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Article

Biological control of root rot and wilt diseases of cucumber using certain bioagents and biocides under greenhouse conditions

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Abstract:

Ten fungal isolates belonging to *Rhizoctonia solani* (4 isolates), *Fusarium semitectum*, *F. solani*, *Macrophomina phaseolina*, *F. oxysporum* f.sp. *cucumerinum* (2 isolates), and *Sclerotium rolfsii* were recovered from infected cucumber roots collected from various localities during the fall (2020) and spring (2021) growing seasons. Pathogenicity tests revealed that all isolates were capable of infecting cucumber plants, causing root rot and wilt diseases. *Rhizoctonia solani* isolate No. 3 was identified as the most destructive, resulting in the highest disease severity. Similarly, *F. oxysporum* f.sp. *cucumerinum* isolate No. 8 was the most virulent among the wilt pathogens. Significant variations were observed among the tested cucumber genotypes regarding their susceptibility to pre- and post-emergence damping-off. *In vitro* studies demonstrated that *Trichoderma asperellum*, *T. harzianum*, *T. album*, and T34 exhibited varying degrees of antagonistic activity against the pathogenic fungi. Additionally, different bacterial bioagents were evaluated under laboratory and greenhouse conditions; *Pseudomonas fluorescens* followed by *Bacillus megaterium* showed the highest efficacy in inhibiting the mycelial growth of *R. solani* and *F. oxysporum* f.sp. *cucumerinum*, whereas *Bacillus subtilis* exhibited moderate effects, and *Paenibacillus polymyxa* was the least effective. Furthermore, commercial biocides (Bio-Arc, Plant Guard, and Rizo-N) were evaluated during the fall (2022) and spring (2023) seasons under greenhouse conditions. Plant Guard was the most effective treatment at all tested concentrations in reducing disease severity caused by *R. solani* and *F. oxysporum* f.sp. *cucumerinum*. Moreover, Bio-Arc, followed by Rizo-N at the highest concentration, resulted in a significant reduction in the incidence of root rot and wilt diseases.

Keywords:

Cucumber, Biological control, Root rot, *Rhizoctonia solani*, *Fusarium oxysporum*.

1. Introduction

Cucumber (*Cucumis sativus* L.) is considered one of the major summer vegetable crops in commercial fields in Egypt. In recent decades, efforts have been concentrated on cultivating the crop in protected systems (greenhouses) during the autumn and winter seasons. Consequently, the cultivated area of cucumber in Egypt is expanding at a relatively fast rate, particularly in newly reclaimed desert lands. However, cucumber plants are attacked by several fungal diseases during various growth stages, causing considerable yield losses under both field and greenhouse conditions. Among these, soil-borne diseases are particularly destructive. Root rot and wilt, primarily caused by *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *cucumerinum*, are the most common diseases affecting cucumber plants, leading to damping-off and significant economic damage (Al-Tuwaijri, 2015; Martinez et al., 2003). Sabbagh et al. (2017) emphasized that damping-off and root rot are serious threats to cucumber at both seedling and adult stages under protected cultivation. Furthermore, Aljawasim et al. (2020) reported that several fungal pathogens, including *R. solani*, *F. oxysporum*, *F. solani*, *Sclerotium rolfsii*, and *Macrophomina phaseolina*, are responsible for damping-off and root rot in cucumber and watermelon, causing severe losses in seed germination and plant survival. Although chemical fungicides have provided satisfactory control of these diseases, they are considered major contributors to environmental pollution and pose health risks. Therefore, to avoid the hazards associated with fungicides, alternative control methods have been investigated. Biological control using antagonistic microorganisms has proven to be a successful, effective, and eco-friendly strategy to manage various plant diseases and reduce crop damage (Fasusi et al., 2021; Wang et al., 2018). In recent years, *Trichoderma* species, beneficial bacteria, and commercial biocides have been extensively used to enhance plant growth and combat diseases (Awad and Fayyadh, 2018; Mahmoud, 2015). For instance, Thabet (2023) evaluated the inhibitory effect of fungal bioagents (*T. harzianum*, *T. asperellum*, *T. album*, and T34) and bacterial isolates against the linear growth of *R. solani*, *F. oxysporum* f.sp. *cucumerinum*, and *Verticillium albo-atrum* in vitro. Additionally, several commercial biocides have been tested for their efficacy in controlling cucumber root rot and wilt diseases under greenhouse conditions, offering a promising alternative to chemical treatments (Thabet, 2023). The present study aims to isolate and identify the causal pathogens of root rot and wilt diseases in cucumber. Furthermore, the investigation intends to evaluate the efficacy of certain antagonistic bioagents (fungal and bacterial) and selected commercial biocides in controlling these diseases under greenhouse conditions, providing a sustainable approach for disease management.

2. Materials and Methods

This study was conducted during the growing seasons of 2021 and 2022 under laboratory and greenhouse conditions at the Department of Agricultural Botany, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Egypt.

2.1 Isolation, Purification, and Identification of Pathogens

Samples were collected from the roots of cucumber and watermelon plants exhibiting typical symptoms of root rot and wilt. These samples were obtained from various locations in Al-Buhaira and Menoufia governorates, Egypt during the autumn growing season of 2019. Diseased roots were washed thoroughly with tap water, cut into small pieces (0.3–0.5 cm), surface-sterilized with 70% ethyl alcohol for 2–3 minutes, and dried between sterile filter papers. The pieces were then transferred onto Potato Dextrose Agar (PDA) medium supplemented with streptomycin to prevent bacterial growth and incubated at 25–27°C for 5-7 days. Hyphal tips or single spores were transferred to PDA slants to obtain pure cultures (Cowan et al., 1999). The isolated fungi were identified based on morphological and microscopic characteristics according to taxonomic keys (Barnett and Hunter, 1986; Nelson et al., 1983; Sneh et al., 1991). Identification was confirmed by the Department of Agricultural Botany, Al-Azhar University, Assiut, Egypt.

2.2 Pathogenicity Tests

Pathogenicity tests were conducted under greenhouse conditions during the spring season of 2020 using the cucumber hybrid 'Hayel'. Plastic pots (25 cm diameter) were filled with 4 kg of soil that had been previously sterilized with a 5% formalin solution and covered with a plastic sheet for 7 days. The soil was then aerated for four weeks to remove formaldehyde residues. The experiment included pots filled with sterilized soil and inoculated with pathogenic fungi, as well as control pots. Five seeds were sown in each pot, with four replicates per treatment. Disease incidence (pre- and post-emergence damping-off) was recorded at 15 and 30 days after sowing, while plant survival was recorded after 45 days. Disease severity was assessed after 60 days using a 0–5 scale described by Liu et al. (1995).

2.3 Susceptibility of cucumber hybrids

Six cucumber hybrids (Mashoor, HCU 096, Jannt, Crystal, G0, and Hayel) were evaluated for their susceptibility to *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *cucumerinum* during the fall 2020 season. The experimental design and disease assessment (pre- and post-emergence damping-off, survival percentage) were carried out as described in the pathogenicity test. The percentage of infection was calculated according to the formula by El-Helaly et al. (1970).

2.4 *In Vitro* Studies

2.4.1 Antagonistic activity of *Trichoderma* spp.

The inhibitory effect of *Trichoderma harzianum*, *T. asperellum*, *T. album*, and T34 (obtained from the Biological Control Unit, ARC, Giza) was evaluated against *R. solani* and *F. oxysporum* f.sp. *cucumerinum* using dual culture technique. A mycelial disc (6 mm) of the pathogen was placed on one side of a PDA plate, and a disc of the antagonist was placed on the opposite side. Plates inoculated with the pathogen alone served as controls. Four replicates were used for each treatment, and plates were incubated at 25±2°C. The percentage of growth inhibition was calculated using the following formula:

$$\text{Inhibition}(\%) = \frac{C - T}{T} \times 100$$

Where: C = Radial growth of the pathogen in the control. T = Radial growth of the pathogen in the treatment.

2.4.2 Antagonistic activity of bacteria

Four bacterial isolates (*Bacillus subtilis*, *B. megaterium*, *Paenibacillus polymyxa*, and *Pseudomonas fluorescens*), obtained from MERCIN (Faculty of Agriculture, Ain Shams University, Egypt), were tested. Bacterial isolates were streaked 2 cm from the edge of PDA plates, and a 6 mm pathogen disc was placed in the center (Abou-Aly, 2008; Landa et al., 1997). Plates were incubated at 25°C for 5 days. The inhibition zone was measured, and the percentage of inhibition was calculated as described above.

2.5 Efficacy of commercial biocides under greenhouse conditions

Three commercial biocides (Bio-Arc, Rizo-N, and Plant Guard) were evaluated during the fall (2022) and spring (2023) seasons. Sterilized soil was infested with *R. solani* or *F. oxysporum* f.sp. *cucumerinum* at a rate of 1% (w/w). The biocides were applied to the infested soil at three rates (1, 2, and 3 g or cm^3/kg soil) before sowing. Control pots contained infested soil without biocides. Each treatment consisted of 5 replicates (one pot per replicate) with 5 seeds of the hybrid 'Hayel' per pot. Pre- and post-emergence damping-off, plant survival, and disease severity were recorded as previously described.

2.6 Statistical analysis

The obtained data were subjected to statistical analysis of variance (ANOVA). The Least Significant Difference (L.S.D.) test was used to compare treatment means at a probability level of 0.05, as described by Gomez and Gomez (1984).

3. Results and Discussion

3.1 Isolation and pathogenicity tests

Ten fungal isolates were recovered from infected cucumber roots collected from different localities. The isolated fungi included *Rhizoctonia solani* (4 isolates), *Fusarium oxysporum* f.sp. *cucumerinum* (2 isolates), *F. semitectum*, *F. solani*, *Macrophomina phaseolina*, and *Sclerotium rolfsii*. Pathogenicity tests revealed that all fungal isolates were capable of infecting cucumber plants ('Hayel' hybrid), causing varying degrees of root rot and wilt symptoms. Data presented in Table (1) indicate that *R. solani* isolate No. 3 was the most destructive pathogen, recording the highest disease severity (88.12%), as well as high pre- and post-emergence damping-off. Among the wilt pathogens, *F. oxysporum* f.sp. *cucumerinum* isolate No. 8 was the most virulent, causing 77.15% disease severity. Significant differences were observed among the isolates compared to the control. *M. phaseolina* (isolate No. 7), *F. semitectum* (isolate No. 5), and *S. rolfsii* (isolate No. 10) exhibited lower disease severity, recording

38.35%, 41.65%, and 42.25%, respectively. These results are in agreement with Martinez et al. (2003) and Al-Tuwaijri (2015), who identified *R. solani* and *F. oxysporum* as the most common and aggressive pathogens on cucumber. Consequently, the most aggressive isolates (*R. solani* No. 3 and *F. oxysporum* No. 8) were selected for further studies.

Table 1: Disease severity of cucumber plants caused by the isolated fungi under greenhouse conditions during the fall 2022 growing season.

Isolate No.	The tested fungi	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survival (%)	Disease Severity (%)
1	*Rhizoctonia solani* (1)	15.00	35.00	50.00	65.35
2	*Rhizoctonia solani* (2)	12.00	22.00	66.00	73.40
3	*Rhizoctonia solani* (3)	22.00	46.00	32.00	88.12
4	*Rhizoctonia solani* (4)	18.00	38.00	44.00	72.35
5	*Fusarium semitectum*	12.00	24.00	64.00	41.65
6	*Fusarium solani*	40.00	22.00	38.00	52.10
7	*Macrophomina phaseolina*	12.00	24.00	64.00	38.35
8	*F. oxysporum* f.sp. *cucumerinum*	20.00	55.00	25.00	77.15
9	*Fusarium oxysporum*	12.00	52.00	36.00	63.75
10	*Sclerotium rolfsii*	16.00	36.00	48.00	42.25
-	Control (Uninfected)	0.00	0.00	100.00	0.00
L.S.D at 5%		4.18	5.36	6.65	7.15

3.2 Reaction of cucumber genotypes

The susceptibility of six cucumber genotypes to the most aggressive isolates was evaluated under greenhouse conditions. Data in Table (2) show that all tested genotypes were susceptible to infection, though significant differences were observed. 'Jannt' and 'Go' were the most susceptible varieties to *R. solani*, recording disease severity of 65.40% and 55.10%, respectively. Conversely, 'Mashhor' and 'Hayel' were the least susceptible to *R. solani*. Regarding *F. oxysporum* f.sp. *cucumerinum*, 'Go' followed by 'Crystal' exhibited the highest disease severity (55.20% and 48.75%, respectively), while 'Mashhor' (28.45%) and 'Jannt' (32.40%) showed the lowest disease severity. These findings are consistent with reports by Al-Tuwaijri (2015) and Thabet (2023), who noted varietal differences in resistance to root rot and wilt pathogens.

Table 2: Response of six cucumber genotypes to root rot and wilt diseases incited by the most aggressive *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *cucumerinum* isolates under greenhouse conditions.

Cucumber genotype	*Rhizoctonia solani* (Isolate 3)				*F. oxysporum* f.sp. *cucumerinum* (Isolate 8)			
	Pre %	Post %	Surv. %	D.S. %	Pre %	Post %	Surv. %	D.S. %
Hayel	25.00	20.00	55.00	35.25	10.00	15.00	75.00	45.15
HCU 096	15.00	20.00	65.00	45.75	10.00	10.00	80.00	38.75
Crystal	18.00	22.00	60.00	46.25	20.00	30.00	50.00	48.75
Go	22.00	28.00	50.00	55.10	15.00	20.00	65.00	55.20
Jannt	30.00	35.00	35.00	65.40	10.00	10.00	80.00	32.40
Mashhor	20.00	10.00	70.00	35.00	10.00	10.00	80.00	28.45
Control	0.00	0.00	100.00	0.00	0.00	0.00	100.00	0.00
L.S.D at 5%	10.50	8.37	5.15	12.11	8.75	7.15	9.75	10.65

Pre = Pre-emergence damping-off; Post = Post-emergence damping-off; Surv. = Survival; D.S. = Disease Severity.

3.3 *In vitro* biological control

3.3.1 Efficacy of *Trichoderma* spp.

The antagonistic activity of *Trichoderma* species against the pathogenic fungi is presented in **Table (3)**. Results indicated that all tested bioagents significantly inhibited the mycelial growth of the pathogens compared to the control. *Trichoderma* spp. grew rapidly over the mycelium of *F. oxysporum* and *R. solani*, preventing their development. **T34** (biocontrol agent) exhibited the highest inhibitory effect, reducing the growth of *R. solani* by 86.33% and *F. oxysporum* by 88.65%. *T. asperellum* followed, showing 78.35% inhibition of *R. solani*. *T. harzianum* recorded the lowest inhibition percentages against both pathogens. These results align with El-Sheshtawy et al. (2009) and Malathi (2015), who reported the efficacy of *Trichoderma* spp. in hyperparasitizing soil-borne pathogens.

Table 3: Antagonistic activity of *Trichoderma* spp. on the mycelial growth inhibition of the pathogenic fungi *in vitro*.

Trichoderma Bioagents	Mycelial Growth Inhibition (%)	
	Against *R. solani*	Against *F. oxysporum* f.sp. *cucumerinum*
Trichoderma asperellum	78.35	82.66
Trichoderma harzianum	66.66	69.25
Trichoderma album	71.25	79.33
T34 (Biocontrol)	86.33	88.65
Control	0.00	0.00
L.S.D at 5%	1.39	2.15

3.3.2 Efficacy of bacterial bioagents

Data in Table (4) demonstrate that all tested bacterial isolates significantly reduced the linear growth of *R. solani* and *F. oxysporum*. *Pseudomonas fluorescens* gave the highest inhibition of *R. solani* (81.75%) and *F. oxysporum* (86.67%), followed by *Bacillus megaterium*. *Bacillus subtilis* showed moderate efficacy, while *Paenibacillus polymyxa* was the least effective. The use of microbial antagonists offers an effective and eco-friendly strategy for controlling soil-borne pathogens, as supported by Gravel et al. (2004).

3.4 Efficacy of commercial biocides under greenhouse conditions

The efficacy of commercial biocides (Bio-Arc, Plant Guard, and Rizo-N) in controlling root rot and wilt was evaluated during the fall (2022) and spring (2023) seasons (Table 5).

Table 4: Effect of antagonistic bacteria on the mycelial growth inhibition of the pathogenic fungi *in vitro*.

Antagonistic Bacteria	Mycelial Growth Inhibition (%)	
	Against *R. solani*	Against *F. oxysporum* f.sp. *cucumerinum*
Bacillus subtilis	72.33	81.66
Bacillus megaterium	79.15	84.33
Paenibacillus polymyxa	68.66	78.10
Pseudomonas fluorescens	81.75	86.67
Control	0.00	0.00
L.S.D at 5%	2.64	1.55

Table 5: Effect of commercial biocides on controlling cucumber root rot and wilt diseases under greenhouse conditions during the fall (2022) and spring (2023) growing seasons.

Commercial Biocides	Rate of Application	Disease Severity (%)			
		Fall (2022)		Spring (2023)	
		R. solani	*F. oxysporum*	*R. solani*	*F. oxysporum*
Bio-Arc	1 g	14.25	16.75	16.25	15.75
	2 g	10.15	11.10	10.35	9.25
	3 g	6.65	5.35	7.14	6.33
Plant Guard	1 cm³	9.62	11.44	10.65	12.33
	2 cm³	6.66	8.54	7.85	7.66
	3 cm³	4.35	3.90	5.25	4.75
Rizo-N	1 g	16.65	14.75	16.40	17.75
	2 g	12.25	10.25	11.50	12.66
	3 g	9.15	6.65	6.67	7.33
Control	--	86.10	77.25	88.90	78.33
L.S.D at 5%		1.05	1.33	1.28	1.18

All tested biocides effectively reduced disease severity compared to the control. Plant Guard was the most effective treatment at all tested concentrations (1, 2, and 3 cm³/kg soil), significantly decreasing the severity of root rot caused by *R. solani* and wilt caused by *F. oxysporum* in both seasons. Bio-Arc, followed by Rizo-N, also provided significant disease control, particularly at the highest concentration (3 g/kg soil). These treatments resulted in the highest reduction of wilt disease compared to lower concentrations and the control. These findings are in accordance with El-Blasy (2006) and Thabet (2023), who confirmed the potential of these biocides in managing cucumber diseases.

4. Conclusion

The present study highlights the significant threat posed by *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *cucumerinum* to cucumber production under greenhouse conditions, as pathogenicity tests confirmed the susceptibility of various cucumber genotypes, particularly 'Jannt' and 'Go'. *In vitro* investigations demonstrated the high antagonistic potential of *Trichoderma* species, especially isolate T34, and bacterial bioagents like *Pseudomonas fluorescens* and *Bacillus megaterium*, while greenhouse experiments provided practical evidence that commercial biocides can effectively manage these diseases. Among the tested treatments, Plant Guard proved to be the most effective at all concentrations, followed by Bio-Arc and Rizo-N at higher application rates. Consequently, this study recommends the integration of these eco-friendly biocides into disease management programs as a sustainable alternative to chemical fungicides, reducing environmental pollution while maintaining cucumber productivity in protected cultivation systems.

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