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FIELD TREATMENTS WITH ANTIOXIDANTS, ESSENTIAL OILS AND TOPSIN-M TO CONTROL SOME STORAGE DISEASES OF ONION

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ABSTRACT: ***Transplants and foliage treatments with Tartaric acid, Citric acid, Anise oil, Clove oil, Eucalyptus oil and Topsin-M, to Giza 20 onion plants, significantly decreased the storage bulb diseases. Black mould (Aspergillus niger) was sharply affected by Clove oil, Anise oil (3000 ppm) and Topsin-M (2.5 g/L); 2-6 months storage. In this respect; twice foliage applications were more effective than the single ones. Basal rot diseased (Fusarium oxysporum f.sp. cepae) was significantly less than control; in response to all tested compounds. Topsin-M and Eucalyptus oil gave the best results. Neck rot disease caused by (Botrytis allii) was only noticed after six months of storage. The best results of disease reduction were recorded when Clove & Eucalyptus oils and Topsin-M were twice applied to onion foliage in the field. Generally, tested antioxidants and essential oils decreased activity of the oxidative enzymes; compared with control. The concentration of phenolic compounds were also lesser than those of control, in response to different treatments.***

Key words: ***Antioxidants, Essential plant oils, Onion storage rots, Oxidative enzymes, Phenolic compounds.***

INTRODUCTION

Onion (*Allium cepa* L.) is a very important crop in Egypt. Onion bulbs suffer from many post-harvest diseases during marketing, transportation and storage. Of those black mould (*Aspergillus niger*), neck rot (*Botrytis allii*) and basal rot (*Fusarium oxysporum* f.sp. *cepae*) are dominant (Hayden and Moude, 1992; Martinez and Granda, 1993 and Sharaf El-Din-Azza, 2000 and 2007).

Antioxidants toxicity toward several pathogens has been reported as indicated by Elad (1992), Galal and Abdu (1996) and Abd-El-Megid *et al.* (2004).

Recently, several publications concerned with plant volatile oils as a safe method to control variable diseases (Singh and Dwivedi, 1987; Singh and

Gupta, 1992; Raju and Naik, 2007; Sharaf El-Din-Azza *et al.* 2007; Rizk-Islam, 2008 and Saleh-Wagida, 2008.

Magro (1984) reported that *B. allii* treatment had no effect on the phenol content, but increased peroxidase activities as a result of release of pre-existing peroxidase.

McLysky *et al.* (1999) observed that granular deposits of reaction material (RM) were formed in onion epidermal cells at sites of attempted penetration by *Botrytis allii*. They also recorded the accumulation of feruloy 1-3-methoxytyramine (FMT) and feruloyltyramine (FT) within challenged tissues. Additional phenolics increasing at infection sites were identified as coumaroyl glucose, coumaroyltyramine (CT) and 2-hydroxy-2-(4-hydroxyphensl) ethylferulate. They also mentioned that formation of RM was associated with early increases in peroxidase activity detected by histochemistry at reaction sites and striking polarization of action microfilaments. No antifungal activity was detected in FMT, FT or CT, neverthess it is proposed that the phenolics have a key role in resistance by preventing fungal degradation of the cell wall.

This study was conducted to evaluate the role of field trials with two antioxidants (Citric acid and Tartaric acid), three plant oils (Anise, Clove and Eucalyptus) and a fungicide (Topsin-M) for controlling onion black mould, basal-rot and neck-rot diseases. The oxidative enzymes and relative concentration of phenolic compounds; in response to different treatments; were also determined.

MATERIALS AND METHODS:

Two antioxidants, i.e., Citric acid and Tartaric acid were used in this study. Two concentrations of each antioxidant were used (20 and 30 mM).

However, three plant essential oils (Table 1) were obtained from El-Gomhouriya Company for Medicins and Chemicals.

Topsin-M (2.5 g/l) was also used in this work to evaluate the efficiency of the five tested compounds, as pre-harvest applications, (during growing season) on the post harvest diseases.

Table (1): Origin plants, scientific names, plant parts and active ingredients of the essential oils.

Origin plant	Scientific name	Plant part	Active ingredient
Anise	*Pimpinella anisum* l.	Seeds	Charicol methyl ether "estragol"
Clove	*Eugenia carpophylla* Thurb	Flower buds	Eugenol
Eucalyptus	*Eucalyptus restrata* Schlechtend	leaves	Cincole "eucalypto"

Field experiments were carried out at Meet-khalaf Agricultural Research Station, Shebin El-Kom during 2004-2005 and 2005-2006 growing seasons. Seedlings of onion cultivar Giza 20 were seperatly dipped for 15 minutes in solutions of Citric acid and Tartaric acid; just before transplanting.

Anise, clove and Eucalyptus oils were also used at the concentrations of 2000 and/or 3000 ppm for dipping onion Giza 20 cv. seedlings before planting as mentioned above.

Treated seedlings were planted in both ridges of 2.5 m long and 50 cm wide rows. Complete block design experiment with three replicates was accomplished every treatment. Three plots were planted with untreated seedling; at the same time; which served as control. All plants were left for natural inoculation conditions and received the recommended doses of fertilizers, irrigation and other treatments.

Solutions of the same tested materials and at the same concentrations were applied as foliar sprays to onion foliage either once or twice at the rate of 400 L/feddan. First application was conducted at 15th of March and the second was carried out 15 days later.

Harvested onion bulbs were separately stored under room conditions and examined after 2, 4 and 6 months to estimate the incidence of black mould, basal-rot and neck-rot diseases. Percentage of infection was estimated and the compounds efficiency were calculated according to the following formula:

$$\text{Efficiency\%} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

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

Biochemical analysis :

1-Determination of oxidative enzymes:

Samples of onion bulbs (just after harvesting) were prepared to determine peroxidase, polyphenol oxidase and catalase activities as described by Maxwell and Bateman (1967).

Peroxidase assay:

Peroxidase activity was estimated according to the methods 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.

Polyphenoloxidase assay :

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

Catalase assay :

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

2-Determination of phenolic compounds:

Phenolic compounds were colourimetrically determined using phosphotungestic-phosphomolybdic acid (Folin and Ciocaltese) reagents according to Snell and Snell (1953). A standard curve of P. hydroxyl benzoic acid was used to calculate the amount of phenolic compounds in different tested samples. The obtained results were expressed as mg P. hydroxyl benzoic equivalent per gram fresh weight.

RESULTS AND DISCUSSION

I-Effect of the tested compounds on storage diseases:

Results present in Tables (2-4) clear that black mould of onion (*A. niger*) significantly decreased than control in response to the seedling and foliage treatments with different compounds. After two months of storage, Clove oil

(3000 ppm) and Topsin-M (2.5 g/L) showed the disease reduction; when applied once to the foliage at 2004-2005 season.

However; twice application, at the same season, resulted superior effect of Topsin-M followed by Tartaric acid, Anise and Eucalyptus oils. Similar effects were also noticed at the second season (2005-2006) with remarkable ones when Topsin solution was twice applied to onion foliage. Generally, twice applications of most of the tested compounds significantly decreased black mould infection than single application.

Table (2): Effect of the antioxidants essential oils and Topsin-M on black mould infection, two months after bulbs storage.

Treatments		2004-2005				2005-2006			
		An application		Twice applic.		An application		Twice applic.	
		I*%	Efficiency	I*%	Efficiency	I*%	Efficiency	I*%	Efficiency
Tartacic acid	20 mM	8.9	38	4.4	69	7.8	50	5.5	65
	30 mM	5.6	61	2.2	85	6.7	60	5.5	65
Citric acid	20 mM	7.8	46	5.6	61	6.7	60	6.7	60
	30 mM	4.4	69	4.4	69	4.2	73	4.2	73
Anise oil	2000ppm	7.8	46	4.4	69	5.5	65	5.5	65
	3000 ppm	5.6	61	2.2	85	5.5	65	4.2	73
Clove oil	2000	4.4	69	5.6	61	6.7	60	5.5	65
	3000	3.3	77	3.3	77	6.7	60	5.5	65
Eucalypyus oil	2000	5.6	61	5.6	61	5.5	65	4.2	73
	3000	4.4	69	2.2	85	5.5	65	3.0	81
Topsin-M	2.5 g/L	4.0	72	1.0	93	5.5	65	2.0	87
Control		14.4	00	14.4	00	15.5	00	15.5	00

*** I%= Disease infection %.**

L.S.D. at 5% :

Between treatments	**=**	**1.19**	**1.42**
Between concentration	**=**	**0.64**	**0.76**
Between applications	**=**	**0.64**	**0.76**
Interaction	**=**	**N.S**	**N.S**

Table (3): Effect of the antioxidants, essential oils and Topsin-M on black mould infection, four months after bulbs storage.

Treatments		2004-2005				2005-2006			
		An application		Twice applic.		An application		Twice applic.	
		I*%	Efficiency	I%	Efficiency	I%	Efficiency	I%	Efficiency
Tartacic acid	20 mM	11.7	78	8.7	83	13.3	78	12.2	80
	30 mM	9.7	81	8.7	83	13.3	78	5.4	91
Citric acid	20 mM	13.0	75	12.2	77	15.6	74	15.6	74
	30 mM	13.3	74	16.7	68	8.9	85	11.1	81
Anise oil	2000 ppm	16.7	68	14.4	72	12.2	80	13.3	78
	3000 ppm	13.3	74	12.2	77	11.1	82	8.9	85
Clove oil	2000	16.7	68	8.9	83	15.6	74	12.2	80
	3000	13.3	74	12.3	76	14.4	76	10.0	83
Eucalypyus oil	2000	13.3	74	11.1	79	13.3	78	11.1	81
	3000	8.9	83	5.6	89	12.2	80	8.9	85
Topsin-M 2.5 g/L		16.1	69	4.3	92	14.4	76	12.2	80
Control		52.0	00	52.0	00	60.0	00	60.0	00

*** I%= Disease infection %.**

L.S.D. at 5% :

Between treatments	=	3.95	2.95
Between concentration	=	N.S	1.57
Between applications	=	2.11	1.57
Interaction	=	N.S	N.S

Table (4): Effect of the antioxidants, essential oils and Topsin-M on black mould infection, six months after bulbs storage.

Treatments		2004-2005				2005-2006			
		An application		Twice applic.		An application		Twice applic.	
		I*%	Efficiency	I%	Efficiency	I%	Efficiency	I%	Efficiency
Tartacic acid	20 mM	28.0	60	17.7	75	26.7	65	21.1	72
	30 mM	21.0	70	17.3	75	23.3	69	18.9	75
Citric acid	20 mM	28.0	60	17.7	75	26.7	65	24.4	68
	30 mM	21.0	70	17.3	75	24.4	68	22.2	71
Anise oil	2000 ppm	27.8	60	15.6	78	24.4	68	21.1	72
	3000 ppm	23.3	67	22.2	68	18.9	75	14.4	81
Clove oil	2000	26.6	62	21.1	70	28.9	62	23.3	69
	3000	28.9	59	17.8	75	28.9	62	18.9	75
Eucalypyus oil	2000	31.1	56	23.3	67	25.6	66	18.9	75
	3000	26.7	62	17.8	75	18.9	75	16.7	78
Topsin-M 2.5 g/L		20.3	71	10.3	85	20.7	73	15.5	79
Control		70.2	00	70.2	00	75.5	00	75.5	00

*** I%= Disease infection %.**

L.S.D. at 5% :

Between treatments	=	3.15	2.71
Between concentration	=	1.68	1.45
Between applications	=	1.68	1.45
Interaction	=	N.S	N.S

Percentage of infection with *A. niger* was more higher after four months storage (Table 3). Tested compounds, however, significantly decreased infection rate with black mould than control. The best result was observed when Eucalyptus oil (3000 ppm) was applied once to the foliage at 2004-2005 season. This was followed by Tartaric acid; 30 mM (an application at 2004-2005), Topsin-M (2.5 g/L) and Eucalyptus oil (3000 ppm) were twice sprayed at the same season. At the second season; the best effect of an application was recorded with Anise oil (3000 ppm). However; twice applications with Tartaric acid (30 mM) followed by Anise and Eucalyptus oils (3000 ppm) also showed good results.

Results shown in Table (4) clear the increasing of black mould infection after six months storage especially those of the untereated control bulbs. Results of both seasons indicate that Topsin-M, Anise and Eucalyptus oils gave the best results of disease reduction.

Basal rot caused by *Fusarium oxysporum* f.sp. *cepae* was also increased by increasing time of storage (Tables 5-7). In general; the best efficiency after two months storage was noticed when Topsin-M and Eucalyptus oil were applied. The tested compounds showed variable effects after 4 months storage; where two applications with Topsin-M, were effective than the others. In this respect; application of the antioxidants and Clove oil gave satisfactory results. Nearly similar results were observed after six months storage. Twice applications was more effective than single application, in most cases.

Symptoms of neck rot disease (*Botrytis allii*) could only noticed after six months storage (Table 8). The best disease reduction, in comparison with control, was noticed when Clove oil (3000 ppm), Eucalyptus oil (2000 and 3000 ppm) and Topsin-M (2.5 g/L) were twice applied to onion foliage at 2004-2005 season. Twice application with Topsin-M, also gave the same efficiency at the second season.

Table (5): Effect of the antioxidants, essential oils and Topsin-M on basal-rot infection, two months after bulbs storage.

Treatments		2004-2005				2005-2006			
		An application		Twice applic.		An application		Twice applic.	
		I*%	Efficiency	I%	Efficiency	I%	Efficiency	I%	Efficiency
Tartacic acid	20 mM	0.56	42	0.56	42	1.22	39	0.89	56
	30 mM	0.44	54	0.33	65	1.00	50	0.78	61
Citric acid	20 mM	0.56	42	0.22	77	1.00	50	0.67	67
	30 mM	0.33	66	0.22	77	0.89	56	0.67	67
Anise oil	2000 ppm	0.56	42	0.33	65	0.89	56	0.65	68
	3000 ppm	0.56	42	0.22	77	1.11	45	0.89	56
Clove oil	2000	0.44	54	0.44	54	0.89	56	0.89	56
	3000	0.44	54	0.33	65	1.00	50	0.78	61
Eucalypyus oil	2000	0.67	30	0.33	65	1.22	39	1.00	50
	3000	0.56	42	0.24	75	0.55	73	0.78	61
Topsin-M	2.5 g/L	0.40	58	0.20	79	0.11	95	0.42	79
Control		0.96	00	0.96	00	2.00	00	2.00	00

*** I%= Disease infection %.**

L.S.D. at 5% :

Between treatments	=	0.15	0.14
Between concentration	=	0.08	N.S
Between applications	=	0.08	0.07
Interaction	=	N.S	N.S

Table (6): Effect of the antioxidants, essential oils and Topsin-M on basal-rot infection, four months after bulbs storage.

Treatments		2004-2005				2005-2006			
		An application		Twice applic.		An application		Twice applic.	
		I*%	Efficiency	I%	Efficiency	I%	Efficiency	I%	Efficiency
Tartacic acid	20 mM	1.73	42	0.87	71	2.56	32	2.00	47
	30 mM	1.40	53	0.87	71	2.00	47	0.78	79
Citric acid	20 mM	1.20	60	0.87	71	2.22	41	2.00	47
	30 mM	1.44	52	1.33	56	1.89	49	1.45	61
Anise oil	2000 ppm	2.33	22	1.67	44	1.89	49	1.56	58
	3000 ppm	1.78	41	1.56	48	1.89	49	1.72	54
Clove oil	2000	2.56	15	0.78	74	2.33	38	1.72	54
	3000	1.78	41	1.56	48	2.33	38	2.22	41
Eucalypyus oil	2000	1.56	48	1.00	67	2.33	38	1.72	54
	3000	1.33	56	1.33	56	2.00	47	1.89	49
Topsin-M	2.5 g/L	1.50	50	0.50	83	2.00	47	1.00	73
Control		3.00	00	3.00	00	3.74	00	3.74	00

*** I%= Disease infection %.**

L.S.D. at 5% :

Between treatments	=	0.02	0.19
Between concentration	=	0.15	0.10
Between applications	=	0.11	0.10
Interaction	=	N.S	N.S

Table (7): Effect of the antioxidants, essential oils and Topsin-M on basal-rot infection, six months after bulbs storage.

Treatments		2004-2005				2005-2006			
		An application		Twice applic.		An application		Twice applic.	
		I*%	Efficiency	I%	Efficiency	I%	Efficiency	I%	Efficiency
Tartacic acid	20 mM	3.89	22	3.11	38	2.63	62	1.50	79
	30 mM	3.22	36	2.44	51	1.63	78	2.33	67
Citric acid	20 mM	3.67	27	3.00	40	2.63	62	1.50	79
	30 mM	3.22	36	3.00	40	2.30	67	1.63	77
Anise oil	2000 ppm	3.45	31	2.78	44	3.44	51	2.78	60
	3000 ppm	3.11	38	2.11	58	2.67	62	2.33	67
Clove oil	2000	4.22	16	2.89	42	3.56	49	2.67	62
	3000	3.89	22	2.78	44	3.44	51	2.33	67
Eucalypyus oil	2000	3.11	38	3.00	40	2.89	59	2.22	68
	3000	2.67	47	2.56	49	2.56	63	2.00	71
Topsin-M	2.5 g/L	2.55	49	1.20	76	2.77	60	1.74	75
Control		5.00	00	5.00	00	7.00	00	7.00	00

*** I%= Disease infection %.**

L.S.D. at 5% :

Between treatments	=	0.22	0.29
Between concentration	=	0.12	0.16
Between applications	=	0.12	0.16
Interaction	=	N.S	N.S

Table (8): Effect of the antioxidants, essential oils and Topsin-M on neck-rot infection, six months after bulbs storage.

Treatments		2004-2005				2005-2006			
		An application		Twice applic.		An application		Twice applic.	
		I*%	Efficiency	I%	Efficiency	I%	Efficiency	I%	Efficiency
Tartacic acid	20 mM	6.3	52	4.0	72	11.1	39	7.6	43
	30 mM	5.0	62	2.0	86	8.9	51	5.4	59
Citric acid	20 mM	7.3	45	3.0	79	11.1	39	7.6	43
	30 mM	5.0	62	4.0	72	10.2	44	5.4	59
Anise oil	2000 ppm	5.5	58	4.5	69	11.1	39	8.9	33
	3000 ppm	5.2	58	2.2	85	5.4	70	2.0	85
Clove oil	2000	3.2	76	2.8	80	11.1	39	7.6	43
	3000	2.2	83	1.1	92	8.9	51	5.4	59
Eucalypyus oil	2000	5.5	58	1.1	92	10.3	44	7.6	39
	3000	3.3	75	1.1	92	7.6	58	5.4	59
Topsin-M	2.5 g/L	4.4	67	1.1	92	4.2	77	1.0	92
Control		13.2	00	14.3	00	18.2	00	13.3	00

*** I%= Disease infection %.**

L.S.D. at 5% :

Between treatments	=	1.67	2.17
Between concentration	=	1.50	2.06
Between applications	=	0.89	1.16
Interaction	=	N.S	N.S

The above results clear that the application of Tartaric acid, Citric acid (20 and 30 mM), Anise, Clove and Eucalyptus oils (2000 and 3000 ppm) as foliar spraying to onion plants, which previously dipped in the same solutions before transplanting, significantly reduced the incidence of storage

diseases, under study, up to six months. These results could be attributed to direct toxicity of the antioxidants to the pathogens as reported by Elad (1992), Galal and Abdu (1996), Abd El-Megid *et al.* (2004) and Saleh Wagida (2008). The volatile tested oils also showed good efficiency in controlling the three storage diseases. Such oils were successfully used for diseases management by Singh and Dwivedi (1987), Singh *et al.* (1992), Raju and Naik (2007), Sharaf El-Din-Azza *et al.* (2007) and Rizk-Islam (2008). However, the application of antioxidants and/or volatile oils to onion plants in the field could improve plant growth which later make them tolerant to the storage diseases.

II. Effect of the tested materials on enzymes activity and phenolic compounds:

The oxidative enzymes activity showed variable results in response to the antioxidants, essential oils and Topsin-M, as compared with control. Peroxidase activity was higher than control when Citric acid, Eucalyptus oil and Topsin-M were applied to onion plants in the field (Table 9). All tested materials decreased polyphenol oxidase activity than control except Topsin-M, which resulted similar activity. However, Clove oil, Tartaric acid and Eucalyptus oil increased catalase activity than control. The other materials decreased the oxidative enzyme activities which detect their efficiency in reducing the disease; Margo (1984); McLusky *et al.* (1999) and Ammar (2003).

Table (9): Enzymes activity and phenolic compounds concentration of onion plants as affected by antioxiadants, essential oils and Topsin-M, applications.

Treatment	Enzymes activity /minute			concn. of phenolic compounds			
	Peroxi-dase	Polyph-enol oxidase	Catalase	Phenol	2, 6-dimethy phenol	3-5 dimethy phenol	Nitroph-enol
Tartaric acid (30mM)	0.89	0.31	9.58	39.59	7.70	1.35	48.01
Citric acid (30mM)	3.02	0.20	3.78	56.92	8.87	7.49	21.55
Anise oil (3000ppm)	0.71	0.29	0.83	39.96	0.00	2.28	7.01
Clove oil (3000ppm)	1.14	0.29	13.08	54.90	7.62	0.00	17.25
Eucalyptus oil (3000ppm)	2.42	0.33	7.48	55.15	10.53	7.39	25.60
Topsin-M (2.5 g/L)	1.58	0.39	1.30	61.46	2.46	0.00	0.00
Control	1.43	0.39	4.27	76.59	0.00	14.31	0.00

On the other hand; phenolic compounds were less than control when any of the tested materials was applied. The best reduction was detected when Tartaric acid or Anise oil was sprayed. Reduction of phenolic compounds could exeplain plant tolerance to infection, as reported by McLucky *et al.* (1999) who mentioned that the phenolics have a key role in resistance by preventing fungal degradation of the cell wall.

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المعاملات الحقلية بمضادات الأكسدة ، الزيوت العطرية ومبيد توبسين ـ م لمكافحة بعض أمراض البصل في المخزن

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الملخص العربى

أدت معاملة شتلات البصل (صنف جيزة ٢٠) وكذلك رش المجموع الخضرى بأى من حمض الطرطريك ، حمض الستريك ، زيت القرنفل ، زيت الينسون ، زيت الكافور والمبيد الفطرى توبسين ـ م إلى نقص معنوى لأمراض البصل في المخزن . فقد تناقص مرض العفن الأسود (أسبرجلس نيجر) بصورة ملحوظة عند استخدام زيت القرنفل أو زيت الينسون (٣٠٠٠ جزء في المليون) والمبيد الفطرى توبسين ـ م (٢.٥ جم/لتر) ، حتى ستة أشهر في المخزن . وقد أثبتت الدراسة أن الرش مرتين ـ بأى من المركبات المستخدمة ـ يؤدى إلى مكافحة أفضل للمرض ، مقارنة بالرش مرة واحدة في الحقل .

كما تناقصت الإصابة بمرض عفن القاعدة في البصل (فيوزاريوم أكسيبورم سيبا) بصورة معنوية كاستجابة لمعاملات المركبات المختلفة المستخدمة ، مقارنة بالنباتات الغير معاملة . وأظهر زيت الكافور والمبيد الفطرى فعالية أكثر من غيرهما في مكافحة هذا المرض .

وعن مرض عفن الرقبة في البصل (بوترايتس ألياى) فلم تظهر الإصابة به ـ في جميع الأبصال المخزنة ـ إلا بعد مضى ستة أشهر في المخزن . وأدى الرش مرتين بزيت الينسون ، زيت الكافور أو المبيد الفطرى توبسين ـ م إلى تحقيق أفضل النتائج لمكافحة المرض .

وقد أثبتت نتائج التحليل الكيميائى أن نشاط إنزيمات الأكسدة وكذا تركيز الفينولات قد تناقصت ـ بصورة عامة ـ عن مثيلاتها في النباتات الغير معاملة بأى من مضادات الأكسدة أو الزيوت العطرية تحت الاختبار .