

## BIOCONTROL OF PEA *FUSARIUM* POD ROT

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### ABSTRACT

Pea pod rots are the most important diseases affecting pea pods and seeds during harvest, transportation and storage. *Fusarium oxysporium* f.sp. *pisi* was the most pathogenic fungus isolated from pea pod rots. Peppermint extract was the most effective in inhibiting the mycelial growth and conidial germination of the tested *Fusarium* followed by anise extract. Clove oil was the most effective against *F. oxysporium* linear growth and conidial germination followed by onion oil. Fennel oil had the least effect *Trichoderma harzianum* caused 82.03% inhibition of *F. oxysporium* linear growth. Pea seeds infected with *F. oxysporium* showed decreased in free amino acids content.

**Keywords:** Pea pod rot, Plant extract, essential oils, biological control

### INTRODUCTION

Post harvest decay of pea pods is a major problem of the pea industry during storage and marketing which reduce the quality and quantity of pods. Seed mycoflora plays an important role in reduction of pea seed yield. The economically important seed mycoflora are: *Alternaria* sp., *Ascochyta pisi*, *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Rhizoctonia* sp., Bean Yellow Mosaic Virus, Seed borne mosaic poty virus and *Pseudomonas syringae pisi* (Hagedorn, 1984). Several higher plants and their constituents have shown great success in plant disease control and are proved to be harmless and non phytotoxic to the host plant (Singh *et al.*, 1986 and Dubey, 1991). The plant extracts has inhibitory effect on germination of fungal spores (Singh and Singh, 1981; Singh *et al.*, 1983; Dubey, 1991; Singh *et al.*, 1991; Ali *et al.*, 1994; Alam *et al.*, 2002 and Dawood *et al.*, 2003a&b). The extracts of plant parts are recommended to control plant disease (Alice and Rao, 1987 and Singh and Dwivedi, 1990). Plant oils and extracts have been used for wide variety of purposes for many thousands of years (Jones, 1996). Effective results of essential oils in plant disease control have been obtained with several researchers (Arras *et al.*, 1995; Zambonelli *et al.*, 1996 and Eloff, 1998). Paran *et al.*, 1996 and Fahmy and Mahmoud, 2001 proved that some higher plant essential oils and extracts have an allelopathic effect on some disease.

Biological control had succeeded to prevent many plant diseases, offering an alternative or supplement to pesticides, reducing density of soil borne pathogens (Kommedahl and Windels, 1978).

The objective of this study was aimed to study the effects of plant extracts, essential oils on the linear growth and conidiospores germination of *F. oxysporium* f.sp. *pisi* *In Vitro* beside their effect if used as biological control agent compared to *Trichoderma harzianum*.

## MATERIAL AND METHODS

### 1- Disease Survey:

Pea pods collected from different local market at Alexandria Governorate (5 samples each sample contains 2 kg.), they were classified in two groups, apparently healthy and infected pods. Infected pea pods were surface sterilized by dipping in 1% Sodium hypochlorite solution (commercially known as chlorox) for two minute, thin rinsed in sterilized distilled water and dried between two sterilized filter papers. Small pieces of the sterilized pods were plated on Petri dishes containing PDA medium and incubated at 25°C. The developing fungi, were isolated and purified using either single spore isolation or by hyphal tip technique. The obtained cultures were kept on PDA slants for further studies. Identification of the obtained isolates was done according to cultural and microscopical characteristics according to Gilman, (1957) and Barnett and Hunter, (1972).

### 2- Effect of the isolated *Fusarium oxysporum* on some leguminous plants:

studies were carried out to determine the pathogenic capabilities of the isolated *Fusarium* on some leguminous plants i.e., bean (*Phaesolus vulgaris* L.), cowpea (*Vigna siriensis* Endi), broad bean (*Vicia faba* L.), lupine (*Lupinus termis* Forsks) and pea (*Pisum sativum* L.) which were planted in sterilized sandy loam soil artificially inoculated with the tested *Fusarium*. Five pots were used for each tested plant and were sown with 5 seeds/pot. Controls (uninoculated soil) were also considered simultaneously. The pots were kept at 22°C in the green house and the results were recorded after three weeks.

### 3- Effect of plant extracts on mycial growth of the obtained *Fusarium oxysporum* f. sp. *pisi*:

Three plant extracts were used during this study i.e., Anise (*Pimpinella anisum*), peppermint (*Mentha piperita*) and cinnamon (*Cinnamum zelanicum*).

The extract were prepared according to the method of (El-Safwani and Ali, 2003). The tested concentrations 0, 100, 200, 400, 500 and 1000 ppm of anise, cinnamon and peppermint extracts were prepared by adding suitable amount of sterilized distilled water. Two ml of each tested concentration was added to PDA medium in Petri dishes before solidification. Inoculated with 5 mm in diameter obtained from the tested *Fusarium* 7 days old culture. Five replicates were used for each tested concentration. All plates were incubated at 25°C for 7 days. Linear growth was recorded. The percentage reduction in mycelial growth were calculated according to Baratta *et al.*, 1998 as follows:

$$\% \text{ Inhibition} = \frac{C - T}{C} \times 100$$

C = linear growth of the control.

T = linear growth in the tested concentrations.

The obtained data were statistically analyzed according to Snedecore and Co/chrans, (1969).

**4- Effect of plant extracts on conidial germination of the tested *Fusarium oxysporum* f. sp. *pisi*:**

The tested *Fusarium* was grown on PDA medium for 10 days at 25°C. Fungal spores were collected by gently rubbing the agar surface after adding 15 ml of sterilized distilled water to the sporulated cultures in each plate. The spore suspension was filtered through sterilized cheese cloth to remove mycelial fragments. The spore concentration was adjusted at  $15 \times 10^4$  spore/ml. Serial concentrations of the tested plant extracts (0, 100, 200, 400, 500 and 1000 ppm) were prepared. The number of *Fusarium* germinated spores were counted and the germination count was taken after 24 hours.

**5-Effect of essential oils on mycelial growth of *Fusarium oxysporum* f. sp. *pisi*:**

Five essential oils were used during this study i.e., black seed (*Nigella sativa*), clove (*Syzygium aromaticum*), Fennel (*Foeniculum vulgare*), Mint (*Mintha viridis*) and onion (*Allium cepa*) kindly provided by El-Captan Company (CAP Pharm.) Al Obor City Cairo (Egypt). The used oil concentrations were 0.0, 10, 20, 40, 60 and 100% incorporated into melted PDA medium just before solidification at the different concentrations and then poured into Petri dishes. Plates were inoculated at the center with 5 mm discs of the tested *Fusarium* and incubated at 25°C for 7 days. Radial growth of the fungus was determined. Five replicates were used/tested oil. Inhibition percent of mycelia growth with respect to the control was calculated.

**6- Effect of essential oils on conidial germination of *Fusarium oxysporum* f. sp. *pisi*:**

Two microscopic slides per test tube containing *Fusarium* conidial spores were made and in each slide, the germination count was taken after 24 hours. The results were expressed as germination percentage of *Fusarium* conidiospores.

**7- Effect of *Trichoderma harzianum* and Rhizolex-T on the tested *Fusarium oxysporum* f. sp. *pisi*:**

Culture of *Trichoderma harzianum* obtained from healthy pea pods, was used in the present work as antagonistic against the isolated *Fusarium*. The inhibitory effect of *Trichoderma* and fungicide Rhizolex-T on the linear growth of *Fusarium* was evaluated *In Vitro*.

The effect of Rhizolex-T on *Fusarium* growth was tested. Seven concentrations of Rhizolex-T, i.e., 0, 50, 100, 200, 400, 500 and 600 ppm based on the active ingredient were prepared in PDA medium poured in Petri dishes. Petri dishes were inoculated with 5mm discs of 7 days old *Fusarium* cultures. Five replicates were used for each treatment. Plates were incubated at 25°C for 7 days. Percentage of reduction in fungal growth was calculated.

**8- Artificial inoculation of healthy pea pods with the isolated *Fusarium oxysporum* f. sp. *pisi*:**

Fresh pea pods (Lincoln cv.) apparently free of any physical damage and disease symptoms were used in this study. Pea pods were surface disinfected with 10% ethyl alcohol for 2 min., then washed several times with sterilized water and allowed to dry then slightly injured by using sterilized scalpel. Pea pods were artificially inoculated with *Fusarium* suspension ( $15 \times$

10<sup>4</sup> spores/ml). the treated pods were placed in a covered plastic container with moistened paper towels to maintain high humidity for disease development. Each one containing 20 pods and five covered plastic containers were used as well as untreated. Check pea pods were monitored daily for 7 days to study the changes in free amino acids contents.

**9- Effect of *Fusarium oxysporum* f. sp. *pisi* on free amino acids content of pea seeds:**

Seeds of lincoln pea cultivar either healthy or infected with *Fusarium* were used to determine free amino acids content. The seeds were dried at 80°C. Portions of dry material 0.5 g. each was used for analyses according to the method of (El-Mahdy and Sebaiy, 1985).

## RESULTS AND DISCUSSION

### 1- Disease Survey:

The results in Table (1) indicated that *Fusarium* was the most prevailing fungus (68.28%), followed by *Rhizoctonia solani* (18.74%), while *Botrytis cinerea* was the least (12.98%). The obtained *Fusarium* was verified as *Fusarium oxysporum* f. sp. *pisi*:

**Table (1): Survey of Fungi isolated from diseased pea pods.**

Sample	Pod decay	Isolated fungus %		
		<i>Botrytis cinerea</i>	<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>
1	29.5	16.2	73.5	10.3
2	41.0	14.2	69.2	16.6
3	30.0	19.8	59.0	21.2
4	27.5	6.2	71.9	21.9
5	35.5	8.5	67.8	23.7
Mean	32.7	12.98	68.28	18.74

### 2- Effect of the d *Fusarium oxysoprum* on some tested leguminous plants:

Pathogenicity of *F. oxysporum* on the tested leguminous plants showed its inability to cause infection to any of the the tested plants i.e., bean, broad bean, cowpea and lupines. Cross sections in roots of all tested plants didn't show any browning of the vascular elements while, the tested *F. oxysporum* caused wilt of pea plants and browning of vascular elements. So that this *F. oxysporum* is specialized to infect pea plants and then, it is *F. oxysorum* f.sp. *pisi*.

### 3- Effect of the tested plant extracts on mycial growth of *F. oxysporum* f. sp. *pisi*:

Data in Table (2) indicate that the mycelia growth of *F. oxysporum* f. sp. *pisi* was inhibited in response to antifungal substance presented in the tested plant materials, and the mycelia growth was decreased with increasing the concentrations of all tested plant extracts. Complete growth reduction of mycelium was observed when applied 1000 ppm of all tested plant extracts. Peppermint extract at 500 and 1000 ppm gave the highest inhibition on the

linear growth, followed by cinnamon which showed a moderate effect. On the other hand, anise extract had the least effect against *F. oxysporum* f. sp. *pisi*.

**Table (2): Effect of some plant extracts on the linear growth of *F. oxysporum* f. sp. *pisi* on PDA medium.**

Plant extract	Concentrations (ppm)											
	0		100		200		400		500		1000	
	L.G. (cm)	I (%)	L.G. (cm)	I (%)	L.G. (cm)	I (%)	L.G. (cm)	I (%)	L.G. (cm)	I (%)	L.G. (cm)	I (%)
Anise	9	0	8.94	0.66	8.94	0.66	8.92	0.88	8.8	2.22	7.58	15.78
Mint	9	0	8.22	8.67	7.55	16.42	7.28	18.91	6.0	33.33	3.78	58.00
Cinnamon	9	0	8.88	1.35	8.52	5.33	8.78	2.44	8.36	7.11	6.22	28.66

Average of 5 replicates

P > F 0.0001

\*L. G. (cm): means of linear growth

\* I (%): percentage of inhibition.

#### 4- Effect of aqueous plant extracts on spore germination of *F. oxysporum* f. sp. *pisi*:

Data in Table (3) show that peppermint aqueous extract was found to be the supereme extract in terms of suppressing spore germination of *F. oxysporum pisi*.

**Table (3): Effect of aqueous plant extracts (1000 ppm) on *F. oxysporum* f.sp. *pisi* spore germination.**

Plant extract (1000 ppm)	% of conidial germination
Anise	51
Cinnamon	38
Peppermint	30

The obtained results are in agreement with results found by (Singh *et al.*,1994 and El-Korashy, 1997) who indicated that *Mentha spicata* completely inhibited the mycelial growth and spore germination of *F. oxysporum*. Also, El-Doksh and Abd El-Mouty, 1997 stated that peppermint extract inhibiting fungal growth and spore germination of *F. oxysporum*. Ismail, 1998 showed that *F. oxysporum* was the most affected fungus with the tested plant extracts of camphor tree, metel, worm wood, ploughmans, menthe, acacia Arabica and salinin. Peppermint fungal inhibition may due to its chemical compounds menthol and terpenoid (Hammer *et al.*, 1999).

Also, Kasem and Rakvidhysastra, 1985 indicated that the crude extract of star anise completely inhibited the growth of 21 phytopathogenic fungi followed by carway, lemongrass, clove tree, garlic nuxvamic tree and derris, respectively.

Dawood *et al.*, (2003a) reported that the antifungal activity of aqueous of *Allium cepa* and *Nigella sativa* reduced fungal growth of *F. oxysporum* but *Mentha viridis* has no effect or increased the fungal growth. On the other hand, the methanolic plant extracts highly reduced the fungal growth of all tested fungi. *Nigella sativa* reduced fungal growth of *F. oxysporum* for 97.7% followed by *Mentha viridis* 74% and *Allium cepa* 41%. Al- Ismail and Aburjia, 2003 reported that the antioxidant activities of water and alcohol extracts of the flowers of chamomile and the seeds of anise and

dill showed marked antioxidant activity in both linoleic acid and liposome model system.

**5-Effect of essential oils on mycelial growth of *F. oxysporum* f.sp. *pisi*:**

Reduction in mycelial growth of *F. oxysporum pisi* according to application of essential oils was presented in Table (4). Results showed that clove oil was the most effective inhibitor of *F. oxysoprurn pisi* growth with all different concentrations where the percentage of inhibition was (59.56%) followed by mint , onion, black seeds and fennel where inhibition percentage of were 29.98%, 20.93%, 16.96% and 9.78%, respectively.

**Table (4): Effect of different essential oils on the linear growth of *F. oxysorum* f. sp. *pisi* on PDA medium.**

Tested oils	Concentrations (%)											
	0		10		20		40		60		100	
	L.G. (cm)	I (%)	L.G. (cm)	I (%)	L.G. (cm)	I (%)	L.G. (cm)	I (%)	L.G. (cm)	I (%)	L.G. (cm)	I (%)
Black seed	9	0	9	0	8.61	4.33	8.10	10	7.66	14.89	7.46	16.96
Clove	9	0	8.1	0	8.0	11.11	6.7	25.55	4.6	48.89	3.6	59.56
Fennel	9	0	9	0	8.9	1.1	8.71	3.22	8.6	4.44	8.12	9.78
Onion	9	0	9	0	8.8	2.2	8.0	11.11	7.92	12.0	7.10	20.93
Mint	9	0	9	0	8.0	11.11	7.8	13.33	7.1	21.11	6.3	29.98

Average of 5 replicates/ tested oil

P> F 0.0001

\*L. G. (cm): means of linear growth

\* I (%): percentage of inhibition.

**6- Effect of essential oils on conidial germination of *F. oxysorum* f.sp. *pisi*:**

It was observed that the clove and mint essential oils were strongly inhibited conidial germination of *F. oxysoprurn pisi* compared with other tested essential oils. Fennel essential oil had th least effect in this respect (Table 5).

**Table (5): Effect of pure essential oils on conidial germination of *F. oxysporum* f.sp. *pisi*.**

Essential oils (100%)	% of conidial germination
Black seed	52
Clove	21
Fennel	100
Mint	32
Onion	89

The obtained results are in agreement with Saksena and Stripathi, 1985 who stated that anise oil inhibited 80% of the mycelial growth of *F. oxysoprurn pisi* at 500 ppm and 100% at 1000 ppm. Essential oils of cinnamon had high fungicidal activity against *F. moniliformae* (Paran *et al.*, 1996). Zambonelli *et al.*, 1996 indicated that a higher concentration of mint oil was more effective and compeletly inhibited the mycelial growth of *F. solani*, *P. ultimum*, *R. solani* and *C. lindermuthianum*.

Clove and cinnamon oils have been reported by many researchers as a good source of antifungal compounds, Baratta *et al.*, 1998 and Ranasinghe *et al.*, 2002 reported that clove and cinnamon oils gave the best

effect against *F. proliferatum*. but Bowers and Locke, 2000 showed that clove oil gave the best effect against *Fusarium*. Beg Arina and Ahmed (2002) found that clove oil was effective against all tested fungi i.e., *Alternaria alternate* and *F. chlamydosporium*, *Rhizoctonia bataticola* and *Helminthosporium oryzae*. Assawah, (2002) found that clove and mint oils reduced mycelial growth of *F. oxysporum* f. sp. *fabae*. The most effect was obtained by clove oil (61.3%) at 1000 ppm. While, mint oil caused 20.4% inhibition of the mycelial growth. McKay and Blumberg, 2006 reported that *Mentha piperita* has significant antimicrobial, antiviral activities and strong antioxidants. Dawood *et al.*, (2003a) mentioned that the aqueous plant extracts of *Allium cepa* caused (15.9%) inhibition of *F. oxysporum* spore germination while, *Mentha viridis* caused (57%) and *Nigella sativa* showed the highest inhibition (78.5%).

**7- Effect of *Trichoderma harzianum* and Rhizolex-T on *F. oxysporum* f. sp *pisi* In Vitro:**

Data in Table (6) revealed that *Trichoderma harzianum* caused reduction in mycelial growth of *F. oxysporum pisi* (82.03%). In case of Rhizolex-T the mycelial growth of *F. oxysporum pisi* was inhibited by increasing the fungicidal concentration in PDA medium and complete inhibition was achieved at 500 ppm.

The results obtained are in line with AbdEl-Moity *et al.*, 1982. The antagonistic effect of *Trichoderma* spp. Against fungal pathogens may be attributed to the fast growth and better saprophytic activity which suppressed

**Table (6): Effect of *Trichoderma harzianum* and Rhizolex-T on the linear growth of *F. oxysporum pisi* on PDA medium.**

Treatment	<i>F. oxysporum pisi</i>	
	Linear growth (mm)	Reduction (%)
<i>Trichoderma harzianum</i>	16.17	82.03
Rhizolex-T (ppm)		
50	73.83	17.97
100	72.8	19.11
200	38.8	56.89
400	21.5	76.11
500	0	100
600	0	100
check (control)	90	0

\* Data average of 5 replicate each for treatment

The growth of the host, (Iqbal and Akhtar, 1987 and El-Kazzaz *et al.*, 2002 a & b) who found that *T. harzianum* produce the antibiotic gliotoxin such antibiotic proved an inhibition action against *F. oxysporum*, *F. solani*, *P. aphanidermatum* and *R. solani*. Abo-Dakika, 1991 found that Rhizolex-T inhibit the linear growth of *F. oxysporum* at 700 ppm, *F. oxysporum lupine* at 800 ppm.

Ali, 2000 found that Rhizolex-T (5/1kg seed) gave the best effect to control *F. oxysporum niveum* while Moustafa, (2001) declared that the yield of sugar beet per plot in *Ammi visnaga* treatment exceeded the yield obtained from seed treatment with the fungicide Rhizolex-T. El-Araby and Ahmed, (2004) found that faba bean seeds treatment with 5gm Rhizolex-T/kg seed gave the best effective control to *F. oxysporum* f.sp. *fabae*.

8- Effect of *Fusarium oxysporum* f. sp. *pisi* on free amino acids contents:

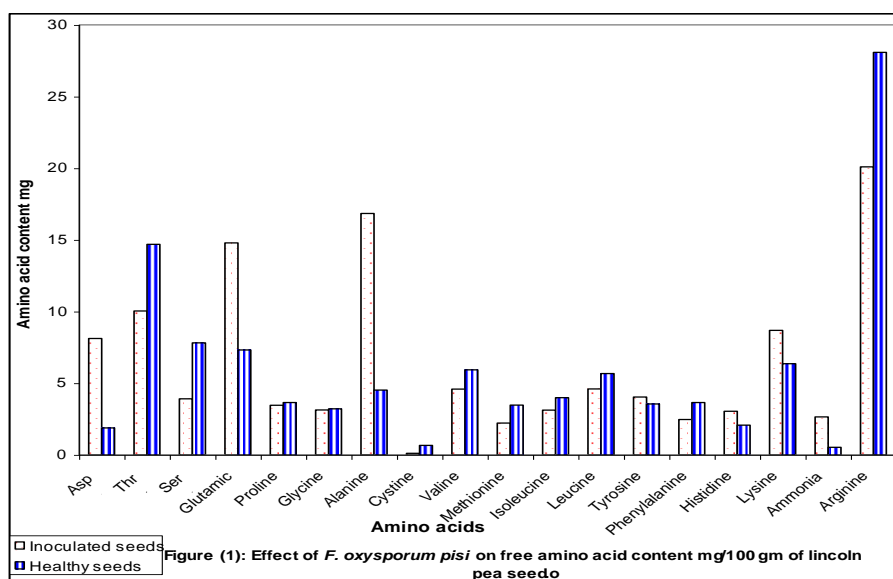
Data in Table (7) and Figure (1) show the followings:

- 1- Seventeen amino acids were found in healthy and infected lincoln pea cultivar.
- 2- Threonine., Serine., glycine, cystine, proline, valine, methionine, isoleucine, leucine, phenyl alanine and arginine were decreased in pea seeds artificially inoculated with *F. oxysporum pisi*.
- 3- Aspartic, glutamic, alanine, tyrosine, histidine and lysine were increased after inoculation with *F. oxysporum pisi*.
- 4- Generally, *F. oxysporum pisi* caused higher reduction in total amino acid content of the artificially infected pea seeds (Table, 7).

**Table (7): Effect of *F. oxysporum pisi* on free amino acids content (mg/100g seeds) of Lincoln pea seeds.**

<b>Amino acids</b>	<b>Inoculated seeds</b>	<b>Healthy seeds</b>
Aspartic.	8.11	
Threonine.	10.03	1.87
Serine.	3.90	14.68
Glutamic	14.79	7.81
Proline	3.45	7.31
Glycine	3.13	3.65
Alanine	16.84	4.513.21
Cystine	0.10	0.66
Valine	4.58	5.92
Methionine	2.21	3.47
Isoleucine	3.11	3.98
Leucine	4.59	5.67
Tyrosine	4.03	3.54
Phenylalanine	2.46	3.64
Histidine	3.04	2.07
Lysine	8.68	6.35
Ammonia	2.64	0.52
Arginine	20.09	28.08





These results are in agreement with Shihata *et al.*, 1988 who stated that the free amino acids increased in cowpea plants infected with *F. oxysporum*. Mahhdy and Eid, 1989 indicated that watermelon cultivars infected with *F. oxysporum niveum* had a low amino acids content specially in susceptible cultivars. Ali, 2000 reported that seed infected with *F. oxysporum niveum* resulted in decrease of most free amino acids contents compared with healthy seeds.

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تتعرض قرون البسلة لأمراض أعفان القرون التي تؤثر على جودة الثمار و صلاحيتها للتسويق و الاستهلاك بعد الحصاد. اظهر العزل من القرون المصابة أن فطر فيوزاريم اوكسيسبورم بيزى هو أعلى الفطريات تكراراً مقارنة بمسببات اعفان القرون الاخرى.

تم فى هذه الدراسة اختبار تأثير ثلاثة من المستخلصات المائية النباتية و هى الينسون و النعناع الفلفلى و القرفة على هذا الفطر المختبر. وأوضحت الدراسة أن النعناع الفلفلى كان أكثر هذه المستخلصات تأثيراً على نمو الميسليوم و كذلك على نسبة الإنبات للجراثيم الكونيدية للفطر يليه مستخلص القرفة ثم مستخلص الينسون.

كما تم اختبار تأثير خمسة زيوت نباتية و هى زيت القرنفل و الشمر والبصل و النعناع و حبة البركة على النمو الميسليومى و نسبة إنبات جراثيم هذا الفطر الكونيدية حيث أكدت هذه الدراسة أن زيت القرنفل كان أكثر هذه الزيوت تثبيطاً بلية النعناع ثم البصل و كان أقلها تأثيراً زيت الشمر.

و تم اختبار تأثير الفطر تريكودرما هارزيانم و المبيد الفطرى ريزولكس -ت على النمو الميسليومى للفطر فيوزاريم اوكسيسبورم بيزى و على نسبة إنبات جراثيمه الكونيدية. و قد أظهرت الدراسة أن الفطر المختبر قد تثبط بنسبة (٨٢,٣%) اما فى حالة المبيد الفطرى المختبر فقد وجد انه بزيادة تركيز المبيد يزداد التأثير التثبيطى للنمو الميسليومى للفطر فيوزاريم اوكسيسبورم بيزى و بلغت نسبة التثبيط ١٠٠% عند تركيز ٥٠٠ جزء فى المليون معملياً.

اوضحت الدراسة أن الإصابة بهذا الفطر فيوزاريم اوكسيسبورم بيزى تؤدي إلى نقص عدد الاحماض الامينية فى بذور البسلة صنف لىكولن المعدة صناعياً بالفطر المختبر.

و تشير الدراسة إلى امكانية استخدام بعض المستخلصات و الزيوت النباتية كبدايل للمبيدات الكيماوية فى مقاومة الفطر فيوزاريم اوكسيسبورم بيزى بعد الحصاد.