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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 аркасында жылыжайдағы қияр дақылдың саңырауқұлақ ауруына қарсы төзімділігін арттырып, фунгицидті аз қолдану.

УДК 635.63:632.4

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_4 at 200mM and $CoSO_4 \cdot 7H_2O$ at 10 ppm) while, $CaSO_4$ and $KMnO_4$ were used at 5000 ppm 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

chemical salts significantly increased the activity of peroxidase, chitinase enzymes and increased lignin content compared to the control

Keywords: Cucumber; *fusarium* wilt, induced resistance and antioxidants.

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).

Plants that are grown in greenhouses may be attacked by a number of plant pathogenic fungi. This way of plant production is very specific due to characteristical temperature conditions, as well as air and soil humidity, which are usually very favourable for development of plant pathogenic fungi (Balaz *et al.* 2009).

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).

Induced resistance against *fusarium* wilt of watermelon using various abiotic inducers included different concentrations of Co as CoSO_4 or ethephon (2-chloroethyl phosphonic acid). Results indicate that the most effective treatment in reducing the percentage of wilted plants were ethephon at 800 ppm, CO^{++} at 0.5 ppm. Treatment with ethephon at 600 ppm was highly effective with cv. Gize 1 only in field experiments, Abd-EL-Kreem *et al.*, (1993).

Application of KMnO_4 solution to the soil provided good control of *Fusarium* wilt of cucumbers. Plots treated with 1:800 or 1:1000 solutions were free from the disease, while the average rate of infected plants following treatment with a 1:1500 solution was 0.88%. The highest yields (112.6 kg) were obtained from plots treated with 1:1000 KMnO_4 , Li *et al.* (1992).

Materials and Methods

1- Effect of treating cucumber seeds some antioxidants on incidence of *Fusarium* wilt disease under protected houses.

Two experiments (during spring and autumn 2009) were conducted to evaluate the effect of four chemical salts (K_2HPO_4 , $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, CaSO_4 and KMnO_4). Cobalt sulphate at 10 ppm, KMnO_4 and Calcium sulphate CaSO_4 at 5000 ppm and K_2HPO_4 at 200mM in controlling *fusarium* wilt disease incidence under protected houses.

Surface sterilized cucumber seeds were soaked for 2.5 hours (Shalaby, 1997) in a known concentration of any of Cobalt sulphate and K_2HPO_4 . While KMnO_4 and CaSO_4 added to the soil. The wetted seeds were spread out in a thin layer and left to 24 hours then they were sown in pathogen-infested potted 40 cm in diameter soils at the rate of 2 seeds/pot. 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 2 seeds/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:

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

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

Determination of enzymes activity and lignin content:

The same chemicals that were previously tested under protected house conditions 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 or treating soil with some resistance inducing chemicals on incidence of *Fusarium* wilt disease.

In this study 4 chemicals (K₂HPO₄, CoSO₄, CaSO₄ and KMnO₄) were used to test their efficacy on controlling wilt disease on tow successive seasons (spring 2009, autumn 2009). The obtained results are presented in Table (1).

The obtained results showed that, in general, both disease incidence and disease severity of *fusarium* wilt disease were reduced as a result of treatment by all chemicals compound compared to the control. In all cases, CaSO₄ was the most effective compound and reducing the disease severity by 93.24 followed by KMnO₄ and K₂HPO₄ their reducing the disease severity by 92.41 and 90.94% respectively.

On the other hand, CoSO₄ was the least effective and reducing the disease severity by 90.35%.

Also, all tested treatments increased the fruit weight/plant. The highest increased in fruit weight/plant was induced by CaSO₄, KMnO₄ and K₂HPO₄ increased fruit weight/plant by 330.77, 311.54 and 299.04% respectively. Whereas CoSO₄ was the least effective and increased fruit weight/plant by 282.69%.

Table(1): Effect of treating cucumber seeds with tested chemicals compound on incidence of *Fusarium* wilt disease.

Efficacy		Mean		Experiment 2 (autumn 2009)		Experiment 1 (spring 2009)		Treatment
Aver- age fruits weight/ plant	Disease severity	Aver- age fruits weight/ plant	Disease severity	Aver- age fruits weight/ plant	Disease severity	Aver- age fruits weight/ plant	Disease severity	
299.04	- 90.94	4.15	7.43	4.45	6.92	3.80	7.94	K ₂ HPO ₄
282.69	- 90.35	3.98	7.92	4.30	7.30	3.65	8.53	CoSO ₄
330.77	- 93.24	4.48	5.55	4.75	5.50	4.20	5.60	CaSO ₄
311.54	- 92.41	4.28	6.23	4.60	6.16	3.95	6.27	KMnO ₄
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				0.26		0.48		
Average fruits weight/plant				1.73		1.72		

2- Effect of treating cucumber seeds with tested chemicals compound in peroxidase activity in cucumber plants:

The results in Table (2) reveal that, all chemicals compound significantly increased peroxidase activity compared with control in all times. CoSO₄ was the best treatments and increased peroxidase activity after 40 and 50 days by (3442.85and 370.45%) respectively, Followed by K₂HPO₄ and CaSO₄ their increased peroxidase activity by (3042.85 and 360.22%) and (2657.14 and 336.36%) respectively. While KMnO₄ increased peroxidase activity by 614.28 and 123.29% respectively.

Table(2): Effect of treating cucumber seeds with tested chemicals compound in peroxidase activity in cucumber plants:

Treatment	Peroxidase activity		Efficacy	
	After 40 days	After 50 days	After 40 days	After 50 days
K ₂ HPO ₄	6.60	8.10	3042.85	360.22
CoSO ₄	7.44	8.28	3442.85	370.45
CaSO ₄	5.79	7.68	2657.14	336.36
KMnO ₄	1.50	3.93	614.28	123.29
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 tested chemicals compound in chitinase activity in cucumber plants:

The results in **Table (3)** show that, all chemicals significantly increased chitinase activity compared with control treatment in all times. The highest activity of chitinase after 40 and 50 days was induced by CoSO₄ (358.34 and 189.87%). After 40 days K₂HPO₄ and CaSO₄ increased chitinase activity by (146.59 and 122.19%) respectively.

While KMnO₄ was the least effective and increased chitinase activity by 96.92%. Whereas After 50 days KMnO₄ and K₂HPO₄ induced the highest activity of chitinase (68.21 and 52.34%) respectively. While CaSO₄ was the least effective and increased chitinase activity by 50.75%.

Table(3): Effect of treating cucumber seeds with tested chemicals compound in chitinase activity in cucumber plants:

Treatment	Chitinase activity		Efficacy	
	After 40 days	After 50 days	After 40 days	After 50 days
K ₂ HPO ₄	5.94	6.05	146.59	52.34
CoSO ₄	11.05	11.51	358.34	189.87
CaSO ₄	5.36	5.99	122.19	50.75
KMnO ₄	4.75	6.68	96.92	68.21
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 tested chemicals compound in lignin content in cucumber plants:

It is clear from Table (4) that all tested chemicals compound significantly increased lignin content compared with control treatment. The highest increased of lignin content was induced CaSO₄ (245.53%) followed by CoSO₄ and KMnO₄ their increased lignin content by (208.30 and 68.44%) respectively. While K₂HPO₄ was the least effective and increased lignin content by 60.80%.

Table(4): Effect of treating cucumber seeds with tested chemicals compound in lignin content in cucumber plants:

Treatment	Lignin	Efficacy
K ₂ HPO ₄	135.38	60.80
CoSO ₄	259.56	208.30
CaSO ₄	290.91	245.53
KMnO ₄	141.81	68.44
Control without Fusarium	175.77	108.78
Control with Fusarium	84.19	00.00

LSD 0.05 = 1.89

Discussion

Disease incidence and disease severity of fusarium wilt disease were reduced as a result of treatment by all chemicals compound compared to the control. In all cases, CaSO₄ was the most effective compound and reducing the disease severity by 93.24 followed by KMnO₄ and K₂HPO₄ their reducing the disease severity by 92.41 and 90.94% respectively.

On the other hand, CoSO₄ was the least effective and reducing the disease severity by 90.35%.

Also, all tested treatments increased the fruit weight/plant. The highest increased in fruit weight/plant was induced by CaSO₄, KMnO₄ and K₂HPO₄ increased fruit weight/plant by 330.77, 311.54 and 299.04% respectively. Whereas CoSO₄ was the least effective and increased fruit weight/plant by 282.69%.

The obtained results are agreed with obtained by **Gado (1997)** Salicylic acid, hydrogen peroxide and cobalt ions were effective for induction of resistant in watermelon against wilt pathogen in four distinct experiments.

All chemicals significantly increased the activity of peroxidase and chitinase enzymes. The present results concerning the increase in peroxidase and chitinase enzymes activity are in agreement with those reported by **Mosa (1997); Reuveni et al. (1997); Abd-El-Kareem (1998) and El-Habbak (2003)**.

All chemicals significantly increased the lignin content The obtained results are agreed with obtained by **Hammerschmidt et al (1982)**. 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**).

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

Rapid lignification in resistant or immunized cucumber plants after penetration by *Cladosporium cucumenmim* or *Colletotrichum lagenarium* and fungal mycelia of both pathogens were lignified in the presence of confiferyl, hydrogen peroxide and peroxidase prepared from immunized cucumber leaves (**Hammerschmidt and Kuc, 1982**).

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Резистентности против *Fusarium* увядании вызванного *Fusarium oxysporum* f.sp. *cucumerinum* в огурцы (сорт Сина 1) исследовалась в защищенном грунте с четырьмя химическими солей в качестве фунгицидов альтернатив. Огурцы Семена замачивания в четыре (K₂HPO₄at 200мм и CoSO₄. 7H₂O в 10 стр/мин), CaSO₄ и KMnO₄ были использоваться при 5000 ПММ, как почва обильно смочить. Результаты показали, что все химические соли снижению заболеваемости тяжести заболевания *Fusarium* увядают и средний вес плодов растение по сравнению с контролем. Все химические соли значительно увеличилась активность пероксидазы, хитиназы ферментов и повышенным содержанием лигнина по сравнению с контролем. Это исследование показало, что мы можем зависеть от химических солях к управлению *Fusarium* увядании болезни, который атакует огурцы растений в теплицах и снижение использования фунгицидов.

Бұл зерттеудің қорытындысы химиялық тұзды белсенділігінің арқасында жылыжайдағы қияр дақылдың саңырауқұлақ ауруына қарсы төзімділігін арттырып, фунгицидті аз қолдану.