Study of Antifungal Effects of Zataria Essence on Morphological Changes of Zygomycetous Fungi

Abdulghaffar Ownagh, Fahimeh Valipouraghdam, Mohadeseh Abohosseini Tabari and Mohammad Reza Yousefi

Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, University of Tehran, Iran
Department of Veterinary Parasitology, Islamic Azad University, Babol Branch, Iran

Abstract: Mucormycosis is an acute and opportunistic mycotic infection caused by mucoral fungi order. These fungi have a wide geographic distribution. The most important predisposing factors in this disease are hyperglycemia, metabolic acidosis, corticosteroids overdose and leukemia. Zataria multiflora is a weed-like and one year plant which its essence have strong disinfectant effect. In this study, for investigating antifungal and morphological changes a mixture of media and the essence in 62.5, 125, 250, 500 and 1000 ppm dilutions was used. Aim of this study was evaluation of Zataria multiflora essence effect on zygomycetous fungi. Evaluation of antifungal effects of this essence in vivo and effects of pH and temperature on it and occurrence of resistance to the essence is also suggested.

Key words: Zataria multiflora essence • Mucor mycosis • Mucor hiemalis • Rhizopus oryzae

INTRODUCTION

Mucor mycosis is an opportunistic mycotic disease which often occurs in secondary form and its primary form is very rare which is characterized by hyphae attacking the blood vessels, infarction and tissue necrosis. The disease can be acute or subacute. Occurrence of the disease is more related to immune system deficiency than age, race or geographic conditions.

Patients with pulmonary mycosis are often affected with underlying diseases such as blood disorders, lymphoma and severe neutropenia or have a history of long term Difoxamine therapy. Renal allograft recipients and other patients with immunodeficiency are at risk of this disease [1, 2]. Metabolic acidosis in patients affected to diabetes mellitus, uremia and starvation has a great role in this disease. Rhizopus oryzae is the most important cause of mucor mycosis in human and after that is Rhizopus microsporus [3, 4].

This group of fungi mostly grow on rotten vegetables, seeds, fruits, manure, soil, animal’s excrement and wet leftover bread. Most of the fungi causing this disease grow on materials containing carbohydrate and produce many spores [5, 6]. Many cases of hospital dermal infections by Rhizopus ssp. are reported. These fungi are able to colonize on wound bandages, around catheters used for peritoneal dialysis and surgical wounds and cause disease in case of immunodeficiency. It is believed that eating foods contaminated with spores can cause gastrointestinal mucor mycosis. Amphothriene B is the drug of choice for mucor mycosis. This drug is an appropriate therapy unless brain is attacked. Another disadvantage of this drug is its nephrotoxic property.

Thyme is a weed-like plant and is a member of Latiatae (mint) family which has many thin, hard, branched stems and is one of the well known medicinal plants in Iran and Europe traditional medicine. Aerial parts of this plant, the parts that grow above the ground and its dried leaves are used as medicinal parts. Essence of this plant contains chemical compounds such as Thymol and Carvacrol, two phenols which are isomers of each other, along with steminal (trace amounts), Pinen and Bornil.

Corresponding Author: Fahimeh Valipouraghdam, Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, P.O. Box 1177
Tel: +98-9126303295, Fax: +98-4412771926, E-mail: fvalipr@gmail.com.

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acetate [7]. Thymes have different species which Zataria
multiflora is one of them [8]. Essence of Zataria is a
potent disinfectant while it has little toxicity.
Thyme essence containing white thyme oil, tincture
and liquid extract of it are used as aromatic compounds in
food. In traditional medicine thyme was used as anti-
spasm and for treatment of pertussis, bronchitis,
pulmonary infection, common cold, influenza, bloat and
muscle cramps. Its infusion is used for treating middle ear
infection, bloat and nausea. Essence of thyme, called
Thymol, is used for treating asthma. It is also effective
against insect bites. Its oil is toxic and is not used orally.
Thyme is used for aphtas and also fungal infections of
mouth and pharynx. Zataria multiflora has a significant
antimicrobial effect so it changes the intestinal flora and
is appetizer. It is anti-inflammatory and is a relief for
spasmodic coughs. It is used for diarrhea and digestive
turbances. Its anti helminth effect is not for sure. In
external use it is excellent for healing of wounds. This
plant is used for treating cutaneous exanthems which are
usually caused by gastrointestinal problems (constipation
and different infections). External therapy must be used
along with internal therapy or at least a laxative [9].

MATERIALS AND METHODS

Thyme essence numbered 88/002/EOF27 was
purchased from Zarinband Company and Mucor hiemalis
(PTCC 5292) and Rhizopus oryzae (PTCC 5176) was
obtained from institute of scientific and industrial
researches.
Organisms were cultured according to protocol in
sabro dextrose agar (SDA) and incubated for 24 hours.
Then number of spores in each milliliter of suspension
was counted by 0.5 Mc Farland opacity measurement
methods. Stocks of fungi used in this study contain 4.2×
10⁶ spores in each milliliter of suspension.

Our purpose was to study the effect of 62.5, 125, 250,
500, 1000 ppm dilutions of the essence on micro
organisms so, considering the 1 to 10 mixture of essence
and medium, essence stock was prepared in 625, 1250,
2500, 5000, 10000 ppm dilutions by dimethyl sulfoxide
(DMSO).

In this study Mucor hiemalis and Rhizopus oryzae
fungi in control group were cultured on sabro dextrose
agar and treatment groups were cultured on this media in
combination with 1000, 500, 250, 125 and 62.5 ppm of
essence. 50 micro liter of fungi spore suspension was
cultured on plate using a sterile swap. Then plates were
incubated in 28°C. Media containing DMSO and
distilled water were used as solvent control and media
containing 1 mg/ml Amphotericin B was used as positive
control. All treatments, positive and negative controls
were repeated 3 times. Morphologic changes of fungi
were detected by macroscopically and recorded daily and
for 7 consecutive days.

Sterile 96 wells microplate was used for determining
minimal inhibitory concentration (MIC) of essence by
microdilution method. A to E wells were used to determine
the effect of different dilutions of the essence on micro
organisms and F to H wells were respectively used as
positive control containing Amphotericin B, negative
control containing distilled water and solvent containing
DMSO. The experience on each fungus was repeated 3
times. 160 micro liter broth media was poured in all of the
wells and then 20 micro liter of spore suspension was
added to A to E wells. Finally essence in 625 to 10000
dilutions was added respectively. 20 micro liter of essence
was also added to F row wells, positive controls, as well.
Instead of adding essence to G row wells, negative
controls, sterile distilled water was added and to H row
wells 20 micro liter diluting solution (DMSO) was added
to study the effects of it. Then the plates were placed in
the 28°C incubator for 3 days. To determine MIC, by
direct observing method, the last well that no opacity was
observed in it after 3 days was chosen. To determine MFC
50 micro liter of the well that was chosen as MIC was
transferred to sabro dextrose agar and studied.

RESULTS

Studying morphologic changes showed that Mucor
hiemalis on first day in presence of 62.5 and 125 ppm
dilutions of thyme essence showed slow and pale and
also nonsporulative colonies. But on 2nd day both
dilutions showed complete growth, gray colonies and
sporelation. 250 ppm dilutions of essence on first day
caus ed no growth, on 2nd day little growth with pale
colonies and no sporelation and on 3rd day complete
growth, gray colonies and spore production. But in media
containing 500 and 1000 ppm of essence no growth was
observed during 7 days.

Rhizopus oryzae on first day in presence of 62.5 and
125 ppm dilutions of essence had pale colonies without
sporelation, on 2nd day both dilutions had complete
growth, white colonies without sporelation and on 3rd day
sporelation began. These fungi in presence of 250 and 500
ppm of thyme essence on first day showed no growth, on
2nd day little growth and pale colonies and on 3rd day
complete white colonies but without sporelation and from
Table 1: MIC results of *Mucor hiemalis*

<table>
<thead>
<tr>
<th>Dilution (ppm)</th>
<th>Day</th>
<th>62.5</th>
<th>125</th>
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<th>500</th>
<th>1000</th>
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<td>First</td>
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<td>Second</td>
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<td>Third</td>
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Table 2: MIC results of *Rhizopus oryzae*

<table>
<thead>
<tr>
<th>Dilution (ppm)</th>
<th>Day</th>
<th>62.5</th>
<th>125</th>
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Results of minimum fungicidal concentration test indicated that, MFC for Mucor hiemalis was 500 ppm and for Rhizopus oryzae was 1000 ppm.

4th day on sporulation was also observed *Rhizopus oryzae* in the media containing 1000 ppm of essence during 7 days of study did not show any growth.

**DISCUSSION**

Considering numerous side effects of chemical antifungal drugs on body cells and their high prices, once again researchers are thinking about the possibility of using medicinal plants for treatment of infectious diseases. Since Ampitriquin B is the only effective therapy for mucormycosis and its toxic effects on kidneys, nephrotoxicity, is proven this study tried to use essence of thyme against mucormycosis and morphologic changes and minimum inhibitory concentration on laboratory species of mentioned organisms were evaluated.

In a study antifungal effect of thyme essence on *Aspergillus niger*, *Candida albicans*, *Microsporum canis* and *Penicillium* was evaluated and a moderate effect was reported [10]. In another study on the antifungal effects of a species of thyme in Senman region good antifungal effects on *Penicillium*, *Candida albicans* and *Microsporum canis* was observed. Thyme essence can also completely inhibited growth of *Aspergillus niger*. In a comparison made between essence of thyme and Nistatin results showed that thyme had a stronger antifungal effect than Nistatin on *Candida albicans*, *Aspergillus flavus* and *Tricophyton mentagrophytes*. Also in another species of thyme which has effective substances such as Thymol, Carvacrol and Paraits stronger effects than Nistatin on *Aspergillus fumigates* was noticed [11]. Results of a research conducted on thyme plants of Lorestan region confirmed very good effect (+4) on *Microsporum canis*, good effect (+3) on penicillium and moderate effect (+2) on *Candida albicans*. In its phytochemistry Saponin and Flavoid is reported [12]. In a study antibacterial effects of different concentrations of essence of a number of medicinal plants of Iran country was reported which thyme had the most antimicrobial effect by MIC method on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* and so it was suggested that using this property it can be used as food conservative to increase food durability and decrease food-associated diseases [13].

In a study antibacterial effect of thyme essence was reported to be the same as Cefazidine and also a strong effect on *Staphylococcus aureus* was reported [14]. During conducted investigations researchers succeeded to use a ointment made of thyme plant in treating acute vaginal candidiasis [15]. During studies aimed to find the component in thyme essence which has analgesic effect, in which Morphine and Diclofenac were used as positive controls, results showed that hydroalcoholic essence can have same effect as Morphine and Diclofenac [16].

In a study that examined analgesic and anti-inflammatory effects of thyme essence in mouse and rat results were confirmative of analgesic effect of this plant, which could be by opioid receptors and also the essence showed anti-inflammatory effects in mice which was stronger against chronic inflammation in comparison with acute inflammation [17].

Conducted investigations in the university of agriculture in Scotland on antibacterial effects of volatile oils of aromatic medicinal plants showed that oregano, a member of thyme family, has antibacterial action against bacteria of different genera. These bacteria include plant and animal pathogens, food toxins and bacterial wastes. Volatile oils had a remarkable inhibitory effect on all examined organisms [17].

In a series of studies aimed to evaluate the anti-Candida effects of *Zataria multiflora* on different species of Candida in laboratory researcher’s evaluated effects of ethanolic and methanolic essence of the wet plant. MIC measurement results showed that methanolic essence has a stronger anti-Candida effect than ethanolic essence and overall the species responsible for genitourinary infections were more sensible [18]. Results of comparing therapeutic effects of mycoconazol gel and *Zataria multiflora* on stomal fungal inflammation showed that *Zataria multiflora* reduces the redness of the palate surface more than mycoconazol gel but does not reduce the number of colonies on the dental surface of palate as mycoconazol gel does [19].
Results of experiments done on benefits of thyme essence for mice affected to intestinal disease experimentally confirmed this plant’s anti oxidant, anti bacterial and anti inflammatory effects [20]. A research study reported thyme essence has growth inhibitory effect in 20.6 µ/ml dilution and fungicidal effect in 41.25 µ/ml dilution on Microsporum canis and Tricophyton mentagrophytes and it also has growth inhibitory effect in 10.31 µ/ml dilution and fungicidal effect in 20.62 µ/ml dilution on Epidermophyton floccosum, however it has no growth inhibitory effect on Microsporum gypseum [21].

Thyme has many biologic effects including todic effects, pain killing, anti bacterial effects, and is a therapy for gastrointestinal problems and cough [22]. In a study on Zataria multiflora’s dried leaves hydroalcoholic essence it was shown that it has anti microbial effect on standard lineage of Staphylococcus aureus [23].

This study was carried out in order to solve problems such as drug side effects and replacement of chemical drugs with herbal antifungal drugs. It is probable that the antifungal effect shown in this study is related to Thymol in the thyme essence, which its inhibitory effect was enhanced in higher concentrations of the essence. As this essence had inhibitory effect on examined micro organisms so it can be used as an effective essence against this group of fungi, but determination of antifungal property of this essence in vivo and effects of temperature, pH and different substances in living organism’s body on it and also occurrence of resistance to this essence needs further investigations.

REFERENCES


