Assessment of growth-inhibiting effect of some plant essential oils on different Fusarium isolates

Évaluation de l’activité antifongique de quelques huiles essentielles de plantes sur divers isolats de Fusarium

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Summary
Objective. — Essential oils (EO) of five medicinal plants were tested for anti-Fusarium activity against 10 non-toxigenic (Fusarium solani and Fusarium oxysporum) and 11 toxigenic (Fusarium verticillioides, Fusarium poae and Fusarium equiseti) isolates.
Materials and methods. — Different dilutions of EO were prepared and 5 x 10^3 conidia of each fungal suspension were inoculated into Sabouraud’s glucose broth tubes. The cultures were incubated at 28 °C for 4 days.
Results. — Mean concentrations of the EO of Zataria multiflora (165.4 and 88.9 μg/ml), Cuminum cyminum (159 and 185.3 μg/ml), Foenicum vulgare (496.4 and 532.9 μg/ml), Pinaceae (869.7 and 852.43 μg/ml) and Heracleum persicum (753.5 and 1492.6 μg/ml) completely inhibit all the non-toxigenic and toxigenic isolates, respectively. Z. multiflora and H. persicum showed the highest and lowest activity against toxigenic Fusarium isolates, whereas C. cyminum and H. persicum had the highest and lowest effect on non-toxigenic isolates, respectively. However, F. vulgare and Pinaceae had moderate effects against tested fungi.
Conclusion. — The results indicated that non-toxigenic and toxigenic Fusarium isolates were sensitive to the five EO, particularly sensitive to C. cyminum and Z. multiflora, respectively.

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Keywords
Fusarium; Essential oils; Fungistatic activity

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Introduction

Many species of fungi are capable of producing fungal infections and mycotoxins in food and feeding. Among them Fusarium species cause seedling blight and root, stalk, and ear rot in maize as well as many of them produce mycotoxins. The most frequently isolated species from maize in temperate climates are Fusarium verticillioides, Fusarium proliferatum and Fusarium graminearum followed by Fusarium subglutinans, Fusarium culmorum and Fusarium equiseti [22].

On the other hand, food-borne diseases are major dilemma in the developing countries, and even in developed nations [23]. Consumption of foods contaminated with some microorganisms represents a serious health risk to humans and animals. The subsistence and growth of fungi in foods may lead to spoilage and formation of toxins. These fungal metabolites are structurally diverse compounds, which represent the most important category of biologically produced natural toxins relative to human health and economic impact worldwide [7].

On the other hand, the widespread indiscriminate use of chemical preservatives has resulted in the emergence of resistant microorganisms leading to fungal diseases [1]. To reduce this problem, there is a need to adopt strategies that are accessible, simple in application, and nontoxic to humans and animals, and that have sustainable broad-spectrum fungitoxicity.

Among natural substances, the essential oils (EO) and herbal extracts have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds [4,28,30]. The antimicrobial and antioxidant activities of EO have formed the basis of many applications including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [7,18,24].

Data concerning the effects of EO against different mycotoxins production in inoculated wheat are limited. A previous study in examination of grain-based foods indicated the food preservative ability of EO (thyme, cinnamon, anise and spearmint) at different concentrations in inhibiting mycotoxins production [25].

In this study, we examined the anti-Fusarium activity of herbs such Zataria multiflora, Heracleum persicum, Pinaceae, Cuminum cyminum and Foeniculum vulgare, which have long been used as spices or important medicinal sources in Iran [13,16]. The objective of this study was to examine the effect of different herb oils on some Fusarium isolates such as Fusarium solani, Fusarium oxysporum, Fusarium verticillioides, Fusarium poae and F. equiseti by determination of the minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC).

Materials and methods

Isolation of Fusarium species and culture

Ten non-toxigenic Fusarium species (F. solani and F. oxysporum) isolated from clinical specimens and maize and 11 toxigenic Fusarium species (F. verticillioides, F. poae and F. equiseti) isolated from maize were included in this study. The selective criteria of toxigenic Fusarium isolates were based on the production of fumonisin B1 by F. verticillioides, T2 toxin by F. poae and zearalenone by F. equiseti. On the other hand, the production of above-mentioned toxins in non-toxigenic Fusarium species such as F. solani and F. oxysporum was non-detectable. Fungal isolates were cultured onto Sabouraud’s glucose agar (Merck Co., Darmstadt, Germany) slant at 28 °C for 7 days. Unless otherwise indicated, all the chemicals were purchased from Sigma Chemical Co. (St. L., MO, USA).

Preparation of the conidial suspension

Conidia were harvested from 7-day-old cultures by pouring a sterile 0.1% aqueous solution of Tween 80 onto the culture plates and scraping the plate surface with a bent glass rod to facilitate the release of conidia. The number of conidia in the suspension was adjusted to approximately $1 \times 10^5$ conidia/ml using a haemocytometer slide.
Essential oils

Standard Z. multiflora (Labiatae), H. persicum (Apiaceae), C. cyminum (Apiaceae), Pinaceae (Pine) and F. vulgare (Apiaceae) EO were obtained from Barij Essence Pharmaceutical Company (Kashan, Iran).

Antifungal assay

The MIC and MFC values of EO for different Fusarium isolates were determined by the broth dilution method using the serially diluted plant oils as described [14]. Briefly, different EO concentrations (0.03—8%) diluted with DMSO 2% was incorporated in Sabouraud’s glucose broth (Merck Co., Darmstadt, Germany). Fungal conidia (5 × 10³ conidia) was added to each tube and incubated at 28 °C for 4 days. Positive control tubes containing only broth media and fungal suspension as well as negative control tubes containing broth media accompanied by each EO and DMSO were prepared and incubated at the same conditions. The lowest EO concentration that did not permit any visible fungal growth was taken as the MIC. The tubes that did not show visible fungal growth were sub-cultured onto oil-free Sabouraud’s glucose agar (Merck Co., Darmstadt, Germany) to determine if the inhibition was reversible. The MFC was the lowest concentration that did not permit growth on the plates.

Statistical analyses

The Mann-Whitney, Kruskal-Wallis and t-Student tests were used. Probabilities of 5% were taken to be statistically significant.

Results

Table 1 show the inhibitory and fungicidal activities on the growth of different non-toxigenic Fusarium species including F. solani and F. oxysporum. The results show that the minimum inhibitory and fungicidal concentrations of different EO were determined to be 164.9 to 1103.8 µg/ml and 166.9 to 1248.7 µg/ml, respectively. C. cyminum (MIC: 164.9 µg/ml and MFC: 166.9 µg/ml) and H. persicum (MIC: 1103.8 µg/ml and MFC: 1248.7 µg/ml) had the highest and the lowest activity.

In addition, the MIC and MFC of different EO were assayed against toxigenic Fusarium species; F. verticillioides, F. poae and F. equiseti. Z. multiflora had the highest activity (MIC: 175.2 µg/ml and MFC: 203 µg/ml), whereas H. persicum showed the lowest activity (MIC and MFC: 2307.7 µg/ml) on fungi tested (Table 1).

Means of MIC/MFC between EO demonstrated significant differences between Z. multiflora and H. persicum as well as H. persicum and C. cyminum against Fusarium species.

Table 1

Antifungal activity of different essential oils of medicinal plants against various Fusarium isolates.

<table>
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<th>Samples</th>
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Z: Zataria multiflora; H: Heracleum persicum; P: Pinaceae; C: Cuminum cyminum; minimum inhibitory concentrations (MIC) (w/v) µg/ml; minimum fungicidal concentrations (MFC) (w/v) µg/ml; F: Foeniculum vulgare; F1, F2, F6, F7 and F9: Fusarium solani isolates; F3, F4, F5 and F8: Fusarium oxysporum isolates; F11, F12, F13, F14, F15, F16, F17, F18, F19, F20 and F21: Fusarium verticillioides isolates; F11 and F21: Fusarium poae isolates; F19 and F20: Fusarium equiseti isolates.
Discussion

Fusarium species mainly colonize cereal grains before harvest and cause fungal infections in human and animals [19]. Various publications have documented the antimicrobial activity of EO including lemongrass, cinnamon leaf, clove, palmarosa and oregano oils against different microbial species [8,11,12,17]. In an old great medical Persian book, ‘‘Al-Aghrazi al — Tibbia val Mabahees al — Alaia’’ by Sayyed Esma’iil Jorjani (1105 BH), more than half of the book has been established to plant pharmacology and pharmacokinetics, which some of his ideas and speculations are accepted in the modern medicine. In fact, pure EO fight infections and are known to exhibit antimicrobial activities, and many applications for controlling the growth of food-borne pathogens have been developed using these EO as natural food preservatives [31].

Expression of different level of antifungal activities may be due to the differences in the content of known antimicrobial compounds in each EO as earlier determined by Amvam Zollo et al. [2] and Tassou et al. [27]. Higher anti-Fusarium activity was found in the EO from C. cyminum and Z. multiflora against non-toxigenic (F. solani and F. oxysporum) and toxigenic (F. verticillioides, F. poae and F. equiseti) isolates studied, respectively. Moderate activity was observed for the EO from Pinaceae and F. vulgare while the EO from H. persicum was less inhibitory. Similar antifungal activities of EO from Z. multiflora and C. cyminum were not reported on Fusarium species. In general, the antimicrobial activity of EO is mostly due to the presence of phenols such as thymol, carvacrol and aldehydes such as geraniol, citronellal as well as alcohols such as geraniol, linalool, citronellol and lavandulol [6,20,26]. Z. multiflora showed the highest activity against toxigenic Fusarium species, which is consistent with several studies that showed EO of the phenol type such as thymol and carvacrol possess antimicrobial activity [3,5]. The EO of C. cyminum was another most effective of the EO against non-toxigenic species and main components, such as pinene, cineole and linalool exhibited strong activity on F. solani and F. oxysporum [21]. Previous studies demonstrated that EO containing aliphatic alcohols and phenols have significant action against Aspergillus aegyptiacus, Penicillium cyclopium, Trichoderma viride and Candida albicans [15,16]. Main constituents of C. cyminum oil were pinene, cineole and linalool, while the main components found in F. vulgare were anethole, limonene, fenchone, and in H. persicum oil were anethol, terpinolene [16]. Although the three EO including H. persicum, C. cyminum and F. vulgare belong to Apiaceae family, the presence of higher contents of alcoholic compounds and different antimicrobial components in C. cyminum was attributed to its higher activity. This may explain the general superiority of the EO from C. cyminum than that of other EO belonged to Apiaceae family in the present study. The superiority of the EO from C. cyminum against F. solani and F. oxysporum in this work is in agreement with the findings of Naeini et al. [16]. Overall, the antifungal activity of the EO is related to the respective composition of the herbal EO, the structural configuration of the constituent components and their functional groups and possible synergistic interactions between components [10].

It is necessary to mention that there is a direct relationship exists between inhibitory effects of EO on fungal growth and fusariotoxins production [9]. In a study conducted by Velluti et al. [29], all EO tested had an inhibitory effect on growth rate of zearalenone and deoxynivalenol produced by Fusarium species. Therefore, EO can also serve as alternative means to prevent or control fungal attack and the presence of mycotoxin in stored corn and upon foodstuff.

In conclusion, our data confirmed that Z. multiflora and C. cyminum possessed higher than in vitro antifungal activity against the species of Fusarium studied.

Conflict of interest statement

The authors have not declared any conflict of interest.

Acknowledgement

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References


