Control of Soft Rot of Onion Bulbs Caused by *Pseudomonas gladioli* pv. *alliicola* Hanaa A.H. Armanious

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Eight isolates of bacteria were isolated from onion bulbs exhibiting symptoms of soft rot disease. The cultural, morphological and physiological characteristics of the selected isolates revealed that the isolated bacteria belong to Pseudomonas gladioli pv. alliicola. Pathogenicity tests indicated that inoculation of whole bulbs or their slices (of Giza-20 or Giza-6 mohasan onion cultivars) developed different degrees of soft rot, that ranging from a colourless soft rot to the dark brown discoloration. Chitosan proved the most effective compound in reducing the growth of onion soft rot pathogen, followed by sodium citrate, seaweed extract and finally salicylic acid. All tested compounds reduced the disease incidence (DI%) and disease severity (DS%) of onion bulbs either under natural or artificial inoculation, compared with untreated control treatment. Seaweed extract (at 0.8 and 1.6%, v:v) was the most affective compound, followed by chitosan, at the same concentrations and sodium citrate (1 or 2 mg/l). Seaweed extract (at 1.6%) and chitosan (at 16 mg/l) treatments caused the highest decrease in both DI% and DS% in wounded bulbs stored 30 days after inoculation at room temperature conditions. Moreover, both seaweed extract and chitosan completely protected the sound onion bulbs against bacterial infection until 30 days of storage, either under artificial or natural inoculation. The percentages of dry matter, total sugars, colouring matter and vitamin C in bulbs of the two tested cultivars of onion were significantly decreased due to infection with Pseudomonas gladioli pv. alliicola (isolate Ps4), whereas total phenols concentration was increased in infected bulbs compared to the healthy ones. Results also indicated that dry matter, total sugars, total phenols, colouring matter concentrations were higher in Giza-20 (red cultivar) than in Giza-6 mohasan (white cultivar), either in healthy or infected onion bulbs. Vitamin C concentration was higher in bulbs of healthy cv. Giza-6 mohasan than in cv. Giza-6, whereas bulbs infection led to decrease Vitamin C concentration in both cultivars. The percentage of reduction was more in bulbs of cv. Giza-20 mohasan.

Keywords: Chitosan, onion, *Pseudomonas gladioli* pv. *alliicola,* salicylic acid, seaweed extract, sodium citrate and soft rot.

Onion (Allium cepa L.) is considered as one of the most important commercial vegetable crops in the world. The crop is used in various ways including cooking, pickling and medicinal purposes. It contains some of the most important human nutrients, *i.e.* proteins, carbohydrates, vitamins and minerals. It is rich in free and glycol-phenols which play an important role in protecting human and plants against infected pathogens. Most harvested onions are stored in a low or at room

temperature. Onion is vulnerable to attack by several diseases caused by fungal and bacterial pathogens such as *Aspergillus niger, Botrytis allii* (Abd-Elrazik *et al.*, 1988a), *Fusarium* sp., *Penicillium* sp. (Kem *et al.*, 2001), *Sclerotium cepivorum* (Abd-Elrazik *et al.*, 1988b), *Pseudomonas* spp. (Tesoriero *et al.*, 1982; Saleh, 1991; Wright *et al.*, 1993 and Abd-Alla *et al.*, 2011), *Bacillus amyloliquefaciens* (Hwang *et al.*, 2012) and *Erwinia* spp. (Saleh, 1991 and Lee *et al.*, 2005).

Disease control is largely based on the use of fungicides, bactericides or synthetic chemical compounds toxic to plant invaders, or victors of plant diseases. However, the hazardous effect of these chemicals or their degradation products on the environment and human health strongly necessitate the search for new and harmless means of disease control. Thus, it is necessary to find out some natural methods of induced resistance to protect plants from disease and also to improve the plant vigour. The plants defence response against pathogens can be elicited by numerous external signals. Commonly tested chemical elicitors are salicylic acid, benzothiadiazole, chitosan and so forth which induce production of various defencerelated enzymes in plants (Thakur and Sohal, 2013). Postharvest decay of onion bulbs in Egypt is primarily controlled by synthetic fungicides (Abd-Elrazik et al., 1988a & b and Naffa and Shanoudy, 2007). Chitosan, a polymer of 1, 4-Dglucoseamine derived from crab-shell chitin, was reported as a fungicidal product (Benhamou and Th rigult, 1992) and used to protect other vegetable and fruit crops against several fungal diseases (Hirano and Nagao, 1989; El-Ghaouth et al., 1992; Du et al., 1997; Morsy et al., 1999 and Ragab et al., 2001). Moreover, various studies concerning the usage of Aspirin (salicylic acid) and other antioxidant compounds for controlling pre- and postharvest diseases were reported (Elad, 1992; Walters et al., 1993; Quarles, 2002; Anonymous, 2003 and Kiraly and Hafez, 2007). Seaweeds have been widely used as food for many centuries in Asia (Darcy-Vrillon, 1993), but in western countries they were employed for the production of valuable chemicals. The bulk of the investigations pertaining to bioactive compounds from seaweeds deals with human pathogens; studies related to phytopathogens are being restricted to pathogens of commercial crops such as tobacco (Caccamese et al., 1980), citrus trees (Kulik, 1995) and rice (Sultana et al., 2005). Moreover, Kumar et al. (2008) found that seaweeds collected from the Tamil Nadu coast of India have been shown to possess a number of biological activities against the phytopathogenic bacterium Pseudomonas syringae, causal of leaf spot disease on the medicinal plant Gymnema sylvestre.

The objectives of this study were (i) to isolate and identify the causal organism(s) of soft rot of onion bulbs during storage, (ii) to assess the potential of some natural chemicals for reducing disease severity and (iii) to determine the effect of infection on some biochemical components of the bulbs.

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Materials and Methods

Samples:

During April and May 2013, apparently healthy onion bulbs (cv. Giza-6 mohasan), were collected from markets located at Giza and Minia Governorates and, however, revealed a high level of internal rot when cut.

Isolation of the pathogen:

Diseased onion bulbs were cut longitudinally and small cubes (1-2 mm³) of infected tissue close to the margins of rots were aseptically removed and macerated with a sterile glass rod in 1ml of sterile water in a sterile mortar and left for 15 min. to allow pellets to settle. A loopfull of resulting supernatants was streaked on yeast nutrient agar (YNA) medium (Dye and Kemp, 1977). After incubation at 25°C for 48 h, representative single bacterial colonies were isolated. Pure colonies were obtained by repeating sub-culturing on YNA several times to ensure purity. The obtained isolates were maintained at 4°C in slants.

Pathogenicity test:

Eight bacterial isolates were selected for pathogenicity tests, which were performed using sound and artificially wounded intact bulbs of Giza 20 and Giza-6 mohasan onion cultivars. For each of the tested bacterial isolates, 50 intact bulbs were surface disinfected by dipping in 95% ethanol and air dried; half of bulbs were artificial wounded by using a sterile scalped at midway between the neck and basal part of the bulbs and another half on bulbs were left sound. All disinfected bulbs were dipped into a bacterial suspension containing ca. 10^8 colony forming units per ml (cfu/ml) of 48 h old cultures. Sound and wounded bulbs were dipped in sterile water which served as control. Inoculated bulbs were immediately placed in sterile plastic boxes supplemented with a sterile piece of wetted cotton, each containing 5 bulbs and incubated in the dark at 25°C for 14 days. After incubation, the bulbs were cut in half longitudinally with a sterile knife and the percentages of infection (DI%) and weight of rotted tissues (DS%) were recorded. To confirm Koch's postulates, bacteria were re-isolated from the artificially inoculated diseased tissues on YNA. In addition, for each of the 8 bacterial isoltes,10 sterile, 3 cm thick, transverse and longitudinal sections through leaf bases were placed on sterile wet filter paper in Petri dishes and inoculated in the centre with 2 loopfuls of bacterial suspension (ca. 10⁸ cfu/ml), and incubated at 25°C for a week. Control treatment received only sterile distilled water.

Identification: Characterization of bacterial cultures:

Four isolates, that were highly virulent to whole onion bulbs in pathogenicity tests, were selected for identification. The cultural, morphological and physiological characteristics of the selected isolates (listed in Table 2) were carried out according to the methods outlined by Stapp (1961), Schaad (1980), Fahy and Hayward (1983) and Lelliot and Stead (1987). The cultures characteristics were described from cultures grown at 27°C for 24h on YNA, nutrient sucrose (5%) agar (NSA), potato sucrose agar (PSA), bullion glucose and King's B media. Potato slices were used to study the ability of bacterial isolates to cause soft rot. Their ability to utilize a range of compounds as sole sources of carbon was assessed in mineral base broth (Palleroni and Doudoroff, 1972) in glass test tubes.

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In vitro effect of some chemicals on growth of bacteria:

The effect of three chemical substances, *i.e.* sodium citrate, salicylic acid and chitosan and seaweed extract (a solution packed by UAD Company, Egypt) were tested *in vitro* at three concentrations as shown in Table (3). Liquid basal medium (Palleroni and Doudoroff, 1972) supplemented with the tested substance was used for determination of their effect on the bacterial growth. Stock solutions of these compounds were added to conical flasks (250 ml) each containing 100ml of sterilized basal medium to obtain the desired concentration (Table 3). Media, contained or free from the tested compounds, were inoculated with 1 ml of bacterial suspension (10^6 cfu/ml) of 48 h old culture of Ps4, Ps5. Ps6 or Ps7 isolate and then incubated at 25° C for 72 h. Bacterial growth was measured turbidimetrically using Carl-Zeiss colorimeter at a wave length of 480nm. Growth inhibition percent was calculated according to the following formula:

Growth inhibition (%) =
$$(1 - T/C) \times 100$$

Whereas, T = OD of bacterial growth in treated medium.

C = OD of bacterial growth in untreated (control) medium.

Effect of chemical treatments on soft rot of onion bulbs incidence under storage conditions

Fresh onion bulbs cv. Giza-6 mohasan; the most susceptible cultivar to bacterial soft rot, apparently healthy, free of physical damage and diseases, were used in this study. Bulbs were sterilized as previously described. Each bulb was wounded using sterile scalpel to obtain small scratch. Two concentrations of sodium citrate, salicylic acid (1 and 2 mg/l), chitosan (8 and 16 mg/l) and seaweed extract (0.8 and 1.6 %, v:v) were tested. Wounded onion bulbs were dipped in the tested chemical solution for 5 min. After air dried, onion bulbs either were left to natural infection under store conditions or artificially inoculated by dipping in 10^6 cfu/ml of bacterial (isolate Ps4) suspension for 5 minutes. Also, surface sterilized sound bulbs were dipped either in different concentrations of chemicals under investigation or in sterilized water and served as control treatment. Treated bulbs were air dried in laminar flow for 2 h, and were held at room temperature, $22-28^{\circ}$ C in plastic boxes. Each box (20x10x7 cm) containing 5 bulbs and 5 boxes were used as a replicates. Bulbs were examined weekly, under sterilized conditions, throughout the storage period up to 30 days.

Disease assessment:

At the end of storage period, bulbs were cut in half longitudinally with a sterile knife and the percentages of infection (DI%) and fresh weight of rotted tissues (DS%) in relative to the whole weight of bulb were calculated.

Changes in biochemical constituents of onion bulbs due to infection with soft rot:

Some biochemical constituents; *i.e.* total soluble sugars (reducing- and non-reducing sugars), total phenols, Vitamin C, and dry and colour matters were determined in both healthy and artificially bacterial infected onion bulbs.

Samples:

For biochemical assay, 10 bulbs of either cvs. Giza-20 or Giza-6 mohasan, *i.e.* 5 healthy and 5 artificially inoculated by isolate Ps4 of isolated bacteria as described before, were randomly selected and analyzed in duplicate.

Method of determination:

The dry matter percent was determined by drying the samples at $70\pm 2^{\circ}$ C till a constant weight. The dried samples were ground to pass through a 40 mesh sieve. The sugars were extracted exhaustively as described by Ackerson (1981) by using ethanol 80% at 70°C. Reducing sugars were determined by using the method of Shales and Schales (1945) by using alkaline potassium ferricyanide reagent. Total soluble sugars (TSS) were estimated in the previous alcoholic extract, according to the methods of Dubois *et al.* (1956), after inversion of non-reducing sugars with HCl (2N), 10 ml, added to 10 ml of sugar extract at 60°C for 30 min. After cooling, the inverted sugars were neutralized by NaOH (2N) using phenolphthalein as indicator, and made the volume up to 25 ml with distilled water. Determination of reducing sugars and TSS (in form of total reducing sugars) were identified colorimetrically at 420 nm by using potassium ferricyanide reagent. A standard curve of glucose (10-100 µg) and expressed as mg g⁻¹ dry weight. Non-reducing sugars were calculated by difference.

Extraction of phenolic compounds was conducted according to the method described by Daniel and George (1972) as follows: 1g of dry onion bulbs was macerated in 5-10 ml 80% ethanol for at least 24 hours at 0°C. The alcohol was clarified; the remained residue was re- extracted with 5 -10 ml 80% ethanol 3 times. At the end, the clarified extract was completed to 50 ml using 80% ethanol. The colorimetric method of Folin-Denis reagent as described by Daniel and George (1972) was employed for the chemical determination of phenolic compounds. The optical density was recorded at 725nm, 0.5 ml 80% ethanol and reagents only were used as a blank. The percent of phenolic compounds was expressed as equivalents of pyrogallol (μ g/g) on a dry weight basis.

Colouring matter was determined calorimetrically as the absorbance of 0.2% aqueous extracts of the dehydrated onions at 520 nm. L- ascorbic acid (vitamin C) was extracted and determined according to the method described by Iqbal *et al.* (2010) as follows:

Accurately weight 1g of each sample in a 25 ml conical flask, add 10 ml of oxalic acid (0.05M) solution and the samples were placed under shade for 24 h for extraction of vitamin C contents. The samples were filtered through filter papers. Then 2.5 ml of each sample were transferred to volumetric brown flask, 2.5 ml of oxalic acid (0.05M) solution. Then added separately meta phosphoric acid with acetic acid 05 ml, sulphuric acid (5% v/v) solution 1ml, and ammonium molybdate (5% m/v) solution 2ml in each flask and make up the volume 25 ml with distilled water. Each sample was then analysed for Vitamin C at 760 nm compared with a pure L. ascorbic acid (0.1w/v) solution as a standard.

Statistical analysis:

All data obtained were statistically analyzed using the one way analysis of variance (ANOVA). The ANOVA was performed according to Steel and Torrie (1980) using the General Linear Models Procedure (Anonymous, 2009). Significant treatment differences were evaluated by using Duncan's multiple-range test (Duncan, 1955).

Results

All inoculated whole bulbs or slices of Giza-20 or Giza-6 mohasan onion cultivars developed soft rot, ranging from a colourless soft rot to the dark brown discoloration (Table 1). Data presented in Table (1) show that all isolates of the pathogen under investigation were able to infect both sound and artificial wounded bulbs of the two tested onion cultivars, *i.e.* Giza 20 and Giza-6 mohasan. A severe rotting of the fleshy scales of onion bulbs was produced in wounded bulbs when inoculated with isolates Ps4, Ps5, Ps6 and Ps7, which were considered as highly pathogenic, whereas isolates Ps1, Ps2, Ps3, and Ps8 were weakly pathogenic ones.

Table 1. Disease incidence (DI%) and disease severity (DS%) on sound and wounded onion bulbs of Giza-20 and Giza-6 mohasan cultivars after inoculation with bacterial isolates

	cv. Giza-6 mohasan					cv. Giza-20					
Isolate	DI%		DS%		Colour	DI%		DS%		Colours	
	Wound	Sound	Wound	Sound	Coloui	Wound	Sound	Wound	Sound	Colour	
Ps1	44	8	19.9	2.8	+	24	8	6.4	2.5	+	
Ps2	28	4	12.8	1.5	+	28	8	6.6	1.9	+	
Ps3	72	28	89.1	7.7	+2	32	0	8.0	0.0	-	
Ps4	100	59	54.4	13.6	+3	556	28	33.47	7.3	+2	
Ps5	92	68	65.1	26.6	+3	64	32	25.7	10.8	+2	
Ps6	88	48	64.8	16.1	+3	772	32	54.8	7.3	+2	
Ps7	100	60	77.1	25.2	+3	68	32	50.4	13.6	+2	
Ps8	28	24	11.7	7.23	+	16	12	2.8	3.5	+1	
Control	0	0	0	0	-	0	0	0	0	-	
LSD 5% for:											
A = 0.5			5.5			0.7		4.9			
	B = 0.2			2.1		0.4		3.2			
A x B= 0.7			6	.4		0.3		9.6			

* (-) = Colourless, (+)= Yellowish, (+2)= Browning and (+3)= Dark brown.

Data in Table (1) show also that the two tested cultivars of onion showed different degrees of susceptibility to infection with bacterial isolates. Onion Giza-6 mohasan cultivar was more susceptible to all bacterial isolates than cv. Giza-20. None of the control bulbs developed soft rot symptoms. The slices of onion bulbs, inoculated with any of the eight bacterial isolates, completely rotted within 4 days after inoculation. Bacteria re-isolated from the diseased bulbs were confirmed as having identical characteristics to the tested isolates

The bacterial cells of all four isolates selected for characterization were rodshaped, non-spore forming, motile, Gram negative, grown at 40°C, grown anaerobically, produced soft rot in potato slices and did not hydrolyze starch. After 4 days of growth on King's B medium at 25°C, colonies were round, 1.0 - 2.0 mm in diameter, creamy-yellowish, non-mucoid, wrinkled with rough edges and produced diffusible non-fluorescent yellowish green pigment. All tested isolates grew well at a wide range of temperature and pH (Table 2). They utilized and produced acid from glucose, sucrose, xylose, arabinose (D, L) but not raffinose, asculin, salicin and rhamnose, hydrolyzed gelatine, reduced nitrate to nitrite and were oxidase positive but were arginine-dehydrolase negative. On the basis of morphological, physiological and biochemical properties, it is clear that the four tested bacterial isolates (Table 2) are closely related to Pseudomonas gladioli pv. alliicola, according to Kishun and Swarup (1981); Tesoriero et al. (1982) and Wright et al. (1993). However, in some minor characters recorded from P. gladioli pv. alliicola, such a utilization of Salicin and Rhamnose, and optimum temperature for growth were conflicted, but these are not essential and do not contradict the pathogen being P. gladioli pv. alliicola.

Data presented in Table (3) show that the growth of the tested bacterium; *P. gladioli* pv. *alliicola*, was inhibited by application of all chemicals tested and seaweed extract. Chitosan was the most effective compound in reducing the growth of onion soft rot pathogen, followed by sodium citrate, seaweed extract then salicylic acid. All tested isolates of the bacterial pathogen were affected by using the different concentrations of the tested chemicals and seaweed. The differences between isolates against treatment, in most cases, were insignificant

Data in Table (4) reveal that all tested treatments reduced the percentages of infection (DI%) and disease severity (DS%) of onion bulbs compared with untreated control ones. Seaweed extract (at 0.8 and 1.6 %) seems to be superior treatment in reducing both DI % and DS %. since it recorded 4% and 12%, in naturally and artificially infected bulbs, respectively, when it applied at the concentration of 1.6%. On the other hand, moderate protection was obtained on wounded bulbs of onion dipped in chitosan (8 or 16 mg/l) or sodium citrate (1 or 2 mg/l) since they caused reduction in DI %, up to 87.5% and DS% more than 95% compared with control treatment under natural infection. Data Table (4) show also that wounded bulbs of onion, artificially inoculated with the soft rot pathogen significantly decreased the percentages of both DI and DS when treated with tested treatments. Seaweed extract (at 0.8%) and chitosan (at 8mg/l) treatments caused the highest decrease in both DI% and DS% in bulbs stored 30 days after inoculation under room temperature conditions. No infection was recorded in sound onion bulbs stored under room conditions and natural infection, whereas the bulbs of control were infected (40% DI and 24.4% DS) when dipped in bacterial suspension (106 cfu/ml) for 5 min. Both DI% and DS% were significantly decreased by applications of sodium citrate or salicylic acid compared with control treatment. Both Seaweed extract and chitosan completely protected the sound onion bulbs against bacterial infection up to 30 days of storage, either under artificial or natural inoculation.

Table	2.	. Morphological, physiological and biochemical characteristics	of							
		Pseudomonas gladioli pv. alliicola in comparison with those	of							
		isolated bacteria (isolates Ps4, Ps5, Ps6 and Ps7)								

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	Resu	lts of Ba	cterial is	olates	Reported P. g. alliicola by					
Classication		Ps5		Ps7	Kishun &	Tesoriero	Wright			
Character	Ps4		Ps6		Swarup	et al.	et al.			
					(1981)	(1982)	(1993)			
Size	0.8	-1.2µm	x 0.5-0.7	μm			(1)			
Hanging drop	+	+	+	+	+	+	+			
Motility at 37°C	-	-	-	-	-	?	?			
Opt. growth temp.		30 -	32°C		25-30°C	?	25°C			
Max. growth temp.		40)°C		?	(2)	40°C			
Min. growth temp.		5	°C		?	?	?			
Thermal death point		45 -	50°C		?	?	?			
•	Non-flu	orescen	t, yellow	ish-	Non-fluor	escent, yello	w,			
Pigmentation	green, c	liffusible	e pigmer	it on	diffusible pigment on King's					
-	King's	B mediu	m at 25°	С	B medium					
Potato soft rot	-	±	±	-	-	?	-			
V.P. test	+	+	+	+	?	?	?			
Methyl red	+	+	+	+	?	?	?			
Ammonia production	+	+	+	+	?	?	?			
H2 S production	+	+	-	-	?	?	?			
Indol formation	±	+	±	+	+	?	+			
Nitrate reduction	+	+	+	+	+	?	+			
Catalase production	+	+	+	+	+	?	?			
Asculin hydrolysis	-	-	-	-	-	?	?			
Levan test on NSA	+	+	+	+	+	-	+			
Arginine dehydrolase	-	-	-	-		-	-			
Optimum pH for		<i>(</i> 0			6 - 8					
growth		6 – 8			6 - 8					
T '4	Alk. is cleared without				9	0	0			
Litmus milk	cc	agulatic	n		?	?	?			
Tobacco							1			
hypersensitivity	-	-	-	-	-	-	-			
Utiliz	zation of carbon sources (within 20 days)									
Glucose, Galactose,	+A	+A	+A	+A	+	+	+			
Fructose, Sucrose,	+A	+A	+A	+A	+	+				
mannose, D-						0	9			
arabinose, L-	+A	+A	+A	+A	+	? 9	? 9			
arabinose, Maltose,	+A	+A	+A	+A	2	<i>'</i>	<i>!</i>			
Lactose, Mannitol,	+A	+A	+A	+A	+	+	+			
Xylose, sorbitol	+A	+A	+A	+A	+	+	+			
Raffinose, Asculin,	-	-	-	-	-	?	-			
Glycerol, Sorbitole	+	+	+	+	+	+	?			
Cellobiose	+	+	+	+	+	?	?			
Salicin, Rhamnose	-	-	-	-	+	-	?			

⁽¹⁾ After 4 days on KB medium at 25°C, bacterial colonies were round, 1.0 - 2.0 mm in diameter ⁽²⁾ Produced turbid growth on oxide nutrient broth in 48h at 40°C

? = not tested

+= positive, -=negative and \pm = delayed or weak positive reaction

		Growth inhibition (%) of <i>P. gladioli</i> pv. <i>alliicola</i> *							
Substrate	Concentration	isolates							
		Ps4	Ps5	Ps6	Ps7				
Codimen	1mg/l	49.2**	41.9	55.5	73.0				
socium	2mg/l	90.7	68.0	62.3	71.8				
cittate	3mg/l	93.5	93.6	92.5	90.2				
Caliardia	1mg/l	39.3	42.4	67.1	61.5				
Sancync	2mg/l	65.7	64.5	79.5	74.1				
aciu	3mg/l	85.0	80.8	88.4	81.0				
	4 g/l	78.6	66.9	80.1	85.1				
Chitosan	6 g/l	75.7	84.9	87.0	90.2				
	8 g/l	97.9	94.2	93.2	96.6				
Commond	0.4 %	65.0	64.5	68.5	66.7				
Seaweed	0.6 %	79.3	83.1	73.3	81.6				
extract	0.8 %	87.9	90.1	86.3	84.5				
Control		0.0	0.0	0.0	0.0				
LSD 5% for: Substrates (S)= 0.2		Concentration	rs(C) = 0.1	Isolates $(I)=0$.	1 $SxCxI =$				

Table 3. Effect of different concentrations of some chemicals and seaweed extract on the growth of 4 isolates of onion soft rot bulbs pathogen

0. * Growth inhibition (%) = $(1 - T/C) \times 100$, Whereas T = OD of bacterial growth in treated medium and C = OD of bacterial growth in untreated (control) medium.

** Each value is an average of 3 replicates.

Table 4. Effect of application some chemicals on soft rot of onion (cv. Giza-6 mohasan) bulbs, 30 days after storage

		W	ounded o	Sound healthy onion bulbs					
Treatments	Como	Dis	ease	Dise	ease	Dis	ease	Disease	
	Colic.	incidence (%)		severity (%)		incidence (%)		severity (%)	
		N.in.	A.in.	N.in.	A.in.	N.in.	A.in.	N.in.	A.in.
Sodium	1mg/l	20.0^{*}	44.0	2.2	13.4	0.0	8.0	0.0	1.3
citrate	2mg/l	8.0	24.0	1.0	5.5	0.0	4.0	0.0	0.5
Salicylic	1mg/l	16.0	28.0	2.2	9.4	0.0	12.0	0.0	3.3
acid	2mg/l	12.0	16.0	1.0	5.0	0.0	4.0	0.0	1.3
Chitasan	8mg/l	20.0	24.0	3.0	4.4	0.0	0.0	0.0	0.0
Chitosan	16mg/l	8.0	12.0	1.2	1.9	0.0	0.0	0.0	0.0
Seaweed	0.8%	12.0	24.0	1.6	9.0	0.0	0.0	0.0	0.0
extract	1.6%	4.0	12.0	0.7	1.5	0.0	0.0	0.0	0.0
Control		64.0	100.0	26.7	52.8	0.0	40.0	0.0	24.4
LSD 5% for:									
Treatments (T)		0.4		2.6		0.3		2.8	
Concentrations (C)		0.2		1.2		0.1		0.2	
Inoculation methods		0.2		1.2		0.1		1.2	
(I)		0.7		4.3		0.4		4.0	
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* Each value represents the mean of 5 replicates.

N.in. = Naturally infected bulbs, A.in. = Artificially infected bulbs

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Data in Table (5) indicate that dry matter (%), total soluble sugars (TSS,%), colouring matter (absorbance at 520 nm), and vitamin C (mg/100g dry matter) in bulbs of the two tested cultivars of onion were significantly decreased due to infection with *Pseudomonas gladioli* pv. *alliicola* (isolate Ps4), whereas total phenol concentration was increased in infected bulbs compared with the healthy ones. Results also revealed that dry matter (%), TSS, total phenols, colouring matter concentrations were higher in Giza-20 than in Giza-6 mohasan, either in healthy or in infected onion bulbs. Data presented in Table (5) show also that reduced sugars concentration was higher in infected bulbs, whereas the non-reduced sugars were the highest in non-infected (healthy) bulbs. Vitamin C concentration was higher bulbs of healthy cv. Giza-6 mohasan than in cv. Giza-20, whereas bulbs infection led to decrease vitamin C concentration in both cultivars. The percentage of reduction was more in bulbs of cv. Giza-20 mohasan than in cv. Giza-6.

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Cultivar	Bulbs	Dry matter (%)	TSS (g/100g d. wt.)	Reducing sugars (g/100g d. wt.)	Non- Reducing sugars (g/100g d. wt.)	Total phenols (µg/g d. wt).	Colouring matter	Vitamin C (mg/100g d. wt.)	
Giza-20 (Red)	Infected bulbs	7.3*	3.50	2.50	1.00	4.1	1.34	6.3	
	Healthy bulbs	13.6	5.43	1.93	3.50	3.0	0.26	12.2	
Giza-6 mohasan (White)	Infected bulbs	7.22	3.03	2.03	1.13	2.5	0.9	3.5	
	Healthy bulbs	12.2	4.33	1.57	2.77	2	0.18	16.2	
LSD at 5% for									
Cultivar (A)		3.2	0.91	0.17	0.26	ns	ns	14.95	
Infection (B)		2.65	0.23	0.24	0.07	0.13	0.05	11.89	
A x B		3.74	0.32	0.34	0.10	0.19	0.72	16.82	

Table 5. Changes in chemical compositions of onion bulbs (cvs. Giza 20 and
Giza-6 mohasan) due to infection with Ps4 isolate of P. gladioli pv.
alliicola, the agent pathogen of soft rot

^{*}Each reading is mean of 3 replicates.

Discussion

Bacterial soft rot of onion bulbs was recorded in several parts of the world, *e.g.* in Egypt (Ouf *et al.*, 1985 and Abd-Alla *et al.*, 2011); in India (Kishun and Swarup, 1979); in Australia (Tesoriero *et al.*, 1982 and Cother and Dowling, 1985); in New Zealand (Watson and Hale, 1984; Fullerton *et al.*, 1986 and Wright *et al.*, 1993); in Japan (Sofokawa and Takikawa, 2004) and in Korea (Hwang *et al.*, 2012).

Bulbs of onion (cv. Giza-6 mohasan) appeared externally sound but when cut longitudinally showed creamy to dark-brown discoloration in internal fleshy scales. In severe cases, the rot extended to outer fleshy scales with an associated sour smell.

Isolation from apparently sound tissues adjacent to infected tissue showed symptoms consistently yielded a Pseudomonad producing diffusible yellowish-green non-fluorescent pigment on King's B medium. Eight isolates (Ps1 to Ps8) of bacteria were taken and purified on NGA medium. Moreover, all isolates produced a soft creamy discoloration within two weeks when whole onion bulbs of cvs. Giza-6 mohasan and Giza-20 were dipped in bacterial suspension (10⁸ cfu/ml), or within 4 days when poured over onion slices in a moist Petri dishes. Isolates Ps4, Ps5, Ps6 and Ps7 were highly pathogenic. Again, cv. Giza-6 mohasan was more susceptible than Giza-20. Isolated bacteria were short rods, non-sporing, Gram negative, did not produce a hypersensitive reaction on tobacco. They were oxidase positive and produced Levan from sucrose. They produced rot on potato tuber slices. On the bases of disease symptoms, cultural and biochemical characteristics and pathogenicity tests, the eight bacterial isolates were closely related to Pseudomonas gladioli pv. alliicola (Burkholder, 1942 and Young et al., 1978). Four bacterial pathogens were reported to cause diseases of onions, i.e. P. gladioli pv. alliicola and P. cepacia (Burkholder, 1942) and P. aeruginosa (Cother et al., 1976). Ouf et al. (1985) found a pectolytic Pseudomonad (P. alliicola Starr and Burkh.) commonly associated with soft rotting onion in Egypt. Recently, Sofokawa and Takikawa (2004) and Hwang et al. (2012) reported that Burkholderia cepacia and Bacillus amyloliquefaciens caused rot symptoms of onion in Japan and Korea, respectively. The soft rot symptoms observed in this investigation were identical to those ascribed to P. gladioli pv. alliicola.

The ratio of bacterial growth was highly inhibited *in vitro*, with chitosan, sodium citrate, seaweed extract and salicylic acid applications, respectively. The growth reduction of *P. gladioli* pv. *alliicola* ranged between 97.9 and 80.8% by application the highest concentrations of the tested compounds. Also, in the present study, dipping onion bulbs in sodium citrate, salicylic acid (1 or 2mg/l), chitosan or seaweed extract (0.8 or 1.6%) solutions reduced the percentages of onion bulbs. The highest reduction percentages in wounded artificially inoculated bulbs were observed with seaweed extract and chitosan (1.6%) applications (DI reduced with 88% and DS reduced the percentages of DI to 24 and 16%, and DS to 77 and 74%, respectively.

Sound onion bulbs, treated with either chitosan or seaweed extract (0.8 or 1.6%) showed complete protection up to 30 days of storage at room temperature against soft rot infection. Whereas the disease incidence reduced by 90% and DS reduced by 97% in sound bulbs when dipped into sodium citrate or salicylic acid then in bacterial suspension (10^6 cfu/ml) comparing with control. Wounded onion bulbs exposed to natural infection were protected against soft rot symptoms up to 30 days storage, when they were treated with either seaweed extract or chitosan (1.6%) which reduced both DI by 93.7 and 87.5% and DS% by 97.7 and 97.2%, respectively. Chitosan was reported as a by-product extracted from seafood industry and considered as one of safe products and it has anti-fungal effect against several fungi (Hirano *et al.*, 1990). The activity of chitosan may be related to its ability to interfere with the plasma membrane function (Leuba and Stossel, 1986) or the

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interaction of chitosan with fungal DNA and RNA and its relation with pathogenesis related protein (PR) genes which regarded as the genes that functionally develop disease resistance (Hadwiger, 1999). On the other hand, using of chitosan as fruit coating decreased postharvest decay of several plants (Hirano and Nagao, 1989; El-Ghaouth et al., 1992 and El-Mougy et al., 2002). Also, the extract of seaweed (SWE) contains all major and minor plant nutrients, and all trace elements, alginic acid, vitamins, auxins at least two gibberellins and antibiotics (Kulik, 1995). Because many algae produce a large number of antibacterial and antifungal materials, are almost never a threat to the environment, and many can be grown in quantity in mass culture, they are suitable candidates for exploitation as biocontrol agents of plant, this author reported that extracts from seaweeds (macroalgae) sprayed on plants reduced the incidence of Botrytis cinerea (gray mould) on strawberries, Erysiphe polygoni (powdery mildew) on turnips, and damping-off of tomato seedlings. Jayaraj et al. (2008) found that carrot plants sprayed with an extract (2%) of the seaweed Ascophyllum nodosum significantly reduced diseases caused by Alternaria radicina and Botrytis cinerea severity at 10 and 25 days after inoculation compared to control plants sprayed with water. They also found that activity of certain defence related enzymes, including peroxidase, polyphenoloxidase, phenylalanine ammonia lyase, chitinase, and -1,3 glucanase were significantly increased in plants treated with SWE and salicylic acid (SA) compared to the control. The results in this study showed an agreement with that found by Jayaraj et al. (2008) who reported that SWE was more effective than SA (100mM) in reducing infection.

The nutritive value, pungency and the quantity of lachrymatory factor, which is related to the tear factor (Bandyopadhyay and Tewari, 1973), of onion bulbs vary in different cultivars and genotypes. The white onion varieties with high dry matter content and pungency are considered suitable for dehydration purposes (Valdia and Holle, 1971), while the varieties with high contents of dry matter and non-reducing sugars and low protein contents are best for storage purposes (Toul and Pospisilova, 1966). According to these specifications, the tested cultivars (Giza-20 and Giza-6 mohasan) should be considered suitable for storage purposes. Phenolic compounds are believed to be responsible for resistance against phytopathogenic pathogens. Phenol concentration was higher in Giza-20 than in Giza-6 mohasan. Infection with P. gladioli pv. alliicola related with increase the total phenolic concentration, so cv. Giza-20 showed more resistance against the pathogen than cv. Giza-6 mohasan. These results are in agreement with Bajaj et al. (1979). Coloury matter in onion scales may be formed by both enzymatic and non-enzymatic oxidation of orthodihydroxy phenolic compounds such as quercetin and protocatechuic acid (Bajaj et al., 1980). These are present in higher amounts in red onion varieties than in the white varieties (Slimestad et al., 2007). High colouring matter in cv. Giza-20 is correlated with high phenolic concentration, whereas, a low value of colouring matter is correlated with low phenolic concentration which was found in cv. Giza-6 mohasan. The amounts of Vitamin C were significantly decreased in infected bulbs of onion in comparison with control (healthy one). The percent of decreasing ranged between 48.4% for cv. Giza-20 and 78.4% for cv. Giza-6 mohasan. These results are in harmony with those found by Dubey (1995) and Amusa et al. (2005).

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مقاومة مرض العفن الطرى البكتيرى Pseudomonas gladioli pv. alliicola

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جمعت عينات من البصل المصاب بمرض العفن الطرى الداخلى من المخازن بمحافظتى المنيا والجيزة، تم عزل ميكروب بكتيرى منها بصورة نقية وطبقت فروض كوخ لإثبات العلاقة بين المسبب المرضى المعزول وحدوث المرض.

عزل ثمانية عزلات من الميكروب متشابهة في الصفات المورفولوجية والمزرعية وبتعريفها وجدت أنها تنتمى إلى الميكروب .*Pseudomonas gladioli* pv صنفى *alliicola*. بتت الدراسه قدرة الميكروب على احداث المرض في صنفى جيزه- و جيزه - مُحَسن، سواء في الأبصال الكاملة المجروحة صناعيا أوفى شرائح البصل التي كانت فيها الإصابة أسرع وأشد. كما تم اختبار تأثير بعض الكيماويات ومستخلص الأعشاب البحرية وثبت من الدراسة أن النمو الميكروبي على البيئة الصناعية قد تأثر من جميع

تثبيط للنمو الميكروبي ظهرت عند آختبار مركب الشيتوزان، ولم تظهرفروق معنوية في نسبة تثبط النمو بين العزلات المختبرة. وفي نفس الوقت أظهرت جميع

المحقونة صناعيا والمخزنة على درجة حرارة الغرفة بالمقارنة بالأبصال غير المعاملة بالكيماويات أو مستخلص الأعشاب البحرية.

شدة إصابة مع استخدام مستخلص الأعشاب البحرية يليه مركب الشيتوزان ديوم.

تأثر المحتوي الكيميائى للأبصال نتيجة الإصابة البكنيرية بشكل واضح حيث نقص بدرجات معنوية كل من النسبة المئوية للمادة الجافة وتركيز السكريات الذائبة والمادة الملونة وكذلك فيتامين C فى الأبصال المصابة بالبكتريا، بينما زاد فيها تركيز الفينولات الكلية. ضح من الدراسة أن نسبة المادة الجافة والسكريات الكلية وتركيز الفينولات والمادة الملونة كانت عموما أعلى فى أبصال الصنف جيزه. () عن الصنف أبيض الأبصال (الصنف جيزه.

سواء السليمة منها أو المصابة. تركيز فيتامين C في أبصال الصنف جيزه. السليمة كان أعلى مما في الصنف جيزه

تركيز فيتامينC في أبصال كلا الصنفين وقد كانت نسبة النقص أ الصنف جيزه -