Ass. Univ. Bull. Environ. Res. Vol. 7 No. 2, October 2004

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IMPROVING CUMIN PRODUCTION UNDER SOIL INFESTATION WITH FUSARIUM WILT PATHOGEN: I-SCREENING OF BIOCONTROL AGENTS

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ABSTRACT :

Twenty fungus and ten bacterium isolates of microorganisms associated with Fusarium wilt disease infected cumin plants were obtained from samples collected from different locations of Assiut Governorate. On the basis of a preliminary antagonism test conducted in vitro, 3 fungal isolates (*Trichoderma harzianum*, *T. humatum*, and *T. viride*) and one bacterial (*Bacillus subtilis*) isolate were screened. These antagonists were further screened by artificially infested potting-soil bioassay including a commercial formulation (biocide 'Plant Guard') in addition to untreated seeds (positive control). The untreated seeds grown in the sterile soil free of the pathogen (negative control) were also used. No wilt symptoms were observed on the cumin plants raised from untreated seeds when grown in sterile soil (negative control) while untreated seeds grown in the infested soil (positive control) showed 90% to 95% infected plants. The plants grown under the latter treatment were significantly shorter with lesser weight than the former. Comparing with the positive control, all isolated antagonists and the biocide had a significantly lower percentage of infection and developed plants similar to the negative control concerning plant length and weight. The lowest percentage of infection was found in pre-sowing treatment with *T. harzianum*. However, there were no differences among the different biocontrol seed treatments (locally isolated antagonists and the biocide) and the negative control treatment. We concluded that efficient bio-control agents might be developed via bioassay for microorganisms associated with local cumin cultures. This research is considered, therefore, a significant step toward finding an efficient environment friendly strategy for the management of Fusarium wilt disease in cumin.

INTRODUCTION:

Cumin (*Cuminum cyminum* L.) is seriously affected by the Fusarium wilt disease incited by the soil borne pathogen *Fusarium oxysporum* f. sp. *cumini*. Fusarium wilt disease usually increases in warm areas and under dried conditions. This disease has been reported as a problem limiting cumin production worldwide including Argentina (Gaetan and Madia, 1993), Egypt (Arafa, 1985), Greece (Pappas and Elena, 1997) and India (Champawat and Pathak, 1990). Infested field may not be replanted with cumin for at least 10 years. Limited control of this pathogen is provided by seed pre-sowing with certain fungicides such as benlate (Champawat and Pathak,1991). Soil solarization (Lodha, 1995) and soil fumigation with methyle bromide (Larkin and Fravel, 1998) can provide a control measure against the disease but may be of limited application value for large scale

production systems in the open field. In addition, methyle bromide is considered an ozone-depleting compound and has potential risk on the living environment and human health.

Varying limited efficiency in controlling the disease has been suggested using combined crop rotation and other cultural practices including plough, irrigation and fertilization (Arafa, 1985; Champawat and Pathak,1990). Potential genetic variability available to conduct conventional breeding for resistance against the fusarium pathogen has been found very limited in cumin (Champawat and Pathak, 1990a). Apparently, there is a need for an efficient control measure that complies with the recent crop production trends of the environmentally sustainable agriculture (Hassan, 1992; Larkin and Fravel, 1998). Great emphasize has been currently directed, therefore, towards biological control measures (Cook,1993; Larkin and Fravel, 1998).

Numerous plant-associated fungi and bacteria are known for their antagonistic activity to soil-borne pathogens and could be utilized as biocontrol agents against wilt disease including Fusarium (Cook, 1993; Weller, 1988). Successful biocontrol application as pre-sowing treatments to control fusarium wilt has been reported in different ornamental plants (Hassan and Tawfik, 1996; Keinath, 1994; Postma and Rattink, 1992). A significant increase in plant growth and development has been noticed to be associated with utilizing biocontrol agents (Baker *et. al*., 1984; Chang *et al.*, 1986; Hassan, 1992; Linderman, 1994; Ousley *et al.* 1994). Progressively, biocides industry is currently developing to establish a realistic alternative to chemicide use in crop production.

Cumin is mainly produced in southern regions of Egypt and the control of the disease incidence is a crucial factor for its production. While cumin is regarded as one of the economically most important spice and medicinal plant for both local consumption and exportation, only few studies have been directed to it especially concerning Fusarium wilt control (Arafa, 1985; Omar *et al.*, 1997). To our knowledge, there has been no report on assessment of biocontrol measurement in cumin under southern Egypt conditions. The present investigation was, therefore, implemented to isolate efficient antagonist(s) obtained from the rhizosphere and roots of local field-grown cumin and screen them for their biocontrol capability towards developing an effective bio-agent against cumin fusarium wilt in southern Egypt.

MATERIALS AND METHODS:

Isolation and identification of the casual pathogen: The causal pathogen was isolated from naturally infected cumin plants grown at the Experimental Station (Ornamental Farm), Assiut University. Isolation of the pathogen was carried out by placing surface sterilized root and stem parts in Petri-dishes containing acidified Potato Dextrose Agar medium (PDA) to prevent bacterial contamination. The fungus was purified using the hyphal tip technique and identified as *Fusarium oxysporum* f. sp. *cumini* according to their morphological characteristics of mycelia and spores as described by Booth (1977). Then, the pathogenic capability of the fungal isolates was tested using potting soil bioassay (Arafa, 1985). The soil used in the pathogenicity test was sterilized before being infested with fungal isolates.

Isolation and identification of microorganisms associated with infected cumin: Fungi and bacteria associated with infected cumin plants with fusarium wilt were

isolated from samples collected from different locations of Assiut Governorate. Fungal isolates were obtained in pure cultures using the hyphal tip technique. Subsequently, they were transferred to Potato Dextrose Agar (PDA) medium slants. The medium composed of 200 g potato, 20 g dextrose, 20 g agar and 1000 ml water. Slants were incubated at 25°C for 7-10 days. Bacterial isolates were obtained in pure culture using the dilution method. Bacteria grown in separate colonies on the diluted plates were transferred to PDA slants at 27°C for 2 days. Isolated fungi (20 isolates) and bacteria (10 isolates) were then kept at 5°C. The isolated fungi were identified according to their morphological characteristics of mycelia and spores as described by Rifai (1969). Identification of bacterial isolate was carried out following the procedures described by Claus and Berkeley (1986).

Antagonistic capability of the isolated microorganisms against *F. oxysporum in vitro*: Preliminary antagonism screening test in dual culture were carried out for all isolated thirty microorganisms. Isolates, which showed a negative antagonism, were discarded, and the remaining isolates, which showed a positive effect, were used in a study to evaluate their differential antagonistic capability. Ten ml of PDA medium in 9 cm Petri-dishes were inoculated with two disks (7 mm in diameter) of five-day old antagonistic fungi or loop full of two-day old suspension of bacterium. In case of bacteria, suspension was streaked at the center of each plate. Two disks of the tested pathogen obtained from four-day old cultures were then placed at the periphery of each plate at the same distance. The inoculated plates in addition to plates inoculated with the pathogen only (control treatment) were kept at 25°C. Four replicates were used for each treatment. Antagonistic effect was evaluated by scoring the width of the inhibition zone (clear area), where 0 expressed no inhibition, 1<10 mm (slight antagonism), 10 mm < 2 > 20 mm (moderate antagonism) and 3 > 20 mm (high antagonism). The score value of 4 expressed mycoparasytism (over growth) of the antagonist. Further test of antagonistic capability of these latter microorganisms showing over growth was conducted using their sterilized culture filtrate (10% of the filtrate was added to PDA medium). Five days later, the pathogen growth reduction percentage was recorded. Based on test of antagonistic capability scoring, three fungal and one bacterial isolates were screened for subsequent study.

Evaluation of biocontrol agent effects on incidence of cumin wilt and the plant growth: A potting soil experiment was carried out in two successive seasons (1998/99 and 1999/2000) at the Agricultural Research Station of Assiut University. Cumin seeds cv. 'Baladi' used in this study were obtained from growers and local seed retailers in Assiut. Healthy disinfested cumin seeds were grown in sterile potting soil during 1997/1998 to produce seed stock for the present study. The evaluated biocontrol treatments of cumin seed inoculation were the use of the three different isolated antagonistic fungi (*Trichoderma harzianum, T. humatum*, and *T. viride*) and the isolated antagonistic bacterium (*Bacillus subtilis*). The 'Plant Guard' biocide was used as a commercial formulation. In addition, untreated seeds (positive control) were included. During the course of the biocontrol evaluation study, seed were planted at depth of 2 cm in clay pots (25 cm). All seeds of the above mentioned treatments were planted in the autoclaved soil after being artificially infested with the Fusarium wilt pathogen. The untreated seeds

grown in the sterile soil free of the pathogen (negative control) were also used. The experiment in both seasons contained seven treatments that were arranged in randomized complete block (RCB) design with 4 replicates. Ten seeds were planted in each pot and two pots presented each treatment. Plants were fertilized with half strength Hoagland's solution twice a week.

The inoculum of the pathogen was prepared by growing the fungus on Barley grain medium for 20 days at 22-25°C. Soil infestation was carried out by mixing the pathogen inoculum thoroughly with steam sterilized sandy soil at the rate of 5% of soil weight. The infested soil was then placed in sterilized pots. Inocula of biocontrol agents were prepared by growing them in liquid Gliotoxin Fermentation medium. Bacterium was grown on liquid Richard medium and incubated at 25°C for 10 days. Cultures were centrifuged for 5 minutes at 3000 rpm and propagules of fungi and the bacterial cells were resuspended in sterile distilled water to get concentration (CFU/ml) equal to those of the recommended doses of the used biocide ($1.5X10^5$). Cumin seeds were soaked in the suspension of each isolated biocontrol agents or biocide for 15 minutes. Control seeds were soaked in distilled water for 15 min. Disease was monitored to the flowering stage. The plants were lifted out and plant length was measured (cm). Disease incidence was assayed as the total percentage of infection. The percentage of reduction for the disease incidence due to the different seed treatments was calculated based on the percentage of infection relative to the positive control (untreated seeds sown in infested soil). The fresh weight (g/plant) was recorded and then the plants were dried in an electric oven at 70°C for 48h. The dry weight (mg/plant) of the plants in different studied treatments was obtained. The data obtained in the present study were subjected to analysis of variance (ANOVA) relevant to the used experimental design as described by Gomez and Gomez (1984). Based on the obtained coefficients of variation (C.V.), original data were used in the ANOVA. Treatment means were compared using Duncan's Multiple Range Test at 0.05 probability level. Simple correlation coefficients between the percentage of the infection and plant growth parameters and among the different plant growth parameters were calculated.

RESULTS AND DISCUSSION:

Pathogenicity tests of isolated *Fusarium* and antagonistic capability of isolates from associated microorganisms: **Five different isolates of fusarium pathogen were identified (Table 1). Pathogenicity tests indicated that these isolates significantly varied in the percentage of infection detected in cumin. The highest percentage of infected cumin plants was produced by isolate #3 of the five pathogenic isolates. Several biological forms differing in pathogenicity and including nonpathogenic *F. oxysporum* commonly can be isolated from same or different cumin fields (Larkin and Fravel, 1998). Variation was reported in mycelial growth in addition to the pathogenicity in 9 isolates of *Fusarium oxysporum* f. sp. c*umini* pathogen in India (Champawat and Pathak, 1989). Wide range of pathogenicity was shown by Arafa (1985) for isolates from infested fields in Egypt (Assiut and El-Minia).**

As shown in Table (2), antagonistic capability of 20 fungal and 10 bacterial isolates against growth of the pathogen in vitro revealed different inhibitory effect. *Trichoderma harzianum* (F5), *T. humatum* (F9) and *T. viridae* (F14) fungi exhibited a mycoparasytism associated with high level of growth reduction

induced by their filterate on the pathogen (Table 3). Antagonism and/or mycoparasitism expressed during the *in vitro* tests for the bio-agents and the pathogen interaction (Hassan, 1992; Keinath, 1994). *Bacillus subtilis* (B7) was the most antagonistic bacterial isolate. Effective antagonistic reaction of *Trichoderma spp.*and *Bacillus subtilis* to the fusarium wilt causal pathogen in different plant species including horticultural crops has been widely documented (Larkin and Fravel, 1998; Taylor *et al.*, 1994). The mechanisms of antagonist to suppress the disease (De Boer *et al.*, 2003) include the production of antibiotics, inducing systematic resistance, siderophore-mediated competition for iron and competition for substrate.

Table (1): Pathogenicity test of 5 isolates of *Fusarium oxysporum* f. sp. *cumini* pathogen using 'Balady' cumin cultivar [1].

Isolate No.	Infection (%)	Isolate No.	Infection (%)
1	52.5	4	63.8
2	71.3	5	45.0
3	87.3		
$LSD_{0.05}$	5.93		

1 Average of two tests.

Table (2): In vitro assessment of antagonistic reaction of 20 fungal and 10 bacterial isolates against the growth of *Fusarium oxysporum* f. sp. *cumini* pathogen [1]

Isolate No.	Antagonistic reaction score	Isolate No.	Antagonistic reaction score
Fungal isolates			
F1	2	F11	2
F2	0	F12	0
F3	2	F13	0
F4	2	F14	4 [2]
F5	4 [2]	F15	1
F6	1	F16	0
F7	2	F17	2
F8	2	F18	1
F9	4 [2]	F19	2
F10	0	F20	2
Bacterial isolates			
B1	2	B6	2
B2	0	B7	3
B3	2	B8	2
B4	2	B9	1
B5	1	B10	2

1 Average of two tests; values from 0 to 3 express growth inhibition zones (clear spaces) where 0 expresses no inhibition, 1 < 10 mm (slight antagonism), 10 mm < 2 > 20 mm (moderate antagonism) and 3 > 30 mm (high antagonism).

2 Value of 4 expresses mycoparasytism (over growth) of the antagonistic bio-control agent.

Table (3): Assessment of growth reduction percentage of *Fusarium oxysporum* f. sp. *cumini* pathogen cultured on medium containing culture filtrate of three fungal isolates showed over growth during in vitro growth antagonistic test with the pathogen.

Antagonistic isolate Number and name	Growth reduction (%) of *Fusarium oxysporum* f. sp. *cumini* pathogen[1]
Trichoderma harzianum (isolate F#5)	88
Trichoderma humatum (isolate F#9)	86
Trichoderma viride (isolate F#14)	80
$LSD_{0.05}$ 4.1	

1 Average of two tests; values were calculated by subtracting the growth (colony diameter) of the pathogen cultured on medium with filtrate from the growth of the control (cultures on medium lacking filtrate) divided by the growth of the control and multiplied by 100.

Effect of biocontrol agents on incidence of cumin wilt and plant growth: No wilt symptoms were observed on the cumin plants raised from untreated seeds when grown in sterile soil (Table 4). On the other hand, untreated seeds grown in the infested soil (positive control) showed 90% to 95% infected plants. Comparing with the positive control, the in vitro identified antagonistic fungi (*T. harzianum, T. humatum* and *T. viride*) and bacterium (*Bacillus subtilis*) produced a significant lower percentage of infection. The used biocide 'Plant Guard' also produced lower infection percentage than the positive control. This result was consistently shown in both years. Among the five different seed treatments, the lowest percentage of infection was found in pre-sowing treatment with *T. harzianum*. Seed treatment with the antagonist *T. harzianum* also produced the highest percentage of reduction for the disease incidence with *T. humatum* being comparable to it in the second year.

Table (4): The percentage of infection *Fusarium oxysporum* f. sp. *cumini* and the percentage of reduction of the disease incidence for 'Baladi' cumin grown in infested soil as affected by seed pre-sowing treatments with fungal or bacterial antagonists, Assiut 1998/99 and 1999/2000.

Seed pre-sowing treatments	Infection [1] (%)	Reduction [2] (%)
Reference treatments	**1998/99**	
Negative control [3]	0.0 e	95.0 a
Positive control [4]	95.0 a	0.0 e
Antagonists		
Trichoderma harzianum (F#5)	52.5 d	44.7 b
T. humatum (F#9)	65.0 c	31.4 c
T. viride (F#14)	72.5 b	23.6 d
Bacillus subtilis (B#7)	65.0 c	31.4 c
Biocide 'Plant guard'	65.0 c	33.9 c
Reference treatments	**1999/2000**	
Negative control[3]	0.0 e	90.0 a
Positive control [4]	90.0 a	0.0 d
Antagonists		
Trichoderma harzianum (isolate F#5)	47.5 d	47.2 b
T. humatum (isolate F#9)	55.0 c	38.9 bc
T. viride (isolate F#14)	67.5 b	30.5 c
Bacillus subtilis (isolate B#7)	60.0 c	33.3 c
Biocide 'Plant guard'	60.0 c	33.3 c

1, 2 Showing wilt symptoms due to the pathogen and the reduction of the wilt disease relative to the positive control; means followed by the same letter(s) are not significantly different using Duncan's Multiple Rage Test at $P < 0.05$.

3, 4 Seeds were sown in sterile soil and infested soil, respectively.

The pre-sowing treatment of the seeds with *T. humatum* and *Bacillus subtils* did not significantly differ from each other or from the seed treatment with the biocide 'PG' with regard to the percentage of the infection and the reduction of the disease incidence. However, all these different seed treatments were inferior when compared with *T. harzianum*. *T. viride* seemed to be less effective concerning the percentage of cumin infection with the fusarium wilt pathogen and the reduction of the disease incidence than all other studied bio-control agents especially in the first year.

***Trichoderma* spp. particularly *T. harzianum* have been shown to be effective against *Fusarium oxysporum* of tomato (Datnoff *et al.*, 1995; Marois *et al.*, 1981). However, other researchers in tomato have reported that *T. humatum* was more effective in reducing the disease than *T. harzianum* and *T. viride* (Larkin and Fravel, 1998). This may be attributed to difference due to biological variation of isolates as Patel and Patel (1998) found differential inhibitory effect of three isolates of *T. harzianum* obtained from different regions in India. Interesting, however, is the superiority of *T. harzianum* over the biocide 'Plant Guard' which has *T. harzianum* in its ingredient. Cook (1993) suggested that isolates from root or rhizosphere of a specific crop may be better adapted to that crop and provide better control of the disease than isolates from other crop species. Because the concentrations used from both *T. harzianum* and 'Plant Guard' were similar, it is assumed that our isolate of the *T. harzianum* is more adapted to cumin and against its wilt pathogen. Screening of such locally adapted strains of antagonists has been reported to improve the efficiency of bio-control in some cases (Cook, 1993).**

Comparing with the sterile soil (negative control), cumin plants grown in infested soil (positive control) were significantly shorter in both years (Table 5). Pre-sowing treatment of cumin seeds with the different antagonists and with the biocide 'Plant Guard' produced plants with similar height to those of the negative control in both years. Plant growth as determined by fresh and dry weight parameters in each of the two years showed similar results giving two separate groups of plants. One group include plants grown in sterile soil (negative control) and plants grown in infested soil but treated with the isolated antagonistic microorganisms or the biocide. The other group contained the untreated seeds when grown in infested soil (positive control). Obviously, the former group of treatments produced cumin plants with enhanced growth. Growth enhancement by biological control methods has been reported in other crop species (Chang *et al.* 1986; Linderman 1994). In the present study, the growth enhancement relative to the positive control ranged (on average for the 2 years) from 163 to 172% for fresh weight. Such average enhancement ranged from 166 to 173% for the dry weight. It is suggested, therefore, that all pre-sowing treatments similarly provided cumin with favorable conditions to grow once the disease is controlled.

Correlation coefficients (Table 6) summarized the overall interrelations among the different growth parameters and between them and the percentage of infection. The growth parameters (plant length and fresh and dry weight) are positively correlated such that an increase of any of them is associated with an increase in the other ones. As implicated by the negative correlation between the percent of infection and each of the studied growth parameters, reduction in the wilt disease incidence improved the plant growth. Out of the present study using bioassay in the potting soil culture, its suggested that application of pre-

sowing treatments with antagonistic microorganisms could produce a great impact in improving cumin stand and consequently seed yield. Therefore, a complementary trial has been considered in wilt-disease infested production field (Tawfik and Allam, 2004).

Of special interest in this study is the possibility of isolating efficient bio-control agents based on the bio-analysis of microorganisms associated with local cumin cultures. This research is considered, therefore, a significant step toward finding an efficient alternative strategy for the management of Fusarium wilt disease of cumin. Further manipulations based on such approach may lead to isolation of more superior bio-control agents towards the development of sustainable environment friendly crop production systems in Egypt.

Table (5) : The percentage of infection with *Fusarium oxysporum* f. sp. *cumini* and plant growth (length and fresh and dry weight) of 'Baladi' cumin grown in infested soil as affected by seed pre-sowing treatments with fungal or bacterial antagonists, Assiut 1998 and 1999

Seed pre-sowing treatments	Plant length (cm) [1]	Fresh weight (g/plant) [1]	Dry weight (mg/plant) [1]
Reference treatments	**1998**		
Negative control [2]	19.5 a	2.84 a	337.2 a
Positive control [3]	14.9 d	1.66 b	196.5 b
Antagonists			
Trichoderma harzianum (F#5)	18.9 a	2.86 a	328.8 a
T. humatum (F#9)	17.6 a	2.65 a	326.5 a
T. viride (F#14)	17.5 a	2.65 a	335.3 a
Bacillus subtilis (B#7)	17.6 a	2.68 a	324.3 a
Biocide 'Plant guard'	18.7 a	2.77 a	336.1 a
Reference treatments	**1999**		
Negative control [2]	22.7 a	3.06 a	374.8 a
Positive control [3]	15.5 b	1.69 b	217.3 b
Antagonists			
Trichoderma harzianum (F#5)	21.5 a	2.98 a	380.8 a
T. humatum (F#9)	21.8 a	2.90 a	374.8 a
T. viride (F#14)	20.9 a	2.87 a	371.5 a
Bacillus subtilis (B#7)	22.6 a	2.81 a	363.2 a
Biocide 'Plant guard'	22.4 a	2.91 a	370.1 a

1 Means followed by the same letter(s) are not significantly different using Duncan's Multiple Rage Test at P < 0.05.
2, 3 Seeds were sown in sterile soil and infested soil, respectively.

Table (6): Correlations coefficients (r) between the percentage of fusarium wilt infection of cumin and each of plant growth parameters (length and fresh and dry weight), and among these growth parameters [1].

Parameters	Infection %	Plant length	Fresh weight	Dry weight
Infection %		-0.816* [2]	-0.655	-0.588
Plant length	-0.637 *		0.941** [3]	0.882 **
Fresh weight	-0.679*	0.964 **		0.981 **
Dry weight	-0.595	0.955 **	0.993 **	

1 Upper right diagonal presents correlation coefficients obtained in 1998 and the lower left diagonal shows these values in 1999.
2, 3 Significant at P < 0.05 and P < 0.01, respectively.

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Ass. Univ. Bull. Environ. Res. Vol. 7 No. 2, October 2004

تحسين إنتاج الكمون تحت ظروف عدوى التربة بالذبول الفيوزارمى: ١- انتقاء عوامل للمقاومة الحيوية

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أجريت هذه الدراسة بغرض تقييم التأثير المضاد لبعض أنواع الفطريات والبكتريا المعزولة من بيئة جذور الكمون المصاب بالذبول الفيوزارمى على تقليل الإصابة بالمرض وعلى تحسين نمو نباتات الكمون، وذلك كمدخل لإيجاد مبيد حيوى فعال ضد فيوزاريم الكمون وآمن للبيئة. تم الحصول على ٢٠ عزلة من الفطريات و ١٠ عزلات من البكتريا والتى وجد من بينها ٣ عزلات فطرية وعزلة واحدة بكتيرية ذات تأثير مضاد قوى على نمو المسبب المرضى للفيوزاريم فى الكمون على بيئة اجار البطاطس. وعند تعريفها وجد أن العزلات الفطرية كانت من جنس ترايكوديرما والأنواع التالية (هاريزيانم، هاماتم، فيردى) بينما كانت البكتريا" باسلس سابتلس". تلى ذلك عمل معلقات من هذه الفطريات والبكتريا لتقييم تأثيرها على تقليل الإصابة بالمرض وعلى نمو الكمون. وكان ذلك فى تجربة قطاعات كاملة عشوائية ضمت بالإضافة لمعاملات البذور بالمعلق للفطريات والبكتريا المعزولة السابقة كل من البذور المعاملة بالمبيد الفطرى الحيوى والبذور بدون معاملة كمعاملة مقارنة موجبة كذلك استخدم بذور كمون بدون معاملة، ولكن فى تربة معقمة خالية من المسبب المرضى كمعاملة مقارنة سالبة. وفى هذه التجربة تم تسجيل بيانات عن نسبة الإصابة لنباتات الكمون والتى منها حسبت نسبة تقليل الإصابة منسوبة لمعاملة المقارنة الموجبة. كما تم أيضا أخذ قياسات على طول نبات الكمون والوزن الطازج والجاف وتم تحليل جميع البيانات إحصائياً واتضح أن معاملة البذور بمعلق الفطريات أو البكتريا أو المبيد الفطرى الحيوى أدى إلى انخفاض معنوى فى نسبة الإصابة بالمرض، وكان أكثر المعاملات فعالية هى معاملة البذور بمعلق فطر الترايكوديرما هاريزيانم حيث أعطى أقل نسبة إصابة وأعلى نسبة تقليل للمرض مقارنة بباقى معاملات الفطريات أو البكتريا أو المبيد الفطرى الحيوى. وكان نمو نباتات الكمون (طول النبات والوزن الجاف والطازج) أفضل معنويا عند استخدام أيا من معاملات البذور مقارنة بالمعاملة المقارنة الموجبة.

وبناء على النتائج السابقة فإنه قد تم استنتاج أنه يمكن أن يكون لفطر الترايكودرما هاريزيانم الذى تم عزله فى هذه الدراسة دور فعال فى تحسين إنتاج الكمون بالأراضى الموبوءة بالذبول الفيوزامى فى صعيد مصر بما يحسن المحصول كماً ونوعاً، ويشكل فى نفس الوقت وسيلة مقاومة آمنة بيئياً.