Env. Soil Security **Vol. 8,** pp: 147- 163 (2024)

Environment, Biodiversity & Soil Security

http://jenvbs.journals.ekb.eg/

Effects of Co-inoculation of *Bradyrhizobium* and Cyanobacteria Strains on Growth Parameters and Yield of Peanut (*Arachis hypogaea* L.) Plants in Sandy Soils

Naeem M. E. Doha[1*], Gehan M. Salem[1], Naayem M. Elgaml[1], Adel A. Awad[1] and Dina Fathi Ismail Ali[2]

[1] *Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza 12112, Egypt.*

[2] *Department of Microbiology, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt.*

PEANUT plants can obtain N from N_2 fixation via symbiosis with rhizobia, and inoculation with selected strains can improve grain yields. The aim of this work is to conduct field trials to compare single inoculation with *Bradyrhizobium* sp., *Anabaena circinalis*, and *A. variabilis* with dual inoculation during the 2021 and 2022 seasons in order to verify whether microbial inoculants may enhance peanut performance, growth, and grain yield as a result of their advantageous effects. The findings demonstrated that T8 treatment (dual inoculation with *Bradyrhizobium* sp, *A. circinalis* and *A. variabilis* and (1:1:1) + 25% N + 100% PK), generally improved peanut plant growth, resulting in significantly higher chlorophylls (mg g^{-1} FW), carotenoids (µg g^{-1} FW), number of nodules and dry weight of nodules (mg $plant^{-1}$). In addition, improved soil fertility by increasing dehydrogenase and CO_2 evolution, in the peanut rhizosphere during the two growing seasons. On the other hand, the percentages of N, P, and K in peanut plant leaves were affected by various inoculations in ways that were statistically significant ($P \leq 0.05$), which were arranged by T8 > T6 > T7 > T5 for dual inoculation treatments and T2 > T4 > T3 > T1 for single inoculation treatments. The same pattern was observed for microelements (Fe, Cu, Mn and Zn). This was reflected in the yield (plant height, pod number, pod weight, yield, and 100-weight seeds), and the quality of the grain (percentage of oil, carbohydrates and protein). According to the current study, cyanobacteria and *Bradyrhizobium* are helpful in enhancing peanut plant development, physiological changes, biological performance, productivity, and seed quality.

Keywords: Biofertilizers, Biological performance, Peanut, Physiological changes, Yield

1. Introduction

Peanut (*Arachis hypogaea* L.) is considered one of the most important oil plants in Egypt since its seeds have a high nutritional value for humans, the cakes and green hay obtained from them can be used as animal feed, and the oil extracted from peanuts can be used as animal feed. The seeds can also be used for industrial purposes, which is important. The most important cultivation areas are located in the north of the country; they include the dry deserts east and west of the Nile Delta. Peanut seeds are characterized by a high oil content (50%), which is used in various industries. They also contain 26-28% protein, 20% carbohydrates, and 5% fiber **(Fageria et al., 1997; Elbaalawy et al., 2020; Zaki et al., 2021).**

The legume-rhizobia symbiosis is a well-known N_2-fixing plant-microorganism interaction that is thought to be the most significant and effective crop production process, improving soil fertility and the adaptability of farming systems **(Abdalla et al. 2018; Abd-Alla et al., 2023).** Several studies have

*Corresponding author e-mail: na3emdoha@gmail.com
Received: 23/11/2024; Accepted: 8/12/2024

DOI: 10.21608/jenvbs.2024.338639.1257

shown that inoculating legumes with efficient and effective rhizobial strains is essential, particularly in cases when the soil lacks the particular *Rhizobium* agents **(Kebede, 2021; Gebremariam and Tesfa 2021; Fahde, et al., 2023).** The function of rhizosphere organisms in promoting plant growth and biologically controlling soil-borne diseases is currently of great interest **(Kloepper et al., 1989; Vargas et al., 2009; Omara et al. 2017, 2018).** There are a number of commercially available PGPR whose plants have been shown to have growth-promoting properties in a number of ways, such as producing iron-sequestering siderophores and antimicrobial compounds that prevent phytopathogens from colonizing their hosts, inducing resistance to systemic diseases in the host, producing ACC deaminase to lower the amount of ethylene in the roots of developing plants, solubilizing precipitated mineral nutrients, producing plant growth hormones, and/or enhancing the ability of roots to absorb water and nutrients (**Saeed et al., 2021; Ouf et al., 2023; Hasan et al., 2024**).

Although mixed findings have been reported, *Bradyrhizobium* spp. have been identified as the most representative symbiotic rhizobial species for groundnuts **(Bouznif et al., 2019).** Only seven out of twenty tests in the USA where groundnuts had never been grown before saw inoculation increase yields **(Lanier et al., 2005).** In Argentina, BNF by native rhizobia allowed for the achievement of maximum yields, while inoculation of specific strains did not enhance yields when compared to those in noninoculated fields **(Bogino et al., 2006).** In these and other instances, the varying reactions to *Bradyrhizobium* inoculation of peanut may be explained by the crop's fluctuating environmental circumstances **(Asante et al., 2020; Jovino et al., 2022).**

Additionally, producers pursuing sustainable development have been drawn to eubacteria of the phylum Cyanobacteria **(Sutherland et al., 2021; Taira et al., 2021)**, due to their potential applications as foliar fertilizers **(Amatussi et al., 2023),** biofertilizers **(Gavilanes et al., 2020; Horácio et al., 2020; Supraja et al., 2020),** and wastewater bioremediation **(Araujo et al., 2021; Melo et al., 2022).** The earliest creatures to evolve photosynthesis were cyanobacteria, and some species, including *Anabaena* and *Nostoc*, fix nitrogen dioxide. A combination of *Rhizobium tropici*, *R. freirei*, and *Azospirillum brasilense* has also been employed as a co-inoculant in common beans **(Horácio et al., 2020)** and with *A. brasilense* in maize **(Gavilanes et al., 2020).** Though they do not fix nitrogen, some cyanobacteria may aid in crop growth through methods akin to those of the most well-known bacteria that promote plant growth **(Singh et al., 2017; Gavilanes et al., 2021).**

To confirm whether microbial inoculants may improve peanut performance, growth, and grain yield due to their beneficial effects, we carried out field experiments to compare single inoculation with *Bradyrhizobium sp*, *Anabaena circinalis* and *A. variabilis* and with dual inoculation the during 2021 and 2022 seasons.

2. Materials and Methods

The current investigation was conducted during two successive summers in the 2021 and 2022 seasons at the Ismailia Agricultural Research Station (ARC), Ismailia Governorate, Egypt (30° 35′ 41.901″ N and 32° 16′ 45. 843″ E) in a field experiment on sandy soil. The goal of this study was to study the effect of single and dual inoculation with cyanobacteria strains (*Anabaena circinalis* and *Anabaena variabilis*), and *Bradyrhizobium* sp. to improve peanut plant growth, physiological modifications, biological performance, productivity, and seed quality.

The following treatments (8 treatments), were employed in a totally randomized block design with three replicates. 25% of nitrogen fertilizer and 100% potassium (K), and 100% phosphorus (p) were added to the treatments inoculated with *Bradyrhizobium* sp. and *Anabaena* spp. T1: control (100% NPK), T2: *Bradyrhizobium* sp, T3: *A. circinalis*, T4: *A. variabilis*, T5: *A. circinalis* and *A. variabilis* (1:1), T6: *Bradyrhizobium* sp. and *A. circinalis* (1:1), T7: *Bradyrhizobium* sp. and *A. variabilis* (1:1), and T8: *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1).

The inoculation treatments of *Bradyrhizobium* sp. (provided from Biofertilizers Unit, Agricultural Research Station, Giza, Egypt), were prepared as peat-based inoculums, 15 mL of 10^8 CFU mL^{-1} from culture per 30 g of the sterilized carrier and mixed carefully with peanut seeds using a sticking material and spread away from direct sun over a plastic sheet for a short time before sowing. On the other hand, *A. circinalis* and *A. variabilis* were inoculated into 500-ml conical flasks filled with Watanabe's liquid medium in order to prepare the algal inocula **Watanabe et al. (1984).** For seven days, cultures were cultured at 28–30°C under constant light from fluorescent white bulbs that were 120 cm long and illuminated with fluorescence (5500–6650 lux). 0.50 ml of the cyanobacterial culture per bottle was used for the cyanobacteria inoculation. To replicate as much of the natural sunshine as possible, the bottles were incubated for 50 days in a temperature-controlled (30 ± 0.5 °C) lighted compartment with neon and regular lamps. The culture was planted on clay soil for 1 month, and then 400 grams (10^6 cells g^{-1}) were taken from each strain and mixed with 2 sand picks, then spread on the lines immediately after sowing.

Peanut seeds (*Arachis hypogaea* L. cv. Giza 6) were provided from the Leguminous Research Department, Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt. Each plot had five ridges (furrows) with 4.5 m long and 60 cm in width. The experimental unit area was 13.5 m^2 and the hills were 20 cm apart. The sowing date was May 20th for the first season (2021) and 20th May in the second season (2022). Peanut seeds were sown on one side of the ridge and thinning was done after 14 days from sowing to two plants per hill. The physicho-chemical properties were illustrated in Table 1.

Nitrogen basal dose as ammonium sulphate (20.5% N) at rate of 70 kg ha^{-1} was applied after thinning. A basal dose of calcium superphosphate (15.5% P_2O_5) at a rate of 720 kg ha^{-1} was added during soil preparation. Potassium fertilization doses in the form of potassium sulphate (48% K_2O) at a rate of 180 kg ha^{-1} were added at three equal doses, after 30, 60 and 90 days from sowing. Standard agricultural practices for growing peanut crops at Ismailia Governorate were followed and weeds were hand-controlled continuously during peanut vegetative growth as well as no pesticides or fungicides were used during the experiments.

Table 1. Physico-chemical characteristics of the soil used in the agricultural experiments

Physical characteristics (%)					
Sand	**Silt**	**Clay**	**Texture**		
87.09	9.88	3.03	Sandy		
Chemical characteristics					
PH (1:2.5 sups.)	**EC (dS m^{-1}, 1:5)**	**Organic matter (%)**	**Available N (mg kg^{-1})**	**Available P (mg kg^{-1})**	**Available K (mg kg^{-1})**
8.11	1.21	0.13	16.18	1.60	88

Measurements

Total chlorophyll and carotenoids

In accordance with **Lichtenthaler (1987),** 0.1 g of five leaf samples were pulverized and extracted in 5 ml of 80% acetone (70 days after sowing), to measure the total amount of carotenoids and chlorophylls. Following a 10-minute centrifugation at 13,000 xg, the supernatant's wavelengths were measured at 663, 645, and 470 nm. Carotenoids (µg g^{-1} FW) and total chlorophylls (mg g^{-1} FW) were measured.

Nodules parameters

At 70 days from sowing, number of nodules plant^{-1} and dry weight of nodules plant^{-1} were determined

Soil biological activities

Dehydrogenase activity (DHA)

The DHA was calculated by **Chander and Brookes (1991),** after adding 2 g of air-dried soil and 2 ml of tetrazolium chloride solution, the mixture was left in the dark for 24 hours at 30 °C. Using a UV spectrophotometer (Model 6705), triphenyl formazan (TPF) was extracted with 10 milliliters of acetone and measured at 485 nm using the TPF standard curve. The result was represented as µg TPF g^{-1} dry soil day^{-1}.

CO_2 evolution

At 70 days after sowing, CO_2 evolution was calculated using the methodology described by **Colema et al. (1978).** Ten g of sample (2-mm sieve) was put into Erlenmeyer flasks (1-L capacity) and incubated for three days after three replicates of each treatment were pre-incubated for six days at 25° C and 60% moisture. CO_2 evolution was absorbed in a 50 ml beaker that had 25 ml of 0.1 N NaOH solution. Titration with an excess of sodium hydroxide to pH 8.3 using 0.10 N HCl solution was used to quantify the total carbon dioxide, which precipitated as $BaCO_3$ with $BaCl_2$.

Macro- and Micro-Nutrients

Seventy days after sowing, 0.5 g of crushed leaf material was digested on a hot plate using 30% H_2O_2 and concentrated sulfuric acid in accordance with **Jones et al. (1991)** guidelines. The nitrogen (%) was calculated using micro-Kjeldahl **(Peters et al. (2003).** The phosphorus (%) was computed using the spectrophotometric techniques of **Page et al. (1982).** The potassium (%) was calculated using a flame photometer and the methodology of **Cottenie et al. (1982).** Additionally, atomic adsorption spectrophotometry (Perkin Elmer 3300) was used to measure micronutrients such as Zn, Mn, Fe, and Cu in milligrams per kilogram using the procedures described by **Cottenie et al. (1982).**

Yield and yield components

At 120 days from sowing, height of plant (cm), No. of pods plant^{-1}, pods weight (g plant^{-1}), yield of pods (kg ha^{-1}) and 100-weight of pods (g) were determined.

Seeds quality

According to **AOAC (1995),** the percentages of oil % of seeds, total carbohydrates and protein % were calculated after harvested at 120 days from sowing.

Statistical Analyses

ANOVA was applied to the obtained experimental data in accordance with the protocols described by **Duncun (1950)**.

3. Results

3.1 Total chlorophyll and carotenoids

Based on inoculation treatments, peanut leaves' levels of total chlorophyll and carotenoids varied significantly ($P \leq 0.05$) after 70 days after sowing (Figure 1). Data from seasons 2021 and 2022 revealed that the T8 treatment (dual inoculation with *Bradyrhizobium* sp., *A. circinalis*, and *A. variabilis* (1:1:1)) and T6 treatment (dual inoculation with *Bradyrhizobium* sp. and *A. circinal*is (1:1)) had the highest total chlorophyll values of 2.71, 2.31, and 2.87, 2.47 mg g^{-1} FW) over control treatment (T1), respectively (Figure 1A). On the other hand, dual inoculation treatments (T5, T6, T7, and T8) showed the highest values for carotenoids when compared to the same treatments but single inoculation (T1, T2, T3, and T4). For instance, T5, T6, T7, and T8 treatments recorded 0.75, 0.94, 0.91, and 0.96 (µg g^{-1} FW) and 0.84, 1.08, 1.00, and 1.05 for carotenoids (µg g^{-1} FW) in seasons 2021 and 2022, respectively, compared to other treatments that were tested (Figure 1B). When comparing different applications of dual inoculation treatments to single inoculation treatments, the previously indicated data shows that T8 > T6 > T7 > T5.

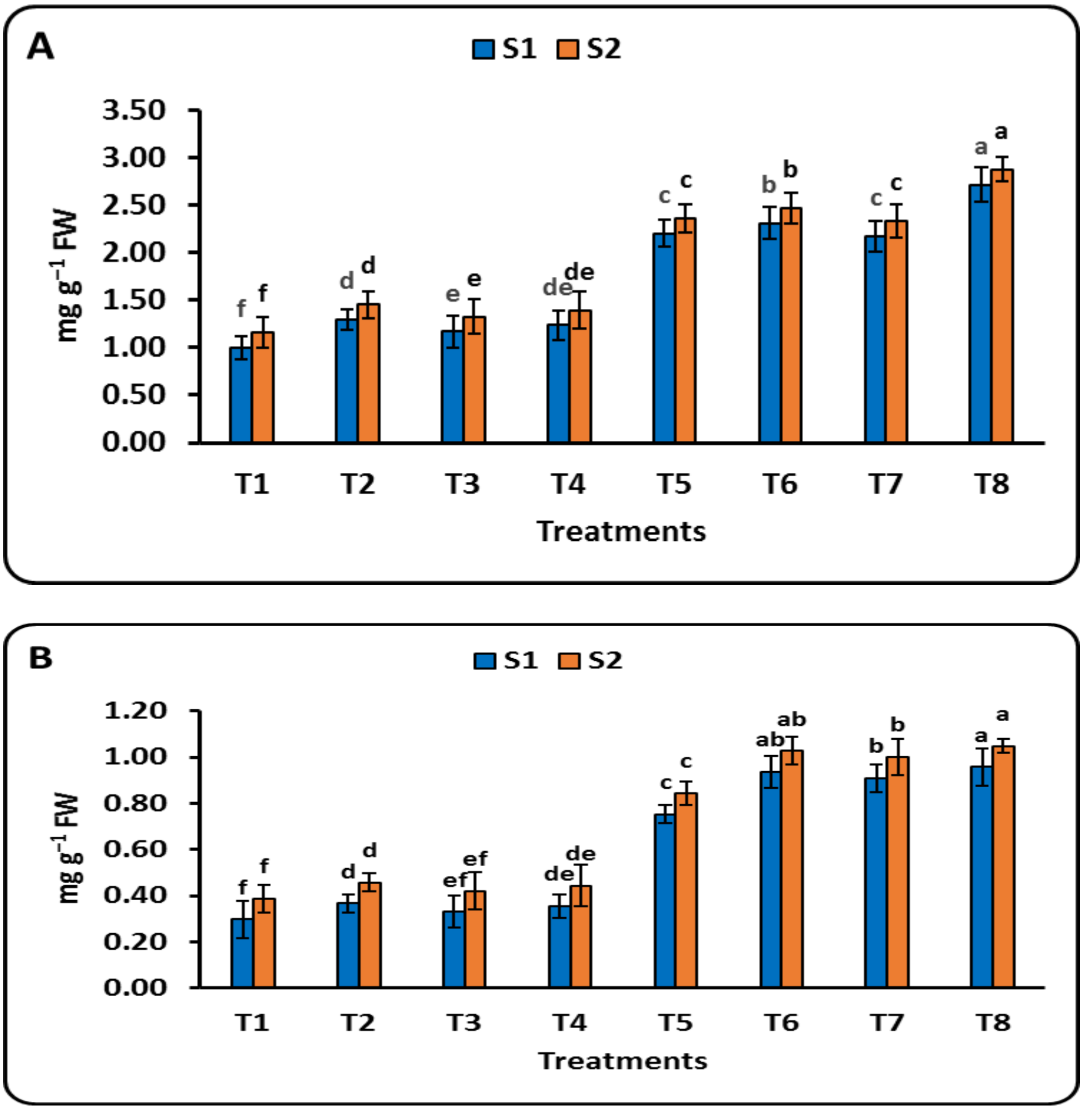

Figure 1: Effect of single and dual inoculation treatments on total chlorophyll (A) and carotenoids (B) in peanuts leaves at 70 days from sowing during 2021 and 2022 seasons. Duncan's test, means with different letters after them denote significant differences ($P \leq 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. **S1**: 2021 season; **S2**: 2022 season. **T1**: control (100% NPK), **T2**: *Bradyrhizobium* sp., **T3**: *A. circinalis*, **T4**: *A. variabilis*, **T5**: *A. circinalis* and *A. variabilis* (1:1), **T6**: *Bradyrhizobium* sp. and *A. circinalis* (1:1), **T7**: *Bradyrhizobium* sp. and *A. variabilis* (1:1), and **T8**: *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1).

3.2 Nodules parameters

After 70 days from sowing, there were notable variations ($P \leq 0.05$) in the number of nodules and dry weight of nodules in peanut plants based on inoculation treatments (Table 2). In seasons 2021 and 2022, data showed that the highest number of nodules values were 201.00 and 204.60 for T8 treatment (dual inoculation with *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1), followed by 166.00 and 169.60 for T6 treatment (dual inoculation with *Bradyrhizobium* sp. and *A. circinalis* (1:1)) over control treatment (T1), respectively (Table 2). In contrast, the T8 treatment showed the highest dry weight of nodules throughout the 2021 and 2022 seasons, with values of 302.00 and 302.60 mg plant-1, respectively, when compared to the other treatments. T8 > T7 > T6 > T5 for dual inoculation treatments and T2 > T4 > T3 > T1 for single inoculation treatments, according to the data previously mentioned (Table 2).

Table 2: Effect of single and dual inoculation treatments on number of nodules and dry weight of nodules in peanuts plants at 70 days from sowing during 2021 and 2022 seasons

Treatments	Number of nodules plant^{-1}		Dry weight of nodules (mg plant^{-1})	
	S1	**S2**	**S1**	**S2**
T1	51.53 ± 2.33 g	55.13 ± 3.01 g	152.53 ± 2.22 g	151.13 ± 1.98 g
T2	109.39 ± 2.78 e	112.99 ± 3.78 e	210.39 ± 3.10 e	208.99 ± 3.76 e
T3	69.67 ± 1.98 f	73.27 ± 2.65 f	170.67 ± 2.98 f	169.27 ± 4.11 f
T4	80.33 ± 2.38 f	83.93 ± 2.13 f	181.33 ± 3.65 f	179.93 ± 3.76 f
T5	139.33 ± 2.65 d	142.93 ± 3.09 d	240.33 ± 3.21 d	238.93 ± 3.56 d
T6	166.00 ± 3.81 c	169.60 ± 3.11 c	267.00 ± 2.87 c	265.60 ± 2.98 c
T7	183.67 ± 3.98 b	187.27 ± 2.87 b	284.67 ± 3.98 b	283.27 ± 4.10 b
T8	201.00 ± 4.67 a	204.60 ± 3.87 a	302.00 ± 3.34 a	300.60 ± 4.25 a
LSD 0.05	11.04	10.87	12.34	11.09

Duncan's test, means with different letters after them denote significant differences ($P \leq 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. **S1**: 2021 season; **S2**: 2022 season. **T1**: control (100% NPK), **T2**: *Bradyrhizobium* sp., **T3**: *A. circinalis*, **T4**: *A. variabilis*, **T5**: *A. circinalis* and *A. variabilis* (1:1), **T6**: *Bradyrhizobium* sp. and *A. circinalis* (1:1), **T7**: *Bradyrhizobium* sp. and *A. variabilis* (1:1), and **T8**: *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1).

3.3 Soil biological activities

The quantities of dehydrogenase (DHA) and CO_2 evolution in the rhizosphere of peanut plants depending on inoculation treatments varied significantly ($P \leq 0.05$) after 70 days after planting (Figure 2). Data from seasons 2021 and 2022 revealed that the T8 treatment (dual inoculation with *Bradyrhizobium* sp, *A. circinalis*, and *A. variabilis* (1:1:1)) and T6 treatment (dual inoculation with *Bradyrhizobium* sp. and *A. circinalis* (1:1)) had the highest DHA values (268.00, 276.40, and 183.33, 191.73 µg TPF g^{-1} dry soil day^{-1}), respectively (Fig. 2A). On the other hand, dual inoculation treatments (T5, T6, T7, and T8) showed the highest values for CO_2 when compared to the same treatments but single inoculation (T1, T2, T3, and T4). For instance, T5, T6, T7, and T8 treatments reported CO_2 evolution in season 2021 at 148.67, 150.34, 148.21, and 155.89 mg CO_2/100 g soil, respectively, compared to other treatments that were evaluated (Figure 2B). The 2022 season had the same pattern. When comparing different applications of dual inoculation treatments to single inoculation treatments, the previously indicated data shows that T8 > T6 > T7 > T5.

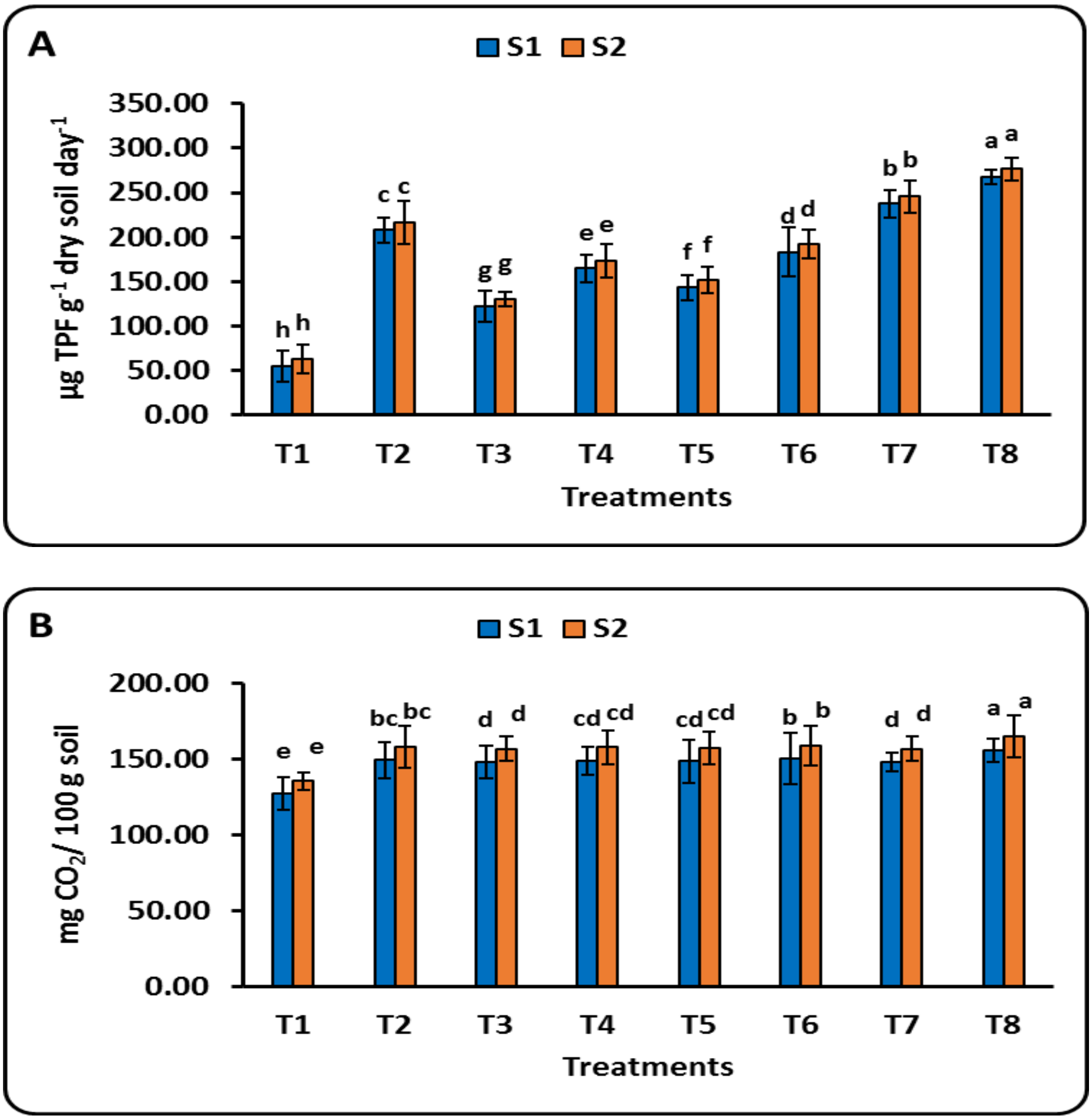

Figure 2: Effect of single and dual inoculation treatments on soil biological activities; DHA (A), and CO_2 evolution (B) in rhizosphere of peanuts plants at 70 days from sowing during 2021 and 2022 seasons. Duncan's test, means with different letters after them denote significant differences ($P \leq 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. **S1**: 2021 season; **S2**: 2022 season. **T1**: control (100% NPK), **T2**: *Bradyrhizobium* sp., **T3**: *A. circinalis*, **T4**: *A. variabilis*, **T5**: *A. circinalis* and *A. variabilis* (1:1), **T6**: *Bradyrhizobium* sp. and *A. circinalis* (1:1), **T7**: *Bradyrhizobium* sp. and *A. variabilis* (1:1), and **T8**: *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1).

3.4 N, P and K in peanut leaves

Throughout both growth seasons, the percentages of N, P, and K in peanut plant leaves were affected by various inoculations in ways that were statistically significant ($P \leq 0.05$) (Table 3). When compared to T1 treatment (control), which yielded 1.42 and 1.50% during the 2021 and 2022 seasons, respectively, T2 treatment produced the highest percentage of N, attained 2.01 and 2.09% under single inoculation with *Bradyrhizobium* sp + 25% N + 100% PK and T4 treatment, which involved single inoculation with *A. variabilis* + 25% N + 100% PK, attained 1.97 and 2.05% (Table 2). In contrast, T8 treatment (dual inoculation with *Bradyrhizobium* sp, *A. circinalis*, and *A. variabilis* (1:1:1)) and T6 treatment (dual inoculation with *Bradyrhizobium* sp. and *A. circinalis* (1:1)) produced the highest percentage of N at 2.98 and 3.08 in seasons 2021 and 2022, respectively (Table 3). On the other hand, during the 2021 and 2022 seasons, the T8 therapy had the greatest P and K percentages compared to the other treatments, with values of 0.90 and 0.94 percent and 2.63 and 2.69 percent, respectively. T8 > T6 > T7 > T5 for dual inoculation treatments and T2 > T4 > T3 > T1 for single inoculation treatments, according to the data previously mentioned (Table 3).

Table 3: Effect of single and dual inoculation treatments on N, P and K % in peanuts leaves at 70 days from sowing during 2021 and 2022 seasons

Treatments	N		P		K	
	S1	S2	S1	S2	S1	S2
T1	1.42 ± 0.19 e	1.50 ± 0.33 e	0.24 ± 0.09 f	0.28 ± 0.02 f	1.07 ± 0.13 e	1.13 ± 0.23 e
T2	2.01 ± 0.15 bc	2.09± 0.18 bc	0.29 ± 0.04 de	0.33 ± 0.03 de	1.66± 0.22 bc	1.72 ± 0.31 bc
T3	1.81 ± 0.22 d	1.89 ± 0.31 d	0.31 ± 0.02 d	0.35 ± 0.04 d	1.46 ± 0.19 d	1.52 ± 0.32 d
T4	1.97 ± 0.28 cd	2.05± 0.27 cd	0.27 ± 0.03 ef	0.31 ± 0.05 ef	1.62± 0.12 cd	1.68 ± 0.27 cd
T5	1.89 ± 0.27 cd	1.97± 0.14 cd	0.69 ± 0.11 c	0.73 ± 0.06 c	1.54± 0.23 cd	1.60 ± 0.26 cd
T6	2.14 ± 0.31 b	2.22 ± 0.25 b	0.88 ± 0.06 ab	0.92 ± 0.07 ab	1.79 ± 0.27 b	1.85 ± 0.35 b
T7	1.82 ± 0.25 d	1.90 ± 0.22 d	0.85 ± 0.09 b	0.89 ± 0.03 b	1.47 ± 0.33 d	1.53 ± 0.33 d
T8	2.98 ± 0.31 a	3.06 ± 0.18 a	0.90 ± 0.04 a	0.94 ± 0.08 a	2.63 ± 0.37 a	2.69 ± 0.17 a
LSD 0.05	0.16	0.14	0.03	0.04	0.16	0.14

Duncan's test, means with different letters after them denote significant differences ($P \leq 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. **S1**: 2021 season; **S2**: 2022 season. **T1**: control (100% NPK), **T2**: *Bradyrhizobium* sp., **T3**: *A. circinalis*, **T4**: *A. variabilis*, **T5**: *A. circinalis* and *A. variabilis* (1:1), **T6**: *Bradyrhizobium* sp. and *A. circinalis* (1:1), **T7**: *Bradyrhizobium* sp. and *A. variabilis* (1:1), and **T8**: *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1).

3.5 Fe, Cu, Mn and Zn in peanut leaves

Plant microelements, such as Fe, Cu, Mn, and Zn, were significantly ($P \leq 0.05$) increased in peanut plants exposed to various inoculation treatments (Table 4). In comparison to the control treatment during the 2021 season, the combined treatment of T8 produced considerably greater levels of Fe, Cu, Mn, and Zn (mg Kg^{-1}) in peanut leaves by day 70 after sowing, reaching record amounts of 91.34, 6.75, 40.76, and 51.84, respectively. During the 2022 season, this pattern was also noticeable (Table 4). Consequently, the T8 treatment (dual inoculation with *Bradyrhizobium* sp, *A. circinalis*, and *A. variabilis* and (1:1:1)) showed the maximum uptake of microelements in peanut plants when compared to other treatments under investigation. Table 4 shows that the treatments were in decreasing order: T8 > T6 > T7 > T5. The highest Fe values were obtained by T2 treatment (single inoculation with *Bradyrhizobium* sp.) attained 66.78 and 69.99 mg Kg^{-1}, followed by T4 treatment (single inoculation with *A. variabilis*) attained 65.70 and 68.91 mg Kg^{-1}, in comparison to T1 treatment (control), which obtained 61.44 and 64.65 mg Kg^{-1} during the 2021 and 2022 seasons, respectively (Table 4). Cu, Mn, and Zn elements showed a similar pattern.

3.6 Yield and yield components

Based on inoculation treatments, peanut plants' yield and yield components varied significantly ($P \le 0.05$) after 120 days of sowing (Table 5). In general, dual inoculation treatments (T5, T6, T7, and T8) showed the highest values for yield and yield components when compared to the single inoculation treatments (T1, T2, T3, and T4). The highest plant height (cm plant^{-1}), number of pods plant^{-1}, pod weight (g plant^{-1}), yield of pods (kg ha^{-1}), and 100-weight of pods (g) were found to be 69.75, 53.76, 49.79, 4.03, and 167.89 for T8 treatment (dual inoculation with *Bradyrhizobium* sp, *A. circinalis*, and *A. variabilis* and (1:1:1)), and 67.90, 51.93, 47.95, 3.19, and 162.43 for T6 treatment (dual inoculation with *Bradyrhizobium* sp. and *A. circinalis* (1:1)) over control treatment (T1), respectively (Table 5). In the 2022 season, the same phenomenon was noted. For different applications of dual inoculation treatments, T8 > T6 > T7 > T5 in comparison to single inoculation treatments, as evidenced by the results previously mentioned (Table 5).

Table 4: Effect of single and dual inoculation treatments on microelements (Fe, Cu, Mn and Zn, mg kg^{-1}), in peanuts leaves at 70 days from sowing during 2021 and 2022 seasons

Treatments	**Fe**		**Cu**		**Mn**		**Zn**	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
T1	61.44 ± 2.32 f	64.65 ± 2.22 f	3.33 ± 0.55 e	3.55 ± 0.54 e	18.07 ± 0.98 f	19.29 ± 0.78 f	21.94 ± 1.03 f	23.13 ± 1.01 f
T2	66.78 ± 3.54 d	69.99 ± 2.87 d	4.61 ± 0.47 bc	4.83 ± 0.56 bc	22.17 ± 0.88 d	23.39 ± 1.03 d	27.28 ± 1.32 d	28.47 ± 1.22 d
T3	64.50 ± 3.11 e	67.71 ± 2.67 e	4.19 ± 0.32 d	4.41 ± 0.55 d	20.42 ± 1.02 e	21.64 ± 0.98 e	25.00 ± 1.11 e	26.19 ± 1.10 e
T4	65.70 ± 3.32 de	68.91 ± 2.11 de	4.52 ± 0.45 cd	4.74 ± 0.34 cd	21.34 ± 1.09 de	22.56 ± 0.94 de	26.20 ± 1.21 de	27.39 ± 1.12 de
T5	83.10 ± 2.87 c	86.31 ± 3.10 c	4.36 ± 0.23 cd	4.58 ± 0.86 cd	34.68 ± 0.84 c	35.90 ± 1.02 c	43.60 ± 1.22 c	44.79 ± 2.11 c
T6	85.08 ± 2.65 b	88.29 ± 3.12 b	4.91 ± 0.34 b	5.13 ± 0.59 b	36.20 ± 1.01 b	37.42 ± 1.23 b	45.58 ± 1.56 b	46.77 ± 2.08 b
T7	82.56 ± 3.01 c	85.77 ± 3.67 c	4.20 ± 0.56 d	4.42 ± 0.87 d	34.27 ± 1.65 c	35.49 ± 1.34 c	43.06 ± 2.01 c	44.25 ± 2.23 c
T8	91.34 ± 3.10 a	94.55 ± 3.27 a	6.75 ± 0.67 a	6.96 ± 0.54 a	40.76 ± 1.45 a	41.98 ± 1.52 a	51.84 ± 2.08 a	53.03 ± 2.43 a
LSD 0.05	1.27	1.23	0.36	0.35	0.97	0.93	1.27	1.25

Duncan's test, means with different letters after them denote significant differences ($P \le 0.05$) between the treatments. SD: means ± standard deviation, which derived from three replicates. S1: 2021 season; S2: 2022 season. **T1**: control (100% NPK), **T2**: *Bradyrhizobium* sp., **T3**: *A. circinalis*, **T4**: *A. variabilis*, **T5**: *A. circinalis* and *A. variabilis* (1:1), **T6**: *Bradyrhizobium* sp. and *A. circinalis* (1:1), **T7**: *Bradyrhizobium* sp. and *A. variabilis* (1:1), and **T8**: *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1).

Table 5: Effect of single and dual inoculation treatments on yield and yield components in peanuts plants at 120 days from sowing during 2021 and 2022 seasons

Treatments	Height (cm plant^{-1})		Pod number		Pod weight (g plant^{-1})		Pod Yield (Kg ha^{-1})		100 pod weight (g)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
T1	61.33 ± 2.45 e	61.53 ± 2.11 e	45.33 ± 1.66 e	46.53 ± 1.34 e	41.33 ± 1.01 e	43.53 ± 1.11 e	2.47 ± 0.22 e	2.52 ± 0.16 e	139.58 ± 1.33 e	144.70 ± 1.22 e
T2	67.61 ± 2.67 bc	68.81 ± 2.37 bc	51.61 ± 2.11 bc	53.81 ± 2.11 bc	47.61 ± 1.56 bc	50.81 ± 1.29 bc	3.06 ± 0.12 bc	3.11 ± 0.17 bc	161.44 ± 1.98 bc	167.36 ± 1.26 bc
T3	67.19 ± 2.34 d	68.39 ± 2.32 d	51.19 ± 1.23 d	53.39 ± 1.76 d	47.19 ± 1.09 d	50.39 ± 1.09 d	2.86 ± 0.19 d	2.91 ± 0.15 d	160.16 ± 1.23 d	166.03 ± 1.65 d
T4	67.52 ± 2.29 cd	68.72 ± 2.56 cd	51.52 ± 1.89 cd	53.72 ± 2.01 cd	47.52 ± 1.22 cd	50.72 ± 1.23 cd	3.02 ± 0.15 cd	3.07 ± 0.14 cd	161.17 ± 1.45 cd	167.08 ± 1.53 cd
T5	67.36 ± 2.88 cd	68.56 ± 2.76 cd	51.36 ± 2.18 cd	53.56 ± 2.89 cd	47.36 ± 1.23 cd	50.56 ± 1.98 cd	2.94 ± 0.21 cd	2.99 ± 0.22 cd	160.67 ± 2.09 cd	166.56 ± 1.42 cd
T6	67.90 ± 2.48 b	69.11 ± 2.73 b	51.93 ± 2.76 b	54.11 ± 2.34 b	47.95 ± 1.26 b	51.11 ± 1.65 b	3.19 ± 0.16 b	3.24 ± 0.23 b	162.34 ± 2.09 b	168.29 ± 1.86 b
T7	67.20 ± 2.54 d	68.40 ± 2.18 d	51.20 ± 2.87 d	53.40 ± 2.87 d	47.20 ± 1.32 d	50.40 ± 1.97 d	2.87 ± 0.23 d	2.92 ± 0.21 d	160.21 ± 1.99 d	166.09 ± 2.01 d
T8	69.75 ± 2.76 a	70.96 ± 2.87 a	53.76 ± 2.34 a	55.96 ± 2.56 a	49.79 ± 1.45 a	52.96 ± 2.02 a	4.03 ± 0.25 a	4.08 ± 0.26 a	167.89 ± 2.14 a	174.05 ± 2.16 a
LSD 0.05	0.36	0.35	0.33	0.36	0.34	0.35	0.16	0.18	1.08	1.12

Duncan's test, means with different letters after them denote significant differences ($P \leq 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. S1: 2021 season; S2: 2022 season. **T1**: control (100% NPK), **T2**: *Bradyrhizobium* sp., **T3**: *A. circinalis*, **T4**: *A. variabilis*, **T5**: *A. circinalis* and *A. variabilis* (1:1), **T6**: *Bradyrhizobium* sp. and *A. circinalis* (1:1), **T7**: *Bradyrhizobium* sp. and *A. variabilis* (1:1), and **T8**: *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1).

3.7 Seeds quality

Throughout both growing seasons, the percentages of oil, carbohydrates, and protein in peanut seeds were affected by various inoculations in ways that were statistically significant ($P \leq 0.05$) (Figure 3). Under single inoculation with *Bradyrhizobium* sp + 25% N + 100% PK, T2 treatment produced the highest percentage of oil at 53.81 and 54.01%. T4 treatment (single inoculation with *A. variabilis*) produced 53.72 and 53.92%, respectively, in comparison to T1 treatment (control), which produced 46.53 and 46.73% during the 2021 and 2022 seasons (Figure 3). In contrast, T8 treatment (dual inoculation with *Bradyrhizobium* sp., *A. circinalis*, and *A. variabilis* and (1:1:1)) produced the highest percentage of oil at 55.96 and 56.16 percent under dual inoculation, while T6 treatment (dual inoculation with *Bradyrhizobium* sp. and *A. circinalis* (1:1)) produced 54.11 and 54.31% in seasons 2021 and 2022, respectively (Figure 3). On the other hand, the T8 treatment had the largest percentage of protein and carbs compared to the other treatments, with values of 14.08 and 14.30 percent and 22.58 and 22.80 percent during the 2021 and 2022 seasons, respectively. T8 > T6 > T7 > T5 for multiple inoculation treatments and T2 > T4 > T2 > T1 for single inoculation treatments, according to the previously mentioned results (Figure 3).

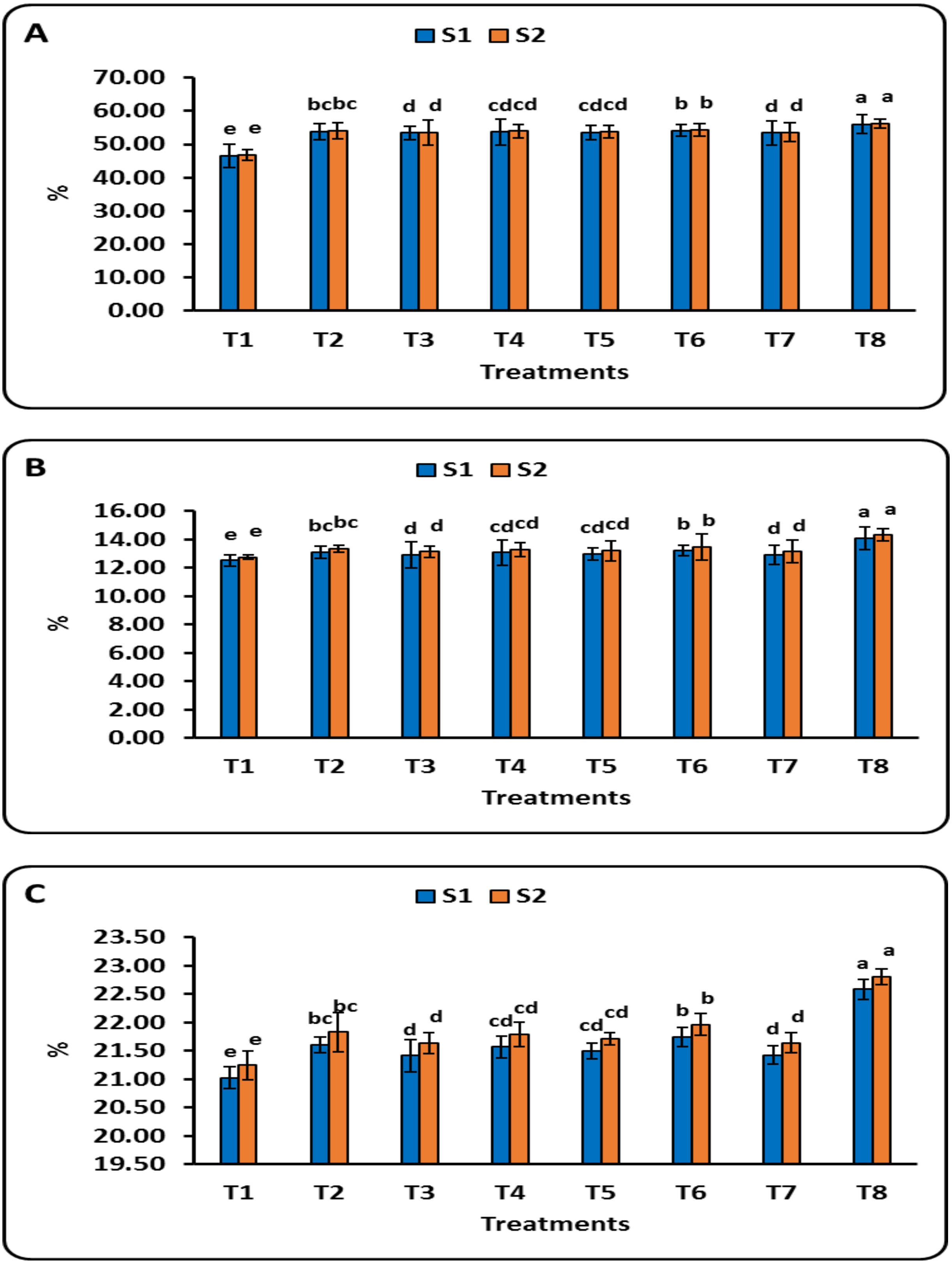

Figure 3: Effect of single and dual inoculation treatments on seed quality (oil, carbohydrates and proteon %), in peanuts seeds at 120 days from sowing during 2021 and 2022 seasons. Duncan's test, means with different letters after them denote significant differences ($P \leq 0.05$) between the treatments. SD: means ± standard deviation which derived from three replicates. S1: 2021 season; S2: 2022 season. **T1**: control (100% NPK), **T2**: *Bradyrhizobium* sp., **T3**: *A. circinalis*, **T4**: *A. variabilis*, **T5**: *A. circinalis* and *A. variabilis* (1:1), **T6**: *Bradyrhizobium* sp. and *A. circinalis* (1:1), **T7**: *Bradyrhizobium* sp. and *A. variabilis* (1:1), and **T8**: *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* (1:1:1).

4. Discussion

This study emphasizes how important it is to use cyanobacteria and *Bradyrhizobium* together to promote peanut plant growth. In addition to boosting soil health, *Bradyrhizobium* helps fix nitrogen in the soil, which enhances soil fertility. Cyanobacteria, on the other hand, promote plant growth. Thus, when these two organisms cooperate, plants consume more nutrients. The amounts of total chlorophyll and carotenoids in peanut leaves showed an increase based on inoculation by T8 treatment (dual inoculation with *Bradyrhizobium* sp., *A. circinalis* and *A. variabilis* and (1:1:1)), over control treatment (Figure 1). The efficiency of *Bradyrhizobium* to fix nitrogen in peanuts resulted in the accumulation of nitrogen in plant tissues, which in turn reflected the synthesis of chlorophyll **(Nageswara, et al. 2001)**. According to **Song et al. (2005),** cyanobacteria play an important role in the maintenance and development of soil fertility, and thus serve as a natural biofertilizer. The primary functions of blue-green algae are make adhesive that are produced in porous soil, excreta of phytohormones (auxin, gibberellins, and so on), vitamins, and amino acids **(Al-Sherif et al. 2015).** On the other hand, our study found significant variations in dehydrogenase and CO_2 levels in peanut plants' rhizosphere after 70 days of sowing, with the highest values observed for T8 and T6 treatments compared to the control treatment in 2021 and 2022 (Figure 2). These increases of dehydrogenase and CO_2 activity might be due to the support of cyanobacteria, either singly or mixed with *Bradyrhizobium*. Such contribution encouraged all endogenous microorganisms in the soil and also stimulated the root growth of the legume plants, since cyanobacteria represented a source of organic matter which favours the activity of the majority of soil microorganisms. Dehydrogenase activity depends on the metabolic state of soil microorganisms **(Nain et al., 2010; Ghazal et al. 2022; Salem et al. 2023).**

Furthermore, this is consistent with earlier research by **Wu et al. (2019),** which discovered that PGPR application raised the microbial biomass's C content. However, elevated DHA indicates that PGPR treatment enhances soil enzyme activity, particularly dehydrogenase activity, as well as soil microbial activity **(Wu et al. 2019; Toor et al. 2024).**

Additionally, our study found significant effects of inoculation on N, P, and K levels in peanut plant leaves during both growth seasons (Table 2). The use of chemical nitrogenous fertilizers in agriculture is a global concern, prompting the search for alternatives like biological nitrogen fixation (BNF), a microbiological process that reduces external inputs and improves internal resources **(Pabbi, 2015)**. Due to complimentary processes of plant growth promotion, coinoculation of *Bradrhizobium* with cyanobacteria has frequently been proposed as an additive strategy for enhancing symbiosis and BNF in legumes **(Ahemad and Kibret, 2014; Gavilanes et al., 2020).** *Bradyrhizobium* and other cooperative microorganisms, such as *Azospirillum brasilense* **(Gericó et al., 2020),** *Bacillus* species **(Figueredo et al., 2014; Preyanga et al., 2021; Kaschuk et al., 2022),** *Serratia marcescens*, and *Trichoderma harzianum* **(Badawi et al., 2011),** have been successfully coinoculated peanuts on a number of nodules. Coinoculation can have direct or indirect effects on nodule development; for instance, a meta-analysis found that *Bradyrhizobium* and cyanobacteria coinoculation greatly increases groundnut root and shoot sizes and this was reflected in the leaves' content of phosphorus and potassium **(Andrade et al., 2024).**

Concurrently, our study highlights how crucial it is to combine cyanobacteria and *Bradrhizobium* to improve peanut nutritional absorption (Table 3). The capacity of PGPR to enhance the absorption, solubility, and bioavailability of vital minerals, such as P, K, Zn, and Fe, is responsible for this improvement in plant nutrition. Furthermore, enhanced nutritional intake is a result of the capacity to physiologically fix atmospheric N_2 (**Acurio Vasconez et al. 2020; Basu et al. 2021).** Additionally, cyanobacteria and *Bradrhizobium* have the capacity to promote the synthesis of gibberellins, which are strongly related to yield enhancement, as well as phytohormones like cytokinins and IAA. To improve overall plant growth and development, these phytohormones are required **(Khatoon et al. 2020; Ikiz et al. 2024),** as well as stimulate the production and elimination of numerous chemical substances such as siderophores, volatile chemical compounds, and hydrolytic enzymes such as cellulases, pectinases, proteases, and chitinases **(Lyu et al., 2023).**

Our findings (Table 4), are consistent with the context that cyanobacteria and *Bradrhizobium* additive inoculation has been demonstrated to enhance the growth and yield of peanut plants, as demonstrated by improvements in key plant growth parameters such as height, number of pod, pods weight, yield and 100-weight of pods **(Abd El-Haliem et al. 2022; Ikiz et al. 2024)**. The symbiotic relationship between bacteria and roots reveals a dynamic interaction demonstrates the ability to produce phytohormones, particularly IAA, which is essential for supporting plants' vegetative growth **(Chauhan et al. 2023).** Additionally, the positive effects of microbes cover important activities like atmospheric N_2 fixation and potassium and phosphate solubilization in the nutritional solution. These mechanisms work together to promote peanut plants' overall growth **(Reid et al. 2021). El-Howeity and Abdel-Gawad (2017),** showed that inoculation with *Bradyrhizobium* or cyanobacteria improved plant growth parameters, but co-inoculation with cyanobacteria significantly enhanced these parameters. Mixed inoculation improved dehydrogenase activity, seed content, and yield, with the highest yield and components recorded. **Andrade et al. (2024)** provided evidence in support of this claim by four field experiments under subtropical that confirmed the effectiveness of microbial inoculants in improving groundnut performance. Single inoculation with *Bradyrhizobium arachidis*, coinoculation with *Arthrospira platensis* or *Synechocystis* sp., or N fertilization increased plant growth, N accumulation, and yield. In addition, increases in the number of pods, dry weight of pods and 100 seeds weight were certainly referred to as microbial coinoculation, which improved soil structure and porosity via secretion of polysaccharides and mucilage **(Nain et al., 2010).** A similar finding, confirming that the coinoculation increased yield of legumes, was observed by **Sanchez et al. (2014)** and **Zimmer et al. (2016)** who reported that, coinoculation with beneficial microorganisms increased yield and protein content of *Phaseolus vulgares* and soybean (Figure 3).

5. Conclusions

According to the current study, cyanobacteria and *Bradyrhizobium* are helpful in enhancing peanut plant development, physiological changes, biological performance, productivity, and seed quality. Therefore, it is advised that cyanobacteria and *Bradyrhizobium* biofertilizers be used in agriculture as natural, renewable nitrogen-fixing fertilizers for a variety of agricultural plants. They use renewable resources in addition to free solar energy, atmospheric nitrogen, and water, and they are affordable and non-polluting. The study's findings unequivocally showed that applying *Bradyrhizobium* and cyanobacteria together produced the best plant growth, yield, and yield qualities when compared to applying either kind alone. In sandy soil conditions, this combination may also lower the quantity and expense of N mineral fertilizer used for peanuts, maintaining soil health in the process. Additionally, by simulating the natural ecological interactions between crop plants and soil organisms, these linkages will aid in the development of agro-ecosystems with less need for pesticide inputs.

Ethics approval and consent to participate: None of the authors of this article have ever conducted any research on humans or animals.

Consent for publication: All authors declare their consent for publication.

Funding: No external funding.

Conflicts of Interest: No conflict of interest.

Contribution of Authors: Each author contributed to the manuscript's composition, editing, and revisions and approved of its release.

Acknowledgements: The support from the Soils, Water, and Environment Research Institute, ARC, Egypt is greatly appreciated by all of the authors.

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