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Effects of Single, Double and Triple Bacterial Formulations on Soil Microbiological Properties in Black Cumin Cultivation

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Abstract: Microorganisms are essential for the function, health, fertility, productivity, quality, and sustainable development of the soil ecosystem. The study was conducted to determine the effects of bacterial formulations on the biochemical and microbial properties of the soils. Possible effects of bacterial formulations in six single strains (*Pseudomonas fluorescens*, *P. fluorescens*, *P. fluorescens*, *Bacillus licheniformis*, *B. megaterium*, and *B. subtilis*), two double combinations (*Pseudomonas fluorescens* + *B. megaterium*, and *P. fluorescens* + *B. subtilis*), and three triple combinations (*P. fluorescens* + *B. megaterium* + *B. subtilis*, *P. fluorescens* + *B. licheniformis* + *B. megaterium*, and *P. fluorescens* + *B. licheniformis* + *B. subtilis*) on the urease, catalase, dehydrogenase and alkaline phosphatase enzyme activities, extractable ammonium and nitrate nitrogen contents, soil respiration and mesophyll aerobic microorganism counts were investigated. Soil biological properties were affected by the bacterial treatments at different levels. Most effective bacterial treatments were ordered as *P. fluorescens* > *P. fluorescens* > *P. fluorescens*. It was concluded that single bacterial treatments were more effective than the double and triple bacterial combinations.

Keywords: Black cumin, microbial fertilization, soil biology, soil enzymes

Çörekotu Yetiştiriciliğinde Tekli, Çiftli ve Üçlü Bakteriyel Uygulamaların Toprağın Mikrobiyolojik Özelliklerine Etkileri

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Öz: Mikroorganizmalar, toprak ekosisteminin işlevi, sağlığı, verimliliği, üretkenliği, kalitesi ve sürdürülebilir gelişimi için gereklidir. Bu çalışma, siyah kimyon olarak da bilinen çörekotunun yetiştirildiği toprakların biyokimyasal ve mikrobiyal özellikleri üzerinde bakteri formülasyonlarının etkilerini belirlemek için yürütülmüştür. Altı tek suş (*Pseudomonas fluorescens*, *P. fluorescens*, *P. fluorescens*, *Bacillus licheniformis*, *B. megaterium* ve *B. subtilis*), iki adet çift kombinasyon (*P. fluorescens* + *B. megaterium* ve *P. fluorescens* + *B. subtilis*) ve üç adet üçlü kombinasyondaki (*P. fluorescens* + *B. megaterium* + *B. subtilis*, *P. fluorescens* + *B. licheniformis* + *B. megaterium* ve *P. fluorescens* + *B. licheniformis* + *B. subtilis*) formülasyonlarının üreaz, katalaz, dehidrogenaz ve alkali fosfataz enzim aktiviteleri, ekstrakte edilebilir amonyum ve nitrat azot içerikleri, toprak solunumu ve mezofil aerobik mikroorganizma sayıları üzerine olası etkileri araştırılmıştır. Toprak biyolojik özelliklerinin farklı düzeylerde uygulanan bakteriyel uygulamalardan etkilendiği belirlenmiş, en etkili bakteriyel uygulamalar *P. fluorescens*, *P. fluorescens*, *P. fluorescens* olarak sıralanmıştır. Tekli bakteriyel uygulamaların ikili ve üçlü bakteriyel kombinasyonlardan daha etkili olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Çörekotu, mikrobiyal gübreleme, toprak biyolojisi, toprak enzimleri

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Introduction

Global climate change has brought forward the concept of sustainable agriculture. Within the concept of sustainable agriculture, environment-friendly inputs, various plant growth-promoting practices, and plant growth-promoting bacteria are widely used.

Some microorganisms within the plant rhizosphere can dissolve organic and chemical substances and thus they can produce the plant growth promoting substances and facilitate nutrient intake of plants (Çakmakçı, 2019a). Bacteria such as *Pseudomonas, Bacillus, Azospirillum, Azotobacter, Arthrobacter, Achromobacter, Micrococcus, Enterobacter, Rhizobium, Agrobacterium, Pantoea* and *Serratia* can be included in the plant growth and development promoting microorganisms (Verma et al., 2019). Besides plant growth-promoting bacteria, chemical fertilizers are enriched with organic matter and microorganisms, and such practices offer more sustainable and economical agriculture (Goenadi et al., 2018). However, efficiency of these microbial products largely depends on the use of local strains, well-developed chelating materials and preservatives and the development of compounds that stimulate and enrich bacteria (Çakmakçı, 2019a).

Soil enzymes play an important role in the mineralization of plant nutrients, decomposition of organic matter and supply of plant nutrients. Soil enzyme activities play a critical role in the sustainability of soil fertility and soil ecosystem (Burns & Dick, 2002).

Little is known about the re-inoculation of the PGPR-derived microorganisms and their effects on different plants and soil enzyme activities (Singh et al., 2021). Soil respiration has been used to estimate the nutrient cycles, fertility and the ability to sustain soil biological activities (Anderson & Domsch, 2010). Among microbial products, numerous studies have focused on the use of plant growth-promoting bacteria (PGPB). In particular, research on drought stress and halotolerant PGPB has gained increasing attention in parallel with climate change (Arora et al., 2020). In addition, substantial research has addressed the management of biotic and abiotic factors affecting PGPB applications for soil nutrient management and sustainable productivity (Ahmed et al., 2019), as well as the combined use of PGPB strains with fungi, especially mycorrhizal fungi (Yadav et al., 2022a).

Many productivity studies have been conducted by researchers applying PGPB to various plants. Examples of recent studies on widely cultivated plants worldwide include wheat (Verma et al., 2014; Valenzuela-Aragon et al., 2019; Chandra et al., 2019; Yadav et al., 2022b), maize (Calzavara et al., 2018; Olanrewaju & Babalola, 2019; Amezquita-Aviles et al., 2022), and rice (Kumar et al., 2021; Wang et al., 2022). However, studies on the application of PGPB to black cumin (Nigella sativa L.), a medicinal and aromatic plant highly beneficial to human health, have been quite limited and generally focused on yield and quality (Shaalan, 2005; Dimitrijević et al., 2018; Egamberdieva et al., 2019; Merajipoor et al., 2020; Akçura & Çakmakçı, 2023).

In this study, local (Turkish origin) PGPB strains were applied for the first time to soils cultivated with black cumin, a crop widely grown in the arid regions of the Mediterranean Basin and Western Asia (D'Antuono et al., 2002). The objective was to determine changes in selected soil biological and biochemical properties through the application of newly developed single, dual, and triple bacterial formulations, in addition to plant yield parameters, which were previously reported by Akçura & Çakmakçı (2023).

Materials and Methods

In present experiments, newly developed single, double and triple bacterial formulations were used as the primary materials of the study. Alluvial soils were used as the growing media and Çameli (*Nigella sativa* L. cv. Çameli) black cumin variety was used as the plant material. There were 12 treatments including six single, two double and three triple bacterial formulations and a control without bacterial treatments (Table 1).

Table 1. Experimental treatments

Number	Treatment	Treatment composition	Symbol
1	Control	No bacteria application	C
2	Single strains	*P. fluorescens* RC24	PF1
3		*P. fluorescens* RC36	PF2
4		*P. fluorescens* RC38	PF3
5		*B. licheniformis* RC33	BL
6		*B. megaterium* RC61	BM
7		*B. subtilis* RC121	BS
8	Mixed double strains	*P. fluorescens* RC24 *B. megaterium* RC61	PF1 + BM
9		*P. fluorescens* RC36 *B. subtilis* RC121	PF2 + BS
10	Mixed triple strains	*P. fluorescens* RC24 *B. megaterium* RC61 *B. subtilis* RC121	PF1 + BM + BS
11		*P. fluorescens* RC36 *B. licheniformis* RC33 *B. megaterium* RC61	PF2 + BL + BM
12		*P. fluorescens* RC38 *B. licheniformis* RC33 *B. subtilis* RC121	PF3 + BL + BS

The single, double, and triple formulations of local the bacterial strains were prepared from the isolates of Prof. Dr. Ramazan Çakmakçı collection, which were isolated from the root rhizosphere of various cultivated and wild plants (Çakmakçı et al., 2010; Çakmakçı, 2019b). The experimental soils were prepared for analysis according to Müftüoğlu et al. (2014), and the analysis methods and results are provided in Table 2.

Table 2. Soil properties of the experimental area

Element/Analysis	Analysis values	Evaluation	Methods
Sand (%) Silt (%) Clay (%)	73.36 14.90 11.74	Sandy-loam	Bouyoucos (1951)
pH (1 / 2.5 soil / water)	7.08	Neutral	Jackson (1958)
Salt (1 / 2.5 soil / water, dS m^{-1})	0.23	Unsaline	Jackson (1958)
Carbonate (%)	1.36	Low	Allison & Moodie (1965)
Organic matter (%)	0.97	Very low	Walkley & Black (1934)
Total nitrogen (%)	0.04	Very low	Bremner (1965)
Available P_2O_5 (kg da^{-1})	25.56	Very high	Olsen (1954)
Available K_2O (kg da^{-1})	28.32	Low	Page et al. (1982)
Field capacity (%)	15.54	Very low	Allmaras & Gardner (1956).
Wilting point (%)	7.70	Very low	
Profitable moisture (%)	7.84	Very low	

The Nigella plant requires 5 kg N da^{-1}, 4 kg P_2O_5 da^{-1} and 3 kg K_2O da^{-1} plant nutrients (Kaya et al., 2021). Based on soil fertility analyses, 8 kg N (24 kg da^{-1}, ammonium nitrate 33%) and 4 kg K_2O (8 kg da^{-1}, potassium sulphate 50%) were applied to the experimental fields and phosphorus fertilization was not applied because phosphorus (25 kg da^{-1} P_2O_5) was found more than the plant requirement. The strains selected from the frozen bacterial isolates were first inoculated on petri dishes containing sterile Nutrient Agar medium and incubated at 27 °C and the fresh and pure cultures were obtained for 24 hours. These fresh cultures were transferred to 500 mL nutrient broth containing liquid media and grown in a horizontal shaker incubator at 150 cycles min^{-1} and 27 °C

for 48 hours. Absorbance of these cultures was measured with a turbidimeter, and the absorbance was equalized with sterile water. Single, double and triple formulations of the bacterial cultures obtained in this way were adjusted in the laboratory to have at least 1×10^8 viable colony units per mL.

Seed sowing was performed manually and the number of seed applied is 1.2 kg da^{-1}. Sprinkler irrigation was used initially to ensure sufficient germination and soil moisture was brought to the field capacity.

Selected single, double and triple bacterial combinations were prepared in 100 mL each and suspensions containing 1×10^8 CFU mL^{-1} bacteria were prepared. This suspension was transferred to the final liquid nutrient medium consisting of natural spring water (4.6 L), sugar beet molasses (300 mL) and sugar beet sugar (30 g), bringing the total volume to 5.0 L. From this final liquid nutrient medium for each bacterial treatment, 200 mL of bacterial suspension was separated per plot. Then, the seeds were inoculated by spraying 50 mL of bacterial suspension in each row during the hours when the sun was not active and the seeds were immediately covered with approximately 3 - 4 cm of soil. Weed control was practiced manually during the experiments. Since the soils of the experimental area were sandy-loam, sprinkler irrigation was practiced 3 times based on the plant water consumption.

Biological fertilizer applications were carried out approximately four months before harvest on black cumin plants, which have a vegetation period of about 9 months. Following the harvest of black cumin plants, soil samples were taken from 0 - 20 cm depth of 36 plots and transported to the laboratory in + 4ºC containers. A part of the samples was subjected to analyses for biological and biochemical properties of the soils. Another part of the samples was subjected to analyses for texture, soil reaction, salinity, carbonate, total nitrogen, and available phosphorus (Table 2). Urease enzyme activity was determined spectrophotometrically at 578 nm in the presence of standard urea solutions after adding substrate to soil samples and incubating them at 37°C for 3 hours, as reported by Hoffmann and Teicher (1961).

Catalase enzyme activity was determined as reported by Beck (1971); after adding 10 ml of phosphate buffer (pH=7) solution to 5 g of pre-prepared soil and incubating at 27°C for 30 minutes, the volume of O_2 released from the 3% H_2O_2 that was broken down in 3 minutes was measured in using Scheibler calcimeter as ml. Soil dehydrogenase activity was determined by adding a 3% triphenyl tetrasodium chloride (TTC) solution to soil samples and incubating them at 27°C for 24 hours and then measuring of the resulting triphenyl formazan (TPF) at 485 nm using a spectrophotometer with a series of standard TPF solutions (Casida, 1977). Alkaline phosphatase enzyme activity was determined by adding buffered (pH=11) sodium p-nitrophenyl phosphate solution (0.025 M) and toluene to the soil, incubating at 37°C for 60 minutes, and measuring the amount of free p-nitrophenol at 410 nm using a spectrophotometer with a series of standard solutions (Tabatabai, 1994). To determine ammonium (NH_4) and nitrate (NO_3) in soil, samples were initially extracted with 2 M potassium chloride (KCl), followed by nitrogen distillation and H_2SO_4 titration (Kacar, 2016).

Soil respiration was determined by measuring the amount of CO_2 released from the soil and retained in an alkaline (BaOH) solution over 24 hours at 27°C in a closed system (Anderson, 1983). For total mesophyll aerobic bacteria counts, soil dilutions were sown in Nutrient Agar media and incubated at 27°C for three days, then a colony counter was used (Wollum, 1982). Experiments were conducted in randomized block design with three replications (12 treatments × 3 replications = 36 plots). Experimental data were subjected to analysis of variance using SAS 9.0 statistical analysis software. Significant means were compared with the use of Duncan's multiple comparison test ($p < 0.05$).

Results and Discussion

The changes in soil urease and catalase enzyme activities with six single, two double, and three triple bacterial combinations are provided in Table 3.

Table 3. Effects of the experimental treatments on the urease and catalase enzyme activities*

Treatments	Urease enzyme activity (µg NH_3 - N g^{-1})			Catalase enzyme activity (mg O_2 5 g^{-1})		
Control	23.95	±0.41	c	4.68	±0.12	d
PF1	35.35	±0.45	a	6.41	±0.06	a
PF2	34.64	±0.98	a	4.74	±0.06	d
PF3	25.48	±0.52	bc	5.20	±0.03	bc
BL	27.86	±0.76	b	6.64	±0.11	a
BM	18.64	±0.50	d	5.31	±0.06	b
BS	27.76	±1.08	bc	4.70	±0.08	d
PF1 + BM	25.59	±0.43	bc	4.64	±0.10	d
PF2 + BS	25.61	±0.59	bc	4.85	±0.12	cd
PF1 + BM + BS	26.70	±0.97	bc	4.95	±0.08	bcd
PF2 + BL + BM	24.32	±1.04	bc	4.60	±0.06	d
PF3 + BL + BS	24.63	±0.74	bc	6.77	±0.11	a

*Values are presented in mean ± SE and means indicated with different letters within the same column are significantly different ($p<0.05$).

The PF1, PF2 and BL treatments significantly increased and the BM treatments significantly decreased the urease enzyme activity as compared to the control treatment. The increases by the other treatments were not found to be significant. Urease enzyme activities were higher in single bacterial treatments, except for BM, than in double and triple mixtures. Soil urease enzyme activities for three hours of incubation are expressed in mg NH_3 - N (100 g^{-1} soil) and classified as: low (< 8 mg), normal (8 - 16 mg), and high (> 16 mg) (Hoffmann & Hoffmann, 1966). Present values varied between 18.64 - 35.35 µg NH_3 - N g^{-1} (1.864-3.535 mg 100 g^{-1} soil), thus classified in low group. Such a case can be attributed to low organic matter levels, sandy-loam texture, low field capacity of the soil and post-harvest hot/dry weather conditions of harvest season.

The PF1, PF3, BL, BM and PF3 + BL + BS treatments significantly increased the catalase enzyme activity as compared to the control treatment and the changes with the other treatments were not found to be significant. Catalase enzyme activity varies with temperature, oxygen, soil moisture, and nutrients, all of which also affect microbial activity (Kandeler, 2007). Catalase enzyme activity also changes with the soil tillage (Erdel, 2021).

It is also closely related to the soil organic matter content and N, P, K fertilization usually increases catalase enzyme activity (Kandeler, 2007). Catalase and dehydrogenase-like enzymes are intracellular enzymes and these enzymes play a vital role in the biodegradation of organic compounds. Enzyme activities are considered to be the most sensitive sensors of microbial activity changes in the soil environment in response to different factors, including drought (Hueso et al., 2012). Türkmen et al. (2013) indicated that there were significant differences in the enzyme activities of different depth segments (3.72 - 7.42 mg O_2 5 g^{-1}) and catalase enzyme activity was higher in the topsoil. Present findings (4.60 - 6.77 mg O_2 5 g^{-1}) comply with the results of that study.

Changes in the soil dehydrogenase and alkaline phosphatase enzyme activities with six single, two double, and three triple bacterial combinations are provided in Table 4.

Table 4. Effects of the experimental treatments on the dehydrogenase and alkaline phosphatase activities*

Treatments	Dehydrogenase (µg TPF g^{-1})			Alkaline phosphatase (µg pNP g^{-1})		
Control	13.12	±0.54	c	21.21	±0.18	e
PF1	7.910	±0.32	ef	28.77	±0.41	a
PF2	13.47	±0.13	bc	25.45	±0.78	bcd
PF3	17.45	±0.43	a	25.63	±0.35	bcd
BL	9.350	±0.19	e	20.94	±0.98	e
BM	7.200	±0.07	f	22.25	±0.49	e
BS	11.07	±0.34	d	23.25	±0.93	de
PF1 + BM	12.02	±0.19	cd	26.26	±0.08	abc
PF2 + BS	9.000	±0.33	e	27.52	±1.04	ab
PF1 + BM + BS	13.44	±0.28	bc	25.26	±0.56	bcd
PF2 + BL + BM	14.69	±0.23	b	23.49	±0.75	cde
PF3 + BL + BS	7.360	±0.17	f	26.13	±0.25	abcd

*Values are presented in mean ± SE and means indicated with different letters within the same column are significantly different ($p<0.05$).

The PF3 and PF2 + BL + BM treatments significantly increased and PF1, BM and PF3 + BL + BS treatments significantly decreased the dehydrogenase enzyme activity. The changes by the other treatments were not found to be significant.

Dehydrogenase enzyme activity can also be used as an indicator of total microbial activity in any ecosystem (Casida, 1977). Hridya et al. (2014) indicated that dehydrogenase enzyme activity increased in the soils treated with the microbial fertilizers and there was a significant relationship between the dehydrogenase enzyme activity and the microbial populations. In addition, soil moisture, soil reaction, soil organic matter, local climate, and soil characteristics also result in significant changes in such enzymatic reactions (Burns & Dick, 2002; Wolińska & Stępniewska, 2012). Okur et al. (2007) reported the dehydrogenase enzyme activity of biofarm, humic acid and leonardite-treated soils as between 22.86 - 119.74 μg TPF g^{-1} and indicated that the effects of plant variety and fertilizers on soil dehydrogenase, alkaline phosphatase, β-glucosidase, and protease activities were significant at 1% level. Present dehydrogenase enzyme activities varied between 7.2 - 17.45 μg TPF g^{-1} and such values were lower than the values reported in the previous studies. Such a case could be attributed to quite low field capacity and available moisture content of the experimental soils (Burns & Dick, 2002; Wolińska & Stępniewska, 2012).

The PF1, PF2, PF3, PF1 + BM, PF2 + BS, PF1 + BM + BS and PF3 + BL + BS treatments significantly increased the alkaline phosphatase enzyme activity as compared to the control treatment and the changes with the other treatments were not found to be significant. Alkaline phosphatase enzyme plays an important role in agricultural production through catalyzing hydrolysis of organic phosphorus. Soil alkaline phosphatase enzyme activity varies with several factors, especially with the soil reaction (Burns & Dick, 2002). Like the other enzyme activities, the alkaline phosphatase enzyme activity is significantly influenced by soil moisture, soil reaction, soil organic matter, climate, and other soil properties (Burns & Dick, 2002; Wolińska & Stępniewska, 2012). Okur et al. (2007) reported the soil alkaline phosphatase enzyme activities as between 306.2 - 475.1 μg pNP g^{-1} h^{-1}. Present alkaline phosphatase enzyme activities (20.94 - 28.77 μg pNP g^{-1}) were quite lower than the findings of the previous studies. As it was in the other enzyme activities, such lower values can be attributed to dry conditions, low organic matter levels and light soil texture of the study area. Changes in the extractable ammonium and nitrate contents of the soil with single, double, and triple bacterial combinations are given in Table 5.

Table 5. Effects of the experimental treatments on the soil extractable ammonium and nitrate contents*

Treatments	Ammonium (μg g^{-1})			Nitrate (μg g^{-1})		
Control	15.04	±0.15	abc	7.34	±0.09	a
PF1	15.12	±0.08	ab	6.27	±0.12	cd
PF2	15.50	±0.05	a	6.54	±0.12	bc
PF3	14.47	±0.17	bcde	7.12	±0.14	ab
BL	14.12	±0.07	def	6.07	±0.13	cde
BM	13.61	±0.12	f	5.60	±0.15	de
BS	14.35	±0.14	cdef	5.61	±0.14	de
PF1 + BM	14.82	±0.12	abcd	5.77	±0.14	de
PF2 + BS	14.40	±0.20	bcde	6.13	±0.17	cde
PF1 + BM + BS	13.87	±0.28	ef	5.48	±0.02	e
PF2 + BL + BM	14.44	±0.16	bcde	6.52	±0.14	bc
PF3 + BL + BS	14.82	±0.02	abcd	7.37	±0.16	a

*Values are presented in mean ± SE and means indicated with different letters within the same column are significantly different ($p<0.05$).

Extractable ammonium contents increased with the PF1 and PF2 treatments as compared to the control treatment, but these increases were not significant. The BL, BM and PF1 + BM + BS treatments significantly reduced the extractable ammonium contents and the changes with the other treatments were not significant. While the changes in extractable nitrate contents were not significant in the PF3 and PF3 + BL + BS treatments, the other treatments caused a significant decrease as compared to the control. Present extractable NO_3 contents varied between 5.48-7.68 μg g^{-1} and extractable NH_4 contents varied between 13.60-15.53 μg g^{-1}. Such changes were similar with the changes reported on below literature.

It was reported that the compost-based organic fertilization treatments in paddy soils caused different effects on N mineralization and these effects may also result from enzyme activities (Yagüe et al., 2023). The enzyme urease plays the upmost active role in the nitrogen cycle and hydrolyses urea up to ammonium and carbon dioxide (Burns & Dick, 2002). Inorganic forms of nitrogen in soil such as NH_4 and NO_3 exhibit continuous variability. These variations are highly correlated with soil moisture, soil salinity, and soil reaction (Zhang & Wienhold, 2002). Most of the nitrogen in the soil is in organic form and with the mineralization of organic matter, nitrogen is constantly released in inorganic forms and NH_3 and NH_4 levels change instantaneously (Paul, 2016). It was reported in an incubation study in which plant growth-promoting bacteria (PGPB) were applied together with the urea fertilizer that NH_4 and NO_3 levels extracted in different weeks could reach the highest value of 29.797 mg k^{-1} and these levels varied based on PGPB applications and time (Akter et al., 2023).

Changes in the soil respiration and microorganism counts with single, double, and triple bacterial treatments are provided in Table 6.

Table 6. Effects of the experimental treatments on the soil respiration and number of mesophyll bacteria*

Treatments	Soil respiration (μg CO_2 g^{-1} h^{-1})			Number of bacteria (number × 10^6 g^{-1})		
Control	23.53	±0.15	bcd	92.60	±0.61	d
PF1	24.06	±0.68	bc	92.11	±0.77	d
PF2	27.11	±0.34	a	99.27	±1.02	c
PF3	25.60	±0.37	ab	126.90	±0.22	a
BL	22.49	±0.64	cde	71.61	±0.91	g
BM	25.38	±0.51	ab	52.19	±0.49	h
BS	23.78	±0.29	bc	77.88	±0.32	f
PF1 + BM	21.57	±0.07	de	86.90	±0.57	e
PF2 + BS	22.56	±0.38	cde	76.96	±1.02	f
PF1 + BM + BS	25.33	±0.27	ab	85.06	±0.53	e
PF2 + BL + BM	24.55	±0.26	bc	108.20	±0.36	b
PF3 + BL + BS	20.81	±0.33	e	87.87	±0.28	e

*Values are presented in mean ± SE and means indicated with different letters within the same column are significantly different ($p<0.05$).

Soil respiration significantly increased only in the PF2 treatment (27.11 μg CO_2 g^{-1} dry soil per hour) as compared to the control treatment. In triple mixture treatments (PF3+BL+BS), respiration decreased significantly (20.81 μg CO_2 g^{-1} dry soil per hour). The changes with the other treatments were not found to be significant.

Soil respiration is a result of microorganism activities and varies with several factors such as soil texture, soil moisture, soil organic matter, and soil temperature (Yigi & Zhou, 2010). Organic matter mineralization is a highly complex process and depends on various variables, thus different materials may undergo different mineralization processes (Yiqi & Zhou, 2010; Qin et al., 2019). Ergün (2017) indicated that hazelnut biochar treatments could affect CO_2 values and a daily average of 11.77 mg CO_2 100 g^{-1} was produced. As well as continuous soil conditions and applications, different expressions of soil CO_2 production (day-hour, gram-microgram, per gram or per 100 grams of CO_2 production) and differences in the measurement methods of researchers make it difficult to discuss CO_2 production levels.

Number of microorganisms increased significantly with the PF2, PF3 and PF2 + BL + BM treatments, while significant decreases were determined in the other treatments. In an incubation study conducted by mixing different doses of sewage sludge into the soils of Çanakkale Dardanos campus, the researchers reported number of microorganisms as between 5×10^6 - 15×10^6 per g dry soil (Uzunboy & Türkmen, 2018).

Conclusion

Present findings revealed that single, double, and triple bacterial combinations applied to a sandy-loam soil in which black cumin seeds were grown had significant effects on the investigated soil properties. *P. fluorescens* RC36 treatments were found to be effective in the extractable ammonium and respiration; *P. fluorescens* RC38 in the dehydrogenase enzyme activity and microorganism count; *P. fluorescens* RC24 in the urease and alkaline phosphatase enzyme activities; *B. lichenifoirmis* RC33 + *B. suptilis* RC121 + *P. fluorescens* RC38 treatments in

the extractable nitrate and catalase enzyme activity. It was determined that the soil biological properties were affected by the bacterial treatments at different strains. The most effective bacterial treatments were ordered as *P. fluorescens* RC36> *P. fluorescens* RC38> *P. fluorescens* RC24 and they all positively affected the biological properties investigated. It was concluded based on present findings that single bacterial treatments provided significant increases in the soil enzyme activities. New local bacterial strains to be identified should be tested in different textured soils, plant species and under different climate conditions. Development of microbial fertilizers is an important issue for sustainable soil fertility, sustainable food production, sustainable environment, and healthy life.

Additional Information and Declarations

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