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Research Article

Biocontrol of *Rhizoctonia solani* (Kühn) and *Fusarium solani* (Marti) causing damping-off disease in tomato with *Azotobacter chroococcum* and *Pseudomonas fluorescens*

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Abstract

Background and Objective: The Damping-off disease is one of the most reasons for low productively of tomato in the world, especially in Iraq. In the current study, two types of bacteria (*Azotobacter chroococcum* and *Pseudomonas fluorescens*) were used to evaluate their efficacy in inhibiting the growth of pathogenic fungi *Rhizoctonia solani* and *Fusarium solani* and protecting the seeds of tomato and increasing their germination percentage. **Materials and Methods:** Dual culture technique and Food poisoning technique were used to study the effect of bacteria on the growth of fungi understudy, and study the effect of bacterial filtrates on germination of tomato seeds. **Results:** *A. chroococcum* showed the strongest antagonistic activity followed by *P. fluorescens* with the percentage of inhibition ranging between 72.9-77.1 and 69.5-70.3% for *R. solani* and *F. solani* respectively after 7 days of incubation. The effect of *A. chroococcum* and *P. fluorescens* filtrates were increased and also increased the inhibition of growth of fungi understudy, *A. chroococcum* filtrate also showed the strongest inhibitory effect followed by *P. fluorescens* with the percentage of inhibition ranging between 86.0-87.0 and 83.0-83.5% for *R. solani* and *F. solani* respectively at 20% concentration of filtrate. The percentage of seeds germination reached 90% in the treatment of *A. chroococcum* filtrate and 80% in the treatment of *P. fluorescens* filtrate. **Conclusion:** It can be concluded that the filtrates of *A. chroococcum* and *P. fluorescens* have antifungal properties against *R. solani* and *F. solani* and provided a high protection and increasing tomato seeds germination percentage.

Key words: Biocontrol, *Rhizoctonia solani*, *Fusarium solani*, Tomato, *Azotobacter chroococcum*, *Pseudomonas fluorescens*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In many countries tomato (*Lycopersicon esculentum* Mill) considered is one of the best common and important vegetable yields, which ranks second in the world in importance following potato[1,2]. There are many regions specialized in the cultivation of this yield but it suffers from many agricultural pests, including pathogenic fungi such as *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani* and *Pythium aphanidermatum* which are specific factors for the success of cultivation of this crop and considered one of the main causes of plant diseases, which can infect plants at different stages of growth[3,4]. Many methods have been used to reduce the economic losses in cultivating the tomato crop due to fungal infections such as the use of chemical pesticides[5]. Which are considered not a successful solution because their incorrect use causes many environmental and health problems and disrupts the natural balance of living microorganisms[6,7]. Also, many of these chemical pesticides have lost their ability to effect because of the development of new strains of pathogens resistant to the action of it[8]. Because of these problems, recent studies have focused on the use of microorganisms including bacteria, in the biological control of various pathogens. The soil contains many microorganisms that affect each other in different relationships such as antagonism, parasitism, and competition among themselves. These relationships have been used to resist various pathogens[9,10]. Due to the importance of this crop and the increase of agricultural regions of the crop of tomatoes in Iraq and the occurrence of many of the fungal infections that cause significant economic losses this study aimed to use biological control using *Azotobacter chroococcum* and *Pseudomonas fluorescens* bacteria as one of the important methods of resistance to damping-off disease in tomato caused by pathogenic fungi *Rhizoctonia solani* and *Fusarium solani*.

MATERIALS AND METHODS

Study area: The study was carried out at the University of Qadisiyah/College of Science/ Department of Biology/Iraq for the period from 2 September to 19 December 2019.

Microorganism

Fungal isolates: Ten samples were taken from tomato plants that showed symptoms of infection of damping-off disease from one of the agricultural fields in the Sinniyah district in Diwaniyah city/Iraq, the tomato plants parts were washed with water for 30 min and then left to dry, these parts were cut into small pieces about 0.5-1 cm, then sterilized with sodium hypochlorite solution at a concentration of 1% for 3 min, then washed with sterile distilled water three times and left to dry, after that put 5 pieces for all part in sterile Petri dishes with a diameter of 9 cm containing the sterile culture medium Potato Dextrose Agar with 5 replicates for each sample and incubated at 27°C for 3-5 days, then the fungi *R. solani* and *F. solani* under study were identified dependent on the macroscopic appearance and microscopic features using the classification keys[11,12].

Bacterial isolates: *A. chroococcum* and *P. fluorescens* were collected from the Graduate Laboratory at the Department of Biology/College of Sciences/University of Al-Kufa.

Dual culture technique: The interaction of the colony among the test pathogenic fungi and bacteria were studied *in vitro* in dual culture technique. The individual test *R. solani* and *F. solani* were grown separately on Potato Dextrose Agar medium and the individual species of *A. chroococcum* and *P. fluorescens* grew separately on Nutrient Agar medium, then the agar blocks (5 mm) cut from the individual just opposed to each other about 2 cm apart, in Petri dishes containing PDA medium. Three duplicates for each treatment were used and control was treated in lone. The place of the colony edge on the back of the disc was noted daily. The calculation was made for the pathogenic fungi and bacteria completed an equilibrium after which there was no more change in the growth since both of the microorganisms were naturally inhibited, the calculation was made according to Barnett and Hunter[13] as follows:

$$\text{Inhibition}(\%) = \frac{r_1 - r_2}{r_1} \times 100$$

where, r_1 is the radial growth of fungus in control, r_2 is the radial growth of fungus in treatment.

Food poisoning technique: The pure culture of fungal species *R. solani* and *F. solani* were grown separately on PDA medium in Petri dishes. Agar blocks (5mm) cut from the actively growing edge of the new colonies of *A. chroococcum* and *P. fluorescens* were inoculated individually in a 250 mL conical flask containing 100 mL of sterilized PDB medium. The flasks were incubated at 37°C for 15 days. After the duration of incubation, the staling materials were filtered using filter paper first through (Whatman No. 40 then through membrane filter). The filtrate was moved aseptically into the conical flasks and kept at 4°C until use.

The filtrates of culture that prepared were added individually to the cooled PDA medium to prepare the concentration 5, 10, 15 and 20%, the PDA medium was distributed in Petri dishes and after solidification, agar blocks (5mm) cut from the actively growing edge of the pathogenic fungi under study (three duplicates for each treatment were used and control was treated in lone) and inoculated at the center of the Petri dishes then incubated at 37°C. The radial growth was measured when the growth in control reached to the edge of the petri dish, the percent of growth inhibition was calculated according to Broekaert *et al.*[14] as follows:

$$\text{Inhibition}(\%) = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Effect of bacterial filtrates on germination of tomato seeds: Some tomato seeds types (Castle Rock) was sterilized by using sodium hypochlorite at 1% concentration for 2 min and washed with distilled water and left to dry, then treated with 15 mL of pre-prepared bacterial filtrates individually for 3 min and distributed 20 seeds for each Petri dishes containing PDA medium after inoculating the center of it with agar blocks (5 mm) cut from the actively growing edge of the colonies of pathogenic fungi under study individually with three duplicates for each treatment, with a control treatment which included non-treated seeds with the bacterial filtrate. The Petri dishes were incubated at 25°C for 10 days. Then calculate the number of germinated seeds and the germination percentage according to Carling *et al.*[15] as follows:

$$\text{Germination}(\%) = \frac{\text{Germination seeds}}{\text{Total seeds}} \times 100$$

Statistical analysis: Analysis of Variance (ANOVA) was used to find significant differences between the rates by Duncan's multiple range test at 5% probability level[16].

RESULTS

The percentage of inhibition of *A. chroococcum* and *P. fluorescens* against test pathogenic fungi *R. solani* and *F. solani* are presented in (Table 1 and 2). It was observed that *A. chroococcum* showed the strongest antagonistic activity followed by *P. fluorescens* with the percentage of inhibition ranging between (72.9-77.1) and (69.5-70.3)% for *R. solani* and *F. solani* respectively after 7 days of incubation. In the present study, the effect of *A. chroococcum* and *P. fluorescens* filtrates was increased and also increased the inhibition of growth of fungi under study, it was observed that *A. chroococcum* filtrate also showed the strongest inhibitory effect followed by *P. fluorescens* with the percentage of inhibition ranging between 86.0-87.0 and 83.0-83.5% for *R. solani* and *F. solani* respectively at 20% concentration of the filtrate, there were also found significant differences between different treatments at the 5% probability level (Table 3 and 4). The

Table 1: Antagonistic effect of *A. chroococcum* against the test pathogenic fungi

	The average diameter of mycelial fungal growth(mm)			The average diameter of mycelial fungal growth(mm)		
Incubation days	Control (*R. solani*)	Treatment (*A. chroococcum* + *R. solani*)	Inhibition (%)	Control (*F. solani*)	Treatment (*A. chroococcum* + *F. solani*)	Inhibition (%)
3	55.2±0.17^{c}	30.3±0.22^{a}	45.1	53.4±0.25^{c}	25.5±0.25^{a}	52.2
5	60.7±0.24^{b}	25.6±0.15^{b}	57.8	58.2±0.12^{b}	21.6±0.19^{b}	62.8
7	69.8±0.28^{a}	18.9±0.32^{c}	72.9	70.9±0.31^{a}	16.2±0.28^{c}	77.1

All values in the table are expressed as a Mean±Standard error, The columns with the same letters indicate no significant differences between means at 5%

Table 2: Antagonistic effect of *P. fluorescens* against the test pathogenic fungi

	The average diameter of mycelial fungal growth (mm)			The average diameter of mycelial fungal growth(mm)		
Incubation days	Control (*R. solani*)	Treatment (*P. fluorescens* + *R. solani*)	Inhibition (%)	Control (*F. solani*)	Treatment (*P. fluorescens* + *F. solani*)	Inhibition (%)
3	55.8±0.15^{c}	35.2±0.24^{a}	36.9	48.7±0.28^{c}	33.5±0.22^{a}	31.2
5	62.6±0.20^{b}	25.6±0.27^{b}	59.1	57.2±0.15^{b}	29.2±0.27^{b}	48.9
7	68.7±0.18^{a}	20.9±0.35^{c}	69.5	71.2±0.24^{a}	21.1±0.33^{c}	70.3

All values in the table are expressed as a Mean±Standard error. The columns with the same letters indicate no significant differences between means at 5%

Table 3: Effect of *A. chroococcum* filtrate against the test pathogenic fungi

		Pathogenic fungi			
		R. solani		*F. solani*	
Bacterial filtrate used	The concentration of the filtrate (%)	The average diameter of mycelial fungal growth (mm)	Inhibition (%)	The average diameter of mycelial fungal growth (mm)	Inhibition (%)
A. chroococcum	5	45.3±0.14[b]	49.6	42.7±0.22[b]	52.5
	10	32.6±0.17[c]	63.7	30.8±0.27[c]	65.7
	15	24.4±0.27[d]	72.8	19.6±0.16[d]	78.2
	20	12.6±0.29[e]	86.0	11.7±0.33[e]	87.0
	Control	90.0±0.1[a]	-	90.0±0.1[a]	-

All values in the table are expressed as a Mean±Standard error, The columns with the same letters indicate no significant differences between means at 5%

Table 4: Effect of *P. fluorescens* filtrate against the test pathogenic fungi

		Pathogenic fungi			
		R. solani		*F. solani*	
Bacterial filtrate used	The concentration of the filtrate (%)	The average diameter of mycelial fungal growth (mm)	Inhibition (%)	The average diameter of mycelial fungal growth(mm)	Inhibition (%)
	5	47.4±0.37[b]	47.3	44.9±0.36[b]	50.1
	10	34.2±0.27[c]	62.0	36.6±0.29[c]	59.3
P. fluorescens	15	22.7±0.21[d]	74.7	24.3±0.18[d]	73.0
	20	15.3±0.14[e]	83.0	14.8±0.25[e]	83.5
	Control	90.0±0.1[a]	-	90.0±0.1[a]	-

All values in the table are expressed as a Mean±Standard error, The columns with the same letters indicate no significant differences between means at 5%

Table 5: Effect of *A. chroococcum* and *P. fluorescens* filtrates on seeds germination

Treatments	Germination (%)
A. chroococcum	90.0
P. fluorescens	80.0
Control	40.0

bacterial filtrates that used provided high protection for seed against pathogenic fungi understudy and showed a high effect in increasing seeds germination percentage, which reached 90% in the treatment of *A. chroococcum* filtrate and 80% in the treatment of *P. fluorescens* filtrate compared with the treatment of control which reached 40% (Table 5).

DISCUSSION

In the current study, *A. chroococcum* and *P. fluorescens* showed significant activity against *R. solani* and *F. solani* damping-off disease *in vitro*. The ability of *A. chroococcum* to inhibit the growth of pathogenic fungi under study may be due to their ability to produce some enzymes that have ability to degrading fungal cell walls such as β-1,3-Glucanase, Laminarinase, and Chitinase and produce antibiotics such as Phenazine, Herbicolin and Pyoluteorin as well as low molecular weight compounds such as HCN, which if are present in high concentrations inhibit the growth of pathogenic fungi[17]. These results correspond with the findings of AL-Azawy[18] that *A. chroococcum* can inhibit the growth of *R. solani* after 96 hrs with the percentage of inhibition 41.33%, and correspond with Muhsin and Al-Kaabi[19] which found that *A. chroococcum* can inhibit the growth of *R. solani* and *F. solani* causing seed decay and seedling damping-off disease in sunflower. As for *P. fluorescens*, the reason for its ability to inhibit the growth of fungi under study may also be the ability to produce some antibiotics such as Lipopeptide cyclic, Amphisin, Siderophores, 2,4-acetylphloroglucinol and Pyrrolnitrin and their production to enzymes such as Endochitinase, Lipase, Protease, β-1,3-Glucanase which degrading fungal cell walls and inhibits the growth of fungi and therefore has a strong resistance against the growth of fungal isolates under study[20]. These results correspond with some previous studies such as Chen *et al.*[21] and Ongena *et al.*[22] who found that *P. fluorescens* has a high inhibitory effect and ability to the protection of Cucumber against *Pythium* root rot and Showkat *et al.*[23] who found 7 isolates of *P. fluorescens* showed high antifungal activity against *F. oxysporum* and *Aspergillus* spp. *A. chroococcum* and *P. fluorescens* have many characters that make them well suited as biocontrol and growth-supporting factors since wide use of fungicides have resulted in the accretion of toxic materials potentially risky to humans and the environment also in the increase of resistance of pathogens, eco-friendly antagonistic bacteria like *A. chroococcum* and *P. fluorescens* could be used in the control of damping-off disease[24]. Therefore, our current study, according to the results obtained, suggests the possibility of

using the filtrates of *A. chroococcum* and *P. fluorescens* to provide a high protection and increasing tomato seeds germination percentage.

CONCLUSION

It can be concluded that the filtrates of *A. chroococcum* and *P. fluorescens* have antifungal properties against *R. solani* and *F. solani*, and provided a high protection and increasing seeds germination percentage, deserving more study for the determine the active materials in these filtrates and effectiveness of both under field conditions needs to be developed before these species can be used successfully in fungal control of damping-off disease in tomato and other fungal diseases.

SIGNIFICANCE STATEMENT

This study discovered the possibility of using the filtrates of *A. chroococcum* and *P. fluorescens* to inhibit the growth of fungi *R. solani* and *F. solani* that causing damping-off disease in tomato that can be useful in using them as safe alternatives to the environment instead of chemical pesticides. This study will help researchers to discover environmentally safe materials that have antimicrobial properties against pathogenic microorganisms that cause significant economic losses in different crops.

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REFERENCES

1. Raiola, A., M.M. Rigano, R. Calafiore, L. Frusciante and A. Barone, 2014. Enhancing the health-promoting effects of tomato fruit for biofortified food. Mediators Inflammation, Vol. 2014. 10.1155/2014/139873
2. Fentik, D.A., 2017. Review on genetics and breeding of tomato (Lycopersicon esculentum Mill). Adv. Crop Sci. Tech., Vol. 5, No.5. 10.4172/2329-8863.1000306
3. Lamichhane, J.R., C. Durr, A.A. Schwanck, M. Robin and J. Sarthou et al., 2017. Integrated management of damping-off diseases. A review. Agron. Sustain. Dev., Vol. 37, No. 10. 10.1007/s13593-017-0417-y
4. Jilani, M.S., K. Waseem, K. Ameer, T.A. Jilani, M. Kiran, A.A. Alizia and A. Parveen, 2013. Evaluation of elite tomato cultivars under the agro-climatic conditions of Dera Ismail Khan. Pak. J. Agri. Sci., 50: 17-21.
5. Mancini, V. and G. Romanazzi, 2013. Seed treatments to control seedborne fungal pathogens of vegetable crops. Pest. Manage. Sci., 70: 860-868.
6. Du Toit, L.J. 2004. Management of diseases in seed crops, in Encyclopedia of plant and crop science. In: Encyclopedia of Plant and Crop Science, Goodman, R.M., CRC Press, New York, pp: 675-677.
7. Lamichhane, J.R., S. Dachbrodt-Saaydeh, P. Kudsk and A. Messéan, 2015. Toward a reduced reliance on conventional pesticides in European agriculture. Plant Dis., 100: 10-24.
8. Kandel, Y.R., K.A. Wise, C.A. Bradley, M.I. Chilvers, A.U. Tenuta and D.S. Mueller, 2016. Fungicide and cultivar effects on sudden death syndrome and yield of soybean. Plant Dis., 100: 1339-1350.
9. Burns, J.R. and D.M. Benson, 2007. Biocontrol of damping-off of Catharanthus roseus caused by Pythium ultimum with Trichoderma virens and Binucleate rhizoctonia fungi. Plant Dis., 84: 644-648.
10. Bakker, P.A.H.M., L.X. Ran, C.M.J. Pieterse and L.C. Van Loon, 2010. Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant diseases. Canadian J. Plant Pathol., 25: 5-9.
11. Skidmore, A.M. and C.H. Dickinson, 1976. Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. Trans. Br. Mycol. Soc., 66: 57-64.
12. Moubasher, A.H. and A.T. Al-Subai, 1987. Soil Fungi in State of Qatar. Scientific and Applied Research Center, University of Qatar, Doha, Pages: 108.
13. Barnett, H.L. and B.B. Hunter, 1998. Illustrated Genera of Imperfect Fungi. 4th Edn., APS Press, Saint Paul, MN., ISBN-13: 978-0-89054-192-0, pp: 150-259.
14. Broekaert, W.F., F.R.G. Terras, B.P.A. Cammue and J. Vanderleyden, 2006. An automated quantitative assay for fungal growth inhibition. FEMS Microbiol. Lett., 69: 55-59.
15. Carling, D.E., D.J. Helm and R.H. Leiner 2007. In vitro sensitivity of Rhizoctonia solani and other multinucleate and Binucleate rhizoctonia to selected fungicides. Plant Dis., 70: 577-578.
16. Armitage, P., G. Berry and J.N.S. Matthews, 2008. Statistical Methods in Medical Research. 4th Edn., John Wiley & Sons, New Jersey, United States, Pages: 832.
17. Bashan, Y. and L.E. de-Bashan, 2005. Plant Growth-Promoting Soil. In: Encyclopedia of Soils in the Environment, Hillel, D. (Ed.). Vol. 1, Elsevier, Oxford, UK., pp: 103-115.
18. Al-Azzawi and I.Q. Wahid, 2010. Efficiency of interaction between Azotobacter SP. and arbuscular Mycorrhizal fungi for their potential to stimulate tomato plant resistance (Lycopersicon Escolentum Mill.)? to root rot disease. Ph.D. Thesis, University of Baghdad, College of Science, Department of Biology, Iraq.
19. Muhsin, M. and A. AL-Kaabi, 2015. Study of the effect of the bacteria "Azotobacter chroococcum" in protection of seeds and seedlings of sunflower (Helianthus annuus L.). J. Kerbala Agri. Sci., 2: 176-185.

20. Andersen, J.B., B. Koch, T.H. Nielsen, D. Sorensen and M. Hansen et al., 2003. Surface motility in Pseudomonas sp. DSS73 is required for efficient biological containment of the root-pathogenic microfungi Rhizoctonia solani and Pythium ultimum. Microbiol., 149: 37-46.
21. Chen, C., R.R. Belanger, N. Benhamou and T.C. Paulitz, 1998. Induced systemic resistance (ISR) by Pseudomonas spp. impairs pre- and post-infection development of Pythium aphanidermatum on cucumber roots. Eur. J. Plant Pathol., 104: 877-886.
22. Ongena, M., F. Daayf, P. Jacques, P. Thonart, N. Benhamou, T.C. Paulitz, P. Cornelis, N. Koedam and R.R. Belanger, 2003. Protection of cucumber against Pythium root rot by fluorescent pseudomonads: predominant role of induced resistance over siderophores and antibiosis. Plant Pathol., 48: 66-76.
23. Showkat, S., I. Murtaza, O. Laila and A. Ali, 2012. Biological control of Fusarium oxysporum and Aspergillus sp. By Pseudomonas fluorescens isolated from wheat rhizosphere soil of Kashmir. J. Pharm. Biol. Sci., 1: 24-32.
24. Houssien, A.A., S.M. Ahmed and A.A. Ismail, 2010. Activation of tomato plant defense response against Fusarium wilt disease using Trichoderma harzianum and salicylic acid under greenhouse conditions. Res. J. Agric. Biol. Sci., 6: 328-338.