

Antimicrobial Activity of the Extract of Garlic and Onions

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ABSTRACT

This study investigated the pathogenicity of two fungi (*A. niger* and *P. digitatum*) and two bacteria (*S. aureus* and *E. carotovora*) on onion bulbs. The inoculated areas of the onion bulbs showed changes in their colour and the tissues macerated then an offensive smell developed. The three onion bulbs responded similarly. The non-autoclaved red onion extracts reduced radial growth of both fungi more than the autoclaved ones. The results on the radial growth indicated that the onion extracts were highly effective at the higher concentration (0.01, 0.1 and 1.0) compared to the lower concentration (0.001) and the control (0.00). Garlic extract at the higher concentration completely inhibited radial growth of both fungi. The yellow onion extract caused a reduction of mycelial dry weight of *A. niger* to about 60.46, 31.09, 23.04 and 5.95% at the concentrations 1.0, 0.1, 0.01 and 0.001, respectively. The white onion extract gave a reduction of about 38.77, 34.55, 20.73 and 4.41% at the concentrations 1.0, 0.1, 0.01 and 0.001, respectively. The red onion extract caused a reduction of about 23.22, 16.51, 14.98 and 0.38% at the concentration 1.0, 0.1, 0.01 and 0.001, respectively. Garlic extract at the higher concentration (1.0) reduced 89.06% of mycelial dry weight of *A. niger* and 38.58, 2.88 and 2.11% at the concentrations 0.1, 0.01 and 0.001, respectively. While, onion extracts promote mycelial growth of the fungus *P. digitatum* garlic extract at the higher concentration reduced 93.29% of mycelial dry weight of that fungus and 25.93, 2.78 and 0.47% at the other concentrations (0.1, 0.01 and 0.001, respectively). The three onion extracts showed no effect on the spore

EDITORIAL

germination of *A. niger*, while, 2.0% of the spores of *P. digitatum* were germinated at the higher concentration of the yellow onion extracts. Garlic extracts completely inhibited spore germination of both fungi at the higher concentration (1.0). The three onion extracts as well as the lower concentrations (0.1, 0.01 and 0.001) of the garlic extract have no effect on both bacteria. Although, the inhibition zone of *S. aureus* at the lower concentration (0.1) of the garlic extract was 0.5 cm, the higher concentration (1.0) of it completely inhibited bacteria.

Keyword: Antimicrobial properties, Garlic, Onions.

INTRODUCTION

Onion (*Allium cepa*) and garlic (*Allium sativum*) are belonging to the lily family. Onion, a biennial, produce single large bulb, whereas the perennial garlic produces a composite bulb, with each clove of garlic called bulbet. Both species produce an inflorescence consisting of small lily like flowers, which also make the attractive ornamentals (Levetin and McMahon, 2006).

Onion and garlic are two of the oldest cultivated plants used for both culinary and medicinal purposes, with a history even predating the ancient Egyptian civilization. In the herbal medicine traditions of both India and China, onions and garlic have been prescribed for centuries as remedies for numerous conditions. Modern research has shown that these folk remedies have a sound scientific basis. The organic sulfur compounds from garlic inhibit the growth of many disease causing bacteria and fungi. Researchers have found that the allicin blocks certain enzymes that infectious organisms use to damage or invade tissues (Levetin and McMahon, 2006). Ground or sliced raw garlic cloves applied directly onto

EDITORIAL

wounds or injuries. Used in this way, allicin, produced enzymatic form alliin, may play an important role in killing bacteria to prevent infection (Kyolic, 2003).

The onion crop seems to be the most popular and indispensable flavor enhancer in the world. These flavor compounds are widely appreciated by many cultures as an important component of their foods. In addition, onions possess many medicinal properties such as lowering of sugar and lipid and enhance Fibrinolysis in the blood (Augusti, 1990).

Spices and herbs have been used for thousands of years by many cultures to enhance aroma and flavor of food. Early cultures also recognized the value of using spices and herbs in preserving foods for their medicinal value (Shelef, 1983; Lubna, 2006). Essential oils, which are concentrated herbal liquid extracts, inhibit the growth of a wide variety of pathogenic microorganisms and may have great potential as antimicrobial agents (Friedman *et al.*, 2002).

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans (Clark, 1996). Secondly, the public is becoming increasingly aware of the problem with the over prescription and misuse of traditional antibiotics. The use of plant extracts, as well as other alternative forms of medical treatment is enjoying great popularity in the late 1990s (Eisenberg *et al.*, 1993).

Aspergillus niger is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments (Staino *et al.*, 2005). However, *A. niger* is less likely to cause human disease than some other *Aspergillus* species, but if large amounts of spores are inhaled, a serious lung disease, aspergillosis can occur (Staino *et al.*,

EDITORIAL

2005).

Various strains of *A. niger* are used in the industrial preparation of citric acid and gluconic acid and have been assessed as acceptable for daily intake by the World Health Organization. Many useful enzymes are produced using industrial fermentation of *A. niger*. Glucoamylase is used in the production of high fructose corn syrup and pectinase are used in cider and wine clarification (Staino *et al.*, 2005).

The fungus *Penicillium digitatum* causes a disease called green mold. The green mold is most prevalent in cold stored fruits where it is able to develop slowly at cold temperature and the fungus can spread from infected to healthy fruits in packed containers (Brown, 1994).

Staphylococcus aureus and related organisms are generally wide spread in nature, their normal habitats being the skin, skin glands and mucus membrane of humans and other mammals (Devries, 1986). They produce a variety of syndromes with clinical manifestation ranging from single pustules to sepsis and death.

Erwinia carotovora, is a major problem in agriculture, the microbes invade crops of potatoes and other vegetables in the fields or in storage that cause plant tissues to become soft and watery which eventually turn slimy and foul-smelling (Wood, 1998).

The present work was designed to investigate the effect of the extracts of three onion types (red, white and yellow) and one garlic type on two bacteria (*Staphylococcus aureus* and *Erwinia carotovora*) and two fungi (*Aspergillus niger* and *Penicillium digitatum*).

MATERIALS AND METHODS

Samples of three types of onion; white, red and yellow as well as one type of garlic were obtained from Wad Medani local market and were used in the following experiments. The fungal (*Aspergillus niger* and *Penicillium digitatum*) and bacterial (*Staphylococcus*

EDITORIAL

aureus and *Erwinia carotovora*) isolates used in this study were obtained from Center of Bioscience and Biotechnology.

Throughout this study potato dextrose agar (PDA) was used as medium for isolation and maintenance of fungi, while, the nutrient agar medium was used for isolation and growth of bacteria.

Inoculation of onion bulbs

The pathogenicity of the isolates of fungi and bacteria mentioned above were tested on healthy onion bulbs. The bulbs were wounded by a cork-borer in the first three scales. The inoculum was placed at the injured region. A solution of *A. niger* or *P. digitatum* and *Staph. aureus* or *E. carotovora* were made separately by mixing each culture with a 10 ml distilled and sterilized water in the Petri-dish. The solution was completed to 50 ml in conical flasks. About one ml of each solution was placed on the cork-borer pore in the onion bulbs (the three types), and the inoculum was covered by a paraffin layer. The rotting areas were measured every two days as was described by Arbab (1984).

Effect of the extracts on fungal growth

For the preparation of the extracts, 500 g of fresh bulbs of the tested plants (red, yellow and white) onion types and garlic were crushed with pestle and mortar separately in 500 ml distilled water. The extract was then filtered through Whatman No. 4 filter papers. The filtrate was divided into two equal parts designated as A and B. Part A was immediately sterilized by being passed through a membrane filter (0.22 μ), while, Part B of the extract was mixed with the ingredients of the PDA, PDB or NA medium and autoclaved. The tests were made as was described by Arbab (1984).

The Effect of the extracts on fungal growth was made by the radial growth and the mycelial dry weight methods, using Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) media, respectively. The methods were used as described by Abdel-Rahim *et al.* (2002). For radial growth, the diameter of growth was measured every 48

EDITORIAL

hours, by taking the average of two crossed diameters for each disc in the Petri dish. The results were then calculated as a percentage from the diameter of the growth of the control treatment. For the mycelial dry weight, the inoculated broth media in the flasks were incubated at room temperature (28 – 30⁰C) for 8 days. After incubation the mycelia were collected by filtering the culture through a Whatman No. 1 filter paper and the fresh weight was recorded. The mycelia mats were then dried at 80⁰C for 24 hours, before being reweighed.

Effect of the extracts on spore germination

Cultures of *A. niger* and *P. digitatum* were grown on PDA slopes in screw-cap bottles at 27° C. Spores were harvested from 4 days slope cultures by covering the culture with sterile distilled water and brushing gently with an inoculation loop. The spore suspension was filtered through a sintered glass funnel to remove hyphae and conidiophores. The spores were finally suspended in sterile deionized water, shaken in a Griffen flask-shaker to dispense clumps of spores and then counted by haemocytometer. For the fungal spore germination, the medium described by Anderson and Smith (1972) was used. Germ-tube production was assessed as was recommended by Manners (1966). Readings were made after 3, 6, 9, 12 and 18hours.

Effect of the extracts on bacterial growth

The effect of the extracts on bacterial growth was made by the Cup-Plate agar (Inhibition zone) method, as described by Barry *et al.* (1970) and Cruickshank *et al.* (1975) and by the Dilution Plate method as was described by Zainal *et al.* (1988). The numbers of bacterial colonies obtained from the dilution plate method were calculated as a log number of colonies per 1 ml. The logs of the numbers were plotted against time. The statistical analysis methods used were the ANOVA and the Duncan's multiple range tests.

RESULTS AND DISCUSSION

The first observation on bulbs inoculated with fungi was the development of a white

EDITORIAL

mycelia on the surface of each bulb at the place of inoculation. The tissues in the inoculated areas became pulpy with a little bit of liquid secretion and an offensive smell was then developed. With further incubation the color of the colored types (Red and Yellow) was changed and the rot condition was spreading throughout the infected bulb (Table 1). However, there was no significant differences between infections of *A. niger* and *P. digitatum*, the rotted areas of the first fungus were more pronounced than that of the second one. These findings were in agreement with those reported by Sharma and Roy (1978) and Arbab (1984). On the other hand, the three onions varieties showed no significant differences toward infection with both *Er. carotovora* and *E. coli* (Table, 1). Wood (1998) stated that *E. carotovora* has the ability to infect a variety of vegetables and plants including onions, carrots, potatoes, cucumbers, tomatoes, lettuce and ornamental plants like iris. The results also showed that there is no significant difference between the three types of onions (Table, 1). However, Walker and Lindergreen (1924) reported that coloured varieties of onions were in general more resistant to smudge (*Colletotrichum circinans*), rottings of *Fusarium*, *Aspergillus sp.*, and *Borytis allii* and other fungi, than the white types.

Table 1. Response of bulbs of different onion varieties to infection with some fungi and bacteria

Variety	Diameter area of infection(cm)			
	<i>A. niger</i>	<i>P. digitatum</i>	<i>S. aureus</i>	<i>E. carotovora</i>
Red	6.30	3.38	6.40	6.48
Yellow	6.54	2.62	7.02	7.18
White	7.08	3.14	8.02	8.12

F = 29.065 (between organisms). F = 0.241 (between onion types)

Effects of the extracts on radial growth of fungi

The effects of autoclaved and non-autoclaved extracts of the red onion variety on radial growth of *A. niger* and *P. digitatum* are shown on Fig. (1). The results indicated that the non-autoclaved extracts were significantly effective in reducing radial growth of both

EDITORIAL

fungi compared to the autoclaved extracts and the controls. Similar results were also reported by Arbab (1984).

Results in Tables (2 and 3) compared the effects of the extracts on radial growth of both fungi (*A. niger* and *P. digitatum*), respectively. The onion extracts were found highly effective at their higher concentrations compared to the control and the lower concentration. Although, the higher concentration of the garlic extract (1.0) was found to give a complete inhibition, its lower concentration (0.001) was insignificantly effective.

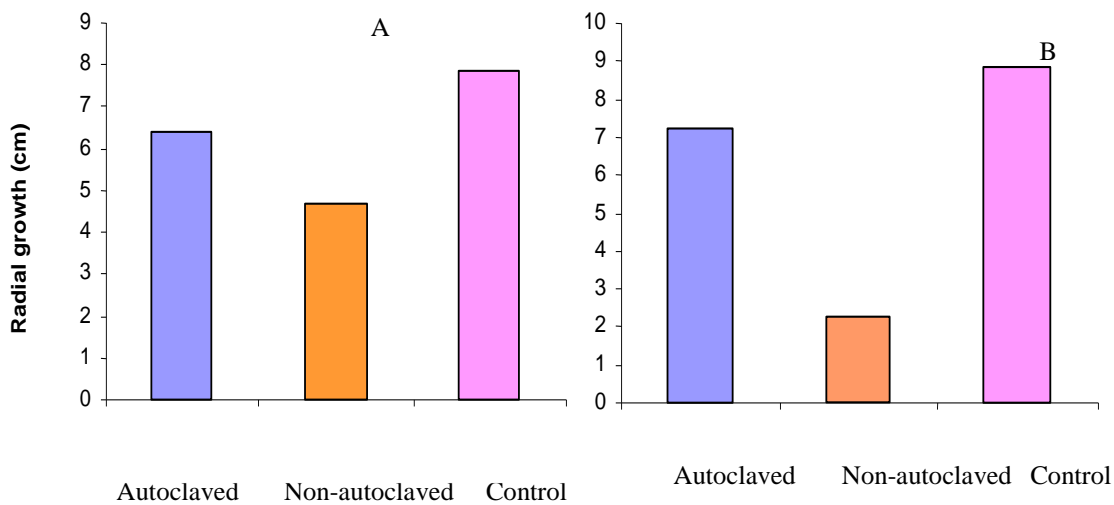


Fig. 1. The effect of autoclaved and non-autoclaved red onion extracts on radial growth of both fungi (A = *P. digitatum* B = *A. niger*).

Table 2. Effect of different concentrations of onions and garlic extracts on radial growth (cm) of

	<i>A. niger</i>				
Concentrations	0.00	0.001	0.01	0.1	1
Sample					

EDITORIAL

Red onion	7.85	5.59	3.63	3.29	3.03
Yellow onion	7.85	4.29	3.33	3.01	2.44
White onion	7.23	4.14	2.26	2.95	2.33
Garlic	7.69	6.98	6.55	5.12	0.00

LSD = 0.7932

Table 3. Effect of different concentrations of onions and garlic extracts on radial growth (cm) of

	<i>P. digitatum</i>				
Concentrations	0.00	0.001	0.01	0.1	1
Sample					
Red onion	8.83	6.53	6.06	3.90	2.48
Yellow onion	8.90	7.31	7.14	3.73	2.39
White onion	8.94	7.45	7.27	4.00	2.12
Garlic	9.00	9.00	9.00	5.31	0.00

LSD = 0.9499

Abdel-Hafez and El-Said (1997), stated that lower concentrations of onion extracts (0.625, 0.250 and 0.125% v/v) did not inhibit fungal growth of *A. niger*. According to Nouredine, *et al.* (2005) radial growth of the fungus was highly reduced at the higher concentration of the onion extracts. Abdel-Hafez and EL-Said (1997) also reported that the extracts of garlic were highly effective in reducing radial growth of the fungus *A. niger*, particularly at the higher concentrations. Garlic extracts were found effective even against microorganisms that have become resistant to antibiotics (Jezowa *et al.*, 1966).

EDITORIAL

Effect of the extracts on mycelial dry weight

Results of the effect of the different concentrations of onions and garlic extracts on mycelial dry weight of *A. niger* are shown on Fig. (2). From the results it was found that the garlic extracts were the best in reducing mycelial dry weight. At the higher concentration, garlic extract reduced 89.06% of the mycelial dry weight of *A. niger*. There was also some reduction at the lower concentration (38.58% reduction). Garlic extract at the concentrations 0.01 and 0.001 caused reduction of 2.88% and 2.11%, respectively.

The onion extract of the yellow type showed the best effect among the onion types on mycelial dry weight of *A. niger*. It caused a reduction of 60.46% at the higher concentration (1.0). However, the lower concentrations 0.1, 0.01 and 0.001 of the yellow onion extracts gave a reduction of about 31.09%, 23.04% and 5.95%, respectively. The white onion extracts gave a reduction of about 38.77% at the higher concentration; and gave about 34.55%, 20.73% and 4.41% at the lower concentrations (0.1, 0.01 and 0.001, respectively). The red onion extract showed the least effect.

EDITORIAL

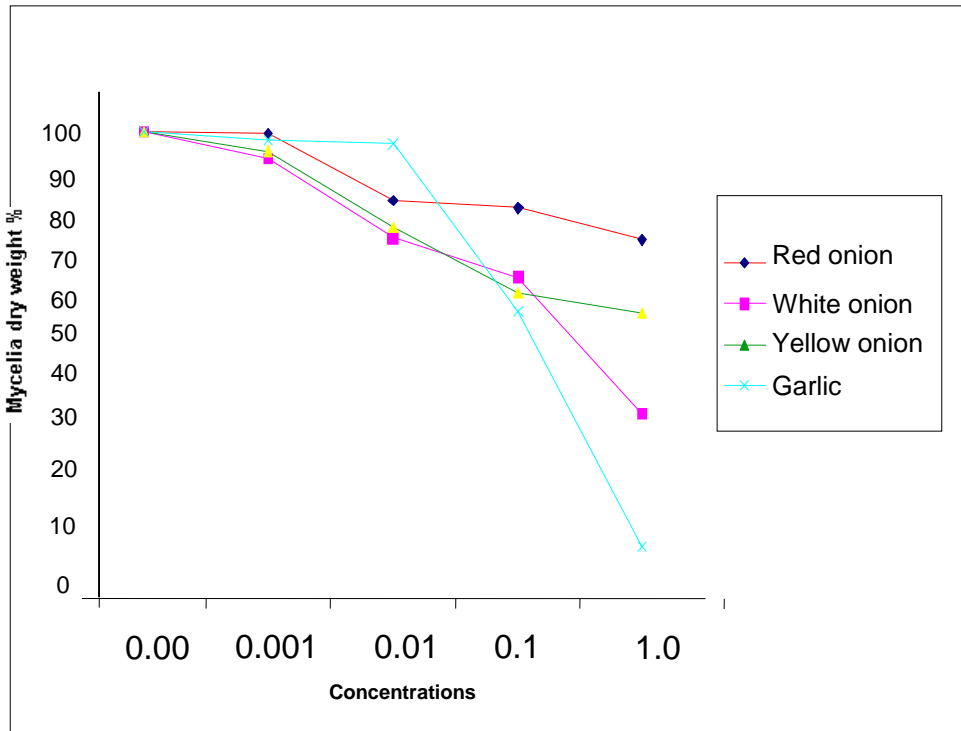


Fig. 2. Effect of different concentrations of onions and garlic extracts on mycelial dry weight of *A. niger*.

On the other hand the three onion extracts promoted growth of *P. digitatum* even at the higher concentrations, as shown on Fig. (3). Garlic extracts were effective causing about 93.29% reduction of the dry weight of *P. digitatum* at the higher concentration (1.0) and 25.93%, 2.78% and 0.47% at the lower concentrations (0.1, 0.01 and 0.001, respectively).

EDITORIAL

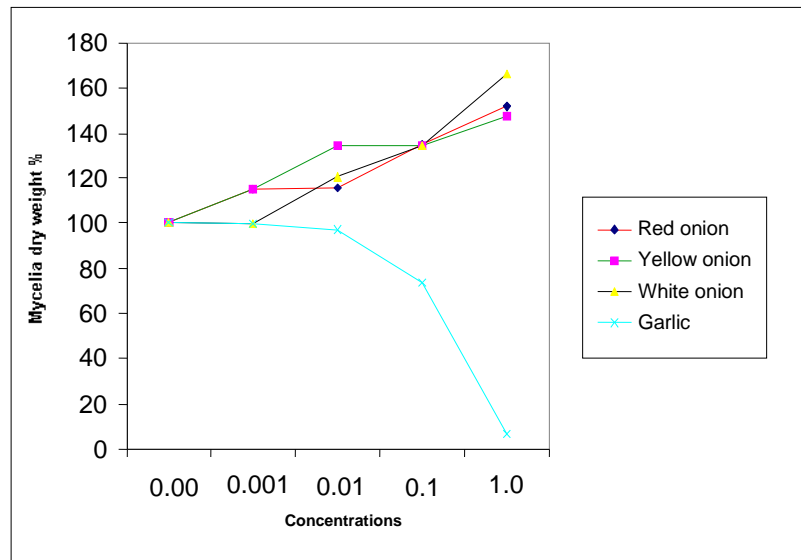


Fig. 3. Effect of different concentrations of onions and garlic extracts on mycelial dry weight of *P. digitatum*.

Zaman *et al.* (1997) reported that the efficacy of garlic, neem, ginger and onion extracts on mycelial growth of seed borne fungi (*A. niger*, *A. flavus*, *Fusarium moniliforme* and *Botryodiplodia theobromae*) declined with increasing dilution. Also Nwachukwu and Umechuruba (2001) reported that the leaf extracts of basil (*Ocimum basilicum*) bitter leaf (*Vernonia amygdalina*), lemon grass (*Cymbopogon citrates*), neem (*Azadirachta indica*) and paw-paw (*Carica papaya*) gave significant reduction of mycelial dry weight of *A. niger* and *A. flavus*.

Effects of the extracts on spore germination

For fungi in general, spore germination is a crucial event in the propagation of the species and for pathogenic fungi in particular, it is a deleting factor in the onset of host colonization (Allen, 1976).

Results in Table (4) show the effect of the different concentrations of the onions and garlic extracts on spore germination of *A. niger* after 18 hours incubation. Garlic extract was highly effective especially at the higher concentration. No spores were germinated at

EDITORIAL

that concentration. Percent germination then increased to about 14.0%, 88.0% and 88.1% at the concentrations 0.1, 0.01 and 0.001, respectively. The three onions extracts on the other hand showed no effect on the spore germination of *A. niger* even at the higher concentration.

Results of the effect of the different concentrations of onions and garlic extracts on the spore germination of *P. digitatum* after 18 hours incubation are shown in Table (5). From the three onions extracts only the yellow onion was showing a remarkable effect on the spore germination of that fungus. Only about 2.0% of the spores were germinated at the higher concentration, then germination increased with dilution. Spore germination of *P. digitatum* was completely inhibited by garlic extracts at the higher concentration, then germination was increasing with dilution (Table 5).

Walker and Lindergreen (1924) found that *A. niger* germinated and grew well in the extracts made from the dry outer scales of the white or colored onion varieties; although, germination and growth of *Botrytis alii*, *Borytis spp.*, *Fusarium lycopersici*, *Fusarium graminearum* and other fungal pathogens were greatly retarded or entirely inhibited when treated with extracts of the dry outer scales of the coloured onion types but not with extracts from the white onion scales.

Table 4. Effect of different concentrations of onions and garlic extracts on spore germination % of *A. niger* (after 18 hours)

Concentrations						
Sample	0.00	0.001	0.01	0.1	1	
Red onion	33.4	75.0	72.5	70.1	70.0	
Yellow onion	31.8	77.9	81.1	85.0	70.3	
White onion	25.2	81.0	80.0	77.7	72.0	

EDITORIAL

Garlic	26.0	88.1	88.0	14.0	0.00
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LSD = 44.58

Table 5. Effect of different concentrations of onions and garlic extracts on spore germination% of *P. digitatum* (after 18 hours)

Concentrations						
	Sample	0.00	0.001	0.01	0.1	1
Red onion	26.1	57.0	56.2	35.0	34.5	
Yellow onion	27.3	58.7	46.6	43.0	2.0	
White onion	30.0	59.4	59.5	29.6	18.8	
Garlic	28.9	37.0	17.0	17.0	000	

LSD = 29.12

Extracts of different plants were found to reduce fungal growth and spore germination. Osman (1986) found that the extracts of fennel were able to inhibit fungal growth of *A. flavus*, while the extracts of karkadi enhanced growth of the same fungus. Many studies were confirming that plant extracts can play an important role in inhibiting fungal growth and aflatoxin production by *A. flavus* (Sharma *et al.*, 1979; Batt *et al.*, 1980; El-Shayeb and Mabrouk, 1984).

EDITORIAL**Antibacterial activity of the extracts**

The results in Tables (6 and 7) show the inhibitory activity of onion and garlic extracts on *S. aureus* and *E. carotovora*, respectively. The three onion extracts have no effect on both bacteria. This is in agreement with Nelson *et al.*, (2007), who reported that the cold water extracts of onion did not inhibit growth of *S. aureus*. However, AL-Delmy and Ali (2006) reported that onion extracts were completely inhibited growth of *S. aureus* at 10^{-6} dilution.

Garlic, extract on the other hand, showed a large inhibition zone toward *S. aureus* at the higher concentration (Table, 6). Chung *et al.* (2003) reported that garlic juice showed bactericidal activity against *S. aureus* at concentrations (0.1 – 2.5% v/w). Nouredine *et al.* (2005) reported that garlic and onions exhibited marked antibacterial activity, with garlic showing the highest inhibition and green onion the lowest. Although staphylococci were reported to possess high resistant ability to antibacterial agents (Kirril and Krivoshein, 1987), garlic was reported to inhibit *S. aureus* growth completely (AL-Delmy and Ali, 2006).

The onions extract were found to have no effect on *E. carotovora* even at the higher concentrations; but the garlic extract was completely inhibited the same bacteria at the higher concentration (Table, 7). There is no report about the antibacterial activity of the onion extract on *E. carotovora*. Dankert, *et al.* (1979) reported that onion juices showed no effect on the gram-negative bacteria, while, Russel (1991), reported that aqueous extracts of garlic and onion were effective against the gram-positive, gram-negative bacteria and fungi.

Table 6. Effects of different concentrations of onions and garlic extracts on *Staphylococcus aureus* (Inhibition zone (cm))

EDITORIAL

Concentrations						
Sample	0.00	0.001	0.01	0.1	1	
Red onion	0.0	0.0	0.0	0.0	0.0	
Yellow onion	0.0	0.0	0.0	0.0	0.0	
White onion	0.0	0.0	0.0	0.0	0.0	
Garlic	0.0	0.0	0.0	0.5	9.0	

Table 7. Effects of different concentrations of onions and garlic extracts on *Erwinia carotovora* (Inhibition zone (cm))

Concentrations						
Sample	0.00	0.001	0.01	0.1	1	
Red onion	0.0	0.0	0.0	0.0	0.0	
Yellow onion	0.0	0.0	0.0	0.0	0.0	
White onion	0.0	0.0	0.0	0.0	0.0	
Garlic	0.0	0.0	0.0	0.0	9.0	

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