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## Synergistic Effects of Plant Growth Promoting Rhizobacteria and Mycorrhizal Fungi on Roselle Performance in Sandy Soils

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ROSELLE cultivation in sandy soils faces major challenges like poor water and nutrient retention, negatively impacting plant growth and yield. This study aimed to evaluate the potential of microbial inoculants to improve roselle productivity in nutrient-deficient sandy soils. A field experiment was conducted in Egypt over two seasons using eight microbial treatments: *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pleurotus ostreatus*, Mycorrhizeen (mycorrhizal) fungi, and combinations thereof, along with a non-inoculated control. Microbial inoculants were applied as seed treatment and soil drench to improve sandy soil fertility. All microbial inoculants significantly increased fresh and dry calyx yield, shoot growth, seed yield, and overall biomass versus the control. *Bacillus subtilis* gave the greatest enhancement in yield parameters. Combining *Bacillus*, *Pseudomonas*, *Pleurotus* and mycorrhizae led to further synergistic yield improvements up to 332% over control. Microbial inoculation also dramatically increased nitrogen and potassium uptake by up to 724% and utilization efficiency compared to control plants. Results demonstrate the excellent potential of microbial inoculants to significantly improve roselle productivity and nutrition in nutrient-deficient sandy soils through synergistic promotion of soil fertility and plant growth. Microbial inoculation could provide a sustainable solution to challenges of roselle cultivation in poor sandy soils.

**Keywords:** Beneficial Microorganisms; Nutrient Use Efficiency; Plant Growth-Promoting Rhizobacteria; Roselle; Soil Fertility.

### 1. Introduction

Roselle (*Hibiscus sabdariffa* L.) is an economically important medicinal and food crop, with its leaves and flowers used extensively in food and pharmaceutical industries (El Naim et al., 2017). Due to population growth and increasing demand for roselle products, enhancing productivity of this crop through sustainable agricultural practices has become imperative (Rehman et al., 2015). However, roselle cultivation in sandy soils faces major challenges like poor water and nutrient retention, negatively impacting plant growth and yield (Attia, 2018). Therefore, novel agricultural strategies to improve sandy soil fertility and support roselle crop growth are urgently needed.

The relevance of medicinal (Hegazi et al., 2023) and aromatic plants (Sarhan and Shehata, 2023) are derived from their extensive range of applications in several domains, including medicine, treatment, nutrition, cosmetics, and the manufacturing of enjoyable beverages. Furthermore, these compounds are employed within the food industry for their natural flavour-enhancing and scent-enhancing properties. These agricultural export commodities hold significant importance in terms of generating foreign currency revenue and meeting the demands of the European market (Gaafar et al., 2021). The agricultural exports of Roselle contribute to around 6.6% of the overall Egyptian agricultural exports (FAO, 2018). Roselle, scientifically referred to as *Hibiscus sabdariffa* L., is a tropical plant belonging to the Malvaceae family. It is often recognised as Karkade in Egypt.

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The Roselle plant is a biennial species that reaches maturity within a span of around six months (Islam et al., 2016).

The phenomenon of substantial population expansion is concomitantly occurring alongside heightened levels of food consumption (Abdallah et al., 2023; Farid et al., 2023). The decline in soil fertility can be attributed to the intense agricultural practises and extensive cultivation of crops. Consequently, the maintenance of soil fertility and preservation of its quality are vital in order to enhance crop productivity, which is presently experiencing a decline (Elsherpiny et al., 2022; Sarhan and Bashandy, 2021). The addition of a specific substance to the soil has the potential to enhance its physical characteristics, transforming it from a poor state to a thriving condition.

The implementation of effective reclamation technologies for managing sandy soils holds significant importance in an environment where the accessible land area for cultivation is continuously diminishing. This is primarily due to the need to enhance crop output and productivity. Sandy soil management presents several challenges, such as its notable permeability and restricted capacity for water and nutrient retention. The fundamental objective of improving sandy soil is to effectively manage the utilization of irrigation water and plant nutrients (Selim et al., 2009). The soils of Egypt are predominantly composed of sand, exhibiting limited fertility and a relatively low capacity to retain water. Additionally, there is a scarcity of mineral fertilizers in the region.

Research has shown beneficial soil microorganisms like plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi can enhance soil fertility and plant nutrient uptake through various mechanisms (Chauhan et al., 2021). For instance, *Bacillus subtilis* stimulates plant growth and stress tolerance by producing beneficial compounds and fixing nitrogen (Liu et al., 2023; Yi et al., 2022). Mycorrhizal fungi increase nutrient absorption like phosphorus via the extensive hyphal network (Smith and Read, 2010).

Soil microorganisms play a crucial role in the soil ecosystem and are believed to facilitate the process of material transformation inside the soil. The assessment of soil quality is contingent upon the structural composition and diversity of microbial communities (Zhao et al., 2022). Soil microorganisms have a crucial role in facilitating the processes of mineralization and nutrient solubilization through biogeochemical cycling. Moreover, they exert significant influence on the chemical and biological characteristics of the soil, ultimately leading to enhanced soil fertility. Plant Growth-Promoting Rhizobacteria (PGPR) exert their effects through direct mechanisms such as enhancing plant nutrient uptake and elevating hormone levels on the plant surface. Additionally, they can also exert indirect effects by mitigating the incidence of diseases caused by bacterial and fungal plant pathogens (Sindhu and Sharma, 2020). The survival, persistence, and establishment of plant PGPR strains in the rhizosphere are greatly influenced by many physicochemical factors of the soil. These parameters include soil pH, release of root exudates, water potential, and partial pressure of $O_2$ (Sindhu and Sharma, 2020). These substances facilitate the growth of plants, enhance the absorption of nutrients, provide resistance against non-living environmental factors, and inhibit the occurrence of diseases. The microbiotas exhibit a self-sustaining nature, necessitating less applications and circumventing the issue of pest and disease resistance to treatments (Chaparro et al., 2012).

*Bacillus subtilis* is a commonly employed plant growth-promoting rhizobacterium (PGPR) that has demonstrated efficacy in enhancing both plant growth and production. The plant is able to mitigate stress through various mechanisms, including induced systemic resistance (ISR), formation of biofilms, creation of lipopeptides, siderophores, and exopolysaccharides. In the agro-ecosystem, this agent serves as a valuable denitrifying agent, contributing to the maintenance of soil health through the implementation of eco-friendly rejuvenation technologies (Mahapatra et al., 2022).
The presence of mycorrhizal hyphae enhances nutrient absorption by optimising soil volume utilization and modulating soil chemical properties. Therefore, the introduction of mycorrhizal inoculation in plants enhances the efficiency of nutrient absorption, namely for phosphorus, nitrogen, magnesium, zinc, iron, copper, potassium, sulphur, and various other ions (Fallahi et al., 2017). Moreover, the presence of Mycorrhizal hyphae serves as an additional mechanism for the absorption of nutrients and water in plants that have been inoculated, especially in the case of elements with limited mobility in the soil, such as phosphorus (Sonar et al., 2013). According to research conducted on the roselle plant, the application of mycorrhizal inoculation resulted in a significant increase of 6% and 19% in the leaf nitrogen and phosphorus levels, respectively (Vashvaei et al., 2015).

*Pleurotus ostreatus* exhibits the capacity to enlarge air spaces inside soil and infiltrate micropores, alongside its ability to co-metabolize non-toxic organic substances. These attributes enable the fungus to sustain its activity in environments that are unfavourable for bacterial proliferation. The synergistic interaction between *Anabaena azolla, Azolla pinnata, Azotobacter sp.*, and *Pleurotus columbinus* is of significant importance in the enhancement of soil fertility (Gaafar et al., 2021). Microorganisms provide several benefits to soil fertility. Firstly, they excrete growth-promoting compounds. Secondly, their death and subsequent decomposition contribute to an increase in soil biomass. This increase in biomass enhances soil biological activity, leading to higher levels of soil $CO_2$ evolution. Ultimately, these processes collectively result in improved soil fertility.

However, the precise mechanisms by which such microbes improve roselle growth and productivity in sandy soils are not fully understood. Therefore, this study aims to evaluate the effect of inoculating sandy soil with a combination of *Bacillus subtilis* and *Pseudomonas fluorescens* PGPR and mycorrhizal fungi on growth and yield parameters of the roselle crop. It is hypothesized that the microbial inoculants will enhance roselle growth, yield, and nutrition by increasing soil fertility and nutrient acquisition.

The findings of this research are expected to provide valuable insights into how to improve roselle cultivation in sandy soils using beneficial microorganisms, contributing to the development of sustainable agricultural practices and enhanced productivity.

## 2. Materials and methods

### 2.1. Experimental site

The field experiment was conducted over two summer growing seasons (2019-2020) at the Agricultural Research Station in Ismailia, Egypt (30°36'46.88″N to 30°37'11.79″N, 32°14'26.66″E to 32°14'51.62″E). The soil was classified as sandy torriorthent with low fertility.

The experimental site has an arid climate characterized by hot, dry summers and mild winters. As shown in Table 1, there was minimal rainfall during the summer months (June-August) in both 2019 and 2020, with no measurable precipitation most months. Average daily relative humidity during the summer was around 50%, and specific humidity ranged from 9.7 to 12.2 g $kg^{-1}$. Surface pressure averaged near 100 kPa. Maximum wind speeds were highest in summer, ranging from 5.1 to 5.8 m $s^{-1}$ on average, while minimum wind speeds were 0.4 to 1.0 m $s^{-1}$. Temperatures were highest in summer, with average maximum temperatures around 40°C in June through August (Fig. 1). Minimum temperatures ranged from 22-25°C. Earth skin temperature reached over 30°C on some days. The dew point and wet bulb temperatures also peaked during summer. Soil moisture was lowest in the summer months (Fig. 2). Volumetric water content at the surface dropped below 5% in both seasons. Root zone moisture content declined to around 10% by August. The soil moisture profile showed very dry conditions (under 5% VWC) below 50 cm depth during summer. Overall, the climate is hot and dry in summer with high temperatures, intense solar radiation, low humidity, and negligible rainfall from June to August. The winter months are milder with lower temperatures and winds, and occasional precipitation events that provide limited soil moisture recharge.

### 2.2. Experimental design

The experiment was laid out in a randomized complete block design with four replications. The treatments consisted of eight microbial inoculation treatments: 1- *Bacillus subtilis* + *Pseudomonas fluorescens* (B), 2- *Pleurotus ostreatus* (P), 3- Mycorrhizal fungi (M), 4- B + P, 5- B + M, 6- P + M, 7- B + P + M, and 8- non-inoculated control. Each experimental unit was seven ridges, 3m long and spaced 60cm apart (Fig. 3). Irrigation was carried out with a drip irrigation system in the experiment.

### 2.3. Microbial inoculant preparation

*Bacillus subtilis* and *Pseudomonas fluorescens* were cultured in nutrient broth media (28°C, 120 rpm, 48 h). Cell suspensions were diluted to 107 CFU/ml. The fungus *Pleurotus ostreatus* was inoculated into glass vials containing sterile PD broth. The bottles underwent agitation at a rotational speed ranging from 250 to 350 revolutions per minute (rpm) for duration of 7 days at a temperature of 25°C on a mechanical shaker. The liquid contents were blended for a duration of 1 minute to achieve homogeneity. This homogenised solution was then utilised as a seed treatment before to planting. Mycorrhizal inoculant (Mycorrhizeen) containing 10 spores/g was obtained commercially. Roselle seeds were soaked in inoculants for each treatment for half an hour before planting. The same inoculants used for seed soaking were applied as a soil treatment for each treatment at 30 days after planting at a rate of 48 L per hectare (Gaafar et al. 2021). All bacterial inoculants and mycorrhizal fungus were obtained from the Microbiology Research Department, Soil, Water and Environment Research Institute, Agricultural Research Center, Egypt.

**Table 1. Average Precipitation (mm day$^{-1}$), Relative Humidity (%), Specific Humidity (g kg$^{-1}$) Surface Pressure (kPa), Maximum Wind Speed (m s$^{-1}$) and Minimum Wind Speed (m s$^{-1}$) of experimental site during cultivar seasons 2019 and 2020.**

| | Date | Precipitation Corrected (mm day$^{-1}$) | | Relative Humidity (%) | | Specific Humidity (g kg$^{-1}$) | | Surface Pressure (kPa) | | Wind Speed Maximum (m s$^{-1}$) | | Wind Speed Minimum (m s$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Dev.± | Mean | Std. Dev.± | Mean | Std. Dev.± | Mean | Std. Dev.± | Mean | Std. Dev.± | Mean | Std. Dev.± |
| 2019 | June | 0.000 | 0.000 | 50.128 | 5.349 | 10.730 | 1.111 | 99.850 | 0.205 | 5.763 | 1.290 | 0.937 | 0.428 |
| | July | 0.000 | 0.000 | 49.214 | 4.779 | 11.246 | 0.935 | 99.609 | 0.196 | 5.317 | 0.790 | 0.950 | 0.478 |
| | August | 0.000 | 0.000 | 49.951 | 6.562 | 11.640 | 1.410 | 99.683 | 0.157 | 5.097 | 0.805 | 0.862 | 0.397 |
| | September | 0.000 | 0.000 | 58.585 | 4.554 | 11.957 | 0.873 | 99.979 | 0.167 | 5.070 | 0.695 | 1.101 | 0.388 |
| | October | 0.310 | 1.157 | 63.880 | 6.095 | 11.114 | 1.051 | 100.206 | 0.217 | 4.638 | 0.868 | 1.192 | 0.517 |
| | November | 0.012 | 0.048 | 58.343 | 15.113 | 8.287 | 2.530 | 100.334 | 0.224 | 4.115 | 1.035 | 1.262 | 0.638 |
| 2020 | June | 0.001 | 0.003 | 51.858 | 7.962 | 9.662 | 1.208 | 99.888 | 0.212 | 5.118 | 1.019 | 1.089 | 0.390 |
| | July | 0.000 | 0.000 | 53.091 | 4.317 | 11.612 | 1.087 | 99.511 | 0.167 | 5.293 | 0.684 | 0.985 | 0.375 |
| | August | 0.000 | 0.000 | 54.596 | 4.476 | 12.227 | 1.064 | 99.535 | 0.137 | 5.129 | 1.000 | 0.765 | 0.360 |
| | September | 0.000 | 0.000 | 61.158 | 5.169 | 13.527 | 1.332 | 99.906 | 0.237 | 4.873 | 0.986 | 1.162 | 0.535 |
| | October | 0.034 | 0.085 | 66.256 | 4.136 | 12.231 | 0.675 | 100.302 | 0.172 | 4.702 | 0.794 | 1.342 | 0.399 |
| | November | 0.645 | 1.587 | 64.201 | 7.634 | 8.409 | 1.233 | 100.473 | 0.246 | 3.786 | 1.448 | 0.946 | 0.540 |

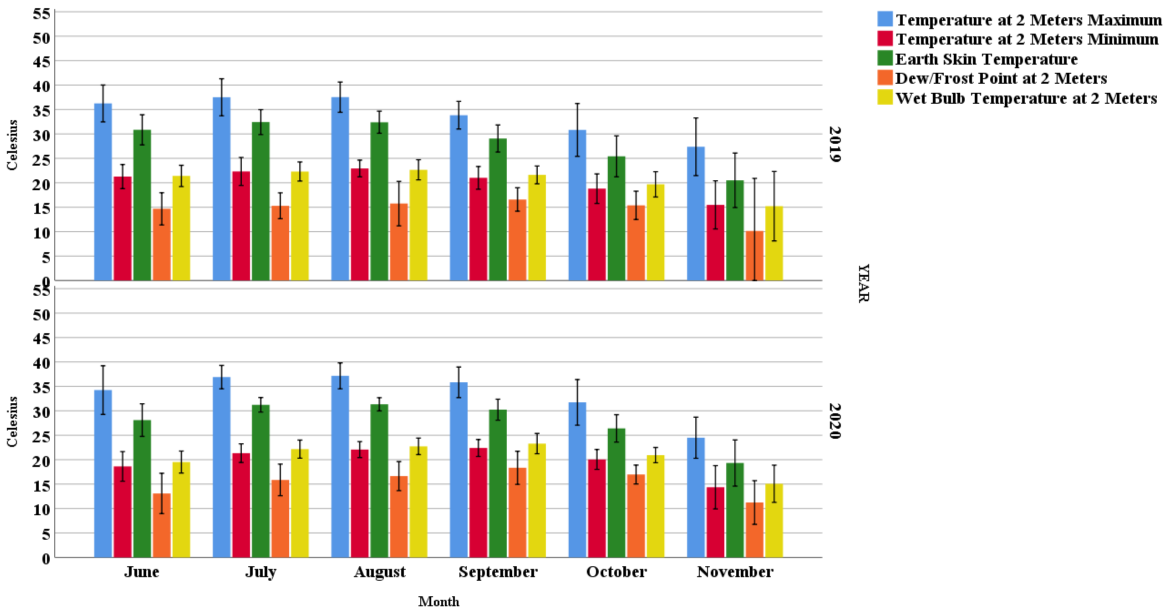


**Fig. 1. Maximum and Minimum Temperature, Earth Skin Temperature, Dew/Forest Point, Wet Bulb and Earth Skin Temperature (°C) of experimental site during cultivar seasons 2019 and 2020.**

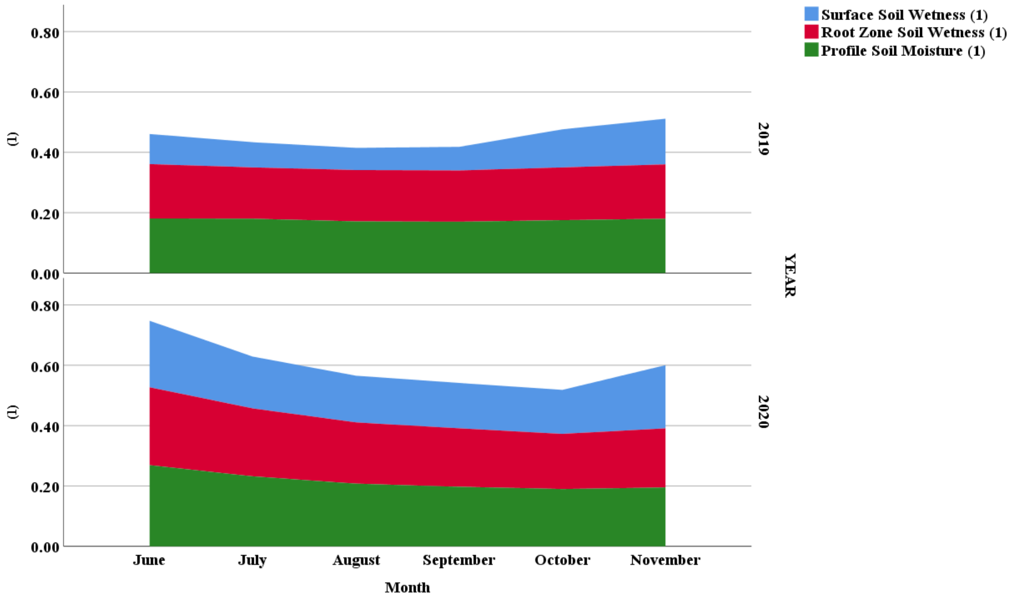


**Fig. 2. Surface, Root Zone Soil Wetness and Profile Soil Moister (1) of experimental site during cultivar seasons 2019 and 2020.**

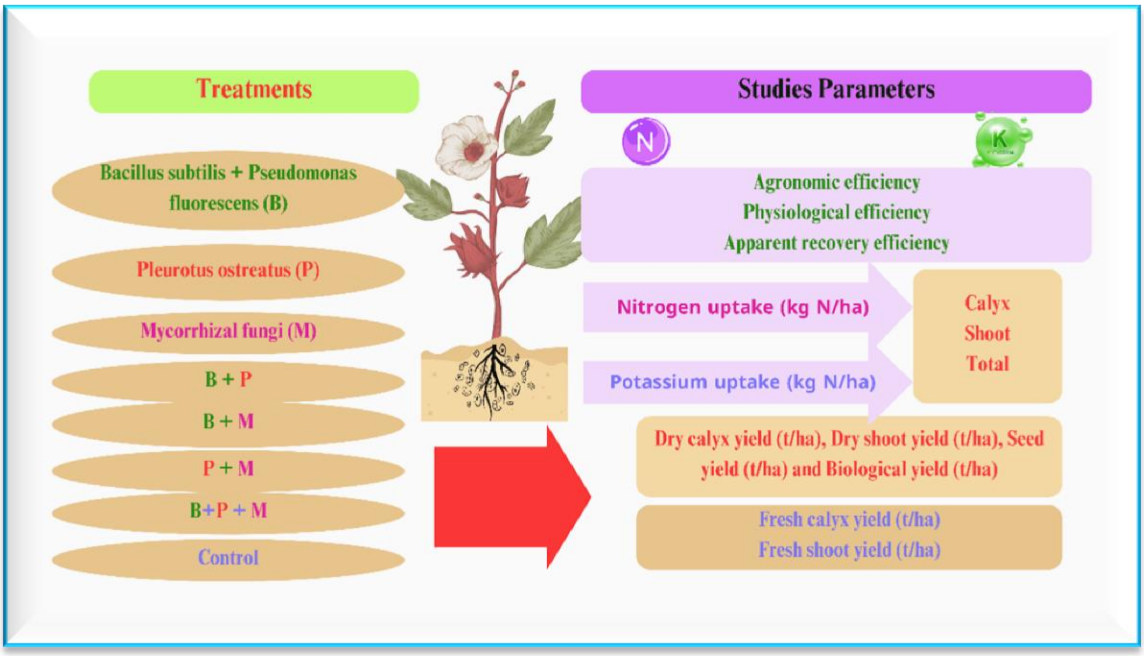


**Fig. 3. General description of the study.**

### 2.4. Crop husbandry

The roselle seedlings were generously provided by the Horticultural Res. Institute of the ARC in Egypt. Roselle seedlings were transplanted at 50cm spacing on 1 June in the both seasons. Basal fertilization was done using calcium superphosphate (42 kg P $ha^{-1}$) and potassium sulphate (80 kg K $ha^{-1}$). Nitrogen as ammonium sulphate (167 kg N $ha^{-1}$) was applied in three equal splits. Irrigation and standard agronomic practices were implemented uniformly.

### 2.5. Data collection

At harvest (160 days after sowing), plant samples were collected to determine fresh and dry weights of calyx, shoot, and seed yield on a per hectare basis. Total nitrogen and potassium uptake were measured following digestion. The investigation focused on the uptake of N and K in a solution derived from digested plants. The measurement of total N form, specifically nitrate ($NO_3^-$) and ammonium ($NH_4^+$), was conducted by the micro Kjeldahl, as stated by (Markus et al., 1985). The quantification of total K was conducted by Jackson (1973) through the utilization of a flame photometer.

Nitrogen and potassium utilization efficiencies were calculated using standard formulae.

Agronomic efficiency (kg $_{\text{biological yield}}$/kg $_{\text{fertilizer applied}}$), physiological efficiency (kg $_{\text{biological yield}}$ /kg $_{\text{nutrient uptake}}$) and apparent recovery efficiency RE (%) of applied fertilizer N and K were calculated according to (Baligar et al., 2001; Dobermann 2007) using the following formulas:

Agronomic efficiency (AE) = (Biological Yield) / (quantity of fertilizer applied)
Physiological efficiency (PE) = (Biological Yield) / (total uptake of nutrient)
Apparent recovery efficiency (RE) = [(total uptake of nutrients)/ (quantity of fertilizer applied)] × 100

### 2.6. Statistical analyses

Analysis of variance was performed using SPSS (v. 24, IBM Inc., Chicago, II, USA) and means were compared using LSD test at 5% probability level. Additionally, the ANOVA method was employed for variance analysis (Snedecor and Cochran, 1989). Correlations were determined using Pearson's coefficients.

## 3. Result

### 3.1. Yield parameters

The data presented in Table 2 and 3 demonstrate that microbial inoculation significantly influenced roselle yield parameters compared to the non-inoculated control. Inoculation with the PGPR *Bacillus subtilis* (B) led to the greatest enhancement of fresh and dry calyx yield, increasing yields by 64% and 221% over the control, respectively. Treatments with *Pleurotus* (P) and mycorrhizal fungi (M) also substantially increased fresh and dry calyx yields compared to the control, but to a lesser degree than B. Moreover, combining the microbial inoculants led to additional synergistic improvements in yield, with the B+P+M treatment improving fresh and dry calyx yields by 106% and 332% over the control.

### 3.2. Macronutrient uptake in roselle calyx and shoot

The results in Table 4 and 5 demonstrate the significant influence of microbial inoculation on nitrogen (N) and potassium (K) uptake in the calyx and shoot of roselle plants. Among individual treatments, B led to the greatest enhancement of N and K uptake compared to the control. Treatments with P and M also improved N and K uptake substantially compared to the control. Furthermore, combining the microbial inoculants led to additional increases in nutrient uptake, with B+P+M treatment increasing total N and K uptake by 322% and 724% over the control, respectively.

**Table 2. Biological influences on fresh calyx and shoot yield parameters of Roselle plant.**

| Treatments | Fresh calyx yield (t ha$^{-1}$) | | Fresh shoot yield (t ha$^{-1}$) | |
|---|---|---|---|---|
| | Mean | Std. E. | Mean | Std. E. |
| Control | 5.238 d | ±0.496 | 16.333 d | ±1.593 |
| B. | 8.571 b | ±0.687 | 24.758 b | ±2.221 |
| P. | 6.190 d | ±0.687 | 17.483 cd | ±2.125 |
| M. | 6.429 cd | ±0.599 | 17.692 cd | ±1.431 |
| B. + P. | 9.206 ab | ±0.346 | 24.807 b | ±0.545 |
| B. + M. | 9.444 ab | ±0.420 | 27.198 ab | ±1.216 |
| P. + M. | 8.095 bc | ±0.765 | 23.188 bc | ±2.745 |
| B. + P. + M. | 10.794 a | ±0.572 | 31.748 a | ±1.298 |
| F. test | ** | | ** | |

B.: Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens,* P.: *Pleurotus ostreatus* and M.: Mycorrhizeen®.

**Table 3. Biological influences on dry calyx and shoot, seed and biological yield parameters of Roselle plant.**

| Treatments | Dry calyx yield (t ha$^{-1}$) | | Dry shoot yield (t ha$^{-1}$) | | Seed yield (t ha$^{-1}$) | | Biological yield (t ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std. E. | Mean | Std. E. | Mean | Std. E. | Mean | Std. E. |
| Control | 0.545 d | ±0.073 | 3.259 c | ±0.348 | 0.700 e | ±0.104 | 4.505 d | ±0.525 |
| B. | 1.753 b | ±0.161 | 6.890 ab | ±0.675 | 2.404 c | ±0.264 | 11.048 bc | ±1.095 |
| P. | 0.708 d | ±0.097 | 3.553 c | ±0.454 | 0.805 e | ±0.108 | 5.066 d | ±0.656 |
| M. | 0.789 d | ±0.092 | 4.211 c | ±0.378 | 0.835 e | ±0.060 | 5.835 d | ±0.518 |
| B. + P. | 1.884 b | ±0.041 | 7.834 a | ±0.155 | 2.599 c | ±0.020 | 12.317 ab | ±0.159 |
| B. + M. | 1.952 b | ±0.121 | 7.998 a | ±0.349 | 3.011 b | ±0.095 | 12.961 ab | ±0.548 |
| P. + M. | 1.384 c | ±0.136 | 6.133 b | ±0.725 | 1.456 d | ±0.174 | 8.974 c | ±1.035 |
| B. + P. + M. | 2.355 a | ±0.134 | 8.166 a | ±0.296 | 3.723 a | ±0.117 | 14.245 a | ±0.535 |
| F. test | ** | | ** | | ** | | ** | |

B.: Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens*, P.: *Pleurotus ostreatus* and M.: Mycorrhizeen®.

**Table 4. Nitrogen uptake as affected by some microbiota in calyx and shoot of Roselle plant.**

| Treatments | Nitrogen uptake (kg N ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Calyx | | Shoot | | Total | |
| | Mean | Std. E. | Mean | Std. E. | Mean | Std. E. |
| Control | 6.325 d | ±0.840 | 30.524 e | ±4.307 | 36.849 e | ±5.015 |
| B. | 26.904 b | ±2.956 | 82.031 bc | ±10.642 | 108.935 cd | ±13.094 |
| P. | 8.443 d | ±1.684 | 34.315 e | ±3.887 | 42.758 e | ±5.555 |
| M. | 11.018 d | ±1.222 | 43.215 de | ±3.188 | 54.234 e | ±4.372 |
| B. + P. | 31.155 b | ±3.492 | 95.122 ab | ±11.072 | 126.277 bc | ±12.329 |
| B. + M. | 33.807 b | ±2.478 | 110.612 a | ±1.897 | 144.420 ab | ±1.251 |
| P. + M. | 19.862 c | ±2.288 | 65.640 cd | ±8.487 | 85.502 d | ±10.582 |
| B. + P. + M. | 42.551 a | ±1.985 | 113.247 a | ±11.383 | 155.798 a | ±9.434 |
| F. test | ** | | * | | ** | |

B.: Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens,* P.: *Pleurotus ostreatus* and M.: Mycorrhizeen®.

**Table 5. Potassium uptake as affected by some microbiota in calyx and shoot of Roselle plant.**

| Treatments | Potassium uptake (kg K ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Calyx | | Shoot | | Total | |
| | Mean | Std. E. | Mean | Std. E. | Mean | Std. E. |
| Control | 8.005 e | ±1.116 | 17.423 e | ±2.680 | 25.428 f | ±3.792 |
| B. | 29.035 c | ±2.355 | 47.435 d | ±4.153 | 76.470 d | ±6.485 |
| P. | 10.503 e | ±1.305 | 18.738 e | ±2.241 | 29.241 f | ±3.342 |
| M. | 11.818 e | ±1.637 | 23.710 e | ±3.111 | 35.528 f | ±4.748 |
| B. + P. | 31.646 bc | ±0.608 | 68.216 c | ±2.963 | 99.861 c | ±2.647 |
| B. + M. | 34.985 b | ±2.563 | 83.036 b | ±2.668 | 118.022 b | ±3.917 |
| P. + M. | 21.131 d | ±1.899 | 40.830 d | ±4.506 | 61.961 e | ±6.307 |
| B. + P. + M. | 49.092 a | ±1.816 | 160.582 a | ±2.120 | 209.674 a | ±2.703 |
| F. test | ** | | ** | | ** | |

B.: Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens,* P.: *Pleurotus ostreatus* and M.: Mycorrhizeen®.

### 3.3. Nitrogen use efficiencies

The data on nitrogen use efficiency parameters (Figures 4-6) provide valuable insights into the effects of microbial inoculation on roselle nitrogen utilization. The microbial treatments substantially increased agronomic nitrogen efficiency compared to the control, suggesting more effective utilization of applied nitrogen fertilizer for biomass production. Physiological nitrogen efficiency was lower with microbial treatments compared to the control, indicating nitrogen uptake outpaced biomass accumulation. Moreover, inoculation dramatically increased percent nitrogen recovery, pointing to enhanced uptake and utilization of applied nitrogen.

#### 3.3.1. Agronomic efficiency of N and K

The agronomic nitrogen efficiency (biological yield per unit of nitrogen fertilizer used) and agronomic potassium efficiency (biological yield per unit of potassium fertilizer applied) for various treatments are shown in Figure 4. For each treatment, mean values and standard errors of the mean are reported. It is noteworthy to note that the data show statistically significant variations in nitrogen and potassium efficiencies among treatments, as indicated by the asterisks and low p-values (0.000). The specific figures in the table show the differences in efficiency between each treatment and the control group.

The nitrogen efficiency of the control group was 26.973 kg biological yield per kilogramme nitrogen fertilizer applied. The Bacteria + *Pleurotus* + Mycorrhiza treatment had the best nitrogen efficiency among the other treatments, with a mean value of 85.297 kg/kg. When compared to the control, the Bacteria treatment had a considerably better nitrogen efficiency (66.153 kg/kg).

The potassium efficiency in the control group was 37.853 kg biological yield per kilogramme potassium fertilizer applied. With a mean value of

119.702 kg/kg, the Bacteria + *Pleurotus* + Mycorrhiza treatment displayed the best potassium efficiency. Similarly, when compared to the control, the Bacteria treatment had significantly higher potassium efficiency (92.836 kg/kg).

### 3.3.2. Physiological efficiency of N and K

Figure 5 depicts the physiological nitrogen and potassium efficiencies of various treatments as measured by biological yield per unit of nitrogen and potassium uptake. For each treatment, mean values and standard errors of the mean are reported. The physiological nitrogen efficiency in the control group, which received no therapy, was 122.841 kg biological yield per kg nitrogen absorption. Similarly, the control group's physiological potassium efficiency was 178.805 kg biological yield per kilogramme potassium absorption. The bacteria treatment had the lowest physiological nitrogen efficiency among the individual treatments (102.223 kg biological yield per kg nitrogen uptake). With 144.120 kg biological yield per kg of potassium uptake, the bacteria treatment likewise had a poorer physiological potassium efficiency. With 118.537 kg biological yield per kg nitrogen uptake, the *Pleurotus* therapy indicated slightly improved physiological nitrogen efficiency. The *Pleurotus* treatment had 172.555 kg biological yield per kilogramme of potassium uptake for physiological potassium efficiency. The Mycorrhiza treatment performed similarly to the bacteria treatment in terms of physiological nitrogen efficiency, with 107.472 kg biological yield per kg nitrogen uptake. The Mycorrhiza treatment, on the other hand, had a greater physiological potassium efficiency, with 166.181 kg biological yield per kg of potassium uptake.

### 3.3.3. Apparent recovery efficiency of N and K

Figure 6 depicts the apparent recovery efficiency of nitrogen and potassium for various treatments as percentages. For each treatment, mean values and standard errors of the mean are reported. The apparent recovery efficiency of nitrogen in the control group, which received no particular treatment, was 22.065%. In the control group, the apparent recovery efficiency of potassium was 21.368%. The bacteria treatment had the highest apparent nitrogen recovery efficiency among the individual treatments, with a mean value of 65.231%. The bacteria treatment likewise had the highest apparent potassium recovery efficiency, with a mean value of 64.260%. The *Pleurotus* therapy had reduced apparent nitrogen recovery efficiency, with 25.603%. The *Pleurotus* treatment had a mean value of 24.573% for apparent potassium recovery efficiency. The Mycorrhiza treatment outperformed the *Pleurotus* treatment in terms of apparent nitrogen recovery efficiency, with 32.475%. Similarly, the Mycorrhiza treatment demonstrated better apparent potassium recovery efficiency, with 29.855%.

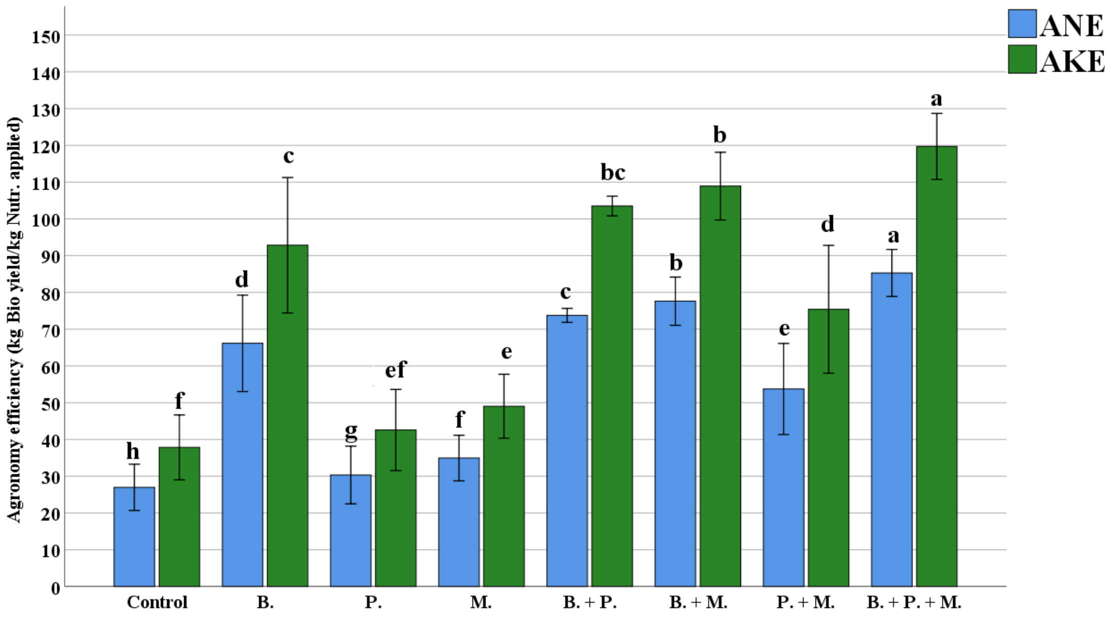


**Fig. 4. Biological influences on Agronomy efficiencies of nitrogen (ANE) and potassium (AKE).**

B.: Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens*, P.: *Pleurotus ostreatus* and M.: Mycorrhizeen®.

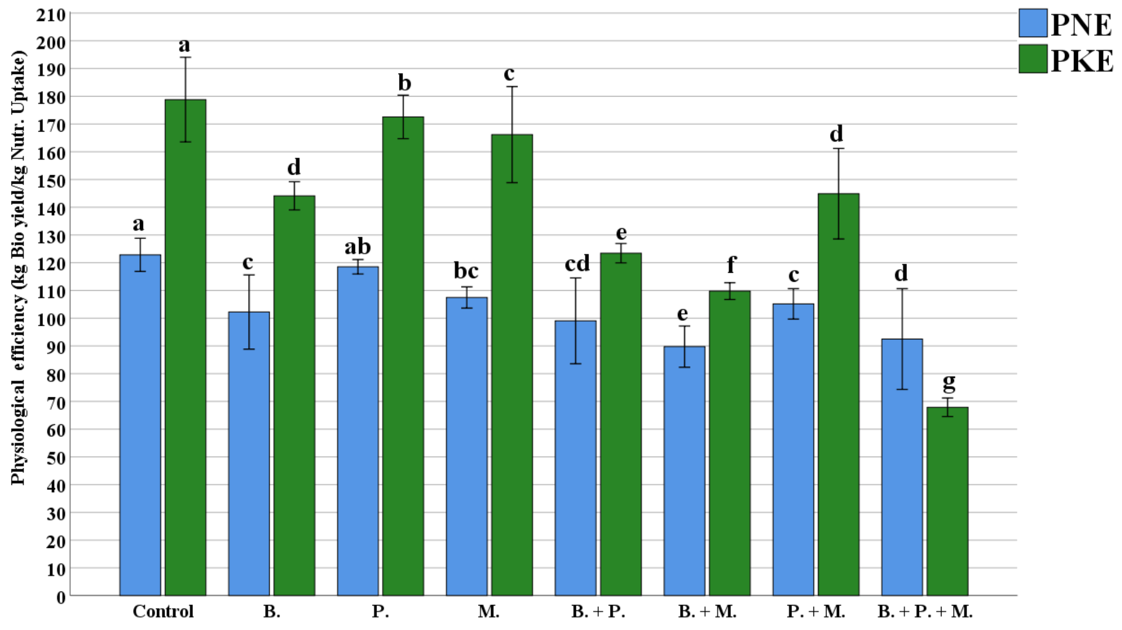


**Fig. 5. Biological influences on Physiological efficiencies of nitrogen (PNE) and potassium (PKE).**

B.: Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens*, P.: *Pleurotus ostreatus* and M.: Mycorrhizeen®.

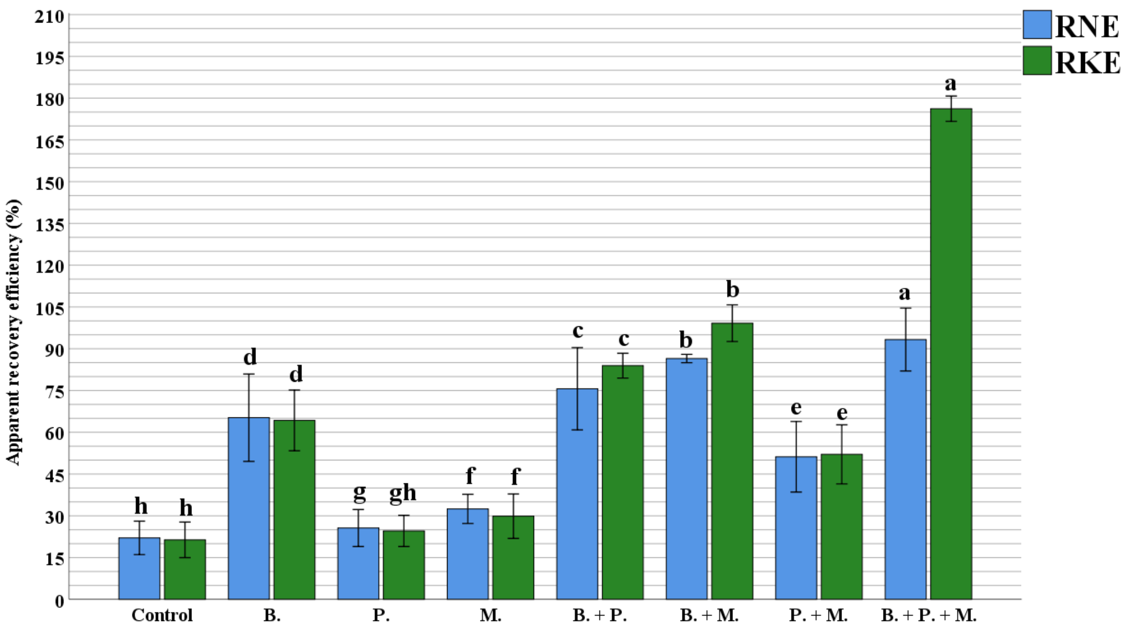


**Fig. 6. Biological influences on apparent recovery efficiencies of nitrogen (RNE) and potassium (RKE).**

B.: Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens*, P.: *Pleurotus ostreatus* and M.: Mycorrhizeen®.

**Correlation results**

Figure 7 showed that there was a significant positive connection ($r = 0.969$, $p < 0.01$) observed between the fresh calyx yield and the dry calyx yield. This suggests that a higher output of fresh calyxes is associated with an increase in the yield of dry calyxes. This observation indicates a reliable trend in the growth and productivity of the calyx under varying environmental circumstances. Both metrics exhibited significant positive relationships with other yield-related variables, including fresh shoot yield ($r = 0.983$, $p < 0.01$), dry shoot yield ($r = 0.963$, $p < 0.01$), and fresh seed yield ($r = 0.929$, $p < 0.01$), suggesting a coordinated growth response. Although The data revealed significant positive associations between the total nitrogen intake per hectare and both fresh shoot yield (r = 0.911, p < 0.01) and biological yield (r = 0.969, p < 0.01), suggesting that nitrogen plays a crucial role in enhancing plant growth and overall production. There was a significant positive correlation between the total uptake of potassium per hectare and the fresh shoot yield (r = 0.888, p < 0.01), as well as the fresh seed yield (r = 0.938, p < 0.01). These findings suggest that potassium plays a crucial role in the growth and development of shoot and seed components. Beneficial microorganisms played a significant role in augmenting specific plant characteristics. The positive connections shown between agronomic efficiency of nitrogen and several yield parameters suggest that microorganisms may play a role in enhancing nitrogen absorption efficiency. In a similar vein, there were favourable associations observed between the agronomic efficiency of potassium and several yield indices, indicating the possibility for enhancing potassium absorption efficiency. The study revealed the presence of negative correlations between phosphorus-related metrics, specifically the physiological efficiency of nitrogen and potassium, and specific yield features. This observation suggests a potential correlation between increased physiological efficiency of phosphorus and diminished plant development under specific circumstances.

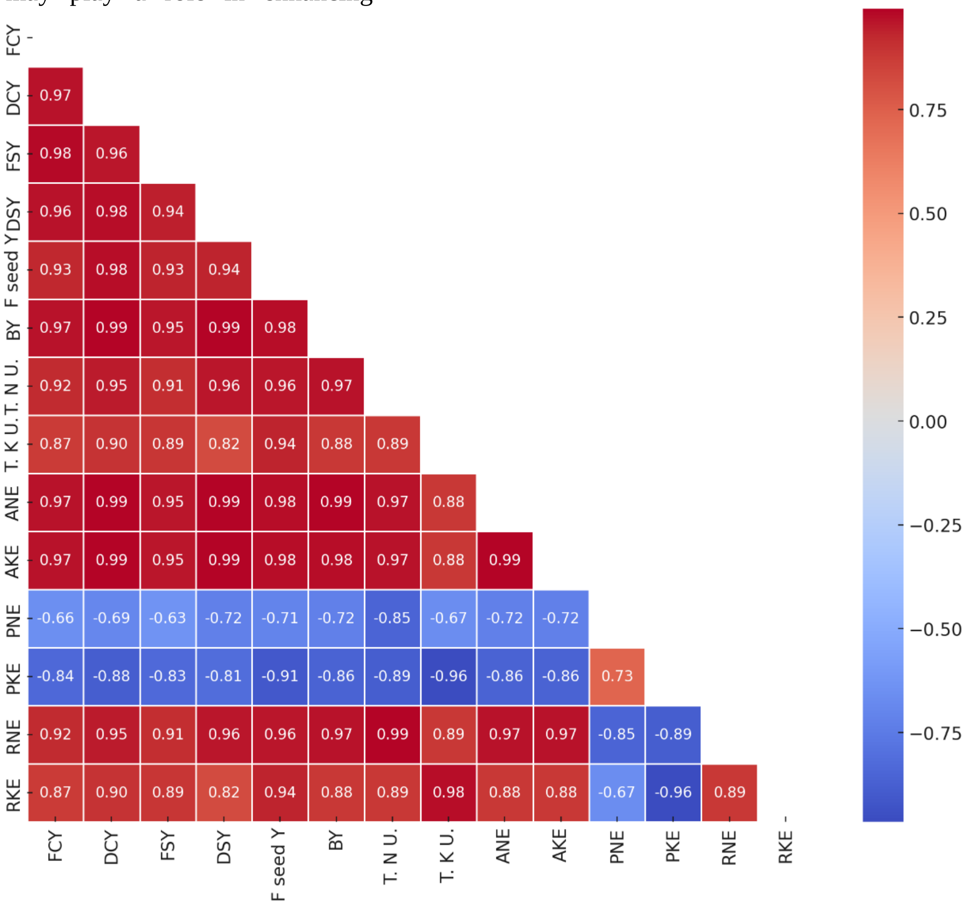


**Fig. 7. Heat map of Pearson correlation coefficients among Roselle plant characters affected by some microbiota.**

**4. Discussion**

The field experiment was conducted at the Agricultural Research Station in Ismailia, Egypt, which has an arid climate characterized by hot, dry summers and mild winters. As shown in Table 1, there was minimal rainfall during the summer months (June-August) in both 2019 and 2020, with no measurable precipitation most months. Average daily relative humidity during the summer was around 50%, and specific humidity ranged from 9.7 to 12.2 g kg-1. Surface pressure averaged near 100 kPa. Maximum wind speeds were highest in summer, ranging from 5.1 to 5.8 m s-1 on average, while minimum wind speeds were 0.4 to 1.0 m s-1. Temperatures were highest in summer, with average maximum temperatures around 40°C in June through August. Minimum temperatures ranged from 22-25°C. Earth skin temperature reached over 60°C on some days. The dew point and wet bulb temperatures also peaked during summer. Soil moisture was lowest in the summer months. Volumetric water content at the surface dropped below 5% in both seasons. Root zone moisture content declined to around 10% by August. The soil moisture profile showed very dry conditions (under 5% VWC) below 50 cm depth during summer.

The hot and dry climate during the summer months, with high temperatures, intense solar radiation, low humidity, and negligible rainfall could have impacted the results of the study. The dry conditions would increase water stress on the plants. The microbial inoculations may have helped the plants better tolerate the dry conditions through mechanisms like increased root development, nutrient uptake etc. This could explain the significant improvement in plant growth and yields observed with the microbial treatments compared to the non-inoculated control under the water-limited conditions of the study area. Therefore, the local climate with hot and dry summers likely played a role in the responses of roselle plants to the different microbial treatments evaluated in this study.

The results of this study provide strong evidence that inoculating sandy soils with beneficial microorganisms like PGPR and mycorrhizal fungi can significantly improve the growth, yield, and nutrition of the roselle crop.

### 4.1. Yield parameters

The current study sought to evaluate the biological effects of various microbiota treatments on Roselle plant production measures. A control group was included in the treatments, as well as combinations of beneficial microbiota, including biological mixture of *Bacillus subtilis* + *Pseudomonas fluorescens* (B.), *Pleurotus ostreatus* (P.), and Mycorrhizeen® (M.). Table 1 shows that there were substantial changes in yield characteristics between treatments, showing the potential impact of these bacteria on plant development and productivity.

The impact of several microbiota treatments on the yield characteristics of Roselle plants begins with a description of the control group's results, which did not receive any special microbiota treatments. The control group had moderate yield metrics, such as fresh and dry calyx yield. However, when various microbial treatments were administered to the Roselle plants, the yield parameters improved. The "B." treatment had the greatest impact among the individual bacterial treatments, resulting in significantly increased fresh and dried calyx yields compared to the control group. Treatments with "P." and "M." considerably improved yield characteristics as compared to the control group. The combined microbiota therapy, which utilised many beneficial bacteria in various combinations. When compared to separate treatments, the combined treatments produced higher yield parameters, with the "B. + P. + M." treatment having the greatest impact on fresh and dried calyx yield. The findings suggest that using certain microbial treatments can improve the growth and development of Roselle plants, resulting in higher yields. The favourable interactions between the applied bacteria and the plants could explain the positive benefits of these treatments. The presence of certain bacteria may have aided nutrient uptake, increased nutrient utilization efficiency, and encouraged plant growth and development via a variety of mechanisms including increased nutrient availability, hormone synthesis, and disease suppression (Lugtenberg and Kamilova, 2009; Vacheron et al., 2013). Additionally, mycorrhizal fungi have been reported to improve nutrient acquisition, particularly phosphorus, through their extensive hyphal network and enhanced root exploration (Smith and Read, 2010).

In accordance with findings of the current study, (El Naim et al., 2017) documented that fresh and dry weight of shoot, calyx and seed weight were significantly enhanced under inoculation of combined bioagents. This may be ascribed to the collaborative effect of bioagents i.e., production of vitamins, amino acids and growth promoting substances for instance, indole acetic acid, cytokinins (Chauhan et al., 2021) and gibberellic acid, synthesis of phytohormones, $N_2$ fixation, synthesis of some enzymes that modulate the level of plant hormones (Gouda et al., 2018; Gupta et al., 2021), solubilization of inorganic phosphate and mineralization of organic phosphate by mycorrhiza (Adeyemi et al., 2020; Bagyaraj, 2018; Fallahi et al., 2016) which makes phosphorus available for plant, which resulted in increasing the surface area per unit root length and enhanced the root hair branching with an eventual enhancement nutrients uptake from the soil, translocation and synthesis of photosynthate assimilates and consequently increased plant growth characters. These results are supported by other authorised papers, (Al-Sayed et al., 2020, 2023).

### 4.2. Macronutrient uptake by roselle calyx and shoot

The current study looked at how different microbiota treatments affected nitrogen (N) and potassium (K) uptake in the calyx and shoot of Roselle plants (*Hibiscus sabdariffa*). A control group was included, as well as other microbiota combinations, specifically a Bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens* (B.), *Pleurotus ostreatus* (P.), and Mycorrhizeen® (M.). The findings revealed significant changes in nutrient intake between treatments. The control group had decreased nitrogen and potassium uptake in both the calyx and the shoot, indicating that the presence of particular bacteria altered plant nutrient acquisition. Among the individual microbiota treatments, B. had the greatest impact on nutrient intake. In comparison to the control, the presence of B. substantially increased both nitrogen and potassium uptake, resulting in higher total nutrient acquisition. This finding is consistent with prior research that found *Bacillus* and *Pseudomonas* species to be favourable to plant nutrient intake and growth (Castrillo et al., 2017; Verma et al., 2010). Similarly, the P. and M. treatments boosted nitrogen and potassium uptake as compared to the control, though their individual effects were less strong than *Bacillus subtilis*. *Pleurotus ostreatus*, a common edible fungus, has been shown to create symbiotic connections with plant roots, which improves nutrient intake and plant growth (Marschner, 2012). On the other hand, Mycorrhizeen® is a commercially available mycorrhizal inoculant that contains arbuscular mycorrhizal fungi, which are well-known for their role in improving nutrient availability and uptake in various plant species (Smith and Read, 2010). Furthermore, the combined microbiota treatments exhibited synergistic effects, leading to even higher nutrient uptake compared to individual treatments. The combined application of B. + P. + M. resulted in the most substantial increase in both nitrogen and potassium uptake, highlighting the potential for synergistic interactions between different microbial species to further enhance plant nutrient uptake. This finding aligns with other studies that have reported synergistic effects of combining beneficial microorganisms for improved plant performance (Nadeem et al. 2014).

### 4.3. Nitrogen use efficiencies

#### 4.3.1. Agronomic efficiency of N and K

In this study, the agronomic nitrogen and potassium efficiencies were evaluated for different treatments, and the results are presented in Figure 3. The agronomic nitrogen efficiency represents the biological yield obtained per unit of nitrogen fertilizer applied, while the agronomic potassium efficiency represents the biological yield per unit of potassium fertilizer applied. The mean values and standard errors of the mean are provided for each treatment. The results showed statistically significant differences among the treatments for both nitrogen and potassium efficiencies, as indicated by the asterisks and the low p-values (p = 0.000). These findings suggest that the presence of specific microbiota significantly influenced the efficiency of nutrient uptake and utilization by the Roselle plants. In the control group, the mean nitrogen efficiency was 26.973 kg of biological yield per kg of nitrogen fertilizer applied. This implies that, on average, the control plants produced 26.973 kg of biomass for every kilogram of nitrogen fertilizer added. However, the application of different microbiota treatments resulted in notable improvements in nitrogen efficiency. The treatment with Bacteria + *Pleurotus* + Mycorrhiza exhibited the highest nitrogen efficiency, with a mean value of 85.297 kg yield /kg N applied. This suggests that the combined application of *Bacillus subtilis, Pseudomonas fluorescens*, *Pleurotus ostreatus*, and Mycorrhizeen® led to a significantly more efficient utilization of nitrogen fertilizers, resulting in substantially higher biomass production per unit of nitrogen applied. The positive impact of *Bacillus* and *Pseudomonas* on nutrient uptake and utilization has been reported in previous studies (Castrillo et al., 2017; Verma et al., 2010), while *Pleurotus ostreatus* and Mycorrhizeen® are known to enhance nutrient availability and uptake (Marschner, 2012; Smith and Read, 2010). The treatment with Bacteria alone also showed significantly higher nitrogen efficiency, with a mean value of 66.153 kg/kg. This indicates that the presence of *Bacillus subtilis* and *Pseudomonas fluorescens* in isolation also promoted efficient nitrogen utilization by the Roselle plants. Regarding potassium efficiency, the control group had a mean value of 37.853 kg of biological yield per kg of potassium fertilizer applied. Like nitrogen efficiency, the treatments with Bacteria + *Pleurotus* + Mycorrhiza and Bacteria alone demonstrated the highest potassium efficiencies, with mean values of 119.702 kg/kg and 92.836 kg/kg, respectively. These findings suggest that the combined application of specific microbiota or *Bacillus subtilis* and *Pseudomonas fluorescens* alone significantly improved potassium utilization, leading to higher biomass production per unit of potassium applied. The observed increases in agronomic nitrogen and potassium efficiencies in the presence of beneficial microbiota have important implications for sustainable agriculture. By enhancing nutrient uptake and utilization, the application of these microbiota can lead to reduced dependency on chemical fertilizers, thereby promoting more environmentally friendly and economically viable agricultural practices. In conclusion, the results of this study demonstrate the significant positive influence of specific microbiota treatments on agronomic nitrogen and potassium efficiencies in Roselle plants. The combination of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pleurotus ostreatus*, and Mycorrhizeen®, as well as the presence of *Bacillus subtilis* and *Pseudomonas fluorescens* alone, led to remarkably higher nutrient efficiencies, indicating the potential for these treatments to improve plant nutrition and overall crop productivity.

#### 4.3.2. Physiological efficiency of N and K

The investigation of the effect of different microbiota treatments on nitrogen (N) and potassium (K) uptake in the calyx and shoot of Roselle plants revealed significant differences among the treatments. The control group exhibited relatively lower nutrient uptake, while the presence of specific microbiota positively influenced nutrient acquisition and utilization, resulting in higher nutrient uptake efficiencies. Among the individual microbiota treatments, the bacterial mixture of *Bacillus subtilis* + *Pseudomonas fluorescens* (B.) showed the highest impact on nutrient uptake. The B. treatment significantly enhanced both nitrogen and potassium uptake in the calyx and shoot of Roselle plants, resulting in substantially higher total nutrient acquisition compared to the control. This finding is consistent with previous studies that have reported the beneficial effects of *Bacillus* and *Pseudomonas* species on nutrient uptake and growth in various plant species (Castrillo et al., 2017). The treatments with *Pleurotus ostreatus* (P.) and Mycorrhizeen® (M.) also led to significantly improved nutrient uptake compared to the control. *Pleurotus ostreatus*, as an edible mushroom, is known to form symbiotic relationships with plant roots, enhancing nutrient uptake and plant growth (Marschner, 2012). On the other hand, Mycorrhizeen® is a commercial mycorrhizal inoculant containing arbuscular mycorrhizal fungi, which are well-known for their role in improving nutrient availability and uptake in plants (Smith and Read, 2010). Moreover, the combined microbiota treatments demonstrated synergistic effects, leading to even higher nutrient uptake compared to individual treatments. The combination of B. + P. + M. exhibited the most substantial impact on nutrient uptake, with significantly higher total nitrogen and potassium uptake compared to the control and other treatments. This suggests that the presence of multiple beneficial microorganisms in combination can enhance nutrient acquisition and utilization, which is consistent with findings from other studies that have reported the synergistic effects of combining different beneficial microorganisms for improved plant performance (Nadeem et al., 2014). In summary, the results of this study demonstrate that the presence of specific microbiota, especially in combination, significantly enhanced nitrogen and potassium uptake in the calyx and shoot of Roselle plants. These findings indicate the potential application of beneficial microbiota to improve nutrient acquisition and overall plant nutrition, with valuable implications for sustainable agriculture and crop productivity enhancement. The utilization of specific microbiota to enhance nutrient uptake efficiency can lead to reduced dependency on chemical fertilizers, mitigating the environmental impacts associated with excessive fertilizer use and promoting more sustainable agricultural practices.

#### 4.3.3. Apparent recovery efficiency of N and K

The results indicate that the application of specific treatments had varying effects on the apparent recovery efficiency of nitrogen and potassium compared to the control group. These findings suggest that the treatments influenced the plants' ability to recover and utilize applied nitrogen and potassium fertilizers. The control group showed relatively low apparent recovery efficiencies for both nitrogen and potassium, indicating substantial losses or inefficiencies in the uptake and utilization of applied fertilizers. These results are consistent with previous studies that have reported suboptimal nutrient recovery in untreated or non-amended soil conditions (Diaz et al., 2022). Among the individual treatments, the bacteria treatment demonstrated the highest apparent recovery efficiencies for both nitrogen and potassium. Bacteria can enhance nutrient availability and uptake through various mechanisms, including nitrogen fixation, solubilization, and mobilization of nutrients (Hartmann et al., 2008). The observed higher apparent recovery efficiencies in the bacteria-treated plants suggest that these mechanisms were effective in promoting nutrient recovery and utilization. The *Pleurotus* treatment showed relatively lower apparent recovery efficiencies compared to the bacteria treatment for both nitrogen and potassium. *Pleurotus* is a type of fungus that can enhance nutrient uptake through its symbiotic association with plant roots. However, the specific mechanisms involved in nutrient recovery and utilization may differ from those facilitated by bacteria. This could explain the differences observed in the apparent recovery efficiencies between the two treatments. The Mycorrhiza treatment exhibited higher apparent recovery efficiencies of nitrogen and potassium compared to the *Pleurotus* treatment. Mycorrhizal associations can improve nutrient uptake by extending the root system and enhancing nutrient absorption capacity (Smith and Read, 2010). The observed higher apparent recovery efficiencies suggest that the mycorrhizal associations facilitated nutrient recovery and utilization in the plants. When combinations of treatments were applied, there were further enhancements in the apparent recovery efficiencies. The combinations of bacteria and *Pleurotus*, as well as bacteria and Mycorrhiza, showed higher apparent recovery efficiencies for both nitrogen and potassium compared to the individual treatments. This shows that these treatments may have synergistic effects, resulting in improved nutrient recovery and utilization. Among all treatments, the combination of bacteria, *Pleurotus*, and Mycorrhiza had the best apparent nitrogen recovery efficiency. However, it had an unusually high apparent potassium recovery efficiency. These therapies' synergistic interactions may have accelerated nutrient absorption, transport, and utilization, leading in dramatically higher recovery efficiencies.

#### Correlation results

The findings of this correlation analysis underscore the complex associations between several characteristics of Roselle plants and their modification by Beneficial Microorganisms. The significance of sufficient nutrient availability in maximising output is underscored by the positive correlations identified between yield metrics and nutrient uptake. The potential application of Beneficial Microorganisms in sustainable agricultural practises is suggested by their ability to boost nitrogen and potassium uptake efficiency. Furthermore, the presence of negative correlations between phosphorus-related metrics prompts inquiries into the intricate interplay between phosphorus and other nutrients in the context of plant growth. Further investigation is warranted to explore the underlying mechanisms that govern these interactions, with the aim of informing nutrient management strategies in a more effective manner. In summary, this research enhances our comprehension of the interaction between plant factors and Beneficial Microorganisms. The correlation between yield indices and nutrient uptake efficiency highlights the potential advantages of utilising these microbes to improve agricultural productivity. The consequences of these findings are relevant for the optimisation of agricultural practises and justify the need for additional investigations into the underlying mechanisms.

## Conclusion

The key findings of this study demonstrate that inoculation with beneficial microorganisms such as PGPR and mycorrhizae can substantially improve the growth, yield, and nutritional quality of roselle grown in nutrient-poor sandy soils. Microbial inoculation significantly increased fresh and dry calyx yield, shoot growth, seed yield and overall biomass production compared to non-inoculated plants. Moreover, inoculation dramatically enhanced nitrogen and potassium uptake and utilization efficiency, overcoming nutrient limitations in the sandy soil. Combining PGPR strains *Bacillus subtilis* and *Pseudomonas fluorescens* with mycorrhizal fungi led to synergistic improvements in all growth, yield and nutritional parameters measured. These results highlight the excellent potential of microbial inoculants as a sustainable agricultural practice to enhance roselle productivity and nutrition in nutrient-deficient sandy soils through synergistic mechanisms of enhanced soil fertility and plant growth promotion. Further optimization of microbial inoculation protocols and evaluation of quality aspects are warranted to translate these findings into effective field-level practices for roselle farmers.

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## Author contribution

Ibrahim S. M. Mosaad (ISMM), Doaa E. M. Gaafar (DEMG), Mohamed A. T. Al-Anoos (MATA) and Ali K. Seadh (AKS) contributed to the study conception and design. DEMG and ISMM conducted the experiments and analysed the data together with MATA and ASA. All authors contributed to the writing of the manuscript with ISMM as the lead. All authors reviewed and approved the final manuscript.

## Statements and Declarations

On behalf of all authors, the corresponding author states that there is no conflict of interest

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