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Araştırma
(Research)

Effect of Bacterial Pre-Treatment on Plant Growth and Dry Matter Accumulation in Lentil*

Sipan SOYSAL [1*], Özge UÇAR [1], Murat ERMAN [2], Fatih ÇIĞ [1*]

[1]Siirt University, Faculty of Agriculture, Department of Field Crops, Siirt/TÜRKİYE
[2]Bursa Uludağ University, Faculty of Agriculture, Department of Field Crops, Bursa/TÜRKİYE

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Sorumlu yazar: Fatih ÇIĞ, e-posta: fatih@siirt.edu.tr

Abstract

Objective: Microbiological fertilizer usage is an expanding phenomenon in agriculture worldwide due to conformity to sustainable agriculture model. Nitrogen fixing and phosphate solubilizing bacteria species that can live symbiotic or free in soils have been used in both legumes and other crops. This experiment was laid out to investigated the effectiveness of rhizobia and plant growth promoting bacteria (PGPB) inoculation on plant growth and dry matter accumulation in different lentil cultivars.

Materials and Methods: The experiment was conducted according to completely randomized factorial design with three replications at growth chamber of Field Crops Department, Siirt University, Siirt. A rhizobia strain, *Rhizobium leguminosarum biover. to vicia*, and two PGPB strains (TV83D and TV119E) were treated to six lentil cultivars and compared with non-inoculated control plants.

Results: Analysis of variance indicated that cultivar, bacterial inoculation and CxB interactions caused statistically significant differences ($p<0.01$) in all characteristics including total biomass, plant and root length, dry matter accumulation in roots and shoots. According to the results, shoot (plumula) fresh weight, root fresh weight, plant length, root length, shoot dry weight and root dry weight were changed between 0.171-0.311 g, 0.150-0.268 g, 12.2-18.5 cm, 11.3-18.8 cm, 0.0186-0.0303 g and 0.0233-0.0381 g, respectively. Out of lentil cultivars, Seyran-96 and Yerli kırmızı exhibited higher growth performance. The TV83D, capable of high nitrogen fixation, promoted plant growth and dry matter accumulation compared with control and other beneficial microorganisms.

Conclusion: Biopriming with nitrogen fixing microorganisms provides to enhance plants during early growing stage, therefore, it might be used as a sustainable strategy in lentil cultivation.

Keywords: Beneficial microorganism, Bio-priming, Biomass, Fertilizer, Growth, Sustainable agriculture

Bakteriyel Ön Uygulamalarin Mercimekte Bitki Gelişimi ve Kuru Madde Birikimi Üzerine Etkisi

Öz

Amaç: Mikrobiyolojik gübre kullanımı, sürdürülebilir tarım modeline uyumlu olması nedeniyle dünya çapında tarımda yaygınlaşan bir fenomendir. Toprakta simbiyotik veya serbest yaşayabilen azot bağlayıcı ve fosfat çözünürlüğünü arttıran bakteri türleri, hem baklagillerde hem de diğer ürünlerde kullanılmıştır. Bu araştırma, farklı mercimek çeşitlerinde rizobium ve bitki büyümesini teşvik eden bakteri (PGPB) inokülasyonunun bitki büyümesi ve kuru madde birikimi üzerindeki etkinliğini araştırmak amacıyla düzenlenmiştir.

Materyal ve Yöntem: Araştırma, Siirt Üniversitesi Tarla Bitkileri Bölümü büyüme odasında üç tekrarlamalı olarak tesadüf parsellerinde faktöriyel deneme desenine göre yapılmıştır. Bir rizobium suşu (*Rhizobium leguminosarum biover. to vicia*) ve iki PGPB suşu (TV83D ve TV119E), altı mercimek çeşidine uygulanmış ve kontrol bitkileriyle karşılaştırılmıştır.

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Araştırma Bulguları: Varyans analizi, çeşit, bakteri inokülasyonu ve CxB etkileşimlerinin tüm karakterlerde (toplam biyo-kütle, bitki ve kök uzunluğu, köklerde ve sürgünlerde kuru madde birikimi dahil olmak üzere) istatistiksel olarak önemli farklılıklara neden olduğunu göstermiştir ($p<0.01$). Sonuçlara göre, sürgün (plumula) yaş ağırlığı, kök yaş ağırlığı, bitki uzunluğu, kök uzunluğu, sürgün kuru ağırlığı ve kök kuru ağırlığı sırasıyla 0.171-0.311 g, 0.150-0.268 g, 12.2-18.5 cm, 11.3-18.8 cm, 0.0186-0.0303 g ve 0.0233-0.0381 g arasında değişmiştir. Mercimek çeşitlerinden, Seyran-96 ve Yerli kırmızı daha yüksek gelişme performansı sergilemiştir. Yüksek azot bağlama kapasitesine sahip olan TV83D, kontrol ve diğer faydalı mikroorganizmalara göre bitki büyümesini ve kuru madde birikimini daha fazla teşvik etmiştir.

Sonuç: Azot bağlayıcı mikroorganizmalarla biyopriming, bitkilerin erken büyüme aşamasında gelişimini artırabilir ve bu nedenle mercimek yetiştiriciliğinde sürdürülebilir bir strateji olarak kullanılabilir.

Anahtar kelimeler: Faydalı mikroorganizma, Biyo-priming, Biyo-kütle, Gübre, Gelişim, Sürdürülebilir tarım

Introduction

Lentil is extensively used in both human and animal nutrition. Lentil contains 18-36% protein in dry grains (Jarpa-parra, 2017). Additionally, it is rich in carbohydrates, fiber, amino acids, antioxidants, and probiotics, along with vitamins A, B, and D (Kumar et al., 2018). According to Anonymous (2023) data, while 5.9 million tons were produced on 5.5 million hectares globally during the 2022-23 season, these figures varied to 5.4 million tons on 5.2 million hectares in the 2023-24 season. Therefore, lentil constitutes a critical link in the food chain, especially in less developed and developing countries.

In plant production, providing essential nutrients at optimum levels plays a crucial role in grain yield and quality. Nitrogen, phosphorus, and potassium are the primary nutrients for plants avoiding yield and quality losses (Mengel et al., 2021; Ceritoglu and Erman, 2020; Barlog et al., 2022). Nitrogen is the most important nutrient in plant production, and leguminous plants can meet their nitrogen requirement throughout the vegetation period through symbiotic nitrogen fixation, provided suitable conditions exist (Neugschwandtner et al., 2021; Ahmad et al., 2022). Effective strains of rhizoba (*Rhizobium leguminosarum biover. to vicia*) in lentil cultivation areas or inoculation techniques can convert atmospheric nitrogen (N_2) into usable forms for plants via nodules formed on the roots (Mahmud et al., 2020; Erman et al., 2022a; Sharma et al., 2023). Lentil meets approximately 75% of their nitrogen needs through this process. Additionally, free-living beneficial microorganisms can fix atmospheric nitrogen into the soil, thereby providing nitrogen gains through biological nitrogen fixation in both leguminous and non-leguminous plants (Erman et al., 2022a; Sharma et al., 2023). Microorganisms that do not require leguminous plants for nitrogen fixation and live freely in the soil are generally defined as plant growth-promoting bacteria (PGPB).

Generally, PGPB is defined as organisms that can convert atmospheric elemental nitrogen into forms that plants can uptake (such as ammonia and subsequently ammonium), facilitate the mineralization of insoluble phosphorus and potassium compounds in the soil, promote plant growth through the secretion of plant hormones, increase stress tolerance in plants through ACC deaminase activity, and facilitate the uptake of plant nutrients through various mechanisms (Glick, 2020; Çığ et al., 2021, 2022; Ajijah et al., 2023). Thus, they support plants not only in terms of important nutrients like nitrogen and phosphorus but also provide protection against biotic and abiotic stress factors. Moreover, PGPB applications play a major role in improving soil physicochemical and biological properties aside from supporting plant growth (Ortega Perez et al., 2023).

Research has shown significant differences in plant-microorganism interactions concerning biological nitrogen fixation (Rosier et al., 2018; Bhat et al., 2020). Therefore, the compatibility between the PGPB strain to be inoculated into seeds before planting and the lentil plant for inoculation is crucial. This study is unique in investigating the effects of two different specific PGPB strains alongside rhizobia inoculation on red lentil varieties. The aim of the study is to examine the effects of symbiotic and non-symbiotic microbial inoculation on plant growth in different lentil varieties.

Material and methods

In the experiment, 6 red lentil varieties (Çağıl, Altıntoprak, Tigris, Fırat-87, Seyran-96, Yerli Kırmızı) were used. These lentil varieties have been cultivated in the region for many years.

The rhizobia strain used in the research was obtained from the Soil Fertilizer and Water Resources Research Institute. The PGPB strains used (TV83D: *Bacillus atrophaeus*, TV119E: *Bacillus* GC-group) were isolated from the Van Lake Basin as part of a TÜBİTAK project conducted in 2010. Among the selected PGPB strains, TV83D has nitrogen-fixing capabilities, while TV119E has phosphate-solubilizing properties (Soysal and Erman, 2020).

The study was conducted as a completely randomized factorial design with three replications. The experiment took place under controlled conditions in the laboratory of the Department of Field Crops at Siirt University. Throughout the experiment, the temperature of the environment was maintained between 18-25 °C. The plants were exposed to 16 hours of light and 8 hours of darkness each day (Tavakoli et al., 2012). The inoculation of symbiotic nitrogen-fixing bacteria, *Rhizobium leguminosarum* bv. Vicieae, was carried out by moistening the seeds with a 4% sugar solution early in the morning (İşler and Coşkan, 2009). Then, 1 kg of *Rhizobium leguminosarum* bv. Vicieae culture per 50 kg of seeds was evenly applied to the seeds, and the inoculated seeds were planted on the same day. As for the PGPB strains, they were initially inoculated onto solid nutrient agar medium from stock cultures. Then, a single colony was transferred to liquid nutrient broth medium. The bacterial density in the obtained solution was determined using turbidimetric methods, and when the solution density reached 10^8 cfu, it was applied to the seeds. Before inoculation, the seeds underwent surface sterilization with 10% sodium hypochlorite for 5 minutes, followed by soaking in the solution for 4 hours and drying on coarse filter paper (Erman et al., 2022b). The thoroughly dried seeds were then planted in 1.2-liter pots after complete surface moisture removal.

Pots were filled with a mixture of sterilized field soil and peat material at a 1:1 ratio. Then, watering was carried out to reach 80% of the field capacity, and 10 seeds were planted in each pot, at a depth of 3 cm. One week after sowing, thinning was performed to leave 5 plants per pot. Throughout the study, irrigation was applied in equal amounts according to the plants' needs. The experiment was completed 30 days after thinning. Upon completion of the study, the plants were carefully removed from the pots along with the soil. After removing coarse soil, roots were carefully washed and cleaned. The plants were then quickly weighed after cutting off the root collar to determine shoot fresh weight and root fresh weight. Subsequently, shoot length and root length were measured using a meter. The samples were then placed in an oven set at 68°C and weighed after 72 hours to determine shoot dry weight and root dry weight.

The obtained data were subjected to analysis of variance to determine the statistical significance levels. The LSD test was used for the grouping of means for the applications. All statistical calculations were performed using the JMP statistical package programs.

Results

Cultivar, bacteria, and CxB interaction led to statistically significant differences in root length at the 1% level. Upon examining all applications, root length ranged from 11.33 to 18.81 cm. The highest root length (18.81 cm) was obtained from TV83D strain on the Yerli Kırmızı, while the lowest value (11.33 cm) was observed in Rhizobium on the Altıntoprak. According to cultivars, the highest root length was observed in the Yerli Kırmızı, while the lowest was obtained from Altıntoprak. Regarding the applications, the TV83D inoculation provided the highest root length, while the Rhizobium and TV119E inoculations were in the same group. All applications resulted in higher root length compared to the control group (Table 1).

Table 1. Effect of bio-priming treatments on root length of lentil cultivars

Treatment/Cultivar	Seyran 96	Yerli Kırmızı	Fırat 97	Tigris	Çağıl	Altıntoprak	Mean
Control	14.80 i-l	15.99 e-i	14.46 j-l	14.83 h-k	14.88 g-k	13.58 kl	14.76 C
TV119E	14.33 j-l	13.38 l	16.09 e-i	16.58 c-f	15.61 f-j	16.24 d-h	15.37 B
TV83D	16.20 e-i	18.81 a	16.98 b-f	18.16 ab	16.27 d-g	17.66 a-d	17.35 A
Rhizobium	16.73 c-f	18.00 a-c	17.38 a-e	15.03 g-j	15.75 f-j	11.33 m	15.70 B
Mean	15.51 B	16.54 A	16.23 AB	16.15 AB	15.62 B	14.70 C	

LSD (Cultivar): 0.71**, LSD (Bacteria): 0.58**, LSD (CxB): 0.02**

The effect of cultivar, bacteria, and CxB interaction on root fresh weight was found to be statistically significant at the 1% level. Upon examining all applications, root fresh weights ranged from 0.150 to 0.268 g. The Yerli Kırmızı, Fırat-87, and Tigris varieties were statistically grouped together and exhibited the highest root fresh weight. The lowest root fresh weight was obtained from the Altıntoprak. Regarding bacterial inoculation, the lowest root fresh weight was observed in control plants, while the highest value was obtained from TV83D. When examining the CxB interaction, the highest value (0.268 g) was observed in the Yerli Kırmızı with the TV83D, whereas the lowest value was obtained from the Altıntoprak with Rhizobium inoculation (Table 2).

Table 2. Effect of bio-priming treatments on root fresh weight of lentil cultivars

Treatment/Cultivar	Seyran 96	Yerli Kırmızı	Fırat 97	Tigris	Çağıl	Altıntoprak	Mean
Control	0.188 g-j	0.221 de	0,193 f-i	0.197 f-h	0.205 e-g	0.169 jk	0.195 C
TV119E	0.183 h-j	0.173 ij	0.221 de	0.238 cd	0.210 ef	0.204 e-h	0.205 B
TV83D	0.205 e-g	0.268 a	0.221 de	0.261 ab	0.212 ef	0.207 e-g	0.229 A
Rhizobium	0.221 de	0.245 bc	0.243 bc	0.206 e-g	0.204 e-h	0.150 k	0.211 B
Mean	0.199 B	0.227 A	0.219 A	0.225 A	0.208 B	0.182 C	
LSD (Cultivar): 0.01**, LSD ((Bacteria): 0.008**, LSD (CxB): 1.42**							

Cultivar, bacteria, and CxB interaction on plant length was found to be statistically significant at the 1% level. Plant length ranged from 12.17 to 18.52 cm. The highest plant length was measured in the Seyran-96, whereas the lowest one was observed in the Tigris and Çağıl. Among the applications, the highest plant length was observed in the TV83D, while the lowest one was observed in the control. Regarding the CxB interaction, the highest plant length (18.52 cm) was obtained from the Rhizobium application on the Seyran-96, while the lowest value (12.17 cm) was obtained from the control application on the Altıntoprak (Table 3).

Table 3. Effect of bio-priming treatments on plant length of lentil cultivars

Treatment/Cultivar	Seyran 96	Yerli Kırmızı	Fırat 97	Tigris	Çağıl	Altıntoprak	Mean
Control	16.70 cd	13.94 i	15.52 d-h	12.31 jk	14.87 f-i	12.17 k	14.25 D
TV119E	16.66 c-e	14.58 g-i	18.22 ab	14.60 g-i	15.75 d-g	15.31 e-h	15.85 B
TV83D	18.48 ab	17.12 bc	17.38 a-c	16.19 c-f	13.55 ij	17.50 a-c	16.70 A
Rhizobium	18.52 a	15.74 d-g	13.94 i	14.26 hi	13.93 i	14.17 hi	15.09 C
Mean	17.59 A	15.35 C	16.26 B	14.34 D	14.52 D	14.79 CD	
LSD (Cultivar): 0.67**, LSD (Bacteria): 0.55**, LSD (ÇxB): 1.35**							

Cultivar, bacteria, and CxB interaction have been observed to cause significant differences in plant fresh weight at the 1% level. The lowest plant fresh weight was determined in the Tigris, while the highest value was obtained from the Seyran-96. Regarding the applications, the highest plant fresh weight was observed in seeds inoculated with TV83D, while the lowest value was found in the control plants. In terms of the CxB interaction, plant weight varied between 0.171 and 0.321 g. Accordingly, the highest plant fresh weight was obtained from the Yerli Kırmızı with TV83D inoculation, while the lowest value was observed in the Tigris without bacterial inoculation (Table 4).

Table 4. Effect of bio-priming treatments on plant fresh weight of lentil cultivars

Treatment/Cultivar	Seyran 96	Yerli Kırmızı	Fırat 97	Tigris	Çağıl	Altıntoprak	Mean
Control	0.278 cd	0.231 ij	0.234 h-j	0.171 p	0.221 j-l	0.182 op	0.219 D
TV119E	0.291 bc	0.255 e-g	0.247 f-i	0.204 l-n	0.225 jk	0.239 g-j	0.244 B
TV83D	0.306 ab	0.321 a	0.264 d-f	0.211 k-m	0.193 m-o	0.253 e-h	0.258 A
Rhizobium	0.311 a	0.269 de	0.194 m-o	0.190 n-p	0.211 k-m	0.200 m-o	0.229 C
Mean	0.296 A	0.269 B	0.235 C	0.194 E	0.212 D	0.218 D	
LSD (Cultivar): 0.009**, LSD (Bacteria): 0.007**, LSD(ÇxB): 0.038**							

Cultivar, bacteria, and CxB interaction have resulted in statistically significant differences in plant dry weight at the 1% level. Among cultivars, the highest plant dry weight (0.0284 g) was observed in the Seyran-96, while the lowest (0.0205 g) was found in the Tigris. Rhizobium and TV119E applications were in the same group, while the highest plant dry weight was observed in plants inoculated with TV83D. The lowest plant dry weight was determined in the control group. Regarding the CxB interaction, the highest plant g dry weight was obtained from the Rhizobium on Seyran-96, while the lowest value was observed in the Tigris without bacterial inoculation (Table 5).

Table 5. Effect of bio-priming treatments on plant dry weight of lentil cultivars

Treatment/Cultivar	Seyran 96	Yerli Kırmızı	Fırat 97	Tigris	Çağıl	Altıntoprak	Mean
Control	0.0267 bc	0.0250 d-f	0.0224 gh	0.0187 k	0.0220 hi	0.0199 jk	0.0224 C
TV119E	0.0267 bc	0.0245 ef	0.0238 fg	0.0217 hi	0.0221 h	0.0230 gh	0.0236 B
TV83D	0.0297 a	0.0279 b	0.0263 cd	0.0222 h	0.0206 ij	0.0227 gh	0.0249 A
Rhizobium	0.0303 a	0.0254 c-e	0.0229 gh	0.0193 jk	0.0219 hi	0.0186 k	0.0231 B
Mean	0.0284 A	0.0257 B	0.0238 C	0.0205 E	0.0216 D	0.0210 DE	
LSD (Cultivar): 0.0007**, LSD (Bacteria): 0.0006**, LSD (ÇxB): 0.0015**							

Cultivar, bacteria, and CxB interaction have resulted in statistically significant differences in root dry weight at the 1% level. Root dry weights ranged from 0.0233 to 0.0381 g. Among cultivars, the lowest root dry weight was obtained from the Çağıl, while the Seyran-96, Yerli Kırmızı, and Fırat-87 varieties were grouped together with the highest root dry weight. Regarding the CxB interaction, the highest root dry weight was obtained from the Rhizobium on the Seyran-96, while the lowest value was observed in the Rhizobium on the Altıntoprak (Table 6).

Table 6. Effect of bio-priming treatments on root dry weight of lentil cultivars

Treatment/Cultivar	Seyran 96	Yerli Kırmızı	Fırat 97	Tigris	Çağıl	Altıntoprak	Mean
Control	0.0288 fg	0.0333 cd	0.0298 fg	0.0292 fg	0.0298 fg	0.0254 h	0.0294 C
TV119E	0.0293 fg	0.0291 fg	0.0320 de	0.0332 cd	0.0298 fg	0.0289 fg	0.0304 B
TV83D	0.0356 b	0.0344 bc	0.0339 b-d	0.0330 cd	0.0301 ef	0.0297 fg	0.0328 A
Rhizobium	0.0381 a	0.0341 bc	0.0328 cd	0.0285 fg	0.0281 g	0.0233 i	0.0308 B
Mean	0.0329 A	0.0327 A	0.0321 A	0.0310 B	0.0295 C	0.0268 D	
LSD (Cultivar): 0.0009**, LSD (Bacteria): 0.0007**, LSD (CxB): 0.0019**							

Discussion

The use of beneficial microorganisms in agricultural production is becoming increasingly widespread and holds growing importance in the context of sustainable agriculture vision. The symbiotic relationship between leguminous plants and specific Rhizobium bacterial species largely fulfills the plant's nitrogen requirements (Dwivedi et al., 2015). Additionally, various researchers have reported significant effects of free-living PGPB bacteria, particularly on plant nutrition, including nitrogen and phosphorus. The findings from the research indicate that bacterial inoculation outperformed control plants in all examined traits. Furthermore, while there was significant variation among cultivars, the responses of these varieties to bacterial application also exhibited substantial differences.

Rhizobium inoculation has resulted in increased shoot and root length, shoot and root fresh weight, as well as shoot and root dry weight in plants compared to control plants. The microorganisms that colonize the plant roots as a result of Rhizobium inoculation convert atmospheric nitrogen into ammonia, facilitating its uptake by the plant. Meeting the nitrogen requirements in plants compared to the control group stimulates cell division, plant growth and development, and increases dry matter accumulation (Sun et al., 2020a). Haque et al. (2014) examined the responses of different lentil genotypes to bacterial inoculation and reported significant variation. Kumar and Chandra (2008) reported that Rhizobium inoculation in lentils increased dry matter accumulation in plants by approximately 47% compared to the control group, based on observations taken 90 days after planting. Erman et al. (2012) reported that Rhizobium inoculation increased shoot and root dry weight in lentils compared to the control. The results obtained from the study are consistent with findings from previous researchers.

The high nitrogen-fixing capability TV83D strain used in the study has demonstrated superior results in all examined traits compared to Rhizobium and other PGPB strains. Previous research indicates that the amount of nitrogen fixed per square meter through symbiotic nitrogen fixation is higher compared to free-living microorganisms (Lucas Garcia et al., 2004; Matse et al., 2020). However, PGPB strains may exhibit various other characteristics besides nitrogen fixation ability, such as phosphate mineralization, indole acetic acid (IAA) and siderophore production, organic acid synthesis, phytohormone, vitamin, and antibiotic production (Santoyo et al., 2021; Reed and Glick, 2023). Since the plants were harvested after 30 days, they may have sufficiently met their nitrogen requirements during the early growth stage through the TV83D strain, and additionally, plant growth may have been promoted due to various growth-promoting properties. Researchers have observed that nitrogen-fixing PGPB strains stimulate plant growth and dry matter accumulation in lentils (Kumar and Chandra, 2008), chickpeas (Soysal and Erman, 2020), wheat (Çığ et al., 2022), cowpeas (Kumar et al., 2022), and barley (Zaib et al., 2023). The findings from the study are consistent with the results of previous researchers.

The TV119E strain used in the study has also promoted growth and development compared to control plants, however, it exhibited less growth compared to Rhizobium and TV83D organisms. Although the TV119E strain has a high capability for phosphate mineralization, it lacks nitrogen fixation ability. Phosphorus plays an important role in plants, however, if there is insufficient nitrogen present, it can lead to significant growth retardation in plants (Sun et al., 2020b). This is because nitrogen is essential for many fundamental metabolites, particularly amino acids and thus proteins, and contributes to their critical functions (Yang et al., 2020; Heinemann and Hildebrandt, 2021). Therefore, although TV119E inoculation led to greater plant growth and dry matter accumulation compared to control plants due to phosphate mineralization, it exhibited lower performance compared to nitrogen-fixing bacteria.

Conclusion

The results indicated significant development differences among cultivars even in the early growth stage. Seyran-96 and Yerli Kırmızı cultivars were found to be superior to other varieties in terms of plant development and dry matter accumulation. Plant growth, dependent on both symbiotic and non-symbiotic bacterial inoculation, outperformed the control group. When applied as a bio-priming agent in the study, the TV83D strain stimulated plant growth more effectively compared to *Rhizobium leguminosarum*. Pre-seeding bacterial inoculation in lentil cultivation is anticipated to be a sustainable approach contributing to higher yield and quality by promoting plant growth.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Author contribution

SS and ME designed the experimental laid out. SS, ÖU and FÇ run the experiments, collected data and wrote Ms. draft. ME made statistical analysis and contributed writing. FÇ edited Ms. draft and gave feedback. All authors read and approved the final manuscript.

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