

EVALUATION OF BIOLOGICAL ACTIVITY OF THREE NON-CHEMICAL TECHNIQUES AGAINST FUSARIUM WILT OF CUCUMBER

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ABSTRACT: Three biotechniques, plant extract (garlic juice), fungal bioagent (*Trichoderma harzianum*), and non-pathogenic strain of *Fusarium oxysporum* were evaluated in controlling cucumber wilt disease caused by *Fusarium oxysporum* f.sp. *cucumerinum* in comparison to fungicidal application with Carboxin I thiram.

Isolation of the causal pathogen was carried out from three different Govern-
orates.

Twelve isolates were obtained for which pathogenesis capabilities were test-
ed. Isolate (Is.3) proved to be the most virulent one.

Although, applying Carboxin I thiram led to the best control of the disease in
comparison to the three tested biotechniques; the non-pathogenic strain of
oxysporum showed high results concerning disease reduction. Garlic juice,
however, proved to be less effective followed by the bioagent *Trichoderma*
harzianum without significant differences between them

Therefore seed soaking in spore suspension of non-pathogenic strain
F.oxysporum for $1/2$ hr before planting is strongly recommended as a safe al-
ternative to fungicides to avoid environmental pollution and to obtain cucum-
ber fruits free of fungicidal residues that are available for exportation.

INTRODUCTION

Fusarium wilt of cucumber (*Cucumis sativus* L.) caused by *Fusarium oxysporum* Schelechtend. Fr. f sp. *cucumerinum* I. H. Owen is one of the most wide spread and economic diseases of this vegetable crop under the Egyptian environmental conditions.

Currently, control of this soil-borne pathogen is provided by seed treatment with fungicides and soil fumigation with methyl bromide which is classified as ozone depleting compound. Accordingly, it is scheduled to be removed from the market by 2005. Due to the great awareness to keep human health and avoid environmental pollution, more attention is paid to the non-chemical methods, specially the biological techniques (Jensen, et al., 1996).

Biological control, as an important component of plant disease management, led to the use of natural products obtained from plants, such as garlic extract (El Shami, 1985, Mohamed, 2002).

Several plant pathologists used bio-control agents, such as *Trichoderma harizianum* as a biocide against *Fusarium* sp. (Borda, Arbelaez, 1993, Ellas, et al., 1993)

Moreover, non-pathogenic strains, collected from suppressive soils, such as *Fusarium* spp.; were the most effective antagonist compared to other common bioagents such as *Trichoderma* spp.; *Gliocladium virens* and *Bacillus subtilis* (Mandel, Barker, 1991, Attiatalla, et al., 1998, Larkin, Fravel, 1998).

Seed treatment with botanical extracts and biological agents are planned to replace fungicides treatment against soil-borne fungal diseases (Berger et al., 1996, Chidburee et al., 1996)

The objective of this study is to evaluate the influence of three biotechniques against *Fusarium* wilt of cucumber in comparison to the fungicide Carboxin / thiram.

MATERIALS AND METHODS

To find an alternative application for the harmful effect of chemicals, this research is designed to evaluate the potential activity of three different biological resources against cucumber *Fusarium* wilt in comparison with the use of the standard fungicide Carboxin / thiram.

This study was conducted during the season of 2002 under greenhouse conditions of Vegetable Disease Department, Plant Pathology Research Institute, ARC, Giza, Egypt. Seed soaking was the method of application throughout this research (Biker & Biker, 1936).

1- Isolation and pathogenesis of the causal pathogen

Cucumber plants (of two months old) showing typical symptoms of wilt disease were collected from open fields of three different Governorates, Ismaillia, Menofia, and Giza.

Fusarium oxysporum f.sp. *cucumerinum* was isolated in pure cultures of PDA medium and incubated at 25-27 °C. The identification was carried out by the author according to Barnet (1960) & Singh (1982).

Pathogenesis capability of the obtained isolates was proven under greenhouse conditions. Ten pots (five replicates x 2) of 25cm in diameter containing soil infested with spore suspension of the pathogen at the rate of 7×10^8 spore/ml, 15ml/ kg soil, were used per each isolate according to Abd El Moity (1985).

Each pot was cultivated with 10 seeds of "Primo", the highly susceptible cultivar of cucumber (Abd El Moneim, 2001). Percentages of wilted seedlings were determined after 4 weeks from sowing date. Data were analyzed statistically according to Gomze & Gomze (1964).

2- Evaluation of three different biotechniques for controlling cucumber wilt of cucumber in comparison to the traditional fungicide treatment :

Three biotechniques were tested for their biological activity to control Fusarium wilt of cucumber in comparison to the traditional fungicide treatment:

a. Plant extract technique:

According to El Shami Mona (1985), mature frozen cloves of Chinese garlic (*Allium sativum* L.) were crushed in a blender and filtered through cheesecloth. The blended extract was sterilized through Zietz filter. Then, seeds of "Primo" cultivar were soaked for 12 hr in the extract, and then planted in soil artificially infested with spore suspension of *F. oxysporum* f. sp. *cucumerinum* (Is. 3) at the same rate as mentioned in the pathogenicity test.

b. Fungal bioagent technique:

The biological agent *Trichoderma harzianum* was isolated from the rhizosphere of healthy cucumber plants grown in the same fields where the causal pathogen was isolated from diseased plants.

Trichoderma pure isolates were grown on gliutoxin fermentation medium (GFM) under complete darkness just to stimulate toxin and enzymes production (Abd El Moity, Shatla, 1981).

Spore suspension of the bioagent was prepared and adjusted to contain 30×10^6 spore I ml according to Abd El Moity (1985).

"Primo" cucumber seeds were soaked in the bioagent suspension for $1/2$ hr, and then cultivated in pots containing soil infested with *Fusarium oxysporum* f. sp. *cucumerinum* isolate (Is.3).

c. Non-pathogenic strain technique:

One of the most important features of the biological agent is the non-pathogenic strains.

Non-pathogenic strain of *Fusarium oxysporum* was isolated from the rhizosphere of cucumber healthy plants as associated fungus during the isolation of *Trichoderma harzianum* particularly from Ismaillia Governorate. The isolate was grown on PDA medium and incubated at 25-27 °C. Its incapability to induce wilt disease to "Primo" cucumber was confirmed under greenhouse conditions when all seedlings grew in full health.

Spore suspension of non-pathogenic strain was adjusted to contain 30×10^6 spore I ml according to Abd El Moity (1985) and "Primo" seeds soaked in the suspension for $1/2$ hr, then planted in soil infested with the pathogen.

d- Fungicidal treatment:

Fungicides application is still the classical practice to control plant diseases. Accordingly, it is necessary to compare its efficiency against the effect of the three tested non-chemical techniques as a check.

Cucumber "Primo" seeds were treated as seed soaking with Carboxin I thiram, one of the most common and effective fungicide, for $1/2$ hr at the rate of 1.5 gm I lit, of water I kg of seeds. The treated seeds were planted in the infested soil.

Untreated "Primo" seeds were planted in pots containing infested soil serving as control for all treatments.

Ten pots were used for each particular treatment, and 10 seeds of "Primo" cultivar were sown in each pot, all pots were kept under greenhouse conditions and irrigated as needed. Disease assessment was carried out by calculating the percentage of wilted seedlings.

RESULTS AND DISCUSSION

1- Isolation and pathogenicity of the causal pathogen:

Twelve isolates of *F.oxysporum* fsp. *cucumerinum* were obtained from the 3 examined Governorates. Their pathogenesis capabilities were investigated and percentage of wilted seedlings was calculated after 4 weeks from planting date and tabulated in Table 1 and fig (1).

Data revealed that from the 5 isolates of Ismaillia Governorate, isolate No.3 (Is.3) was the most pathogenic; while the least effect resulted from isolate No.2 (G.2) of Giza Governorate. Other isolates, however, varied in their pathological characters. This variation may be attributed to the differences between the chemical, organic, and biotic soil contents of the cultivated area.

According to the above results, (Is.3) was chosen for further studies.

Table 1: Pathogenesis capability of *F. oxysporum* f. sp. *cucumerinum* isolates obtained from three examined Governorates:

Isolate No.	Is. 1	Is.2	Is.3	Is.4	Is.5	M 1	M 2	M 3	M 4	G1	G 2	G3
% of wilted seedlings	62	70	85	34	47	56	32	54	72	76	30	52
Means	12.4	14.0	17.0	6.8	9.4	11.2	6.4	10.8	14.4	15.2	6.0	10.4

LSD at 5% = 3.4

Is. = Ismaillia

M. = Menofia

G. = Giza

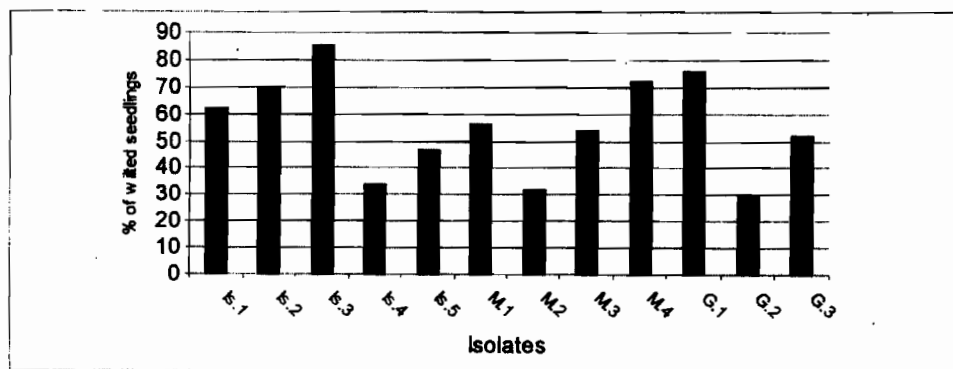


Fig (1): Pathogenesis capability of *F. oxysporum* f. sp. *cucumerinum* isolates.

2- Evaluation of three biotechniques for controlling cucumber wilt disease:

Data in Table 2 and Fig. (2) shows the biological potentiality of three tested biotechniques to control Fusarium wilt of cucumber represented as percentage of wilted seedlings calculated after 4 weeks from planting date.

Table 2: Influence of three biotechniques on percentage of cucumber wilted seedlings, 4 weeks after planting:

Tested biotechniques	Percentage of wilted seedlings	Means	Efficiency %
Plant Extract (garlic extract)	40	8	55.06
Fungal bioagent <i>T. harzianum</i>	43	8.6	51.68
Non-pathogenic strain <i>F. oxysporum</i>	25	5	71.91
Fungicide treatment Carboxin / thiram	10	2	88.76
Control Untreated seeds	89	17.8	---

LSD at 5% = 2.1

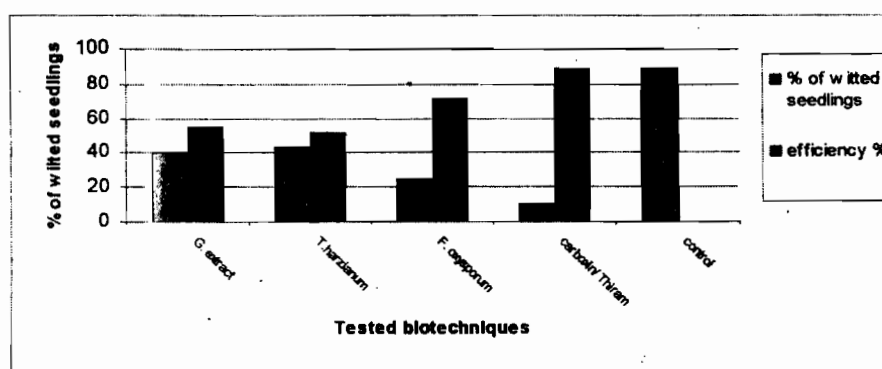


Fig. (2): Influence of three biotechniques on percentage of cucumber wilted seedlings, 4 weeks after planting:

The above data indicated that all tested techniques significantly decreased the percentage of wilted seedlings compared with the control (untreated seeds).

Seeds soaked in Carboxin / thiram produced the least percentage of wilted seedlings followed by seeds treated with non-pathogenic strain *F. oxysporum*, while garlic extract treatment occupied the third position followed by *T. harzianum* treatment without significant difference between them.

Results obtained were in harmony with Mandeel & Barker (1991).

The high effect of non-pathogenic strain on disease reduction could be attributed to the quick germination of conidia spores. This led to the speedy occupation of germ tubes to the infection sites. The agent could also be capable of competing with the pathogen on the limited amounts of oxygen and nutrition in the rhizosphere. Moreover, the author suggests that the agent might have induced resistance to the cucumber host. This suggestion agrees with Fuchs et al. research on tomato. (1997)

As regards the effectiveness in decreasing the percentage of wilted seedlings (from 98% in control to 40%), when cucumber seeds were soaked in garlic extract; it is due to the fact that garlic contains a number of substances with an inhibitory effect on spore germination and mycelial growth of a wide range of microorganisms. Some of these antibiotic-like compounds had been isolated by the author in previous studies.

The reduction of wilted cucumber seedlings, grown from seeds soaked in spore suspension of *T. harzianum*, could be attributed to its capability to involve mycoparasitism against great number of causal pathogens in the rhizosphere throughout toxins and enzymes production such as B- (1, 3) -glucanase, which decompose the cell wall of the pathogen's hypha. Additionally, this agent acts as fungi static and inhibits the pathogen's growth when produces a compound like siderophores, which chelates iron present in the soil, that will not be available to the surrounded pathogens.

The author assumes that *T.harzianum* treatment showed the lowest effect on disease reduction compared with other non-chemicals because of its low potential to compete with the pathogen and its need for time to affect it. Moreover, this agent is very sensitive to the soil unfavorable conditions.

Application of the fungicide Carboxin / thiram, as a standard treatment, clearly minimized the percentage of wilted seedlings because of its inherent toxicity against the *Fusarium* wilt pathogen.

From the above results it could be recommended that non pathogenic strain treatment could replace the use of chemicals and serve as an environmental friendly solution for cucumber wilt disease. Additionally, with the recent boom in the field of organic farming, and the increasing interest in chemical free fruits, there could be an excellent potential for exportation.

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تقييم النشاط البيولوجي لثلاث طرق غير كيميائية ضد مرض الذبول الفيوزاري يومي على الخيار

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مركز البحوث الزراعية بالجيزة

• أجرى هذا البحث لتقييم فعالية النشاط البيولوجي لثلاث طرق غير كيميائية حيث تم استخدام المستخلصات النباتية ممثلة في مستخلص فصوص الثوم الصيني واستخدام عوامل مكافحة الحيوية ممثلة في الفطر ترايكودرما هارزيانم واستخدام السلالات الفطرية غير الممرضة ممثلة في الفطر فيوزارييم أوكسى سبورم في مكافحة مرض الذبول الفيوزاري على الخيار المتسبب عن الفطر فيوزارييم أوكسى سبورم فورما إسبشيسكيس كيوكيومرينم وذلك مقارنة بمعاملة قياسية باستخدام مادة كيميائية تقليدية مثل كريبوكسين / ثيرام .

• أمكن الحصول على ١٢ عزلة من ثلاث محافظات مختلفة ، وتم إثبات قدرتهم المرضية على الصنف بريمو (القابل للإصابة بشدة) حيث تبين أن العزلة رقم ٣ المعزولة من محافظة الإسماعيلية كانت أكثرهم تفوقاً في إحداث الإصابة بالمرض . لذا اختيرت هذه العزلة في إجراء تجارب هذا البحث .

• بمقارنة جميع المعاملات المذكورة فيما بينها تبين أن مادة كريبوكسين / ثيرام هي الأكثر فعالية في مكافحة المرض ، يليها استخدام السلالة غير الممرضة ، ثم مستخلص فصوص الثوم ، بينما كانت المعاملة بالفطر ترايكودرما هارزيانم أقلهم كفاءة في تثبيط المرض ، وقد كانت الفروق معنوية بين جميع المعاملات المختبرة ومعاملة المقارنة (البذور غير المعاملة) .

• بالرغم من أن الكفاءة العالية للمادة الكيميائية في تثبيط المرض قد وصلت الي ٨٨,٧٦ % إلا أن استخدام السلالة غير الممرضة أدى الى تثبيط نسبة الإصابة من ٨٩% (في معاملة المقارنة) الي ٢٥ % بكفاءة قدرها ٧١,٩١ % ، فقد أدى ذلك الى التوصية بمعاملة بذور الخيار بيولوجيا بنقعها في معلق جراثيم سلالة الفطر غير الممرض لمدة نصف ساعة قبل الزراعة لمكافحة مرض الذبول الفيوزاريومي بدلا من معاملتها بالمبيدات ، ذلك للحصول علي ثمار خالية من المتبقيات للحفاظ علي صحة الإنسان والبيئة وإتاحة فرصة أكبر للتصدير .