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Eco-friendly control of common fungi grown on food items

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ABSTRACT

During antifungal screening of the essential oils of some angiospermic plants, oil of *Artemisia maritima* exhibited the strongest activity, completely inhibiting the mycelia growth of common food spoilage and mycotoxin producing fungi (*viz. Aspergillus flavus, A. fumigates, A. niger, Rhizopus nigricans). Aspergillus* and *rhizopus spp*. are frequentely present on food and thus can be an indirect source of airway or digestive tract colonization. *The* mycelia growth inhibition of test fungi was found at the concentration of 0.7µl/ml against *Aspergillus flavus*, 0.8µl/ml against *A. fumigatus* and *A. niger* and 0.9µl/ml against the *Rhizopus nigricans*. The essential oil of *Artemisia maritima* was also found to be fungicidal at the concentration of 0.9µl/ml against *Aspergillus flavus, A. fumigates, A. niger and* 1.0µl/ml against *Rhizopus nigricans*. The fungicidal activity of the oil was found to be thermo stable up to 70^0C, with no decrease in activity up to 24 months of storage. The oil also showed a broad fungi toxic spectrum, inhibiting the mycelia growth of many other fungi. As such, the essential oil has a potential effect against the food spoilage and mycotoxin producing fungi. This indicates the possible ability to prevent the food spoilage and use as a food preservative.

KEY WORDS: *Artemisia maritima;* essential oil; *Aspergillus*, *Rhizopus,* food spoilage

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INTRODUCTION

Fungal contaminate food items are very common in country like India where temperature and moisture prevalent. High temperature and moisture are good conductive for the multiplication and spreading of fungi. The degree and type of fungal contamination varied a lot according to the type of food items. The heaviest and most frequent fungal contamination occurred in food items are spores of *Asergillus* and Mucorales. Sample from several type of food items were selected at random from the kitchens, hostel mess and foods were usually stored. Food included regular tea bags, herbal tea bags, chocolate powder, chicory, ground pepper bags, bread, sandwich, rusks, ketchup, mustard, cereals, potato, biscuits, sponge cake, cheeses, and fruits like apple banana, lemon, melon, orange and papaya.

Aspergillus species are frequent present on food items and thus can be an indirect source of airway or digestive tract colonization[1]. Aspergillosis is usually acquired by inhalation of conidia[2.] One typical example is pepper which has been found to be massively contaminated by *Aspergillus flavus, A. fumigates, and A. niger*[3, 4, 5]. As pepper is usually sprinkled on food just before eating, there is major risk of producing an aerosol of *Aspergillus* spores and exposing the persons to an air bourn contamination[6]. Regular tea was also consistently contaminated by molds[7]. Similarly uncertainty applies to freeze-dried soups, which may also contain spores of *Aspergillus* and Mucorales.

Mostly synthetic antifungal (fungicides) are used to control these fungi, which are largely non-renewable petro-products that are non-biodegradable and cause adverse effects and residual toxicity. Thus, in a meaningful search for new treatments with better and cheaper substitutes, plant resources are the natural choice. Naturally occurring fungi toxicants described to date are biodegradable[8] and are devoid of side effects compared with commercially available antifungal. Recently, essential oils of higher plant origin have been shown to be an effective bioactive agent without undesirable side effects and with strong fungicidal activity.[9] The findings prompted us to explore other plant products (essential oils), which could be utilize as effective fungi toxicants. We here report on the result of our investigation of the essential oil of *Artemisia maritima* L. *a* member of the family Compositae (Asteraceae) as an effective antifungal again common food spoilage and mycotoxin producing fungi (*Aspergillus flavus, A. fumigates, A. niger, Rhizopus nigricans).*

EXPERIMENTAL SECTION

Plant *Artemisia maritima* samples were collected during the month of September from the Kullu Valley of Himanchal Pradesh, India. The essential oil was extracted from the aerial parts of collected plant by hydro distillation using a Clevenger's apparatus.[10]A clear light-yellow-colored oily

layer was obtained on the top of the aqueous distillate, which was separated from the latter and dried over anhydrous sodium sulphate.

In *in vitro* studies, the minimum inhibitory concentrations (MICs) of the oil against test pathogens were determined following the poisoned food technique[11] with slight modification.[12] The requisite quantity of the oil samples were mixed in acetone (2% of the required quantity of the medium) and then added in pre-sterilized potato dextrose agar (PDA) medium, pH 5.6. In control sets, sterilized water (in place of the oil) and acetone were used in the medium in appropriate amounts. Mycelial discs of 5mm diameter, cut out from the periphery of 7 day old cultures, were aseptically inoculated upside-down on the agar surface of the medium. Inoculated Petri plates were incubated at 27 ± 1^0C and the observations were recorded on the seventh day. Percentage of mycelial growth inhibition (MGI) was calculated according to the formula:

$$MGI= (dc - dt) \times 100/dc$$

Where dc = fungal colony diameter in control sets. dt = fungal colony diameter in treatment sets.

The minimum fungi static /fungicidal concentrations of the oil at minimum inhibitory concentrations (MICs) were ascertained by the method of Garber and Houston.[13] This was done by reinoculated the inhibited fungal discs at MICs on PDA medium. Observations were recorded after 7 days of incubation. Fungal growth on the seventh day indicated a fungi static nature, while the absence of fungal growth denoted fungicidal action of the oil. The effect of inoculums density (increased progressively up to 30 discs in multiples of five, each of 5 mm diameter) of the test pathogens on MICs of the oil was determined following the procedure outlined by Dikshit and Dixit.[14] The effect of physical factors viz. temperature and expiry of toxicity during storage of the oil, was evaluated according to Shahi *et al.*[15] Five lots of oil were kept in small vials, each containing 5 ml oil; these were exposed to different temperatures (30, 50 and 70^0C) in an incubator for 1 h. Antifungal activity was then tested at MICs by the poisoned food technique.[16] Expiry of toxicity of the oil was determined by storing the oil at room temperature and testing antifungal activity at MICs at regular intervals of 60 days up to 24 months, following the poisoned food technique.[16]

The minimum killing time (MKT) of the oil was determined by the mycelial disc killing technique (MDKT) of Shahi *etal.*[15] Two treatment sets were maintained, one with pure oil (PO) and the other with the minimum fungicidal concentrations (MCCs) of the oil. The treatment set using MCCs of the oil was prepared by mixing the required quantity of the oil samples in acetone (5% of the total quantity of the treatment solution) and then adding this to the appropriate quantity of distilled water. Simultaneously, controls were maintained using sterilized water (in place of the oil) and acetone, adding into the distilled water in appropriate quantities.

Mycelial discs of 5 mm diameter, cut out from the periphery of 7 day-old cultures of the test pathogens, were aseptically placed in the culture tubes of different treatment and control sets. These mycelial discs were taken out of the tubes at different time intervals and washed immediately in the washing solution (containing acetone: sterilized distilled water, ratio 1:2) to remove the treatment solution. These washed mycelial discs were aseptically transferred upside-down to the PAD medium (pH 5.6) in the Petri plates. The same procedure was followed with the control sets. The inoculated Petri plates were incubated at 27 ± 1^0C and the observations recorded as an average value of five replicates on the seventh day. The percentage of fungal growth inhibition (FGI) was calculated by the formula of Shahi *et al.*[15] All the experiments were repeated twice, each containing five replicates, and the data presented here are based on their mean values.

Table-1. Physicochemical properties of *Artemisia maritima* oil.

Properties Studied	Observation
Plant Height	Up to 1.0 m.
Oil Yield (%)	0.60
Colour	Light Yellow
Specific Gravity at 29.5 ^{0}C	0.8835-0.9457
Refractive Index at 20 ^{0}C	1.4799-1.4925
Optical Rotation	$+4^0$ 25'-16^0 75'
Acid Value	7.535-27.07
Ester Value	71.0-175.0
Cineole (%)	14.0-22.0
Solubility	Acetone

Table-2. Minimum fungistatic concentration (s) and minimum fungicidal concentration (c) of the oil *Artemisia maritima* against test fungi.

Concentration (µl/ml)	Mycelial growth inhibition (MGI) (%)			
	Aspergillus flavus	*Aspergillus fumigates*	*Aspergillus niger*	*Rhizopus nigricans*
1.2	100[c]	100[c]	100[c]	100[c]
1.1	100[c]	100[c]	100[c]	100[s]
1.0	100[c]	100[c]	100[c]	100[s]
0.9	100[c]	100[c]	100[s]	100[s]
0.8	100[s]	100[s]	100[s]	90
0.7	100[s]	90	90	80
0.6	90	80	80	60
0.5	80	70	70	40
0.4	60	50	50	30
0.3	50	40	40	20
0.2	40	30	30	10
0.1	10	10	10	0.0

[c] Fungicidal, [s] Static

Table-3 Minimum killing times (mkt) of the oil *Artemisia maritima* against test fungi:

Minimum killing time (mkt)	Mycelial growth inhibition (MGI) (%)							
	Aspergillus flavus		*Aspergillus fumigates*		*Aspergillus Niger*		*Rhizopus nigricans*	
	PO	**MCCs**	**PO**	**MCCs**	**PO**	***MCCs***	***PO***	***MCCs***
90m	100	100	100	100	100	100	100	100
80m	100	100	100	100	100	100	100	90
70m	100	100	100	90	100	90	100	80
60m	100	90	100	80	100	80	100	70
50m	100	70	100	70	100	75	100	55
40m	100	50	100	50	100	45	100	40
30m	100	40	100	30	100	-	100	25
60s	100	-	100	-	100	-	100	-
30s	100	-	100	-	100	-	100	-
20s	100	-	100	-	100	-	100	-
10s	80	-	90	-	90	-	85	-
5s	50	-	40	-	40	-	40	-
1s	30	-	25	-	20	-	0.0	-

PO (pure oil), MCCs (minimum fungicidal concentration)

RESULTS AND DISCUSSION

The essential oil was extracted from the aerial parts of *Artemisia maritima* by hydro distillation using Clevenger's apparatus.[17]A clear light-yellow-colored oil on hydro distillation, yielded 0.6 % essential oil. The physicochemical properties of the oil are shown in Table 1. The minimum inhibitory concentration (MIC) was found at 0.7µl/ml against *Aspergillus flavus*, 0.8µl/ml against *A. fumigatus* and *A. niger* and 0.9µl/ml against the *Rhizopus nigricans* (Table -2). The essential oil was also found to be fungicidal at 0.9µl/ml against *Aspergillus flavus, and A. fumigatus*, 1.0µl/ml against *A. niger and* 1.20µl/ml against *Rhizopus nigricans* concentrations.(Table-2). The oil inhibited heavy doses of inocula which exhibited 100% mycelial growth at their respective fungicidal concentrations. The activity of the oil did not expire even up to 24 months storage and persisted up to 70^0C. The pure oil (100%) killed the fungi in just 15-20 seconds, while at its minimum fungicidal concentration it required 70 minutes against *Aspergillus flavus*, 80 minutes against *A. fumigatus* and A. *niger* and 90 minutes against *Rhizopus nigricans* respectively (Table-3).

The oil also showed a broad fungi toxic spectrum, inhibiting the mycelia growth of many other fungi *viz. Trchophyton rubrum, T. mentarophytes, Epidermatophyton floccosum, Microsporum gypseum, M. canis, Trichophyton tonsurans and T. violaceum*. As such, the essential oil has a potential effect against the food spoilage and mycotoxin producing fungi as well as human pathogenic fungi. This result shows the potential ability of *Artemisia maritima* oil to prevent the food spoilage caused by the fungi and can be used as a food preservative.

CONCLUSION

The essential oil of *Artemisia maritima* exhibiting strong toxicity against the test fungi causing food spoilage. The findings suggest that potential ability of *Artemisia maritima* oil to prevent the food spoilage caused by the fungi. The oil can be use as a food preservative because no any adverse effect on the human health.

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