3. Голобородько С.П., Ковтун Н.Н., Влащук А.Н. и др. Совершенствовать семеноводство люцерны// Селекция и семеноводство, 1987, 3.- С. 38-42.
4. Коренев Г.В., Щедрина Д.И., Потапова А.А. Совершенствовать технологию производства семян люцерны//Селекция и семеноводство, 1987, 2. - С. 30-32.

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Мақалада жоңышқаның тұқым өнімділігін жоғарылату үшін себу мерзімдерін зерттеу нәтижелері жазылған. Жылдың ауа райына байланысты жоңышқаның тұқымдығын өсіру үшін көктемде және жазда себу ұсынылады.

The experiment results of crop′s terms of alfalfa to increase its seed′s productivity were described in this article. Depending on conditions of year, it is recommended to plant the alfalfa seed′s in spring and summer period.

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BIOLOGICAL CONTROL OF CUCUMBER FUSARIUM WILT DISEASE USING CHAETOMIUM AND PENICILLIUM ISOLATES UNDER PROTECTED HOUSES.

БИОЛОГИЧЕСКИИ КОНТРОЛЬ К ФУЗАРИОЗНОМУ УВЯДАНИЮ БОЛЕЗНИ ОГУРЦА ИСПОЛЬЗОВАНИЕ CHAETOMIUM И PENICILLIUM ИЗОЛЯТОВ В УСЛОВИЯХ ЗАЩИЩЕННОГО ГРУНТА.

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Abstract

Treating cucumber seeds with three *Chaetomium* isolates and *Penicillium* spp. on incidence of Fusarium wilt disease was studied under protected houses conditions. The obtained results revealed that, all isolates were effective in reducing disease severity and increasing cucumber yield compared with the control. *Cheatomium bostrycoides***,** *Penicillium spp.* and *Cheatomium globosum* were the best isolates respectively. All antagonistic isolates significantly increased the activity of peroxidase, chitinase enzymes and lignin content. This study revealed that, we can depend on *Cheatomium* and *Penicillium* to control of *Fusarium* wilt disease that attack cucumber plants under greenhouses and reducing the use of fungicides.

Keywords: *Chaetomium***,** *Penicillium, Fusarium* wilt and Cucumber.

Introduction

Cucumber **(***Cucumis sativus* L.) is one of the most important economical crops, which belongs to family cucurbitaceae. Cucumber is grown either in the open field or under protected houses **(Hanam et al., 1978).** Fusarium wilt caused by *Fusarium oxysporum* f.sp. *cucumerinum* is one of the most devastating diseases in cucumber production worldwide **(Zhou et al. 2008).** *Fusarium oxysporum f. sp. cucumerinum* is a destructive pathogen on cucumber **(** *Cucumis sativus* L.) seedlings and the causal organism of crown and root rot of cucumber plants, **(Chen Fang et al. 2010).** Mechanisms of biological control of Fusarium wilt by beneficial microorganisms are complex. Most studies conducted previously have focused on using nonpathogenic fusaria or other antagonists **Baker et al. (1978); Kroon et al. (1991); Paulitz et al. (1987)** and **Shimotsumaet *et al.* (1972)** that exert biological control through mechanisms such as competition for nutrients or iron **Duijff *et al.* (1990),** competition for infection sites on roots **(Mandeel and Baker**

(1991), or production of antibiotics **Hebber *et al.* (1992); Lambert *et al.* (1987); Sneh *et al.* (1984) and Yuen *et al.* (1985).** Mycelial growth of *F. oxysporum* was inhibited more than *M. phaseolina* by the antagonistic fungi. *Trichoderma spp***.** particularly *T***.** *viride* was the most effective in this regard followed by *Gliocladium penicilloides* and *Chaetomium bostrycoides***.** *T. harzianum* followed by *C. bostrycoides* were the best for reducing root-rot and/or wilt disease incidence on sesame and increased percentage of healthy plants compared with other antagonistic fungi, **Khalifa (1997).** Recently, there were many reports about the application of antagonistic fungi in controlling plant disease such as the use *Penicillium* species, *Trichoderma* species and *Chaetomium* species by Soytong ***et al.*** (2005).

Materials and Methods

1- Effect of treating cucumber seeds with some antagonistic *Trichoderma* fungi on incidence of *fusarium* wilt disease under protected houses:

Two experiments (during spring and autumn 2009) were conducted to evaluate the effect of coating cucumber seeds with suspension of any of the following antagonistic *Cheatomium bostrycoides***,** *Cheatomium globosum***,** *Cheatomium spp.* 0 *Penicillium spp.* to evaluate their efficiency in controlling *fusarium* wilt disease incidence under protected houses. A known amount of surface sterilized cucumber seeds placed in plastic bags was thoroughly mixed and shacked slowly for 5 minutes with mixture consisted of 2 ml spore suspension plus 1 ml of 1% Arabic gum solution as sticker (modified from **Harman *et al*., 1980).**

Cucumber seeds whether treated or non-treated with antagonistic fungi were sown in potted (40 cm in diameter) soils infested by *Fusarium oxysporum* f. sp. *cucumerinum* at the rate of 2seeds/pot. Three replicates were used for each particular treatment. The average weight of fruit/plant was measured and the *fusarium* wilt disease was recorded using a scale containing 6 grades suggested by **Liu *et al*., (1995):**

- Disease severity percent was determined according to equation:

$$\textbf{Disease severity (\%)} = \frac{[\Sigma\,(\text{rating no.})\,(\text{no. plants in rating category})\,(100)\,]}{(\text{Total no. plants})\,(\text{highest rating value})}$$

$$\textbf{Reduction (\%)} = \frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

Determination of enzymes activity and lignin content:

The same antagonistic fungi that were previously tested under protected house conditions on incidence of *Fusarium* wilt disease in addition to untreated control treatment on peroxidase, polyphenol-oxidase and chitinase activity were determined.

Samples were taken at 40 and 50 days after seeding.

Extraction of enzymes:

Samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM b-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine enzyme activities **(Tuzun *et al.* 1989).**

1. Peroxidase assay:

Peroxidase activity was spectrophotometrically measured (as optical density 425nm/g fresh wight/15min) according to the method of **(Abeles *et al.,* 1971).**

2. Chitinase assay:

Chitinase activity was spectrophotometrically measured (as optical density 540nm/g fresh wight/15min) according to the method of **Monreal and Reese (1969).**

3. Determination of lignin content:

Cucumber root was taken after 50 days from seeding as samples. The determination was carried out according to the method of **Bjorkman (1956).**

Results

1- Effect of treating cucumber seeds with antagonistic fungi on severity of *fusarium* wilt disease.

The obtained results in Table (1) revealed that, all isolates were effective in reducing disease severity and increasing the average weight of fruit/plant compared to the control. *Cheatomium bostrycoides* and *Penicillium spp.* were the best isolates and reduced disease severity by 84.62 and 83.48% and increasing the average weight of fruit/plant by 261.54 and 248.08% respectively. In the other hand *Cheatomium spp.*

was the lowest effective one and reduced disease severity by 81.72% and increasing the average weight of fruit/plant by 153.85%.

Table(1): Effect of treating cucumber seeds with antagonistic fungi on severity of Fusarium wilt disease.

Efficacy		Mean		Experiment 2 (autumn 2009)		Experiment 1 (spring 2009)		Treatment
Average fruits weight/plant	Disease severity	Average fruits weight/plant	Disease severity	Average fruits weight/plant	Disease severity	Average fruits weight/plant	Disease severity	
261.54	-84.62	3.76	12.62	4.15	12.03	3.36	13.21	Cheatomium bostrycoides
193.27	-82.90	3.05	14.03	3.78	13.92	2.31	14.14	Cheatomium globosum
153.85	-81.72	2.64	15.00	3.18	14.76	2.10	15.25	Cheatomium spp.
248.08	-83.48	3.62	13.55	4.09	13.18	3.15	13.91	Penicillium spp.
00.00	00.00	1.04	82.04	1.32	80.76	0.75	83.33	Control
L.S.D. at 0.05 for:				Spring 2009		Autumn 2009		
Disease severity								
Average fruits weight/plant								

2-Effect of treating cucumber seeds with cell suspension of antagonistic fungi isolates in peroxidase activity in cucumber plants:

The results in **Table (2)** reveal that, all treatments significantly increased peroxidase activity compared with control treatment in all times. The highest activity of peroxidase was induced after 40 days by *Cheatomium bostrycoides* **(2000.00%)** followed by *Cheatomium spp.* and *Penicillium spp.* their increased peroxidase activity by (1828.57 and 1257.14%) respectively. While, *Cheatomium globosum* was the least effective and increased peroxidase activity by 757.14%. Whereas After 50 days *Cheatomium spp.* and *Cheatomium bostrycoides* induced the highest activity of peroxidase (336.36 and 334.65%) respectively. followed by *Cheatomium globosum* that increased peroxidase activity by 217.04%. While, *Penicillium spp.* was the least effective and increased peroxidase activity by 215.34%.

Table(2): Effect of treating cucumber seeds with cell suspension of antagonistic fungi isolates in peroxidase activity in cucumber plants:

Treatment	Peroxidase activity		Efficacy	
	After 40 days	After 50 days	After 40 days	After 50 days
Cheatomium bostrycoides	4.41	7.65	2000.00	334.65
Cheatomium globosum	1.80	5.85	757.14	217.04
Cheatomium spp.	4.05	7.68	1828.57	336.36
Penicillium spp.	2.85	5.55	1257.14	215.34
Control without Fusarium	0.93	1.63	342.86	-7.39
Control with Fusarium	0.21	1.76	00.00	00.00

3- Effect of treating cucumber seeds with cell suspension of antagonistic fungi isolates in chitinase activity in cucumber plants:

The results in **Table (3)** reveal that, all antagonistic fungi significantly increased chitinase activity compared with control treatment in all times. The highest activity of chitinase was induced after 40 days

by *Penicillium spp* (311.28%) followed by *Cheatomium globosum* and ***Cheatomium spp.*** their increased chitinase activity by (271.20 and **196.26%**) respectively. While, *Cheatomium bostrycoides* was the least effective that increased chitinase activity by 84.73%. Whereas After 50 days *Penicillium spp* and *Cheatomium globosum* induced the highest activity of chitinase (179.29and 165.54%) respectively. followed by *Cheatomium spp.* that increased chitinase activity by 156.02%. While *Cheatomium bostrycoides* was the least effective and increased chitinase activity by 92.54%.

Table(3): Effect of treating cucumber seeds with cell suspension of antagonistic fungi isolates in chitinase activity in cucumber plants:

Treatment	Chitinase activity		Efficacy	
	After 40 days	After 50 days	After 40 days	After 50 days
Cheatomium bostrycoides	4.45	7.64	84.73	92.54
Cheatomium globosum	8.95	10.54	271.20	165.54
Cheatomium spp.	7.14	10.16	196.26	156.02
Penicillium spp.	9.91	11.09	311.28	179.29
Control without Fusarium	2.58	4.72	7.05	18.89
Control with Fusarium	2.41	3.97	00.00	00.00

4- Effect of treating cucumber seeds with cell suspension of antagonistic fungi isolates in lignin content in cucumber plants:

It is clear from **Table (4)** that all tested antagonistic fungi significantly increased lignin content compared with control treatment. The highest increased of lignin content was induced *Cheatomium bostrycoides* (279.07%) followed by *Penicillium spp.* and *Cheatomium spp.* their increased lignin content by (229.24 and 146.39%) respectively. While *Cheatomium globosum* was the least effective and increased lignin content by 53.12%.

Table(4): Effect of treating cucumber seeds with cell suspension of antagonistic fungi isolates in lignin content in cucumber plants:

Treatment	**Lignin**	**Efficacy**
Cheatomium bostrycoides	**319.14**	**279.07**
Cheatomium globosum	**128.92**	**53.12**
Cheatomium spp.	**207.44**	**146.39**
Penicillium spp.	**277.19**	**229.24**
Control without Fusarium	**175.77**	**108.78**
Control with Fusarium	**84.19**	**00.00**

L.S.D. at 0.05: 1.84

Discussion

All isolates were effective in reducing disease severity and increasing the average weight of fruit/plant compared to the control. *Cheatomium bostrycoides* and *Penicillium spp.* were the best isolates and reduced disease severity by 84.62 and 83.48% and increasing the average weight of fruit/plant by 261.54 and 248.08% respectively. In the other hand *Cheatomium spp.* was the lowest effective one and reduced disease severity by 81.72% and increasing the average weight of fruit/plant by 153.85%.

The obtained results are agree with obtained by, **Amemiya *et al.*, (1994)** they found that, *Chaetomium* isolates from soil reduced the development of wilt caused by *Verticillium dahliae* and were antagonistic against the pathogen in dual culture. *Chaetomium globosum* completely inhibited spore germination of *V. dahliae* at 32µg/ml. was also active against *V. albo-atrum* and *Rhizoctonia solani.*

Chaetomium globosum produced antibiotic substances inhibitory to *Verticillium dahliae* growth; producing 1.1-0.7 cm diam, **Castrejon Sanguino, (1994).**

All isolates significantly increased the activity of peroxidasee and chitinase enzymes. The present results concerning the increase in peroxidase and chitinase enzymes activity are in agreement with those reported

by **Matta *et al.* (1988)** and **Yurina *et al*. (1993)** In this respect, **Smith** and **Hammerschmidt (1988)** found that induced resistance in cucurbit plants accompanied by a marked increase in intercellular peroxidase isozymes. Induced resistance in cucumber plants increased the activity of peroxidase and chitinase enzymes **(Irving and Kuc 1990).**

All isolates significantly increased lignin content. The present results concerning the increase in lignin content are in agreement with those reported by **Kuc (1982)** Lignification play its role as defense mechanisms, increasing the mechanical resistance of the host cell wall, restricting the diffusion of pathotoxins and nutrients and inhibiting growth of the pathogens by the action of toxic lignin precursors and lignifications of the pathogen.

1. Abeles, F.B.; Bosshart, R.P.; Forrence, L.E. and Habig, W.H. (1971) Preparation and purification of glucanase and chitinase from bean leaves. Plant Physiol., 47:129-134.
2. Amemiya, Y.; Kondo, A.; Hirano, K.; Hirukawa, T. and Kato, T. (1994) Antifungal substances produced by *Cheatomium globosum***.** Technical Bulletin of Faculty of Horticulture, Chiba University, 48: 13 – 18.
3. Baker, R., Hanchey, P., and Dottarar, S. D. (1978) Protection of carnation against *Fusarium* stem rot by fungi. Phytopathology 68:1495-1501.
4. Bjorkman, A. (1956) Studies on finely divided wood. Part 1. Extraction of lignin with neutral solvents. Svensk Papperstidn., 59:447-485.
5. Castrejon Sanguino, A. (1994) Detection, *in vitro***,** of fungi antagonistic to *Verticillium dahliae* Kleb. race T.9. ITEA Produccion Vegetal, 90 (2):129-131.
6. Chen Fang; Wang Min; Zheng Yu; Luo JianMei; Yang XiuRong; Wang XueLian (2010) Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber Fusarium wilt by Bacillus subtilis B579. World Journal of Microbiology & Biotechnology. 2010. 26: 4, 675-684. 43 ref.
7. Duijff, B. J., Bakker, P. A. H. M., and Schippers, B. (1990) Suppression of Fusarium wilt of carnation by *Pseudomonas* in soil: Mode of action. Pages 152-157 in: Plant Growth Promoting Rhizobacteria, Progress and Prospect. C. Keel, B. Knoller, and G. Defago, eds. 10BCAVPRS Bull. 14/8.
8. Hanam, J.J.; Holley, W.D. and Goldsberry, K.L. (1978) Greenhouse management- Springer-Verlag, Berlin.
9. Harman, G.E.; Chet, I. and Baker, R. (1980) *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium* spp. or *Rhizotonia solani***.** Phytopathology, 70 (12): 1167-1172.
10. Hebber, K. P., Davey, A. G., and Dart, P. J. (1992) Rhizobacteria of maize antagonistic to *Fusarium moniliforme,* a soil-borne fungal pathogen: Colonization of rhizosphere and roots. Soil Biol. Biochem. 24:989-997.
11. Irving, H. R. and Kuc, J. (1990) Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by potassium phosphate monobasic. Physiological and Molecular Plant Pathology, 37: 355-366.
12. Khalifa, M. M. A., (1997) Studies of root-rot and wilt diseases of sesame plants. M.Sc.Thesis, Fac. Agric., Moshtohor, Zagazig Univ. Benha branch, pp.158.
13. Kroon, B. A. M., Scheffer, R. J., and Elgersma, D. M. (1991) Induced resistance in tomato plants against Fusarium wilt invoked by *Fusarium oxysporum* f. sp. *dianthi*. Neth. J. Plant Pathol. 97:401-408.
14. Kuc, J. (1982) Induced immunity to plant disease. Bioscience, 32: 854-860.
15. Lambert, B., Leyns, F, Van Rooyen, L., Gossele, F, Papon, Y, and Swings, J. (1987) Rhizobacteria of maize and their antifungal activities. Appl. Environ. Microbiol. 53:1866-1871.
16. Liu, L. Kloepper, J. W. and Tuzun, S. (1995) Induction of systemic resistance in Cucumber against Fusarium wilt by plant growth-promoting rhizobacteri. Phytopathology 85:695-698.
17. Mandeel, Q., and Baker, R. (1991) Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. Phytopathology 81:462-469.
18. Matta, A.; Abattista Gentile, I, and Ferraris, L. (1988) Stimulation of β,1,3-glucanase and chitinase by stresses that induce resistance to fusarium wilt in tomato. Phytopath. Medit., 27: 45-50.
19. Monreal. J. and Reese, E.T. (I969) The chitinase of Serratia marcescens. Canadian J. of Microbiology, 15: 689-696.
20. Paulitz, T. C, Park, C. S., and Baker, R. (1987) Biological control of Fusarium wilt of cucumber with nonpathogenic isolates of *Fusarium oxysporum*. Can. J. Microbiol. 33:349-353.
21. Shimotsuma, M., Kuc, J., and Jones, C. M. (1972) the effects of prior inoculations with non-pathogenic fungi on *Fusarium* wilt of watermelon. HortScience 7:72-73.
22. Smith, J. and Hammerschmidt, R. (1988) Comparative study at acidic peroxidases associated with induced resistance in cucumber, muskmelon and watermelon. Physiological and Molecular Plant Pathology, 33: 255-261.
23. Sneh, B., Dupler, M., Elad, Y, and Baker, R. (1984) Chlamydospore germination of Fusarium oxysporum f. sp. cucumerinum as affected by fluorescent and lytic bacteria from a Fusarium-suppressive

soil. Phytopathology 74:1115-1124.
24. Soytong, K., Srinon, W., Ratanacherdchai, K, Kanokmedhakul, S., Kanokmedhakul, K (2005). Application of antagonistic fungi to control anthracnose disease of grape. Agricultural Technology 1: 33-42.
25. Tuzun, S.; Rao, M. N.; Vogeli, U.; Schardl, C. L.; Ku, J. A. (1989) Induced systemic resistance to blue mold: early induction and accumulation of b,1,3-gluconases, chitinases, and other pathogenisis-related proteins (b-proteins) in immunized tobacco. Phytopathology, 79: 979-983.
26. Yuen, G. Y, Schroth, M. N., and McCain, A. H. (1985) Reduction of Fusarium wilt of carnation with suppressive soil and antagonistic bacteria. Plant Dis. 69:1071-1075.
27. Yurina, O.V.; Yurina and T.P. Anikina, L. (1993) Peroxidase activity of the leaves in cucumber as a test for resistance to mildew. SeI'Skokhozyaistvennaya-Biology, I: 113-117(c.f. Data Base of CAB International).
28. Zhou XinGang; Wu FengZhi; Wang;XueZheng and Yuan Ye (2008) Progresses in the mechanism of resistance to Fusarium wilt in cucumber (Cucumis sativus L.). Journal of Northeast Agricultural University (English Edition). 2008.

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Обработка семян огурцов с тремя Chaetomium изолятов и *Penicillium spp.* по заболеваемости увядания Fusarium изучали в условиях защищенного грунта. Полученные результаты показали, что все изоляты были эффективными в снижении тяжести заболевания и повышению огурец, выход по сравнению с контролем. *Cheatomium bostrycoides, Penicillium spp***.** и *Cheatomium globosum* были лучшие изолятов соответственно. Все антагонистических штаммов значительно увеличилась активность пероксидазы**,** хитиназы ферментов и содержание лигнина**.** Это исследование показало, что мы можем зависеть от Cheatomium и Penicillium к управлению Fusarium увядании болезни**, который атакует** огурцы растений в теплицах и снижение использования фунгицидов.

Бұл зерттеудің қорытындысы биологиялық жолмен Cheatomium және Penicillium белсенділігінің арқасында жылыжайдағы қияр дақылының саңырауқұлақ ауруына қарсы төзімділігін арттырып, фунгицидті аз қолдану.

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EFFICACY OF SOME CHEMICAL INDUCERS FOR CONTROL OF CUCUMBER FUSARIUM WILT DISEASE UNDER PROTECTED HOUSES

ЭФФЕКТИВНОСТЬ НЕКОТОРЫХ ХИМИЧЕСКИХ ИНДУКТОРОВ ДЛЯ КОНТРОЛЯ К ФУЗАРИОЗНОМУ УВЯДАНИЮ ОГУРЦОВ В УСЛОВИЯХ ЗАЩИЩЕННОГО ГРУНТА

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Abstract

Inducing resistance against *fusarium* wilt caused by *fusarium oxysporum f.sp. cucumerinum* in cucumber (cv. Sina 1) was investigated under protected houses conditions with four chemical salts as fungicides alternatives. Cucumber seeds were soaking in four (K_2HPO_4at 200mM and $CoSO_4$. $7H_2o$ at 10 ppm) while, $CaSO_4$ and KMnO4were used at 5000 pmm as soil drench. Results showed that, all chemical salts reduced disease severity of *fusarium* wilt disease and the average weight of fruit/plant compared to the control. All