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In vitro antifungal activity of some essential oils against some filamentous fungi of rainbow trout (*Oncorhynchus mykiss*) eggs

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Abstract. One application of medicinal plants is their use as antimicrobial agents. The aim of this study was to investigate the effect of essential oils on four species of fungi in rainbow trout (*Oncorhynchus mykiss*) eggs, including *Saprolegnia parasitica, Aspergillus niger, Aspergillus fumigates, Aspergillus flavus*. Essences minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFC), were determined using Microdilution methods. For other fungi, best effect gained in 20 µL mL^{-1} by *Satureja bachtiarica*. The results of the ELISA showed growth of fungal isolates inhibited completely in the presence of 10-20 µL mL^{-1} of most essential oils. Based on the results the MIC values against *S. parasitica* fungi for essence oils of *Eucalyptus globulus, Myrtus communis*, *Thymus daenensis, Matricaria recutita, Mentha longifolia* and *S. bachtiarica* was obtained equal to 2.5, 10, 5, 5, 10 and 5 mL, respectively. Based on this results the numbers of the fungal colonies for 3 fungal isolates greatly decreased with increase of the essence oil concentration from 2.5 µL mL^{-1} to 5 µL mL^{-1}. For *A. flavus* the best antifungal effect was obtained (not growth) at 20 µL mL^{-1} concentration in the presence of the essence oils of pennyroyal, savory, chamomile, thyme and *Myrtus*. For *S. parasitica* the best antifungal effect was obtained at 5 µL concentration in the presence of pennyroyal essence oil and then at 10 µL concentration of *T. daenensis* and *Myrtus* essence oil. Hence, these plants can be used to produce natural products that may serve as key in the development of new pharmaceuticals research activities.
Key Words: essential oil, antifungal activity, minimum inhibitory concentration, *Saprolegnia*.

Introduction. New antibiotics and drugs were introduced and produced by pharmacological companies in the last three decades. However, these antibiotics/drugs have failed to inhibit and control the growth of many pathogenic organisms that have genetic ability to transmit and acquire resistance to drugs (Plakas et al 1996). Essential oils (also called volatile or ethereal oils (Guenther 1948) are aromatic oily liquids obtained from plant tissues such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or various extractions but for commercial production of EOs the method of steam distillation is most commonly used (Van de Braak & Leijten 1999). The term essential oil is thought to derive from the name coined in the 16th century by Paracelsus von Hohenheim; he named the effective component of a drug Quinta essential (Guenther 1948). Essential oils of herbs and their components, which are products from the secondary metabolism of plants, have many applications in ethno-medicine, food flavoring and preservation as well as in the fragrance and pharmaceutical industries (Fabian et al 2006). The antimicrobial properties of essential oils have been described (Pinto et al 2006, 2009). As natural products from aromatic and medicinal plants, essential oils are known to possess antiviral, antibacterial, antifungal and antioxidant activities (Kordali et al 2005; Baratta et al 1998; Bakkali et al 2008) and there is an increasing interest in medicinal plants as an alternative to synthetic preservatives and antibiotics (Edris 2007).

The extracts and essential oils of many plants/herbs have been shown to exert biological activity in vitro and in vivo, which justified research on traditional medicine focused on the characterization of antimicrobial activity of these plants. Iran, India,

Pakistan, Turkey, Jordan, Brazil and Mexico are examples of countries that have a diverse flora and a rich tradition in the use of medicinal plants for both antibacterial and antifungal applications (Mahasneh et al 1999; Navarro et al 1996). Since plants produce a variety of compounds with antimicrobial properties, it is expected that screening programs for some under-represented targets, such as antifungal activity, may yield candidate compounds for developing new antimicrobial drugs (Ahmad & Beg 2001). In addition, it is expected that plant compounds showing target sites other than those currently used by antibiotics will be active against drug-resistant microbial pathogens (Duarte et al 2005). Plants and their essential oils are potentially valuable sources of antimicrobial compounds. Several studies have been published on the antimicrobial activities of plant compounds against many different types of microorganisms (Friedman et al 2002; Tassou et al 1995; Rančić et al 2005).

Eucalyptus (*Eucalyptus globulus* - Myrtaceae) is indigenous to Australia and Tasmania and it is cultivated in some subtropical regions of southern Europe, Africa, Asia, and America. Eucalyptus oil is obtained from the leaves of the plant. The oil contains over 80% cineol, with other constituents as p-cymene, alpha-pinene, limonene, geraniol and camphene. The oil is widely used in curing headache, body pains, fever, chronic bowel complaints and dysentery (Esmort 1997; Juergens et al 2003; Sefidkon et al 2007). It is greatly valued for its antiseptic and disinfectant properties and is used especially in the treatment of infection of upper respiratory tract and in certain skin diseases (Rasooli et al 2006; Sefidkon & Jamzad 2000). In vitro, eucalyptus oil has an antibacterial and fungicidal effect (Osawa et al 1995).

Myrtle (*Myrtus communis* - Myrtaceae) grows from the Mediterranean region to the northwestern Himalayas. The medicinal parts of myrtle are the leaves (dried and as a source of oil), twigs and fresh, flowering branches. The oil contains eucalyptol (15-45%), alpha-pinene (15-38%), myrtenol (1-5%), myrtenylacetate (4-20%), limonene (4-10%), alpha-terpineol (2-12%), geraniol (0.5-1.5%), geranylacetate (1-5%), myrtol (a myrtle oil fraction that boils between 160 and 180°C, and chief components are eucalyptol and alphapinene). The essential oil showed antibacterial, fungicidal and disinfectant activities (Yadegarinia et al 2006; Owlia et al 2007).

The essential oils of thyme (*Thymus daenensis* - Lamiaceae) are extracted from the fresh, flowering herb, the dried leaves, the stripped and dried leaves, and the fresh aerial pan of the flowering plant. Chief components are: thymol (20-55%), p-cymene (14-45%), carvacrol (1-10%), gamma-terpinene (5-10%), borneol (up to 8%), linalool (up to 8%). Thyme is a bronchial antispasmodic and an expectorant. It has shown antibacterial, antifungal, antiviral, antiprotozoal, and antioxidant properties. In animal experiments, a spasmolytic effect was demonstrated for the flavone fraction and an expectorant effect on ciliary activity for the terpenes (Sajjadi & Khatamsaz 2003).

Chamomile (*Matricaria recutita* - Asteraceae) is indigenous to Europe and northwest Asia, naturalized in North America and elsewhere (Salamon 1992). The essential oil was shown to be a potential antiviral agent against herpes simplex virus type 2 (HSV-2) in vitro (Koch et al 2008). Potentially active chemical constituents of *M. chamomilla* including α-bisabolol (56.86%), trans-trans-farnesol (15.64%), cis-β-farnesene (7.12%) and guaiazulene (4.24%) which are believed to be responsible in part for such awide range of biological activities (Tolouee et al 2010).

Savory (*Satureja bachtiarica* - Lamiaceae) is found in southern and central Europe, the Caucasus and the southern mountains of Asian Russia, as well as in the northern U.S. (Cantino et al 1992). Analysis of the essential oil was showed in the identification of 20 constituents, carvacrol (44.8%), ɣ-terpinen (18.7%) and thymol (14.95%) being the main components (Babadi et al 2012).

Pennyroyal (*Mentha longifolia* - Lamiaceae) is native to Europe, western and central Asia and northern and southern Africa. Pennyroyal is commonly used to reduce abdominal cramps, relieve flatulence and settle an upset stomach (Gulluce et al 2007). High contents of antimicrobial components such as piperitenone, pulegone and piperitenone oxides in the essential oil were recorded (Ghoulami et al 2001; Gulluce et al 2007).

The rainbow trout (*Oncorhynchus mykiss*) has been widely introduced into suitable lacustrine and riverine environments around the world (MacCrimmon 1971) and becomes one of the most widely cultured species in Iran due to their adaptability to artificial rearing. Diseases cause the largest econobiologic losses in animal and human life and fungal infections are second only to bacterial diseases in economic importance of aquaculture industry (Srivastava 1987). Fish egg quality is defined as the capability of an egg to become fertilized and subsequently develop into a normal embryo, while Saprolegniaceae is very common pathogenic fungus infecting in cold water fish such as salmonids and their eggs (Sudova et al 2007).

Using chemichal agents (i.e. Malachite green, formalin, oxygen proxide, sodium hypochloride) have been limited or banned in aquaculture industries (Sudova et al 2007). Furthermore, necessity of an eco-friendly antifungal agent promoted to introducing of herbal drugs to aquatic farms. Therefore, the aim of this study was to assess in vitro antifungal activity of 6 essential oils against some filamentous fungi isolated from rainbow trout eggs.

Material and Method. The plants that are used in this research: *E. globulus*, *M. communis*, *T. daenensis*, *M. recutita*, *S. bachtiarica*, and *M. longifolia* were collected from mountain areas of Zagross, Iran, during May-November, 2014.

Fungal strains and culture. Fungal species were isolated from infected rainbow trout eggs from a commercial aquaculture farms in Lorestan Provice, Iran and transported to the Fisheries Science Laboratory of the Science and Research Branch, Islamic Azad University, Tehran, Iran. All of the fungal strains were cultured, purified and identified according to Tampieri et al (2003) and Ghasemi-Pirbalouti et al (2009).

Isolation of essential oils. Leaves and flowers of the herbs were air-dried (100 g from each herb) and ground to semi-powdered state. Then they were subjected to hydro distillation (2 L of distilled water) for 4 hours using a Clevenger-type apparatus to produce essential oils in accordance with the British pharmacopoeia (Basiri et al 2007). The oil samples were stored in universal bottles and refrigerated at 4°C prior to use.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The micro dilution assay was used in order to determine the minimum inhibitory concentration (MIC). For this purpose, 5 mL of RPMI1640 and DMSO 3% of medium with 40 µL mL^{-1} essence oil concentration was used in Venoject tubes. The fungal suspension was prepared equal to McFarland No. 0.5 with density of cfu/mL 1.5×10^{8} out of studied fungi. To ensure and eliminate the possible errors the numbers of condies were counted by Neubauer Slide and 96 cells were added to each of the plate sockets by eight-channel sampler to 100 µL of 2% of RPMI1640 medium, then the desired serial dilutions in 10 dilutions were prepared out of the solution of the slightly essence oil in flat micro plate with 96 cells and 100 µL was added to each of the sinks, so that it was added to the first socket of the left side and well mixed with the medium and next 100 µL was removed and transferred from the first socket to the second one, and so on the 2.1 dilution was prepared of each essence oil in each socket and finally 100 µL of essence oil was extracted from the tenth socket. In this method the first and tenth sinks had the highest and the lowest concentration, respectively. Sockets 11 and 12 were free of essence oil solution and standardized fungal suspension was added to each of the sockets to a constant rate of 100 µL so the final volume of the solution became equal to 200 µL which is different from its essence oil concentration. The socket 11 contains 100 µL of the medium and 100 µL of the fungal suspension and the socket 12 contains only the medium were considered as positive and negative control respectively. This test will be twice repeated for each fungal isolates in the separated horizontal row. In this test the highest concentration is in the first socket equal to 20 and the lowest concentration in the tenth socket is equal to 0.039 mcg mL^{-1}. These plates were covered by Para film to prevent the evaporation and pollution and were incubated at 25°C for 24 hours. After this time the micro plates were removed from the incubator

and were read both with eye and by ELISA Reader at the wavelength of 490 nm. The sockets which prevented the fungal growth were considered as the MIC. In order to determine the minimum fungicidal concentration (MFC) of the extracts using sampler, we removed the 10 µL MIC dilution of each of the extracts and also a dilution before and after it (MIC), and cultured it on the potato dextrose agar (PDA) medium at 25°C and after that was incubated for 30 hours. After this time, each dilution which was prevented the full fungal growth and there was a growth in the range of less than 3 colonies (approximately equal to 99-99.95% of fungicidal activity) was considered as MFC. If the difference between the MFC and MIC value is high, then the desired fungus is resistant to the fungicidal action and if the difference value between them is small then the studied strains were considered sensitive.

Determination of fungal strains growth pattern by ELISA method. In order to obtain the growth pattern, the optical absorption level is read before the incubation of fungal strains by ELISA machine and also the optical absorption level is read after the incubation and is subtracted of the initial absorption level to obtain the growth pattern of the fungi.

Results and Discussion. The antimicrobial activity of the extracts was studied in different concentrations. In this study, antifungal activity of some plants' essential oils were evaluated and summarized in Tables 1 to 4. Based on the results the MIC values against *Saprolegnia parasitica* fungi for essence oils of eucalyptus, myrtle, thyme, chamomile, pennyroyal and savory was obtained equal to 2.5, 10, 5, 5, 10 and 5 µL mL^{-1}, respectively. The MFC values for the above essence oils have been respectively equal to the MIC values (Table 1). Also the MIC and MFC related results to these essence oils against the *Aspergillus fumigatus* are given in Table 2. Accordingly, the MIC values of the above essence oils have been <20, 20, 10, 20, 20 and 20 µL mL^{-1} respectively. These values obtained 99% for MFC. The related results to the MIC and MFC 99% of the above essence oils against *Aspergillus flavus* are listed in Table 3 as well, so that the values of MIC and MFC 99% of these essence oils estimated 200>, 200, 20>, 20> and 10 µL mL^{-1}, respectively. And the related results to the MIC and MFC 99% of the above essence oils against *Aspergillus niger* were respectively 20, 20, 20> and 20 µL mL^{-1} (Table 4).

Table 1

MIC and MFC of essential oils against *Saprolegnia parasitica*

Essential oil	*Essential oil concentration (µL mL^{-1})*			
	2.5	5	10	20
Eucalyptus globulus	7	5	3 MFC99, MIC	0
Myrtus communis	4	3 MFC99, MIC	0	0
Thymus daenensis	5	1 MFC99, MIC	0	0
Matricaria recutita	8	6	2 MFC99, MIC	0
Mentha longifolia	2 MFC99, MIC	0	0	0
Satureja bachtiarica	5	3 MFC99, MIC	1	0

*MFC not seen. Need to higher extract concentration.

Table 2

MIC and MFC of essential oils against *Aspergillus fumigatus*

Essential oil	*Essential oil concentration (µL mL^{-1})*			
	2.5	5	10	20
Eucalyptus globulus	30	20	13	10
Myrtus communis	32	30	6	3 MFC99, MIC
Thymus daenensis	10	7	3 MFC99, MIC	0
Matricaria recutita	20	10	4	1 MFC99, MIC
Mentha longifolia	20	10	6	1 MFC99, MIC
Satureja bachtiarica	14	9	7	0 MFC99, MIC

*MFC not seen. Need to higher extract concentration.

Table 3

MIC and MFC of essential oils against *Aspergillus flavus*

Essential oil	*Essential oil concentration (µL mL^{-1})*			
	2.5	5	10	20
Eucalyptus globulus	18	10	7	5
Myrtus communis	30	23	18	10
Thymus daenensis	13	8	4	1 MFC99, MIC
Matricaria recutita	20	15	11	7
Mentha longifolia	21	12	7	3 MFC99, MIC
Satureja bachtiarica	12	8	1 MFC99, MIC	0

*MFC not seen. Need to higher extract concentration.

Table 4

MIC and MFC of essential oils against *Aspergillus niger*

Essential oil	*Essential oil concentration (µL mL^{-1})*			
	2.5	5	10	20
Eucalyptus globulus	25	17	13	10
Myrtus communis	35	25	12	2 MFC99, MIC
Thymus daenensis	20	10	6	0 MFC99, MIC
Matricaria recutita	23	15	8	2 MFC99, MIC
Mentha longifolia	32	16	7	0 MFC99, MIC
Satureja bachtiarica	33	20	4	1 MFC99, MIC

*MFC not seen. Need to higher extract concentration.

The results of the fungal isolates growth behavior of *A. flavus*, *A. fumigatus*, *S. parasitica*, *A. niger* in presence of different dilutions of the essence oils were measured by the ELISA method and sumerized in Tables 5 to 8. The results showed that by increasing the essence oils concentration the optical absorption level is decreased which represents the fungi growth is decreased.

The obtained results of the effect of essence oils on fungal isolates growth pattern are shown in Figures 1 to 4. Based on this results the numbers of the fungal colonies for 3 fungal isolates greatly decreased with increase of the essence oil concentration from 2.5 µL mL^{-1} to 5 µL mL^{-1}. The number of grown fungal colonies in the presence of most of the essence oils and in the presence of the 10-20 µL concentration is zero.

For *A. flavus* the best antifungal effect was obtained (not growth) at 20 µL mL^{-1} concentration in the presence of the essence oils of pennyroyal, savory, chamomile, thyme and myrtus.

For *S. parasitica* the best antifungal effect was obtained at 5 µL concentration in the presence of pennyroyal essence oil and then at 10 µL concentration of *T. daenensis* and Myrtus essence oil.

In the case of *A. fumigatus* the most appropriate antifungal effect was obtained in the presence of the essence oils of thyme, myrtus, chamomile, pennyroyal and savory at 20 µL mL^{-1} concentration.

For *A. niger* the best antifungal effect was obtained in the presence of Savory at 10 µL mL^{-1} and then in the presence of the *T. daenensis* at 20 µL mL^{-1}.

Table 5

The results of *Aspergillus flavus* growth behavior in the presence of different dilutions of the essence oils

Number of socket	*Concentration (mL)*	*Eucalyptus globulus*	*Myrtus communis*	*Thymus daenensis*	*Matricaria recutita*	*Mentha longifolia*	*Satureja bachtiarica*
1	20	0.587	0.537	2.183	2.198	0.516	1.162
2	10	0.827	0.535	1.984	0.623	0.480	0.652
3	5	0.952	0.585	0.859	0.717	0.549	0.814
4	2.5	1.024	0.806	0.532	0.743	0.780	0.870
5	1.25	0.894	0.881	0.867	0.852	0.890	0.861
6	0.625	0.869	0.866	0.809	0.856	0.901	0.868
7	0.312	0.909	0.864	0.855	0.897	0.922	0.932
8	0.156	1.007	0.952	0.823	0.854	0.875	0.866
9	0.078	1.029	0.931	0.869	0.912	0.861	0.942
10	0.039	0.935	0.871	0.859	0.871	0.898	0.933
11	control +	0.846	0.897	0.898	0.815	0.839	0.874
12	control -	0.427	0.436	0.422	0.443	0.421	0.415

Table 6

The results of *Aspergillus fumigatus* growth behavior in the presence of different dilutions of the essence oils

Number of socket	*Concentration (mL)*	*Eucalyptus globulus*	*Myrtus communis*	*Thymus daenensis*	*Matricaria recutita*	*Mentha longifolia*	*Satureja bachtiarica*
1	20	0.530	0.570	2.090	1.700	0.562	2.068
2	10	0.772	0.521	1.173	0.709	0.524	0.927
3	5	0.855	0.771	0.846	0.745	0.701	0.587
4	2.5	0.874	0.810	0.757	0.772	0.759	0.773
5	1.25	0.894	0.792	0.803	0.792	0.820	0.795
6	0.625	0.892	0.823	0.835	0.829	0.826	0.802
7	0.312	0.900	0.825	0.814	0.830	0.846	0.812
8	0.156	0.863	0.881	0.812	0.838	0.841	0.806
9	0.078	0.860	0.850	0.816	0.824	0.840	0.850
10	0.039	0.874	0.878	0.817	0.824	0.851	0.830
11	control +	0.923	0.873	0.875	0.858	0.879	0.832
12	control -	0.406	0.403	0.402	0.400	0.407	0.402

Table 7

The results of *Saprolegnia parasitica* growth behavior in the presence of different dilutions of the essence oils

Number of socket	*Concentration (mL)*	*Eucalyptus globulus*	*Myrtus communis*	*Thymus daenensis*	*Matricaria recutita*	*Mentha longifolia*	*Satureja bachtiarica*
1	20	0.519	0.541	1.757	1.842	0.597	1.103
2	10	0.543	0.547	1.081	0.625	0.557	0.635
3	5	0.551	0.855	0.618	0.588	0.549	0.565
4	2.5	0.531	0.556	0.549	0.580	0.549	0.574
5	1.25	0.531	0.532^{MIC}	0.526^{MIC}	0.552^{MIC}	0.544	0.547^{MIC}
6	0.625	0.523^{MIC}	0.539	0.530	0.559	0.530^{MIC}	0.556
7	0.312	0.519	0.540	0.531	0.554	0.522	0.559
8	0.156	0.502	0.548	0.529	0.542	0.523	0.549
9	0.078	0.517	0.545	0.529	0.553	0.528	0.546
10	0.039	0.517	0.545	0.527	0.435	0.510	0.530
11	control +	0.557	0.549	0.558	0.575	0.560	0.589
12	control -	0.454	0.406	0.404	0.409	0.413	0.425

Table 8

The results of *Aspergillus niger* growth behavior in the presence of different dilutions of the essence oils

Number of socket	*Concentration (mL)*	*Eucalyptus globulus*	*Myrtus communis*	*Thymus daenensis*	*Matricaria recutita*	*Mentha longifolia*	*Satureja bachtiarica*
1	20	0.517	0.569	2.420	1.068	0.541	0.551
2	10	0.590	0.576	2.173	0.588	0.522^{MIC}	0.619
3	5	0.587^{MIC}	0.607^{MIC}	0.640	0.588^{MIC}	0.524	0.647
4	2.5	0.614	0.820	0.544^{MIC}	0.594	0.564	0.650
5	1.25	0.623	0.628	0.547	0.610	0.569	0.629^{MIC}
6	0.625	0.667	0.633	0.581	0.615	0.602	0.804
7	0.312	0.635	0.654	0.593	0.633	0.605	0.672
8	0.156	0.652	0.660	0.615	0.649	0.623	0.697
9	0.078	0.642	0.634	0.613	0.907	0.595	0.919
10	0.039	0.634	0.664	0.607	0.617	0.653	0.662
11	control +	0.605	0.667	0.625	0.697	0.616	0.682
12	control -	0.419	0.415	0.412	0.409	0.411	0.412

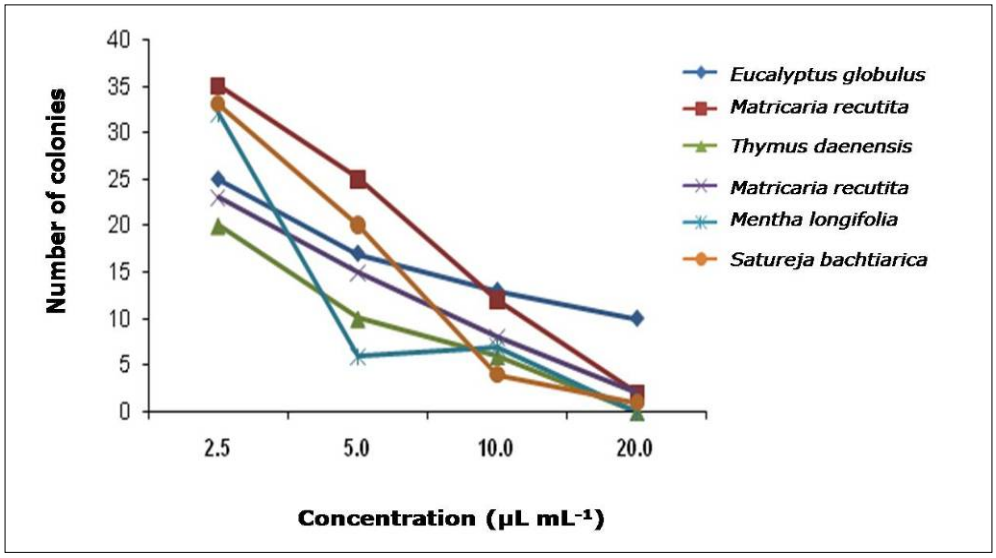

Figure 1. Comparison number of colonies by *Aspergillus flavus* effective of essential oils.

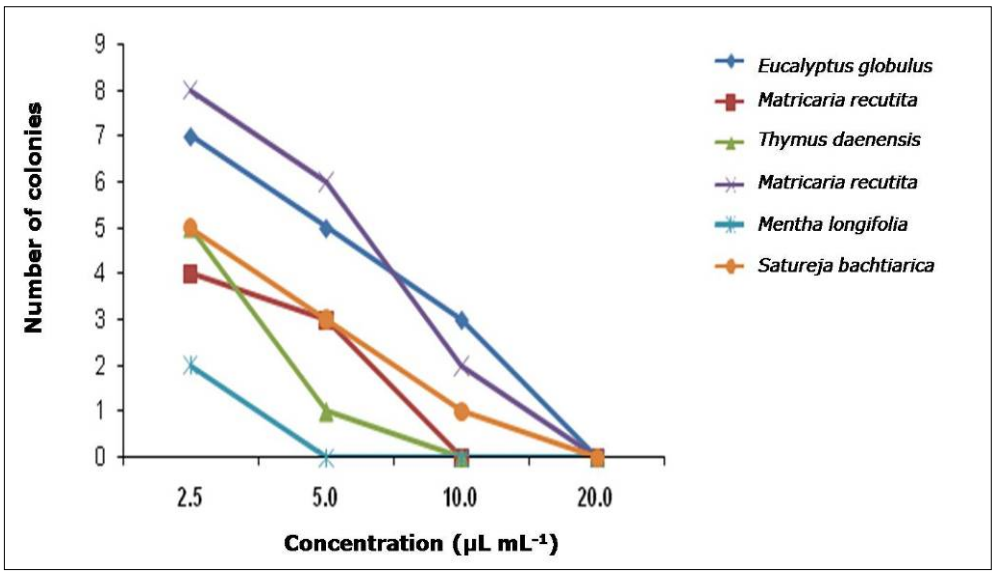

Figure 2. Comparison number of colonies by *Saprolegnia parasitica* effective of essential oils.

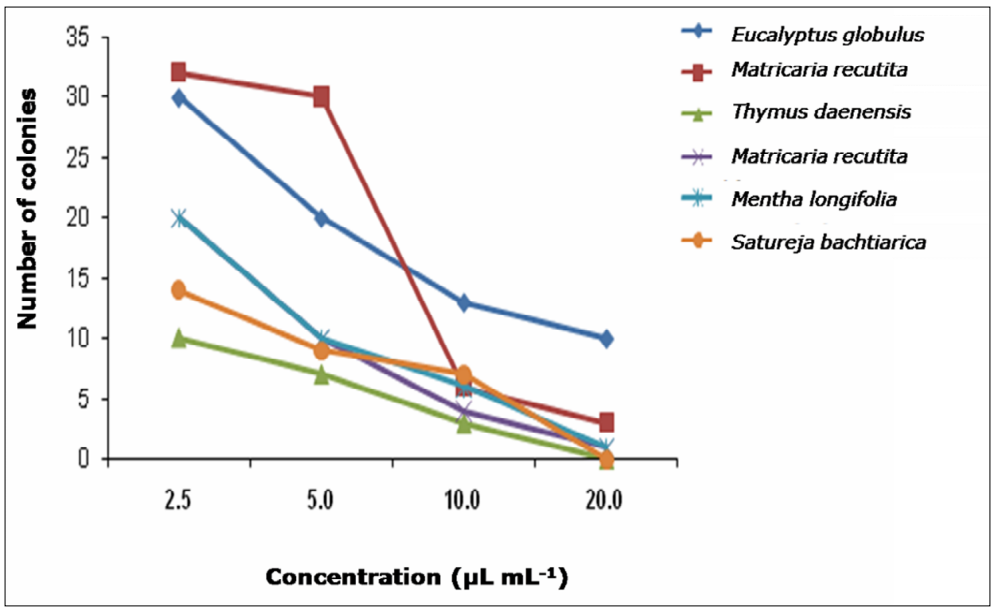

Figure 3. Comparison number of colonies by *Aspergillus fumigatus* effective of essential oils.

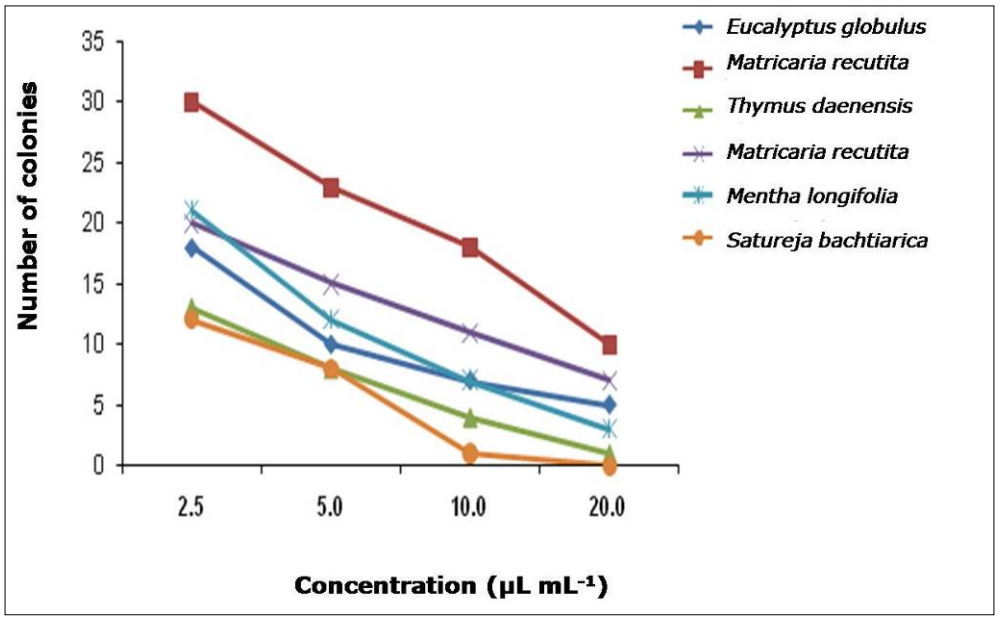

Figure 4. Comparison number of colonies by *Aspergillus niger* effective of essential oils.

Plant essential oils are a potentially useful source of antimicrobial compounds (Mondello et al 2006; Pyun & Shin 2006). The antimicrobial activity of the pure essential oil is higher than those found in the aqueous or hydroalcoholic extracts. It is often quite difficult to compare the results obtained from different studies, because the compositions of the essential oils can vary greatly depending upon the geographical region, species, age of the plant, method of drying and extraction of the oil.

The antimicrobial activity of essential oils from *Achillea setacea* (Unlu et al 2002), *Pimpinella anisum* (Kosalec et al 2005), *Sesuvium portulacastrum* (Magwa et al 2006), *Melaleuca alternifolia* (Mondello et al 2006; Hammer et al 2002; Hammer et al 2004), *Juniperus* spp. (Cavaleiro et al 2006), *Allium* spp. (Pyun & Shin 2006; Ledezma & Apitz-Castro 2006) and *Thymus* spp. (Pina-Vaz et al 2004; Pinto et al 2006) were fully studied.

Previous researches have suggested that several essential oils show important antifungal activity in vitro, with varied MIC and MFC values, against dermatophytes, yeasts and other fungi (Brito et al 2007; Pinto et al 2006; Cavaleiro et al 2006; Unlu et al 2002; Hammer et al 2002; Viana et al 2000).

Similar inhibitory effects of essential oils from five plants from Asteraceae family were studied and reported by Rai et al (2002) against the fungus, *Saprolegnia ferax.* In vitro antibacterial activity of *Andrographis paniculata* evaluated by Zaidan et al *(*2005) using disc diffusion. Bobbarala et al (2009) investigated the antimicrobial activities of *A. paniculata* against phytopathogenic bacteria and fungi. Although literature review showed that there is no research about antifungal activity of these tested oils against *S. parasitica*, some investigators demonstrated the inhibitory effects of different plants on some fungi in the Saprolegniaceae such as *Artemisia verlotiorum* and *Santolina etrusca* extracts against *S. ferax* (Macchioni et al 1999), *Blumea balsamifera, B. mollis, Eupatorium triplinerve, Guizotia abyssinica*, and *Tagetes erecta* essential oils against *S. ferax* (Rai et al 2002) and *Alpinia galanga* essential oil against *S. parasitica, S. diclina, Achlya bisexualis, A. diffusa,* and *Aphanomyce spiscicida* (Chukanhom et al 2005).

Many herb and spice essential oils for example *Satureja bachtiarica* contained high levels of phenolic compounds and showed antimicrobial activity. The result of a study by Sefidkon & Jamzad (2000) showed that the essential oil of *S. bachtiarica* contained thymol (44.5%) and *γ*-terpinene (23.9%). Sonboli et al (2004) reported that essential oil of *Satureja laxiflora* has antimicrobial activities against *Candida albicans* ATCC 5027, *A. niger* ATCC 16404, *Saccharomyces cerevisiae* ATCC 9763, *Klebsiella pneumoniae* ATCC 3583 and *Enterococcus faecalis* ATCC 15753. Chemical studies confirmed that a major part of this antimicrobial activity is due to thymol (64%) presence in the oil. Previous studies by Rasooli et al (2006) on antimicrobial activity of the essential oils from some *Thymus* spp. showed that most of them containing large quantities of phenolic monoterpenes, that have activity against viruses, bacteria, food-derived microbial strains and fungi. Ghasemi-Pirbalouti et al (2009) showed that essential oils of *T. daenensis* and *Thymbra spicata* leaves and flowers developed antibacterial activities against *L. monocytogenes* bacteria from chicken meat. Fazeli et al (2007) evaluated the antimicrobial activity of two medicinal plants (*Rhus coriaria* and *Zataria multiflora*) used in Iranian traditional medicine against some pathogenic food-borne bacteria. The MIC of *R. coriaria* and *Z. multiflora* were determined against several strains of Gram-positive and Gram-negative bacteria. The essential oil and extract of some aromatic plants (for example mint family, Lamiaceae) with a higher percentage of carvacrol and thymol (natural monoterpene phenol isomers) have a higher efficacy against microbial agents (Rasooli et al 2006).

Bansod & Rai (2008) reported that all oils tested in their study displayed different degrees of antifungal activity against *A. fumigatus* and *A. niger*. The maximum antimycotic activity was determined by *Cymbopogon martinii* followed by *Cymbopogon citratus*, *Eucalyptus globulus* and *Cinnamomum zeylanicum*. Aggarwal et al (2000) reported antimycotic activity of *C. martinii* against *A. niger*. The oil of *C. citratus*was effective against fungal pathogens causing diseases in plants and human beings (Singh 2000). Quale et al (1996) treated infections caused by *Candida* in AIDS patients with a drug based on Cinnamon. In our study we also found that essential oil extracted from *C. zeylanicum* had strong antifungal activity on both species of *Aspergillus*. The antimycotic activity of cinnamon bark due to presence of cinnamaldehyde is wellknown (Viollon & Chaumont 1994). Similarly, in vitro antimicrobial activity of *C. zelyanicum* against human pathogenic fungi and commensally bacteria was studied by Chaumont (2003) and Matan et al (2006). The oils of *Mentha spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera* and *Zingiber officinale* exhibited moderate activity. The essential oil of mint was found to have strong antimycotic activity against *C. albicans* (Kishore et al 1993). It has been reported that essential oil of mint showed high activity against *Cuminum cyminum, Allium sativum, Ocimum sanctum, Trachyspermum copticum, Foeniculum vulgare* and *Elettaria cardamomum* showed comparatively low effectiveness against *A. niger and A. fumigates* as compared to control. Antifungal mechanisms of mint essential oil may be associated with the fungal cell wall composition and/or construction (Godwin & Michniak 1999). Nigam & Rao (1977) reported that oil of *C. cyminum* was toxic to *Aspergillus.* Similarly, Singh (2001) reported high antifungal activity of *C. cyminum.* Essential oil of *A. sativum* has significant growth inhibition ability against fungi including dermatophytes (Singh 2000). Singh & Pandey (1998) found antimycotic activity

in oil of *Ocimum sanctum* against pathogenic fungi. Plant oils are important source of fungitoxic compounds and they may provide a renewable source of useful fungicides that can be used in antimycotic drugs against *A. fumigates* and *A. niger* infection in patients suffering from pulmonary tuberculosis. Among the plant oils tested in researches, *C. martini*, *C. citrates, E. globulus* and *C. zeylanicum* showed high antimycotic activity. Also, a combination of some of the metabolites found with others types of agents extracted by other popular remedy preparations can form a natural mixture that elevate the final antimicrobial spectrum by some kind of synergy. Mixed oils were inhibited the *A. fumigates* and *A. niger* at lowest MICs. On the other hand, the essential oil exhibited a good antimicrobial spectrum when pure, but its relative low concentrations in common folk preparations do not allow for any good activity in these extracts. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Conclusions. This study suggests that natural products derived from some medicinal plants have the potential to be used as health rainbow trout eggs against fungal contamination. The results will form the basis for selection of plant species as a non-hazardous material and environmentally friendly which is as effective as chemical agents to control fungal disease. Further investigation in the potential discovery of new natural bioactive compounds is needed. Among the essential oils derived from some of the medicinal and aromatic plants (Asteraceae, Myrtaceae and Lamiaceae), *Mentha longifolia* and *Thymus daenensis* may be proposed for control of fungal diseases. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated. In addition, further trials are required to study the effect and toxicity of these esseances in experimental animals (in vivo).

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