

Relationship between Some Plants Species Belonging to Brassicaceae, Chenopodiaceae and Urticaceae Families, and Arbuscular Mycorrhizal Fungi and Rhizobacteria

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ABSTRACT

This study was conducted to investigate the effects of some plant growth-promoting rhizobacteria (PGPR) on the [Brassica fungi (AMF) formation on (cauliflower), Spinacia oleracea (spinach), and Urtica urens (stinging nettle)] belonging to Brassicaceae, Chenopodiaceae and Urticaceae families, which are known to have a negative influence on the symbiotic life formation with AMF. Two PGPR isolates that contributed to the plant's growth and served as a "mycorrhizal helper" in various hosts were predetermined at the initial stage; then they were applied to three plant species with AMF species [Gigaspora margarita and commercial AMF (ERS)]. The obtained results revealed that combined AMF x PGPR treatments improved the growth and morphological development parameters of cauliflower, spinach, and nettle plants. PGPR bacteria had different effects on AMF root colonization depending on the plant species. The highest root colonization rate was achieved in spinach plants with the commercial AMF treatments. Commercial AMF isolate, alone or in combination with PGPR strains, was also found to increase AMF spore density and mycorrhizal dependency in cauliflower and spinach plants. There was no significant difference in total phosphorus content in cauliflower and nettle compared to the control group, and only one application group (G. margarita x PGPR) in spinach plants had an increase in phosphorus content.

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Keywords

Arbuscular mycorrhizal fungi (AMF),

Brassica oleracea, Rhizobacteria, Spinacia oleracea, Urtica dioica

Brassicaceae, Chenopodiaceae ve Urticaceae Familyalarına Ait Bazı Bitki Türlerinin Arbusküler Mikorhizal Fungus (AMF) ve Rhizobacteria Arasındaki İlişki

ÖZET

Bu çalışma, AMF ile simbiyotik yaşam oluşumunu olumsuz etkileyen Brassicaceae, Chenopodiaceae ve Urticaceae familyalarından bazı bitkilerin gelişimini teşvik eden rizobakterilerin (PGPR) arbusküler mikorizal fungus (AMF) oluşumuna etkilerini araştırmak amacıyla yapılmıştır. Bitki gelişimine katkıda bulunan ve çeşitli konukçularda 'mikorhizal helper' olarak adlandırılan iki PGPR izolatı ilk aşamada belirlenmiş; daha sonra AMF türleriyle [Gigaspora margarita ve ticari AMF (ERS)] birlikte üç bitki türüne uygulanmıştır. Çalışma sonucunda; AMF x PGPR interaksiyonun ıspanak, karnabahar ve ısırgan otunda bitki gelişim parametreleri açısından teşvik edici olduğu ortaya konmuştur. Bitki türlerine göre PGPR'in kök kolonizasyonuna etkisi değişkenlik göstermiştir. En yüksek kök kolonizasyon oranı, ticari AMF + ıspanak kombinasyonundan elde edilmiştir. Ticari AMF'nin tek başına veya PGPR ile interaksiyonu karnabahar ve ıspanağın toprak spor yoğunluğunu ve mikorhizal bağımlılığını arttırdığı görülmüştür. Bitkilerde toplam fosfor içeriği açısından karnabahar ve ısırgan otu bitkilerinde kontrol grubuna göre önemli bir farklılık olmadığı, ıspanak bitkilerinde ise sadece bir uygulama grubunun (G. margarita x PGPR) fosfor içeriğinde artış olduğu ortaya konmuştur.

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INTRODUCTION

Many practices such as the protection of water and resources, integrated pest and management, organic fertilizers and beneficial microorganisms are being performed in order to achieve a sustainable agriculture (Turhan, 2005). Among these, Arbuscular Mycorrhizal Fungi and Plant Growth Promoting Rhizobacteria (PGPR) have important effects on maintaining the balance of the soil ecosystem and eliminating the negative effects of climate change (Bellgard and Williams, Erzurumlu and Kara, 2014). Mycorrhiza, which means "root fungus", has a symbiotic relationship with plant roots in the soil (Fiorilli et al. 2015). This association is generally considered a mutualistic symbiosis because of the highly interdependent relationship established between both partners (Peterson and Farguhar, 1994). The function of all mycorrhizal systems depends on the ability of the fungal symbiont in the absorption of nutrients available in inorganic and/or organic forms in soil . In most mycorrhizal types, organic C, which is derived from photosynthesis, is also transferred from the plant to fungus (Demir, 1998; Erzurumlu and Kara, 2014; Cakmakci et al. 2017). Such a relationship has not, however, been predicted regarding Brassicaceae, Chenopodiaceae and Urticaceae families (Smith and Read, 2008; Brundrett, 2009; Tushar and Satish, 2013). Lack of colonization of these families is associated with four different factors: 1- These plants are not able to secrete some basic substances to initiate root colonization. 2- The plants cannot recognize the fungus in the early stage of symbiotic life. 3- The plant creates physical obstacles for the formation of fungi colonies and 4-the plant inhibits the development of arbuscular mycorrhizal fungi (AMF) by secreting some antifungal compounds (Sosa-Rodriguez et al., 2013). AMF also interacts with the other useful microorganisms found in the rhizosphere. This interaction is in the form of competition or a synergistic effect (Akköprü and Demir, 2005). Some specific bacteria stimulate the germination of arbuscular mycorrhiza spores, as well as provide faster and more intensive colonization as well. They can compete with AMF and (PGPR), or together. Saprophytic microorganisms interact colonized on the plant roots within the rooting area of the plants (rhizosphere) and establish a positive relationship between them (producing hormones, helping plants to take nutrients from the soil); these are called root bacteria (rhizobacteria) (Kloepper, 2003; Soylu, 2011). It has been reported that the pathogen population in the rhizosphere region is decreased with the metabolites produced by PGPRs; since these such metabolites promote plants' resistance to pests and diseases (Ciftci and Altınok, 2019). PGPRs also affect plants' sensitivity to stress conditions even though such effects has been reported to vary with the plant types, growing regions, and ecological conditions (Telek et al., 2019; Vargas et al., 2019). It has been stated that bacterial species have antagonistic effects, promoting plants' growth and development, like PGPRs. Thus, these species are used as biological fertilizers or biological control agents (Bayrak and Okmen, 2014).

In this study, the effects of PGPRs on the formation of AMF in cauliflower, spinach and nettle species were investigated. The research was carried out in two stages. To identify the two most successful bacteria that could promote plants' growth, five different bacterial isolates were inoculated to three different plant species. Initially, the selection was done according to the growth parameters; then to promote plants' growth and development and to determine the effects of PGPRs on mycorrhizal colonization, two different types of AMF were inoculated with PGPRs. Thus, the interactions of these two biological control agents with each other and the effects of these interactions were investigated.

MATERIALS and METHODS

Plant materials and growth medium

In the study, *Brassica oleracea* (cauliflower), *Spinacia oleracea* (spinach) and *Urtica dioica* (nettle) were used as the plant materials. In the first stage of the study, cauliflower, spinach, and nettle seedlings were grown in 45 plastic vials with one eye of 4.7 x 6.0 cm. In the second one, 16 x 18 cm plastic pots that could hold 3.5 kg of the mixture were used. In both stages, the material consisting of a 1:1 rate of the sterile peat-perlite mixture was used. The plants were cultivated in a climate room with a light intensity of 4000-6000 lux, 12-hour exposure time, a temperature of 22°C, and a proportionate humidity of 60-70 percent.

AMF isolates and applications

Gigaspora margarita widely used AMF species obtained from YYU Plant Protection Department, consisting of spores, extraradical mycelium, and mycorrhizal roots; and a commercial AMF composed

of different *Glomus* spp. [Endo Roots Soluble(ERS)]. These AMF inoculums were placed 5 cm below the seed depth as 10 g (25-150 spores g-1) in each pot. Sterile sand was left on the seed bed in the pots without AMF application.

PGPR bacterial isolates and applications

Ochrobactrum sp. (CB36/1), Bacillus thuringiensis (CA41/1), Pseudomonas fluorescens (14/1Y), Pseudomonas fluorescens (30/1m) and Pseudomonas putida TR21/1K, which is a "mycorrhizal helper bacterium" administered with AMF were used as PGPR isolates. These isolates were obtained from Bacteriology Laboratory Stocks, Van Yuzuncu Yil University, Faculty of Agriculture (Akköprü et al., 2005). King B medium was used for the growth of PGPRs. Diluted nutrient solutions were also used to supply plant nutrient requirements (Hoagland and Arnon, 1950).

The bacteria were applied to the plants three times, as previously described by Akköprü et al. (2021).

The first application (seed coating): the selected PGPR isolates were incubated in the King-B medium for 24–48 hours. Developed cultures were supplemented with 1.5% carboxymethyl cellulose (CMC). Surface-disinfected cauliflower, spinach, and stinging nettle seeds were then coated with the prepared bacterial suspension by keeping the seed within the solution for 1 hour. The coated seeds were preserved in a fridge at +4 °C overnight between drying papers and made ready for planting.

The second application (soil drenching). The bacterial suspensions were prepared as 24-hour PGPR cultures developed at the King-B medium. The concentrations of the bacterial suspensions were adjusted at 10⁸ CFU ml⁻¹ by a spectrophotometer. The bacterial suspension was applied using the drenching method with 30 mL⁻¹ seedling of the suspension when the plant reached the first leaves stage.

Third application (soil drenching): This was performed similarly one week after the second application.

AMF x PGPR application

At the end of the first stage of the study, two PGPRs were selected based on the plant development parameters, such as fresh and dry weight, and plant height for the next stage. The selected PGPRs and AMF were used together on the target plants to detect their effect on AMF colonization and plants' development. Seeds treated with AMF and PGPR as described above were planted in the growing medium. Drenching method was also used in the second and third PGPR applications.

Plants growth parameters and phosphorus analysis

The plants were kept in the climatic room at 22 ± 2 °C, 60%–70% relative humidity, and 12 h of fluorescent illumination for 8 weeks; they were harvested and the roots of the plants were cleaned by washing under tap water. Plants' weights, heights and root lengths were measured; then the dry weight of each plant was determined. The total amount of phosphorus was also determined based on the vanadomolybophosphoric yellow method (Barton, 1948). For this purpose, 5 g of each plant was dried at 70 °C for 48 h.

then 0.5 g of the extracts was weighed and 1 mL of ethyl alcohol (Merck 818,760, Germany) was added and burned. Then, 4 mL of hydrochloric acid (Merck 1.05590.2500, Germany) was added to the samples and kept at 90 °C for 15 min. The extracts were filtered and measured by a spectrophotometer (Jenway 6505 UV/vis, UK) at 430 nm (Jenway 6505 UV/VIS, UK).

Determination of AMF root colonization

Approximately, 0.5 g of the cleaned roots were weighed and cut into 1-2 cm pieces; it was made ready for fixation and staining. Roots were kept in the AFA (Ethyl Alcohol: Formaldehyde: Acetic Acid) solution until staining. Staining was performed to determine the percentage of AMF colonization (Phillips and Hayman, modified from 1970). To determine the colonization percentage of AMF in the roots stained with lactophenol blue, investigated under the light microscope (Olympus, Japan); Grid-Line Intersect Method was used for this purpose (Giovannetti and Mosse, 1980). During microscopic observations, each root fragment containing any fungal reproductive structure (hyphae, chlamydospore, vesicle arbuscular) was considered colonized by the fungus. % AMF colonization (AC) was calculated by equation 1 (Giovannetti and Mosse, 1980);

$$AC \% = AC_R/R \times 100$$
 [1]

 AC_R = number of roots colonized with AMF; R = total number of roots

Determination of AMF spore density in soil

AMF spore density in the soil of the rhizosphere region of the plants in which AMF was inoculated was determined with the help of a fresh sieving method. Fresh rhizosphere soils were sieved through a 2 mm sieve to remove stones and plant residues, as compared to the samples passed through 80 μm and 45 μm sieves. After the liquid in the tube was centrifuged at 2000 rpm for 3 minutes, the remaining liquid was removed. Later, it was poured into a petri dish and healthy-looking spores were determined under a stereoscopic microscope; then the density of the spores in the soil was determined (g). Dependency

of application (MD) was determined with the aid of equation 2 (Declerck et al., 1995):

$$MD\% = A - B/A \times 100$$
 [2]

A = total dry weight of the application plant

B = total plant weight of the non-application plant

Statistical analyses

This study was carried out as a completely randomized experimental design with replications. Descriptive statistics for the studied variables are presented as Mean (x) and standard deviation (SD). One-way Factorial ANOVA was applied to the data. Treatments at different (Mycorrhiza and Rhizobacteria) concentrations were considered as the factors. Duncan's Multiple Range Test comparisons were also used to determine different treatment levels. The statistical significance level was set at 5%, and all statistical analyses were conducted using the SAS (2018) statistical program.

RESULTS

Selection of PGPR isolates

In the study, firstly, it was revealed that the effects of PGPR isolates on the morphological growth

parameters of plants differ depending on the isolates. As compared to negative control treatments, an increase was observed in some parameters with the PGPR isolates. The differences in the development parameters of the treatment groups of cauliflower plants were not, however, significant (p > 0.05) (Table 1). Despite this, differences in the development parameters of the treatment groups of spinach plants were significant. Among the treatment groups, CA41/1 bacteria exhibited better development on spinach plants than on the other bacterial isolates. However, the difference with CB36/1 was not statistically significant (Table 1). Despite this, the differences in the development parameters of the treatment groups of the nettle plants were significant. 14/1Y bacteria exhibited less development on the nettle plants, as compared to the other bacterial isolates among the treatment groups. The difference between other treatments was not statistically significant (p>0.05) (Table 1). According to the assessments conducted on the PGPR isolates, CB36/1 and TR21/1 bacteria isolates were decided to be used in the AMF x PGPR treatments of the second stage of the study (Table 1).

Table 1. The effect of five PGPR isolates applied to the cauliflower, spinach and stinging nettle plants on the shoot length, shoot fresh weight, root fresh weight, root length, total fresh weight and total dry weight *Çizelge 1. Karnabahar, ıspanak ve ısırgan otu bitkilerine uygulanan beş PGPR izolatının sürgün uzunluğu, sürgün yaş ağırlığı, kök yaş ağırlığı, kök uzunluğu, toplam yaş ağırlık ve toplam kuru ağırlık üzerine etkisi*

			Shoot	Root Fresh	Root Length	Total	Total Dry
Plants	Treatment	Shoot Length	Fresh	Weight (g)	(cm)	Fresh	Weight (g)
	Groups	(cm)	Weight (g)			Weight (g	
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
	CB36/1**	10.83±2.62a*	0.78 ± 0.13^{a}	0.03 ± 0.02^{a}	5.00 ± 2.17^{a}	0.82 ± 0.13^{a}	0.05 ± 0.01^{a}
ìr	CA41/1	11.88 ± 1.26^{a}	0.76 ± 0.21^{a}	0.04 ± 0.03^{a}	5.00 ± 2.46^{a}	0.79 ± 0.24^{a}	0.04 ± 0.01^{a}
We	14/1Y	12.15 ± 1.91^{a}	0.78 ± 0.24^{a}	0.04 ± 0.01^{a}	3.55 ± 1.30^{a}	0.82 ± 0.25^{a}	0.04 ± 0.01^{a}
Jauliflower	TR21/1K	11.55 ± 1.13^{a}	0.79 ± 0.16^{a}	0.05 ± 0.01^{a}	3.27 ± 1.27^{a}	0.84 ± 0.16^{a}	0.05 ± 0.01^{a}
u. Ji	30/1m	11.38±1.31a	0.78 ± 0.22^{a}	0.03 ± 0.01^{a}	3.72 ± 1.37^{a}	0.82 ± 0.22^{a}	0.04 ± 0.01^{a}
	NC	12.16±1.80a	0.73 ± 0.13^{a}	0.04 ± 0.01^{a}	3.55 ± 1.15^{a}	0.78 ± 0.13^{a}	0.04 ± 0.01^{a}
	CB36/1	10.00 ± 1.05 ab*	$2.06 \pm 0.55^{\mathrm{a}}$	0.12 ± 0.07^{a}	11.10 ± 4.22^{ab}	2.18 ± 0.58^{a}	0.10 ± 0.03^{a}
	CA41/1	10.80 ± 1.22^{a}	2.20 ± 0.51^{a}	0.27 ± 0.32^{a}	12.10 ± 2.96^{a}	2.36 ± 0.51^{a}	0.10 ± 0.03^{a}
_	14/1Y	9.66 ± 1.11^{ab}	1.94 ± 0.29^{ab}	0.12 ± 0.05^{a}	10.33 ± 3.67 ab	2.05 ± 0.33^{ab}	$0.08\pm0.01^{\rm ab}$
Spinach	TR21/1K	9.12 ± 1.35^{b}	1.50 ± 0.41^{ab}	0.07 ± 0.02^{a}	8.50 ± 2.56^{ab}	1.58 ± 0.41^{b}	0.06 ± 0.01^{b}
iñ	30/1m	8.90 ± 1.79^{b}	1.78 ± 0.62^{ab}	0.19 ± 0.29^{a}	10.80 ± 3.93^{ab}	1.87 ± 0.66 ab	0.08 ± 0.03^{ab}
$\overline{\mathrm{Sp}}$	NC	9.90 ± 1.44^{ab}	$1.83\pm0.45^{\rm ab}$	0.09 ± 0.05^{a}	8.00 ± 3.33^{b}	1.92 ± 0.46 ab	0.08 ± 0.02^{ab}
е	CB36/1	$20.61 \pm 5.42 $ a*	3.30 ± 1.59^{a}	0.80 ± 0.52^{a}	16.90 ± 7.83 ab	4.09 ± 2.06^{a}	0.22 ± 0.10^{ab}
nettle	CA41/1	21.80 ± 2.74^{a}	$2.20 \pm 0.97^{\mathrm{ab}}$	0.40 ± 0.22^{b}	$12.40\pm5.46^{\rm ab}$	3.23 ± 1.11^{ab}	$0.20\pm0.07^{\rm ab}$
ne	14/1Y	15.50 ± 5.98 b	$1.83 \pm 1.47 ^{\mathrm{b}}$	$0.42 \pm 0.51^{\mathrm{ab}}$	10.70 ± 10.69 b	2.25 ± 1.95 b	0.14 ± 0.12^{b}
ng	TR21/1K	20.20 ± 3.04^{a}	2.80 ± 1.19^{ab}	$0.55 \pm 0.27^{\mathrm{ab}}$	$17.50\pm6.36^{\mathrm{ab}}$	3.34 ± 1.38 ab	0.19 ± 0.05 ab
ngi.	30/1m	22.60 ± 2.67^{a}	3.70 ± 1.26^{a}	$0.86 \pm 0.39 ^{\mathrm{ab}}$	18.90 ± 6.67^{a}	4.52 ± 1.63^{a}	0.23 ± 0.08 ab
Singing	NC	21.55 ± 5.0^{a}	3.65 ± 1.37^{a}	$0.69 \pm 0.55^{\mathrm{ab}}$	19.66 ± 9.47^{a}	4.43 ± 1.86^{a}	0.24 ± 0.08^{a}

^{*} Plants were evaluated among themselves, and the means represented with the same letter in the same column are not significantly different according to Duncan's multiple comparison tests at p<0.05 ** TR21/1K: *P. putida*, CB36/1: *Ochrobactrum* sp, CA41/1: *B. thuringiensis*, 14/1Y: *P. fluorescens*, 30/1m: *P. fluorescens*, NC: Negative Control.

Effects of PGPR x AMF combinations on cauliflower, spinach and stinging nettle plants

In the study, the effects of CB36/1 and TR21/1 bacterial isolates, selected according to the **AMF** morphological growth parameters. on colonization. mycorrhizal total dependency, phosphorus content, and plant growth were determined (Table 1). The differences in the total phosphorus contents and root dry weights of the cauliflower plants were not significant (p>0.05). However, the differences in the other developmental parameters were significant (p<0.05). The commercial mycorrhiza x CB36 / 1 and mycorrhiza AMF x TR21/1 treatment groups had greater total fresh-dry weights, in comparison to the others. Combined application of the bacterial species with the commercial AMF increased all parameters, except the shoot length. The greatest shoot diameter (4.12 cm) was observed in the commercial AMF x CB36 / 1 treatment group. Effects of the G. margarita AMF isolate on the morphological development parameters varied with the bacterial species (Table 2).

The differences in the shoot length, shoot diameter and root length parameters of the spinach plants were not, however, significant (p>0.05). Despite this, differences in $_{
m the}$ other developmental parameters were significant (p<0.05). Further, the difference in the total phosphorus contents of G. margarita x TR21/1 and the other treatment groups was also significant (p<0.05). Total phosphorus content was increased with G. margarita x TR21/1 bacterial isolate treatments. Bacterial isolate treatments (CB36/1 and TR21/1) had greater values, as compared to single G. margarita and commercial AMF isolate ones. Bacterial isolates increased the investigated parameters, both alone and combination (Table 3). The commercial AMF x TR21/1 had treatment group greater morphological development parameters related to the nettle plants, as compared to the other treatment groups. Bacterial isolates (TR21/1 and CB36/1), when applied together with the commercial AMF, promoted the plant's growth and development (p<0.05). The differences in the totally fresh and dry weights of the commercial AMF x CB36 / 1 and commercial AMF x TR21 / 1 treatment groups were found to be significant (p<0.05). The greatest total phosphorus content was observed in the control group, but the difference between the treatment groups was not significant (Table 4).

AMF root colonization, spore density, and dependency on applications

In cauliflower plants, the greatest AMF root colonization (13.68%) was observed in single commercial AMF treatments, while the lowest value

(0.48%) was recorded in single *G. margarita* treatments. It was noticed that CB36/1 and TR21/1 bacterial species increased the soil spore density. The greatest dependency (+46.34) was observed in the commercial mycorrhiza x CB36/1 treatment group. Another striking detail, as shown in Table 5, was that bacteria species (CB36/1 and TR21/1) increased dependency when applied to the commercial AMF species. Dependency was not, however, observed in the single *G. margarita* treatments (Table 5).

In spinach plants, dependency (+15.53) was observed only in the commercial mycorrhiza x CB36/1 treatment group. The differences in the AMF root colonization of AMF and G. margarita treatment groups were significant (p<0.05). There was an increase in AMF root colonization with commercial AMF treatments (Figure 1). The highest soil spore density (38.0 spores / g soil) was seen in the G. margarita treatment group, while the lowest value (20.4 spores / g soil) was observed in G. margarita x TR21/1 treatments (Table 5). In stinging nettle plants, the greatest dependency (+43.26) was seen in the commercial AMF x TR21/1 treatment group. The differences in the AMF root colonization of the treatment groups were not, however, significant (p>0.05). The greatest AMF root colonization were determined in the commercial AMF treatment group. The differences in the soil spore density of the treatment groups were found to be significant (p<0.05) (Table 5).

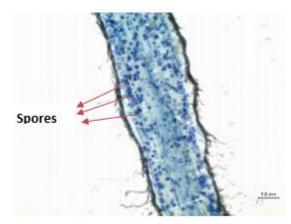


Figure 1- AMF spores of commercial AMF in root of spinach plant

Şekil 1- İspanak bitkisinin kökündeki ticari AMF'nin AMF sporları

DISCUSSION

This study attempted to determine the effects of PGPR on the development of cauliflower, spinach, and stinging nettle plants, as well as revealing its effects on the formation and development of AMF. Thus, the interactions of these two biological agents with each other and the effects of these interactions

Table 2. The effect of AMF species and PGPR isolates applied to cauliflower plant on the shoot diameter, shoot length, root length, shoot fresh weight, root fresh weight, total fresh weight, total dry weight, total dry weight and total phosphorus content

Çizelge 2. Karnabahar bitkisine uygulanan AMF türleri ve PGPR izolatlarının sürgün çapı, sürgün uzunluğu, kök uzunluğu, sürgün yaş ağırlığı, kök

yaş ağırlığı, toplam yaş ağırlık, sürgün kuru ağırlığı, kök kuru ağırlığı, toplam kuru ağırlık ve toplam fosfor içeriğine etkisi

Treatment	Shoot	Shoot	Root	Shoot	Root Fresh	Total Fresh	Shoot Dry	Root Dry	Total Dry	Total
Groups	Diameter (cm)	Length (cm)	Length (cm)	Fresh Weight (g)	Weight (g)	Weight (g)	Weight (g)	Weight (g)	Weight (g)	Phosphorus Content(ppm)
	$\bar{\mathbf{x}} \pm \mathrm{SD}$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\vec{x} \pm SD$	$\bar{x} \pm SD$	$\bar{\mathbf{x}} \pm \mathrm{SD}$	$\bar{x} \pm SD$	$\vec{x} \pm SD$	$\vec{x} \pm SD$
Control Commercial AMF	3.50±0.16 ^{bc} 3.76±0.58 ^{ab}	24.15±1.56 ^c 29.51±1.62 ^a	24.50±1.92bc 25.46±3.28b	9.82±1.24 ^b 13.37±2.77 ^a	0.98±0.37 ^c 1.10±0.36 ^{bc}	10.80±1.47 ^b 14.47±3.09 ^a	0.76±0.07 ^{cd} 1.22±0.36 ^{ba}	0.11±0.26 ^a 0.11±0.05 ^a	0.88±0.09 ^{cd} 1.33±0.41 ^{ab}	3494.5±366.6 ^a 3371.7±137.4 ^a
G. margarita CB36/1 TR21/1K Commercial	3.32±0.27 ^c 3.30±0.35 ^c 3.57±0.13 ^{bc} 4.12±0.18 ^a	$26.75{\pm}2.43^{b} \\ 22.53{\pm}0.89^{cd} \\ 20.40{\pm}1.44^{d} \\ 28.40{\pm}1.75^{ab}$	23.35±2.85 ^{bc} 24.90±2.24 ^{bc} 22.75±3.49 ^{bc} 29.30±0.81 ^a	9.22±0.81bc 8.32±0.70bc 7.21±0.83c 13.51±1.36a	0.97±0.51° 0.67±0.32° 0.93±0.21° 1.76±0.61°	10.17±1.06 ^{bc} 9.00±0.97 ^{bc} 8.14±0.94 ^c 15.28±1.71 ^a	$0.69\pm0.05^{\rm cd}$ $0.61\pm0.14^{\rm d}$ $0.72\pm0.09^{\rm cd}$ $1.49\pm0.36^{\rm a}$	0.10±0.03a 0.09±0.04a 0.13±0.01a 0.14±0.06a	$0.80\pm0.08^{\rm cd}$ $0.71\pm0.18^{\rm d}$ $0.85\pm0.10^{\rm cd}$ $1.64\pm0.41^{\rm a}$	3834.9±416.4 ^a 3173.7±345.5 ^a 3129.4±444.0 ^a 3238.7±308.4 ^a
AMFxCB36/1 Commercial AMFxTR21/1K	3.91±0.14ab	28.20±1.65ab	29.15±0.97ª	13.34±1.95ª	1.59±0.32ab	15.17±1.94ª	1.27±0.22ª	0.14±0.02a	1.41±0.23ab	3361.4±371.9a
G. margarita xCB36/1	4.03±0.30a	20.35 ± 2.16^{d}	22.30 ± 2.74 ^{cb}	9.19±1.09bc	1.07±0.35bc	10.26±1.36bc	0.97 ± 0.14 bc	0.13±0.02ª	1.12±0.16bc	3132.7±412.8a
G. margarita xTR21/1K	4.08±0.25a	$22.70 \pm 1.79^{\text{cd}}$	21.80 ± 2.05^{c}	9.72±1.00b	0.74 ± 0.33^{c}	10.46±1.08bc	$0.85 \pm 0.12^{\rm cd}$	0.11±0.02a	$0.96\pm0.14^{\rm cd}$	3465.0±523.7a

^{*} Means represented with the same letter in the same column are not significantly different according to Duncan's multiple comparison tests at p<0.05.** TR21/1K: P. putida, CB36/1: Ochrobactrum

Table 3. The effect of AMF species and PGPR isolates applied to the spinach plant on the shoot diameter, shoot length, root length, shoot fresh weight, root fresh weight, total fresh weight, total dry weight, total dry weight and total phosphorus content

Çizelge 3. Ispanak bitkisine uygulanan AMF türleri ve PGPR izolatlarının sürgün çapı, sürgün uzunluğu, kök uzunluğu, sürgün yaş ağırlığı, kök yaş

ağırlığı, toplam yaş ağırlık, sürgün kuru ağırlığı, kök kuru ağırlığı, toplam kuru ağırlık ve toplam fosfor içeriğine etkisi

Treatment Groups	Shoot Diameter (cm)	Shoot Length (cm)	Root Length (cm)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Total Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Total Dry Weight (g)	Total Phosphorus Content
	$\vec{x} \pm SD$	$\vec{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\vec{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\vec{x} \pm SD$	$\bar{x} \pm SD$
Control	3.71±0.37a	17.21±2.36	16.61±2.4	10.06±1.55	0.58 ± 0.13^{ab}	10.63±1.64	0.78±0.14a	0.08 ± 0.02^{a}	0.87 ± 0.15^{a}	5054.1±1046.
Commercial AMF	3.80 ± 0.32^{a}	$18.10{\pm}0.97$	17.85±0.9 6 ^a	9.18 ± 0.83^{d}	$0.48{\pm}0.05^{\rm bc}$	9.66 ± 0.84^{c}	$_{\rm d}^{0.60\pm0.09^c}$	0.04 ± 0.01^{c}	$_{\rm c}^{0.65\pm0.09^{ m b}}$	5620.5±727.2b
G. margarita	$3.53 \pm 0.25 ^{\mathrm{b}}$	17.30 ± 3.33	15.80 ± 1.5	6.86 ± 1.03^{d}	$0.44 \pm 0.06^{\rm cd}$	7.30 ± 1.08^{c}	$0.47{\pm}0.07^{\rm d}$	0.04 ± 0.01^{c}	0.52 ± 0.07^{c}	5296.4 ± 791.0 b
CB36/1	4.13 ± 0.62^{a}	21.33 ± 3.18	20.15 ± 6.3	10.12 ± 2.09	0.54 ± 0.16^{bc}	10.67 ± 2.20	0.71 ± 0.25^{b}	0.06 ± 0.01^{a}	0.78 ± 0.26^{a}	4996.1 ± 1075 .
TR21/1K	4.05 ± 0.33^{a}	21.05 ± 3.68	16.10 ± 2.9	17.80 ± 4.21	0.64 ± 0.18^{ab}	16.41 ± 5.35	0.93 ± 0.30^{a}	0.08 ± 0.03^{a}	1.02 ± 0.31^{a}	5415.8 ± 1582 .
Commercial AMFxCB36/1	4.17±0.57 ^a	$20.50{\pm}3.04$	19.75±3.4 3 ^a	13.88 ± 3.27	0.71 ± 0.09^{a}	15.23±2.58 a	0.97±0.21ª	$_{\rm c}^{0.05\pm0.02^{\rm b}}$	1.03±0.23a	4794.7±406.8b
Commercial AMFxTR21/1	4.09±0.23a b	$\underset{\mathrm{ab}}{20.43 \pm 1.70}$	19.13±4.9 8 ^a	13.30±1.46 bc	0.59 ± 0.06^{ab}	13.87±1.43	0.74±0.13ª bc	0.06±0.03ª bc	0.81±0.10a b	5238.3±639.8 ^b
G. margarita xCB36/1	3.96±0.62a b	19.90±1.15	16.40±1.0 4 ^a	9.66 ± 3.56^{d}	$0.47 \pm 0.11^{\rm cd}$	10.13±3.63	$_{\rm d}^{0.66\pm0.08^c}$	$0.05 \pm 0.02^{\rm b}$	0.72 ± 0.09^{b}	5330.5±864.5b
<i>G. margarita</i> xTR21/1K	4.19±0.19 ^a	$18.25{\pm}1.91$	18.41 ± 4.0 7^{a}	9.72 ± 1.69^{d}	0.42 ± 0.05^{d}	10.13±1.76 bc	0.74 ± 0.11^{a}	$0.04\pm0.01^{\rm b}$	0.79 ± 0.12^{a}	6961.7±810.4ª

^{*} Means represented with the same letter in the same column are not significantly different according to Duncan's multiple comparison tests at p<0.05 ** TR21/1K: *P. putida*, CB36/1: *Ochrobactrum* sp.

Table 4. The effect of AMF species and PGPR isolates applied to the stinging nettle plant on the shoot diameter, shoot length, root length, shoot fresh weight, root fresh weight, total fresh weight, shoot dry weight, root dry weight, total dry weight and total phosphorus content

Çizelge 4. Isırgan out bitkisine uygulanan AMF türleri ve PGPR izolatlarının sürgün çapı, sürgün uzunluğu, kök uzunluğu, sürgün yaş ağırlığı, kök

yaş ağırlığı, toplam yaş ağırlık, sürgün kuru ağırlığı, kök kuru ağırlığı, toplam kuru ağırlık ve toplam fosfor içeriğine etkisi

Treatment groups	Shoot Diameter (cm)	Shoot Length (cm)	Root Length (cm)	Shoot Fresh Weight (g)	Root Fresh Weight	Total Fresh Weight (g)	Shoot Dry Weight	Root Dry Weight (g)	Total Dry Weight (g)	Total Phosphorus Content
	$\vec{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\vec{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{\mathbf{x}} \pm \mathrm{SD}$
Control	6.00 ± 1.28^{bc}	56.55±9.63ab	29.25±7.12	$9.56 \pm 0.84^{\rm cde}$	1.71±0.18	11.31±1.01c	1.11±0.15	0.16 ± 0.04^{c}	1.28±0.19	3016.7±137.2
Commercial AMF	$_{\rm c}^{7.07\pm0.72^{\rm ab}}$	$52.63\pm7.12^{\rm ab}$ c	32.83±5.57	16.42±3.84 ^a	2.86±1.16	$19.24{\pm}4.75^{\mathrm{a}}$	1.60±0.30	0.20 ± 0.03^{b}	1.80±0,33	2675.5±269.4
G. margarita	6.28 ± 1.85^{bc}	55.25 ± 2.03^{ab}	28.45 ± 1.02	$7.93 \pm 0.62^{\rm cd}$	1.24 ± 0.20	9.16 ± 0.71^{c}	1.11 ± 0.06	0.14 ± 0.02^{c}	1.26 ± 0.07	2399.1 ± 308.2
CB36/1	6.36 ± 0.66 bc	53.08 ± 5.41 ab	27.51 ± 7.13	10.53 ± 1.84^{c}	1.65 ± 0.66	12.18 ± 2.44^{c}	1.11 ± 0.15	0.16 ± 0.05^{c}	1.28 ± 0.19	2358.1 ± 391.0
TR21/1K	$5.94 \pm 0.13 $ bc	51.00 ± 6.58 bc	28.30 ± 1.37	$9.92{\pm}1.35^{\rm cde}$	1.79 ± 0.14	11.71 ± 1.47^{c}	1.09 ± 0.21	$0.19\pm0.01^{\rm b}$	1.29 ± 0.23	2289.9 ± 477.7
Commercial AMFxCB36/1	7.28 ± 0.78 ab	56.81 ± 2.82 ab	40.88±9.17 a	14.31±1.51b	3.41 ± 0.25	17.68±1.31 ^b	1.65 ± 0.16	$0.25{\pm}0.10^{ m a}$	1.91±0.19	2593.6±325.9
Commercial AMFxTR21/1	7.96 ± 1.02^{a}	60.95±3.73ª	41.20±3.75	16.87±1.18 ^a	4.90±1.07	21.01±1.22a	1.97±0.14 a	0.28±0.03a	2.26±0.13	2808.6±531.5
G. margarita xCB36/1	$5.75\pm0.30^{\circ}$	46.05±5.93°	29.90 ± 5.08	7.53±1.01e	1.65 ± 0.15	9.16±1.14°	$_{\rm d}^{0.82\pm0.08}$	0.16 ± 0.02^{c}	0.98 ± 0.10	2610.6±368.9
<i>G. margarita</i> xTR21/1K	$5.81 \pm 0.42^{\circ}$	56.31±5.61ab	27.60 ± 3.89	10.10 ± 1.79^{c}	1.90 ± 0.52	12.00±2.29°	1.12 ± 0.14	0.18±0.03°	1.30 ± 0.17	2392.2±513.5

^{*} Means represented with the same letter in the same column are not significantly different according to Duncan's multiple comparison tests at p<0.05 ** TR21/1K: P. putida, CB36/1: Ochrobactrum sp.

Table 5. Effects of PGPR (TR21 / 1, CB36 / 1) isolates inoculated with the AMF species on the root colonization rate (%), soil spore density (spores g⁻¹ soil) and dependency (%) of the cauliflower, spinach and stinging nettle plants

Çizelge 5. AMF türleri ile inokule edilen PGPR (TR21/1, CB36/1) izolatlarının karnabahar, ıspanak ve ısırgan otu bitkilerinin kök kolonizasyonu oranı (%), toprak spor yoğunluğu (spor/g-1 toprak) ve mikorhizal bağımlılık oranı (%)'na etkisi

	sagiiiiiiii eraiii (70) na etnisi				
	_	AMF Root	Soil Spore Density	Dependency (%)	
Plants	Treatment Groups	Colonization (%)	(spore g ⁻¹ soil)		
		$\bar{x} \pm SD$	$\bar{x} \pm SD$		
	Commercial AMF	13.68±2.27a*	17.80 ± 6.30^{ab}	+(33.83)**	
Ħ	G. margarita	0.48 ± 0.66 ^c	7.80 ± 3.42^{c}	-(10)***	
Cauliflower	Commercial AMF x CB36/1****	2.10 ± 2.88^{c}	12.60 ± 1.67 bc	+(46.34)	
Ęľo	Commercial AMF x TR21/1K	2.45 ± 2.66^{c}	14.40 ± 7.89^{abc}	+(37.58)	
ulji	G. margarita x CB36/1	8.33 ± 3.19^{b}	20.60±4.21ª	+(21.42)	
	G. margarita x TR21/1K	1.77 ± 2.74^{c}	17.00±4.35ab	+(8.33)	
	Commercial AMF	50.51±8.58a*	30.80±11.00ab	-(33.84)	
	G. margarita	13.25 ± 5.48^{b}	38.00 ± 9.13^{a}	-(67.30)	
_	Commercial AMF x CB36/1	41.69 ± 30.06^{ab}	$32.40 \pm 9.07^{\mathrm{ab}}$	+(15.53)	
Spinach	Commercial AMF x TR21/1K	32.06 ± 28.98^{ab}	31.20 ± 12.96 ab	-(7.40)	
in?	G. margarita x CB36/1	29.62 ± 11.80^{ab}	34.80 ± 16.84^{ab}	-(20.83)	
$^{ m Sp}$	G. margarita x TR21/1K	31.93 ± 22.33^{ab}	20.40 ± 8.11^{b}	-(10.12)	
Je	Commercial AMF	3.45±3.17a*	2.00 ± 1.87^{d}	+(28.88)	
ett	G. margarita	1.38 ± 1.89^{a}	$12.20 \pm 3.27^{\rm cd}$	-(1.58)	
g D	Commercial AMF x CB36/1	1.17 ± 1.61^{a}	42.20 ± 15.02^{a}	+(32.98)	
Stinging nettle	Commercial AMF x TR21/1K	$0.77 \pm 1.06 ^{\mathrm{a}}$	26.00 ± 8.51^{b}	+(43.26)	
ng	G. margarita x CB36/1	2.58 ± 3.53^{a}	19.40 ± 4.39 bc	-(30.61)	
$\mathbf{S}_{t:}$	G. margarita x TR21/1K	1.17 ± 1.61^{a}	$10.40 \pm 3.13^{\rm cd}$	+(1.53)	

^{*} Plants were evaluated among themselves, and the means represented with the same letter in the same column are not significantly different according to Duncan's multiple comparison tests at p<0.05 ** (-): No dependency *** (+): Dependency exists **** TR21/1K: *P. putida*, CB36/1: *Ochrobactrum* sp.

were examined. In general, in all plant species, when both biological control agents were applied, an increase was achieved in the plants' growth parameters, as compared to the control plants (Tables 2, 3 and 4). Egamberdieva and Adesemoye (2016) also reported that PGPR x AMF combinations not only increased the plant growth and yield but also raised the plants' height and dry weight. It has been suggested that it could be enriched by N, P and K nutrients. In addition, the presence of a synergistic effect between PGPR x AMF combinations confirmed these results (Pérez-de-Luque et al., 2017).

Although AMFs do not have host selectivity, it has been reported that they have negative effects on forming a symbiotic life in plant species belonging to Brassicaceae, Caryophyllaceae, Chenopodiaceae and Urticaceae families (Tester et al., 1987; Smith and Read, 2008; Brundrett, 2009; Lambers and Teste, 2013; Tushar and Satish, 2013; Zuccarini and Savé, 2016).

The root secretion of these plants shows an allelopathic effect, with an adverse effect on the development of AMF (Sosa-Rodriguez et al., 2013). In addition to the allelopathic effect, non-host plant genes have been noted to be effective in these families, which are described as "non-host plants"

(Fiorilli et al., 2015). In the recent years, it has been suggested that, especially in the plants belonging to the Brassicaceae family, the loss of genes related to possible AMF symbiosis and/or the ability of plants to recognize AMF effectors can lead to the loss of their ability to form AMF symbiotic relationships during evolution (Poveda et al., 2019).

This study revealed that the treatment groups with the commercial AMF isolate improved AMF root colonization and soil spore density of cauliflower and spinach plants (Table 5) It was observed that AMF colonization rates were very low in nettle plants, as compared to the other plants (Table 5). Similar to the present study, Vierheilig et al. (1996) stated that agglutinin, a protein similar to the root structures found in the rhizomes of stinging nettle, prevented the formation of AMF hyphae and colonization, further, a mycorrhizal symbiotic relationship was not observed. In another study conducted by Gunes et al. (2019) it was revealed that the commercial AMF (ERS) isolate inoculated into spinach plant was effective in mycorrhizal dependency. Non-mycorrhizal plants harm mycorrhizal fungus with chemical weapons having an allopathic effect since they perceive mycorrhizal fungi as pathogens that cannot establish a symbiotic relationship (Lambers and Teste, 2013). Similarly, in the present study, the effect of the development parameters (root colonization, dependency and soil spore density) of AMFs on cauliflower, spinach and nettle, in common applications with both single and PGPR, varied, depending on the plant species. However, when PGPR and AMF are applied simultaneously, the former can provide a significant increase in the root of the plant, thus supporting the presence of AMF in the roots of these plants (Poveda et al., 2019).

Conclusions

According to the results of the present study, plants not hosting AMF may be under stress because they perceive mycorrhizal fungi as pathogens when inoculated with AMF. It was noticed that the effect of bacterial species in the rhizosphere on different plants was greater than that of AMF colonization. Therefore, it is very difficult to determine clearly how the relationship between the two microorganisms in the rhizosphere region can affect each other and how this can have an impact on the plant. However, proper AMF x PGPR x host combinations seem to contribute positively to soil health and fertility, thus helping to ensure sustainable agriculture. In addition, these findings could have a significant agronomic impact, as the combined use of both types of microorganisms can significantly productivity of these important plant groups.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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