The Effect of Inoculation With *Amycolatopsis* Strains On Yield and Nutrient Content of Wheat (*Triticum Aestivum* L.) and Soil Microbiological Properties

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Abstract

In this study, the yield and nutrient content of wheat (*Triticum aestivum* L.) grown in greenhouse conditions and soil microbiological properties were investigated. Inoculating *Amycolatopsis* strains, including *A. magusensis* DSM 45510^T, *A. orientalis* DSM 40040^T, and *A. azurea* DSM 43854^T was considered. The mixture of wheat straw (WS) was used to increase soil organic matter content by 5%. It was determined that the grain and straw yield of wheat increased significantly ($P < 0.001$) in inoculation with *Amycolatopsis* strains in soils without WS. However, inoculation with *Amycolatopsis* strains in soil with WS significantly decreased grain and straw of wheat yield. Also, it was found that soil microbial biomass and soil basal respiration (SBR) increased in inoculation with *Amycolatopsis* strains in both soils with and without WS. While it was established that $C_{mic}:C_{org}$ ratio of the control group in the soil samples at the end of the harvest was 1.23, infusing with *Amycolatopsis* strains was observed to be around 2.95–3.31. Moreover, inoculation with *Amycolatopsis* strains in soils with WS varied between 0.32–0.40. In the same way, it was determined the microbial metabolic quotient (qCO_2) was 2.58 in the control group. This meaning was between 5.67–5.82 in infusing with *Amycolatopsis* strains and 6.04–6.41 in inoculating with *Amycolatopsis* strains in soils mixed with ground wheat stalk. As a result, it suggested that the yield of wheat could be increased inoculation with *A. magusensis*, *A. orientalis*, and *A. azurea*, from *Amycolatopsis* strains, in soils with low soil organic matter content and soils that cannot be shown an increment in terms of organic matter content.

Introduction

Agricultural systems commonly put excess pressures on the soils, leading to environmental problems and the exhaustion of natural resources. In the long run, the rapid increase in the yield of production by adding chemical inputs replaces with the gradual declining trend. Eventually, farming systems that produce healthy foods would collapse. Production of healthy foods without using agricultural chemicals is compulsory for the sustainable use of natural resources. For the sustainability, some actions that need to be considered, including prevention of environmental pollution, maintaining agricultural sustainability, ensuring the sustainability of natural resources, reducing agricultural expenses, and using plant hormone-producing and nitrogen-fixing microorganisms.

Many microorganisms, such as plant growth-promoting rhizobacteria (PGPR), can naturally promote plant growth. The usage of a biological fertilizer as a sustainable approach to increasing crop yield provides many benefits to the host plant after the infusing (Gouda et al., 2018; Backer et al., 2018). Apart from the species belonging to PGPR microorganism, using bacterial species including *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Pseudomonas*, *Bacillus*, and *Paenibacillus* are reported by several researchers (Saubidet et al., 2002; Lucy et al., 2004). Bacteria that stimulate plant growth by controlling harmful microorganisms are called biopesticides (Banerjee et al., 2005). However, it is known that some PGPRs act as both biofertilizers and biopesticides to stimulate plant growth. For example, while *Burkholderia cepacia* species has biocontrol properties against *Fusarium* species, they also contribute to the development of corn with the formation of siderophores in iron-poor conditions (Bevivino et al., 1998).

The effects of PGPRs on crop yield can vary according to the climate and ecological conditions of the grown plants and the region. Soil properties can also impact their performance. Many physicochemical properties such as texture, pH, salinity, and nutrient content play an essential role in the PGPRs on yield (Hussain et al., 1999; Valarini et al., 2003). Increasing soil organic substance improves the efficiency of PGPRs by providing the carbon and energy source for PGPRs (Fageria, 2012).

So far, PGPRs are used for an antibacterial activity such as balhimycin, vancomycin, and rifamycin (Kumari et al., 2016), synthesis of vanillin (Fleige et al., 2016), and polycyclic aromatic hydrocarbons degradation capacities (Ortega-González et al., 2015). In this study, the possibility of using *Amycolatopsis* strains as PGPR was investigated. For this purpose, the effect of wheat (*Triticum aestivum* L.) grown in greenhouse conditions on yield, nutrient content, and soil microbiological properties were evaluated. Microbial inoculating was carried out with *A. magusensis* DSM 45510^T, *A. orientalis* DSM 40040^T, and *A. azurea* DSM 43854^T, from *Amycolatopsis* strains. Besides, soils were mixed with and without ground wheat straw (WS) to consider the factor of organic substance input. The results of wheat yield and soil microbiological properties were studied in detail.

Material And Methods

Soil and wheat straw

Soil samples used in this study were taken from the most biologically active part of soil (0–20 cm). The soil was collected from arable agriculture field for a long time. The field is located on the Black Sea Region of Northern Turkey (latitude, 41°21'N; longitude, 36°15'W). The region has a semi-humid climate with temperatures ranging from 3.8°C in February to 27°C in August, with an annual mean temperature of 14.5°C. The annual mean precipitation is 718 mm. The field has no background in receiving pesticide and fertilizer treatments within the last six months before the soil sampling. The soil in the field was classified as Vertic Haplustoll, according to the FAO (2006). The soil sample was homogenized, dried, gently crumbled, and screened from 2 mm opening sieve to remove stones, gravels, and root material. Basic soil properties were analyzed according to the method proposed by Rowell (1996). The obtained results were measured to be as follows: pH (1:1 w:v) 7.10; total organic matter 1.73%; conductivity (1:1 w:v) 0.94 dSm⁻¹, lime (CaCO₃) content 11.74%; total nitrogen (Kjeldahl N) %0.18; available P (Olsen-P) 23.52 mg kg⁻¹; exchangeable K 1.12 cmol kg⁻¹; and exchangeable Ca: 23.08 cmol kg⁻¹. The soil sample used in this study had a clay loam textural class, including 29.55% clay, 43.06% silt, and 27.39% sand.

In this research, wheat straw (WS) was obtained from the Experimental Field of Agricultural Faculty, Ondokuz Mayıs University, Samsun, Turkey. The WS, with no diseases or contaminating insects, was dried at 65°C. Whole WS materials, including stalks and leaves, were chopped into 10 cm pieces, grounded, sieved from 0.50 mm, and stored in polyethylene bags at 5°C. The properties of WS were expressed on a dry basis and analyzed according to standard procedures given by Jones (2001). The WS

had approximately 79% of oxidizable organic matter. The organic fraction of WS had 45.9% C and 0.89% N, while the inorganic fraction of WS had 2.60% P, 5.01% K, 1.10% Ca, and 1.3% Mg by weight.

Bacterial culture and inoculant preparation

Three *Amycolatopsis* strains (i.e., *A. magusensis* DSM 45510^T, *A. orientalis* DSM 40040^T, and *A. azurea* DSM 43854^T) were obtained from Soil Microbiology Laboratory in Ondokuz Mayıs University, Samsun, Turkey. *Amycolatopsis* strains are known as an essential source of various bioactive compounds such as antibacterial, antifungal, and antiviral. Pure cultures of three *Amycolatopsis* strains used for inoculation were grown in SM2 medium at 28°C for seven days. To prepare selective medium SM2, the procedure given by Tan et al. (2006) was performed as follows: 67.0 g yeast nitrogen base and 100 mg casamino acids were added to a liter of distilled water, and the solution sterilized using 0.20 µm cellulose filters prior to the addition of sterilized K₂HPO₄ (200 ml, 10% w/v). Thereafter, 100 ml of basal medium was added to 900 ml of sterilized molten agar (1.5% w/v) followed by filter-sterilized solutions of D(+)melezitose (1% w/v) (final concentration 1% w/v), 50 µg cycloheximide ml⁻¹, 4 µg neomycin sulfate ml⁻¹, and 50 µg nystatin ml⁻¹.

A single colony from each strain was transferred to a 50 mL flask, including N and agar free SM2 medium. The resulting medium was grown aerobically in flasks for 168 hours while on a rotating shaker (125 rpm) at 28°C. *Amycolatopsis* strains grown liquid SM2 medium was then diluted with sterile distilled water, containing 0.025% Tween 20 to obtain a final concentration of 10⁹ CFU ml⁻¹. Before sowing seeds into unsterilized soil, spring wheat (*Triticum Aestivum* L.) seeds were placed in bacterial suspensions of 10⁹ CFU ml⁻¹ for 30 min under sterilized conditions.

Greenhouse experiment

A pot experiment was carried out in the greenhouse with the spring wheat (*Triticum Aestivum* L.) in order to investigate the effects of inoculation with *Amycolatopsis* strains. The experiment consisted of eight treatments with three replications, and the pots were distributed in a completely randomized design. The experiments consisted of three treatments of *Amycolatopsis* strains (*A. Magusensis* DSM 45510^T, *A. Orientalis* DSM 40040^T, and *A. Azurea* DSM 43854^T) and a control treatment without *Amycolatopsis* strains inoculation. Twenty-five seeds were sown to the soil in each 5 L pot. After the emergence of the first leaf, plants were thinned to fifteen per pot. The pots were daily irrigated to maintain soil moisture level at 60% of water holding capacity for 124 days after sowing. The greenhouse experiment was designed according to a complete randomized plot with three replicates. The 72 experiment pots were performed in accord with the eight treatments appended below:

(1)	Control (without WS, without <i>Amycolatopsis</i> inoculation)
(2)	With addition WS, without <i>Amycolatopsis</i> inoculation
(3)	<i>Amycolatopsis magusensis</i> DSM 45510 ^T
(4)	<i>Amycolatopsis magusensis</i> DSM 45510 ^T + 50 g kg ⁻¹ WS
(5)	<i>Amycolatopsis orientalis</i> DSM 40040 ^T
(6)	<i>Amycolatopsis orientalis</i> DSM 40040 ^T + 50 g kg ⁻¹ WS
(7)	<i>Amycolatopsis azurea</i> DSM 43854 ^T
(8)	<i>Amycolatopsis azurea</i> DSM 43854 ^T + 50 g kg ⁻¹ WS

Harvest and plant analyses

Studied plant parameters included grain yield, straw yield, and nutrient concentration in grain and straw at the end of the pots experiment in the greenhouse. The dry matter of the plant was determined after oven drying to constant mass at 65°C. The dried samples were ground in a hammer mill to pass through a 0.5 mm mesh sieve. Then, the plant samples were analyzed to measure total N, P, K, and Ca. Whole pot was used for biomass determination at harvest. All parameters were measured according to the method suggested by Jones (2001).

Soil microbiological analyses

The soil samples taken from the pots were transferred to the laboratory on the same day and kept in the refrigerator at 4°C. The soil microbiological analysis was carried out no longer than 72 h prior. All microbiological properties were analyzed for the soil samples in triplicate, and all values were reported as a mean of three replications expressed on an oven-dried sample basis at 105°C for 24 h.

The soil samples were sieved through a 150 µm mesh before determining the total organic carbon (C_{org}) by the wet oxidation method (Walkley-Black) with K₂Cr₂O₇ (Rowell, 1996). Soil basal respiration (SBR), CO₂ production at 22°C without the addition of glucose at field capacity, was measured according to the method given by Anderson (1982). Microbial biomass carbon (C_{mic}) was determined by the substrate-induced respiration (SIR) method according to the method given by Anderson and Domsch (1978). The ratio of C_{mic}/C_{org} was estimated as a percentage (%). Specific respiration of the microbial biomass (the microbial metabolic quotient, *q*CO₂) was calculated as the ratio of BR to C_{mic} by following formula (BR/C_{mic} = *q*CO₂, µg CO₂-C mg⁻¹ C_{mic} h⁻¹).

Statistical analysis

All experimental data were analyzed for analysis of variance (ANOVA) using SPSS 11.0 statistical software (SPSS Inc.). ANOVA was carried out according to the completely randomized design, where

significant F -values were obtained. The significance between the means was tested using the DUNCAN test at the level of $P < 0.01$. All tables presented, including standard errors of the data and F -values. The asterisks, *, **, and *** were used to indicate the significance level at $P < 0.05$, 0.01, and 0.001, respectively.

Results And Discussion

Wheat yield

In order to determine the effects of grain and straw yield of wheat, inoculating *Amycolatopsis* strains alone and together with WS was performed. The obtained results are summarized in Table 1. According to the results, significant increases in wheat straw and grain yield after applying only 5% WS to the soil ($P < 0.001$) were observed relative to the control. Regarding the control without any amendment, an increase of 24% in straw yield and 49% in grain yield were determined (Table 1). Similar to studies carried out by Brock et al. (2011) and Zhao et al. (2016), significant increases were noted in plant crop yield as a result of the application of organic compounds to the soil. Two main reasons could explain these increases in crop yield. First, the nutrient content of the soil increases after the amendment as plant nutrients are full of organic compounds in the structure, and thus, contributing to an increase in crop yield (Espe et al., 2015). Secondly, there is an increase in plant crop yield due to organic compounds applied to soils. It could improve soil properties such as aggregate stability, water retention capacity, and cation exchange capacity (Murphy, 2015).

It was determined that *Amycolatopsis* strains inoculated into the soil significantly increased grain and straw yield of the wheat compared to control (Table 1). The increase was mostly observed for the inoculation of *A.azurea*. In this case, grain yield and straw yield were increased by 274% and 74%, respectively, compared to the control. The associated reasons for the increase in plant production in microbial inoculation to soils could be due to i) fixation of atmospheric N by microorganisms (Kızılkaya, 2009), ii) increase in the solubility of organic and inorganic P in the soil by microorganisms (Ogut and Er, 2016), and iii) hormones that increase plant development such as auxins (Egamberdiyeva, 2005), cytokinins (Garcia de Salamone et al., 2001), gibberellins (Gutiérrez-Mañero et al., 2001) by microorganisms such as the production of microbial metabolites and hydrolytic enzymes, and vitamins (Ahmad et al., 2018). In addition to those, it could be due to creating a better plant growth environment by increasing the aggregation by microorganisms or indirect effects such as antibiotic production. There is no scientific evidence that *Amycolatopsis* strains would carry out N fixation, increases the solubility of organic and inorganic P in soil, or directly affect the yield of plant crops by synthesizing hormones such as gibberellins, auxin. However, *Amycolatopsis* strains could synthesize antibiotics such as balhimycin, vancomycin, rifamycin (Nigam et al., 2014; Xu et al., 2014; Frascch et al., 2015; Kumari et al., 2016), vanillin (Fleige and Steinbüchel, 2014; Fleige et al., 2016) and therefore, causing polycyclic aromatic hydrocarbons to degrade (Ortega-González et al., 2015). In this study, the fact that *A.magusensis*, *A.orientalis*, and *A.azurea* increased the grain and straw yield of the wheat, could be attributed to the antibiotic synthesis properties of *Amycolatopsis* strains.

Table 1

The effects of inoculating *Amycolatopsis* strains alone and together with WS on grain and wheat yield

Treatments	Weight (gr/pot)		Change (%)	
	Straw	Grain	Straw	Grain
Control	10.26 ± 0.32 d	2.44 ± 0.10 e	–	–
WS (50 g kg ⁻¹)	12.74 ± 1.43 c	3.64 ± 0.21 d	24.20 ± 13.91 d	49.04 ± 8.53 d
<i>A. magusensis</i>	15.53 ± 0.56 b	7.54 ± 0.21 c	51.36 ± 5.42 c	208.88 ± 8.62 c
<i>A. orientalis</i>	16.16 ± 0.61 b	8.67 ± 0.20 b	57.47 ± 5.94 b	255.46 ± 8.20 b
<i>A. azurea</i>	17.92 ± 1.01 a	9.14 ± 0.15 a	74.63 ± 9.84 a	274.45 ± 5.97 a
<i>A. magusensis</i> + 50g WS kg ⁻¹	2.15 ± 0.16 e	1.21 ± 0.03 f	-79.01 ± 1.58 e	-50.55 ± 1.25 e
<i>A. orientalis</i> + 50g WS kg ⁻¹	2.86 ± 1.50 e	0.98 ± 0.03 f	-72.12 ± 1.57 e	-59.97 ± 1.03 f
<i>A. azurea</i> + 50g WS kg ⁻¹	2.54 ± 0.06 e	1.20 ± 0.02 f	-75.21 ± 0.54 e	-50.96 ± 0.63 e
F-value	172.346***	1862.946***	1982.910***	175.361***
*** <i>P</i> < 0.001				

As given in Table 1, *Amycolatopsis* strains applied to the soil together with WS decreased the grain and straw yield dramatically ($P < 0.001$). Compared to the control, the reduction in the grain yield by *Amycolatopsis* strains varied between 50–60%. Moreover, the straw yield was found to be between 72–79%. The wheat yield increased when WS and *Amycolatopsis* strains alone were applied to the soil. It is interesting to note that wheat yields drop significantly after the application of these together. *Amycolatopsis* strains inoculated with WS affected more significantly by the soil microbiological properties. It was found that C_{mic} and SBR were more available in pots with these combinations (Table 3). This may be due to heterotrophic *Amycolatopsis* strains, which supply the C from the organic C compound of WS. Thus, they were the dominant microflora in the environment. In this context, the growing population could synthesize more antibiotics, and this may result from the lack of positive effects of microflora on wheat yield by suppressing other microflora. Especially, bacteria populations such as nitrifier bacteria involved in N transformation might disappear from the environment due to the synthesized antibiotics. Eventually, this might prevent the formation of nutrients that can be taken as mineral N, and plant yield may have decreased.

Nutrient contents of wheat

The application of *Amycolatopsis* strains alone and together with WS was studied to understand the effects of grain and straw of wheat within different scopes. The obtained results within the scope of N

and P are given in Table 2. The corresponding results within the scope of K and Ca are presented in Table 3. It was found that the nutrient content of the wheat increased in general as a result of the application of WS and *Amycolatopsis* strains alone and together. It was observed that the application led to increases in the N and P content in the grain and straw of the wheat compared to the control. When WS applied with *Amycolatopsis* strains (Table 2), this application also increased the content of grain K and stem Ca content of the plant (Table 3). Jones et al. (1991) reported that the nutrient adequacy levels of the leaves of the wheat plant should be 1.75–3% N, 0.2–0.5% P, 1.5-3% K, and 0.2-1% Ca. According to these values, it was found that in the control application, the N content of the plant samples at harvest was insufficient. However, P, K, and Ca contents were found to be sufficient.

Similarly, Orhan et al. (2006), Çakmakçı et al. (2001), Kızılkaya (2008), Esitken et al. (2010) found that the product yield and nutrient content of the plant such as N, P, and K significantly increase with the inoculation of different microorganisms to the soil, in raspberry, sugar beet and barley, spring wheat, strawberry, respectively. In this study, the main reason why P, K, Ca contents of the plant in the control application without any fertilizer application are determined at a sufficient level is, without a doubt, due to the fact that the test soil contains sufficient levels of P (23.52 mg kg^{-1}), K ($1.12 \text{ cmol(+)kg}^{-1}$) and Ca ($23.08 \text{ cmol(+)kg}^{-1}$) to meet the plant's need. It is thought that the N (0.18%), which was initially found in the soil at an average level, did not fully meet the requirements of the plant during the vegetation period. With microbial inoculations in the soil, increased nutrient content in the plant is related to promotes the mineral uptake of plants, and microorganisms promote mineral uptake with different mechanisms (Gouda et al., 2018; Backer et al., 2018). However, it may not be due to the normal ion uptake of mineral uptake by plants but due to volume increase of root system that is made of root amount, thickness, and length. Increased number of lateral roots by bacteria and widening of the root tips increases root surface area and it causes the plant to absorb more water and nutrients (Pinton et al., 2007). In inoculation with *Amycolatopsis* strains, including antibiotics secreted by the bacterial species, secretions do not only protect the plant root system against pathogenic organisms but also increases plant root development and eventually increases mineral intake by plants so that more nutrients may be absorbed by grain and straw of wheat.

Table 2

The effects of inoculating *Amycolatopsis* strains alone and together with WS on N and P contents of wheat

Treatments	N (%)		P (%)	
	Straw	Grain	Straw	Grain
Control	0.68 ± 0.04 g	1.72 ± 0.08 e	0.35 ± 0.01 c	0.46 ± 0.01 b
WS (50 g kg⁻¹)	0.82 ± 0.03 f	2.65 ± 0.06 c	0.45 ± 0.02 b	0.44 ± 0.01 c
A. magusensis	1.28 ± 0.09 b	3.38 ± 0.07 a	0.56 ± 0.02 a	0.51 ± 0.02 a
A. orientalis	1.23 ± 0.05 bc	3.19 ± 0.06 b	0.54 ± 0.01 a	0.52 ± 0.01 a
A. azurea	1.39 ± 0.06 a	2.27 ± 0.10 d	0.55 ± 0.01 a	0.52 ± 0.01 a
A. magusensis + 50g WS kg ⁻¹	1.00 ± 0.06 e	2.25 ± 0.10 d	0.33 ± 0.01 c	0.31 ± 0.01 d
A. orientalis + 50g WS kg ⁻¹	1.08 ± 0.03 de	2.37 ± 0.14 d	0.34 ± 0.02 c	0.33 ± 0.02 d
A. azurea + 50g WS kg ⁻¹	1.16 ± 0.07 cd	2.24 ± 0.09 d	0.34 ± 0.01 c	0.34 ± 0.02 d
F-value	59.463***	116.798***	138.806***	105.018***
*** <i>P</i> < 0.001				

Table 3

The effects of inoculating *Amycolatopsis* strains alone and together with WS on K and Ca contents of wheat

Treatments	K (%)		Ca (%)	
	Straw	Grain	Straw	Grain
Control	1.75 ± 0.06 a	4.46 ± 0.13 ab	0.31 ± 0.01 bc	0.35 ± 0.01
WS (50 g kg ⁻¹)	1.55 ± 0.11 b	4.48 ± 0.10 a	0.30 ± 0.02 c	0.33 ± 0.02
<i>A. magusensis</i>	1.45 ± 0.02 cd	4.47 ± 0.06 a	0.41 ± 0.01 a	0.33 ± 0.01
<i>A. orientalis</i>	1.46 ± 0.01 cd	4.52 ± 0.05 a	0.42 ± 0.01 a	0.37 ± 0.01
<i>A. azurea</i>	1.47 ± 0.02 bc	4.26 ± 0.12 b	0.42 ± 0.01 a	0.36 ± 0.01
<i>A. magusensis</i> + 50g WS kg ⁻¹	1.37 ± 0.02 de	3.52 ± 0.12 c	0.32 ± 0.01 b	0.33 ± 0.01
<i>A. orientalis</i> + 50g WS kg ⁻¹	1.36 ± 0.01 e	3.23 ± 0.19 d	0.32 ± 0.01 b	0.33 ± 0.01
<i>A. azurea</i> + 50g WS kg ⁻¹	1.34 ± 0.04 e	3.05 ± 0.09 d	0.32 ± 0.01 b	0.33 ± 0.02
F-value	20.579***	86.854***	107.357***	3.887 ^{ns}
*** <i>P</i> < 0.001				

Soil microbiological properties

As a result of the inoculation with *Amycolatopsis* strains alone and together with WS, and its effects on C_{org} and some microbiological properties are given in Table 4. It is determined that inoculating *Amycolatopsis* strains alone to soil and the change in C_{org} content is not statistically significant, but as a result of infusing *Amycolatopsis* strains with WS, the most increase in C_{org} content occurs (*P* < 0.001). Similarly, it was found that WS application added to the soil at the level of 5% significantly increased the C_{org} content (*P* < 0.001). This is a clear indication that there will be more increases in C_{org} content with microbial inoculation when there is an adequate source of organic matter in the soil. When compared to the control and application of WS only, the main reason for the increase in C_{org} content is related to the microbial excretions are more common in this environment and contributions of capillary roots to C_{org} content with an increase in root secretions of better growing plant roots contribute (Powlson et al., 2001; Pinton et al., 2007). However, obtained results are limited to the 124-day greenhouse trial. Without a doubt, organic compounds in the soil will be used as a C source by the growing microorganism population after the experiment, and C_{org} content will also decrease (Li et al., 2018).

Table 4
The effects of inoculating *Amycolatopsis* strains alone and together with WS on C_{org} and soil microbiological properties

Treatments	C _{org}	SBR	C _{mic}	C _{mic} /C _{org}	qCO ₂
Control	0.42 ± 0.10 d	13.04 ± 1.64 e	121.37 ± 5.28 f	1.23 ± 0.27 b	2.58 ± 0.36 c
WS (50 g kg⁻¹)	3.01 ± 0.03 c	22.22 ± 0.27 d	182.91 ± 2.94 e	0.25 ± 0.01 c	2.92 ± 0.04 c
A.magusensis	0.34 ± 0.12 d	55.74 ± 3.19 c	230.03 ± 9.46 d	3.06 ± 0.98 a	5.82 ± 0.44 b
A.orientalis	0.31 ± 0.05 d	57.74 ± 0.66 c	244.22 ± 9.53 c	3.31 ± 0.36 a	5.67 ± 0.27 b
A.azurea	0.35 ± 0.16 d	59.12 ± 2.39 c	247.22 ± 11.30 c	2.95 ± 0.01 a	5.75 ± 0.37 b
A.magusensis + 50g WS kg ⁻¹	3.66 ± 0.16 a	75.73 ± 2.39 b	283.75 ± 11.33 b	0.32 ± 0.01 c	6.41 ± 0.37 a
A.orientalis + 50g WS kg ⁻¹	3.76 ± 0.18 a	81.26 ± 3.00 a	321.82 ± 9.19 a	0.36 ± 0.03 c	6.06 ± 0.20 ab
A.azurea + 50g WS kg ⁻¹	3.29 ± 0.07 b	80.14 ± 4.14 ab	318.63 ± 8.01 a	0.40 ± 0.02 c	6.04 ± 0.44 ab
F-value	702.180***	299.638***	221.191***	28.778***	66.318***
***P < 0.001;					
C _{org} : total organic carbon (%); SBR: basal soil respiration (mgCO ₂ -C gr ⁻¹ .24 h); C _{mic} : microbial biomass C (mg C g ⁻¹ 24 h); C _{mic} /C _{org} : %; qCO ₂ : the microbial metabolic quotient (µg CO ₂ -C mg ⁻¹ C _{mic} h ⁻¹)					

Soil Basal Respiration (SBR) is defined as a constant respiration rate in the soil caused by the mineralization of organic matter, and it is estimated based on CO₂ evolution or O₂ uptake (Dilly and Zyakun, 2008). The determination of SBR measurement is a widely used indicator in the assessment of both microbial respiration in the soil and mineralization of organic matter (Creamer et al., 2014). In this study, the most SBR among applications was obtained by inoculation with *Amycolatopsis* strains with WS (Table 4). This is followed by inoculation with *Amycolatopsis* strains alone and WS, respectively (P<0.001). This reveals that organic matter mineralization and microbial CO₂ production in soils are mostly obtained in inoculation with *Amycolatopsis* strains. Similarly, studies carried out by Rui et al. (2016) and Mierzwa-Hersztek et al. (2018), it has been determined that SBR has significant increases by adding organic matter to the soil or by microbial inoculation.

In this study, Substrate Induced Respiration (SIR) method was used to evaluate microbial biomass (C_{mic}). Microbial population in soil produces CO_2 from an easily degradable substrate in a simple structure added to soils. As a result of this, maximal respiration occurred at the beginning is determined, and this is widely used in C_{mic} estimation (Anderson and Domsch, 1978; Nakamoto and Wakahara, 2004). In this study, it was determined that all applications significantly increased C_{mic} in soil ($P < 0.001$) when compared to the control application. Just like in SBR, the most C_{mic} is obtained in infusion with *Amycolatopsis* strains with WS. The obtained results show that microbial inoculation to soil and the addition of WS increases microbial biomass (Table 4). Similarly, with the studies carried out by Beare et al. (1990) and Rui et al. (2016), it has been determined that microbial inoculations and organic waste applications to soil significantly increases C_{mic} . Besides, it is known that antibiotics synthesized by *Amycolatopsis* strains reduce populations by suppressing other microflora in the soil and rhizosphere region (Thomashow et al., 2008). Therefore, it can be said that *Amycolatopsis* strains inoculated with increased microbial activity in the experiment are dominant microorganisms in the soil environment.

The ratios of C_{mic} in C_{org} were calculated based on C_{org} and C_{mic} data of soils. According to the results obtained, the highest C_{mic}/C_{org} ratios were obtained in applications where *Amycolatopsis* strains were inoculated (2.95–3.31). The lowest C_{mic}/C_{org} rates were found in applications where WS and WS with *Amycolatopsis* strains were applied (Table 4). Although the ideal C_{mic}/C_{org} ratio varies between 1–4% in agricultural soils, the ideal rate is intended to be around 5%, and the C_{mic}/C_{org} ratio alone provides a more sensitive assessment opportunity compared to the evaluation of C_{org} and C_{mic} alone (Sparling, 1992). This case reveals that the most appropriate application in terms of balance and approach to ideal conditions that will occur at the end of C_{org} and infusion in soils can only be obtained in applications where *Amycolatopsis* strains are inoculated.

The microbial metabolic quotient or qCO_2 is a microbial respiration rate for one C_{mic} unit and it represents the capacity of microorganisms in the soil to use C_{org} (Chao et al., 2019). According to the results obtained from the study, the highest qCO_2 values were obtained in applications where *Amycolatopsis* strains were inoculated with WS (6.04–6.41). This is followed by *Amycolatopsis* strains inoculations and WS application, respectively (Table 4). This case is a clear indication that the inoculation of *Amycolatopsis* strains in the soil also uses WS which is added to the soil. Because in these applications, not only qCO_2 was determined high but also C_{mic} and SBR of the soils were found high (Table 3).

Conclusions

A combination of microorganisms and microbial fertilizer applications into the soil has comprehensive and multiple functions to promote sustainable agriculture and reducing environmental pollution and long-term adverse effects on soil fertility. However, not all types of microorganisms could serve for these purposes. Hence, it is essential to determine the microorganism species that can be used for such as microbial fertilizer purposes, the soil improving characteristics. Since microorganisms that can be used

for microbial fertilizer are mostly heterotrophic, an increase in soil organic substance level will increase the effectiveness of these organisms and has more effect on plant crop yield. Besides, soil organic substance plays a critical role in these organisms to survive in the soil. In this study, it was determined that inoculation of *A. magusensis* DSM 45510^T, *A. magusensis* DSM 45510^T, and *A. orientalis* DSM 40040^T, from *Amycolatopsis* strains, significantly increased yield and nutrient content of the wheat. Although organic substances amendment increased the activity of these microorganisms, it was found that it significantly reduced the yield of plant crops. Contradictory, the greenhouse experiment proved that yield could be increased with *Amycolatopsis* strains inoculation, especially in soils without organic material input with insufficient soil organic substance located in the arid/semi-arid climate zones. However, it is necessary to expand the study results with field experiments by including different plant species. The next step will be microbial fertilizer production. The effect of microbial inoculation on plant growth should be assessed using the changes in soil microbial properties and yield parameters together.

Declarations

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RK and MK conceived of the presented idea, verified the analytical methods. RK developed the field design and performed the computations, participated in the sequence alignment and drafted the manuscript. MK carried out the all field and laboratory experiments, supervised the findings of this work. All authors discussed the results and approved the final manuscript.

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