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مكافحة مرضى العفن الأسود وعفن الرقبة فى البصل باستخدام بعض المستحثات الكيميائية والعلاج التجفيفى

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الملخص العربى :

أدى رش نباتات البصل صنف جيزة ٢٠ تحت ظروف الحقل مرة واحدة قبل ٤٠ يوم من الحصاد أو مرتين قبل ٤٠ ، ٢٠ يوم من الحصاد بحمض الساليسليك وحمض الأكساليك أو فوسفات البوتاسيوم أحادية الهيدروجين كمركبات كيماوية مستحثة إلى مكافحة مرضى العفن الأسود وعفن الرقبة اللذان يُهاجمان البصل تحت ظروف التخزين وكان حمض الساليسليك بتركيز ٨ ملليمول هو الأفضل فى مكافحة المرضين حيث كان تأثيره قريباً من تأثير مبيد ريدوميل الفطرى فى مقاومة المرضين .وقد أدى أيضاً رش نباتات البصل صنف جيزة ٢٠ مرتين قبل الحصاد بكيماويات الاستحثاث المختبرة إلى تحسين القدرة التخزينية للبصل مقارنةً بالغير معامل أو المعامل برشة واحدة . فضلاً عن ذلك ، فقد أدت المعالجة التجفيفية للبصل لمدة ١ – ٣ أسابيع بعد الحصاد مباشرةً إلى نقص معنوى فى حدوث كلا المرضين مع أفضلية فى التأثير بزيادة مدة التجفيف . وقد لوحظ أن المعاملة بالمستحثات أو المبيد الفطرى ريدوميل فى الحقل قد أدت إلى نقص كبير فى السكريات الكلية الذائبة فى الأبصال . ومن ناحيةٍ أخرى لوحظت زيادة كبيرة فى النشاط الإنزيمى لإنزيمات بيروأكسيديز ، بولى فينول أكسيديز ، شيتينيز كاستجابة للمعاملات المختلفة .

CONTROL OF ONION BLACK MOULD AND NECK-ROT DISEASES USING SOME CHEMICAL INDUCERS AND CURING PERIOD

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ABSTRACT: *Spraying onion plants cv. Giza 20 under field conditions either 40 or 40 and 20 days before harvesting individually with salicylic acid (SA), Oxalic acid (OA) and Potassium monohydrogen phosphate as chemical inducers with different concentrations controlled effectively the storage diseases i.e., black mould and neck rot attacking onion bulbs. SA with 8 mM was the best for controlling both storage diseases on onion bulbs, where its effect was nearly equal to the effect of Ridomil fungicide. Also, spraying onion plants cv. Giza 20 with the tested chemical inducers as twice applications significantly improved the store-ability of onion bulbs. Moreover, curing onion bulbs for 1 – 3 weeks directly after harvesting, significantly decreased the incidence of both diseases with superiority of increasing the curing period. High significant reduction of total soluble sugars (TSS) was observed in response to the application of the inducers and / or Ridomil. On the other hand, peroxidase, polyphenol oxidase and chitinase activities were more higher in the treated plant bulbs than those of nontreated control.*

Key words: *Black mould, neck-rot, Aspergillus niger, Botrytis allii, chemical inducers, curing, sugars and enzymes.*

INTRODUCTION

Onion "*Allium cepa* L." is very important vegetable winter crop in Egypt which cultivated for both local consumption and exportation. Early harvesting of onion yearly in March is a good time for exportation to markets of Europe and Arab countries. The cultivated area of this crop reached 115,295 feddan which produced 1,504,600 tons in 2011 (Ministry of Agriculture Report, Economic and Statistics Department, 2010).

Black mould caused by *Aspergillus niger* and neck-rot caused by *Botrytis allii* are the most destructive diseases affecting onion bulbs during storage, marketing and transportation (Hayden and Moude, 1992, Martinez and Granda, 1993; Sharaf El-Din Azza, 2000 and Sharaf El-Din Azza *et al.,* 2007).

Antioxidant toxicity against different pathogens had been reported by Galal and Abdu (1996) and Abd El-Megid *et al.* (2004).

Magro (1984) reported that, *Botritis allii* increased peroxidase activity as a result of releasing pre-existing peroxidase. Tanaka (1991) found that germination of *A. niger* spores was stimulated by onion bulb sap. He also mentioned that infection increased the concentrations of oxalic, citric and lactic acids in onions. McLusky *et al.* (1999) indicated that *Botritis allii* infection was associated with early increases in peroxidase activity and sugar compounds. Nawar and Kuti (2003) found that infection of *Vicia faba* leaves with *B. fabae* increased peroxidase activities both in resistant and susceptible cultivars.

Ali *et al.* (1994) found that growth regulators and ascorbic acid combination showed great reduction of onion neck-rot disease. However, Hussein *et al.* (2007) reported that chemical inducers and potassium phosphate treatments resulted in significant reduction in stemphylium blight of onion. Saleh, Wagida *et al.* (2008) reported that foliage treatments of onion cv. Giza 20 with different inducer resistant compounds and / or Topsin-M significantly decreased the storage bulb diseases.

Curing onion bulbs before storage

resulted in reduction of different diseases as reported by El-Shabrawy *et al.* (1987), Rajapakse *et al.* (1992), Tyson *et al.* (2002) and Lars *et al.* (2006).

This study aimed to find out some safe field and post-harvest treatments which could be apply in controlling the infection with some storage diseases i.e., black mould and neck rot on onions. Also, studying the effect of these tested safe compounds on some chemical changes in stored onion bulbs.

MATERIALS AND METHODS

The present investigation was carried out at Sers El-Layan Experimental Station during 2009 and 2010 growing seasons. Seeds of onion cv. Giza 20 were cultivated in nursery at the middle of September with complete care up to 60 days. Onion seedlings were transplanted in 20th of December in plots of 3 × 3.5 m (1 / 400 feddan) as complete randomize block design with three replicates. The recommended agricultural practices were applied all over growth periods.

I. Effect of chemical inducers:

The concentrations of 2, 4 and 8 mM of either Salicylic acid or Oxalic acid were individually tested. While those of Potassium monohydrogen phosphate were 5, 10 and 15 mM. Grown onion plants were pre-sprayed with each compound 40 days before harvesting. Some other plots were sprayed again 20 days before harvesting. Ridomil fungicide (2.5 g / l) was also used at the same dates, as comparative treatment. Nontreated control plots were sprayed with water. The harvested bulbs (without topping) were separately stored at room temperature and examined every two months for the incidence of both black mould and neck-rot diseases.

II. Chemical analysis:

Just after harvesting, onion bulbs were used for the following determinations:

1. Total soluble sugars (TSS):

Fifty ml of 95% ethanol were added to 0.2 g dry weight of bulb tissue in test tube and kept for 3 days in freezing conditions. Each sample was then filtered through filter paper (Watman No. 1). One ml of the filterate received 1 ml of 5% phenol and 5 ml of concentric sulfuric acid, shacked for 2 m and left for cooling before measuring at 490 nm using Spectrophotometer apparatus (Michel *et al.,* 1956).

2. Peroxidase assay:

Peroxidase activity was estimated according to the method of Allam and Hollis (1972) by measuring the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 at 425 nm. The sample cuvette contained 0.5 ml of 0.1 M potassium phosphate buffer (pH 7), 0.1 ml enzyme extract, 0.3 ml of pyrogallol and 0.1 ml H_2O_2 brought to 3 ml with distilled water.

3. Polyphenol oxidase assay:

The activity of polyphenol oxidase was measured using colorimetric method described by Maxwell and Bateman (1967). The reaction mixture contained 0.2 ml enzyme extract, 0.5 ml sodium phosphate buffer (pH 7) and 0.5 ml of catechol and brought to final volume of 3.0 ml with distilled water. The activity of polyphenol oxidase was expressed as the change in absorbance / ml of the extract per minute at 495 nm.

4. Chitinase assay:

Chitinase activity was determined using spectrophotometer according to Maxwell and Bateman (1967). The sample cuvette contained 0.5 ml of 0.2 M sodium phosphate buffer (pH 7.6), 0.3 ml of 0.5% H_2O_2, 0.4 ml tissue extract and brought to final volume of 3.0 ml with distilled water. Results were expressed as the change in absorbance / ml of the extract per minute at 240 nm.

III. Effect of curing period:

Onion cv. Giza 20 was grown in plots (1 / 400 f.) during 2009 and 2010 seasons and subjected to the recommended cultural practices. After full ripening, random samples (120 bulbs / each replicate) have been taken, where control samples (4 replicates) were stored without topping; just after harvesting. However, curing periods were 1, 2 and 3

weeks where the bulbs were left in the open conditions before storage at room temperature. Onion bulbs were examined, after 4 months, for the incidence of either black mould and / or neck-rot diseases.

All the obtained data were statistically analyzed according to the analysis of variance "ANOVA" using statistical analysis system (SAS, 1996).

RESULTS AND DISCUSSION

Under field and natural infection conditions; onion cv. Giza 20 plants were treated once or twice with any chemical inducers and / or Ridomil (40 and / or 40 & 20 days before harvesting time). The harvested bulbs and those of untreated control plants were stored at room temperature and examined every two months for the incidence of black and / or neck-rot diseases.

Results in Table (1) clear that an application of Salicylic acid at the concentrations of 4 and 8 mM significantly reduced the incidence of black mould infection than control when determined two months after storage; both at 2009 and 2010. The same result was achieved with the application of Oxalic acid, but at the concentration of 8 mM only. Meanwhile, an application of potassium phosphate at the concentrations of 10 and 15 mM insignificantly decreased the disease incidence than control. On the other hand, twice applications showed much better results of all tested compounds. However, Ridomil application (s) gave better results of disease reduction than the tested inducers.

Four months storage after harvesting increased the percentage of infection with black mould disease on both treated and nontreated samples. Also, all tested concentrations of SA and OA significantly decreased the infection comparing to control. Potassium phosphate also gave the same result when applied at the concentrations of 10 and 15 mM. The best results could be noticed with SA applications during the growing seasons 2009 and 2010 as clear in Table (2).

The same trend was observed after storage for six months, but the highest increase of infection was recorded in the nontreated control plant bulbs with significant variation with all tested compounds at all concentrations (Table 3).

Table (1). Effect of chemical inducers and Ridomil gold plus, fungicide on black mould infection (%), two months after bulbs storage.

Treatments	Tested concentrations	2009 season		2010 season	
		I*	II**	I*	II**
Salicylic acid	2 mM	23.33	20.00	26.67	23.33
	4 mM	13.33	10.00	16.67	13.33
	8 mM	6.67	3.33	10.00	6.67
Oxylic acid	2 mM	23.33	20.00	30.00	26.67
	4 mM	16.67	13.33	20.00	16.67
	8 mM	13.33	10.00	16.67	13.33
Potassium monohydrogen phosphate (K_2 HPO_4)	5 mM	26.67	26.67	33.33	26.67
	10 mM	23.33	23.33	26.67	20.00
	15 mM	20.00	16.67	20.00	16.67
Ridomil	2.5 g/litre	10.00	6.67	13.33	10.00
Control		30.00	26.67	33.33	30.00

L.S.D.	at 5%	12.15	13.83	16.93	14.44

I*: Plants were treated once in the field, 40 days before harvesting.
II**: Plants were treated twice in the field, 40 and 20 days before harvesting.

Table (2). Effect of chemical inducers and Ridomil gold plus, fungicide on black mould infection (%), four months after bulbs storage.

Treatments	Tested concentrations	2009 season		2010 season	
		I*	II**	I*	II**
Salicylic acid	2 mM	20.00	16.67	23.33	20.00
	4 mM	13.33	13.33	16.67	10.00
	8 mM	10.00	6.67	10.00	6.67
Oxylic acid	2 mM	33.33	26.67	40.00	30.00
	4 mM	20.00	16.67	23.33	20.00
	8 mM	16.67	13.33	16.67	13.33
Potassium monohydrogen phosphate (K_2 HPO_4)	5 mM	40.00	33.33	43.33	36.67
	10 mM	23.33	23.33	23.33	23.33
	15 mM	20.00	16.67	20.00	16.67
Ridomil	2.5 g/litre	6.67	6.67	6.67	6.67
Control		50.00	50.00	53.33	53.33
L.S.D.	at 5%	12.85	16.93	13.51	17.69

I*: Plants were treated once in the field, 40 days before harvesting.
II**: Plants were treated twice in the field, 40 and 20 days before harvesting.

Table (3). Effect of chemical inducers and Ridomil gold plus, fungicide on black mould infection (%), six months after bulbs storage.

Treatments	Tested concentrations	2009 season		2010 season	
		I*	II**	I*	II**
Salicylic acid	2 mM	26.67	20.00	33.33	23.33
	4 mM	16.67	13.33	20.00	16.67
	8 mM	10.00	10.00	13.33	10.00
Oxylic acid	2 mM	26.67	23.33	30.00	26.67
	4 mM	20.00	16.67	23.33	16.67
	8 mM	16.67	13.33	16.67	13.33
Potassium monohydrogen phosphate (K_2 HPO_4)	5 mM	33.33	30.00	36.67	33.33
	10 mM	23.33	20.00	26.67	23.33
	15 mM	20.00	16.67	16.67	16.67
Ridomil	2.5 g/litre	10.00	10.00	10.00	10.00
Control		60.00	60.00	63.33	63.33

L.S.D.	at 5%	11.42	16.67	18.41	14.74

I*: Plants were treated once in the field, 40 days before harvesting.
II**: Plants were treated twice in the field, 40 and 20 days before harvesting.

The above observations were also noticed by Ali *et al.* (1994), Hussein *et al.* (2007) and Saleh, Wagida *et al.* (2008). However, antioxidant toxicity against different pathogens had been reported by Galal and Abdu (1996) and Abd El-Megid *et al.* (2004).

Results illustrated in Tables (4, 5 and 6) indicate that the infection with *B. allii* was increased gradually by increasing storage period (2, 4 and 6 months) or both treated and nontreated onion plants. However, different treatments of the used chemical inducers decreased neck-rot disease incidence than control insignificantly in most cases; after two months of storage. Only the highest concentration of SA (8 mM) showed significant variation compared with control during 2009 and 2010 seasons (Table 4). After four and six months; neck-rot disease infection was greatly increased in the nontreated control plant bulbs; and so significant variations was noticed between them and those treated with the inducers. Generally; application of Ridomil followed by double treatments with the inducers resulted the least infection rate with *B. allii*. However, onion storage diseases reduction by different inducers and / or fungicides were also obtained by Ali *et al.* (1994), Hussein *et al.* (2007) and Saleh, Wagida *et al.* (2008).

Data in Table (7) clear that total soluble sugars (TSS) were significantly decreased than control in response to separate application of Salicylic acid, Oxalic acid, Potassium phosphate and / or Ridomil to the plants in the field. The best action was noticed when SA was applied followed by Potassium monohydrogen phosphate which was nearly equal to Ridomil influence. In comparison to control, TSS were 0.018, 0.250, 0.150 and 0.148%, respectively with SA, OA, PP and Ridomil. It is clear that such reductions of TSS are unfavourable to rot pathogens infection (Ammar, 2003).

Table (4). Effect of chemical inducers and Ridomil gold plus, fungicide on neck rot infection (%), two months after bulbs storage.

Treatments	Tested concentrations	2009 season		2010 season	
		I*	II**	I*	II**
Salicylic acid	2 mM	16.67	13.33	20.00	16.67
	4 mM	13.33	10.00	13.33	10.00
	8 mM	10.00	6.67	10.00	6.67
Oxylic acid	2 mM	20.00	16.67	26.67	23.33
	4 mM	16.67	13.33	20.00	16.67
	8 mM	13.33	10.00	16.67	13.33
Potassium monohydrogen phosphate (K_2 HPO_4)	5 mM	23.33	20.00	33.33	30.00
	10 mM	20.00	16.67	23.33	20.00
	15 mM	16.67	13.33	20.33	16.67
Ridomil	2.5 g/litre	6.67	6.67	6.67	6.67
Control		26.33	26.67	30.00	30.00

L.S.D.	at 5%	14.14	15.87	13.83	12.15

I*: Plants were treated once in the field, 40 days before harvesting.
II**: Plants were treated twice in the field, 40 and 20 days before harvesting.

Table (5). Effect of chemical inducers and Ridomil gold plus, fungicide on neck rot infection (%), four months after bulbs storage.

Treatments	Tested concentrations	2009 season		2010 season	
		I*	II**	I*	II**
Salicylic acid	2 mM	20.00	16.67	23.33	20.00
	4 mM	13.33	10.00	16.67	13.33
	8 mM	10.00	6.67	13.33	10.00
Oxylic acid	2 mM	23.33	20.00	23.33	20.00
	4 mM	20.00	16.67	20.00	16.67
	8 mM	16.67	13.33	16.67	13.33
Potassium monohydrogen phosphate (K_2 HPO_4)	5 mM	26.67	23.33	23.33	23.33
	10 mM	23.33	20.00	20.00	16.67
	15 mM	20.00	16.67	20.00	16.67
Ridomil	2.5 g/litre	6.67	6.67	6.67	6.67
Control		40.00	40.00	43.33	43.33
L.S.D.	at 5%	10.63	15.60	14.74	17.44

I*: Plants were treated once in the field, 40 days before harvesting.
II**: Plants were treated twice in the field, 40 and 20 days before harvesting.

Table (6). Effect of chemical inducers and Ridomil gold plus, fungicide on neck rot infection (%), six months after bulbs storage.

Treatments	Tested concentrations	2009 season		2010 season	
		I*	II**	I*	II**
Salicylic acid	2 mM	26.67	20.00	23.33	20.00
	4 mM	20.00	13.33	20.00	16.67
	8 mM	16.67	10.00	16.67	10.00
Oxylic acid	2 mM	33.33	23.33	26.67	23.33
	4 mM	23.33	16.67	23.33	20.00
	8 mM	20.00	13.33	20.00	13.33
Potassium monohydrogen phosphate (K_2 HPO_4)	5 mM	36.67	30.00	30.00	26.67
	10 mM	26.33	20.00	23.33	20.00
	15 mM	23.67	16.67	20.00	16.67

Ridomil	2.5 g/litre	10.00	10.00	10.00	10.00
Control		43.33	43.33	50.00	50.00
L.S.D.	at 5%	13.83	17.44	13.18	11.90

I*: Plants were treated once in the field, 40 days before harvesting.
II**: Plants were treated twice in the field, 40 and 20 days before harvesting.

Table 7

On the other hand, individual application of the chemical inducers and / or Ridomil increased the activity of peroxidase, polyphenol oxidase and chitinase enzymes in onion bulbs. In general, Ridomil application gave the highest increment of all determined enzymes. Peroxidase activity was 150, 139, 128 and 116%, compared to control, when Ridomil, SA, DA and / or PP were individually applied to onion plants in the field. In the same respect, PPO activity was 224, 218, 209 and 130%, in comparison with control. Chitinase activity was so high where it reached 216, 213, 201 and 169%; with the above mentioned respect; compared to the untreated control plants.

Such results clear that increasing the oxidative enzymes (PO and PPO), in response to the chemical inducer application, increase respiratory rate and sugar dissimilation which consequently reduce the infection with rot diseases (Ammar, 2003). In the meantime, the high chitinase activity could be a good factor for analysis of the pathogen(s) cell wall. These results were also observed by Magro (1984), Tanaka (1991), McLusky *et al.* (1999) and Nawar and Kuti (2003).

Results in Table (8) indicate that curing onion bulbs before storing significantly decreased the percentage of infection with both *A. niger* and *B. allii* compared with control four months after storage. It was also noticed that increasing the period of curing (1, 2 and / or 3 weeks) significantly decreased percentage of infection with either pathogens. The obtained results of two successive years (2009 and 2010) clear that curing onion bulbs after harvesting minimized rot diseases significantly which could be attributed to the reduction of bulbs water content which consequently become unfavourable to rot disease pathogens. Such results were also obtained by El-Shabrawy *et al.* (1987), Rajapakse *et al.* (1992), Tyson *et al.* (2002) and Lars *et al.* (2006).

Table (8). Effect of curing period on onion black mould and neck-rot diseases incidence (%) after four months of storage.

Curing period after harvesting	Black mould disease incidence (%)		Neck-rot disease incidence (%)	
	2009 season	2010 season	2009 season	2010 season
A week	59.00	50.00	47.00	45.00
Two weeks	36.00	33.00	22.00	20.00
Three weeks	18.00	17.00	13.00	10.00
Control (stored without curing)	73.00	74.00	62.00	61.00
L.S.D. at 5%	7.69	6.36	5.34	4.23

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مكافحة مرضى العفن الأسود وعفن الرقبة فى البصل باستخدام بعض المستحثات الكيميائية والعلاج التجفيفى

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الملخص العربى :

أدى رش نباتات البصل صنف جيزة ٢٠ تحت ظروف الحقل مرة واحدة قبل ٤٠ يوم من الحصاد أو مرتين قبل ٤٠ ، ٢٠ يوم من الحصاد بحمض الساليسليك وحمض الأكساليك أو فوسفات البوتاسيوم أحادية الهيدروجين كمركبات كيماوية مستحثة إلى مكافحة مرضى العفن الأسود وعفن الرقبة اللذان يُهاجمان البصل تحت ظروف التخزين وكان حمض الساليسليك بتركيز ٨ ملليمول هو الأفضل فى مكافحة المرضين حيث كان تأثيره قريباً من تأثير مبيد ريدوميل الفطرى فى مقاومة المرضين .وقد أدى أيضاً رش نباتات البصل صنف جيزة ٢٠ مرتين قبل الحصاد بكيماويات الاستحثاث المختبرة إلى تحسين القدرة التخزينية للبصل مقارنةً بالغير معامل أو المعامل برشة واحدة . فضلاً عن ذلك ، فقد أدت المعالجة التجفيفية للبصل لمدة ١ – ٣ أسابيع بعد الحصاد مباشرةً إلى نقص معنوى فى حدوث كلا المرضين مع أفضلية فى التأثير بزيادة مدة التجفيف . وقد لوحظ أن المعاملة بالمستحثات أو المبيد الفطرى ريدوميل فى الحقل قد أدت إلى نقص كبير فى السكريات الكلية الذائبة فى الأبصال . ومن ناحيةٍ أخرى لوحظت زيادة كبيرة فى النشاط الإنزيمى لإنزيمات بيروأكسيديز ، بولى فينول أكسيديز ، شيتينيز كاستجابة للمعاملات المختلفة .

Table (7). Effect of some chemical ind ucers and Ridomil fungicide on total soluble sugar content (TSS) and enzymes activity of onion bulbs cv. Giza 20.

Treatment	Con.	TSS (mg/100 g d.w.)	% to control	Peroxidase activity / min	% to control	Polyphenol-oxidase activity / min	% to control	Chitinase activity / min	% to control
Salicylic acid	8 mM	0.44	0.018	3.25	139	2.18	218	6.18	213
Oxylic acid	8 mM	5.92	0.250	3.00	128	2.09	209	5.82	201
Potassium monohydrogen phosphate (K_2 HPO_4)	15 mM	3.70	0.150	2.70	116	1.30	130	4.90	1.69
Ridomil	2.5 g/litre	3.64	0.148	3.50	150	2.24	224	6.27	216
Control	-	23.30		2.33		1.00		2.89	