Control of *Fusarium* Wilt Disease of Cucumber Using Rhizospheric Antagonistic Fungi

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Abstract

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Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cucumerinum* is a severe disease of cucumbers causing yield losses worldwide. Various experiments were conducted to assess the antifungal potential of fungal bio-agents isolated from soil and cucumber seed (bio-priming). *Trichoderma koningii* seemed to be the most effective bio-agent against *F. oxysporum* f. sp. *cucumerinum* with mycelial inhibition rate above 90%, followed by *Aspergillus niger* (87.70%) under laboratory conditions. Results showed that *T. koningii* was found effective to enhance the germination rate (95.07%). In greenhouse experiments, *T. koningii* applied preventively on cucumber inoculated with *F. oxysporum* f. sp. *cucumerinum* generated the lowest disease severity index (0.42), mortality (13.33%) and wilting rate (23.33%) and the highest fresh (0.857 g) and dry (0.180 g) weight of root, and root volume (2.04 cm^3). Based on these results, *T. koningii* applied preventively against *F. oxysporum* f. sp. *cucumerinum* on cucumber plants could be recommended as a biocontrol agent. However, although *A. niger* was effective against the tested phytopathogen, it is not recommended for biological control due to its carcinogenic properties.

Keywords: Biological control**,** *Cucumis sativus*, *Fusarium oxysporum* f. sp. *cucumerinum*, antifungal potential.

Introduction

In Iraq, cucumbers (*Cucumis sativus* L.) contribute significantly to the agricultural economy. The total area under cucumber cultivation was reported to be around 45,533 ha with an approximate production of 413.7 thousand tons during 2012 with a yield of 7.6 tons/ha of cucumber and gherkins productions in 2017. The total national production of cucumber in greenhouses was 156 thousand tons in 2016, and cv. Dhi Qar ranked 8th with 6.850 thousand tons (4.4% of the total production) (Jongerden *et al.*, 2019; Rhouma *et al.*, 2020).

However, important yield fluctuations were recorded from year to year mainly due to several factors such as biotic and abiotic stresses. *Fusarium oxysporum* (Schlechtend) f. sp. *cucumerinum* (Owen) Snyder & Hansen is the causal agent of *Fusarium* wilt disease of cucumbers and reduces both yield and fruit quality (Hussein, 2016, 2018). Symptoms appear typically on the host plant as necrotic lesions, foliar yellowing and wilting, vascular tissue damage, and finally plant death (López-Orona *et al.*, 2019). Control of *Fusarium* wilt disease is currently based on integrating different approaches such as crop rotation, soil fumigation or solarization, using resistant cultivars, organic amendment fertilization, biological control and grafting on resistant rootstocks. However, the most common practice used by Iraqi farmers the application of fungicides; which often is expensive, do not provide the control level anticipated in spite of its negative effect on the environment and human health. That's why alternative strategies for the management of *Fusarium* wilt disease are required by using an eco-friendly approach (Avinash & Ravishankar, 2017).

Biological control of *Fusarium* wilt diseases demonstrated high efficiency in controlling several *F. oxysporum* globally, throughout using multiple strategies and tactics (natural plant extracts, antagonistic fungi and bacteria, etc.) to maintain pathogen population under the economic injury level (Hussein, 2018). The use of these antagonistic fungi reduced the mycelium growth of certain soil-borne fungi and reduced disease severity. *Trichoderma* spp. was applied widely as a biocontrol agent against many plant diseases since 1920s (Harman *et al.*, 2004). The treatment of cucumber seeds and plants with beneficial microorganisms including *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., etc., could mitigate a wide variety of biotic, abiotic, and physiological stresses. Moreover, these bio-agents possessed a good antifungal potency and can enhance and increase seed germination and subsequent plant growth (Hussein, 2016, 2018; Mei *et al.*, 2019).

The aim of the current study was to: i) isolate and identify antagonistic fungi from rhizosphere of cucumber, ii) study the *in vitro* and *in vivo* interaction between antagonistic fungi and *F. oxysporum* f. sp. *cucumerinum* to assess their antifungal potentialities and consequently their potential use as biocontrol agents for several fungal diseases affecting cucumber in the field.

Material and Methods

Fungal community in cucumber rhizosphere

Soil samples were collected from three greenhouses (9 m x 60 m) located in Basra Iraq (Shatt al-Arab, Abu al-Khasib and Karmat Ali) planted with cucumber. Soil samples were taken using a 7-cm-diameter soil auger at 2-m intervals along

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transect randomly in a zigzag fashion at 10-20 cm depth and each sample was a composite of ninety soil cores. For each greenhouse, all samples were mixed together into a single sample. 9 samples (200 g) per replicate (3 replicates) were collected from each greenhouse (Rhouma *et al.,* 2019). Samples were collected in sterile polythene bags and brought to the laboratory for isolation of antagonistic fungi (*Trichoderma koningii*, *Aspergillus niger*, *A. flavus* and *Mucor* sp.), and determine their relative occurrence in different greenhouses by the dilution-plate method according to Matrood *et al.* (2021a).

Fungal population was determined by colonies count: only Petri dishes containing between 30 and 300 colonies at two successive dilutions were retained. The number of colony forming units (CFU) was determined by the following formula: CFU/g of soil = ((Tn)/(0.1 x (N1+ 0.1 x N2) x Df); where: Tn is the total number of colonies, N1 is number of Petri dishes retained for the first dilution, N2 is Number of Petri dishes retained for the second dilution, Df is dilution factor (Matrood & Rhouma, 2021a).

Following macroscopic and microscopic observations of fungal structures, the fungal species were identified based on different identification keys (Chen *et al.,* 2012; Cwalina-Ambroziak & Wierzbowska, 2011; Harman *et al.,* 2004; Hussein, 2016; Ratna Kumar *et al.,* 2015; Rhouma *et al.,* 2021; Rosas-Medina *et al.,* 2020).

In vitro* antifungal potential of fungal bio-agents against pathogenic *Fusarium oxysporum* f. sp. *cucumerinum

Antifungal activities of the four fungal antagonists (*T. koningii*, *A. niger*, *A. flavus* and *Mucor* sp.) on radial mycelial growth of *F. oxysporum* f. sp. *cucumerinum* were determined by dual confrontation technique on potato dextrose agar (PDA) according to Rhouma *et al.* (2018). The inhibition of radial mycelial growth of the pathogen was evaluated according to the formula of Matrood & Rhouma (2021b). I (%) = (1- C_n/C_0) x 100; where: C_n is the diameter of radial growth of the pathogen in the presence of the antagonist, whereas, C_0 is the diameter of growth of the pathogen in the control treatment.

***C. sativus* seed bio-priming with different conidial suspension of fungal bio-agents**

Cucumber (cv. Maymon) seeds were sterilized by soaking in 3% solution of sodium hypochlorite (NaOCl) for 2 min and washed with sterilized distilled water 3 times. Application of each conidial suspension (10^8 CFU/mL) of fungal antagonist was carried out separately after seed drying. The assay was carried out by spraying cucumber seeds by the same amount of each fungal antagonist separately. Two controls were used for comparison; a positive control (seeds inoculated only with the pathogen) and a negative control (seeds treated only with sterilized and distilled water). The treated cucumber seeds were placed on the surface of Petri dishes (9 cm in diameter) containing cotton balls soaked in sterilized distilled water. In each Petri dish, 25 seeds were placed (with total of 10 Petri dishes for each replicate). Conidial suspension of *F. oxysporum* f. sp. *cucumerinum* (isolated from cucumber roots) was adjusted to 10^6 CFU/mL with hemocytometer. The plates were incubated in the dark at 25±2°C for 10 days, and then examined for germination rate (El Hartiti *et al.,* 2016). Cucumber seed germination rate (PG) was determined referring to the formula as followed: PG (%) = (Ng/Nt) x 100; where: Ng is number of germinated seeds; Nt is total number of seeds (250 seeds for each replicate) (Mukhtar, 2008; Rahman *et al.,* 2012).

***In vivo* antifungal potential of the fungal bio-agents**

Germinated cucumber seeds were placed in a pot containing a mixture of peat and vermiculite (1:1) at the rate of 3-5 seedlings in each pot. This experiment was carried out in February, 2019 in the greenhouse to assess preventive treatments. The preventive assay was carried out by dipping roots of 15 days old cucumber seedlings (cv. Maymon) into a flask containing a conidial suspension of the different antagonists (10^8 CFU\mL each) for 30 min. Plants were treated with the same antagonistic agent as in the seed stage. 50 ml (10^6 CFU\mL) of *F. oxysporum* f. sp. *cucumerinum* were applied 7 days after. The pots were then placed in a greenhouse for 30 days. The same negative and positive controls were used in the treated seeds assay. For each treatment of pathogen and fungal antagonist, and controls; cucumber plants were randomly distributed, with 10 plants per replicate (3 replicates), and the entire experiment was repeated twice (Matrood *et al.,* 2021b; Matrood & Rhouma, 2021c).

The parameters evaluated were measured within 30 days following inoculation. Three parameters used for disease assessment were: (i) disease severity index (DSI) using a 0-5 scale, where 0=healthy plant; 1=plant with brown vessels in the first internode region, without other visible symptoms; 2=plant with brown vessels up to the height of the first leaf, with yellowing of at least one leaflet; 3=plant showing vessel browning up to half of the stem length, with yellowing of two or more leaves; 4=plant showing vessel browning nearly to the leader shoot, with most leaves wilted, except the leader shoot; 5=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot (Silva & Bettiol, 2005); (ii) mortality rate (%) calculated by using the following formula: Mortality rate(MR) (%) = (Tdp/T) x 100; where: Tdp is the total number of dead plants; T is the total number of plants: and (iii) wilting rate (%) measured by using the following formula: Wilting rate (WR) (%) = (Twp/T) x 100; where: Twp is the total number of wilted plants, and T= total number of plants.

Other horticultural parameters were measured such as root fresh and dry weight (RFW, RDW) (Boughalleb-M'Hamdi *et al.,* 2017; Rhouma *et al.,* 2018). Root volume (RV) (ml) was determined by the immersion method as described by Rhouma *et al.* (2018).

Statistical analysis

Statistical analysis was carried out on the mean values of replicates using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA) followed by one-way ANOVA. Homogeneity of variances and normality were performed by applying Duncan multiple range test. Differences between treatments were determined by Duncan multiple range test at P=0.05.

Results

Fungal community in cucumber rhizosphere

The results obtained revealed that *T. koningii*, *A. niger*, *A. flavus* and *Mucor* sp. were recovered from all sampling sites at 10-20 cm depth. The antagonistic fungi concentration isolated from soil ranged from 38.15 x 10^5 (Shatt Al-Arab) to 46.08 x 10^5 (Karmat Ali) CFU per gram of soil. Statistical analysis revealed a significant difference of fungal densities ($P < 0.05$). Highest fungal density was recorded for *A. flavus* (17.48 x 10^5 CFU per gram of soil), followed by *A. niger* (10.76 x 10^5 CFU per gram of soil). However, the lowest concentration was recorded for *Mucor* sp. with a value of 3.70 x 10^5 CFU per gram of soil (Table 1). Another antagonistic fungi identified in the soil of the three sites were: *A. terreus*, *A. fumigatus*, *A. nidulans*, *Penicillium* spp., *Chaetomium* spp., *Gliocladium* spp., *Paecilomyces* spp. and *Cladosporium* spp., as well as many pathogenic fungi (*Fusarium* spp., *Rhizoctonia* spp., *Macrophomina* spp., *Colletotrichum* spp., *Alternaria* spp. and *Sclerotinia* spp.).

In vitro* antifungal potential of fungal bio-agents against the fungal pathogen *Fusarium oxysporum* f. sp. *cucumerinum

The four antagonistic fungal species exerted highly significant reduction ($P<0.01$) on radial mycelial growth of *F. oxysporum* f. sp. *cucumerinum* five days after incubation. Statistical analysis revealed high significant interaction between *F. oxysporum* f. sp. *cucumerinum* and the antagonists ($P<0.01$). The linear decrease of growth of the pathogenic fungi ranged from 90.13% (*T. koningii*) to 55.07% (*Mucor* sp.) (Table 2). *Trichoderma* spp. showed a good ability to limit the mycelial growth of *F. oxysporum* f. sp. *cucumerinum in vitro*. In fact, the mycelial growth decreased in presence of *T. koningii* with values of 90.13%. Moreover, *in vitro* assay noted that *A. niger* possessed a good antifungal potency with mycelial inhibition rate of 87.70% (Table 2). The antagonistic efficacy was not only on the mycelial growth reduction, but also on the microscopic hyphal growth. Compared to controls, *F. oxysporum* f. sp. *cucumerinum* treated with *T. koningii* and *A. niger* showed strong mycelium lyses, in addition to induction of mycelial cords via anastomosis between hyphal filaments and mycelium winding.

***C. sativus* seed bio-priming with different conidial suspension of fungal bioagents**

The effect of six treatments on *C. sativus* seed germination under *in vitro* conditions are presented in Table 3. Statistical analysis showed significant differences between treatments ($P<0.01$). All treatments increased significantly seed germination rate as compared with the positive control (40.40%). Results obtained also showed that *Trichoderma* was effective in enhancing seed germination rate compared to the negative control (86.53%). However, among the treatments with the four fungal antagonists, *T. koningii* significantly enhanced cucumber seed germination rate (95.07%) followed by *A. niger* (79.53%) and *A. flavus* (68.07%).

***In vivo* antifungal potential of the fungal bio-agents**

All tested antagonists for *in vitro* competition with *F. oxysporum* f. sp. *cucumerinum* were used for the *in vivo* experiment. Statistical analysis indicated that cucumber plants' growth inoculated with *F. oxysporum* f. sp. *cucumerinum* and treated preventively with the four antagonistic fungi (each separately) was significantly higher ($P<0.01$) than non-inoculated plants. The preventive application with some fungal antagonists reduced disease incidence and improved some agronomic traits (Table 4).

Cucumber plants treated with *Trichoderma* sp. produced less symptoms on roots and foliage of cucumber plants. Results obtained confirmed the efficiency of *T. koningii* in decreasing significantly the disease severity index (DSI = 0.42), the mortality rate (MR = 13.33%) and wilting rate (WR = 23.33%). However, *Mucor* sp. was less efficient, with a DSI value of 2.50 ($DSI_{Positive\ control}$ = 4.83; $DSI_{Negative\ control}$ = 0), mortality rate of 51.67% ($MR_{Positive\ control}$ = 100%; $MR_{Negative\ control}$ = 0%) and wilting rate of 61.67% ($WR_{Positive\ control}$ = 100%; $WR_{Negative\ control}$ = 0%) (Table 4).

The efficiency of antagonistic fungi, applied preventively, on growth parameters was also evaluated under *in vivo* conditions. The results revealed that *T. koningii* increased significantly the root fresh weight (0.857 g) and dry weight (0.180 g), and root volume (2.04 ml). Similarly, the treatment with *A. niger* produced the highest value for root fresh weight (0.680 g) and dry weight (0.143 g) (Table 4).

Table 1. Mean densities of *Trichoderma koningii*, *Aspergillus niger*, *A. flavus* and *Mucor* sp. isolated from soil samples from three locations in Basrah, Iraq. Values in the table are the average of three replicates (with nine soil samples per replicate).

	Mean densities of some antagonistic fungi (x 10^5 CFU / g soil)					
Location	***T. koningii***	***A. niger***	***Mucor* sp.**	***A. flavus***	**Sum**	***P-value*** [a]
Shatt Al-Arab	6.93 bC	10.76 bB	3.43 bD	17.03 abA	38.15	<0.01
Abu al-Khasib	5.30 cC	11.95 abB	3.50 bD	16.33 bA	37.07	<0.01
Karmat Ali	9.06 aC	13.78 aB	4.16 aD	19.07 aA	46.08	<0.01
Mean	7.10	12.16	3.70	17.48	Nd	Nd
P-value [a]	<0.01	<0.05	<0.05	<0.05	Nd	Nd

[a] Probabilities associated with individual F tests.

Nd= not determined.

Means followed by the same capital letters in the same row or small letters in the same column are not significantly different according to Duncan multiple range test.

Table 2. Effect of direct confrontation of four fungal antagonists on mycelial growth inhibition of *Fusarium oxysporum* f. sp. *cucumerinum* five days after incubation at 25°C. Values in the table are the average of three replicates (with five Petri dishes per replicate).

Fungal antagonists	Mycelial growth inhibition rate (%)
T. koningii	90.13 a
A. niger	87.70 a
Mucor sp.	55.07 c
A. flavus	72.17 b
Mean	**76.27**

Means followed by the same small letters are not significantly different at P=0.01 according to Duncan multiple range test for comparison of means in the same column.

Table 3. Effect of seed treatment with fungal antagonists on *Cucumis sativus* as measured by seed germination rate.

Treatments	Seed germination (%)
Positive control[a]	40.40 e
Negative control[b]	86.53 b
T. koningii	95.07 a
A. niger	79.53 c
Mucor sp.	66.53 d
A. flavus	68.07 d
Mean	**72.69**

[a] Seeds inoculated with pathogen only.
[b] Seeds treated with sterilized distilled water only.
Means followed by the same small letters are not significantly different at P=0.01 according to Duncan multiple range test.
Values in the table are the average of three replicates (with 10 Petri dishes per replicate).

Discussion

The soil-borne pathogens control was difficult as they produce viable structures resistant to adverse environmental conditions such as ascospores, chlamydospores, etc. However, the misuse of fungicides to control *F. oxysporum* f. sp. *cucumerinum* had negative effects to the ecosystem (microbial population, soil, water quality, etc.) and human's health (Hussein, 2016, 2018). Biological control, which received more attention recently, involves the use of fungal antagonistic fungi to manage *Fusarium* wilt disease (Mei *et al.*, 2019).

The prevalence of a diversified fungal community in cucumber rhizosphere is in agreement with previous investigations (Boughalleb-M'Hamdi *et al.*, 2017; Cwalina-Ambroziak & Wierzbowska, 2011; Matrood *et al.*, 2021a; Matrood & Rhouma, 2021a), which pointed out that *Aspergillus* species were the most frequent in eggplants rhizosphere, followed by *Mucor* spp. and *Penicillium* spp.; and the lowest frequency was recorded for *Alternaria* species. Sangeetha *et al.* (2020) reported more than 15 species belonging to more than 6 genera from cultivable fields, where the dominant species were *Aspergillus* spp. and *Penicillium* spp., followed by *Trichoderma* spp. and *Fusarium* spp. Ratna Kumar *et al.* (2015) demonstrated that *Aspergillus* spp. and *Penicillium* spp. were dominant in all agricultural fields due to high sporelation capacity. The soil-borne fungal species diversity varied according to the specific conditions and the ecological factors (Rosas-Medina *et al.*, 2020) such as climatic conditions, geographical area, specificity of colonized plant tissues, host physiology, etc. (Sangeetha *et al.*, 2020).

In the current study, using *Trichoderma*, *Aspergillus* and *Mucor* species as biocontrol agents against *F. oxysporum* f. sp. *cucumerinum*, revealed that *T. koningii* inhibited the growth of this phytopathogen. Al-Tuwaigri (2008) has reported earlier that *T. viride* is able to suppress the growth and colonization of *F. oxysporum* f. sp. *cucumerinum*. Among the five antagonistic fungal isolates, *T. reesei* and *T. koningii* were effective bio-agents against *F. oxysporum* pathogen (Otadoh *et al.*, 2011). Saravanakumar *et al.* (2016) examined 100 isolates of *Trichoderma* against *F. oxysporum* under laboratory conditions, but only ten isolates decreased the growth of this pathogen with an inhibition rate of 85%. Similarly, Boughalleb-M'Hamdi *et al.* (2018) noted that *A. flavus*, *T. harzianum*, *A. terreus*, *T. viride* and *A. niger* were the most effective (>50%) against *F. oxysporum*. Nasrin *et al.* (2018) reported that *Trichoderma* spp. reduced the growth of *F. oxysporum* mycelia (82%). Abro *et al.* (2019) reported that *Trichoderma* spp. inhibited *in vitro* the mycelial growth of *F. oxysporum* f. sp. *Cucumerinum* through hyperparasitism. Furthermore, Mei *et al.* (2019) reported that *Trichoderma* spp. were able to produce antimicrobial substances and synthesize antibiotics and hydrolytic enzymes. Rhouma *et al.* (2018) reported that *T. viride* and *T. harzianum* exhibited the highest inhibitory activity (90%) against *Monosporascus cannonballus* mycelial growth.

Treatment with *T. koningii* led to early seed germination and highest germination rate. Similar results were documented by many workers for different seed species and varieties using *Trichoderma* sp. Matrood & Rhouma (2021a) documented that seed treatment with *T. koningii* increased shoot and root length, and leaf size. They also reported increase in the germination rate of *Zea mays*, *Phaseolus vulgaris*, *Pennisetum glaucum*, *Spinacia oleracea*, *Beta vulgaris*, *Solanum lycopersicum* and *Cichorium intybus*, and vigour index of *Capsicum annuum* and *P. glaucum* (Okoth *et al.*, 2011; Rahman *et al.*, 2012). Furthermore, *Trichoderma* spp. improved shoot length and seed germination, and boosted yield (Asaduzzaman *et al.*, 2010). Mukhtar (2008) reported that *Abelmoschus esculentus* seeds treated preventively with *T. harzianum* increased the germination index and reduced germination delay. Furthermore, *T. viride*, *T. pseudokoningii* and *T. harzianum* extracts applied preventively as a watermelon seed treatment enhanced seed germination and seedling vigour, and decreased the severity of *Fusarium* spp. and *Didymella bryoniae* (Bharath *et al.*, 2006). Moreover, Mogle & Maske (2012) revealed that the treatment with *Trichoderma* spp. against many seed-borne fungal pathogens were more efficient than fungicides, leaf extract and other antagonistic fungi (*Penicillium* spp. and *Aspergillus* spp.).

Table 4. Disease Severity Index, mortality and wilting percent, root fresh and dry weight and root volume values recorded by *Cucumis sativus* plants inoculated with *Fusarium oxysporum* f. sp. *cucumerinum* and treated preventively by four antagonistic fungi *in vivo* assay. Values in the table are the average of three replicates (with 10 plants per replicate).

Treatments	DSI	MR (%)	WR (%)	RFW (g)	RDW (g)	RV (ml)
Positive control[a]	4.83 a	100.00 a	100.00 a	0.298 d	0.037 d	0.39 e
Negative control[b]	0.00 d	0.00 e	0.00 e	0.938 a	0.238 a	2.89 a
T. koningii	0.42 d	13.33 d	23.33 d	0.857 a	0.180 ab	2.04 b
A. niger	1.83 bc	36.67 c	43.33 c	0.680 b	0.143 bc	1.05 cd
Mucor sp.	2.50 b	51.67 b	61.67 b	0.348 d	0.099 cd	0.91 d
A. flavus	1.67 c	31.67 c	48.33 c	0.538 c	0.111 bcd	1.21 c
Mean	**1.88**	**38.89**	**46.11**	**0.61**	**0.135**	**1.417**

[a] Plants inoculated with pathogen only.
[b] Plants treated with sterilized distilled water only.
DSI= Disease Severity Index; MR= Mortality rate; WR= Wilting rate; RFW=Root fresh weight; RDW=Root dry weight; RV= Root volume.
Means followed by the same small letters in the same column are not significantly different at P=0.01 according to Duncan multiple range test.

T. koningii applied as a preventive treatment against cucumber *Fusarium* wilt improved growth parameters. Cheng *et al.* (2010) demonstrated that *Trichoderma* sp. protected cucumber against *F. oxysporum* f. sp. *cucumerinum*. Zhuang *et al.* (2005) revealed thatcucumber seedlingsshowed a reduction in disease intensity (<33.69%) following treatment with chlamydospores and conidia of *Trichoderma* sp. Chen *et al.* (2012) demonstrated that the use of *Trichoderma* sp. protected cucumber against *Fusarium* wilt (>75%). Data presented in this paper documented a high efficiency of *Trichoderma* compared to published results, bringing up the potential value of *T. koningii* as a bio-control agent in the field.

The use of *Trichoderma* species led to the best growth parameters of cucumber plants. Yedidia *et al.* (2001) showed that *Trichoderma* spp. applied in the soil increased cucumber leaf area (80%), root surface area (95%), plant height (45%), root length (75%) and plant dry weight (80%). Mei *et al.* (2019) reported that *Trichoderma* spp. significantly increased the root specific surface area, total root absorption area, root activity, nitric nitrogen content and chlorophyll content. Matrood & Rhouma (2021a) documented that *T. koningii* improved seedling fresh and dry weights, and plant length. The same authors revealed an increase of peroxidase activity and chlorophyll content when eggplants were treated with *T. koningii* alone or in combination with *Mucor* sp. and *A. niger*.

Many mechanisms (competition, antibiosis, mycoparasitism mediated by hydrolytic enzymes) attributed to *Trichoderma* spp. could adversely influence phytopathogen growth and disease development (Munir *et al.*, 2014), but also activate the induced systemic resistance (ISR) in the host plant (Mei *et al.*, 2019). *Trichoderma* spp. improves the synthesis and stimulation of phytohormone production. The cytokinin-like molecules production (zeatyn and gibberellin GA3) could improve plant biofertilization (Osiewacz, 2002). Most *Trichoderma* species acidified their surroundings by producing many organic acids such as fumaric acid, citric acid or gluconic acid capable of dissolving mineral cations (magnesium, manganese and iron), micronutrients and phosphates (Harman *et al.*, 2004).

Synergism between *T. koningii* action modes and cucumber plant against *F. oxysporum* f. sp. *cucumerinum*was evaluated. It is usually noted that the abiotic (soil pH, water potential, soil type, soil temperature, and such like) and biotic environmental factors (plant varieties and species, microbial density and their activity in the soil), the biocontrol agents and their method and time of application method has a great influence on the effectiveness in biological control.

It can be concluded that *T. koningii* protects cucumber plants against *F. oxysporum* f. sp. *cucumerinum*, and improves plant growth, and consequently can be recommended for biocontrol. The systemic resistance induction of cucumber plants by *Trichoderma* spp. against *F. oxysporum* f. sp. *cucumerinum* is a subject of future research. However, although *A. niger* and *A. flavus* were effective against *Fusarium* wilt disease, these antagonistic fungi are not recommended due to their carcinogenic properties.

الملخص

مطرود، عبد النبي عبد الأمير، عبد الحق رحومة وذكاء فايز محمد. 2022. مكافحة مرض الذبول الفيوزاريومي للخيار باستخدام الفطريات المضادة المعزولة من الوسط الجذري. مجلة وقاية النبات العربية، 40(1): 62−69. https://doi.org/10.22268/AJPP-040.1.062069

يسبب مرض الذبول الفيوزاريومي الناتج عن الفطر *Fusarium oxysporum* f. sp. *cucumerinum* خسائر كبيرة في محصول الخيار في جميع أنحاء العالم. أجريت تجارب مختلفة تضمنت المكافحة الحيوية وذلك تحت ظروف شبه مُحكمة في المختبر والحقل. اختبرت القدرة المضادة للفطور باستخدام العوامل الحيوية الفطرية المعزولة من التربة (التربة نفسها المعزول منها الفطر الممرض). أكّدت النتائج أن الفطر *Trichoderma koningii* هو الأكثر فعالية كعامل حيوي مضاد للفطر الممرض *Fusarium oxysporum* f. sp. *cucumerinum* بنسبة تثبيط للميسيليوم الفطري فاقت 90%، تلاه الفطر *Aspergillus niger* بنسبة تثبيط بلغت 87.70% تحت

ظروف المختبر. أظهرت النتائج أن الفطر *T. koningii* أدى إلى زيادة نسبة الإنبات (95.07%). في تجارب الدفيئة، أدى استخدام الفطر *T. koningii* بشكل وقائي على نبات الخيار المُلقَّح بالفطر الممرض *F. oxysporum* f. sp. *cucumerinum* إلى تقليل شدة مؤشر المرض (0.42)، ونسبة موت النباتات (13.33%)، والذبول (23.33%)، وزيادة الوزن الرطب (0.857 غ) والجاف (0.180 غ) للجذر وحجم الجذر (2.04 مل). يمكن التوصية باستخدام الفطر *T. koningii* وقائياً في المكافحة الحيوية للسيطرة على الفطر الممرض *F. oxysporum* f. sp. *cucumerinum*. وعلى الرغم من فعالية الفطر *A. niger* في المكافحة الحيوية، إلاّ أنّه لا ينصح باستخدامه نظراً لخصائصه المسرطنة.

كلمات مفتاحية: مكافحة حيوية، *Cucumis sativus*، *Fusarium oxysporum* f. sp. *cucumerinum*، الإمكانات المضادة للفطور.

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