9-12 mm, partially active; 13-18 mm, active; > 18 mm very active [7].

2.4. Assessment of Minimum Inhibitory Concentration

MIC (Minimum Inhibitory Concentration) of essential oil was further examined by standard two fold microdilution broth methodology [8]. A stock solution of essential oil was serially diluted in 96 wells microtitre plate with Sabrourd Dextrose Broth (fungi) so as to obtain a decreasing concentration ranging from 4096µg/ml to 08 µg/ml. A standardized inoculums for fungal strain was prepared so as to give an inoculum size of ≥ 0.4 O.D at 530nm respectively. Microtitre plates were then incubated 25± 2^0C for 5-7 days (fungi). Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial and fungal strain.

2.5. Determination of Minimum Fungicidal Concentration (MFC).

Minimum fungicidal concentration is the lowest concentration of antifungal agent that prevents the growth of an organism after subculture on an antimicrobial free agent. To determine MFC, 100 µL aliquot from the microtitre wells showing MIC and from wells having more than MIC were subcultured on SDA (fungi) and incubated at 25±2^0C for 5-7 days (fungi). The plates were observed for the development of the colonies to determine if the inhibition was reversible or permanent. MFC was determined as the lowest concentration at which no growth occurred on the plates [9].

2.6. Statistical analysis

All the experiments were done in triplicates and results are the mean of three independent values ± Standard Deviation.

3. RESULTS

3.1.Food Poisoned Technique (FPT): *F.vulgare* seeds essential oil in pure as well as diluted form (1:10) totally inhibited the growth of *A.flavus* and *A.niger* showing 100% activity (Table 1)

3.2.Agar well diffusion assay: *F.vulgare* essential oil also showed significant zone of inhibition with average zone size of 17.66 (Pure) and 12.66mm (diluted) against *A.niger* and 14.00 (Pure) and 2.33 (diluted) against *A.flavus* (Table 2)

3.3.MIC and MFC: MIC of *F.vulgare* essential oil are shown in Table 3 & 4. The MIC values were found to be 128µg/ml for both the fungi, *A.flavus* and *A.niger* and the MFC values were recorded as 256 µg/ml (Table 3 & 4).

Table 1: Antifungal activity of Essential Oil of *F.vulgare* seeds by FPT

Essential Oil	Radial growth			
	A.flavus		A.niger	
	Pure	1 : 10	Pure	1 : 10
Foeniculum vulgare	-	-	-	-
Sodium propionate (positive control)	+	ND	-	ND
DMSO(Negative control)	++++	++++	++++	++++

(-) 'No growth '; (+) 'limited growth' ; (++) 'Growth' ; (+++) 'Abundant growth' ; (++++) 'Maximum growth';*spore production was inhibited.

Table 2: Antifungal activity of Essential Oil of *F.vulgare* seeds

Essential Oil	Zone of inhibition (in mm) Mean± S.D.			
	A. flavus		A.niger	
	Pure	1 : 10	Pure	1 : 10
Foeniculum vulgare	14.00 ± 3.46	2.33 ± 0.577	17.66 ± 2.88	12.66 ± 1.154
Sodium propionate (Positive control)	11.00 ± 1.00	ND	12.00 ± 0.00	ND
DMSO (Negative control)	-	-	-	-

Table 3: MIC of Essential Oil of *F.vulgare* seeds against test fungi

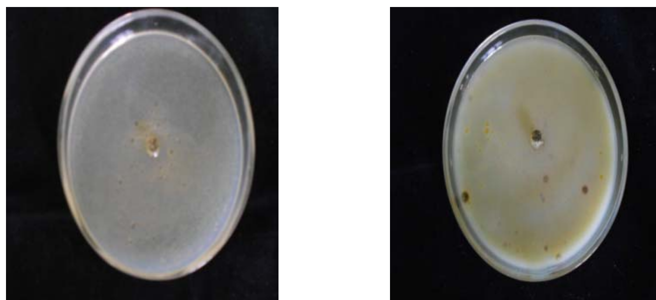
Test Fungi	Concentration of Extracts (µg/ml)							MIC (µg/ml)
	512	256	128	64	32	16	08	
A. flavus.	-	-	-	+	+	+	+	128
A.niger.	-	-	-	+	+	+	+	128

(-) 'No growth ' ; (+) 'growth '.

Table 4: MFC of Essential Oil of *F.vulgare* seeds against test fungi

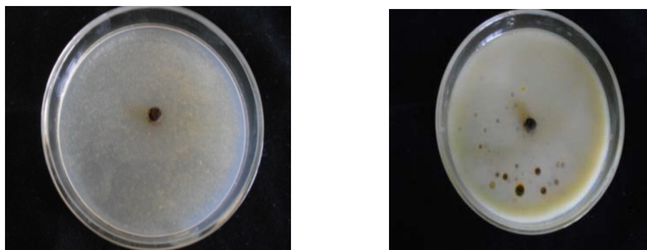
Test Fungi	Concentration of Extracts (µg/ml)							MFC (µg/ml)
	512	256	128	64	32	16	08	
A. flavus.	-	-	-	+	+	+	+	256
A.niger.	-	-	-	+	+	+	+	256

(-) 'No growth '; (+) 'growth '.



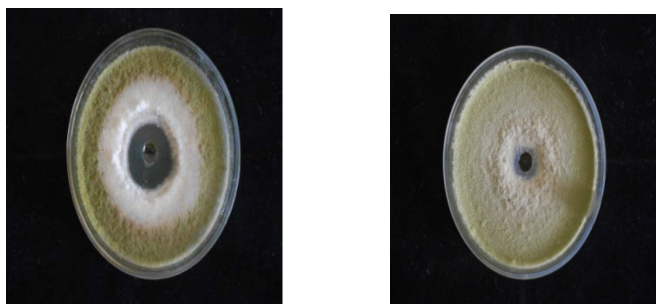
Pure 1:10

Fig.1.Antifungal activity of *F.vulgare* essential oil against *A.flavus* (FPT).



Pure 1:10

Fig. 2.Antifungal activity of *F.vulgare* essential oil against *A.flavus* (FPT)



Pure 1:10

"Fig.3.Antifungal activity of *F.vulgare* essential oil against *A.flavus* (Agar Well Diffusion Assay)".

Pure 1:10

Fig. 4: Antifungal activity of *F.vulgare* essential oil against *A.flavus* (Agar Well Diffusion Assay)

4. DISCUSSION

Essential oils are natural products which accumulate in specialized structures such as oil cells, glandular trichomes, oil or resin ducts. Chemically, the essential oils are primarily composed of mono and sesquiterpenes and aromatic polypropanoides.. The essential oils from the aromatic plants are the most volatile and thus lend themselves to several methods of extraction such as hydrodistillation, water and steam distillation. The specific extraction method employed depends upon the plant material to be distilled and desired end product. Here, the hydrodistillation method is employed which utilizes the Clevenger type apparatus. The potential of essential oil was evaluated in pure as well as diluted form and found that both preparations fennel essential oil totally inhibited both the fungi while in Agar Well Diffusion Assay, the zone of inhibition varied from 17 (pure)-12.66 (diluted) mm for *A.niger* and 14 (pure)-2.33 (diluted) mm for *A.flavus* respectively. Similar results were reported by Duarte *et al.*,2005, Ozcan *et al*.,2006 and Freire *et al.*,2011[10-12]. Jelica *et al,* (2013) [13] examined the antifungal properties of *F.vulgare*, *Carum carvi* and Eucalyptus essential oils against *Candida albicans* strains and found that the essential oil of *F.vulgare* showed good antifungal activity against *Candida albicans* strains.

5. CONCLUSION

The result of the present study are quite encouraging and the study opens up the possibility for the search of new antimicrobials as an alternative to the antibiotics. The study provided us a strong basis for the development and utilization of *F.vulgare* as both pharmaceuticals and dietry supplement. Also, it can be used as adjuvant with spices to check the microbial growth of food borne microorganisms.

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