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Research Article

Effects of Different Biological Fertilizers Formulated with Multiple Bacteria and Carriers in Pazar 20 Tea Clone on Leaf Enzyme Activity

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Abstract. This study was performed on 2-year-old seedlings of the Pazar-20 tea clone pots in 2014-2015. Twelve different bacterial isolates (*Bacillus atrophaeus*RC11, *Bacillus megaterium*RC07, *Bacillus megaterium*42/4, *Bacillus megaterium*21/3, *Pseudomonas fluorescens*RC77, *Pseudomonas fluorescens* 8/4, *Pseudomonas fluorescens*8/6, *Pseudomonas fluorescens*9/7, *Bacillus subtilis*RC63, *Bacillus subtilis* 39/3, *Bacillus subtilis*36/10, *Bacillus subtilis*RC521), which were isolated from acidic tea soils and characterized and all laboratory tests were performed. With biological fertilizers formed by triple combinations and 7 different carrier formulations (K-tea compost, T-peat, P-perlite, L-leonardite, Z-zeolite, V-vermiculite and S-liquid carrier formulation) on the enzyme activities (peroxidase-POD, Polyphenol oxidase-PPO, Urease, 5-Dehydroxyshikimate reductase, Alchol dehydrogenase-ADH, Glucose 6-phosphate dehydrogenase-G6PD, 6-phosphogluconate dehydrogenase-6PGD, Glutathione reductase-GR and Glutathione S-transferase-GST) of tea leaves were determined. All bacterial formulations used in the study positively affected the enzyme activities in the tea leaves at different rates compared to the control. This activity was found to be statistically significant. Additionally, it was determined that the efficiency of the carrier was important in both years (2014-2015) statistically, liquid formula and peat carriers had the highest effect.

Çoklu Bakterilerle Oluşturulmuş Farklı Biyolojik Gübre Formulasyonlarının Pazar 20 Çay Klonunda Yaprak Enzim Aktivitesine Etkileri

Anahtar kelimeler:
Camellia sinensis L., PGPR formülleri, enzim aktivitesi, taşıyıcılar

Özet. Bu çalışma, 2014-2015 yıllarında potlarda, Pazar-20 çay klonuna ait 2 yaşlı fidanlarda yürütülmüştür. Asidik çay topraklarından izole edilerek tanılanan ve karakterize edilip tüm laboratuvar testleri yapılan, biyolojik gübre adaylarından 12 farklı bakteri izolatının (*Bacillus atrophaeus*RC11, *Bacillus megaterium*RC07, *Bacillus megaterium*42/4, *Bacillus megaterium*21/3, *Pseudomonas fluorescen* RC77, *Pseudomonas fluorescens*8/4, *Pseudomonas fluorescens*8/ 6, *Pseudomonas fluorescens*9/7, *Bacillus subtilis*RC63, *Bacillus subtilis*39/3, *Bacillus subtilis*36/10, *Bacillus subtilis*RC521) üçerli kombinasyonları ile oluşturulmuş biyolojik gübre formulasyonları ile 7 farklı taşıyıcının (K-çay kompostu, T-torf, P-perlit, L-leonardit, Z-zeolit, V-vermikülit ve S-likit taşıyıcı formulasyonu) birlikte uygulanmalarının çay yapraklarının enzim aktivitelerine (Peroksidaz-PO, Polifenol oksidaz- PPO, Üreaz, 5-Dehidroksişikimat Redüktaz, Alkol dehidrogenaz-ADH, Glikoz 6 fosfat dehidrogenaz-G6PD, 6 Fosfo glukonat dehidrogenaz-6PGD, Glutatyon redüktaz-GR, Glutatyon S-transferaz-GST) etkileri belirlenmiştir. Araştırmada kullanılan tüm bakteriyel formulasyonlar, çay yaprağındaki enzim aktivitelerini kontrole kıyasla farklı oranlarda olumlu yönde etkilemiştir. Bu etkinlik istatistiki olarak önemli bulunmuştur. Ayrıca, yapılan değerlendirmelerde, her iki yılda da (2014-2015) taşıyıcıların da etkinliğinin önemli olduğu, likit formüllü taşıyıcı ile torf olarak kullanılan taşıyıcıların en yüksek etkiye sahip oldukları belirlenmiştir.

INTRODUCTION

Tea (*Camellia sinensis* L. Kuntze), which is one of the most popular beverages around the world with its characteristic aroma and flavor, is an important horticultural plant that has become widespread in subtropic and tropic fields and its leaves are used. Especially the development period, which needs a lot of nitrogen, has also caused the use of intensive inorganic fertilizers for yield increase. Fertilization (especially nitrogen) is of great importance in the development of tea leaves, which are harvested at least 3 or 4 times a year due to their herbal characteristics (Kacar, 2010). Most of the fertilizer in agricultural fields are lost due to immobilization, evaporation, and washing. Particularly, a significant part of the nitrogen applied in tea growing regions is washed away due to effective precipitation, high humidity, and sloping areas, and fertilizers pollute the surface and ground waters. Additionally, the high cost of chemical fertilizers, the deep gap between supply and demand, and their negative impact on the environment have led to the search for alternative strategies. Also, extensive use of agrochemicals to meet the global requirement of tea resulted in an alteration of the microbial community associated with the tea plants (Cernava *et al*., 2019).

As it is known, the soil is a perfect ecosystem where living and non-living components form a special harmony. Both It meets the structural needs of plants and provides living space for many living populations. Among these organisms, bacteria (non-pathogenic), which are highly effective in the root rhizosphere of plants, are positively affecting the growth and development through many direct and indirect mechanisms, which constitute an important part. For this reason, this type of bacteria is very commonly called plant growth-promoting rhizobacteria (PGPR) and is stated to serve as an environmentally friendly substitute for chemical fertilizers. The fact that its positive effects are valid for many plants has allowed this type of bacteria to be called biofertilizer.The direct effects of PGPRs on plant growth are the fixation of atmospheric nitrogen into the soil occurs by dissolving phosphate, potassium and iron in insoluble form in the soil and producing many phytohormones. In indirect mechanisms; It is most important to protect plants from biotic and abiotic stress factors. Moreover, hydrolytic enzyme production by PGPRs, production of polysaccharides, bioremediation of heavy metals and stimulation of induced systemic resistance (by mechanisms such as the biosynthesis of defense principle related molecules, increasing the levels of defense proteins) are critical contributions (Çakmakçı, 2019).

Biotic and abiotic stresses cause many different physiological changes in plant cells, including the production of reactive oxygen species (ROS). The accumulation of high concentrations of ROS in plant cells leads to oxidative damage and causes disruption of cellular homeostasis. Plant cells are equipped with advanced antioxidant mechanisms. Some of these include antioxidant defense enzymes such as Ascorbate peroxidase (APX), catalase (CAT), peroxidase (PO), superoxide dismutase (SOD), glutathione reductase, glutathione S-transferase, and guaiacol peroxidase. These enzymes are involved in scavenging and converting ROS into non-toxic end products, thereby protecting cells from oxidative damage. In addition, plant cells also produce various antioxidant molecules such as carotenoids and phenylpropanoids to overcome oxidative damage. Furthermore, PGPR-assisted ISR prepares host plants to resist the pathogen invasion through the production of defense-related antioxidative enzymes and molecules (Saravanakumar *et al.,* 2007; Çakmakçı *et al*., 2016; Bhattacharyya *et al.,* 2020).

The first study on the rhizosphere microbiome of tea plantations in Turkey started in 2007 with a project supported by TUBITAK. It has been determined that the tea rhizosphere is composed of very different PGPR isolates that can be used as biological fertilizers in different locations, depending on different cultivar/clone characteristics (Çakmakçı *et al*., 2010 and 2016; Çakmakçı, 2019). Similar evaluations were made for tea plantations in different countries (Chakraborty *et al*., 2009; 2015, Mishra *et al*., 2014; Dutta *et al*., 2015). At the end of the studies, it was determined that the most characteristic bacterial species of the tea rhizosphere were *Bacillus*, *Pseudomona*s, and *Paenibacillus* in the diagnosis made by the MIDI system. In addition, *Bacillus, Pseudomonas, Paenibacillus, Stenotrophomonas, Brevibacillus*, and *Arthrobacter* genera include the most prominent culturable isolates in rhizosphere and soil populations in tea plantations in Trabzon and Rize provinces in the Eastern Black Sea Region. In the acidic tea rhizosphere, *Bacillus cereus, Pseudomonas fluorescens, Bacillus megaterium*, *S. maltophilia*, *Pseudomonas putida, Bacillus licheniformis*, *Bacillus pumilus, Bacillus subtilis* and *Paenibacillus polymyxa* are the most dominant nitrogen fixing- phosphate solubilizing, ACC deaminase producing species (Çakmakçı *et al*., 2010; Ertürk *et.al*., 2010 and 2014; Çakmakçı, 2019).

This research was conducted by applying 12 different biological fertilizer candidates, which were obtained from tea plantations formed by soils with pH levels between 3.5-6.1, and for which all tests were performed, as triple bacterial combinations, with 7 different carriers, on 2-years-old seedlings belonging to Pazar-20 tea clone. This study was carried out to determine the effects of these applications on the leaf enzymes of the plants.

MATERIAL AND METHOD

In this study, *Bacillus atrophaeus*RC11, *Bacillus megaterium*RC07, *Bacillus megaterium*42/4, *Bacillus megaterium*21/3, *Pseudomonas fluorescens*RC77, *Pseudomonas fluorescens*8/4, *Pseudomonas fluorescens*8/ 6, *Pseudomonas fluorescens*9/7, *Bacillus subtilis*RC63, *Bacillus subtilis*39/3, *Bacillus subtilis*36/10, *Bacillus subtilis*RC521, in total 12 different isolates (belonging to 4 different species), 5 different biological fertilizer formulations were formed, 3 for each formulation. Along with these applications, 1 standard biofertilizer, standard NPK fertilizer dose (1400 mg 25:5:10/seedling), and control (without fertilizer and bacteria) were included in the study. In the study, these formulations were used in combination with 7 different carriers. Thus, the study was planned according to the factorial experiment design with 4 replications and 5 seedlings (2 years-old seedlings in pots) in each replication (8 applications x 7 carriers). Some laboratory test results of the isolates used in the study are given in Table 1.

Table 1. Some characteristics of bacteria in the combinations used in the experiment with two-year-old seedlings in the Pazar-20 tea clone.

Çizelge 1. Bakteri kombinasyonlarının laboratuvar test sonuçları.

Strain No	MIS Diagnostic Result	Comb.	OK test	CAT test	N fixation	Sucroz test	Phosphate Solubilization	ACCD
RC11	*Bacillus atrophaeus*	F1	Z+	K+	+	+	+	2
RC07	*Bacillus megaterium*		Z+	K+	+	-	K+	TY
RC77	*Pseudomonas fluorescens*		K+	+	Z+	-	+	2
RC63	*Bacillus subtilis*	F2	+	K+	K+	-	Z+	3
21/3	*Bacillus megaterium*		-	+	K+	-	K+	2
8/4	*Pseudomonas fluorescens*		K+	Z+	+	K+	+	3
36/10	*Bacillus subtilis*	F3	-	K+	K+	+	+	6
42/2	*Bacillus megaterium*		-	+	+	-	+	TY
8/6	*Pseudomonas fluorescens*		K+	K+	+	Z+	K+	2
39/3	*Bacillus subtilis,*	F4	Z+	K+	K+	+	Z+	4
42/4	*Bacillus megaterium*		-	+	K+	-	+	8
9/7	*Pseudomonas fluorescens*		+	+	K+	K+	+	2
RC521	*Bacillus subtilis*	F5	-	K+	K+	+	-	3
42/4	*Bacillus megaterium*		-	+	K+	-	+	8
9/7	*Pseudomonas fluorescens*		+	+	K+	K+	+	2

OK: oxidase; CAT: catalase; ACC: aminocyclopropane carboxylate deaminase activity (2 strong positive, 8 weak positive); TY: not tested; +: positive, K+: strongly positive; Z+: weak positive

The carrier formulas used in the experiment and some of their components (comprehensive ingredients and compositions are unique to this research and carrier determination studies have been continued until the most suitable liquid and solid carriers are concretely revealed):

1. (K): Tea compost-based carrier (compost, animal manure, clampe, etc.)
2. (T): Solid peat-based carrier (peat, glycerol, etc.)
3. (P): Solid perlite-based carrier (perlite, peat, clampe, glycerol, etc.)
4. (L): Solid leonardite-based carrier (leonardite, clampe etc.)
5. (Z): Solid zeolite-based carrier (zeolite, vermiculite, clampe, etc.)
6. (V): Solid vermiculite-based carrier (vermiculite, clampe, etc.)
7. (S): Liquid-based organic carrier (whey, seaweed, grass juice, etc.)

Extraction Preparation, Enzyme Activity, and Determination of Protein Content

Approximately 2 g leaf samples taken from tea plants were wrapped in aluminum foils and frozen at -80°C until used. For analysis, 2 g of leaf sample was pulverized with liquid nitrogen, 10 mL buffer was added (50 mM Tris-HCl and 1 mM EDTA, pH 7.5), the mixture was centrifuged at 4°C for 20 minutes (15,000 g) and macroparticles were removed. precipitated and the precipitate was discarded. The supernatant was used as a crude extract for measuring of enzyme activities and protein determinations. Each enzyme activity was determined spectrophotometrically (Shimadzu Spectrophotometer UV-1208) at 25°C. the protein concentration was calculated according to the Bradford method (1976) using bovine serum albumin as a standard using 595 nm absorbance measurement. Leaf enzyme measurements were repeated three times in each sample.

Assay Peroxidase (POD)

Peroxidase (POD) activity determination is based on monitoring the absorbance increase at 470 nm, caused by the colored compound, which is the product of the reaction in which guaiacol H_2O_2 is the substrate (Angelini *et al*., 1990). Various fresh plant materials were extracted in 0.1 M pH=7.0 cold phosphate buffer by applying 100 mg fresh weight/mL ratio, the extracts were centrifuged at 10 000 rpm for 15 minutes and the supernatants were taken and used for enzyme determination (Smith *et al*., 1971). In addition, 100 mL of 0.1 M phosphate buffer with pH 5.8 was taken and 15 mM guaiacol, 5 mM H_2O_2 were added and this reagent was freshly prepared and used. 3 mL of this reagent and 50 µL of the sample according to enzyme activity were taken from the extracts and the oxidation product was measured at 470 nm at 10-second intervals for 2 minutes. The increase in absorbance was recorded at 1-minute intervals, and the increase in absorbance in the part where the absorbance increased linearly was proportional to 1 minute. The amount of enzyme that increases the absorbance by 0.01 in 1 minute at 25°C was accepted as 1 enzyme unit, and the results were expressed as enzyme units per g of leaf (EU g $leaf^{-1}$) (Yee *et al*., 2003) POD (EU g $leaf^{-1}$) = (5 mL homegenate/0.5 g leaf) / 10µl ingested homogenate) x 2 x (1/0.01) x Absorbance value; POD (EU g $leaf^{-1}$) = 1000 x 100 x Absorbance value. Secondly, in the POD reaction system, 0.05 mL enzyme extract, 2 mL water, 1 mL guaiacol as a donor, and 1 mL H_2O_2 as substrate was kept in 35 °C water for 5 minutes and measured spectrophotometrically. POD activity was defined as 0.1 units of absorbance change per minute (Mei *et al*., 2009).

Assay Polyphenol oxidase (PPO)

Catechol was used as a substrate. The reaction mixture; It was formed from 2 mL of phosphate buffer solution (pH 6.2, 0.05 M) + 0.5 mL of "enzyme-containing solution" + 0.5 mL of substrate (0.5 M) solution (Lee *et al*., 1991). After the reaction mixture was incubated for 5 minutes at 30°C, absorbance measurements were made at a wavelength of 410 nm at 15-second intervals. For this purpose, the a spectrophotometer was used. The slope of the curve reflecting the absorbance x time relationship was calculated in terms of "Absorbance min-1 mL-1" and expressed as "activity level" (Lee *et al*., 1991). In addition, in the reaction system of PPO enzyme extract, 1 mL of enzyme-containing 2 mL of citrate and phosphate buffer solution (pH=5.6, 0.1 M), 0.4 mL of proline (10 mg/mL) and 1 mL of catechol were 10 in 35 °C water. minutes and measured spectrophotometrically. The unit of PPO activity is defined as 0.1 absorbances per minute (Mei *et al*., 2009).

Assay Urease

For the at 37°C for 5 minutes. 0.5 mL (0.05 M) of urea was added to this sample and the entire mixture was left to stand for 20 minutes. 0.2 mL (1%) indophenol solution was added and 0.2 mL (0.5 M NaOH and 0.075% NaOCl) reagent was added, it waited for 30 minutes and the increase in absorbance was measured by spectrophotometer at 640 nm (Weatherburn, 1967).

Assay 5-Dehydroxyshikimate reductase

The absorbance of NADPH2, which is formed according to the reaction Shikimat + NADP + > 5-dehydroshikimate + NADPH", is based on spectrophotometric monitoring at 340 nm (Sanderson, 1966). The reaction mixture contains 1 mM shikimic acid, 170 µM NADP+ and 0.1 mL enzyme solution in 2.5 mL 0.1 M glycine buffer (pH=10). An enzyme unit is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of shikimic acid in 1 minute.

Assay Alcohol dehydrogenase activity (ADH)

For the enzyme activity determination, the tea sample was homogenized with 10 mL of phosphate buffer (0.1 M, pH=7.0) and centrifuged for 15 minutes at 3000 rpm at +4°C (Smith *et al*., 1971). 0.2 mL of the supernatant was taken and NAD+ (nicotinamide adenine dinucleotide) (2.5 mM) and ethyl alcohol (10 mM) solution were added to the reaction medium to form the concentrations in the measuring cuvettes, and the change in absorbance per minute was measured in a spectrophotometer at 340 nm at 25°C. Enzyme activity was expressed as optical density change per minute (DOD 340/g tea/min) (Smith *et al*., 1971; Hatanaka *et al*., 1974).

Assay Glucose 6-phosphate dehydrogenase (G6PD)

G6PD activity in tea leaf samples was determined according to the Beutler method (Beutler, 1984). The procedure contains 0.1 mM Tris-HCl buffer (pH=7.5), 0.5 mM EDTA, 0.2 mM NADP+, and 0.6 mM G6P for G6PD, 0.6 mM 6PGA for 6PGD, and the volume is 1 mL. In the system, the enzyme unit is defined as 1 µmol NADP+ reduction per minute.

Assay 6-phosphogluconate dehydrogenase (6PGD)

6PGD activity in tea leaves was determined according to the Beutler method (Beutler, 1984). The procedure contains 0.1 mM Tris-HCl buffer (pH=7.5), 0.5 mM EDTA, 0.2 mM NADP+, and 0.6 mM G6P for G6PD, 0.6 mM 6PGA for 6PGD, and the volume is 1 mL. In the system, the enzyme unit min is defined as 1 µmol NADP+ reduction. Leaf enzyme activity was determined spectrophotometrically.

Assay Glutathione reductase (GR)

GR activity in tea leaves was determined according to the method developed by Carlberg and Mannervik (1985). Leaf enzyme activity was determined spectrophotometrically. The system contains a total volume of 1 mL of 0.75 mM Tris-HCl buffer (pH=7.0), 1 mM EDTA, 1 mM GSSG and 0.1 mM NADPH. One enzyme unit is defined as the oxidation of 1 µmol of NADPH per minute.

Assay Glutathione S-transferase (GST)

Leaf GST enzyme activity was determined spectrophotometrically. Glutathione S-transferase activity in tea leaves prepared by using the method given above, Habig *et al*. (1974), 1 mL volume of reaction medium contained 0.1 M potassium phosphate buffer (pH=6.5), 1.0 mM GSH, 1.0 mM CDNB, and 1% pure ethanol. An enzyme unit was defined as the formation of 1 µmol of GS-DNB (glutathione-dinitrobenzene complex) per minute at 340 mn.

Statistical Evaluations

After the data determined in the pot experiments were statistically analyzed using STATISTICA (StatSoft-2003) and SPSS (IBM SPSS Statistics 20) programs (especially by making variance, correlation, and multiple comparison tests), significant differences between treatments were determined using Duncan's multiple range test with a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

In this experiment, polyphenol oxidase, peroxidase and urease, alcohol dehydrogenase, and 5-de-hydroxy shikimate reductase enzyme activity tests were completed in the samples taken in May. Although the inoculated bacteria varied depending on the fertilizer applications and the carriers used, the inoculated bacterial formulations, fertilizer applications, and carriers significantly affected the leaf enzyme activity in tea seedlings (Table 2).

There are different enzymes in the root as well as in the leaves and shoots of the tea plant. Polyphenol oxidase (PPO) in tea leaves plays a role in the oxidation of flavonols, formation of taste and color, peroxidase (POD) oxidation of flavonols and alcohol dehydrogenase (ADH) in the formation of some alcohols and the development of aroma (Çalıkoğlu and Bayrak, 2009). The polyphenol oxidase (PPO) and peroxidase (POD) enzymes in the leaf play an important role in oxidation reactions and provide the formation of substances that give black tea its color and smell. Aroma compounds are formed as a result of the oxidation of tea flavonols by the enzyme polyphenol oxidase. In black tea production, catechins are oxidized under the catalysis of polyphenol oxidase enzyme and turn into theaflavin (TF) and thearubigin (TR) pigments, which give black tea its typical color and taste (Ertürk *et al*., 2010; Çakmakçı *et al*., 2017). According to the carriers averages, all treatments except the F1 formulation significantly increased the PPO and POD activities measured as enzyme units per leaf weight (EU g leaf^{-1}) compared to the control. PPO activity per unit protein increased with all treatments, but the rate of increase was found to be significant only with mineral fertilizer applications. Inoculations of F2, F3, and F4 formulations, especially the F5 formulation, significantly increased the enzyme activity in unit protein while urease activity in tea leaves increased significantly with applications other than F1 in terms of unit leaf weight, applications other than F1 and F2 increased urease activity per unit protein, and the rate of increase was found to be significant. The applications tested in this trial set did not have any effect on the increase in alcohol dehydrogenase activity. The applications in the trial set increased the activity of the 5-5-de-hydroxy shikimate reductase enzyme measured in the leaves, and the increasing rates were found to be significant in F4 and F5 applications compared to the control. All treatments significantly increased the enzyme content per protein (EU mg protein^{-1}). According to the fertilizer application averages, compost, leonardite, peat, and liquid carriers gave the most appropriate results in terms of enzyme activity measurements in 2014 (Table 2,3).

Table 2. The effect of different carriers, bacteria combinations and NPK application on polyphenol oxidase, peroxidase and urease enzyme activity in Pazar-20 tea clone (2014).

Çizelge 2. Farklı taşıyıcı, bakteri kombinasyonları ve NPK uygulamasının Pazar-20 çay klonunda polifenol oksidaz, peroksidaz ve üreaz enzim aktivitesi üzerine etkisi (2014).

		PPO		POD		Ürease	
Treatment	**Carrier**	**(EU g leaf^{-1})***	**(EU mg Protein^{-1})**	**(EU g leaf^{-1})**	**(EU/mg protein)**	**(EU g leaf^{-1})**	**(EU mg Protein^{-1})**
Control	K	7.78 k-n	0.073 cd	15.55 h-l	0.115 d-g	0.93 l-n	0.0117 h-j
	T	8.32 i-n	0.078 cd	16.62 e-l	0.126 d-g	0.99 i-n	0.0125 f-j
	P	7.38 l-n	0.069 cd	14.73 i-l	0.107 e-g	0.88 n	0.0111 ij
	L	8.11 j-n	0.076 cd	16.21 g-l	0.122 d-g	0.97 j-n	0.0122 f-j
	Z	8.25 j-n	0.077 cd	16.47 e-l	0.125 d-g	0.98 j-n	0.0124 f-j
	V	8.14 j-n	0.076 cd	16.27 g-l	0.123 d-g	0.97 j-n	0.0122 f-j
	S	7.82 k-n	0.073 cd	15.63 g-l	0.116 d-g	0.93 l-n	0.0118 g-j
	Average	**7.97 e**	**0.074 b**	**15.93 d**	**0.119 d**	**0.95 e**	**0.0120 e**
NPK	K	9.56 g-j	0.249 a	19.18 d-l	0.137 d-g	1.17 b-n	0.0140 c-j
	T	9.05 e-k	0.087 cd	18.35 d-l	0.131 d-g	1.12 d-n	0.0134 c-j
	P	8.17 j-n	0.079 cd	16.56 e-l	0.118 d-g	1.01 g-n	0.0121 f-j
	L	8.34 i-n	0.079 cd	17.32 d-l	0.124 d-g	1.06 f-n	0.0126 e-j
	Z	8.69 g-n	0.084 cd	17.63 d-l	0.126 d-g	1.08 d-n	0.0129 d-j
	V	9.67 c-j	0.093 cd	19.60 d-l	0.140 c-g	1.20 b-m	0.0143 c-i
	S	10.13 c-h	0.097 cd	20.54 c-i	0.147 b-g	1.25 b-k	0.0150 a-h
	Average	**9.09 b-d**	**0.110 a**	**18.45 bc**	**0.132 b-d**	**1.13 b-d**	**0.0135b-d**
F1	K	8.58 h-n	0.059 cd	22.74 b-c	0.139 c-g	1.13 c-n	0.0130 d-j
	T	8.10 j-n	0.078 cd	16.42 f-l	0.117 d-g	1.00 h-n	0.0120 f-j
	P	7.26 n	0.070 cd	14.32 j-l	0.102 g	0.87 n	0.0104 j
	L	8.71 g-n	0.084 cd	17.66 d-l	0.126 d-gj	1.08 d-n	0.0129 d-j
	Z	9.16 d-k	0.088 cd	18.58 d-l	0.133 d-g	1.13 c-n	0.0136 c-j
	V	7.74 k-n	0.074 cd	15.70 g-l	0.112 d-gj	0.96 k-n	0.0115 h-j
	S	9.23 d-k	0.089 cd	18.71 d-l	0.134 d-g	1.14 c-n	0.0137 c-j
	Average	**8.40 e**	**0.077 ab**	**17.73 cd**	**0.124 cd**	**1.05 de**	**0.0124 de**
F2	K	7.28 mn	0.195 ab	33.91 a	0.226 a	1.01 g-n	0.0121 f-j
	T	10.66 cd	0.102 cd	21.62 b-g	0.155 b-e	1.32 a-g	0.0158 a-f
	P	7.94 k-n	0.076 cd	16.10 g-l	0.115 d-g	0.98 j-n	0.0118 g-j
	L	10.30 c-g	0.099 cd	20.89 c-h	0.149 b-g	1.27 b-j	0.0152 a-h
	Z	9.13 d-k	0.088 cd	18.52 d-l	0.132 d-g	1.13 c-n	0.0135 c-j
	V	7.29 l-n	0.070 cd	13.98 l	0.105 fg	0.88 n	0.0104 j
	S	9.34 d-k	0.090 cd	18.95 d-l	0.136 d-g	1.16 c-n	0.0138 c-j
	Average	**8.85 cd**	**0.103 ab**	**20.57 ab**	**0.145 ab**	**1.11 cd**	**0.0132 c-e**
F3	K	9.03 e-k	0.087 cd	18.31 d-l	0.131 d-g	1.12 d-n	0.0134 c-j
	T	9.90 c-i	0.095 cd	20.07 d-k	0.144 b-g	1.23 b-m	0.0146 c-i
	P	9.08 d-k	0.087 cd	18.40 d-l	0.132 d-g	1.12 c-n	0.0134 c-j
	L	12.14 ab	0.161 b-d	22.60 bc	0.162 b-d	1.38 a-d	0.0165 a-d
	Z	8.59 h-n	0.083 cd	17.43 d-l	0.125 d-g	1.06 e-n	0.0127 d-j
	V	9.36 d-k	0.090 cd	18.98 d-l	0.136 d-g	1.16 b-n	0.0138 c-j
	S	10.28 c-g	0.099 cd	20.84 c-h	0.149 b-g	1.27 b-j	0.0152 a-h
	Average	**9.77 ab**	**0.100 ab**	**19.52 bc**	**0.140 a-c**	**1.19 a-c**	**0.0142 a-c**
F4	K	12.63 a	0.122 b-d	26.63 b	0.190 ab	1.58 a	0.0186 a
	T	10.19 c-h	0.098 cd	20.65 c-i	0.148 b-g	1.26 b-k	0.0151 a-h
	P	7.93 k-n	0.076 cd	16.09 g-l	0.115 d-g	0.98 j-n	0.0117 g-j
	L	9.63 c-j	0.093 cd	19.53 d-l	0.140 c-g	1.19 b-m	0.0142 c-i
	Z	10.57 c-e	0.102 cd	21.43 b-h	0.153 b-f	1.31 a-h	0.0156 a-f
	V	10.01 c-h	0.096 cd	20.30 d-j	0.145 b-g	1.24 b-l	0.0148 b-i
	S	11.06 bc	0.106 b-d	20.43 b-e	0.160 b-d	1.37a-e	0.0164 a-e
	Average	**10.29 a**	**0.099 ab**	**21.01 ab**	**0.150 ab**	**1.28 a**	**0.0152 a**
F5	K	7.27 n	0.043 d	36.98 a	0.225 a	1.46 ab	0.0183 ab
	T	8.89 f-l	0.095 cd	18.03 d-l	0.129 d-g	1.10 d-n	0.0132 d-j
	P	7.36 l-n	0.069 cd	14.32 j-l	0.102 g	0.87 n	0.0104 j
	L	12.82 a	0.123 b-d	25.99 bc	0.186 a-c	1.59 a	0.0185 a
	Z	9.16 d-k	0.092 cd	18.17 d-l	0.130 d-g	1.11 d-n	0.0133 d-j
	V	11.00 bc	0.106 b-d	22.31 b-f	0.160 b-d	1.36 a-f	0.0163 a-e
	S	10.48 c-f	0.101 cd	21.25 b-h	0.152 b-g	1.30 b-i	0.0155 a-g
	Average	**9.57 a-c**	**0.088 ab**	**22.44 a**	**0.155 a**	**1.26 a**	**0.0151 a**

Table 2. Continue.
Çizelge 2. Devamı.

		PPO		POD		Ürease	
Treatment	**Carrier**	**(EU g leaf^{-1})***	**(EU mg Protein^{-1})**	**(EU g leaf^{-1})**	**(EU/mg protein)**	**(EU g leaf^{-1})**	**(EU mg Protein^{-1})**
Biological Fert.	K	7.35 l-n	0.136 b-d	14.15 kl	0.122 d-g	1.43 a-c	0.0170 a-c
	T	8.60 h-n	0.083 cd	17.45 d-l	0.125 d-g	1.06 e-n	0.0127 d-j
	P	9.89 c-i	0.095 cd	20.05 d-k	0.143 b-g	1.22 b-m	0.0146 c-i
	L	11,03 bc	0.106 b-d	22.37 b-f	0.160 b-d	1.37 a-f	0.0163 a-e
	Z	8.88 f-m	0.085 cd	18.01 d-l	0.129 d-g	1.10 d-n	0.0131 d-j
	V	9.56 c-j	0.092 cd	19.38 d-l	0.139 c-g	1.18 b-n	0.0141 c-j
	S	9.88 c-i	0.095 cd	20.04 d-k	0.143 b-g	1.22 b-m	0.0146 c-i
	Average	**9.31 bc**	**0.099 ab**	**18.78 bc**	**0.137 a-d**	**1.23 ab**	**0.0147 ab**
Treatment Average	K	8.68 cd	0.120 a	23.43 a	0.161 a	1.23 a	0.0148 a
	T	9.21 bc	0.088 b	18.65 bc	0.134 b	1.14 ab	0.0137 ab
	P	8.13 d	0.078 b	16.32 c	0.117 c	0.99 c	0.0120 c
	L	10.14 a	0.102 ab	20.32 b	0.146 ab	1.24 a	0.0148 a
	Z	9.06 bc	0.087 b	18.28 bc	0.132 bc	1.11 b	0.0134 b
	V	9.10 bc	0.087 b	18.32 bc	0.132 bc	1.12 b	0.0134 b
	S	9.78 ab	0.094 ab	19.80 b	0.142 b	1.21 ab	0.0145 ab

*The differences between the means indicated with the same letter are not significant ($p < 0.05$) in their group.

Table 3. The effect of different carriers and treatments on alcohol dehydrogenase and 5-dehydroxyshikimate reductase enzyme activity in Pazar-20 tea clone (2013).

Çizelge 3. Farklı taşıyıcı ve uygulamaların Pazar-20 çay klonunda alkol dehidrogenaz ve 5-dehidroksişikimat redüktaz enzim aktivitesi üzerine etkisi (2013 yılı).

		Alchol dehidrogenase		5-dehydroxyshikimate reductase	
Treatment	**Carrier**	**(EU g leaf^{-1})***	**(EU mg protein^{-1})**	**(EU g leaf^{-1})**	**(EU mg protein^{-1})**
Control	K	1.50 ab	0.056 a	2.32 f-l	0.033 h
	T	1.60 a	0.060 a	2.48 d-l	0.036 h
	P	1.42 a-d	0.053 ab	2.20 h-l	0.054 fh
	L	1.56 a	0.058 a	2.42 d-l	0.065 d-h
	Z	1.59 a	0.059 a	2.46 d-l	0.066 d-h
	V	1.57 a	0.059 a	2.43 d-l	0.067 d-h
	S	1.51 ab	0.056 a	2.33 e-l	0.060 e-h
Average		**1.54 a**	**0.057 a**	**2.37 c**	**0.055 c**
NPK	K	0.89 g-k	0.029 h-l	2.54 d-l	0.081 c-g
	T	0.85 g-k	0.028 h-l	2.43 d-l	0.078 c-g
	P	0.77 h-k	0.025 i-l	2.19 h-l	0.070 d-h
	L	0.81 g-k	0.026 h-l	2.29 g-l	0.074 d-g
	Z	0.82 g-k	0.027 h-l	2.33 e-l	0.075 c-g
	V	0.91 g-j	0.030 h-l	2.58 d-k	0.083 c-g
	S	0.96 g-i	0.031 h-k	2.72 d-k	0.087 b-g
Average		**0.86 cd**	**0.028 cd**	**2.44 c**	**0.078 b**
F1	K	0.91 g-k	0.027 h-l	3.92 a	0.124 ab
	T	0.76 h-k	0.025 j-l	2.17 i-l	0.070 d-h
	P	0.67 jk	0.022 l	1.90 l	0.061 e-h
	L	0.82 g-k	0.027 h-l	2.34 e-l	0.075 c-g
	Z	0.86 g-k	0.028 h-l	2.46 d-l	0.067 f
	V	0.73 i-k	0.024 kl	2.08 kl	0.067 d-h
	S	0.87 g-k	0.028 h-l	2.48 d-l	0.079 c-g
Average		**0.80 d**	**0.026 d**	**2.48 c**	**0.079 b**
F2	K	1.44 a-c	0.048 bc	3.08 a-d	0.103 b-e
	T	1.01 e-h	0.033 f-g	2.86 c-h	0.092 b-g
	P	0.75 h-k	0.024 j-l	2.13 j-l	0.068 d-h
	L	0.97 f-i	0.032 g-k	2.77 d-c	0.089 b-g
	Z	0.86 g-k	0.028 h-l	2.45 d-l	0.079 c-g
	V	0.65 k	0.022 l	1.89 l	0.059 e-h
	S	0.88 g-k	0.029 h-l	2.51 d-l	0.080 c-g
Average		**0.94 bc**	**0.031 bc**	**2.53 bc**	**0.081 ab**

Table 3. Continue.
Çizelge 3. Devamı.

Treatment	Carrier	Alchol dehidrogenase		5-dehydroxyshikimate reductase	
		(EU g leaf^{-1})*	(EU mg protein^{-1})	(EU g leaf^{-1})	(EU mg protein^{-1})
F3	K	0.85 g-k	0.028 h-l	2.42 d-l	0.078 c-g
	T	0.93 g-i	0.030 h-l	2.66 d-k	0.085 c-g
	P	0.86 g-k	0.028 h-l	2.44 d-l	0.078 c-g
	L	1.05 e-g	0.034 e-h	2.99 a-e	0.096 b-f
	Z	0.81 g-k	0.026 h-l	2.31 f-l	0.074 c-g
	V	0.88 g-k	0.029 h-l	2.51 d-l	0.081 c-g
	S	0.97 f-i	0.032 g-k	2.76 d-j	0.088 b-g
Average		**0.91 bc**	**0.030 bc**	**2.58 a-c**	**0.083 ab**
F4	K	1.24 c-e	0.040 c-f	3.53 ab	0.113 bc
	T	0.96 g-i	0.031 g-k	2.73 d-k	0.088 b-g
	P	0.75 h-k	0.024 j-l	2.13 j-l	0.068 d-h
	L	0.91 g-k	0.030 h-l	2.59 d-k	0.083 c-g
	Z	1.00 f-h	0.033 f-k	2.84 c-i	0.091 b-g
	V	0.94 g-i	0.031 h-k	2.69 d-k	0.086 c-g
	S	1.04 e-g	0.034 e-h	2.97 b-f	0.095 b-f
Average		**0.98 b**	**0.032 b**	**2.78 ab**	**0.089 ab**
F5	K	1.32 b-d	0.044 cd	4.00 a	0.153 a
	T	0.84 g-k	0.027 h-l	2.39 e-l	0.077 c-g
	P	0.67 jk	0.022 l	1.90 l	0.061 e-h
	L	1.21 d-f	0.039 d-g	3.44 a-c	0.110 b-d
	Z	0.85 g-k	0.028 h-l	2.41 e-l	0.077 c-g
	V	1.04 e-g	0.034 e-h	2.95 b-g	0.095 b-f
	S	0.99 f-i	0.032 g-k	2.81 d-i	0.090 b-g
Average		**0.99 b**	0.032 b	**2.84 a**	**0.095 a**
Biological	K	1.21 d-f	0.041 c-e	1.88 l	0.063 e-h
	T	0.81 g-k	0.026 hl	2.31 f-l	0.074 c-g
Fertilizer	P	0.93 g-i	0.030 h-l	2.65 d-k	0.085 c-g
	L	1.04 e-g	0.034 e-h	2.96 b-g	0.095 b-f
	Z	0.84 g-k	0.027 h-l	2.38 e-l	0.076 c-g
	V	0.90 g-k	0.029 h-l	2.57 d-k	0.082 c-g
	S	0.93 g-i	0.030 h-l	2.65 d-k	0.085 c-g
Average		**0.95 bc**	**0.031 bc**	**2.49 c**	**0.080 b**
Treatment	K	1.17 a	0.039 a	2.96 a	0.094 a
Average	T	0.97 bc	0.033 bc	2.50 bc	0.075 bc
	P	0.85 c	0.029 c	2.19 d	0.068 c
	L	1.05 ab	0.035 ab	2.72 b	0.086 ab
	Z	0.95 bc	0.032 bc	2.45 c	0.077 bc
	V	0.95 bc	0.032 bc	2.46 c	0.078 bc
	S	1.02 b	0.034 a-c	2.65 bc	0.083 ab

*The differences between the means indicated with the same letter are not significant ($p < 0.05$) in their group.

The effects of bacteria and chemical fertilizer applications on leaf antioxidant and pentose phosphate pathway enzyme activities in seedlings of Pazar-20 tea clone are given in Table 4. As can be seen from the relevant table, enzyme activities increased in all applications except the control. This increase was generally higher in biological fertilizers. In addition, the F3 formulation was found to be quite effective in terms of GST, G6PD, and 6PGD enzyme activities. When an evaluation is made in terms of the effect of the carriers used on the leaf enzyme activities, it has been observed that the peat-based carrier is promising and the liquid carrier also affects the leaf enzyme activity (Table 4). According to the 2015 results of the leaf polyphenol oxidase, peroxidase, and urease enzyme contents in Pazar-20 tea clone seedlings, all applications increased the activity of the mentioned enzymes compared to the control (Table 5).

Table 4. The effect of different carriers, mineral fertilization and bacterial combinations on leaf GR, GST, G6PD and 6PGD enzyme activity in Pazar-20 tea clone (2014).

Çizelge 4. Farklı taşıyıcı, mineral gübreleme ve bakteri kombinasyonlarının Pazar-20 çay klonunda yaprak GR, GST, G6PD ve 6PGD enzim aktivitesi üzerine etkisi (2014).

Variation Sources	Enzym activity EU mg protein^{-1}*			
	GR**	GST	G6PD	6PGD
Treatments (n=28)				
Control	1.53 f	1.31 e	1.60 d	1.09 d
NPK	2.66 b	2.36 ab	1.72 cd	1.51 c
FI	2.39 c	2.27 bc	1.88 c	1.60 bc
F2	2.17 e	2.13 cd	1.91 bc	1.51 c
F3	2.27 de	2.36 ab	2.38 a	1.86 a
F4	2.35 cd	2.05 d	2.14 ab	1.64 ac
F5	2.25 de	2.11 cd	1.92 bc	1.81 ab
Biological Fertilizer	2.87 a	2.45 a	1.82 cd	1.74 ab
Carriers (n=32)				
Compost	2.29 bc	2.18 ab	1.85 ab	1.66 a
Peat	2.26 c	2.32 a	1.79 b	1.71 a
Perlite	2.13 d	2.04 b	1.91 ab	1.44 b
Leonardite	2.31 bc	2.12 ab	1.91 ab	1.58 ab
Zeolite	2.27 c	2.11 ab	2.07 a	1.61 ab
Vermiculite	2.40 ab	2.02 b	1.93 ab	1.59 ab
Liquid Formula	2.51 a	2.11 ab	1.98 ab	1.59 ab

*GR: Glutation reductase, GST: Glutation S-transferase, G6PD: Glucoz-6 phosphat dehidrogenase (G6PD), 6PGD: 6-Phosphogluconat dehidrogenase (EC 1.1.1.44).

**The differences between the means indicated with the same letter are not significant ($p < 0.05$) in their group.

In this study, especially the F4 formulation gave the highest leaf enzyme activity among the applications. This formulation was followed by F3 and F5 in terms of effectiveness. In terms of carriers used in the study, leonardite and compost leaf gave the highest values in terms of enzyme activities (Table 5). The analysis results regarding the effect of the applications on the alcohol dehydrogenase and 5-de-hydroxy shikimate reductase enzymes in the leaf are given in Table 6. As can be seen from the table, all applications increased leaf enzyme activities compared to the control. The F5 formula increased the enzyme activities at the highest level. According to the application averages, the compost carrier gave the highest values in terms of POD, urease, ADH, and DHSK enzyme activities, followed by leonardite (Table 5,6).

Table 5. The effect of different carriers, bacterial combinations and NPK fertilizer application on polyphenol oxidase, peroxidase and urease enzyme activity in Pazar-20 tea clone (2015).

Çizelge 5. Farklı taşıyıcı, bakteri kombinasyonları ve NPK gübre uygulamasının Pazar-20 çay klonunda polifenol oksidaz, peroksidaz ve üreaz enzim aktivitesi üzerine etkisi (2015).

		PPO		POD		Urease	
Treatment*	Carrier	(EU g leaf^{-1})*	(EU mg Protein^{-1})	(EU g leaf^{-1})	(EU mg Protein^{-1})	(EU g leaf^{-1})	(EU mg Protein^{-1})
Control	K	8.29 i	0.088 a-e	16.55 f	0.144 d-h	1.20 d-g	0.0153 a-f
	T	8.69 g-i	0.082 de	17.52 ef	0.133 e-h	1.06 g	0.0135 d-f
	P	8.30 i	0.081 e	16.58 f	0.124 h	1.10 fg	0.0129 ef
	L	8.85 f-i	0.083 c-e	17.84 ef	0.136 d-h	1.10 fg	0.0138 d-f
	Z	8.84 f-i	0.083 c-e	17.81 ef	0.136 d-h	1.08 fg	0.0138 d-f
	V	8.94 d-i	0.084 b-e	18.03 d-f	0.138 d-h	1.09 fg	0.0139 d-f
	S	8.30 i	0.081 e	16.93 f	0.125 h	1.06 g	0.0124 f
	Average	**8.60 d**	**0.083 d**	**17.32 c**	**0.134 d**	**1.10 d**	**0.0137 d**
NPK	K	10.60 a-h	0.098 a-e	21.53 b-f	0.155 b-h	1.37 a-g	0.0169 a-f
	T	9.27 d-i	0.092 a-e	19.42 c-f	0.140 d-h	1.21 d-g	0.0146 b-f
	P	8.93 d-i	0.087 a-e	18.28 d-f	0.132 f-h	1.14 d-g	0.0137 d-f
	L	9.06 d-i	0.087 a-e	18.99 d-f	0.137 d-h	1.18 d-g	0.0142 b-f
	Z	9.59 c-i	0.093 a-e	19.63 c-f	0.142 d-h	1.22 d-g	0.0147 a-f
	V	10.79 a-g	0.103 a-e	21.66 b-f	0.156 b-h	1.35 a-g	0.0162 a-f
	S	11.10 a-e	0.099 a-e	23.04 a-e	0.155 b-h	1.35 a-g	0.0159 a-f
	Average	**9.91 bc**	**0.094 ac**	**20.36 b**	**0.145 cd**	**1.26 bc**	**0.0152 bc**

Table 5. Continue.
Çizelge 5. Devamı.

F1	K	8.32 i	0.088 a-e	23.86 a-d	0.153 c-h	1.24 cg	0.0147 a-f
	T	8.87 f-i	0.086 a-e	17.71 ef	0.128 gh	1.10 fg	0.0128 ef
	P	8.30 i	0.088 a-e	16.72 f	0.122 h	1.07 fg	0.0129 ef
	L	9.57 c-i	0.093 a-e	20.57 c-f	0.141 d-h	1.32 a-g	0.0147 a-f
	Z	9.63 c-i	0.093 a-e	19.70 c-f	0.142 d-h	1.22 d-g	0.0148 a-f
	V	8.52 hi	0.085 b-e	17.85 ef	0.129 gh	1.11 e-g	0.0134 d-f
	S	9.55 c-i	0.093 a-e	21.77 b-f	0.147 d-h	1.12 d-g	0.0135 d-f
	Average	**8.97 d**	**0.089 cd**	**19.74 b**	**0.137 d**	**1.17 cd**	**0.0138 cd**
F2	K	8.92 e-i	0.081 e	27.42 a	0.194 a	1.30 a-g	0.0158 a-f
	T	10.97 a-f	0.106 a-c	22.42 a-f	0.162 a-h	1.39 a-g	0.0168 a-f
	P	9.20 d-i	0.089 a-e	18.83 d-f	0.136 d-h	1.17 d-g	0.0141 c-f
	L	10.84 a-g	0.105 a-e	22.19 a-f	0.160 a-h	1.38 a-g	0.0166 a-f
	Z	8.98 d-i	0.091 a-e	19.24 d-f	0.139 d-h	1.20 d-g	0.0144 b-f
	V	8.56 hi	0.081 e	16.57 f	0.124 h	1.06 g	0.0125 f
	S	9.83 b-i	0.095 a-e	20.12 c-f	0.145 d-h	1.25 c-g	0.0151 a-f
	Average	**9.61 c**	**0.093 bc**	**20.97 ab**	**0.151 bc**	**1.25 bc**	**0.0151 bd**
F3	K	10.39 b-i	0.095 a-e	20.35 c-f	0.147 d-h	1.26 b-g	0.0153 a-f
	T	10.59 a-h	0.101 a-e	21.67 b-f	0.156 b-h	1.35 a-g	0.0162 a-f
	P	10.60 a-h	0.100 a-e	21.12 b-f	0.152 c-h	1.31 a-g	0.0158 a-f
	L	12.51 a	0.109 ab	23.38 a-e	0.171 a-f	1.45 a-f	0.0175 a-e
	Z	9.41 d-i	0.091 a-e	19.26 d-f	0.139 d-h	1.20 d-g	0.0144 b-f
	V	10.34 b-i	0.100 a-e	21.17 b-f	0.153 c-h	1.32 a-g	0.0159 a-f
	S	11.80 ab	0.110 a	23.84 a-d	0.174 a-d	1.50 a-d	0.0181 a-d
	Average	**10.81 a**	**0.101 a**	**21.54 ab**	**0.156 ac**	**1.34 ab**	**0.0162 ab**
F4	K	12.56 a	0.102 a-e	27.42 a	0.195 a	1.64 a	0.0188 a-c
	T	10.62 a-h	0.102 a-e	21.55 b-f	0.155 b-h	1.34 a-g	0.0162 a-f
	P	8.91 ei	0.086 a-e	18.23 d-f	0.132 f-h	1.13 d-g	0.0137 d-f
	L	10.56 a-h	0.104 a-e	22.02 a-f	0.160 a-h	1.37 a-g	0.0165 a-f
	Z	10.95 a-f	0.106 a-d	22.36 a-f	0.161 a-h	1.39 a-g	0.0168 a-f
	V	11.13 a-d	0.108 ab	23.08 a-e	0.160 a-h	1.42 a-g	0.0171 a-f
	S	10.84 a-g	0.101 a-e	25.11 a-c	0.190 a-c	1.60 a-c	0.0190 ab
	Average	**10.80 a**	**0.101 a**	**22.82 a**	**0.165 a**	**1.41 a**	**0.0169 a**
F5	K	9.07 d-i	0.089 a-e	27.46 a	0.190 a-c	1.50 a-d	0.0189 ab
	T	9.23 d-i	0.089 a-e	18.68 d-f	0.135 d-h	1.16 d-g	0.0140 d-f
	P	9.88 b-i	0.089 a-e	18,46 d-f	0.133 e-h	1.15 d-g	0.0138 d-f
	L	12.56 a	0.110 a	26.54 a-b	0.192 ab	1.65 a	0.0194 a
	Z	10.36 b-i	0.105 a-e	20.76 c-f	0.150 d-h	1.29 a-g	0.0156 a-f
	V	11.62 a-c	0.110 a	23.88 a-d	0.172 a-e	1.48 a-e	0.0181 a-d
	S	10.96 a-f	0.097 a-e	20.63 c-f	0.149 d-h	1.34 a-g	0.0156 a-f
	Average	**10.53 ab**	**0.098 ab**	**22.35 a**	**0.160 ab**	**1.37 ab**	**0.0165 ab**
Biological Fertilizer	K	8.34 i	0.099 a-e	17.03 f	0.146 d-h	1.63 ab	0.0189 a-c
	T	9.13 d-i	0.088 a-e	19.21 d-f	0.139 d-h	1.19 d-g	0.0144 b-f
	P	10.93 a-f	0.105 a-e	23.00 a-e	0.166 a-g	1.43 a-g	0.0172 a-f
	L	11.01 a-f	0.106 a-d	23.17 a-e	0.167 a-g	1.44 a-g	0.0174 a-e
	Z	9.45 d-i	0.091 a-e	19.90 c-f	0.144 d-h	1.24 c-g	0.0149 a-f
	V	10.04 b-i	0.097 a-e	21.14 b-f	0.153 c-h	1.31 a-g	0.0159 a-f
	S	10.37 b-i	0.100 a-e	19.66 c-f	0.147 d-h	1.36 a-g	0.0164 a-f
	Average	**9.89 bc**	**0.098 ab**	**20.45 b**	**0.152 bc**	**1.37 ab**	**0.0164 ab**
Treatment Average	K	9.56 b	0.092 ab	22.70 a	0.165 a	1.39 a	0.0168 a
	T	9.67 b	0.093 ab	19.77 cd	0.143 cd	1.23 cd	0.0148 cd
	P	9.38 b	0.091 b	18.90 d	0.137 d	1.19 d	0.0143 d
	L	10.62 a	0.100 a	21.84 ab	0.158 ab	1.36 ab	0.0163 ab
	Z	9.65 b	0.094 ab	19.83 cd	0.144 cd	1.23 cd	0.0149 b-d
	V	9.99 ab	0.096 ab	20.42 b-d	0.148 b-d	1.27 b-d	0.0154 b-d
	S	10.34 a	0.097 ab	21.39 a-c	0.154 a-c	1.32 a-c	0.0157 a-c

*The differences between the means indicated with the same letter are not significant ($p < 0.05$) in their group.

Table 6. The effect of different carriers, bacterial combinations and fertilizer application on alcohol dehydrogenase and 5-dehydroxyshikimate reductase enzyme activity in Pazar-20 tea clone (2015).

Çizelge 6. Farklı taşıyıcı, bakteri kombinasyonları ve gübre uygulamasının Pazar-20 çay klonunda alkol dehidrogenaz ve 5-dehidroksişikimat redüktaz enzim aktivitesi üzerine etkisi (2015).

		Alchol dehidrogenase		5-Dehydroksişikimat redüktase	
Treatment	Carrier	(EU g leaf^{-1})*	(EU mg protein^{-1})	(EU g leaf^{-1})	(EU mg protein^{-1})
Control	K	1.54 a-d	0.052 a-c	2.73 c-f	0.040 d
	T	1.30 a-g	0.045 a-f	2.62 d-f	0.070 cd
	P	1.24 bg	0.043 a-f	2.46 ef	0.085 a-d
	L	1.41 a-f	0.045 a-f	2.67 c-f	0.067 cd
	Z	1.50 a-e	0.047 a-f	2.60 d-f	0.073 cd
	V	1.43 a-f	0.053 ab	2.70 c-f	0.094 a-c
	S	1.31 a-g	0.044 a-f	2.37 f	0.039 d
Average		**1.39 b**	**0.047 b**	**2.59 c**	**0.067 c**
NPK	K	1.38 a-g	0.050 a-d	3.01 a-f	0.095 a-c
	T	1.28 a-g	0.047 a-f	2.58 d-f	0.083 a-d
	P	1.16 fg	0.036 ef	2.43 f	0.078 b-d
	L	1.22 c-g	0.045 a-f	2.53 ef	0.081 a-d
	Z	1.24 b-g	0.046 a-f	2.61 d-f	0.084 a-d
	V	1.32 a-g	0.048 a-f	2.88 b-f	0.093 a-c
	S	1.29 a-g	0.046 a-f	3.37 a-c	0.101 a-c
Average		**1.27 c**	**0.046 b**	**2.77 bc**	**0.088 ab**
F1	K	1.06 g	0.035 f	2.85 b-f	0.115 a-c
	T	1.06 g	0.045 a-f	2.36 f	0.076 b-d
	P	1.17 e-g	0.035 f	2.36 f	0.071 cd
	L	1.08 g	0.039 c-f	2.60 d-f	0.084 a-d
	Z	1.07 g	0.038 d-f	2.62 d-f	0.084 a-d
	V	1.07 g	0.036 ef	2.37 f	0.076 b-d
	S	1.23 c-g	0.036 ef	2.89 b-f	0.088 a-c
Average		**1.10 d**	**0.038 c**	**2.58 c**	0.085 b
F2	K	1.53 a-d	0.056 ab	3.27 a-d	0.115 a-c
	T	1.31 a-g	0.046 a-f	2.98 a-f	0.096 a-c
	P	1.20 d-g	0.042 b-f	2.50 ef	0.081 a-d
	L	1.38 a-g	0.048 a-f	2.95 b-f	0.095 a-c
	Z	1.31 a-g	0.046 a-f	2.56 d-f	0.082 a-d
	V	1.20 d-g	0.043 a-f	2.38 f	0.074 b-d
	S	1.31 a-g	0.046 a-f	2.68 c-f	0.086 a-d
Average		**1.32 bc**	**0.047 b**	**2.76 bc**	**0.090 ab**
F3	K	1.37 a-g	0.049 a-e	2.71 c-f	0.087 a-c
	T	1.34 a-g	0.048 a-f	2.88 b-f	0.093 a-c
	P	1.26 b-g	0.045 a-f	2.81 c-f	0.090 a-c
	L	1.38 a-g	0.049 a-e	3.18 a-e	0.103 a-c
	Z	1.28 a-g	0.046 a-f	2.56 d-f	0.083 a-d
	V	1.23 c-g	0.044 a-f	2.82 c-f	0.091 a-c
	S	1.31 a-g	0.047 a-f	3.26 a-d	0.106 a-c
Average		**1.31 bc**	**0.047 b**	**2.89 ab**	**0.093 ab**
F4	K	1.56 a-c	0.051 a-d	3.66 a	0.128 a
	T	1.49 a-f	0.049 a-e	2.87 b-f	0.092 a-c
	P	1.33 a-g	0.044 a-f	2.42 f	0.096 a-c
	L	1.56 a-c	0.051 a-d	2.93 b-f	0.103 a-c
	Z	1.52 a-d	0.050 a-d	2.97 b-f	0.096 a-c
	V	1.48 a-f	0.048 a-f	3.06 a-f	0.098 a-c
	S	1.58 ab	0.052 ac	3.35 a-c	0.108 a-c
Average		**1.50 a**	**0.049 ab**	**3.04 a**	**0.103 a**
F5	K	1.60 a	0.056 a	3.66 a	0.122 ab
	T	1.48 a-f	0.050 a-d	2.48 ef	0.080 a-d
	P	1.34 a-g	0.046 a-f	2.46 ef	0.099 a-c
	L	1.61 a	0.056 a	3.53 ab	0.114 a-c
	Z	1.46 a-f	0.050 a-d	2.76 c-f	0.089 a-c
	V	1.51 a-e	0.052 a-c	3.18 a-e	0.102 a-c
	S	1.44 a-f	0.049 a-e	2.78 c-f	0.090 a-c

Table 6. Continue.
Çizelge 6. Devamı.

Average		**1.49 a**	**0.051 a**	**2.98 ab**	**0.099 ab**
Biological Fert.	K	1.62 a	0.056 a	2.35 f	0.074 b-d
	T	1.30 a-g	0.046 a-f	2.56 d-f	0.082 a-d
	P	1.31 a-g	0.046 a-f	3.06 a-f	0.099 a-c
	L	1.43 a-f	0.051 a-d	3.08 a-f	0.099 a-c
	Z	1.29 a-g	0.046 a-f	2.62 d-f	0.086 a-d
	V	1.36 a-g	0.048 a-f	2.81 c-f	0.091 a-c
	S	1.31 a-g	0.046 a-f	2.90 b-f	0.093 a-c
Average		**1.37 bc**	**0.048 ab**	**2.77 bc**	**0.089 ab**
Treatment Average	K	1.46 a	0.051 a	3.03 a	0.097 a
	T	1.32 bc	0.047 ab	2.67 c	0.084 a
	P	1.25 c	0.042 c	2.56 c	0.087 a
	L	1.38 ab	0.048 ab	2.93 ab	0.093 a
	Z	1.34 bc	0.046 b	2.66 c	0.085 a
	V	1.32 bc	0.046 b	2.77 bc	0.090 a
	S	1.35 bc	0.046 bc	2.95 ab	0.089 a

*The differences between the means indicated with the same letter are not significant ($p < 0.05$) in their group.

Table 7. The effect of different carriers, bacterial combinations and mineral fertilizer application on leaf GR, GST, G6PD and 6PGD enzyme activity in Pazar-20 tea clone (2015).
Çizelge 7. Farklı taşıyıcı, bakteri kombinasyonları ve mineral gübre uygulamasının Pazar-20 çay klonunda yaprak GR, GST, G6PD ve 6PGD enzim aktivitesi üzerine etkisi (2015).

Variation Sources	Enzym activity EU mg protein^{-1}			
	GR	GST	G6PD	6PGD
Treatments (n=28)				
Control	1.65 e	1.41 d	1.72 e	1.18 d
NPK	2.87 b	2.55 ab	1.86 d	1.64 c
FI	2.61 c	2.45 b	2.03 c	1.72 b
F2	2.40 d	2.31 c	2.10 bc	1.63 c
F3	2.45 d	2.54 ab	2.57 a	1.93 a
F4	2.54 cd	2.21 c	2.20 b	1.76 b
F5	2.42 d	2.27 c	2.07 c	1.87 a
Biological Fertilizer	3.04 a	2.65 a	1.96 cd	1.89 a
Carriers (n=32)				
Compost	2.50 bc	2.35 b	2.00 bc	1.70 a
Peat	2.44 cd	2.50 a	1.95 c	1.76 a
Perlite	2.32 d	2.21 c	2.06 a-c	1.56 b
Leonardite	2.50 bc	2.29 bc	2.07 a-c	1.70 a
Zeolite	2.45 c	2.28 bc	2.13 a	1.75 a
Vermiculite	2.59 ab	2.18 c	2.09 ab	1.71 a
Liquid formula	2.69 a	2.28 bc	2.16 a	1.73 a

*The differences between the means indicated with the same letter are not significant ($p < 0.05$) in their group.

The values related to the effects of the applications on the enzyme activity of the leaves in the seedlings of the Pazar-20 tea clone are given in Table 7. While GR, GST, G6PD, and 6PGD activities gave the highest values especially in the F3 formulation, the application of biological fertilizers also increased the enzyme activity significantly. All of the bacterial formulations increased leaf enzyme activity compared to the control. In terms of different carriers used to commercialize bacterial formulations, the liquid carrier gave the best values in terms of the effect on leaf enzyme values. While peat was the best carrier for GST and 6PGD enzymes, zeolite carrier gave the highest values for G6PD and 6PGD enzymes (Table 7). F4, F3, and F5 formulations provided the highest leaf PPO, POD, and urease values in 2015, respectively. In the same year, F4, F5, and F3 rankings were valid in terms of Alcohol Dehydrogenase and 5-Dehydroxyshikimate enzyme values (Tables 5 and 6). In terms of carriers, the efficiency of different enzyme activities was determined in the same year, especially in carriers such as liquid formula and peat (Table 7).

CONCLUSION

The regular continuation of the chemical reactions taking place in the cells is with the help of the enzymes in the cell, and the complex molecules are broken down into simple molecules by the effect of the enzymes. The enzymes found in the young leaves and shoots of the tea plant create biochemical transformations during the processing stage, giving the tea its characteristic taste and smell. The production of different types and qualities of black tea occurs thanks to the enzymes in the young tea leaves and buds. Especially oxidative enzymes serve in the production of black tea. In addition, some of the enzymes discussed are those that have direct or indirect effects on the development of the plant's resistance to stress factors (Savanakumar *et al*., 2007; Mishra *et a*l., 2014).

In many studies conducted in similar ecology, as in this study, it has been determined that there is a close relationship between the enzyme activities in plant leaves and the growing support of the PGPR isolates used (Çakmakçı, 2016; Çakmakçı *et a*l., 2017). Also, the changes in enzyme activities are affected differently by the bacterial isolates and carriers in the formulas used. Therefore, it is expected that there will be increased in many growth parameters with PGPR applications of enzymes responsible for both the quality of the finished tea and many growth reactions (enzymes are also active in nitrate assimilation). However; failures in these applications may be due to the inability to select the appropriate bacterial association a combination of carriers. That's why; many studies are needed to determine the activities of PGPRs in the tea rhizosphere. In the future, studies to determine the effects of PGPR strains on different enzyme activities will contribute to the search for alternatives that can provide efficacy against biotic and abiotic stress conditions encountered in tea cultivation. The effect of the tested isolates on the enzymes in plant leaves can be explained by the interaction of plant X microorganism X carrier in a particular plant environment in particular ecology, as well as the classical mechanism of action. Although the effectiveness of the fertilizer dose used in the studies disappears after a period of time, it is reported that the bacterial activity (although it varies depending on many factors) may be longer and permanent (Ramkumar *et al*., 2015; Çakmakçı, 2016; Çakmakçı *et al*., 2011 and 2017; Ertürk *et al*., 2011 and 2014).

The use of effective bacteria with multiple characteristics, such as the bacterial isolates used in this research, may ultimately contribute to reducing the environmental pollution. In short, it is a very important and expected result that soils inoculated with PGPRs have a higher yield potential.

CONFLICT OF INTEREST

There is no conflict of interest between the authors

DECLARATION OF AUTHOR CONTRIBUTION

Yaşar ERTÜRK; Planning, field studies, evaluation, writing
Ramazan Çakmakçı; Planning, field studies, statical analysis, evaluation,
Meral KUTLU; Field studies

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