African Journal of Agriculture Vol. 1 (4), pp. 057-059, October, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Control of *Aspergillus niger* with garlic, onion and leek extracts

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Accepted 7 August, 2013

Antifungal activity of "*Allium*" vegetables that is garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and leek (*Allium porrum* L.) were investigated against *Aspergillus niger*. Minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) of aqueous, ethyl alcohol and acetone extracts were determined by disc diffusion and broth dilution methods in the test tubes. Onion extract with ethyl alcohol (275 mg/mL MFC), aqueous garlic extract (325 mg/mL MFC) and aqueous leek extract (900 mg/mL MFC) found the most inhibitory against *A. niger*.

Key words: *Aspergillus niger*, garlic, onion, leek, antifungal activity.

INTRODUCTION

Aspergillus sp. are the most common fungal species which are able to produce mycotoxins in food and feed-stuffs. Mycotoxins are known to be potent hepatocarcino-gens in animals and humans. The presence and growth of fungi may cause spoilage and result in a reduction in quality and quantity of foods (Paster et al., 1995; Belt-mont and Carjaval, 1998; Sahin and Korukluoglu, 2000; Candlish et al., 2001; Galvano et al., 2001; Juglal et al., 2002; Soliman and Badeaa, 2002; Rasooli and Abyaneh, 2004).

Natural plant extracts may provide an alternative to chemical preservatives. Over the years much effort has been devoted to the search for new antifungal materials from natural sources for food preservation (Karapınar, 1989; Topal, 1989; Paster et al., 1995; De et al., 1999; Yin and Tsao, 1999; Nielsen and Rios, 2000; Galvano et al., 2001; Juglal et al., 2002; Soliman and Badeaa, 2002; Onyeagba et al. 2004; Boyraz and Ozcan, 2005; Hacise-ferogullari et al., 2005)

Allium genus has over 500 members, each differing in maturing, color and taste, but with similar biochemical, phytochemical and neutraceutical content. *Alliums* were revered to possess anti-bacterial and anti-fungal activities and include the powerful antioxidants, sulfur and other numerous phenolic compounds which arouse significant interests (Block, 1985; Topal, 1989; Yin and Cheng, 1998; Phay et al., 1999; Harris et al., 2001; Kyung and Lee, 2001; Rivlin, 2001; Griffiths et al., 2002; Benkeblia, 2004; Haciseferogullari et al., 2005).

The aim of this study was to investigate minimal inhibi-tory concentrations (MIC) and minimal fungicidal concen-trations (MFC) doses of garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and leek (*Allium porrum* L.) extracts (aqueous, acetone and ethyl alcohol) against to *Asper-gillus niger*.

MATERIALS AND METHODS

Allium samples and water content

Onions (*A.* cepa L.) and leeks (*A. porrum* L.) were cultivated in Yenice-Canakkale region and garlic (*A.* sativum L.) was obtained from Balikesir regions of Turkey during harvesting season. Samples freshly harvested were classified for homogeneity and lack of flaws and prepared for analysis. Water content of fresh *Allium* samples were determined by using the official methods of AOAC (1990).

Microorganism

Aspergillus niger strain was isolated from tulum-cheese in Uludag University, Department of Food Engineering in Bursa, Turkey and identified using standard fungi determination procedures.

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Table 1. Water content of *Allium* fresh samples.

***Allium* sample**	**Water content (%) ± SD**
Garlic bulb (*Allium sativum* L.)	76.1±2.2
Onion bulb (*Allium cepa* L.)	89.3±0.65
Whole Leek (*Allium porrum* L.)	87.9±0.36

Preparation of material extracts

270 g of fresh onion, leek and garlic were peeled and then was chopped with 300 mL distilled water, ethanol (Panreac 121086.1612, 99.5%) and acetone (Merck 1.00013.2500, 99.5%) (w/v) by using a domestic blender (Braun model 4259, Germany) for 1 min at average speed. The mixture were macerated during 24 h at the + 4ºC. After that, resulting extracts of materials were filtered and sterilized using a 0.45 µm pore size cellulose acetate membrane filter (Cole-Parmer-47 mm) under nitrogen gas pressure. The extracts were used directly. Dilutions were prepared from 900 to 100 mg/mL (by 25 mg/mL intervals). These dilutions were used in antifungal analysis. Ethyl alcohol, acetone and distilled water served as control.

Preparation of inoculum

A. niger was cultured on Sabouraud Dextrose Broth-SDB-(Oxoid CM0147) at 30ºC for 22 h. Test fungi in SDB were enumerated by using serial dilution method. Final cell concentration of culture was 10^4- 10^5 cfu/mL.

Antifungal activity tests

Disc diffusion method was used as an antimicrobial method (Yin and Tsao, 1999; Karaman et al., 2003; Benkeblia, 2004). Sterile Sabouraud Dextrose Agar-SDA-(Oxoid CM0041) at 43-45ºC and poured into the petri plates (9 cm diameter). Then the agar was allowed to solidify at + 4ºC for 1 h. 0.2 mL of *A. niger* culture inoculum applied of each plate. Inoculum was evenly spread on agar using a glass L- rod spreader. The petri dishes were left at + 4ºC for 1 h to allow agar surface to dry. Sterile filter papers (Schleicher and Schüll 2668, Germany, 6 mm diameter) were placed on the culture mediums and were impregnated with 50 µL material extracts between 100 - 900 mg/mL concentrations and placed on the inoculated plates. Distilled water and other solvents were added at the same concentrations on the discs to provide a control. After 30 min, plates were turned upside down and incubated at 30ºC for 24 h. At the end of the period, inhibition zones, formed in the medium were measured in millimeters (mm). All experiments were done in three replicates.

Determination of minimal ınhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

5 mL of sterile extracts at different concentrations were taken in to the sterile empty tubes and 1 mL of *A. niger* culture was added in to the extracts and mixed. After that 1 mL of (extract + *A. niger*) culture was added in to the 5 mL of sterile SDB in the tubes. Then all the tubes were incubated at 30ºC for 15 days. Observations were made for visible growth of fungi. The highest dilution (lowest concentration) showing no visible growth was regarded as Minimal Inhibitory Concentrations (MIC) during 15 days. Cells from the tubes showing no growth were subcultured on SDA plates and incubated at 30ºC for 5 days to determine if the inhibiton was reversible or permanent. Minimal Fungicidal Concentration (MFC) was determined as the highest dilution (lowest concentration) at which no growth occured on the plates. In control tubes 1 mL (extract and A.niger culture) were added in to the 5 mL of solvent; ethyl alcohol or acetone or water seperately All the tests were done in three replicates (Abbasoglu, 1996; Yin and Tsao, 1999; Flörl et al., 2003; Rasooli and Abyaneh, 2004).

RESULTS AND DISCUSSION

Water contents of fresh *Allium* samples are listed in Table 1. The MIC and MFC (mg/mL) concentrations of *Allium* plant extracts and inhibitory zones (mean ± SD) against to *A. niger* are presented in Table 2. It is seen that *Allium* plants have antifungal effects to *A. niger*. The most inhibitory plant was garlic, followed by onion and leek. Ethyl alcohol extracts of the garlic and onion significantly show inhibitor effect against *A. niger*. Also, inhibitor activities were observed for aqueous extracts of garlic and leek. Acetone extracts of onion and leek did not show any effect on *A. niger*. Ethyl alcohol extract of the onion has the highest inhibitory activity when their MIC and MFC values are compared.

The inhibitory activity of *Allium* vegetables extracts against mould have been reported by numerous authors. It has also been observed that alliicin, thiosulfonates and other compounds show fungistatic activities against *A. niger* , *Rhodotorula nigricans*, *Penicillium italicum*, *Penicillium cyclopium*, *Aspergillus flavus, Cladosporium macrocarpum, Aspergillus fumigatus, Aspergillus alutaceus, Aspergillus terreus* and *Penicillium chryogenum* (Wei et al., 1967; Graham and Graham, 1987; Topal, 1989; Hafez and Said, 1997; Ankri and Mirelman, 1999; Harris et al., 2001). Similarly, ajoene compound which is a deri-vative of alliicin and obtained from garlic with ethyl alcohol extraction is very inhibitory against *A. niger*, *Candida albicans* and *Paracoccidioides brasiliensis* (Naganawa et al., 1996). Yoshida et al. (1987) reported that ajoene compound from garlic have stronger antifungal activity than alliicin. They are determined that ajoene damages the cell walls of fungi. Yin and Tsao (1999) studied antifungal effects of various *Allium* plants. They found 35 ± 3 µg/mL MFC for garlic bulb, 748 ± 15 µg/mL MFC for on-ion bulb and 91 ± 10 µg/mL MFC for chinese leek against *A. niger*. These authors observe that garlic shows the highest antifungal activity against three *Aspergillus* species.

Antifungal effects of onion and garlic essential oils against some fungi have also been investigate. *A. niger* is less inhibited by low concentrations of essential oils of green and yellow onions but red onion and garlic essential oils show strong inhibitory effects against *A. niger* (Benkeblia, 2004). Fistulosin, an antifungal compound isolates from onions, shows antifungal activities against several fungal species (Phay et al.,1999). Combined extract of corni fructus, cinnamon and chinese chieve (1:6:6, v/v/v) also exhibit high inhibitor activity against *A. niger*

Table 2. The minimal inhibitory and minimal fungicidal concentrations (MIC and MFC) and inhibitory zones diameters of *Allium* plant extracts against *A. niger*.

Materıals	Aqueous MIC (mg/mL)	Aqueous MFC (mg/mL)	Ethyl alcohol MIC (mg/mL)	Ethyl alcohol MFC (mg/mL)	Acetone MIC (mg/mL)	Acetone MFC (mg/mL)
Garlic (*Allium sativum* L.)						
MIC/MFC	325	325	425	450	800	875
Inhibition zones*	15.5±0.9	15.5±0.9	15±1.0	16.8±0.8	13.7±1.6	16.5±0.5
Onion (*Allium cepa* L.)						
MIC/MFC	> 900	> 900	250	275	> 900	> 900
Inhibition zones*	-	-	11.8±0.3	16.7±0.6	-	-
Leek (*Allium porrum* L.)						
MIC/MFC	900	> 900	>900	>900	>900	>900
Inhibition zones*	12.3±1.1	-	-	-	-	-

*Inhibition zones (mm, mean ± SD).

(Mau et al., 2001).

A. niger, *P. italicum*, *Tryptophyton gypseum* and *Microsporon audouini* are inhibited by thiosulfonates compounds in onions. However, antifungal studies about onions are very limited. Researchers have observed that ether extracts of onion show inhibitor effect against *A. flavus* and *A. parasiticus* (Wei et al., 1967; Sharma et al., 1979; Pruthi, 1980). Topal (1989) reported that onion exhibits more inhibitor activity against bacteria than yeast and fungi. Researches on antifungal effects of leek are also very limited. In Kivanc and Kunduhoglu (1997) study, leek was found to have the lowest inhibitory vegetable against yeasts compared to onion, cabbage, radish and garlic. Tsao and Yin (2001) obtained 32 mg/L MIC for chinese leek oil and 20 mg/L MIC for garlic oil against *A. niger*.

It is concluded from the results that extracts of *Allium* vegetables can inhibit mould growth. Effectiveness of this inhibition was related to the solvent used in the extraction. The relative percentages for different alkyl groups and ratios of sulphur compounds can vary with the part of the plant. Also growing conditions of the plant affect the ratio of inhibitor of compounds (Block et al., 1992; Kyung and Lee, 2001; Benkeblia, 2004). In food preparations, *Allium* vegetables may provide a good inhibitory effect against fungal growth. However, despite the fact that *Allium* plants could be used as a potential inhibitory source because of instability of alliicin, thiosulfonates and related compounds, the strong odor may limit their use as food additives, necessitating further research.

ACKNOWLEDGMENT

The authors thank Uludag University Research Foundation for the support of 2002/19 project**.**

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