اثر ضد قارچی عصاره های آبی، متانولی و اتانولی *آلیوم ساتیووم* بر قارچ های ساپروفیتی و سم-زای جدا شده از مایونز

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تاریخ دریافت: ۱۳۹۹/۱۱/۲۵ تاریخ پذیرش: ۱۴۰۰/۳/۱۴

چکیده

سیر بدلیل طعم دهندگی، ویژگی های پیشگیری و درمانی، ضدقارچی و ضد باکتریایی یکی از مهم ترین گیاهان مورد استفاده در غذاها است. برخی از قارچ ها در مواد غذایی رشد کرده و می توانند باعث آلودگی مواد غذایی شوند. آسپرژیلوس، پنی سیلیوم، آلترناریا و فوزاریوم از جمله رایج ترین قارچ های تولید کننده مایکوتوکسین هستند و اغلب از مواد غذایی جدا می شوند. هدف از این مطالعه، ارزیابی فعالیت ضد قارچی عصاره های *آلیوم ساتیووم* بر روی قارچ های رشته ای جدا شده از سس مایونز بود. قارچ های رشد یافته بر روی مایونز در محیط سابورودکستروز آگار محتوی کلرآمفنیکل کشت داده شد. قارچ ها بر اساس ویژگیهای ظاهری و ریزبینی شناسائی گردیدند. روش های چاهک پلیت و دیسک انتشاری برای اندازه گیری اثرات ممانعت کننده عصاره ها علیه سویه های هدف مورد نظر در آزمایش، بکار گرفته شدند، همچنین حداقل غلظت ممانعت کننده هر عصاره تعیین گردید. قارچهای *آسپرژیلوس نیجر*، *آ. فومیگاتوس*، *آ. فلاووس*، *پنی سیلیوم*، *موکور*، *رایزوپوس*، *کلادوسپوریوم*، *فوزاریوم*، *آلترناریا* و *ژئوتریکوم* شناسایی گردید. عصاره های آبی فعالیت ضدقارچی بیشتری نسبت به عصاره های متانولی و اتانولی نشان داد. حداقل غلظت ممانعت کننده برای *آ. نیجر*، *آ. فلاووس*، *آ. فومیگاتوس*، *پنی سیلیوم*، *موکور*، *رایزوپوس* و *آلترناریا* برابر با ۳۵۰ و برای *کلادوسپوریوم*، *فوزاریوم* و *ژئوتریکوم* ۳۰۰ میکروگرم بر میلی لیتر تعیین شد. حداقل غلظت ممانعت کننده عصاره های متانولی و اتانولی بالاتر از مقادیر مربوط به عصاره آبی بود. نتایج نشان داد که سیر میتواند به عنوان یک عامل ضد قارچ طبیعی در مواد غذایی استفاده شود.

کلید واژه ها: سیر، آلودگی مواد غذایی، آلودگی قارچی ، کپک ها.

Antifungal effect of aqueous, methanolic and ethanolic extracts of *Allium sativum* L. on saprophytic, and toxigenic fungi isolated from mayonnaise

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Received: 13 February 2021 Accepted: 4 June 2021

Abstract

Allium sativum (*A. sativum*) is one of the most important plants used in foods for its flavouring, prophylactic and therapeutic properties. It has been revealed that garlic has activity against bacteria and fungi. *Aspergillus, Penicillium, Alternaria,* and *Fusarium* are among the most common fungi that are mycotoxin producers, frequently isolated from foods. This study aimed to evaluate the antifungal activity of *A. sativum* extracts against saprophytic and toxigenic fungi isolated from contaminated mayonnaise sauce. The fungi grow on mayonnaise were transferred to sabouraud's dextrose agar supplemented with chloramphenicol and were identified based on macroscopic and microscopic features. Disc and well diffusion methods were applied to measure the inhibitory effects of the extracts against all targeted strains tested in the experiment; also, the minimum inhibitory concentration of each extract was determined. *Aspergillus niger, A. flavus, A, fumigatus, Penicillium sp., Mucor sp., Rhizopus sp., Cladosporium sp., Fusarium sp., Alternaria sp.,* and *Geotrichum sp.* were identified. Aqueous extracts showed higher antifungal activity than the methanolic and ethanolic extracts. *Fusarium* and *A. niger* showed the highest and lowest sensitivity to all extracts, respectively. The MIC of the aquatic extract was determined to be 350 µg/ml for *A. niger, A.fumigatus, A. flavus, Mucor sp., Rhizopus sp., Penicillium sp.*, and *Alternaria sp.* and 300 µg/ml for, *Cladosporium sp., Fusarium sp.* and *Geotrichum sp.* The MIC of methanolic and ethanolic extracts was higher than the corresponding figures for aqueous extracts. It seems that *A. sativum* can be used as an antifungal agent in some food products.

Keywords: Garlic, food contamination, fungal contamination, molds.

Introduction

Recently, due to their active antifungal components, some plants were commonly used to protect foods and agricultural products from fungal contamination (wang et al., 2018). *Allium sativum*, which is commonly known as garlic, is among the most important species of the Liliaceae family. It contains water, carbohydrate, protein, amino acids, organosulfur compounds, and fibre (Meriga et al., 2012; Cheng and Huang, 2018, Raissy et al., 2020), that has many benefits for human health, and nowadays, it has been considered by researchers due to its prophylactic and therapeutic properties both in traditional and modern medicine (Queiroz et al., 2009; Lanzotti et al., 2012; Martins et al., 2016; Shoshi and Akter, 2017; Chen et al., 2018). Two main groups of bioactive substances can be found in garlic. The first one is a sulfur-containing compounds such as allin and its derivatives such as allicin and ajoene, which have antimicrobial activity, and the second group are sulfur-free polyphenolic compounds such as anthocyanins, flavonols, tannins, flavonoids, phenolic acids, phytosterols, carotenoids, and saponins, which have an important role in the human diet. *Allium sativum* has been used for its antibacterial, antiviral, and antifungal activities (Najda et al., 2016), and it is believed that it has bactericidal and fungicidal properties (Majewski, 2014).

Fungi are ubiquitous microorganisms that are found in many different environments, especially when organic material is available (Darko et al., 2017). Various reports have shown the contaminations in different types of foods (Lemons et al., 2018; Pirali Khairabadi et al., 2020), although few reports are available about the fungal contaminations.

Although most of the fungi isolated from foods are not toxigenic and could not produce mycotoxins, some fungal species belong to different genera such as *Aspergillus, Fusarium,* and *Penicillium*, causing food contamination. They produce secondary metabolites such as mycotoxins (Alshannaq and Yu, 2017; Wang et al., 2018).

Sodium benzoate is commonly used in foods (pH= 2.4-4), especially in mayonnaise, to control the growth of yeasts and bacteria, but its inhibitory power for the prevention of moulds growth is low. Consequently, mayonnaise is exposed to mouldy spoilage. Therefore, unfortunately, sometimes Para-hydroxybenzoic acids (i.e., Parabens) are used as a preservative to overcome mouldy spoilage in mayonnaise (Faraji et al., 2016). Since there are a few studies on the effect of garlic as one of the most important agricultural products having antibacterial and antifungal properties on common food contaminant moulds, especially toxigenic moulds, the present study aimed to investigate antifungal activities of the aqueous, methanolic, and ethanolic extracts of *Allium sativum* on some saprophytic and toxigenic fungi isolated from mayonnaise, and to evaluate the feasibility of using garlic in mayonnaise as a natural benefit preservative.

Material and methods

Preparation of *Allium sativum* extracts

Garlic bulbs were cut into small pieces and dried for a week at room temperature, and then powdered using an electric blender. The method mentioned in Gull et al, 2012 with some minor modifications, was used to prepare garlic extracts. Ten grams of garlic powder was soaked in 100 ml sterile distilled water, methanol, and ethanol separately and incubated at room temperature for 72 hours, shaking at 120 rpm. The crud extracts were centrifuged at 454 ×g for 10 minutes at 25˚C. The extracts were evaporated in a rotary evaporator at 45˚C, and dried extracts were dissolved in Dimethyl sulfoxide (DMSO) to obtain the final concentration of 100 mg ml^{-1} and centrifuged again at 5040 ×g to remove them in dissolved residues. The extract solutions were stored at 4 ˚C (Gull et al., 2012).

Isolation and identification of fungi

Mayonnaise with and without preservatives was used to check for fungal contamination. The mayonnaise was kept at room temperature and investigated daily in terms of creating fungal colonies. In mayonnaise without preservatives, fungal colonies appeared after 7 to 10 days, while in mayonnaise containing preservatives, this time lasted more than 30 days. The fungal elements were cultured on sabouraud's dextrose agar supplemented with chloramphenicol (SC) and identified based on their macroscopic and microscopic features.

Preparation of fungal suspensions

The fungal conidia were washed with sterile distilled water and centrifuged at 5000 rpm for 5 min, and then the supernatant was removed, and the conidia were mixed with sterile distilled water. Then the fungal conidia concentration in the suspension was standardized using 0.5 McFarland solution and spectrophotometer at 640 nm wavelength to obtain approximately 1×10^8 conidia per ml of suspension.

Agar disc diffusion method

The antifungal effect of the extracts was investigated using the agar disc diffusion method. Streaking the conidia is not an appropriate method for filamentous fungi because of their growth characteristics; therefore, 100 µl of the fungal suspension from each fungus containing live conidia was added to sterile Petri dishes and over layered with cooled sterile potato dextrose agar (PDA). The Petri dishes were rotated to mix the content and allowed to solidify at room temperature. The blank discs (6 mm diameter) were impregnated for 10 minutes in the extracts, placed on the center of Petri dishes, and incubated at 27˚C. The sensitivity of fungi to the extracts was determined by measuring the diameter of the growth inhibition zone in mm. All tests were done as triple.

Agar well diffusion method

One hundred µl of the fungal suspensions were separately placed on the center of sterile Petri dishes (15 cm diameter) using a sterile pipette, and molten cooled PDA medium was then added to it and mixed well. After the solidification of the medium, the wells with 10 mm in diameter and 5 mm in depth were created on a solid medium using gel puncture under sterile conditions. Wells were filled with

200 µl of different concentrations (from 25 µg ml^{-1} to 300 µg ml^{-1}) of the extracts. One of the wells was filled with distilled water and used as a control. The Petri dishes were incubated at 27˚C for 4-5 days and observed daily. The inhibition zones were measured with a caliper. All experiments were done as triple.

Determination on minimum inhibitory concentration (MIC)

Serial dilutions of each extract were made in a concentration range from 25 µg ml^{-1} to 400 µg ml^{-1}in PDA media. The media without any extract was used as a control. The fungal suspensions were cultured separately on media and incubated at 27˚C. The cultures were observed daily, and the results were recorded. The MIC was defined as the lowest concentration of the extract that was able to inhibit fungal growth after 4-5 days.

Results

Disk diffusion method

The results given in Table 1**,** showed that the aqueous extract of *Allium sativum*was more effective against all tested fungi than methanolic and ethanolic extracts. *Fusarium* and *Geotrichum* were more susceptible to aqueous extract, while, *Fusarium* and *Alternaria* showed more sensitivity to both methanolic and ethanolic extracts.

Table 1- Mean ±SD diameter of the inhibition zones (mm) after using aqueous, methanolic, and ethanolic extracts in disk diffusion method

	Aqueous extract	Methanolic extract	Ethanolic extract
A.niger	20 ± 0.56^{g}	15.2 ± 0.36^{h}	13.3 ± 0.17^{h}
A.fumigatus	25 ± 0.10^{de}	18.6 ± 0.17^{d}	16.5 ± 0.00^{e}
A. flavus	25.3 ± 0.17^{d}	17.8 ± 0.23^{f}	15.3 ± 0.00^{f}
Penicillium	25.5 ± 0.10^{d}	18.2 ± 0.00^{e}	16.3 ± 0.44^{e}
Alternaria	28 ± 0.56^{b}	21.3 ± 0.26^{b}	20.8 ± 0.30^{b}
Cladosporium	24.7 ± 0.35^{c}	17.7 ± 0.10^{f}	17.5 ± 0.00^{d}
Fusarium	33.4 ± 0.30^{a}	26.8 ± 0.10^{a}	26 ± 0.10^{a}
Geotrichum	27.2 ± 0.27^{c}	20.5 ± 0.35^{c}	19.7 ± 0.10^{c}
Mucor	21.3 ± 0.17^{f}	16 ± 0.20^{g}	14.8 ± 0.26^{g}
Rhizopus	21.5 ± 0.00^{f}	16 ± 0.10^{g}	15.1 ± 0.45^{fg}

Means within a column with different superscript letters are significantly different ($p< 0.05$)

Well diffusion method

The inverse relationship was observed between extract concentration and fungal colony growth in media, as the growth of fungal colonies increased with decreasing extract concentration. According to the results obtained that are shown in Table 2, aqueous extracts of garlic could affect the growth of all tested fungi at a minimum concentration of 50 µg/ml except for *A. niger*, *Mucor sp.*, and *Rhizopus sp.* The higher concentrations could cause clear zones of inhibition for all tested fungi. *Fusarium* was found to be more sensitive (33.2 mm) than the others. The highest antifungal activity was seen at a concentration of 300 µg/ml of aqueous extract. Zones of inhibition on methanolic and ethanolic extracts against tested fungi are shown in Tables 3 and 4. The antifungal effect

of both extracts was lower than aqueous extracts. *Fusarium* and *A. niger* showed the highest and lowest sensitivity to extracts respectively.

Table 2- Mean ±SD diameter of the inhibition zones (mm) after using different concentrations of **aqueous extract (µg/ml)** in well diffusion method

	50	**100**	**150**	**200**	**250**	**300**
A.niger	0.00 ± 0.00^{f}	11 ± 0.26^{g}	12 ± 0.20^{g}	15.5 ± 0.35^{f}	20.1 ± 0.17^{g}	$25.2 \pm .010^{g}$
A. fumigatus	11 ± 0.00^{e}	13.3 ± 0.10^{e}	15.2 ± 0.34^{e}	19.3 ± 0.00^{d}	23.2 ± 0.26^{e}	27.8 ± 0.20^{d}
A. flavus	11.3 ± 0.10^{d}	14.1 ± 0.00^{d}	15.7 ± 0.10^{d}	19.5 ± 0.00^{d}	23.7 ± 0.53^{d}	27.2 ± 0.26^{e}
Penicillium	11 ± 0.00^{e}	13.5 ± 0.10^{e}	15.7 ± 0.26^{d}	19.3 ± 0.26^{de}	22 ± 0.00^{f}	26.6 ± 0.36^{f}
Alternaria	12 ± 0.26^{b}	15.6 ± 0.00^{c}	19 ± 0.00^{c}	22.1 ± 0.30^{c}	25.3 ± 0.10^{c}	29.1 ± 0.10^{c}
Cladosporium	11.7 ± 0.15^{c}	16 ± 0.30^{b}	20 ± 0.17^{b}	24.3 ± 0.17^{b}	27 ± 0.00^{b}	30.7 ± 0.30^{b}
Fusarium	13 ± 0.10^{a}	18 ± 0.10^{a}	22 ± 0.17^{a}	25.2 ± 0.10^{a}	28 ± 0.26^{a}	33.2 ± 0.10^{a}
Geotrichum	11 ± 0.10^{e}	12.3 ± 0.20^{f}	15.6 ± 0.20^{d}	18.9 ± 0.26^{e}	22 ± 0.00^{f}	27.6 ± 0.50^{de}
Mucor	0.00 ± 0.00^{f}	11 ± 0.00^{g}	12.6 ± 0.30^{f}	15.3 ± 0.00^{f}	20.4 ± 0.00^{g}	25.7 ± 0.30^{g}
Rhizopus	0.00 ± 0.00^{f}	11 ± 0.00^{g}	12.8 ± 0.10^{f}	15.5 ± 0.10^{f}	20.4 ± 0.00^{g}	25.6 ± 0.30^{g}

Means within a column with different superscript letters are significantly different ($p< 0.05$)

Table 3- Mean ±SD diameter of the inhibition zones (mm) after using different concentrations of methanolic extract (µg/ml) in well diffusion method

	100	**150**	**200**	**250**	**300**
A.niger	0.00 ± 0.00^{e}	11 ± 0.44^{d}	12 ± 0.00^{f}	15.5 ± 0.50^{e}	18 ± 0.10^{f}
A. fumigatus	11 ± 0.20^{d}	12.3 ± 0.30^{c}	15 ± 0.53^{bc}	19 ± 0.30^{d}	22.3 ± 0.26^{d}
A. flavus	11.7 ± 0.10^{c}	12.4 ± 0.53^{c}	15 ± 0.17^{bc}	18.7 ± 0.20^{d}	22.6 ± 0.26^{cd}
Penicillium	12 ± 0.00^{b}	13.3 ± 0.20^{b}	15.3 ± 0.10^{b}	20 ± 0.50^{c}	23 ± 0.50^{bc}
Alternaria	11 ± 0.10^{d}	12.5 ± 0.10^{c}	14.9 ± 0.20^{bcd}	21.2 ± 0.10^{b}	23.5 ± 0.10^{b}
Cladosporium	11 ± 0.00^{d}	12.3 ± 0.20^{c}	14.5 ± 0.20^{d}	19 ± 0.56^{d}	22.7 ± 0.00^{cd}
Fusarium	13 ± 0.20^{a}	16 ± 0.00^{a}	18.2 ± 0.40^{a}	22 ± 0.00^{a}	25.6 ± 0.20^{a}
Geotrichum	11 ± 0.44^{d}	12.6 ± 0.40^{c}	14.8 ± 0.26^{cd}	19.3 ± 0.10^{d}	23 ± 0.26^{bc}
Mucor	0.00 ± 0.00^{e}	11 ± 0.00^{d}	13 ± 0.20^{e}	16 ± 0.00^{e}	19 ± 0.70^{e}
Rhizopus	0.00 ± 0.00^{e}	11 ± 0.26^{d}	13 ± 0.00^{e}	16.1 ± 0.40^{e}	19.2 ± 0.10^{e}

Means within a column with different superscript letters are significantly different ($p< 0.05$)

Table 4- Mean ±SD diameter of the inhibition zones (mm) after using different concentrations of ethanolic extract (µg/ml) in well diffusion method

	100	**150**	**200**	**250**	**300**
A.niger	0.00 ± 0.00^{d}	11 ± 0.10^{c}	11 ± 0.00^{f}	14 ± 0.00^{e}	17.8 ± 0.17^{e}
A. fumigatus	11 ± 0.26^{c}	12 ± 0.00^{b}	13.9 ± 0.10^{b}	16 ± 0.00^{d}	19 ± 0.00^{d}
A. flavus	11.2 ± 0.00^{c}	12 ± 0.00^{b}	13.8 ± 0.17^{b}	16 ± 0.00^{d}	19 ± 0.00^{d}
Penicillium	11 ± 0.00^{b}	11.8 ± 0.20^{b}	13.6 ± 0.17^{c}	16.2 ± 0.20^{c}	21 ± 0.00^{c}
Alternaria	11.3 ± 0.10^{c}	12 ± 0.00^{b}	13.9 ± 0.00^{b}	16.9 ± 0.17^{b}	21.2 ± 0.10^{c}
Cladosporium	11 ± 0.10^{c}	11.9 ± 0.00^{b}	13.5 ± 0.00^{c}	17 ± 0.00^{b}	21.5 ± 0.30^{b}
Fusarium	12.3 ± 0.20^{a}	14.2 ± 0.26^{a}	17 ± 0.10^{a}	20 ± 0.10^{a}	25 ± 0.26^{a}
Geotrichum	11 ± 0.00^{c}	11.8 ± 0.00^{b}	14 ± 0.00^{b}	17 ± 0.00^{b}	21 ± 0.00^{c}
Mucor	0.00 ± 0.00^{d}	11 ± 0.20^{c}	11.8 ± 0.00^{d}	14.1 ± 0.20^{e}	18 ± 0.00^{e}
Rhizopus	0.00 ± 0.00^{d}	11 ± 0.00^{c}	11.6 ± 0.20^{e}	14.1 ± 0.00^{e}	18 ± 0.00^{e}

Means within a column with different superscript letters are significantly different ($p< 0.05$)

Minimal inhibitory concentration

The Mic values of aqueous extract were determined to be 300 µg/ml for *A. niger, A. fumigatus, A. flavus, Mucor sp., Rhizopus sp., Penicillium sp.,* and *Alternaria sp.,* and 350 µg/ml for *Cladosporium sp., Fusarium sp.,* and *Geotrichum sp.* The MIC values of methanolic and ethanolic extracts were higher than the amounts of aqueous extract. These values were 350 and 400 for methanolic and methanolic extracts, respectively.

Discussion

Garlic is one of the most commonly used spices in the food industries for its taste, smell, and health properties and is used in the manufacturing of many food products such as meat preserves, mayonnaise sauces, and dressings (Klebukowska *et al.,* 2015). The antimicrobial activity of garlic was recognized by Louis Pasteur (1822-1895), and then Albert Schweitzer (1875-1965) used it for the treatment of amoebic dysentery. People used garlic for its strong antimicrobial properties to preserve meat and fish for a long time (Fujisawa et al., 2009). Many previous studies reported that garlic has a broad range of antifungal activity against both pathogenic and nonpathogenic fungi (Samuel et al., 2000; Suleiman & Abdallah,. 2014; Aala et al., 2014; Sittisart et al., 2017; Burian et al., 2017).

Moulds are among the most common microorganisms that are widely distributed in the environment and can contaminate a variety of food products (Hakim & Alarousy, 2015). Fungal contamination of foods causes food corruption and has adverse effects on human health since some fungi are capable of producing mycotoxins causing cases of food poisoning (Brr et al., 2004; Alshannaq & Yu., 2017; Rico-Munos *et al.,* 2019).

This study aimed to compare the antifungal potential of aqueous, methanolic and ethanolic extracts of *Allium sativum* against ten isolates belonging to eight genera of fungi which were isolated from mayonnaise samples stored outside the refrigerator and to determine the MIC of each extract.

The fungi grow at a pH range 3 to 8. Spoilage moulds can be classified into four important groups; A: Zygomycetes, which are widespread and grow rapidly on simple carbon sources. *Mucor* and *Rhizopus* are the most common spoilage species. B: *Penicillium* and related genera, which many species are among common important spoilage fungi and some of them, are mycotoxin producers. C: *Aspergillus* and related moulds generally grow faster than *Penicillium* and are more resistant to high temperatures. Many species produce different types of mycotoxins. D: Other moulds belonging to several genera, such as *Fusarium* and *Alternaria,* have been isolated from spoiled food. Several mycotoxins are produced by these fungi (Kabak et al., 2006; Rawat., 2015).

In our study, three species of the genus *Aspergillus* were the most frequent isolates, followed by *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Cladosporium* sp., *Fusarium* sp., *Alternaria* sp., and *Geotrichum* sp. respectively. In a study conducted in some hotels in Ghana, several specific fungi including *Eurotium, Herbariroum,*

Cladosporium, Penicillium, Alternaria, Aurobasidium, Aspergillus, and *Fusarium* were isolated from different food including fried rice, beef, vegetable sauce, tomato sauce, chicken and fish (Darko et al., 2017).

In our study, disc and well diffusion methods were used to evaluate the antifungal activity of the extracts again above mentioned fungi. The efficacy of the extracts was evaluated at various concentrations on the growth of the fungi tested. The results obtained showed that aqueous extracts had higher levels of growth inhibition than methanolic and ethanolic extracts, which is by the report of Chen et al, (2018). However, the results of another study showed that methanol extract of garlic was active against tested bacteria while other extracts did not show considerable antimicrobial activity (Meriga et al., 2012).

In our study, the mean diameter of inhibition zones (mm) at the concentration of50 µg/ml, in the good diffusion method was greater than the disc diffusion method (Tables 1-4).

In a study conducted by Avesthi et al, (2010), *Allium sativum* showed 100% inhibition of the mycelial growth of *A. niger* at 20% concentration, while, in our study, *A. niger* showed lower sensitivity to the extracts than the others. Differences in the results of various studies can be due to the chemical composition and the content of bioactive compounds, which are different between the various available garlic formulations (Martins et al., 2016).

It is approved that allicin which is produced by the action of allinase on allin and has similar activity to ketoconazole against dermatophytes (Paudel., 2010; Touloupakis & Ghanotakis., 2010), is the main component of garlic and it is responsible for antibacterial and antifungal activities of garlic (19, 31). Allicin exhibits strong antifungal activity both *in vivo* and *in vitro* against many pathogenic fungi (Sittisart et al., 2017). In our study, all extracts at concentrations of 150 µg/ml and higher could inhibit the growth of all fungi tested, as, with increasing the concentrations of the extracts, the inhibitory levels increased. These results are following Sittisart et al, (2017) study, which reported that the inhibitory level on mycelial growth of shallot and garlic extracts increased in a dose-dependent manner in all isolates of *Phomopsis* spp.

Also, in a study regarding the antifungal effect of the aqueous extract of *A. sativum* against pathogenic fungus, *Trichophyton rubrum,* it was detected that there is a positive correlation between the volume of the aqueous extract loaded on the disks and the diameter of the inhibition zone formed around the disks (Samuel et al., 2000).

In another study, the antifungal activity of *A. ascalonicum* was evaluated against seven genera of saprophytic fungi, including *Syncephalastrum*, *A. niger*, *Penicillium* sp, *Paecilomyces* sp, *Scopulariopsis* sp, *Cladosporium* sp, *Alternaria* sp, *Drechslerasp,* by agar well diffusion method. By increasing the concentration of the extract, its antifungal activity increased (Zarei Mahmoudabadi & Gharib Nassery., 2016).

The results of a study showed that the aqueous extracts of garlic inhibited the growth of *Candida albicans, A. niger, A. flavus, Curvularia lunata, Microsporum audoinii,*

*Trichophyton soudanense*and *Trichophyton mentagrophytis* at a concentration of 10 mg/ml, while the methanol extract of garlic did not affect fungi tested. They stated this might be due to the insolubility of the plant ingredients in methanol (Suleiman & Abdallah., 2014).

Other studies have shown the antifungal effect of aqueous garlic extract against plant pathogenic such as *Fusarium, Botrytis, verticillium,* and *phytophthora,* which were inhibited by 28 garlic cultivars (Chen et al., 2018). Burien et al, reported that garlic has antifungal activity against *Sporothrix schenkii* and garlic extracts aqueous displayed a MIC value of 0.62 mg/ml (Burrian et al., 2017).

Conclusion

In conclusion, the results of our study suggest that garlic is an important source of ingredients having antifungal properties and can be used as a natural preservative in foods.

Acknowledgment

The authors wish to thank from Vice-Chancellor's Office for Research of Isfahan (Khorasgan) Branch, Islamic Azad University, for providing the financial and technical supports for the completion of this research.

Conflict of interest

The author declares no conflict of interest in this paper.

References

1. Aala F., Yusuf U.K., Nulit R., Rezaie S. 2014. Inhibitory effect of allicin and garlic extracts on growth of cultured hyphae. Iran J Basic Med Sci 17: 150-154. PMCID: PMC4016684

2. Alshannaq A., Yu J.H. 2017. Occurrence, toxicity, and analysis of major mycotoxins in food. Int J Environ Res Public Health 14(6): E632. doi:10.3390/ijerph14060632.

3. Avasthi S., Gautam A.K., Bhadauria R. 2010. Antifungal activity of plant products against *Aspergillus niger:* A potential application in the control of a spoilage fungus. Biol Forum 2(1): 53-55.

4. Brr A.H., Mustafa N.Y., Edris A.M. 2004. Incidence of moulds and aflatoxins in some meat products. Benha Vet Med J 15(2): 65-75.

5. Burian J.P., Sacramento L.V.S., Carlos I.Z. 2017. Fungal infection control by garlic extracts (*Allium sativum* L.*)* and modulat*ion* of peritoneal macrophages activity in murine model of sporotrichosis. Braz J Biol 74(4): 848-855. doi:10.1590/1519-6984.03716.

6. Cao H., Huang C., Wang X. 2016. Allicin inhibits transient outward potassium currents in mouse ventricular myocytes. Exp Ther Med 11: 1896-1900. doi:10.3892/etm.2016.3116

7. Chen C., Liu C.H., Cai J., Zhang W., Qi W.L., Wang Z., Liu Z.B., Yi Y. 2018. Broad-spectrum antimicrobial activity, chemical composition and mechanism of garlic (*Allium sativum*) extracts. Food Control 86: 117-125. doi:10.1016/j.foodcont.2017.11.015

8. Cheng H., Huang G. 2018. Extraction, characterization and antioxidant activity of *Allium sativum* polysaccharide. Int J Biol Macromol 114: 415-419. doi: 10.1016/j.ijbiomac.2018.03.156.

9. Darko S., Mills-Robertson F.C., Wierko-Manu F.D. 2017. Fungal contamination of food prepared in some hotels in Kumasi metropolis. Int Food Res J 24(2): 860-867.

10. Faraji M., Rahbarzare F. 2016. Simultaneous determination of four preservative in foodstuffs by high performance liquid chromatography. Nutr Food Sci Res 3(2): 43-50. doi:10.18869/acadpub.nfsr.3.2.43

11. Fujisawa H., Watanabe K., Suma K., Origuchi K., Matsufuji H., Seki T., Ariga T. 2009. Antibacterial potential of garlic-derived allicin and its cancellation by sulfhydryl compounds. Biosci, Biotechnol, Biochem 73(9): 1948-1955. doi:10.1271/bbb.90096

12. Gull I., Saeed M., Shukat H., Aslam S.M., Samra Z.Q., Atar A.M. 2012. Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. Ann Clin Microbiol Antimicrob 11(8): 1-6. doi:10.1186/1476-0711-11-8.

13. Hakim A.S., Alarousy R.M. 2015. Incidence of fungal infections and mycotoxicosis in pork meat and pork by-products in Egyptian markets. . Int J Nut & Food Eng 9(7): 816-819.

14. Kabak B., Dobson A.D.W., Var I. 2006. Strategies to prevent mycotoxin contamination of food and animal feed: a review. Crit Rev Food Sci Nutr 46(8): 593–619. doi:10.1080/10408390500436185

15. Klebukowska L., Zadernowska A., Chajecka-Wierzchowska W. 2015. Microbiological contamination of dried and lyophilized garlic as a potential source of food spoilage Food Sci Technol 52(3): 1802-1807. doi:10.1007/s13197-013-1169-6.

16. Lanzotti V., Barile E., Antgnani V., Bonanomi G., Scala F. 2012. Antifungal saponin from bulbs of garlic. Phytochemistry 78: 126-134. doi:10.1016/j.phytochem.2012.03.009.

17. Lemons J.G., Garcia M.V., Mello R.O., Copetti V. 2018. Consumers complain about moldy foods in a Brazilian website. Food Control 92: 380-385. doi:10.1016/j.foodcont.2018.05.017.

18. Majewski M. 2014. *Allium sativum*: Facts and myths regarding human health. Rocz Panstw Zakl Hig 65(1): 1-8.

19. Martins N., Petropoulos S., Ferreira I.C.F.R. 2016. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre-and post-harvest conditions: A review. Food Chem 211: 41-50. doi:10.1016/j.foodchem.2016.05.029

20. Meriga B., Mopuri R., Muralikrishna T. 2012. Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum.* Asian Pac J Trop Med 5(5): 391-395. doi:10.1016/S1995-7645(12)60065-0.

21. Najda A., Blaszczyk L., Winiarczyk K., Dyduch J., Tchorzewska D. 2016. Comparative studies of nutritional and health-enhancing properties in the "garlic-like" plant *Allium ampeloprasum* var. *ampeloprasum* (GHG-L) and *A. Sativum.* Sci Hortic 201: 247-255. doi:10.1016/j.scienta.2016.01.044

22. Paudel K.R. 2014. Pharmacological effects of traditional herbal garlic (*Allium sativum)*: A review. Kathmandu Univ Med Coll J 3(4): 158-160.

23. Pirali Khairabadi, E., Sedigheh Mousavi, S., Momtaz, H., Nikokhah, F., Hosseini Shekarabi, S., Raissy, M. 2020. Prevalence and phylogenetic analysis of *Listeria monocytogenes* isolated from the fillets of two farmed fish in Shahrekord in 2018. J Food Microbiol, 7(4), 81-93.

24. Queiroz Y.S., Ishimoto E.Y., Bastos D.H.M., Sampaio G.R., Torres E.A.F.S. 2009. Garlic and ready-to-eat garlic products: *In vitro* antioxidant activity. Food Chem 115: 371-374. doi:10.1016/j.foodchem.2008.11.105.

25. Raissy, M., Keyhani, K., Pirali, K. 2020. Comparison of the effects of *Geranium*, *lavender* and garlic extracts on *Ichthyophthirius multifiliis* in naturally infected *Capoeta damascina*. J Anim Environ, 12(4), 307-310. doi: 10.22034/aej.2020.121630

26. Rawat S. 2015. Food spoilage: Microorganisms and their prevention. Asian J Plant Sci & Res 5(4): 47-56.

27. Rico-Munoz E., Samson R.A., Houbraken J. 2019. Mould spoilage of foods and beverages: Using the right methodology. Food Microbiol 81: 51-62. doi:10.1016/j.fm.2018.03.016.

28. Samuel J.K., Andrews B., Jebashree H.S. 2000. *In vitro* evaluation of the antifungal activity of *Allium sativum* bulb extract against *Trichophyton rubrum,* a human skin pathogen.

World J Microbiol Biotechnol 16: 617-620. doi:10.1023/A:1008972016316
29. Shoshi S.J., Akter H. 2017. Effects of Garlic (*Alium sativum)* on blood glucose level in type 2 diabetes mellitus patients treated with metformin. J Enam Med Coll 7(3)*:* 151-155. doi:10.3329/jemc.v7i3.34075
30. Sittisart P., Yossan S., Prasertsan P. 2017. Antifungal property of chili, shallot and garlic extracts against pathogenic fungi, *Phomopsis* spp., isolated from infected leaves of para rubber (Hevea brasiliensis Muell. Arg). Agric & Nat Resour 51(6): 85-491. doi:10.1016/j.anres.2018.03.005
31. Suleiman E.A., Abdallah W.B. .2014. *In Vitro* activity of garlic *(Allium sativum)* on some pathogenic fungi. European J Med Plants 4(10): 1240-1250. doi:10.9734/EJMP/2014/10132
32. Touloupakis E., Ghanotakis D.F. 2010. Nutraceutical use of garlic sulfur-containing compounds. In: Giardi M.T., Rea G., Berra B. (eds) Bio-Farms for Nutraceuticals. Advances in Experimental Medicine and Biology. Springer, Boston, MA 698: 110-121. doi:10.1007/978-1-4419-7347-4_9.
33. Wang H., Yang Z., Ying G., Yang M., Nian Y., Wei F., Kong W. 2018. Antifungal evaluation of plant essential oils and their major components against toxigenic fungi. Ind Crops Prod 120: 180-186. doi:10.1016/j.indcrop.2018.04.053
34. Zarei Mahmoudabadi A., Gharib Nassery M.K. 2009. Antifungal activity of shallot, *Allium ascalonicum* Linn (Liliaceae), *in vitro*. J Med Plants Res 3(5): 450-453. Article Number - 50D1BA915299.