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Effect of Rhizospheres Bacteria Isolated from Kahramanmaraş Pepper Fields Against *Phytophthora capsici*

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ABSTRACT
For the study, 36 soil samples with roots parts were taken from root zone of better grown healthy pepper plants from the pepper fields of Kahramanmaras province. Bacterial isolations were made from collected soil samples. Overall, 713 isolates were obtained from the bacteria colonies based on the morphological characteristics differences. By measuring the radius of inhibition zone, ZHA17 was determined to be most effective isolate. Fifteen isolates were selected from these bacteria in such a way to establish a zone to inhibit the growth of *Phytophthora capsici* and used in pot experiments under controlled conditions. The isolates were identified as *Bacillus pumilus, B. subtilis* ss *subtilis, Mycobacterium confluentis, M. immunogenum, Paenibacillus castaneae, Pseudomonas fluorescens, P. viridilivida* and *Tsukamurella paurometabola* bacteria species according to Biolog GEN III identification system.

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Kahramanmaraş Biber Alanlarından İzole Edilen Toprak Bakterilerinin *Phytophthora capsici*'ye Karşı Etkisinin Belirlenmesi

ÖZET
Kahramanmaraş ilindeki biber ekim alanlarındaki biber bitkilerinden daha iyi gelişmiş sağlıklı bitkiler seçilerek kök bölgelerinden köklerle birlikte 36 toprak örneği alınmıştır. Bu toprak örneklerinden bakteri izolasyonu yapılarak elde edilen bakteri kolonilerinden morfolojik karakter farklılıkları göz önüne alınarak 713 izolat elde edilmiştir. Engelleme zonlarının yarıçapları ölçülmek suretiyle en etkili izolatın ZHA17 olduğu belirlenmiştir. *Phytophthora capsici*'ye karşı bu bakterilerden engelleme zonu oluşturan 15 izolat seçilerek saksı çalışmasında kullanılmıştır. Saksı çalışmasında ZHA57, ZHA88, ZHA178, ZHA212, ZHA215, ZHA287 ve ZHA579 izolatları kök boğazı yanıklığı hastalığının gelişimini engellemiştir. Biolog Gen III tanılama sistemine göre izolatlar *Bacillus pumilus, Bacillus subtilis* ss *subtilis, Mycobacterium confluentis, Mycobacterium immunogenum, Paenibacillus castaneae, Pseudomonas fluorescens, Pseudomonas viridilivida* ve *Tsukamurella paurometabola* bakteri türleri olduğu belirlenmiştir.

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INTRODUCTION
Many disease and pests lead to significant economic losses in pepper. *Phytophthora capsici* Leon., which is the agent causing the root-crown rot disease (*Phytophthora* blight of pepper), is one of the major diseases affecting pepper. The disease agent is seen at almost anywhere around the world, where pepper is grown, and leads to significant losses. It was detected on pepper in New Mexico for the first time in the world; and then the presence of the agent was reported all around the world. *P. capsici* was detected in Marmara, Aegean, Mediterranean, Black Sea, Central Anatolia and Southeastern Anatolia regions of Turkey leading to significant yield losses (Abak and Pitrat, 1981; Leonian, 1922). *Phytophthora* is a genus in Oomycota group and contains pathogens, which cause epidemics on many plants from time to time. There are more than fifty species of *Phytophthora*, which cause disease on

plants (Tsao, 1983).

Biological control studies have distinct importance in controlling the diseases, which have no chemical control such as *Phytophthora*. It is known that certain bacteria and fungus in saprophytic character living on the rhizosphere zone of the plant are competitive and that they produce certain metabolic material so as to make repressive impact on the pathogenic agents. These include microorganisms such as fluorescent *Pseudomonas, Bacillus, Trichoderma, Gliocladium, Taloromyces* and avirulent *Fusarium*. Such microorganisms was started to be produced commercially due to their adaptation to the existing ecosystem and their positive effects on the plant growth.

In this study, beneficial bacteria isolates were recovered from the rhizosphere of healthy pepper plants in areas where crown rot disease is observed in the Kahramanmaras province and the antagonistic species were determined through in vitro tests and pot experiments under controlled conditions.

MATERIAL and METHODS

The bacteria and fungi strains

Bacteria isolates were isolated from the soil around the root zone of the pepper plants grown in the Kahramanmaras province. In order to isolate the bacteria, the soil samples were taken from the pepper plants with a good growing trend from a depth of 15-20 cm together with the root parts. The dilution series of the isolates, which was isolated from the soil, were prepared according to Aysan et al. (2003). The planting was made into Petri dishes containing Nutrient Agar (NA) nutrient media taking 100 µl with the help of a glass stirrer from 10^3, 10^5 and 10^7 dilution series. At the end of 2-4 days incubation of the Petri plates in 25°C, different colonies, which have developed in the NA nutrient media, were taken and pure cultures were developed on NA nutrient media.

The agent of the stem and fruit blight of peppers, *P. capsici* was obtained from the Eastern Mediterranean Transitional Zone Research Institute (Kahramanmaraş).

Determination of the effects of the bacterial isolates against P. capsici

Phytophthora capsici isolate was planted on a disk area at a radius of 6.0 mm in the middle of a Petri dish containing HRA nutrient media. After planting the *P. capsici* isolate, different bacterial isolates were planted in spots with a toothpick at four sides of the isolate with a distance of 3 cm at each side. On the other hand, sterile water was used at the control treatment instead of bacteria. The development radius of the fungus in control and bacterial treatments was measured with a ruler on the 6th day of planting. In order to identify the inhibition zone, colony radius length of the fungus in bacterial treatment was deducted from the colony radius length of the fungus in control application. This experiment was established according to the randomized trial design of four replication.

Identification of the bacteria isolates

Fifteen bacteria isolates selected according to the length of the inhibition zone were identified. Identification was made according to Anonymous (2008) and Rosvid et al. (2011) using Biolog GEN III MicroPlate (BIOLOG 21124 Cabot Blvd Hayward, CA 94545).

Biological control in pot experiment

In order to determine the efficiency of 15 isolates, which were isolated from the soil and selected according to their antagonistic effects against *P. capsici* in the growth chamber, a pot trial was established with the pepper seedlings in five repetitions. The density of the bacteria isolates, which were developed at 25°C in NA nutrient medium for a period of 24 hours, was adjusted to a concentration of 10^8 cell/ml with physiologic water (0.85 % NaCl). Then the roots of the pepper plants were merged into suspension of bacteria isolates and awaited in such suspension for a period of 10 minutes and then transferred into the pots. However for the control application, the pepper seedlings were kept in physiologic water without bacteria for a period of 10 minutes and then transferred into the pots. *P. capsici* mycelium was inoculated into the peat until one petri plate (10 cm) was filled. The developing micelles were mixed to 1 l torf and 1 petri dish. The plants, which were tranfered into the pots, were grown in climate chamber at 25°C. The scalds on the stem was measured with a ruler 5 days after bacteria treatment. The results were compared by Duncan's Multiple Range Test at 5% importance level.

RESULTS

The effect of the soil bacteria against *P. capsici*

During the study, 713 bacteria were isolated from the soil around the root zone of pepper plant. Fifteen isolates having the highest inhibition zone were selected among the isolates isolated from the soil. The inhibition zone of the selected bacteria varied between 17 mm and 29 mm. ZHA212 isolate (29 mm) was found to have the highest inhibition zone, and followed by ZHA17 (24 mm), ZHA90 (23.2 mm) and ZHA296 (23 mm) respectively. ZHA579 (17 mm), ZHA215 (17.3 mm) and ZHA191 (18 mm) were observed to have the lowest inhibition zone among the selected isolates. All the selected bacterial isolates were found to be negative in the tobacco hypersensitivity reaction and pectolytic activity in potatoes.

Identification of the bacteria isolates via BIOLOG identification system

The metabolic profiles of the selected bacteria were obtained using Biolog's Microbial Identification System Software program. The metabolic profiles of fifteen bacteria isolates, which were selected according to their zone radius, were identified according to the isolates with their metabolic profiles present in the system library. Two of the bacteria isolates were identified as *Mycobacterium immunogenum* (similarity rates of ZHA17 and ZHA57 isolates were 50% and 56 % respectively); 5 isolates were identified as *Paenibacillus castaneae* (similarity rates of ZHA88, ZHA178, ZHA212, ZHA215 and ZHA296 isolates were 54%, 67%, 67%, 68% and 64% respectively); 1 isolate was identified as *Bacillus pumilus* (similarity rate of ZHA90 isolate was identified as 51%); 1 isolate was identified as *Pseudomonas fluorescens* (similarity rate of ZHA91 isolate was 58%); 1 isolate was identified as *Mycobacterium confluentis* (similarity rate of ZHA246 isolate was 51%); 1 isolate was identified as *B. subtilis* ss *subtilis* (similarity rate of ZHA287 isolate was 68%); 1 isolate was identified as *P. viridilivida* (similarity rate of ZHA308 was 63%) and 1 isolate was identified as *Tsukamurella paurometabola* (similarity rate of ZHA569 isolate was 50%) (Table 1). Two of the abovementioned bacteria didn't show similarity with the bacteria recorded on BIOLOG GEN III system.

Biological control in pot experiments

It was detected in pot experiments that ZHA57, ZHA88, ZHA178, ZHA296, ZHA308 and ZHA569 isolates, which were obtained from the bacteria isolated from the soil, inhibit development of Phytophthora blight of pepper significantly ($F_{4,84}$= 9,30; $p<0.05$) (Table 2).

Table 1 The bacteria isolates identified with Biolog GEN III automatic identification system.
Çizelge 1. Biolog GEN III otomatik tanılama sistemi ile tanılanan bakteri izolatları

Species of the Bacteria *Bakteri Türü*	*Similarity Rate (%)* *Benzerlik Oranı*	*Isolate Number* *İzolat No*
Bacillus pumilus	51	ZHA90
Bacillus subtilis ss *subtilis*	68	ZHA287
Mycobacterium confluentis	51	ZHA246
Mycobacterium immunogenum	50-56	ZHA17, ZHA57
Paenibacillus castaneae	54-68	ZHA88,ZHA178,ZHA212,ZHA215,ZHA296
Pseudomonas fluorescens	58	ZHA191
Pseudomonas viridilivida	63	ZHA308
Tsukamurella paurometabola	50	ZHA569
Non-identified		ZHA235, ZHA579

Table 2 Effect of Rhizobacteria on development of the disease
Çizelge 2. Rhizobacteriaların hastalık gelişimi üzerine etkisi

Isolates *İzolatlar*	*Length of the necrosis (cm)* *Nekroz Uzunluğu (cm)*		*Species of the Bacteria* *Bakteri Türü*
Negative Control *(Negatif Kontrol)*	00.0	a	
Positive Control *(Pozitif Kontrol)*	10.0	e	
ZHA246	10.0	e	*Mycobacterium confluentis*
ZHA235	10.0	e	Unidentified
ZHA90	10.0	e	*Bacillus pumilus*
ZHA17	09.1	de	*Mycobacterium immunogenum*
ZHA212	08.9	de	*Paenibacillus castaneae*
ZHA215	08.6	cde	*Paenibacillus castaneae*
ZHA287	08.3	cde	*Bacillus subtilis* ss *subtilis*
ZHA579	08.0	bcde	Unidentified
ZHA191	07.8	bcde	*Pseudomonas fluorescens*
ZHA308	07.3	bcd	*Pseudomonas viridilivida*
ZHA178	06.9	bcd	*Paenibacillus castaneae*
ZHA57	06.6	bcd	*Mycobacterium immunogenum*
ZHA569	06.5	bcd	*Tsukamurella paurometabola*
ZHA296	06.0	bc	*Paenibacillus castaneae*
ZHA88	05.5	b	*Paenibacillus castaneae*

It was observed that ZHA17, ZHA212, ZHA215, ZHA287 and ZHA579 isolates inhibited Phytophthora blight of pepper partly, but such inhibition was not significantly important statistically. ZHA246, ZHA235 and ZHA90 isolates among the soil bacteria gave the same result with positive control and they were unable to inhibit development of the disease.

CONCLUSION and DISCUSSIONS

Rhizosphere soil samples were taken from 36 different pepper fields in Kahramanmaras province in order to isolate antagonistic bacterial agents, which may be used for biological control against *P. capsici*. Bacteria isolates. With this purpose a total of 713 bacterial isolates were obtained from the soil samples. An inhibition zone was determined against *P. capsici* in Petri plates on nutrient media for the rhizosphere bacteria isolates wherein 15 isolates which formed the greatest inhibition zone, were identified and these isolates were further tested in pot experiments. When the radius of the inhibition zones were examined 6 days after inoculation of the Rhizobacteria and the fungal pathogen together in Petri plates, the greatest inhibition zone was observed for *Paenibacillus castaneae* ZHA212 (29 mm) and *Mycobacterium immunogenum* ZHA17 (24 mm) isolates; and the smallest inhibition zone was observed for ZHA579 (17 mm) isolate, whose type could not be identified. Ling et al. (2014) reported that the efficiency of *Paenibacillus* spp. could be increased on terra rossa through different fertilization regimes and hence the number of the soil borne pathogens could be decreased through encouragement of the plant growth. Some species of *Paenibacillus* genus wereidentified as nitrogen fixing species (Anand and Chanway, 2013; Anand et al., 2013). They prevent germination and development of the plant pathogens on the soil; and hence they contribute soil productivity and plant health as they may be used for biological control (Ling et al., 2011; Gua and Liao, 2013).

Efficiency of the soilborne bacteria isolates against *P. capsici* were also reported in previous studies (Jee et al., 1988; Akgül and Mirik, 2008; Kim et al., 2009). Jee et al. (1988) observed *Trichoderma harzianum*, *Pseudomonas cepacia* and *Bacillus polymyxa* species forming an inhibition zone against *Phytophthora capsici* during their study.

Analyzing the results of Biolog Gen III identification system, 9 of the 15 isolates matched with different bacteria species (*B. pumilus, B. subtilis* ss *subtilis, M. confluentis, M. immunogenum, Paenibacillus castaneae*, *P. fluorescens*, *P. viridilivida, Tsukamurella paurometabola*) and 2 isolates (ZHA235, ZHA579) could not be identified. The highest similarity rate was observed as 68% between ZHA287 and *B. subtilis* ss *subtilis*.

In pot experiment conducted in order to determine the efficiency of the 15 isolates selected against *P. capsici*, *M. immunogenum* ZHA57, *Paenibacillus castaneae* ZHA88, ZHA178, ZHA296, *Pseudomonas viridilivida* ZHA308 and *Tsukamurella paurometabola* ZHA569 isolates were detected to inhibit development of *P. capsici L. (*Phytophthora blight of pepper*)* significantly ($F_{4,84}$= 9,30; $p<0.05$). Among these bacteria, *M. immunogenum* ZHA17, *Paenibacillus castaneae* ZHA212, ZHA215, *B. subtilis* ss *subtilis* ZHA287 and unidentified ZHA579 isolates were detected to inhibit *P. capsici (*Phytophthora blight of pepper*)* partly. Uppal et al. (2006) detected in their study that *P. viridilivida* inhibited development Verticillium wilt disease caused by *Verticillium dahliae*. Daafy et al. (2003) reported that *P. viridilivida* isolate was effective against potato late blight caused by *Phytophthora infestans*. Kim et al. (2009) reported in their study that 15 isolates of *Paenibacillus polymyxa* showed antimicrobial activity against *P. capsici*, which was similar to in this study. Soil borne bacteria not only inhibit development of certain plant disease agents but also increase resistance of plants. Tran et al. (2007) reported that *P. flourescens* not only inhibited infection against potato late blight caused by *Phytophthora infestans*, but also inhibited formation of spore structures. Zang et al. (2010) determined that certain isolates of soil borne *Bacillus* bacteria decreases the severity of *P. capsici* at a significant level at greenhouse conditions. It was reported in many studies that *Mycobacterium immunogenum* species, which display a similarity rate of 50-56% in GEN III identification, is a pathogen bacteria for people (Loots et al., 2006; Selvaraju et al., 2005; Gordon et al., 2008). However, identification of *M. immunogenum* ZHA17 and ZHA57 isolates, which were reported to inhibit development of pathogens during the inhibition studies conducted against *P. capsici*, must be supported with molecular identification methods; and it must be detected whether these isolates are pathogens for people. If they are not hazardous in terms of being human pathogen, they can be studied for biological control studies.

There are studies indicating that C-924 isolate of *Tsukamurella paurometabola* species shows antagonistic effect against plant pathogenic fungi and that they may be used for biological control of nematodes (Bruzos et al., 2013; Marin et al., 2013; Hernandez et al., 2008).

It is reported based on the studies conducted that induced systemic resistance (ISR) of soil borne bacteria representing various species including *Pseudomonas* and *Bacillus* encourages systemic acquired resistance (SAR) (Raupach and Kloepper 1998; Kloopper et al., 2004; Mirik, 2005; Zhang et al., 2010). It is proved via the soil borne bacteria that signal transmission in ISR is independent from salicylic acid but dependent on ethylene and jasmonic acid (Tran et al., 2007). Kone at al. (2009) treated their plants in their study with

systemic acquired resistance (SAR) indicators including acibenzolar-S-methyl (ASM); and determined that it showed resistance in pumpkin against *P. capsici*. As a result, certain isolates, which were isolated from soil samples taken from pepper fields from the Kahramanmaras province, was determined to be effective against *P. capsici*. In future studies, effective isolates should be selected and the inhibition mechanism of soil borne bacteria against *P. capsici* and efficiency of different combinations of these bacteria against *P. capsici* must be explored, and finally the optimum combination could be determined.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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