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Research Article/Araştırma Makalesi

Isolation of bacterial isolates from soil samples and determination of their *in vitro* antagonistic potential against chickpea wilt disease agent *Fusarium oxysporum* f. sp. *ciceris*

Toprak örneklerinden bakteri izolatlarının izolasyonu ve nohut solgunluğu hastalığı etmeni Fusarium oxysporum f. sp. ciceris'e karşı in vitro antagonistik potansiyellerinin belirlenmesi

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ABSTRACT

In 2021, post-harvest soil samples were taken from fields where chickpea and wheat were grown in Yozgat province to isolate bacteria. Of 74 isolates obtained from soil samples, 14 nonpathogenic isolates were evaluated for their inhibitory potential against two pathogenic isolates (YBUFoc9 and YBUFoc2) of *Fusarium* wilt disease agent *Fusarium oxysporum* f. sp. *ciceris*. Antagonistic bacterial isolates were identified as *Bacillus* spp. by Blastn analysis based on their 16S rDNA nucleotide sequences. Among them, 2 isolates were identified as *B. amyloliquefaciens* (BM23 and BM40), 3 isolates as *B. subtilis* (BM8, BM32, and BM105), 6 isolates as *B. cereus* (BM10, BM69, BM70, BM104, BM111 and BM215), 2 isolates as *B. megaterium* (BM44 and BM135) and 1 isolate as *B. pumilus* (BM28). No effective isolate of any genus other than *Bacillus* spp. was found. They inhibited mycelial growth of isolates YBUFoc9 and YBUFoc2 from 0 to 78.50% and from 0 to 58.33%, respectively. Among the 5 different species, *B. amyloliquefaciens* (BM23 and BM40) and *B. subtilis* (BM8, BM32, and BM105) showed the strongest inhibitory activity against the two fungal isolates under *in vitro* conditions. In further studies, the biocontrol properties of the most effective isolates (BM23, BM40, BM8, BM32 and BM105) will be investigated and compared with approved fungicides for their fungicidal activity.

Key Words: Fusarium wilt, Chickpea, *Bacillus* spp., Biological control

ÖZ

2021 yılında Yozgat ilinde nohut ve buğday yetiştirilen tarlalardan bakteri izolasyonu için hasat sonrası toprak örnekleri toplanmıştır. Toprak örneklerinden izole edilen 74 izolat arasından patojenik olmayan 14 aday bakteri izolatın *Fusarium* solgunluğu etmeni *Fusarium oxysporum* f. sp. *ciceris*'in iki izolatına (YBUFoc9 and YBUFoc2) karşı engelleme potansiyelleri *in vitro* koşullarda değerlendirilmiştir. Antagonist bakteri izolatların tanısı 16S rDNA nükleotid dizilerine dayanan Blastn analizi ile *Bacillus* spp. olarak belirlenmiştir. Elde edilen izolatların 2'si *B. amyloliquefaciens* (BM23 ve BM40), 3'ü *B. subtilis* (BM8, BM32 ve BM105), 6'sı *B. cereus* (BM10, BM69, BM70, BM104, BM111 ve BM215), 2'si *B. megaterium* olarak (BM44 ve BM135) ve 1'i *B. pumilus* (BM28) olarak tanılanmıştır. Antagonistik etkinlik bakımından *Bacillus* spp. dışında etkili bir cinse rastlanılmamıştır. Bakteriyel izolatlar, YBUFoc9 ve YBUFoc2 izolatlarının misel büyümesine sırasıyla %0-%78.50 ve %0-%58.33 oranında inhibisyon sağlamışlardır. Beş farklı tür arasından *B. amyloliquefaciens* (BM23 ve BM40) ve *B. subtilis*'in (BM8, BM32 ve BM105) *in vitro* koşullar altında patojenin iki izolatına karşı en güçlü inhibisyon aktivitesi gösterdiği belirlenmiştir. Gelecekte yapılması planlanan çalışmalarda, en etkili izolatların (BM23, BM40, BM8, BM32 ve BM105) biyokontrol özellikleri araştırılacak ve fungisidal aktiviteleri için onaylanmış fungisitlerle karşılaştırılacaktır.

Anahtar Kelimeler: *Fusarium solgunluğu, Nohut, Bacillus* spp.*, Biyolojik mücadele*

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important sources of vegetable protein for human and animal nutrition which is one of the edible legumes that have long been cultivated in almost all parts of the world (Muehlbauer and Sarker, 2017). It is a traditional legume with a production of 0.63 million tons on 0.514 million hectares of land in Turkey (Anonymous, 2019a). Turkey ranks 4th with an area of 518 thousand hectares and accounts for about 4% of the total cultivated area in the world (Anonymous, 2019b).

Chickpea production and productivity exhibit high yield instability that fluctuates over the years due to biotic and abiotic factors (Houasli et al., 2021). *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is considered one of the major diseases of chickpea (Zaim et al., 2016). The pathogen causes wilting and yellowing of chickpea plants, resulting in losses of 10-40% of the annual crop depending on the variety and severity of the disease and can completely destroy crops if conditions are favorable for the disease development (Khan et al., 2014; Guerrero et al., 2015). Since the pathogen is widespread in cultivated areas, survives in the soil for a long time, and has different pathogenic races in its populations, it is very difficult to control the disease incidence (Jiménez-Díaz et al., 2015; Oliva-Ortiz et al., 2017). According to Akgün-Yıldırım and Güldür (2019) *F. oxysyporum* f. sp. *ciceris* invades the plant through the root and root neck region of susceptible chickpea cultivars and develops in the xylem tissue of the plant, and blockage of the plant's water transport system results in general wilting and yellowing of the plant. The *Fusarium* wilt disease was detected in 7 regions and 37 provinces of Turkey, indicating *F. oxysporum* f. sp. *ciceris* is an emerging pathogen of chickpea (Kocalar et al., 2020). In a previous study pathogenic isolates of *F. oxysporum* f. sp. *ciceris* isolated in Turkey were assigned to races 0, 2 and 3 by Bayraktar and Dolar (2012). These data are very important for the development of resistant varieties and complicate the management of the pathogen, which varies regionally depending on climatic factors and the susceptibility of the variety.

Both in Turkey and worldwide, the main control methods used by farmers are short crop rotation with wheat and the use of chemical pesticides but their effectiveness in reducing disease inoculum in pathogen-infested soils is low (Elbouazaoui et al., 2022). Biological control is one of the best, most cost-effective, and environmentally sustainable methods of controlling plant diseases caused by soilborne pathogens such as *Sclerotinia*, *Fusarium*, *Rhizoctonia*, and *Pythium* spp. (Stirling, 1991; Abbas et al., 2019; Kara et al., 2020). Moreover, the identification of indigenous antagonistic microbial strains is critical to optimize their success as biological control agents for chickpea *Fusarium* wilt, as they have been shown to be adapted to the source environment.

Bacillus species are Gram-positive and rod-shaped, and their isolates can produce endospores (Sülü et al., 2016). They are the most abundant bacterium in the agricultural ecosystem and one of their most important reasons is their use in the biological control of plant pathogenic fungi (Akhtar et al., 2010; Zaim et al., 2013; Abed et al., 2016; Villarreal-Delgado et al., 2018; Soylu et al., 2020). Strains of *Bacillus* taxa can synthesize a variety of antibiotics, toxins, siderophores, and lytic enzymes and induce systemic resistance and plant growth (Rangel-Montoya et al., 2022), also important plant growth-promoting bacteria (PGPB) that protect plants from biotic and abiotic stresses (Abbas et al., 2019).

Yozgat province located in the Central Anatolian region of Turkey is the second largest chickpea-growing area in Turkey with 713 thousand hectares (Anonymous, 2021). In recent years, the occurrence of wilt disease has been reported in many fields in different districts where the use of fungicides without any benefit of solution through seed and foliar application has been widely used by farmers in the region.

In this study, considering the success in disease

management with antagonistic bacteria, soil samples were collected from the fields after chickpea harvest in Yozgat province to isolate and identify putative isolates for *in vitro* antagonistic activity against *Fusarium* wilt disease agent *Fusarium oxysporum* f. sp. *ciceris*.

Materials and Methos

Soil samples and Fungal isolates

For isolation of bacterial isolates, 119 soil samples were collected from Merkez, Sorgun, Sarıkaya, and Yerköy districts of Yozgat province after harvesting wheat and chickpea between August and October 2021.

Two pathogenic isolates with different colony morphology (isolate YBUFoc9 in white color and isolate YBUFoc2 with violet color) of *F. oxysporum* f. sp. *ciceris* were isolated from diseased chickpea plants showing wilting and root rot in 2019-2021. Fungal isolates were identified according to their morphological characters described by Nelson et al. (1983) and Leslie and Summerell (2006). Pathogenicity tests were performed according to the soil inoculation method as described by Nene and Haware (1980). These isolates (YBUFoc9 and YBUFoc2) were stored as pure cultures at +4˚C or -20°C for long periods during the experiment.

Bacterial isolation from soil samples

Ten grams (g) of the soil sample was added to 90 ml of sterile distilled water in 250 ml flasks. After shaking the flasks at 150 rpm for 30 min in an orbital shaker, serially diluted (10^{-1} to 10^{-6}) suspensions (100 µl) were spread on Nutrient Agar (NA, Merck, Germany) medium and incubated for 48 h at 26˚C. Based on the colony morphology of the isolates, candidate isolates representing different population growth on the medium were randomly selected and subsequently purified.

Hypersensitive reaction on tobacco

To determine the pathogenic/saprophytic status of purified candidate bacterial isolates, a hypersensitivity test (HR) was performed with tobacco (Lelliot and Stead, 1987). Two-day-old bacterial cultures grown on NA medium were suspended in sterile distilled water at a density of 10^6 cfu ml^{-1} (McFarland, 0.5) and injected into tobacco leaf tissue. As a negative control, leaves were inoculated with sterile distilled water. The Evaluation was performed 2 days after inoculation. Isolates that caused tissue collapse at the inoculation site were accepted as HR (+).

Potato soft rot test

Healthy potato tubers were washed in rinse water with a brush for surface disinfection and then they were soaked in 1% sodium hypochlorite (NaOCI) for 3 minutes. They were rinsed 3 times with sterile distilled water to remove sodium hypochlorite. The potato tuber was sliced with a sterile scalpel and placed in petri dishes with sterile filter paper. Bacterial culture was spread on the potato slice and the petri dishes were moistened with sterile water. The evaluation was performed after two days of incubation at 25°C. Softening (maceration) on the inoculated area was positive and the absence of softening indicated a negative reaction (Öztürk and Soylu, 2022).

Antagonism Assay

The antagonistic potential of the bacterial isolates against the pathogen was determined by dual culture tests in petri dishes containing Potato Dextrose Agar (PDA) as described by Soylu et al. (2022). In these tests, the bacterial isolate to be tested was grown on one side of each petri dish and incubated at 26°C for 2 days. After bacterial growth, for both *Fusarium* isolates (white and violet morphology), mycelial disks 5 mm in diameter taken from the ends of a 10-day-old culture grown on PDA medium were placed 4 cm away from the growing bacteria and incubated at 25°C. No bacterial isolate was added to the PDA petri dishes that served as controls. When mycelial growth in the control plates reached the end of the plates (at the 10th day of incubation), mycelial growth of the fungus was measured, and percent inhibition rates were calculated based on

mycelial growth in the control petri dishes using the %Abbot formula (inhibition % = [(MGc-MGb) / MGc) * 100] as described by Soylu et al. (2020).

The experiment was repeated with four biological replicates. Mycelial inhibition values in the petri dishes were analyzed by one-way analysis ANOVA using the SPSS statistical program (SPSS Statistics 17.0), and the difference between isolates was analyzed using the Duncan multiple range test (P≤0.05) as described by Kara and Soylu (2022).

Identification of bacterial antagonist isolates

Gram staining, oxidase, catalase, and growth ability at 37°C tests were performed for bacterial isolates (Lelliot and Stead, 1987). For molecular identification, the 16S rRNA gene region of the isolates was amplified with primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'TACGGTTACCTTGTTACGACTT-3') for Blastn analysis. For the PCR reaction, a single colony was collected with a sterile pipette tip and added directly to the prepared PCR reaction tube with a final volume of 25 µl containing 12.5 µl 2x master mix (MyTaq Mix, Bioline, UK), 1 µl forward primer, 1 µl reverse primer, 1 µl DMSO and 9.5 µl sterile water. PCR amplifications were performed using a thermal cycler (Bio-Rad, T100) and touch-down PCR conditions were set as follows: 95 °C for 4 minutes, 94 °C for 10 cycles for 30 seconds, 65°C for 30 seconds, at 56 °C (1 °C reduction per cycle), 1 minute at 72 °C and 5 minutes at 72 °C, plus 24 cycles at constant 56 °C using the same parameters (Aksoy et al., 2017).

PCR products were separated by electrophoresis in 1x TAE buffer using an agarose gel stained with 1.5% ethidium bromide (w/v). The length of PCR products was estimated using the DNA molecular weight marker Hyperladder, 1 kb (Bioline, UK).

For Blastn analysis, consensus sequences for the 16S rDNA gene were generated from the forward and reverse strand sequences processed and assembled using Chromas pro (version 1.7.6), and the partial nucleotide sequences obtained were searched in NCBI GenBank to determine the taxonomic identification of the isolates. Nucleotide sequences of the 16S rDNA of 14 isolates were deposited in NCBI GenBank under accession numbers OP964829 to OP964842.

Results and Discussion

From soil samples, we obtained 74 bacterial isolates representing the general population with different morphological structures. Among the bacterial isolates, 27 isolates were HR-positive on tobacco and caused maceration on potato tuber slices. Due to the growth of 13 isolates at 37°C, they were excluded from the trials considering the possibility of potential plant and human pathogens. Forty isolates were not included in the *in vitro* dual culture test because they may be plant or human pathogens. It was decided to conduct detailed studies for *in vitro* conditions with the remaining 34 bacterial isolates that were negative for inducing HR on tobacco maceration of potato slices and growth at 37°C.

In the dual culture tests, fourteen bacterial isolates among 34 tested bacterial cultures that showed antagonistic effects against both isolates of pathogen. These isolates were found to inhibit mycelial growth of *Fusarium oxysporum* f. sp. *ciceris* YBUFoc9 isolate (white colony) from 0 to 78.50% and YBUFoc2 isolate (violet colony) from 0 to 58.33% at the 10th day of fungal incubation (Table 1). Among the isolates with high antagonist activity against the fungal agent, 14 different isolates representing the soils used in isolation were selected for molecular identification studies.

16S rDNA nucleotide sequences were used to identify 14 out of 34 isolates which were Gram-positive and catalase-positive and showed inhibition of fungal growth on the 7th day of the experiment. In Blastn analysis, all isolates were found to belong to *Bacillus* spp. Of the 14 isolates, 2 were identified as *B. amyloliquefaciens* (BM23 and BM40), 3 as *B. subtilis* (BM8, BM32, and BM105), 6 as *B. cereus* (BM10, BM69, BM70, BM104, BM111, BM215), 2 as *B. megaterium* (BM44 and BM135) and 1 as *B. pumilus* (BM28). According to the results of *in vitro* tests, no effective isolate was found that could belong to a genus other than *Bacillus* spp. (Table 1).

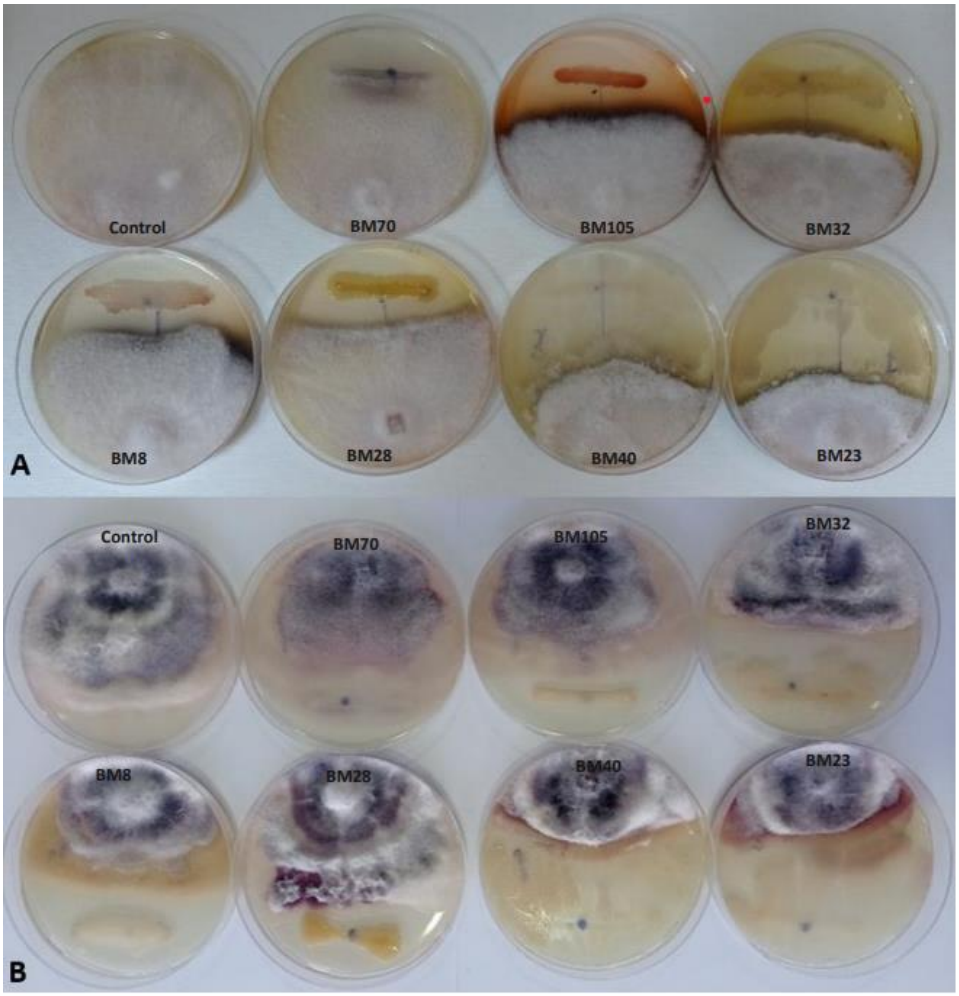

Figure 1. *In vitro* antagonistic effect of bacterial isolates A. inhibition of isolates on mycelial growth of YBUFoc9 (white colony) B. inhibition of isolates on mycelial growth of isolate YBUFoc2 (violet colony). (From left to right, control, BM70, BM105, BM32, BM8, BM28 BM40, BM23 for Figure A and B).

Şekil 1. Bakteriyel izolatların in vitro antagonistik etkisi A. İzolatların, YBUFoc9' nin misel gelişimini engellemesi (beyaz koloni) B. İzolatların, YBUFoc2'nin misel gelişimini engellemesi (mor koloni). (Şekil A ve B için soldan sağa; kontrol, BM70, BM105, BM32, BM8, BM28 BM40, BM23).

Considering the effectiveness of the tested antagonists on the inhibition of mycelial growth of *F. oxysporum* f. sp. *ciceris*, *B. amyloliquefaciens* BM 23 had the highest antagonistic effect on inhibiting fungal mycelial growth among the bacterial isolates, with a rate of 78.6% (white colony) and 58.33% (violet colony) ($p>0,05$). After BM 23 isolate, the highest inhibition of mycelial growth, 63.80% ($p<0,05$) and 53.88% ($p>0,05$), was caused by isolate BM40, which was identified as belonging to the same species as BM23. It was found that after the isolates of *B. amyloliquefaciens* BM23 and BM40, *B. subtilis* BM8, BM32 and BM105 were the most successful isolates for inhibiton of mycelial growth (Fig. 1). Other isolates showed only mild or no activity at all. Of the isolates, *B. cereus* BM70 was not found to be effective in inhibiting mycelial growth of both fungal isolates compared to the control, while *B. cereus* 111 was not able to inhibit the growth of YBUFoc2 isolate of pathogen ($p>0,05$).

It is known that chemical pesticides have harmful effects on humans and their constant use can also cause pathogen resistance. Therefore, there is an urgent need to develop environmentally friendly and hygienically safe crop protection methods based on alternative crop protection strategies to protect crops produced by intensive production in agriculture from yield-reducing factors and also to pay more attention to the microbial quality of the products (Warrior, 2020). Determination of the

effectiveness of bacterial isolates from several sources of nature on the progress of pathogens (Özyılmaz, 2019; Soylu et al., 2020) and their use as biological control agents are important for integrated crop management and organic farming, and their value in controlling fungal diseases is important (Kara and Soylu, 2022).

Table 1. Inhibition (%) potential of *Bacillus* spp. isolates on mycelial growth of isolates of *F. oxysporum* f. sp. *ciceris*
Çizelge 1. Bacillus spp*. izolatlarının F. oxysporum* f. sp. *ciceris'nin misel gelişimini engelleme potansiyelleri*

Bacterial isolates	**Species name**	**Fungal isolates**			
		YBUFoc9 isolate (white colony)		**YBUFoc2 isolate (violet colony)**	
		Mycelial growth (cm)	**Inhibition of fungal growth (%)**	**Mycelial growth (cm)**	**Inhibition of fungal growth (%)**
BM8	*Bacillus subtilis*	3,50^{d}	50,00	3,50^{b}	41,66
BM10	*B. cereus*	5,66^{g}	19,04	4,93^{de}	17,77
BM23	*B. amyloliquefaciens*	1,50^{a}	78,57	2,50^{a}	58,33
BM28	*B. pumilus*	3,76^{e}	46,19	4,80^{de}	20,00
BM32	*B. subtilis*	3,00^{c}	57,14	2,70^{a}	55,0
BM40	*B. amyloliquefaciens*	2,53^{b}	63,80	2,76^{a}	53,88
BM44	*B. megaterium*	5,00^{f}	28,57	5,10^{ef}	15,00
BM69	*B. cereus*	6,50^{i}	7,14	5,33^{f}	11,11
BM70	*B. cereus*	7,00^{j}	0,00	6,00^{g}	0,00
BM104	*B. cereus*	6,50^{i}	7,14	4,93^{de}	17,77
BM111	*B. cereus*	6,50^{i}	7,14	6,00^{g}	0,00
BM105	*B. subtilis*	3,63^{de}	48,10	3,63^{de}	48,09
BM135	*B. megaterium*	6,00^{h}	14,28	3,93^{c}	34,44
BM215	*B. cereus*	6,00^{h}	14,29	4,83^{de}	19,44
Control	*F. oxysporum* f. sp. *ciceris*	7,00^{j}	-	6,00^{g}	-

The mean mycelial growth (cm) of fungal agent determined was based on the measurements of 4 replicate plates, recorded at 10 days after inoculation. Mean values followed by different small letters within the column are significantly different according to Duncan Multiple Range Test ($p<0.05$).

Several bacterial species of *Bacillus* spp. have been reported to have antagonistic effects against disease caused by *Fusarium* spp. on crops. *Bacillus* spp. are well-known antibiotic producers that have an advantage over other biocontrol microorganisms due to their inherent ability to form endospores and their resistance to extreme conditions (Landa et al., 2001; Johri et al., 2003; Kara and Soylu, 2022). Previous biological control studies reported that antagonistic isolates of various *Bacillus* spp. are effective on the mycelium of soil-borne pathogenic fungal species such as *Sclerotinia* spp., *Fusarium* spp., *Rhizoctonia* spp., *Macrophomina* spp. (Fravel, 2005; Soylu et al., 2020; Kara and Soylu, 2022; Özkaya and Soylu, 2022). In this study, isolates were obtained from 4 different *Bacillus* species that exhibited varying levels of antagonistic activity. The most effective isolates were determined to belong to the species *B. amyloliquefaciens* and *B. subtilis*.

We found that six of the 14 isolates tested were found to be effective in controlling the mycelial growth of both fungal isolates by more than 30%. Similar results were obtained by Abed et al. (2016) that reported *Bacillus* strains were highly effective for the inhibition of pathogenic fungi. it has been reported that antagonistic bacterial isolates possess various mechanisms to suppress mycelial growth, such as antibiotics, siderophores, hydrolytic enzymes, and volatile extracellular metabolites (Aktan and Soylu, 2020). They may also produce secondary metabolites and compete for nutrients that contribute to plant growth enhancement and induced resistance in plants.

Biological control of *Fusarium* wilt has shown promise in previous studies because of its low environmental impact and its potential to reduce growers' dependency on chemicals, thereby slowing the development of fungicide resistance in pathogen populations. Production of antifungal compounds is an important mechanism of bacterial biocontrol agents, and they are also known to promote plant growth (Edwards and Arancon, 2004; Akhtar et al., 2010). Zaim et al. (2013) investigated 29 potentially antagonistic strains *in vitro* assays and reported that five antagonistic *Bacillus* spp. showed the most inhibitory effect with inhibition of mycelial growth from 25.63 to 71.11% against chickpea *Fusarium* wilt pathogen *F. oxysporum* f. sp. *ciceris*. Elbouazaoui et al. (2022) reported that 12 of 40 strains inhibited the growth of *F. oxysporum* f. sp. *ciceris* by more than 25% *in vitro* and found that the most antagonistic activity was caused by *B. amyloliquefaciens* and *B. subtilis* isolates. In this study, we also found that the most successful antagonistic isolates were *B. amyloliquefaciens* and *B. subtilis*. Moreover, Jamali et al. (2004) reported that *Bacillus subtilis* strains reduced *Fusarium* wilt of chickpea in both seed and soil treatments. As reported by Zaim et al. (2013), *Bacillus* spp. has been reported to possess several biocontrol properties, including secondary metabolites, colonization potential, and competitor production. Moreover, these properties are very significant to produce bacterial formulations including effective isolates that possess several of these mechanisms to control fungal pathogens (Abed et al., 2016; Soylu et al., 2020; Özkaya and Soylu, 2022).

Although the pathogen continues to more spread in chickpea growing areas, there is no effective chemical control in Yozgat province and neighboring plantations. In addition to these effective bacterial isolates, research will be expanded and plant samples will be used for the next studies to find more effective isolates against pathogen. The fact that two different *Bacillus* species showed higher antagonistic activity against the two fungal isolates encourages us to extend the studies to a wider diversity by isolating antagonistic endophytic and epiphytic bacterial isolates from other genera. Akgün-Yıldırım and Güldür (2019) investigated resistance to different isolates of the pathogen but was not found resistance in any of the 34 varieties registered in Turkey. For this reason, antagonistic bacteria should be part of integrated management in production systems because antagonistic isolates of *Bacillus* spp. reduce the severity of wilting in crops, rapidly colonize plant roots, and promote plant growth (Akhtar et al., 2010).

Conclusion

The objective of this study was to isolate antagonistic bacterial isolates from soil samples for control of *Fusarium* wilt pathogen of chickpea. Obtaining indigenous and beneficial microorganisms from the same environmental conditions to which the pathogen adapts and causes plant infection increases the success of biological control. Based on the results of this study's conducted *in vitro* experiment, further studies will investigate the mechanisms of the most effective isolates of *Bacillus* spp. in comparison with approved fungicides.

Control of soilborne fungal pathogens cannot be suppressed by a single management strategy. Considering the environmental and plant health benefits of antagonistic isolates and the reduction of chemical use, it is possible to reduce crop losses in chickpea, an important crop in Yozgat province, by using antagonistic microorganisms. The success of *Bacillus* spp. isolates that have shown activity against fungal pathogen in *in vitro* tests should also be investigated under *in vivo* and field conditions. Beside, efforts should be made to determine their efficacy against different *Fusarium* wilt disease agents and other chickpea fungal pathogens by preparing commercial biological preparations for future studies.

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Author Contributions: MO collected soil samples, performed bacterial isolation, purification, and identification tests and carried out bacterial inoculation for antagonism assay, prepared the data, and wrote the whole manuscript. AE supplied fungal isolates and inoculated fungal disks on PDA plates for this work.

References

Abed, H., Rouag, N., Mouatassem, D., & Rouabhi, A. (2016). Screening for *Pseudomonas* and *Bacillus* antagonistic rhizobacteria strains for the biocontrol of *Fusarium* wilt of chickpea. *Eurasian Journal of Soil Science*, 5(3), 182-191. DOI: https://doi.org/10.3906/biy-0809-12

Abbas, A., Khan, S. U., Khan, W. U., Saleh, T. A., Khan, M. H. U., Ullah, S., Ali, A., & Ikram, M. (2019). Antagonist effects of strains of *Bacillus* spp. against *Rhizoctonia solani* for their protection against several plant diseases: *Alternatives to chemical pesticides*. *Comptes Rendus Biologies*, 342(5-6), 124-135. DOI: https://doi.org/10.1016/j.crvi.2019.05.002

Akhtar, M. S., Shakeel, U., & Siddiqui, Z. A. (2010). Biocontrol of *Fusarium* wilt by *Bacillus pumilus*, *Pseudomonas alcaligenes* and *Rhizobium* sp. on lentil. *Turkish Journal of Biology*, 34(1), 1-7. DOI: 10.3906/biy-0809-12.

Aksoy, H.M., Kaya, Y., Ozturk, M., Secgin, Z., Onder, H., & Okumus, A. (2017). *Pseudomonas putida*–Induced response in phenolic profile of tomato seedlings (*Solanum lycopersicum* L.) infected by *Clavibacter michiganensis* subsp. *michiganensis*. *Biological Control*, 105, 6–12.https://doi.org/10.1016/j.biocontrol.2016.11.001

Aktan, Z.C., & Soylu, S. (2020). Diyarbakır ilinde yetişen badem ağaçlarından endofit ve epifit bakteri türlerinin izolasyonu ve bitki gelişimini teşvik eden mekanizmalarının karakterizasyonu. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 23 (3), 641-654.

Aydın, M. H. (2019). The biological control of *Fusarium oxysporum* causing wilting in chickpea (Cicer arietinum L.). *Türkiye Tarımsal Araştırmalar Dergisi*, 6(1), 65-72. DOI: https://doi.org/10.19159/tutad.475915

Akgün Yıldırım, Ü., & Güldür, M.E. (2019). Tescilli nohut çeşitlerinde *Fusarium* dayanıklılığının belirlenmesi. *Harran Tarım ve Gıda Bilimleri Dergisi*, 23(2): 218-225. DOI: https://doi.org/10.29050/harranziraat.461816

Anonymous (2019a). TUİK Bitkisel Üretim İstatistikleri https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr, (Retrieved 20.04.2021)

Anonymous (2019b). Food and Agriculture Organization of the United Nations (FAO), http://www.fao.org/site, (Retrieved 20.04.2021).

Anonymous (2021) Tarimsal Ekonomi ve Politika Geliştirme Enstitüsü TEPGE, Ürün Raporu Nohuthttps://arastirma.tarimorman.gov.tr/tepge/Belgeler/PDF%20%C3%9Cr%C3%BCn%20Raporlar%C4%B1/2021%20%C3%9Cr%C3%BCn%20Raporlar%C4%B1/Nohut%20%C3%9Cr%C3%BCn%20Raporu%202021-342%20TEPGE.pdf. (Retrieved 08.04.2022).

Bayraktar, H., & Dolar, F. S. (2012). Pathogenic variability of *Fusarium oxysporum* f. sp. *ciceris* isolates from chickpea in Turkey. *Pakistan Journal of Botany*. 44(2): 821-823.

Edwards, C. A., & Arancon, N. Q. (2004). Interactions among organic matter, earthworms, and microorganisms in promoting plant growth. Soil organic matter in sustainable agriculture, 327-376.

Elbouazaoui, A., Sijilmassi, B., Maafa, I., Allal, D., & Ahmed, S. (2022). Biocontrol activity of *Bacillus*, *Paenibacillus* and *Pseudomonas* against *Fusarium* wilt of chickpea in Morocco, *Acta Agriculturae Scandinavica*, Section B — Soil & Plant Science, 72:1, 847-859, DOI: https://doi.org/10.1080/09064710.2022.2100819

Guerrero-Aguilar, B. Z., Acosta-Gallegos, J. A., Sánchez-García, B. M., Ortega-Murrieta, P. F., & González-Chavira, M. M. (2015). Razas patogénicas de *Fusarium oxysporum* f. sp. *ciceris* en garbanzo cultivado en Guanajuato, México. *Revista Fitotecnia Mexicana*, 38(2), 183-190.

Houasli, C., Sahri, A., Nsarellah, N., & Idrissi, O. (2021). Chickpea (*Cicer arietinum* L.) breeding in Morocco: genetic gain and stability of grain yield and seed size under winter planting conditions. *Euphytica*, 217(8), 1-14. DOI: https://doi.org/10.1007/s10681-021-02885-x

Jamali, F., Sharifi-Tehrani, A., Okhovvat, M., Zakeri, Z., & Saberi-Riseh, R. (2004). Biological control of chickpea *Fusarium* wilt by antagonistic bacteria under greenhouse condition. *Communications in agricultural and applied biological sciences*, 69(4), 649-651.

Jiménez-Díaz, R. M., Castillo, P., del Mar Jiménez-Gasco, M., Landa, B. B., & Navas-Cortés, J. A. (2015). *Fusarium* wilt of chickpeas: Biology, ecology and management. *Crop Protection*, 73, 16-27. DOI: https://doi.org/10.1016/j.cropro.2015.02.023

Johri, B. N., Sharma, A., & Virdi, J. S. (2003). Rhizobacterial Diversity in India and Its Influence on Soil and Plant Health. *Advances in Biochemical Engineering/Biotechnology*, 84, 49-89. DOI: https://doi.org/10.1007/3-540-36488-9_2

Kara, M., Soylu, S., Kurt, Ş., Soylu, E.M., & Uysal, A. (2020). Determination of antagonistic traits of bacterial isolates obtained from apricot against green fruit rot

disease agent *Sclerotinia sclerotiorum*. *Acta Horticulturae*, 1290:135-142.

Kara, M., & Soylu, S. (2022). Isolation of endophytic bacterial isolates from healthy banana trees and determination of their *in vitro* antagonistic activities against crown rot disease agent *Fusarium verticillioides*. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, 27(1), 36-46.

Kocalar, H., Kafadar, F.N., Ozkan, A., Talapov, T., Demirel, O., Anay, A., Mart, D., & Can, C. (2020). Current Distribution and Virulence of *Fusarium oxysporum* f. sp. *ciceris* in Turkey. *Legume Research*, 43(5): 735-741. DOI: https://doi.org/10.18805/LR-520

Khan, M. R., Ashraf, S., Rasool, F., SALATI, K., Mohiddin, F. A., & Haque, Z. (2014). Field performance of Trichoderma species against wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani*. *Turkish Journal of Agriculture and Forestry*, *38*(4), 447-454. DOI: https://doi.org/10.3906/tar-1209-10

Landa, B. B., Navas-Cortés, J. A., & Jiménez-Díaz, R. M. (2004). Influence of temperature on plant–rhizobacteria interactions related to biocontrol potential for suppression of *Fusarium* wilt of chickpea. *Plant Pathology*, 53(3), 341-352. DOI: https://doi.org/10.1111/j.0032-0862.2004.01008.x.

Lelliot, R.A., & Stead, D.E. (1987). Methods for the diagnosis of bacterial diseases of plants. (T.F. Preece. Editör). In: Methods in plant pathology. Vol 2. Blackwell Scientific Publications, pp. 176-177. Oxford.

Leslie, J.F., & Summerell, B.A. (2006). The *Fusarium* laboratory Manual Blackwell Publishing. Ames, Iowa, 230-239.

Muehlbauer, F.J., & Sarker, A. (2017). Economic importance of chickpea: production, value, and world trade. In: Varshney RK, Thudi M, Muehlbauer F, editors. The chickpea genome. *Springer*; p. 5–12. DOI: https://doi.org/10.1007/978-3-319-66117-9_2.

Nene, Y.L., & Haware, M.P. (1980). Screening chickpea for resistance to wilt. *Plant Disease*, 66, 379-380.

Nelson, P. E., Toussoun, T.A., & Marasas, W.F.O. (1983). *Fusarium* species: an illustrated manual for identification. *Pennsylvania State University Press*, University Park. 193 pp.

Oliva-Ortiz, L. D. C., Velázquez-Alcaraz, T. D. J., Sosa-Pérez, R., Partida-Ruvalcaba, L., Díaz-Valdés, T., Arciniega-Ramos, J., & López-Orona, C. A. (2017). Controlling *Fusarium* wilt of chickpea (Cicer arietinum L.) with native microorganisms of Sinaloa, Mexico. *Agrociencia*, 51(6), 683-695.

Öztürk M., & Soylu, S. (2022). Yozgat ve Kırşehir illerinde tüketime sunulmuş patates yumrularında bakteriyel yumuşak çürüklük etmeni *Pectobacterium* izolatlarının izolasyonu ve tanılanması. *Tekirdağ Ziraat Fakültesi Dergisi*, 19 (2), 332-342. DOI: https://doi.org/10.33462/jotaf.974350

Özkaya S, & Soylu, E.M. (2022). Kompostlardan elde edilen antagonist bakteri izolatlarının kavun solgunluk hastalığı etmeni *Fusarium oxysporum* f.sp. *melonis*'e karşı in vitro antagonistik etkilerinin belirlenmesi. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, 27(3), 565-577. DOI: https://doi.org/10.37908/mkutbd.1139502

Özyılmaz, Ü. (2019). Biberde *Phytophthora* Yanıklığına karşı antagonist bakterilerle biyolojik mücadele. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 29(4), 652-661. DOI: https://doi.org/10.29133/yyutbd.597443

Rangel-Montoya, E. A., Delgado-Ramírez, C. S., Sepulveda, E., & Hernández-Martínez, R. (2022). Biocontrol of *Macrophomina phaseolina* using *Bacillus amyloliquefaciens* strains in cowpea (*Vigna unguiculata* L.). *Agronomy*, 12(3), 676. DOI: https://doi.org/10.3390/agronomy12030676

Soylu, E.M., Soylu, S., Kara, M., Kurt, Ş. (2020). Sebzelerde sorun olan önemli bitki fungal hastalık etmenlerine karşı vermikomposttan izole edilen mikrobiyomların *in vitro* antagonistik etkilerinin belirlenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 23 (1), 7-18. DOI: https://doi.org/10.3390/agronomy12030676

Soylu, S., Kara, M., Soylu, E.M ., Uysal, A., & Kurt, Ş. (2022). Geotrichum citri-aurantii'nin sebep olduğu turunçgil ekşi çürüklük hastalığının biyolojik mücadelesinde endofit bakterilerin biyokontrol potansiyellerinin belirlenmesi. *Tekirdağ Ziraat Fakültesi Dergisi*, 19 (1), 177-191.

Villarreal-Delgado, M. F., Villa-Rodríguez, E. D., Cira-Chávez, L. A., Estrada-Alvarado, M. I., Parra-Cota, F. I., & Santos-Villalobos, S. D. L. (2018). The genus *Bacillus* as a biological control agent and its implications in the agricultural biosecurity. *Revista Mexicana de Fitopatología*, 36(1), 95-130. DOI: https://doi.org/10.18781/r.mex.fit.1706-5

Zaim, S., Belabid, L., Bayaa, B., & Bekkar, A. A. (2016). Biological control of chickpea *Fusarium* wilts using rhizobacteria "PGPR". In Microbial-mediated Induced Systemic Resistance in Plants (pp. 147-162). *Springer*, Singapore. DOI: https://doi.org/10.1007/978-981-10-0388-2_10