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Inhibitory Effects of Essential Oil of *Psamogeton canescens* on Asexsual Reproduction of Toxigenic Fungi (Strains of *Aspergillus*)

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Abstract: The effects of essential oil of the plant species *Psernogeton canescens* (a member of family Umbelliferae) and of an antifungal medicine Taeniafex on spore germination, mycelium elongation and sporulation were studied on five toxigenic fungi. All the three stages of fungal asexual reproduction were effected but the mycelium elongation was the most sensitive followed by sporulation and the spore germination. *Aspergillus oryzae* and *Aspergillus flavus* were found to be the most sensitive, followed by *Aspergillus niger*, *Aspergillus awamori* and the *Aspergillus foetidus.* The antifungal activity at all the three stages of asexual reproduction was shown to be co-related to the oil composition.

Key words: Toxigenic fungi, essential oil, asexual reproduction, sporulation, mycelium growth

Introduction

Pakistan because of its geographical conditions possesses an enormous wealth of natural flora. About 167 species belonging to 56 genera of the family Umbelliferae have been reported to occur in different parts of the country (Nasir and Ali, 1972). Our school of research on essential oils have carried out chemical analysis of nearly half of the species grown in the country (Bhatty, 1982; Ahmad *et al*., 1987). Investigations have revealed that the essential oils exhibited fungistatic and fungicidal activities. Antifungal properties of numerous spices and herbs have been reported and recognized to be due, at least partially to their essential oils (Benjilali *et al.,* 1984; Thompson, 1989), Hitokoto *et al.* (1980) have determined the action of spices on fungal mycelium elongation, while the inhibition of fungal spore germination has been studied by Thompson (1986). The antifungal activities studies appear in the literature as toxinogenesis (Bullerman *et al.,* 1977; Tantaoui-Elaraki *et al.,* 1992), lethal effect (Hajji *et al.,* 1993), growth inhibition (Hmamouch *et al.,* 1990) or growth retardation (Es-Safi *et al.,* 1991).

Materials and Methods

Cultures: Five strains of *Aspergillus* namely, *Aspergillus awamori*, *A. flavus, A. foetidus*, *A. niger* and *A. oryzae* were used. These cultures were maintained on Czapek Dos Agar and subcultured after every 20 days.

Essential oil: The essential oil of *Psamogeron canescens* was obtained by steam distillation. The percentage of oil was 2.271%.

Antifungal studies: The basic medium used for the studies was Yeast Extract Agar. There were three different treatments of oil, i.e., 0.1, 0.05, 0.01 percent and one treatment for standard antifungal drug taeniafex 0.01%. The oil treatments were prepared by dispersing the calculated amount of oil in 0.3 percent agar solution and then adding to the rest of the medium. The standard of the antifungal drug was obtained by mixing the known suantity of Taeniafex powder in the medium. Control received no oil or antifungal drug.

Effects of essential oil on spore germination: The fungi ware cultivated in slants for five days to obtained substantial number of spores. The spores were collected by adding 10 mL of 0.9% saline in the slant. The spores were suspended in saline by gentle shaking. 500 μL of the spore suspension was evenly spreaded on the surface of medium in Petri plates. The Petri plates were incubated at 30°C for 7 hours for *A. niger* and 10 hours for *A. awamori, A. fiatars, foetidus* and *A. oryzae.* These times were previously determined for maximum germination. The germination tube equal to the spore size was considered as spore germination index. The percentage of germinated spores was calculated by counting at least 500 spores in various microscope fields at random, using microscope with video attachment Model OLYMPUS BH-2. The germination % was calculated by the formula given as under:

$$\text{Germination, \% (G \%)} = \frac{\text{No. of germinated spores}}{\text{Total No. of spores}} \times 100$$

During the counting of spores, the non germinated spores were also observed for lysis.

Effects of essential oil on mycelial elongation: The angled tubes were used for studying the effects of essential oil on mycelial growth. The fungus was grown on Yeast extract agar for 24 hours at 30°C. After 24 hours a piece of mycelium was taken from growth front and transferred to the entry of the angled tube containing yeast extract agar having different concentrations of oil and antifungal drug. The tubes were then incubated at 30°C for 10 days. The increase in the mycelial length was recorded after every 2 days.

Effects of essential oil on sporulation: The fungus was grown on yeast extract agar for 24 hours. 5 mm diameter disks covered with mycelium were cut and transferred into the same size wells in Petri plates containing Yeast extract agar with different concentrations of oil and drug. After an incubation of seven days at 30°C the spores were harvested in 50 mL 0.9% saline. For counting viable spores, 100 μt of the spore suspension was transferred to 50 mL Yeast extract broth and incubated at 30°C on rotatory shaker at 150 rpm for 36 hours, after than the pellets formed were counted. Each pellet was considered to be formed from single spore. The results were expressed as number of spores per mL of suspension.

Results and Discussion

Composition of oil: The essential oil of the plant species *Psamogeton canescens* was analyzed by gc/ms technique and the

Table 1: Inhibition of fungal spore germination by the essential oil of *Psamogeton canescens*

Treatments	Concentrations %	Spore		Germination	Percent	
		Aspergillus awamori	*Aspergillus flavus*	*Aspergillus foetidus*	*Aspergillus niger*	*Aspergillus oryzae*
Control	0.00	100.00	100.00	100.00	100.00	100.00
Drug	0.01	011.06	074.80	032.87	085.69	082.16
Essential oil	0.01	055.12	094.89	098.60	057.12	026.00
	0.05	048.26	065.00	081.77	011.67	000.00
	0.10	032.25	000.00	045.22	000.99	000.00

Table 2: Inhibition of mycelial growth of *Spergillus* sp. On yeast extract agar by the essential oil of *Psamogeton canescens*

Treatments	Concentrations %	Thallus length (mm)														
		Aspergillus awamori					*Aspergillus flavus*					*Aspergillus foetidus*				
		2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
Control	0.00	2	6	9	13	18	2	5	8	13	17	1	3	7	11	15
Drug	0.01	0	0	2	5	7	0	0	1	3	4	0	0	2	2	7
Essential oil	0.01	0	2	5	9	13	0	0	2	5	8	0	0	2	5	9
	0.05	0	1	3	7	10	0	0	1	4	6	0	0	2	4	7
	0.10	0	0	2	6	11	0	0	0	1	3	0	0	0	0	2

Treatments	Concentrations %	*Aspergillus niger*					*Aspergillus oryzae*				
		2	4	6	8	10	2	4	6	8	10
Control	0.00	2	5	10	15	21	1	3	7	12	16
Drug	0.01	0	0	2	3	6	0	0	0	2	3
Essential oil	0.01	1	3	5	8	12	0	0	2	3	7
	0.05	0	2	3	5	8	0	0	2	3	5
	0.10	0	0	1	3	5	0	0	0	1	5

major components reported *were* limonene, myriticin, methyl eugenol, linallool and terpineol (Ahmed, 1995). The composition of the essential oil was considered in order to establish a relationship between the antifungal activity at all the three stages of asexual reproduction of toxigenic fungi and the essential oil.

Effects of the essential oil on the germination of spores: When tested at the 0.10% level, the essential oil totally inhibited the spore germination in *A. nevus* and *A. oryzae* after 10 hours incubation at 30°C and also at 0.05% level, there was total inhibition in spore germination in *A. oryzae.* Whereas the G% was 0.99 in case of fungus *A.niger* after 7 hours incubation at 30°C (Table 1). However, *A.awarnori* and *A. foetidus* spores showed some germination at 0.1% essential oil concentration i.e., 32.25 and 45.22, respectively.

Lower concentrations of the essential oil showed small effects on all the toxigenic fungi causing variable spore germination percentages. The essential oil at 0.01% level had almost no effect on spore germination in case of *A. flaves* (G% 94,89) and *A. foetidus* (G%98.60), whereas in case of *A. awarnori. A. niger* and *A. oryzae* the germmation, % was less than 60. At 0.05 level of the essential oil, The spare inhibition *was* maximum in case of *A. oryzae* and minimum in case of *A. foetidus.* The effect of spore germination inhibition was 18.23% on *A. foetidus,* 35.00% on *A. flavus,* 51.74% on *A. awarnori,* 88.33% on *A. niger* end 100% on *A. oryzae.*

It is noteworthy that the essential oil was more effective at a level of 0.1% in the inhibition of spore germination (Table 1). A ranking could be established for the five toxigenic fungi studied as follows:

A. flavus>A. oryzae>A. niger>A. awamori>A. foetidus

The spores of *Aspergillus* strains were not sensitive and no lysis was observed in these studies. All the controls showed significant growth. The effect of the essential oil an the inhibition of spore , germination at levels of 0.01, 0.05 and 0.1% were compared with the antifungal medicine, Taeniafex. The medicine showed effect at 0+01% level in the inhibition of spore germination of which ranking could be:

A. awarnori>A. foetidus>A. flavus>A. oryzae>A. niger

This medicine has no comparison with the effectiveness of the essential oil of the plant species *Psamogeton canescens* which is clear from the ranking patterns.

While studying the effect of the oil of cinnamon, thyme and clove (at concentrations of 100, 50 and 25 ppm) on the germination of *Aspergillus, Mucor* and *Rhizoptis* spores. Thompson (1986) reached on a similar conclusions as he noticed different inhibitory effects depending upon the type of, the amount used and the fungus tested. Tantaoui-Elaraki *et al.* (1992) reported the inhibition of germination and loss in germination power of *A. niger* and *Zygorrhynchus* sp. spores when exposed to different concentrations of various oils.

Effect of the essential oil on the mycelium elongation: The results of the mycelium elongation of the five toxigenic fungi on Yeast extract agar medium treated with different concentrations of the essential oil of *Psamogeton canescens* and the antifungal drug as measured over a ten days period of incubation are given in Table 2. The results reported are the averages of three determinations. All controls showed significant growth since the first day of incubation.

Table 3: Inhibition of spore formation by the essential oil at *Psamogeton canescens* in yeast extract medium

Treatments	Concentrations	*Aspergillus awamori*	*Aspergillus flavus*	*Aspergillus foetidus*	*Aspergillus niger*	*Aspergillus oryzae*
Control	0.00	407×10^3	1322×10^3	375×10^3	2425×10^3	642×10^3
Drug	0.01	267×10^3	0135×10^3	0.00	1752×10^3	530×10^3
Essential Oil	0,01	340×10^3	0300×10^3	165×10^2	0417×10^3	340×10^3
	0.05	172×10^3	0150×10^3	015×10^7	0195×10^3	053×10^3
	0.10	150×10^2	0052×10^3	0.00	0575×10^2	027×10^3

A. awamori: The pattern of the effects of 0.01% level of antifungal drug was similar to that of 0.1% level of essential oil upto 6 day showing a delay in the initiation of mycelial elongation upto 4th day. The rate of mycelial elongation was higher in *case* of 0.1% level of essential oil than 0.01 antifungal drug after 6th day. The essential oil 0.01% level showed inhibition in elongation of mycelium upto 2 day. The pattern of elongation of mycelium was more or less similar to that of control from 1st to 8th day. At 0.05% level the inhibition effect on mycelium growth was same to that of 0.01% but the growth rate was lesser (Table 2).

A. flavus: The inhibition in mycelium elongation was effective till the 4th day in case of essential oil concentrations 0.01% and 0.05% levels and the antifungal drug whereas the mycelium growth was delayed till the 6th day at 0.1% level of essential oil. Thallus formation initiated after 4th day, where the rate was higher in case of 0.01% level but was quite slow in case of 0.05% and antifungal drug as compared to control. The rate of mycelium growth was similar till the 6th day of 0.05% level of the essential oil and the drug and then there was a sudden increase in the rate at 0.05% level than the antifungal drug. The mycelium elongation started after 6th day at 0.1% level but the rate was very slow as compared to the other concentrations of essential oil and the drug (Table 2). The ranking in the mycelium elongation inhibition could be:

0.1% oil>0.01% Drug>0.05% oil>0.01% oil

A. foetidus: There has been a delay in mycelium elongation for four days at 0.01 96 and 0.05% level of the essential oil and the antifungal drug. After the fourth day till the 6th day, there has been mycelium growth and the rate of formation of thallus was the same in all the three concentrations but after 6th day the drug has the minimum effect of all.
The mycelium elongation was inhibited at 0.1% level of the essential oil till 8th day of the incubation and after that there was formation of thallus at very slow rate as compared to all (Table 2).

A. niger: No inhibition of mycelium elongation was observed at 0.01% level of the essential oil from the first day of incubation but the rate of thallus formation was slow as compared to the control. At 0.05% level the essential oil, the thallus formation was inhibited till 2nd day of incubation and after that it started growing in size.
The antifungal drug and the 0.15% level of the essential oil inhibited the mycelium elongation tilt the 4th day of incubation and the formation of thallus initiated after that but the rate of growth was slow in case of 0.1% level of essential oil than the antifungal drug (Table 2). The ranking of rate could be:

0.01% oil>0.05% oil>0.01% Drug>0.1% oil

A. oryzae: The 0.1% level of the essential has better inhibition capability as compared to the antifungal drug and other lower concentrations. There has been complete inhibition till the 6th day and after that the mycelium elongation was at lower rate in 0.1% level of essential oil than in the drug. At levels of 0.01 and 0.05% of the essential oil, there was a delay in mycelium growth till the 4th day and after that the thallus formation started and the rate in both the cases was the same but then there was a sharp increase at 0.01% level (Table 2). The rate ranking could be:

0.01% oil>0.05% oil>0.01% drug>0.1% oil

Thus there has been no complete control of the mycelium elongation but at 0.1% level of the essential oil, there has been partial inhibition better than the antifungal drug. The other concentrations of the essential oil also showed no good results.
Effects of essential oil on aporulation: As shown in Table 3, the essential oil of *Psamogeton canescens* at 0.1% level totally inhibited the sporulation of *A. foetidus* but at 0.01% and 0.05% levels and even the antifungal drug Taeniafex, there was partial inhibition of sporulation. The sporulation was significantly *better* at 0.05% essential oil in case of *A. awarnori* and *A. oryzae,* at 0.01 96 in case of *A. niger* and at 0.10% essential oil in case of *A. foetidus* and *A. flavus* than antifungal drug 0.01 96 level. The partial inhibition of sporulation could be attributed either to the mycelial destruction (Table 2) or inhibition of the fungal growth (Table 1).
The extent of these studies revealed that the essential oil at 0.1% level was more effective to inhibit spore germination and sporulation which are the survival sources of fungi. These two can easily be controlled by the essential oil of *Psarnogeton canescens* however the required concentration is high.

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