# EFFECT OF SOME PLANT EXTRACTS AND ESSENTIAL OLIS ON CONTROLLING NECK-ROT DISEASE OF ONION.

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#### ABSTRACT

Onion plant were attacked by neck rot disease caused by *Botrytis allii* (Munn). The modern trends were directed to study the effect of various natural substance against neck rot disease. The effect of plant extract and essintal oil on the incidence of plant disease was studied. Plant extracts of six plant extracts, clove (*Dianthus caryophyllus*), *cinnamam (Cinnamomum zeylamicum*), thyme (*Thymus vulgaris L.*) fenugreek (*Trigonella, fonicum*), amme (*Ammi visnagal*), black pepper (*Piper nigrum*) and three oil essential gernium (*Pelargonium gravedens*), black cumin seeds (*Nigella sativa L.*) and blue gum (*Eucalyptus globulus*).

The six plant extracts and the three oil essential were evaluated as antifungal substance on the mycelial growth and incidence and disease severity of neck rot disease. The antifungal proprieties of cloves extract was more effective than black pepper then Fenugreek extract on inhibiting mycelial and disease, incidence, while amme, thyme cinnamam showed the lowest effect. On the other hand oil essential of geranium was more effective than blue gum and oil black cummon seeds on inhibiting mycelial growth and disease incidence.

Keywords: Botrytis allii, Plant Extracts and Essential olis.

#### INTRODUCTION

Onion (*Allium cepa. L.*) is one of the most economical field crops in Egypt. Neck rot disease caused by *Botrytis allii* Munn is one of the most serious diseases which attacks onion plants grown for bulb production and during storage. (El-Helaly *et al.*, 1966, Hussein *et al.*, 1977, Maude *et al.*, 1982 and Farrag, Eman 2005). The modren trends are directed to study the effect of various natural substances against some pathogenes causes plant diseases. Medical and aromatic plants as anitifungal substances were also documented (Saksena and Tripathi, 1987; Agha, 1992; Zedan *et al.*, 1994; Sivropoulou *et al.*, 1995). Wilson et al., 1997 tested some plant extracts and essential oils for their antifungal activity against *Botrytis allii*. The aim of the present work was to study the effect of some plant extracts and some essential oils on controlling neck rot disease of onion.

# **MATERIALS AND METHODS**

#### 1- Isolation and identification of the causal pathogen:

Samples of diseased onion plants showing neck rot disease were collected from different localities of Assiut Governorate. Isolation procedures was carried out using infected onion bulbs Giza 6 cultivar. Soaking the infected plant parts in 1% sodium hypochlorite solution for 10 minutes followed by complete washing in sterile distilled water was used for surface disinfestation of infected sample before plating on PDA medium at 20°C for 5-7 days. Pure culture of the developing fungi was obtained by single spore isolat. Primary identification was carried out according to Owen *et al.*, (1950)

and Barnett (1960) then was confirmed by Assiut University Mycological center (AUMC).

#### 2- Pathogenicity test:

Apparantly healthy onion bulbs, free from obvious infection by B.allii Giza 6 cultivar, were washed thoroughly with tap water, surface sterilized by dipping in 1% sodiumn hypochlorite solution for 2-3 minutes. Then rewashed with sterile distilled water and left for arial during at room temperature. Inoculum was prepared by growing the three tested isolates on 100ml Potato Dextrose liqued in 250ml. concical flasks, then incubated at 20°C. After 6 days incubation culture filtrates were decanted and the growing mycelium of each tested isolate was washed using sterile distilled water, suspended in 100ml of distilled water and blended in warning blender for two minutes. Healthy onion bulbs were sprayed with the mycelial suspension 4  $\times$  10<sup>5</sup> propagulus. Fungal suspension was replaced by distilled water and the bulbs were treated in the same manner as a control treatment. Twenty five of bulbs were put in 30 × 40cm. Jute sacks for each treatment replicated four times. Sacks were kept in rows at room temperature about 32°C for three months. A piece of wet cotton with sterilized distilled water was put in each bag to maintain suitable relative humidity around the bulbs. The percentage of infection were recorded after 3 months and disease index of bulbs were recorded after the end of experiment by using scale of oto 4 was used by AICRIP (1968).

## 3- Plant extract preparation:

Plant extracts were prepared from the indicated parts of the following 6 plant species collected from Assuit Government. Buds of clove, stem of cinnamom, thyme (seeds), Amme (seeds) and black pepper (seeds) and fenugreek (seeds).

According to natural medicine these parts of the tested plant were known to have antifingal and or other dermal qualities (Krispel 1986).

Samples were extracted with steril destilled water by autoclaving at 121°C for 10 min. The concentration of dried material was 10% (w/v). The filtrate was used as test extract as described by Maoz and Neeman (1998).

#### 4- Essential oils extracts:

Three oils of geranium, blue gum and black cumin at the rat of 1.5ml/L were prepared by method described by Baraka *et al.*, (2003).

# Effect of some plant extracts on the linear growth of B.allii in vitro.

The concentration 10% of clove, black pepper, fenugreek and thymes extracts was prepared by adding suitable amount of sterilized distilled water to the crude extracts of plant (w/v). One ml of each tested plant extract were added to 9ml PDA medium in Petri dishes. Inoculation was done with fungal discs, 5mm in diameter obtained from *B.allii* 7 days old culture. Three replicates were used for each tested concentration.

Another group of PDA plates free from plant extracts, inoculated with the fungus as control. All plates were incubated at 20°C for 7 days. Linear growth was recorded. The obtained data were statistically analyzed, according to Snedecor and Cochracn, (1967).

# Effect of three essential oils on the Linear growth of *B.allii* in *vitro*.

The concentrations 1.5ml/Later of gernium, black cumin seeds and blue gum essential olis were prepared by adding suitable amount 0.015 of each tested concentration to 10ml PDA medium in Petri dishes.

Inoculation was done with fungal discs, (5mm) in diameter obtained from *B.allii* 7 days old culture. Three replicates were used for each tested concentration. Another group of PDA plates free from essential oils inoculated with the fungus as control. All plates were incubated at 20°C for 7 days. Linear growth was recorded. The percentages of reduction in the mycelial growth were calculated. The obtained data were statistically analyzed, according to Snedecor and Cochracn, (1967).

# Effect of plant extracts and essential oil on the disease incidence of neck rot disease during storage:

This experiment was carried out in 2004 and 2005. Two isolates of were used *Botrytis allii*. Healthy onion bulbs were sprayed with the mycelial suspension  $4\times 10^5$  propagulus/ml and were soaked in plant extracts all essential oils with the same concentration used in *vitro* for 5 minutes. The previously treated bulbs were kept in  $30\times 40$ cm. Jute sacks each containing 25 bulbs and a piece of wetted cotton to maintain suitable humidity around bulbs. Then kept at room temp. (about  $32^\circ$ ). Four replicates were used for each treatment a healthy onion bulbs were sprayed with the fungal suspension only used as control. After 90 days from inoculation, percent of infection and disease index of bulbs were recorded after the end of experiment by using scale of 0 to 4 was used by A.I.C.R.I.P. (1968).

## Statistical analysis:

The collected data were statistically analyzed using two factor Completely Randomized Block Design. Treatments were compared at 0.05 and 0.01 level of probability L.S.D. (Fisher 1984).

Table (1): Scientific English, and Arabic names of the source used plant extract, Essential oils and their natural component:

Scientific plant extracts and essential oils	English name	Natural component	Arabic name
Dianthus caryophyllus	clove	Eugenol	قرنفل
Cinnamomum zylanicum	cinnamom	Cuminaldehyde	قرفة
Thymus vulgaris, L.	thyme	Thymol and carvacrol	ز عتر
Tigonella fonicum	fenugreek	Tergeneol and Colein	حلبة
Ammi visnagal	Khella amme	Khilyen and Glycocid	خله
Piper nigrum	black pepper	Pippren Kaloyed	الفلفل الأسود
Pelargonium graveolens	geranium	Geraniol	العتر
Nigella sativa, L.	black cumin seeds	Bachlon	حبة البركة
Eucalyptus globulus	Blue gum camphor	Cineole Thynoleycalptus	الكافور

# **RESULTS AND DISCUSSION**

Data presented in Table (2) indicated that all isolates of *Botrytis allii* causes neck rot disease of onion during storge. Data also show that isolate (No.3) was the highly pathogenic followed by isolate (No.2)then isolate (No.3).

These results were in agreement with the finding of El-helaly *et al.*, 1966 and Hussein *et al.*, 1997 Farrag, Eman 2005.

Table (2): Pathogencity test of *Botrytis allii* isolates causal pathogen of neck rot of onion.

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Isolates	Disease incidence %	Disease severity %		
l <sub>1</sub>	40	32.5		
l <sub>2</sub>	46	37.37		
l <sub>3</sub>	54	42.25		
Control	0	0		
L.S.D. 5%	2.1328	3.5941		
1%	3.0640	5.1634		

Data in Table (3) indicate that all plant extracts inhibited linear growth of *Botrytis alli* in *vitro*. compared with control untretd with plant extract. Clove extract was the most active plant extract in inhibiting linear growth of *Botrytis alli in vitro* followed by, black *pepper, feunogrec; amme, cinnamom* and *thyme* subsequently.

Table (3): Effect of plant extracts on the linear growth of *Botrytis allii* in *vitro*.

in vitro.		
Plant extracts	Isolates	Linear growth (mm)
Cloves	<b>I</b> <sub>1</sub>	27.5
	$I_2$	20
Mean		23.75
Cinnamom	I <sub>1</sub>	84.1
	$I_2$	82
Mean		83.05
Thyme	I <sub>1</sub>	85
	$I_2$	85
Mean		85
Feunogreek	I <sub>1</sub>	67.5
	$I_2$	60
Mean		63.75
Amme	I <sub>1</sub>	76
	$I_2$	70
Mean		73
Black pepper	I <sub>1</sub>	57.5
	$l_2$	51.6
Mean		54.55
Control	I <sub>1</sub>	89
	$I_2$	89
Mean		89
L.S.D. 5%		I 4.430
		P 8.288
		IXP 11.720
1%	·	I 5.976
		P 11.180
		IXP 15.810

Isolates

P Plant extracts. IXP Isolates interaction × Plant extracts.

The present work is similar to that reported by Nadia and Nasif 2002. He found that clove and *eucalyptus* oils strongly reduced the mycelial growth of the *Macrophomina phaseolina, Fusarium solani, Rhizoctonia solani* and *Fusarium semitectum*. Dawood *et al* 2003 found that the extract of (*Nigella sativa* seeds) reduce the dry weight of the *Alternaria alternata, Botrytis cinerea, Fusarium oxysporum Fusarium solant, Rhizoctonia solani, Sclerotinia sativa, Sclerotinia bataticola* and *Sclerotium rolfsii*.

Data in Table (4) indicate that all essential oils inhibited linear growth of *Botrytis allii* in *vitro* when compared with the control. The most reduction was obtained when geranium was used followed by blue gum then black cumin seeds.

Theses result are agree with Singh and Gupta (1992) and Soltan (1998) essential oils have been recognized as having good fungitoxic Pattnaik *et al.*, (1996) they found that *eucalyptus* and peppermint oils were effective against *Fusarium. solani*, *Fusarium. oxysporum* and *M. phaseolina* in *vitro*.

The three essential oil was more effective in isolate 1 than isolate2.

The tested medical and aromatic plants extracts could suppress some plant pathogenic microorganisms due to their activities which include mycelial growth, reduce.

Table (4): Effect of essential oils on the linear growth of *Botrytis alli* in *vitro*.

Essential oils	Isolates	Linear growth (mm)
Geranium	I <sub>1</sub>	50
	$I_2$	45
Mean		47.5
Black cumin	I <sub>1</sub>	55.8
seeds	l <sub>2</sub>	51.3
Mean		53.55
Blue gum	I <sub>1</sub>	53
	<b>l</b> <sub>2</sub>	48.3
Mean		50.65
Control	I <sub>1</sub>	89
	l <sub>2</sub>	89
Mean		89
L.S.D. 5%		I 1.797
		O 2.542
		IXO 3.595
1%		I 2.479
		O 3.502
		IXO 4.953

I Isolates.

O Essential oil. IXO Isolates interaction × Essential oil.

Formation and morphological maltformation However, these antimicrobial effects depends on many factors such as chemical structure methods of determination, concentration, plant species, plant age and other biotic (Omar 2000).

Data presented in Table (5) indicated that the tested plant extracts significantly reduced the effect of the two testing isolates developing of onion neck root disease after 90 days of storage when compared with the control. Plant extracts clove was the most effective treatment in controlling disease incidence and severity through the two tested growing seasons followed by black pepper, fenugreek and thyme respectively.

Table (5): Effect of plant extracts on disease incidence and severity of onion neck-rot after 90 days storage through the growing seasons 2004 and 2005.

		Season 2004		Season 2005	
Plant extracts	Isolates	Disease	Disease	Disease	Disease
		incidence	severity	incidence	severity
Clove	I <sub>1</sub>	9	8.25	11	10.25
	$I_2$	16	14	18	14.5
Mean		12.5	11.12	14.5	12.37
Thyme	I <sub>1</sub>	12	10.75	14	13
	$I_2$	21	17.75	24	20.25
Mean		16.5	14.25	19	16.62
Feunogreek	I <sub>1</sub>	11	10	13	12
	<b>l</b> <sub>2</sub>	20	16.5	23	19
Mean		15.5	13.25	18	15.5
Black pepper	I <sub>1</sub>	10	9.5	12	11.25
	$I_2$	19	15.5	22	18.5
Mean		14.5	12.5	17	14.87
Control	I <sub>1</sub>	40	32.5	42	35.25
	$I_2$	50	39.5	54	42.25
Mean		45	36	48	38.75
L.S.D.		I 1.827	I 1.963	I 2.370	I 2.456
5%		P 2.885	P 3.104	P 3.747	P 3.883
		IXP 4.085	IXP 4.390	IXP 5.299	IXP 5.491
L.S.D.		I 2.460	I 2.644	I 3.191	I 3.307
1%		P 3.889	P 4.180	P 5.046	P 5.22
		IXP 5.500	IXP 5.912	IXP 7.136	IXP 7.394

I Isolates.

P Plant extracts. IXP I Isolates interaction × Plant extracts.

Role in the ability of black pepper, blue gum in control of *Botrytis allii* may be attributed to the presence of sterols  $\infty$  triterpens, phenolic, glycosides, alkaloids and carbohydrates  $\infty$  glycosides, alkaloides and carbohydrates  $\infty$  glycosides, anthraquinone, glycosides, tannins, saponin, flavonoids, cardiac, glycosides (Abd El-All, *et al.*, 2003). Effect of thyme extracts may be attributed to the antifungal activity of the natural components, thymoll Muller-Riebau *et al.*, 1995) gallic acid (Cowan, 1999) and phenolic alcohol, polyphenols and flavones (Vokou *et al.*, 1984). Hassanein and El doksch, 1997) reported that thyme, pepper, ment and carawy oils showed high antimicobial activity against *Agrobacterium tumefaciens*, *Pseudomonas solanacearum* and *Erwinia carotovora* in *vitro* and in *vivo*. Medicinal plant

treatments. Akhtar and Mahmood 1996 and Amin, 1999). Finally, it could be of great impact on the future of biotic or and organic farming approach in Egypt treated with some ground plant parts (aromatic and medicinal plants) in order to have clean, save, low cost and toxic free agricultural commodities. It could be concluded that use of natural plant extracts to control fungal plant disease may be extended in the future, instead of fungicides which destroy the material equilibrium and cause sever environmental pollution.

Data presented in Table (6) indicated that natural essential oils tested significantly reduced the development of neck rot disease of onion during the two tested seasons when compared with the control in case of the two tested isolates of Botrytis allii. Geranium showed the most effective treatment in controlling neck rot (disease incidence and severity) through 2004, 2005 seasons followed by blue gum and Black cumin seeds. The obtained data are in agreement with those of Singh and Gupta (1992) and Soltan (1998). The tested essential oils extracts may contain a variety of substances such as a fixed or volatile oils, tannins, glucoside, alkaloids, flavonoids and saponins while could suppress some plant pathogenic microorganisms, medical plant aromatic possess fungicidal activity and may have a potential for the control of post harvest diseases (Prasad and Stadel backer, (1974) and Singh et al., (1980). It is also agree with the obtained data also are in agreement with Baraka et al., 2003 theses which mentioned that majoram (Geranium) at the rate 1.5ml/L was the most effective treatment in controlling fruit rots followed by cinnamon and camphor (Blue gum).

Table (6): Effect of essential oils on disease incidence and severity of neck rot disease of onion 90 days, after storage under growing seasons 2004 and 2005.

growing seasons 2004 and 2003.						
			Season 2004		Season 2005	
Essentia	al oil	Isolates	Disease	Disease	Disease	Disease
			incidence	severity	incidence	severity
Geranium		<b>I</b> <sub>1</sub>	11	8.75	13	10.5
		$I_2$	14	12	16	13.7
Mean			12.5	10.37	14.5	12.1
Black	cumin	I <sub>1</sub>	15	13.5	18	17
seeds		$I_2$	20	19	22	18.5
Mean			17.5	16.25	20	17.75
Blue gum		<b>I</b> <sub>1</sub>	13	9	15	13.25
		$I_2$	15	13	18	16.5
Mean			14	11	16.5	14.87
Control		I <sub>1</sub>	40	32.5	42	32.25
		$I_2$	50	39.5	54	42.25
Mean			45	36	48	37.25
L.S.D.	5%		I 2.979	I 3.055	I 3.181	I 1.905
			O 4.213	O 4.321	O 4.498	O 2.698
			IXO 5.958	IXO 6.110	IXO 6.361	IXO 5.170
L.S.D.	1%		I 4.037	I 4.140	I 4.310	I 2.585
			O 5.709	O 5.855	O 6.096	O 3.656
			IXO 8.074	IXO 8.251	IXO 8.621	IXO 5.170

I Isolates.

O. Oil essential IXO Isolates interaction × Oil essential.

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تاثير زيوت ومستخلصات بعض النباتات في مقاومة فطر البوتريتس المسبب لمرض عفن الرقبة في البصل.

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تم دراسة تأثير المستخلص المائي لكل من القرنفل والقرفة والزعتر والحلبة والخله والفلفل الأسود وزيوت نباتات العتر وحبة البركة والكافور على النمو الميسليومي للمسبب المرضي بوتريتس آلياي، نسبة الإصابة وشدة الإصابة بالمرض. وقد وجد من النتائج المتحصل عليها إن المستخلص المائي لنبات القرنفل كان ذو تأثير أكثر فعالية من المستخلص المائي للفلف الأسود يليه مستخلص الحلبة في تثبيط النمو الميسليومي للفطر بينما كان تأثير المستخلص المائي للخلة والقرفة والزعتر أقل فعالية في تثبيط النمو الميسليومي للفطر. كما أظهرت الدراسة أن زيت العتر كان أكثر فعالية في تثبيط النمو الميسليومي للفطر يليه الكافور ثم زيت حبة البركة المسبب للمرض. وقد وجد أن النتائج المتحصل عليها في المعمل أعطت نتائج مماثلة لها في المخزن. قد أظهر المستخلص المائي لنبات القرنفل تأثير أفضل من المستخلص المائي للفافل الأسود ثم الحلبة في تقليل نسبة الإصابة وشدة الإصابة بالمرض في حين أن زيت العتر كان أفضل من زيت الكافور ثم زيت حبة البركة في خفض نسبة الإصابة وكذلك شدة الإصابة بالمرض.