

Keratinolytic and keratinophilic fungi of mangrove's soil and air in the city of Qena and their response to garlic extract and onion oil treatments

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Forty-eight species and 1 variety belonging to 25 genera were collected from 50 soil samples (41 species and 1 variety and 21 genera) and the atmosphere (27 species and 1 variety and 14 genera) of mangrove in the city Qena using hair baiting technique at 28°C. Twenty of these species was dermatophytes and closely related fungi. The most common and frequent species of the latter fungi were *Aphanoascus fulvescens* (telemorph of *Chrysosporium keratinophilum*), *A. terreus* (*C. indicum*), *Aphanoascus* sp. (*C. tropicum*) and *Chrysosporium xerophilum*. Sixty-eight isolates were tested for the abilities for growth on hair – sand medium. Most (73.5 %) had moderate growth rate. All keratinophilic fungi recovered in the present investigation were sensitive to garlic extract and onion oil.

Key words: Keratinolytic and keratinophilic fungi, antifungal, garlic extract, onion oil.

INTRODUCTION

Soil rich in keratinic residues constitute a permanent or occasional reservoir for dermatophytes and keratinolytic and keratinophilic fungi, and is a source of potential infection for man and animals. Several investigations have been made on the contribution of these fungi in soil and air of many countries all over the world (Papavassilion, Bartzokas, 1975; Alteras, Lehrer, 1977; Acosta, Roberstad, 1979; Sur et Gosh, 1980; Calvo et al., 1984; Marsella, Marcantini, 1986; Sundaram, 1987; Della-France, Caretta, 1984; Chabasse, 1988). In Arab countries few surveys were carried out on keratinophilic fungi from soil and air (Amer et al., 1975; Jana et al., 1979; Youssef et al., 1980, 1989; Bagy, 1982; Abdell-Fattah et al., 1982; Abdell-Mallek et al., 1989; Abdell-Hafez et al., 1989, 1991; Karam El-Din et al., 1990; Moubasher et al., 1990; El-Said, 1993, 1994; El-Maghraby, 1994).

The distinction between keratinophilic and keratinolytic fungi is based on the proposal of Majchrowicz, Dominik (1969) and Dominik et al. (1973), already adopted by Filipello Marchisio, Luppi Mosca (1980, 1982). Keratinolytic species are defined as those only able to destroy keratin, while keratinophilic species are those only able to use materials naturally associated with keratin or resulting from its breakdown. The keratinolytic activity of dermatophytes using guinea-pig hair as substrate was measured by Yu et al. (1968).

Garlic (*Allium sativum* L.) extract and onion oil have a long history of reputed value and actual use for their medicinal, antimicrobial and pesticidal properties (Amonkar, Banerji, 1971; Shekhawat, Prasad, 1971; Fliermans, 1973; Appleton, Tansey, 1975; Tansey, Appleton, 1975; Moore, Atkins, 1977; Lewis et al., 1977; Deshmukh, 1984; Yoshida et al., 1987; Gherbawy, 1989; Singh et al., 1990; Zohari et al., 1992).

The present investigation aimed to study intensively composition and frequency of occurrence of keratinophilic fungi of mangrove's soil and air in the city Qena and their keratinolytic activity. Also, preliminary study on antifungal effect of garlic extract and onion oil on the keratinophilic fungi that had been isolated.

MATERIALS AND METHODS

Fifty localities were collected from different localities of mangrove in Qena, according to the method described by Johnson et al. (1959). The soil samples were analysed chemically for the estimation of total soluble salts and organic matter. A pH-meter (WGPYE model 290) was used for the determination of soil pH.

Isolation of keratinophilic fungi from soil samples

The hair baiting technique was employed as recommended by Van Breusegheem (1952), and as employed by Abdel-Fattah et al. (1982): 100 g of soil were put in sterile plate and a sufficient quantity of sterile distilled water (about 20-25 % moisture content) was added and mixed thoroughly. Pieces of sterile hairs of horse were sprinkled on the surface of the moistened soil. Two plates were used for each sample: the plates were incubated at 28°C for 6-8 weeks, and the soil in plates were remoistened whenever necessary. The moulds which appeared on the baits were transferred to the surface of Sabouraud's dextrose agar medium (Moss, McQuown, 1969) which was supplemented with 20 unit/ml of sodium penicillin, 40 µg/ml of dihydrostreptomycin and 0.05 % cycloheximide (Actidione). Before adding to the agar, the first 2 antibiotics were dissolved separately in sterile distilled water while the third was dissolved in methanol. The plates were incubated at 28°C for 3-4 weeks and the developing colonies were identified. Frequency of occurrence as percentage of samples and the relative importance value (RIV) was calculated for each species (Shearer, Webster, 1985; Ali-Shtayeh, Asa'd Al-Sheikh, 1988).

Estimation of airborne fungi

Plates of 9 cm diameter containing each 100 g soil were moistened with distilled water to about 25-30 %. Horse hair fragments were scattered on the soil surface. The plates were autoclaved (three times) at 121°C for 30 min. One plate was exposed for 1 h to the atmosphere of mangrove at 50 different sites. Plates were incubated at 28°C for 10-12 weeks and remoistened whenever necessary. Five hair fragments from each plate were transferred to the surface of Sabouraud's dextrose agar medium. The plates were incubated at 28°C for 3-4 weeks and the developing colonies were identified. Frequency of occurrence as percentage of samples and the relative importance value (RIV) was calculated for each species (Shearer, Webster, 1985; Ali-Shtayeh, Asa'd Al-Sheikh, 1988).

Keratinolytic activity

Sixty-eight isolates of keratinophilic fungi recovered during the current study were used for keratinolysis tests, the method English (1976) was used. Hair-sand cultures were made by scattering 1 cm long pieces of autoclaved hair over the surface of 9 cm Petri dishes containing moist twice-autoclaved sand from the mangroves, and inoculating with 5 ml aqueous spore suspensions of each fungus. The hair of fair horse was used in all experiments. After an incubation period 20 days at room temperature, amount of fungal growth and sporulation was rated: + for weak growth, ++ for moderate growth and +++ for heavy sporing and preading cultures.

Test for the antifungal activities of some natural products

Twenty fungal isolates of keratinophilic fungi recovered in the present investigation were used to study the antifungal effect of garlic extract and onion oil.

G a r l i c (*Allium sativum* L.) e x t r a c t. Twenty g of garlic bulbuls free of scaly leaves were washed several times with sterile distilled water. Bulbuls were homogenised in sterile blender in 100 ml ethanol (70 %) and then completed to 200 ml with distilled water to obtain 10 % of garlic extract. The extract was then added to the autoclaved medium (Sabouraud's liquid medium) at 3 concentrations 1000, 2000 and 3000 ppm in addition to control one (garlic extract free). Cultures were incubated at 28°C for 15 days.

O n i o n (*Allium cepa* L.) o i l. The oil of onion obtained from El-Nasr Company for dehydrating agricultural products (A.R.E.). The oil was added to the medium (except the control one) to give concentrations of 100, 200, 300 ppm. Cultures were incubated at 28°C for 15 days.

RESULTS AND DISCUSSION

Soil samples fungi

The organic matter content and total soluble salts in soil samples tested fluctuated between 3.5-8.4 % and 0.5-4.1 %, respectively. All soil samples were in alkaline side (7.4-8.5).

Forty-one keratinophilic and cycloheximide resistant species in addition to 1 variety which belong to 21 genera were collected from 50 mangrove's soil samples baited with horse hair fragments at 28°C.

Aphanoascus (teleomorph of *Chrysosporium*) was the most common genus, occurring in 86 % of the samples and had RIV of 119.7. It was represented by 3 species, these were *A. fulvescens* (teleomorph of *C. keratinophilum*), *A. terreus* (*C. indicum*) and *Aphanoascus* sp. (*C. tropicum*). They were represent in 60 %, 24 % and 30 % of the soil samples and possessed RIVs of 73.3, 32.4 and 40.5, respectively. *Aphanoascus fulvescens* has been shown to cause skin infections (Albala et al., 1982, Rippon et al., 1970). The above species were previously isolated from soil samples, but with different frequencies in many parts of the world (Piontelli, Caretta, 1974; Mostafa, 1977; Todaro, 1978; Jana et al., 1979; Sur, Ghosh, 1980; Abdel-Fattah et al., 1982; Calvo et al., 1984; Filippello Marchisio, 1986; Abdel-Hafez et al., 1991; El-Said, 1993).

Chrysosporium was the secon most frquent genus and was encountered in 40 % of the samples tested and had RIV of 54.7. From the genus 6 species were collected of which *C. xerophilum* (28 %) was the most common species. The remaining *Chrysosporium* species were rarely recovered and these were *C. asperatum* (4 %), *C. carmichaelii* (10 %), *C. lucknowense* (4 %), *C. pannicola* (6 %) and *C. prunosum* (2 %). All these species were isolated from the soil samples of Oman by El-Said, (1993) and were emerged from 6, 12, 10, 10 and 10 %, respectively. In Egypt, *C. asperatum* and *C. pannicola* were isolated from Egyptian soils by Abdel-Hafez et al. (1989, 1991). Filippello Marchisio (1986) isolated *C. pannicola* (3.5 %) and *C. xerophilum* (7.1 %) from children's sandpits in Italy.

Arthroderma occupied the third place with regard to the number of cases of isolation of fungal genera and it was recovered from 28 % of samples examined and had RIV of 37.4. Four species of *Arthroderma* were isolated and these were *A. ciferii* (teleomorph of *Chrysosporium georgii*), *A. cuniculi*, *A. curreyi* and *A. lenticulare* (*Trichophyton terrestre*). In Italy Filippello Marchisio (1986) isolated *A. cuniculi* and *A. curreyi* from children's sandpits. In Oman El-Said (1993) isolated all the above *Arthroderma* species from soil samples.

Aspergillus (3 species + 1 variety) occupied the fourth place and it encountered in 26 % of the soil samples. Among *Aspergillus* species, the most commonly collected were *A. flavus* and *A. terreus*. The remaining *Aspergillus* species were scarcely recovered and these were *A. flavus* var. *columnaris*, *A. fumigatus* and *A. niger*. Aspergillosis due to *A. fumigatus* and *A. flavus* has a world-wide distribution (Frey

et al., 1979). K h a l l i l, A b d e l - S a t e r (1991) isolated *A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus* from the soils of mangroves at Egyptian red sea coast. Most of the above species had been previously encountered, but with different incidence from various types of soil from many parts of the world (S u n d a r a m, 1987; A b d e l - H a f e z et al., 1989; E l - S a i d, 1993, 1994).

Trichophyton encountered from 16 % of the samples tested. It was represented by *T. ejelloi* and *T. mentagrophytes*. This two species have a wide distribution and was recovered from different substrates from different places of the world (T o d a r o, 1978; F i l i p e l l o M a r c h i s i o, 1986; A b d e l - H a f e z et al., 1989; E l - S a i d, 1994). The above 2 species were found as saprophytes in man and animals, but also have been recognised as the causal agent of tinea, onychomycosis and ring-worm (F e r y et al., 1979).

Microsporum gypseum was emerged in 14% of the samples. A b d e l - F a t t a h et al. (1982) isolated *M. gypseum* from Egyptian soils, it was encountered in 8.5, 2.9 and 7.1 % of the soil samples baited with human hair, animal and feathers, respectively. This species is cosmopolitan and it was encountered from different parts of the world (S t e p a n i s h c h e v a, 1965; B e l u k h a, L i k y a n o v a, 1969; P a d h y e et al., 1967; M e i n h o t, G r a b o w s k i, 1972; A l i l o u s, A s g a r, 1973; A b d e l - H a f e z et al., 1989). It has been reported from skin lesions, feathers and pellets of free-living birds, the hair and skin of monkeys, dogs, mice, rats and other small mammals. It has been recognised as the causal agent of dermatomycosis in cattle and man from different parts of the world (D o m s c h et al., 1980).

The remaining isolated 13 genera and 15 species were recovered in rare frequencies as present in Table (1).

Airborne fungi

The concentration and the composition of the airspora trapped at 1.5 m above the ground level are shown in Table (1).

Twenty-nine species and 1 variety belonging to 15 genera were collected from the atmosphere of mangrove by using hair baiting technique at 28°C.

Aphanoascus, a closely related fungus to dermatophytes, was common in the air and emerged in 90 % of the number of exposures comparing 31.3 % of total fungal catches and had RIV of 121.3. Of the genus three species were collected: *A. fulvescens*, *A. terreus* and *Aphanoascus* sp. They occurred in 60 %, 42 % and 52 % of the number of exposures comparing 11.2 %, 9.5 % and 10.3 % of total fungi and had RIVs of 71.2, 51.5 and 62.3, respectively. *Aphanoascus terreus* and *Aphanoascus* sp. were recovered previously from the air of Hibis Temple at El-Kharga Oasis in Egypt, emerging in 25 % and 33 % of the number of exposures matching 3.1 % and 16.1 % of total fungi, respectively (I s m a i l, 1990). Also, other closely related fungi to dermatophytes were isolated but with different incidences: *Aphinisia queenslandica*, *Chrysosporium asperatum*, *C. purinosum*, *C. xerophilum*, *Microsporum gypseum*, *Myceliophthora vellerea*, *Trichophyton equinum* and *T. mentagrophytes*.

Table 1

Total isolatus (TI, calculated per 500 hair fragments in all soil samples and per 250 hair fragments in one exposure of 1 h) unber of cases of isolation (NCI, out of 50 cases), occurrence remarks (OR), relative importance values (RIV) and frequency (% F, calculated per 50 samples) of various fungal genera and species recovered from mangrove's soil and air using hair baiting technique at 28°C

Genera et species	Soil				Air			
	TI	NCL, OR	RIV	% F	TI	NCL, OR	RIV	% F
<i>Acremonium strictum</i> W. Gams	20	5R	12.8	10	-	-	-	-
<i>Alternaria alternata</i> (Firs) Keissler	12	4R	9.7	8	3	2R	4.2	4
<i>Aphanoascus</i>	240	43H	119.7	86	591	45H	121.3	90
<i>A. fulvescens</i> (Fr.) Apinis	95	30H	73.3	60	211	30H	71.2	60
<i>A. terreus</i> (Randhawa et Sandhu) Apinis	60	12M	32.4	24	180	21M	51.5	42
<i>Aphanoascus</i> sp.	75	15M	40.5	30	195	26M	62.3	52
<i>Apinisia queenslandica</i> Apinis et Rees	3	2R	4.4	4	5	2R	4.3	4
<i>Arthroderma</i>	67	14M	37.4	28	-	-	-	-
<i>A. ciferrii</i> Varsavsky et Ajello	8	2R	5.1	4	-	-	-	-
<i>A. cuniculi</i> Dawson	25	6L	15.5	12	-	-	-	-
<i>A. curreyi</i> Berk.	29	7L	18.1	14	-	-	-	-
<i>A. lenticulare</i> Pore, Tsao et Plunkett	5	1R	2.7	2	-	-	-	-
<i>Aspergillus</i>	87	18M	48.2	36	631	47H	127.4	94
<i>A. flavus</i> Link	33	13M	30.6	26	140	36H	79.4	72
<i>A. flavus</i> var. <i>columnaris</i> Raper et Fennel	11	5R	11.5	10	6	2R	4.3	4
<i>A. flumigatus</i> Fresenius	13	5R	11.8	10	160	28H	64.7	56
<i>A. niger</i> Van Tieghem	7	3R	7	6	210	29H	69.1	58
<i>A. sydowii</i> (Bain. et Sart) Thom et Church	-	-	-	-	53	16M	34.8	32
<i>A. terreus</i> Thom	23	8L	19.2	16	62	10L	23.3	20
<i>Chaetomium globosum</i> Kunze	16	5R	12.2	10	-	-	-	-
<i>Cladosporium</i>	-	-	-	-	45	16M	34.4	32
<i>C. cladosporioides</i> (Fres.) De Vries	-	-	-	-	28	13M	27.5	26
<i>C. sphaerospermum</i> Penzig	-	-	-	-	17	4R	8.9	8
<i>Chrysosporium</i>	105	20M	54.7	40	15	7L	14.8	14
<i>C. asperatum</i> J. W. Carmichael	4	2R	4.6	4	3	1R	2.2	2
<i>C. carmichaelii</i> Van Oorschot	22	5R	13.1	10	-	-	-	-
<i>C. luckenowense</i> Gare	9	2R	5.3	4	-	-	-	-

<i>C. pannicola</i> (Corda) Van Oorschot, Staplers	12	3R	7.7	6	-	-	-	-
<i>C. pruinosa</i> Gilman et Abbott	3	1R	2.4	2	2	1R	2.1	-
<i>C. xerophilum</i> Pitt	55	14M	35.7	28	10	5R	10.5	2
<i>Cochliobolus spicifer</i> Nelson	5	3R	6.4	6	110	13M	31.8	10
<i>Cunninghamella echinulata</i> Thaxter	-	-	-	-	50	16M	34.6	26
<i>Fusarium oxysporum</i> Schlecht.	7	4R	9	8	-	-	-	32
<i>Gibberella fujikuroi</i> (Sowada) Ito	8	5R	11.1	10	-	-	-	-
<i>Microsporium gypseum</i> (Bodin) Guiart et Grigorakis	24	7L	17.4	14	3	1R	2.2	-
<i>Mucor racemosus</i> Fresenius	8	2R	5.1	4	-	-	-	2
<i>Myceliophthora velleera</i> (Sacc. et Speg.) Van Oorschot	2	1R	2.3	2	5	2R	4.3	-
<i>Mycosphaerella tassiana</i> (De Not.) Johanson	-	-	-	-	120	26H	58.3	4
<i>Nectria haematococcoa</i> Berk. et Br.	3	3R	6.4	6	-	-	-	52
<i>Pacilomyces variotii</i> Bain	5	2R	4.7	4	-	-	-	-
<i>Penicillium</i>	38	5R	15.3	10	251	32H	77.3	-
<i>P. chrysogenum</i> Thom	8	2R	5.1	4	115	25H	56.1	64
<i>P. citrinum</i> Thom	6	2R	4.8	4	15	8L	16.8	50
<i>P. corylophilum</i> Dierckx	-	-	-	-	28	15M	31.5	16
<i>P. funiculosum</i> Thom	13	5R	11.8	10	63	26H	55.3	30
<i>P. puberulum</i> Bainier	5	1R	2.7	2	30	10L	21.6	52
<i>P. variable</i> Sopp	6	1R	2.8	2	-	-	-	20
<i>Rhizostolonifer</i> (Ehrenb.) Lind	3	2R	4.4	4	-	-	-	-
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier	15	5R	12.1	10	23	13M	27.2	-
<i>Syncephalastrum racemosum</i> (Cohn) Schroeter	-	-	-	-	30	10L	36.5	26
<i>Trichophyton</i>	40	8L	21.6	16	9	3R	6.5	36
<i>T. ajelloi</i> (Vanbreuseghen) Ajello	6	2R	4.8	4	-	-	-	6
<i>T. equinum</i> (Martuchot, Dassonville) Goedelst	11	4R	9.5	8	3	1R	2.2	-
<i>T. mentagrophytes</i> (Robin) Blanchard	18	6L	14.5	12	6	2R	4.3	2
<i>T. rubrum</i> (Castellani) Sabouraud	5	2R	4.7	4	-	-	-	4
<i>Verticillium lateritium</i> Berkeley	5	3R	6.7	6	-	-	-	-
Total isolates	713				1891			
Number of genera	21				15			
Number of species	41+ 1 Var.				29 + 1 Var.			

Occurrence remarks: H = high occurrence, isolated from 25-50 cases (out of 50); M = moderate occurrence, from 13-24 cases; L = low occurrence, from 6-12 cases; R = rare occurrence, from 1-2 cases

Few numbers of keratinophilic fungi had been encountered previously from the air in some parts of the world (Papavassilion, Bartzokas, 1975; Altaras, Lehrer, 1977; Acosta, Roberstad, 1979; Patil, Kulkarni, 1981; Della-France, Caretta, 1984; Moubasher et al., 1990; El-Maghraby, 1994).

Other moulds were also isolated from the by using horse fragments as bait and these include members of *Alternaria* (1 species), *Aspergillus* (5 + 1 variety), *Cladosporium* (2), *Cochliobolus* (1), *Cunninghamella* (1), *Mycosphaerella* (1), *Penicillium* (5), *Scopulariopsis* (1) and *Syncephalastrum* (1). From the above genera the most common species were: *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *Cladosporium cladosporioides*, *Cochliobolus spicifer*, *Cunninghamella echinulata*, *Mycosphaerella tassiana*, *Penicillium chrysogenum*, *P. corylophilium*, *P. funiculosum*, *Scopulariopsis brevicaulis* and *Syncephalastrum racemosum*. These findings are almost in agreement with those reported by El-Maghraby (1994) during her study on the atmosphere of some schools at Hurghada City, she reported that the most common species were: *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Candida* sp., *Cladosporium sphaerospermum*, *Cochliobolus spicifer*, *Cunninghamella echinulata*, *Eurotium chevalieri*, *Mycosphaerella tassiana*, *Syncephalastrum racemosum*, *Talaromyces flavus* and *Torula herbarum* on plates of Sabouraud's dextrose agar and using goat hair fragments as baits. Several of these fungi have been known to be allergenic (Plutarco, 1958; Masatomo et al., 1991), causing asthma (Beaumont et al., 1985), ocular infection (Sehgal et al., 1981), hyper-sensitivity pneumonitis and pulmonary infection (Tregger et al., 1985 and Arianyagam et al., 1986).

Keratinolytic activity

Table (2) indicate that isolates of different or of the same species had variable rates of growth. Most of the isolates (50) showed a reasonable rate (++) with abundant vegetative growth, while only the rest (8) isolates showed little and scarce growth. Some of isolates have keratin-degrading enzyme(s), but they differ in their capabilities for the production of these enzymes. Peyton et al. (1965) recorded a significant keratinophilic activity of *M. canis* and *M. gypseum*. Filipello Marchisio (1986) reported that the members of *Microsporum*, *Trichophyton*, *Mariannaea*, *Aphanoascus*, *Chrysosporium*, *Malbranchea* and *Geomyces* were the most active keratinolysis. In Egypt, Mahmood (1990) reported that *T. mentagrophytes* was able to grow actively on horse hairs.

Effect of garlic extract and onion oil on the isolated keratinophilic fungi

A - Garlic extract. All tested fungi were sensitive to garlic extract. The mycelial dry weight of *Aphanoascus fulvescens*, *A. terreus*, *Apinisia queenslandica*, *Arthroderma ciferrii*, *A. cuniculi*, *A. curreyi*, *Chrysosporium asperatum*, *C. carmichaelii*, *C. lucknowense*, *C. pannicola*, *C. purinosum*, *Microsporum gypseum*,

Trichophyton ajelloi and *T. mentagrophytes* was significantly retarded by the three levels used. The mycelial dry weight of *Aphanoascus* sp., *Arthroderma lenticulare*, *Chrysosporium xerophilum* and *Trichophyton equinum* significantly depressed by 2000 and 3000 ppm, whereas that of *T. rubrum* was decreased by the 3000 ppm only (Table 3).

Appleton, Tansley (1975) reported that *Epidermophyton floccosum*, *Microsporum canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum* and *Scopulariopsis brevicaulis* did not grow in a concentration of 5×10^{-3} garlic extract. Prasad et al. (1982) observed that the topical application of the crude extract of garlic at a 1:10 concentration in distilled water could combat dermatophytosis produced in rabbits with *Microsporum canis* without causing any apparent side effects.

Table 2

Growth of fungal isolates on hair-sand medium

Fungal isolates	Rate of growth		
	+	++	+++
<i>Aphanoascus fulvescens</i> (10)*	—	5	4
<i>A. terreus</i> (6)	2	4	—
<i>Aphanoascus</i> sp. (1)	—	5	3
<i>Apinisia queenslandica</i> (1)	—	1	—
<i>Arthroderma ciferrii</i> (2)	—	2	—
<i>A. cuniculi</i> (4)	1	3	—
<i>A. curreyi</i> (6)	2	4	—
<i>A. lenticulare</i> (1)	—	1	—
<i>Chrysosporium asperatum</i> (1)	—	1	—
<i>C. carmichaelii</i> (4)	—	4	—
<i>C. luckenowense</i> (1)	—	1	—
<i>C. pannicola</i> (2)	—	2	—
<i>C. pruinatum</i> (1)	—	1	—
<i>C. xerophilum</i> (8)	2	6	—
<i>Microsporum gypseum</i> (5)	—	3	2
<i>Myceliophthora vellerea</i> (1)	—	1	—
<i>Trichophyton ajelloi</i> (1)	—	1	—
<i>T. equinum</i> (2)	1	1	—
<i>T. mentagrophytes</i> (3)	—	2	1
<i>T. rubrum</i> (1)	—	1	—
Total (68)	8	50	10
Percentage (100)	11.8	73.5	14.7

* The number between parentheses indicate the number of isolates tested.

+ indicates weak growth; ++ indicates moderate growth; +++ indicates abundant growth.

Table 3

Effect of various concentrations of garlic extract and onion oil on the mycelial dry weight (calculated as percentage of the control) of the test fungi

Species	Garlic extract			Onion oil		
	L (1000 ppm)	M (2000 ppm)	H (4000 ppm)	L (100 ppm)	M (200 ppm)	H (400 ppm)
<i>Aphanoascus fulvescens</i>	60*	43*	0*	75*	33*	15*
<i>A. terreus</i>	74*	52*	12*	82	62*	20*
<i>Aphanoascus</i> sp.	86	74*	68*	92	81*	70*
<i>Apinisia queenslandica</i>	71*	62*	0*	87	63*	22*
<i>Arthroderma ciferrii</i>	53*	42*	0*	66*	54*	0*
<i>A. cuniculi</i>	62*	35*	12*	73*	42*	13*
<i>A. curryei</i>	50*	0*	0*	65*	41*	0*
<i>A. lenticulare</i>	85	73*	25*	92	87	62*
<i>Chrysosporium asperatum</i>	72*	60*	45*	84	63*	23*
<i>C. carmichaelii</i>	69*	71*	63*	73*	39*	10*
<i>C. luckenowense</i>	76*	53*	25*	86	65*	18*
<i>C. pannicola</i>	68*	32*	0*	75*	52*	0*
<i>C. pruinatum</i>	65*	43*	0*	70*	35*	0*
<i>C. xerophilum</i>	85	70*	35*	90	84	60*
<i>Microsporum gypseum</i>	51*	48*	25*	85	65*	22*
<i>Myceliophthora vellerea</i>	95	84	71*	97	90	85
<i>Trichophyton ajelloi</i>	77*	72*	33*	86	66*	24*
<i>T. equinum</i>	83	75	55	89	72*	65*
<i>T. mentagrophytes</i>	59*	10*	0*	40*	0*	0*
<i>T. rubrum</i>	92	80*	69*	75*	60*	12*

*Means significant difference comparable with the control

B – O n i o n o i l. The three levels of onion oil inhibited the mycelial growth of *Aphanoascus fulvescens*, *Arthroderma ciferrii*, *A. cuniculi*, *A. curryei*, *Chrysosporium carmichaelii*, *C. pannicola*, *A. pruinatum*, *Trichophyton mentagrophytes* and *T. rubrum*. The mycelial growth of *Aphanoascus terreus*, *Aphanoascus* sp., *Apinisia queenslandica*, *Chrysosporium asperatum*, *C. lucknowense*, *Microsporum gypseum*, *Trichophyton ajelloi* and *T. equinum* was significantly retarded by medium and high doses. However, *Arthroderma lenticulare* and *Chrysosporium xerophilum* were significantly retarded by high doses only. On the other hand *Myceliophthora vellerea* was not significantly affected by any level of onion oil (Table 3).

S h k h a w a t, P r a s a d a (1971) reported that boiled water extracts of onion caused inhibition to the growth of *Alternaria tenuis*, *Helminthosporium* sp. and *Curvularia perniseta*. More recently **Z o h a r i et al.** (1992) noticed that onion oil (200 ppm) completely inhibited the growth of *Microsporum canis*, *M. gypseum* and *Trichophyton simii*, but *Chrysosporium queenslandicum* and *Trichophyton mentagrophytes* were completely inhibited by 500 ppm of onion the culture media.

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