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: II- FIELD TRIAL OF DIFFERENT LANDRACES AND SEED TREATMENTS

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ABSTRACT :

Two landraces of the 'Balady' cumin (Qina and Assiut) and seven seed treatments were tested in this field study. The treatments included two fungal antagonists (*Trichoderma harzianum,* and *T. humatum)*, one bacterial antagonist (*Bacillus subtilis*), 2-days water priming, combined water priming with *T. harzianum*, biocide 'PlantGuard' and control seed treatment. The fungi and the bacteria were isolated from infected cumin plants collected from Assiut. A significantly reduced percentage of infection and increased seed yield/plant than the control occurred in both years as a result of pre-sowing seed treatments. The lowest percentage of infection was exhibited by the plants raised from seeds that received combined treatment of two-day water-priming and *T. harzianum*. Use of *T. harzianum* alone or in combination with water priming tended to produce a high seed yield/plant in both years. The increased seed yield was significantly associated with decreased percentage of infection in the two landraces and in both years. Significant positive correlation coefficients were found between the seed yield and each of the number of the main and secondary branches, the number of umbels and the weight of the mature dry plants. The cumin landrace from Qina showed an overall lower percentage of infection and higher seed yield/plant than Assiut landrace in both years. We recommend utilizing combined 2-days water priming with *T. harzianum* antagonist for pre-sowing seed treatment. This treatment seemed to combine complementary mechanisms of action against the pathogen enabling a consistent control of the disease. It would establish a better plant stand in the field and, consequently, increased seed yield per production area unit.

INTRODUCTION:

Cumin (*Cuminum cyminum* L.) is an annual plant belongs to Family Apiaceae and it is grown for production of the dry ripe fruits. It was known to the ancient Egyptian as a spice and medicinal plant. In addition to its common use as spice in our daily life, recent studies have indicated its pharmaceutical and medicinal importance (Aruna and Sivaramakrishnan, 1996). Cumin is produced in the warm regions of the world, mainly in India, China and southern Egypt. There has been a recent increased demand on cumin while its production is limited and decreased (Abu-Nahoul and Ismail, 1995). Significant lose in cumin yield can be attributed to the adverse effects caused by biotic stresses of which the

Fusarium wilt disease is the most serious one (Omar *et al.,* 1997). The chlamidiospores of Fusarium may persist in soil for many years in the absence of a susceptible host (Agrios, 1991). Traditional control measures of the Fusarium wilt disease include crop rotation, pre-sowing treatments for the seeds with certain chemical fungicides and management of cultural practices (Agrios, 1991) or soil fumigation (Larkin and Fravel, 1998). Due to the limited effectiveness or applicability and/or environmental concerns using these methods in controlling the disease, there is an interest to search for other approaches of methods (Alabouvette *et al.*, 1998; Weller, 1988).

Breeding of resistant cultivars is desirable approach for sustainable agriculture but studies in this direction (Champawat and Pathak, 1990; Gour and Agrawal, 1988) showed a limited genetic variation among different accessions. Therefore, in vitro methods as a prerequisite for creating new genetic variability or developing genetically modified resistant cultivars with gene introgression from other species have been manipulated (Tawfik and Noga, 2001a and 2002). A recent study by Mandavia *et al.*, (2000), however, have indicated an existence of differential susceptibility within a number of cumin varieties in India. Some lines have been described as being tolerant to the infection with the Fusarium wilt of cumin. The growers with no practicing of plant rouging produce seeds of the local cultivar 'Baladi' cumin in southern regions of Egypt. Therefore, this cultivar can be considered a variety of different landraces developed mainly by natural selection against biotic and abiotic stresses under the different conditions of various regions and fields of production (Chang, 1985). The different landraces of the Egyptian cumin may be useful in improving the production of this cultivar (Ehdaie and Waines, 1989). Information is needed on the potential of this approach in cumin.

Analysis of root, stem and leaf tissues of tolerant cumin varieties (Mandavia *et al.*, 2000) suggested that tolerance can be attributed to higher level of phenolic compounds such as hydroquinone and umbelliferone. Of a special interest is the increase found for salicylic acid in the tolerant cumin varieties. It is widely documented in higher plants that different phenolic compounds (Hassan, 1994), salicylic acid (Raskin, 1992) and several antioxidant compounds and/or enzymes (Noga and Schmitz, 1998) induces a level tolerance to biotic and abiotic stresses. Noticeable is that seed osmopriming has been suggested to enhance the production of antioxidant compounds and/or enzymes such superoxide dismutase (SOD) (Baily *et al.*, 2000). Tawfik and Noga (2001) developed a simple water priming method for cumin seeds and have found a dramatic improvement in acceleration of cumin germination and plant vigor using water-primed seeds. Pre-sowing water priming for cumin seeds can be hypothesized to improve tolerance to certain pathogens. This may be useful to be tested for controlling Fusarium wilt disease incidence in cumin.

In addition, one of the other approaches that are progressively developed in complying with sustainable environment issues during recent years is the biological control method (Hassan, 1992; Hassan and Tawfik, 1996; Larkin *et al.*, 1996; Weller, 1998; Whipps, 1997). Indeed, biocides patents has been deposited (Kurze *et al.*, 2001) and commercially delivered. Primary data obtained from potting bioassay of pre-sowing treatments of cumin seeds with suspension of antagonistic fungal (*Trichoderma harzianum, T. humatum,* and *T. viride*) and bacterial (*Bacillus subtilis*) isolates showed up to 47% reduction in the wilt disease

incidence (Tawfik and Allam 2004). A local isolate of *T. harzianum* from cumin grown in southern Egypt was shown to be superior over other isolates and commercial formulation. This needs to be tested under the complex of the natural conditions in the infested production field (De Boer *et al.*, 1999). The objective of our present field study was to assess the incidence of the Fusarium wilt disease and the seed yield of two cumin landraces from southern Egypt (Assiut and Qina) combined with pre-sowing seed treatments including water priming and biological antagonists.

MATERIALS AND METHODS:

Plant materials and seed treatments:

The present study was conducted at the Agricultural Research Station of Assiut University during 2002/2003 and 2003/2004. Two landraces of the 'Balady' cumin were used in this study. These two landraces were obtained from growers of two Governorates (Qina and Assiut) in southern Egypt. Seven seed treatments were tested in this study for their effectiveness on reducing the incidence of Fusarium wilt disease. The treatments included two fungal antagonists, one bacterial antagonist, 2-days water priming, combined water priming with an antagonist, biocide 'Plant Guard' (commercial formulation, check treatment) and control seed treatment.

The seed treatments with fungal and bacterial antagonists were assigned based on a previous screening conducted both in vitro and in potting soil bioassay (Tawfik and Allam, 2004). The utilized fungal antagonists were *Trichoderma harzianum,* and *T. humatum*. The bacterial antagonist was *Bacillus subtilis*. The inoculum of the pathogen was prepared as described by Tawfik and Allam (2004). In brief, inocula of fungal antagonists were grown in liquid Gliotoxin Fermentation medium while the 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 (1.5 X10^5). Cumin seeds were soaked in the suspension of the antagonists or the biocide for 15 minutes while the control seeds were soaked in distilled water for 15 min.

The procedure of the two-days water priming for cumin seeds was carried out as described by Tawfik and Noga (2001) with minor modifications. Briefly, the seeds were soaked for 1 hour in distilled water. Then these seeds were maintained within paper towels saturated with distilled water and kept for 2 days at room temperature (on average, 26 °C) inside punched plastic pages. Combined water priming with antagonist was achieved by soaking the seeds at the end of the second day of water priming in the suspension of *T. harzianum* antagonist for 15 min. All treated seeds with each antagonist and priming or combined priming/antagonist were then placed to air dry that is to enable conventional handling for sowing.

Experiment layout and the measured parameters:

The experiment was conducted in naturally infested production field. The treatments in the field were arranged as a split-plot in randomized complete-blocks (RCB) with four replicates. The main plots were assigned for the seven pre-sowing seed treatments. Each main plot (seed treatment) contained the two landraces (Qina and Assiut, subplots). The choice of seed treatments in the main plots was to avoid possible contamination among different

seed treatments with different fungal or bacterial antagonists. Also, no information was available on the reaction of the two landraces while we had preliminary knowledge about the differential efficiency of antagonists (Tawfik and Allam, 2004) as shown by the in vitro and the potting soil bioassays.

Each main plot consisted of four rows 3 m long and 50 cm wide. Seeds were sown on the northern side of the row and each landrace (sub-plot) had two rows. The experiment was conducted twice (2002/2003 and 2003/2004). The sowing dates were on November 3 during 2002 and on November 8 during 2003. Standard cultural practices for cumin production were followed during the course of this study (Abu-Zaid, 1992). Data were recorded for the percentage of infected plants. The harvested plants were used to determine the plant height (cm), the number of main and secondary branches per plant, average herbal weight (g) of mature dry plant, the number of umbels per plant, the average 1000-seed weight (g) and seed yield (g) per plant.

Statistical analysis and data presentation:

Separate analysis of variance (ANOVA) relevant to split-plot in RCB design for the obtained data in each year (2002/2003 or 2003/2004) was conducted as described by Gomez and Gomez (1984). Based on the values of the coefficients of variation (C.V.), the original data were used for the ANOVA of all measured plant parameters. Homogeneity of error variances was then tested. Subsequently, combined ANOVA for the data of both years was processed to get the effect of years and the year interaction with the tested treatments. In the present study, means for each year was separately presented. Based on the results of significance for the interaction between landraces and the seed treatments (Gomez and Gomez, 1984), two values for the 'Least Significant Difference' (LSD) were calculated. These LSD values were to compare 1) means of the main effect of seed treatments (i.e., averaged overall landraces) and 2) means of the main effect due to the landrace (averaged overall seed treatments). Total correlation coefficients (r) were also calculated between the total seed yield and each of the other measured parameters.

RESULTS:

Disease incidence:

A significant reduction in the percentage of infection with the Fusarium wilt occurred in both years as a result of pre-sowing treatment of the cumin seeds (Table 1). Both landraces exhibited a similar response for the reduction in the percentage of infection as affected by the pre-sowing seed-treatments. Therefore, the variance due to landrace and seed treatment interaction was not significant. However, cumin landrace from Qina showed an overall lower percentage of infection than the landrace from Assiut.

There were no differences among *T. harzianum*, *Bacillus subtilis* and the tested biocide concerning the percentage of the infection with the wilt disease. Lower percentage of the disease infection was produced by seed treatment with the *T. harzianum*, *Bacillus subtilis* and the biocide than *T. humatum* and the two days water-priming. The percentage of infection with the fusarium wilt was similar in the cumin plants produced from seeds treated with *T. humatum* and the two days water-priming. However, the lowest percentage of infection with the disease was exhibited by the cumin plants raised from seeds that received combined treatment with two-day water-priming and *T. harzianum*.

Table (1): Percentage of infected cumin plants for two landraces grown in Fusarium wilt infested soil when received different pre-sowing seed treatments.[1]

Factors	Infection (%)		
	Landrace		Mean
	Qina	Assiut	
Seed treatments	**2002/2003**		
A- Reference treatments			
Control	72.5	80.0	76.3
Biocide ('Plant Guard')	47.5	50.0	48.8
B- Two days water priming	60.0	67.5	63.8
C- Tested antagonists			
Trichoderma harzianum	47.5	50.0	48.8
T. humatum	55.0	60.0	57.5
Bacillus subtilis	47.5	50.0	48.8
D- Combined water-priming/*T. harzianum*	25.0	32.5	28.8
Mean	50.7	55.7	
$LSD_{0.05}$			
Seed treatments[2]	7.0		
Landraces[3]	3.8		
Interaction	ns[4]		
	2003/2004		
A- Reference treatments			
Control	75.0	85.0	80.0
Biocide ('Plant Guard')	50.0	50.0	50.0
B- Two days water priming	57.5	67.5	62.5
C- Tested antagonists			
Trichoderma harzianum	47.5	52.5	50.0
T. humatum	57.5	60.0	58.5
Bacillus subtilis	50	55.0	52.5
D- Combined water-priming/*T. harzianum*	27.5	37.5	32.5
Mean	52.1	58.2	
$LSD_{0.05}$			
Seed treatments[2]	7.5		
Landraces[3]	3.5		
Interaction	ns[4]		
Combined Significance			
Year effect = ns & Year X treatments = ns			

1 Seed pre-sowing treatment with the biocide and the antagonists were by soaking for 15 min while the control seeds were soaked in distilled water.

2,3 To compare means of seed treatments averaged over landraces and means of landraces averaged over seed treatments, respectively.

4 Nonsignificance.

Plant growth and development:

An increased plant height over the control was found during the first year in cumin plants that received a pre-sowing using *T. harzianum* alone or when preceded by a 2-days water priming (Table 2). These two seed treatments were not significantly different. None of the other seed treatments significantly affected the plant height when comparing to the control treatment. In the second year, seeds treated with the *T. harzianum*, the biocide, primed for 2 days with water or received combined priming and *T. harzianum* treatment produced plants showing significant increased plant height over the control. No differences were found among all these treatments. Difference due to the landrace and the variance for the interaction between landrace and the seed treatments were not significant for plant height in both years.

Table (2): Plant height of two cumin landraces grown in Fusarium wilt infested soil when received different pre-sowing seed treatments.[1]

Factors	Plant height (cm)		
	Landrace		Mean
	Qina	Assiut	
Seed treatments	**2002/2003**		
A- Reference treatments			
Control	16.2	16.7	16.5
Biocide ('Plant Guard')	17.8	17.8	17.8
B- Two days water priming	17.4	17.1	17.2
C- Tested antagonists			
Trichoderma harzianum	17.8	19.8	18.8
T. humatum	15.9	16.3	16.1
Bacillus subtilis	16.7	17.3	16.9
D- Combined water-priming/*T. harzianum*	18.9	20.6	19.7
Mean	17.2	16.9	
$LSD_{0.05}$			
Seed treatments[2]	1.5		
Landraces	Ns[3]		
Interaction	Ns		
	2003/2004		
A- Reference treatments			
Control	15.7	15.9	15.8
Biocide ('Plant Guard')	17.5	17.6	17.5
B- Two days water priming	18.2	17.1	17.6
C- Tested antagonists			
Trichoderma harzianum	17.0	19.0	18.0
T. humatum	16.2	15.8	15.9
Bacillus subtilis	16.1	16.6	16.3
D- Combined water-priming/*T. harzianum*	18.4	19.8	19.1
Mean	17.0	17.4	
$LSD_{0.05}$			
Seed treatments[2]	1.6		
Landraces	Ns[3]		
Interaction	Ns		
Combined Significance			
Year effect = ns & Year X treatments = ns			

1 Seed pre-sowing treatment with the biocide and the antagonists were by soaking for 15 min while the control seeds were soaked in distilled water.
2 To compare means of seed treatments averaged over landraces.
3 Nonsignificance.

Significantly greater number of main branches was developed on cumin plants raised from seeds treated with the biocide, *T. harzianum*, water priming or combined priming and *T. harzianum* in both years than the control treatment (Table 3). Seed treatment with *T. humatum* did not affect the number of main branches in both years while the treatment using *B. subtilis* showed an increased number of main branches only in the second year. Obviously, the greatest number of main branches was produced by treatments of *T. harzianum* or combined priming and *T. harzianum* in both years. These two treatments along with the water priming alone had the greatest number of main branches in the second year. The number of secondary branches increased in cumin plants grown during both

years by seeds that received treatments with the biocide, *T. harzianum* or the combined priming and *T. harzianum* (Table 4). Consistently, *T. harzianum* and the combined water priming with *T. harzianum* treatment had the greatest number of secondary branches in both year. The two landraces were similar with regard to the number of the main and the secondary branches. Variance component of the interaction between the seed treatments and the landraces was not significant for these traits in both years.

Table (3) : The number of main branches per cumin plant in two landraces grown in Fusarium wilt infested soil when received different pre-sowing seed treatments.[1]

Factors	Number of main branches/plant		
	Landrace		
	Qina	Assiut	Mean
Seed treatments	2002/2003		
A- Reference treatments			
Control	4.4	3.6	4.0
Biocide ('Plant Guard')	5.0	4.9	4.9
B- Two days water priming	5.0	4.8	4.9
C- Tested antagonists			
Trichoderma harzianum	4.7	5.6	5.2
T. humatum	4.2	4.3	4.2
Bacillus subtilis	4.5	4.5	4.5
D- Combined water-priming/*T. harzianum*	5.7	5.9	5.8
Mean	4.8	4.8	
$LSD_{0.05}$			
Seed treatments[2]	0.7		
Landraces	ns[3]		
Interaction	Ns		
	2003/2004		
A- Reference treatments			
Control	4.1	3.3	3.7
Biocide ('Plant Guard')	4.7	4.7	4.7
B- Two days water priming	5.2	4.7	5.0
C- Tested antagonists			
Trichoderma harzianum	4.7	5.1	4.9
T. humatum	3.9	4.1	4.0
Bacillus subtilis	4.3	4.4	4.3
D- Combined water-priming/*T. harzianum*	5.4	5.4	5.4
Mean	4.6	4.5	
$LSD_{0.05}$			
Seed treatments[2]	0.6		
Landraces	ns[3]		
Interaction	Ns		
Combined Significance			
Year effect = ns & Year X treatments = ns			

1 Seed pre-sowing treatment with the biocide and the antagonists were by soaking for 15 min while the control seeds were soaked in distilled water.
2 To compare means of seed treatments averaged over landraces.
3 Nonsignificance.

Table (4): The number of secondary branches per cumin plant in two landraces grown in Fusarium wilt infested soil when received different pre-sowing seed treatments.[1]

Factors	Number of secondary branches/plant		
	Landrace		Mean
	Qina	Assiut	
Seed treatments	**2002/2003**		
A- Reference treatments			
Control	**8.5**	**7.7**	**8.1**
Biocide ('Plant Guard')	**10.0**	**9.6**	**9.8**
B- Two days water priming	**9.2**	**9.1**	**9.1**
C- Tested antagonists			
Trichoderma harzianum	**9.8**	**11.6**	**10.7**
T. humatum	**8.5**	**8.8**	**8.6**
Bacillus subtilis	**9.0**	**9.3**	**9.1**
D- Combined water-priming/*T. harzianum*	**11.0**	**11.3**	**11.2**
Mean	**9.4**	**9.6**	
$LSD_{0.05}$			
Seed treatments[2]	**1.1**		
Landraces	**ns[3]**		
Interaction	**Ns**		
	2003/2004		
A- Reference treatments			
Control	**7.9**	**7.0**	**7.5**
Biocide ('Plant Guard')	**9.6**	**9.3**	**9.4**
B- Two days water priming	**9.4**	**8.7**	**9.1**
C- Tested antagonists			
Trichoderma harzianum	**9.7**	**10.9**	**10.3**
T. humatum	**8.1**	**8.5**	**8.3**
Bacillus subtilis	**8.7**	**8.9**	**8.8**
D- Combined water-priming/*T. harzianum*	**10.5**	**10.9**	**10.8**
Mean	**9.1**	**9.2**	
$LSD_{0.05}$			
Seed treatments[2]	**1.2**		
Landraces	**ns[3]**		
Interaction	**Ns**		
Combined Significance			
Year effect = ns & Year X treatments = ns			

1 Seed pre-sowing treatment with the biocide and the antagonists were by soaking for 15 min while the control seeds were soaked in distilled water.
2 To compare means of seed treatments averaged over landraces.
3 Nonsignificance.

The weight of mature dry plants after harvesting their dry mature seeds (fruits) was affected by both the landrace and the pre-sowing seed treatment (Table 5). The interaction between these two factors was not significant. Cumin landrace form Qina seemed to have plants with a significantly increased weight comparing with that landrace of Assiut. Plants from seeds treated with the biocide, *T. harzianum*, water for priming, or combination of water priming and *T. harzianum* had greater weight than the control treatment in both years. There were no differences among these treatments in the second year. The number of umbels counted on the mature plants was affected only by the seed treatments (Table 6). All seed treatments resulted in a significantly increased number of umbels over the control in both years. Among the different seed treatments, *T. harzianum* and water priming with subsequent *T. harzianum* treatment tended to produce large number of umbels in both years.

Table (5): The weight of mature dry cumin plants for two landraces grown in Fusarium wilt infested soil when received different pre-sowing seed treatments.[1]

Factors	Weight of mature dry plant (g/plant)		
	Landrace		Mean
	Qina	Assiut	
Seed treatments	**2002/2003**		
A- Reference treatments			
Control	**1.65**	**0.865**	**1.257**
Biocide ('Plant Guard')	**2.185**	**1.710**	**1.948**
B- Two days water priming	**2.135**	**1.850**	**1.993**
C- Tested antagonists			
Trichoderma harzianum	**1.978**	**2.415**	**2.196**
T. humatum	**1.793**	**1.580**	**1.686**
Bacillus subtilis	**1.875**	**1.500**	**1.687**
D- Combined water-priming/*T. harzianum*	**2.352**	**2.553**	**2.452**
Mean	**1.995**	**1.782**	
$LSD_{0.05}$			
Seed treatments[2]	**0.448**		
Landraces[3]	**0.203**		
Interaction	**Ns[4]**		
	2003/2004		
A- Reference treatments			
Control	**1.525**	**0.765**	**1.145**
Biocide ('Plant Guard')	**2.135**	**1.585**	**1.860**
B- Two days water priming	**2.085**	**1.775**	**1.930**
C- Tested antagonists			
Trichoderma harzianum	**1.852**	**2.165**	**2.009**
T. humatum	**1.568**	**1.430**	**1.499**
Bacillus subtilis	**1.750**	**1.475**	**1.612**
D- Combined water-priming/*T. harzianum*	**2.127**	**2.303**	**2.215**
Mean	**1.863**	**1.642**	
$LSD_{0.05}$			
Seed treatments[2]	**0.523**		
Landraces[3]	**0.220**		
Interaction	**ns[4]**		
Combined Significance			
Year effect = ns & Year X treatments = ns			

1 Seed pre-sowing treatment with the biocide and the antagonists were by soaking for 15 min while the control seeds were soaked in distilled water.

2,3 To compare means of seed treatments averaged over landraces and means of landraces averaged over seed treatments, respectively.

4 Nonsignificance.

Table (6): The number of umbels per plant for two cumin landraces grown in Fusarium wilt infested soil when received different pre-sowing seed treatments.[1]

Factors	Number of umbels/plant		
	Landrace		Mean
	Qina	Assiut	
Seed treatments	**2002/2003**		
A- Reference treatments			
Control	17.4	12.1	14.8
Biocide ('Plant Guard')	19.6	19.1	19.3
B- Two days water priming	19.8	17.9	18.6
C- Tested antagonists			
Trichoderma harzianum	19.1	23.6	21.4
T. humatum	17.7	18.3	18.0
Bacillus subtilis	19.7	18.2	19.0
D- Combined water-priming/*T. harzianum*	20.8	22.0	21.4
Mean	19.2	18.7	
$LSD_{0.05}$			
Seed treatments[2]	2.6		
Landraces	Ns[3]		
Interaction	Ns		
	2003/2004		
A- Reference treatments			
Control	16.1	10.9	13.5
Biocide ('Plant Guard')	18.9	18.8	18.8
B- Two days water priming	20.1	17.4	18.7
C- Tested antagonists			
Trichoderma harzianum	18.8	21.9	20.4
T. humatum	17.1	17.6	17.3
Bacillus subtilis	18.9	17.8	18.4
D- Combined water-priming/*T. harzianum*	20.3	20.5	20.4
Mean	18.6	17.8	
$LSD_{0.05}$			
Seed treatments[2]	2.2		
Landraces	Ns[3]		
Interaction	Ns		
Combined Significance			
Year effect = ns & Year X treatments = ns			

1 Seed pre-sowing treatment with the biocide and the antagonists were by soaking for 15 min while the control seeds were soaked in distilled water.
2 To compare means of seed treatments averaged over landraces.
3 Nonsignificance.

Average weight of 1000-seeds and the seed yield:

Greater average 1000-seed weight was affected with the landrace and the pre-sowing seed treatment (Table 7). The Qina landrace exhibited greater weight than Assiut landrace in both years. All seed bio- and priming treatments in the first year while all except the biocide and *B. subtilis* in the second year produced greater

1000-seed weight than the control one. There were no differences among the various biocontrol and priming treatments of the seeds. As shown in Table (8), variation in cumin seed yield per plant was affected by both the landrace and the pre-sowing seed treatments. The Qina landrace produced higher seed yield than Assiut landrace in both years. Also, all seed treatments increased the seed yield. The greatest seed yield in the first year was produced by *T. harzianum* and water primed seeds when treated with *T. harzianum*. In the second year, there was no significant difference among the different biocontrol or priming treatments for the seeds before sowing.

Table (7): Average weight of 1000-seed for two cumin landraces grown in Fusarium wilt infested soil when received different pre-sowing seed treatments.[1]

Factors	1000-seed weight (g)		
	Landrace		Mean
	Qina	Assiut	
Seed treatments	**2002/2003**		
A- Reference treatments			
Control	2.670	2.238	2.454
Biocide ('Plant Guard')	2.777	2.530	2.654
B- Two days water priming	2.845	2.650	2.747
C- Tested antagonists			
Trichoderma harzianum	2.825	2.787	2.806
T. humatum	2.873	2.745	2.809
Bacillus subtilis	2.740	2.665	2.703
D- Combined water-priming/*T. harzianum*	2.868	2.738	2.803
Mean	2.800	2.622	
$LSD_{0.05}$			
Seed treatments[2]	0.191		
Landraces[3]	0.100		
Interaction	Ns[4]		
	2003/2004		
A- Reference treatments			
Control	2.545	2.167	2.356
Biocide ('Plant Guard')	2.627	2.555	2.591
B- Two days water priming	2.945	2.625	2.785
C- Tested antagonists			
Trichoderma harzianum	2.900	2.742	2.821
T. humatum	2.773	2.645	2.709
Bacillus subtilis	2.640	2.515	2.577
D- Combined water-priming/*T. harzianum*	2.743	2.663	2.703
Mean	2.739	2.559	
$LSD_{0.05}$			
Seed treatments[2]	0.271		
Landraces[3]	0.122		
Interaction	Ns[4]		
Combined Significance			
Year effect = ns & Year X treatments = ns			

1 Seed pre-sowing treatment with the biocide and the antagonists were by soaking for 15 min while the control seeds were soaked in distilled water.

2,3 To compare means of seed treatments averaged over landraces and means of landraces averaged over seed treatments, respectively.

4 Nonsignificance.

Table (8): Total seed yield per plant for two cumin landraces grown in Fusarium wilt infested soil when received different pre-sowing seed treatments.[1]

Factors	Total seed yield (g/plant)		
	Landrace		
	Qina	Assiut	Mean
Seed treatments	2002/2003		
A- Reference treatments			
Control	1.85	1.1	1.48
Biocide ('Plant Guard')	2.43	2.14	2.28
B- Two days water priming	2.49	1.79	2.14
C- Tested antagonists			
Trichoderma harzianum	2.46	2.32	2.39
T. humatum	2.17	1.99	2.08
Bacillus subtilis	2.36	2.00	2.18
D- Combined water-priming/*T. harzianum*	2.88	2.44	2.66
Mean	2.38	1.97	
$LSD_{0.05}$			
Seed treatments[2]	0.29		
Landraces[3]	0.15		
Interaction	Ns[4]		
	2003/2004		
A- Reference treatments			
Control	1.78	0.98	1.38
Biocide ('Plant Guard')	2.36	2.08	2.22
B- Two days water priming	2.35	1.74	2.04
C- Tested antagonists			
Trichoderma harzianum	2.33	2.17	2.25
T. humatum	2.07	2.14	2.11
Bacillus subtilis	2.23	1.85	2.04
D- Combined water-priming/*T. harzianum*	2.66	2.29	2.47
Mean	2.25	1.89	
$LSD_{0.05}$			
Seed treatments[2]	0.44		
Landraces[3]	0.19		
Interaction	Ns[4]		
Combined Significance			
Year effect = ns & Year X treatments = ns			

1 Seed pre-sowing treatment with the biocide and the antagonists were by soaking for 15 min while the control seeds were soaked in distilled water.

2,3 To compare means of seed treatments averaged over landraces and means of landraces averaged over seed treatments, respectively.

4 Nonsignificance.

Seed yield and the percentage of infection with the disease were significantly and negatively correlated in both landraces and years (Table 9). Significant positive correlation coefficients were found between the seed yield and each of the number of the main and secondary branches, the number of umbels and the weight of the mature dry plants. Correlation coefficients were also positive between the seed yield and the 1000-seed weight but achieved significance only in Assiut landrace. Correlations between the seed yield and plant height were fluctuating. Correlation coefficient showed significant positive value in Qina landrace in the first year but was rather low and insignificant in the second year. Also, the coefficient of correlation was positive in the first year for Assiut landrace but negative in the second year.

Table (9): Correlations coefficients between seed yield and the percentage of infection with the Fusarium wilt disease in cumin and between the seed yield and some of its main components for two landraces grown in two years.

Trait	Total seed yield/plant			
	Landrace		Landrace	
	Qina	Assiut	Qina	Assiut
	2002/2003		2003/2004	
Infection percentage	-0.895 **	-0.928 **[2]	-0.933 **	-0.916 **
Plant height	0.891 **	0.711	0.394	-0.358
Main branches	0.862 *[1]	0.894 **	0.848 *	0.831 *
Secondary branches	0.887 **	0.881 **	0.948 **	0.837 *
Mature plant weight	0.930 **	0.889 **	0.873 *	0.827 *
Number of umbels	0.932 **	0.956 **	0.935 **	0.935 **
1000-seed weight	0.668	0.860 *	0.455	0.921 **

1, 2 Significant at 0.05 and 0.01 levels of the probability, respectively.

DISCUSSION:

The present study demonstrates an appreciable influence of both landraces and pre-sowing treatments of cumin seeds on the reduction of the Fusarium wilt disease incidence that was associated with an enhance seed yield. Principally, the two landraces did not seem to be different in most of the main studied traits of plant growth and yield components (plant height, the number of main and secondary branches and the number of umbels). However, they exhibited differential levels of disease incidence especially when the untreated (control) seeds were used. This differential tolerance could have been developed by the natural selection for plant survival under the conditions of infestation with the causal pathogen where those plants are grown (Chang, 1985; Ehdaie and Waines, 1989). In this study, the used pathogen strain was isolated from Assiut Governorate. There is a possibility that more virulent strains exist in Qina (Larkin and Fravel, 1998). The disease incidence is affected not only by the pathogen virulence but also with the prevailing complex of environmental conditions in the different growing regions (Handelsman and Stabb, 1996; Kurze *et al.*, 2001; Larkin and Fravel, 1998). Therefore, the landrace from Qina may have been adapted to more severe biotic and abiotic stresses. The relative superiority of the landrace from Qina suggests that the alternate use of different landraces may be introduced as new control measure in production of cumin under Fusarium wilt infestation conditions in Assiut. This is considered a new result out of the present study.

The reduction of disease incidence can be expected to enhance the plant stand and consequently the seed yield per area unit especially when supported by enhanced plant productivity (seed yield per plant). In this study, cumin landrace from Qina exhibited enhancement in both plant stand (reduced infected plants) in the field and seed yield per plant. The produced higher seed yield per plant was accompanied by an apparent increased plant biomass. The enhanced seed yield per plant seemed, therefore, to be mainly due to an enhanced photosynthetic status that led to a better seed (fruit) filling. Assessments of mechanisms underlying the tolerance for Fusarium wilt in some cumin accessions in India (Mandavia *et al.*, 2000) indicated that tolerance could be attributed to an increased production level of certain compounds. Among these compounds was the salicylic acid, which is known to enhance physiological status and induce tolerance to biotic and abiotic stresses (Raskin, 1992). The dual enhanced plant stand

and seed yield per plant under disease infestation conditions, such that of Qina landrace, could be conditioned by biochemical defense mechanisms of stress tolerance as a result of natural selection.

The biocontrol agents (antagonist microorganisms) suppress disease infection not only by external protection of the plant though the inhibition (competition for substrates and/or production of antibiotics) of the pathogen growth (De Boer *et al.*, 2003) but also via induction of systematic resistance (Leeman *et al.*, 1995; Van Loon *et al.*, 1998). In addition, production of stimulus for plant growth is also suggested (Hofflich *et al.*, 1994; Linderman, 1994). Seed priming increases the extra-embryo enzyme activity (Karssen *et al.*, 1989) and enhances antioxidant compounds and/or enzymes production (Baily *et al.*, 2000). Antioxidants activate and/or act as a defense system for the host (Guetsky *et al.*, 2002). Thus these pre-sowing treatments could result in both lessening the number of infected plants coupled with enhanced growth and yield of the protected plants regardless of their differential initial tolerance to the disease. The overall results of using seed treatments in the present study showed that the increased seed yield per plant was expressed in both landraces. The increased seed yield per plant was associated with reduced percentage of the disease infection, and increased number of the main and secondary branches, weight of mature dry plants and number of umbels (Table 9). While the biocontrol has been widely documented to improve resistance to diseases and to enhance plant growth (Postma and Pattink, 1992; Taylor *et al.*, 1994; Zang *et al.*, 1996), the result obtained in this study with regard to the water priming of the cumin seeds that reduced the incidence of wilt disease is considered new.

Several researchers have observed an improved disease control using various combinations of multiple compatible biocontrol organisms (Duffy *et al.*, 1996; Duffy and Weller, 1995; Dunne *et al.*, 1998). This is attributed to providing multiple mechanisms of action especially of different antagonists (Jetiyanon and Kloepper, 2002; Lemanceau *et al.*, 1993). With regard to the percentage of infection, combination of 2-day water priming and *T. harzianum* in the present study magnificently improved the control of the Fusarium wilt in cumin. Comparing with the use of water priming alone the reduction in the disease incidence using the combined treatment ranged from 48% to 55% with an average of 51.5%. In contrast with the percentage of infection when using *T. harzianum* alone, the reduction utilizing the combined treatment ranged from 35% to 42% and averaged 38.5%. This suggests an existence of different mechanisms for protecting plants against infection with the disease. Possibly this resulted from complementation of external type of action of the antagonists and internal mechanism types of the water priming.

Concerning the seed yield per plant, however, the combination of 2-day water priming and *T. harzianum* did not differ from using *T. harzianum* alone. The enhancement of this combination over the water priming treatment was inconsistent in the different years of the study. The same inconsistency was expressed for almost all the plant growth parameters (plant height, the number of main branches, weight of the mature dry plants and the number of umbels). Most likely is that the internal mechanisms of these seed treatments are similar and did not, therefore, induce appreciable enhancement in plant growth over the use of either one alone.

Form the application point of view, however, combinations of seed treatments are

recommended for several possible advantages. These include a consistent control of the disease over a wide range of environmental conditions and control of different pathogen strains and/or multiple pathogens (Larkin and Fravel, 1998). In this study, the lessened percentage of infected plants obtained using combination of 2-day water priming and *T. harzianum* than either one alone would magnificently increase the stand of the plants in the field and consequently the yield per area unit. Coupling with an enhanced seed yield of individual plants then a greatly improved total seed yield of cumin can be obtained in the wilt-infested fields in Assiut.

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

تحسين إنتاج الكمون تحت ظروف عدوى التربة بالذبول الفيوزارمى
٢- تقييم حقلى لسلالات أرضية ومعاملات للبذور مختلفة

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أجريت هذه الدراسة تحت ظروف العدوى الطبيعية فى حقل موبوء بالفطر المسبب لمرض الذبول الفيوزارمى للكمون بغرض التعرف على جدوى استخدام بعض معاملات البذور ما قبل الزراعة، والتى تم اختبارها على أساس دراسة سابقة فى الظروف المعقمة وظروف الزراعة بالأصص، كذلك شملت الدراسة سلالتين من الصنف البلدى من محافظتى قنا وأسيوط للتعرف على مدى وجود تباين لتحمل المرض فى الصنف البلدى والمتأقلم على الزراعة والإنتاج فى مناطق مختلفة بصعيد مصر. تضمنت معاملات البذور معاملتين بمعلق الفطر المضاد "التراكودرما هاريزيانم" و" الترايكودرما هاماتم" ومعاملة بمعلق البكتريا المضادة "باسلس سبتلس" ومعاملة بالمبيد الحيوى " البلانت جارد"، كل هذه المعاملات الأربع كان يتم فيها نقع البذور فى المعلق لمدة ١٥ دقيقة، بالإضافة لهذه المعاملات استخدمت معامله خامسة بالنقع فى الماء (Water-Priming) لمدة ٤٨ ساعة ومعاملة سادسة (مختلطة)، وهى النقع فى الماء ٤٨ ساعة(Water- Priming) يليها معاملة بمعلق الفطر المضاد "التراكودرما هاريزيانم" هذا بالإضافة إلى المعاملة المقارنة (الكونترول).

تمت التجربة فى وحدات منشقة فى تصميم القطاعات الكاملة العشوائية، وكانت الوحدات الرئيسية لمعاملات البذور والوحدات المنشقة (الفرعية) للسلالات الأرضية المختلفة من الكمون. وشملت بيانات على نسبة الإصابة وطول النبات وعدد الفروع الرئيسية والثانوية وعدد النورات ووزن النبات الناضج الجاف ومتوسط وزن ١٠٠٠ بذرة ومحصول البذور للنبات. وتم التحليل الإحصائى لكل البيانات والتى وجد من خلالها أن كل معاملات البذور قللت الإصابة بالمرض مقارنة بالمعاملة المقارنة (الكونترول). وأن أقل نسبة إصابة بالمرض كانت من المعاملة المختلطة (الماء ٤٨ ساعة ثم فطر الترايكودرما هاريزيانم) فى عامى الدراسة، وكانت نسبة إصابة السلالة قنا أقل من نظيرتها السلالة أسيوط. أما فيما يخص الإنتاج فقد كان محصول النبات من البذور (الثمار) أعلى عند استخدام معاملات البذور مقارنة بمعاملة الكونترول. وتميزت المعاملة المختلطة بأعلى محصول، وقد ظهر أن هذه الزيادة يصاحبها بالإضافة لنقص نسبة الإصابة زيادة فى عدد الفروع الرئيسية والثانوية ووزن النبات الجاف وعدد النورات فى عامى الدراسة وفى كل من السلالتين قنا وأسيوط، مع تفوق السلالة قنا على سلالة أسيوط فى محصول البذرة. وقد فسر تفوق المعاملة المختلطة على أنه راجع لتكامل ميكانيكية تأثير المعاملة بالماء كبادئ مع ميكانيكية تأثير المعاملة بفطر ترايكودرما هاريزيانم.

وقد استخلص من هذه الدراسة إمكانية استخدام معاملات البذور ما قبل الزراعة، وبصفة خاصة المعاملة المختلطة لمقاومة مرض الذبول الفيوزارمى للكمون وتقليل ضراوته فى الأراضى الموبوءة به. فالمعاملة المختلطة والتى استخدمت فى هذه الدراسة كانت الأفضل فى تقليل نسبة الإصابة فى عامى الدراسة مما يعطى فرصة للحصول على أكثر عدد من النباتات فى المساحة المنزرعة مما يمكن أن ينعكس إيجابيا على الإنتاج الإجمالى. وهذه المعاملة تعتبر إضافة جديدة فى هذه الدراسة، والتى أشارت أيضا إلى وجود تباين بين سلالتين من الصنف البلدى للكمون المتأقلمة لمناطق إنتاج مختلفة بما يمهد لإيجاد طرق جديدة لمقاومة هذا المرض تتناسب مع الاتجاهات الحديثة للحفاظ على البيئة، وتقليل التلوث الناتج عن الأنشطة الزراعية للإنتاج النباتى.