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# Microbial Fertilizer Effects on Grain Yield and Quality Traits of Maize Hybrids

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ABSTRACT
Maize (*Zea mays* L.) is a globally significant cereal crop, serving as a fundamental source of food, feed, and industrial raw material. This two-year field study investigated the effects of different doses of a microbial fertilizer on the yield, yield components, and grain quality traits of three maize hybrids under field conditions in Afyonkarahisar, Türkiye, during the 2021 and 2022 growing seasons. The experiment was arranged in a split-plot design with three replications, where maize cultivars (P0937, P0573, and Sy Andromeda) were assigned to the main plots and microbial fertilizer doses (0 (control), 5, 10, 15, and 20 L $ha^{-1}$) to the subplots.

Significant main and interaction effects of year, cultivar, and microbial fertilizer dose were observed on ear morphology, grain yield, thousand-kernel weight, and grain quality traits including ash, protein, fat, starch, acid detergent fiber (ADF), and neutral detergent fiber (NDF). Cultivar P0937 demonstrated superior grain yield (up to 19.57 t $ha^{-1}$) and kernel number per ear, especially under the 2.0 L $ha^{-1}$ microbial fertilizer dose ($MF_2$), whereas Sy Andromeda showed higher protein (9.12%), starch (75.08%), and fiber contents (ADF: 5.29%, NDF: 17.05%). The $MF_2$ dose consistently enhanced yield and quality traits across cultivars and years.

Climatic variation notably influenced results; increased rainfall and humidity in 2022 improved grain yield, kernel number, and fat and starch accumulation compared to 2021. Genotype-specific responses to microbial fertilizer doses highlight the importance of considering cultivar × environment × input interactions. These findings emphasize the agronomic potential of microbial fertilizers to improve maize productivity and grain quality when applied according to cultivar and environmental conditions. Further research is needed to assess long-term soil health impacts and integration with conventional fertilization strategies.

Keywords: Biofertilizer, Sustainable agriculture, Plant nutrition, Grain yield, *Zea mays* L.

## 1. Introduction

Maize (*Zea mays* L.), one of the most important cultivated crops within the *Poaceae* family, is widely utilized in human nutrition, both directly as grain and indirectly in the form of flour and starch derived from its kernels. In animal feeding, maize serves as a valuable feed resource through both its grain and silage. Moreover, maize is a key raw material in various industrial sectors, including vegetable oil production, frozen food processing, and bioethanol manufacturing (Kun 1994). Due to its broad adaptability and high yield potential, the cultivation area of maize has been steadily expanding both globally and in Türkiye. According to 2023 data, maize ranks second after wheat in terms of global cultivation area (20.8 million hectares), but it ranks first in terms of total production, reaching 1.2 billion tons (FAO 2023). In 2024, maize was the third most extensively cultivated cereal crop in Türkiye, following wheat and barley, occupying 789,000 hectares and yielding 8.1 million tons of grain (TUIK 2024). Additionally, in Türkiye, there are 500,000 hectares dedicated to maize cultivation for silage purposes. In the last decade, the area cultivated with grain maize in Türkiye has increased by approximately 50%, while the cultivation area for silage maize has grown by 66%.

To increase maize grain yield per unit area, it is essential not only to develop high-yielding hybrid varieties but also to ensure the timely and proper implementation of all agronomic practices. As a C4 plant, maize is particularly responsive to irrigation and fertilization, with nitrogen fertilization notably enhancing both yield and quality (Kun 1994). However, excessive nitrogen fertilizer application raises input costs and causes environmental problems such as water and soil pollution. Recently, the commercial production and use of microbial fertilizers have been rapidly expanding to reduce chemical fertilizer usage, improve crop yield and quality (Gao et al. 2020; Li et al. 2022), and enhance plant tolerance to adverse environmental stresses including drought, heavy metal toxicity (Islam & Sadhi 2023), salinity (Altas 2016), low temperature (Bradocava et al. 2016), and high temperature (Ismail et al. 2020).

Microbial fertilizers consist of fungi, cyanobacteria, algae naturally present in the soil and capable of forming symbiotic relationships with plants, as well as bacteria isolated from soil and verified for their efficacy. These microorganisms

collectively enhance microbial activity in the soil, accelerating the decomposition of complex organic compounds and converting macro- and micronutrients into plant-available forms (Ayyildiz & Kayikcioglu 2019; Li et al. 2022). Additionally, they stimulate seed germination (Bakonyi et al. 2013). Nonetheless, the majority of microbial products applied in agriculture, both in Türkiye and globally, are composed primarily of bacteria and fungi.

Due to their environmentally friendly nature, microbial fertilizers offer significant advantages over chemical fertilizers and can also function as biopesticides in preventing certain plant diseases and pests (Avan & Kotan 2021). By minimizing the residue risks associated with conventional pesticides, microbial fertilizers help reduce threats to both human and animal health (Li & Zang 2001; Tozlu et al. 2018). Thus, microbial fertilizers have broad applications not only in plant nutrition but also in plant protection. In addition to improving yield and quality in crop production, microbial fertilizers also contribute to reducing production costs (Kotan 2020).

In this study, the effects of increasing doses of a microbial fertilizer containing *Pseudomonas* spp., *Rhizobium* spp., *Azotobacter* spp., *Bacillus* spp., *Serratia* spp., *Aspergillus* spp., and *Penicillium* spp. on the yield and selected quality traits of different maize cultivars were investigated.

## 2. Material and Methods

In this study, two maize hybrids—P0573 (FAO 500) and P0937 (FAO 550)—provided by Pioneer Seed Distribution and Marketing Ltd. Co., and Sy Andromeda (FAO 600), developed by Syngenta Agricultural Industry and Trade Inc., were used as plant materials. As the microbial fertilizer source, EMA PLUS, containing *Pseudomanas spp., Rhizobium spp., Azotobacter spp., Bacillus spp., Serratia spp., Aspergillus spp., Penicillium spp.* was applied. According to the manufacturer, EM Active Plus includes six main microbial groups: photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes, molds, and *Penicillium* strains known for their antibiotic production. The microbial fertilizer (EMA Active Plus) was applied at five different total seasonal doses of 0 (control), 5, 10, 15, and 20 L $ha^{-1}$. All treatments were applied as foliar sprays using a constant carrier water volume of 200 L $ha^{-1}$. To improve spray retention and ensure uniform distribution across the leaf surface, a surfactant was added to the spray solution. Foliar applications were carried out evenly over the crop canopy while avoiding runoff (Anonymous 2023).

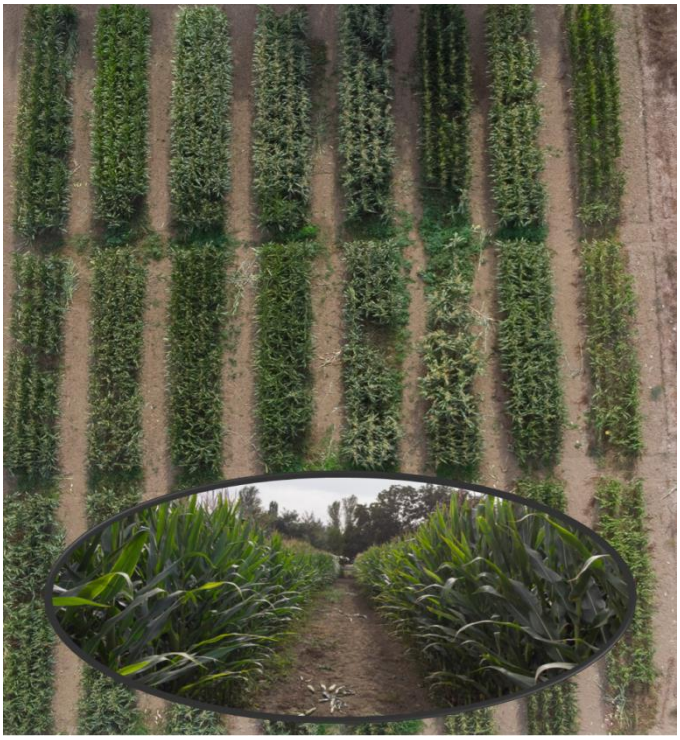


**Figure 1- The maize hybrids microbial fertilizer experiment was conducted at Afyonkarahisar, Türkiye**

The study was conducted in a farmer's field located in Yarıkkaya village of Emirdag district, Afyonkarahisar province, in Türkiye, during the 2021 and 2022 growing seasons (Figure 1). The experiments were arranged in a split-plot design with three replications. Maize cultivars (P0937, P0573, and Sy Andromeda) were assigned to the main plots, while microbial fertilizer doses (0 (control), 5, 10, 15, and 20 L $ha^{-1}$) were allocated to the sub-plots. Each plot consisted of six rows, 6 meters in length, with a row spacing of 70 cm and an intra-row spacing of 16 cm. Seeds were sown at a depth of 5–6 cm using a pneumatic planter. In the first year of the experiment, sowing was performed on April 25, 2021, and in the second year on May 5, 2022.

In both years, 200 kg N and 100 kg $P_2O_5$ per hectare were applied as fertilizers. Diammonium phosphate (DAP) was used as a basal fertilizer and applied to the soil at sowing. Top-dressing was carried out using urea fertilizer, applied in two equal splits: the first half at the 4–6 leaf stage, and the second half at the 8–10 leaf stage. Foliar application of the microbial fertilizer at different doses was carried out using a backpack sprayer at the at the 4–6 leaf stage of maize. The applications were conducted on 6 July 2021 and 24 July 2022, when the plants were at the same phenological stage in both growing seasons. Drip irrigation was used for watering the plants, and irrigation was applied ten times at one-week intervals. In 2021, post-sowing emergence irrigation was performed using a sprinkler system; however, in 2022, it was not required due to sufficient natural rainfall. Harvest was carried out on November 23 in 2021 and on October 25 in 2022. Weed control and hoeing were conducted manually. At harvest, border effects were minimized by discarding one row from each side of the plot and 50 cm from both ends. Harvesting was performed by hand. Morphological and yield-related measurements were conducted on 10 randomly selected plants and ears per replicate. Following the measurements, kernels were oven-dried to a constant weight at 12% moisture content for subsequent physical analyses. For chemical analyses, grain samples were ground to pass through a 0.5 mm sieve and stored at +4 °C until analysis. Climate data for the 2021 and 2022 corn growing seasons are given in Figure 2.

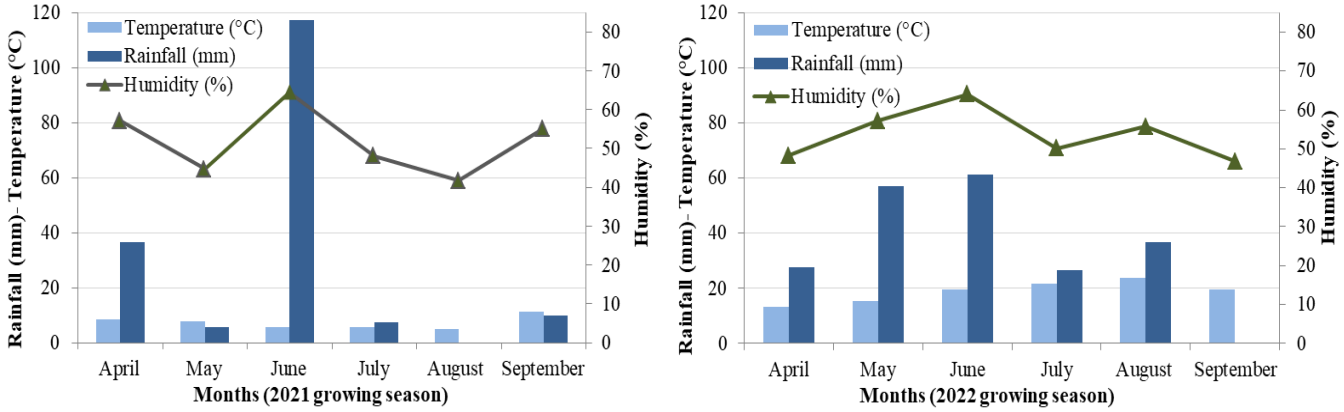



**Figure 2- Meteorological conditions in the experimental area during the 2021 and 2022 growing seasons. (In 2021, the total rainfall, average temperature, and relative humidity were 176.3 mm, 18.7 °C, and 51.8%, respectively, whereas in 2022, these values were 209.3 mm, 18.9 °C, and 53.7%, respectively.) (Data obtained from the Bilecik Regional Directorate of Meteorology)**

Prior to sowing, soil analyses were conducted at the experimental site to characterize the physical and chemical properties of the soil. The soil was classified as loamy in texture. It was non-calcareous, with a calcium carbonate ($CaCO_3$) content of 1.67%, and exhibited a slightly alkaline reaction (pH 7.54). The electrical conductivity was 0.71 mS $cm^{-1}$, indicating the absence of salinity problems. The organic matter content was low (1.43%). Available phosphorus, potassium, and magnesium concentrations were 29.12 ppm, 468.80 ppm, and 517.10 ppm, respectively, and were classified as high.

Morphological characteristics, including ear length and ear diameter, were measured on 10 randomly selected ears per plot. Ear length was measured from the base to the tip after husk removal, while ear diameter was measured at the midpoint using a caliper. Thousand-kernel weight was determined by weighing four subsamples of 100 kernels each, averaging the weights, and multiplying the result by 10. Hectoliter weight was measured according to AACC Method 55-10.01 (AACC 2020). Grain yield per hectare was calculated by threshing and weighing the ears harvested from each plot. Ash content was determined in ground grain samples following AACC Method 08-01.01 (AACC 2020). Oil content was analyzed by Soxhlet extraction using diethyl ether for 6 hours, and crude oil content was calculated on a dry matter basis (Welch 1977). Protein content was estimated using the Kjeldahl method by determining nitrogen concentration and multiplying by a factor of 5.78 (AACC 2020). Starch content was analyzed using commercial kits according to AACC Method 76-33.01 (AACC 2020). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were measured using an ANKOM 200 Fiber Analyzer following the procedures described by Van Soest et al. (1991).

The combined analysis of variance across years was conducted based on a split-plot design with three replications using the MSTAT-C statistical software. Treatment means were compared using Tukey's multiple comparison test at the 0.05 probability level ($P<0.05$).

## 3. Results

This study was conducted in Afyonkarahisar, Türkiye, during the 2021 and 2022 growing seasons to evaluate the effects of different microbial fertilizer doses on the yield, yield components, and selected quality traits of various maize cultivars. Analysis of variance and mean values for the examined traits are presented in Tables 1-4, Figures 3-4.

**Table 1- Mean squares and significance levels from the combined years' analysis of variance for the examined traits**

| *Source of variation* | *SD* | *EL* | *ED* | *NKE* | *GY* | *TGW* | *HW* |
|---|---|---|---|---|---|---|---|
| Year (Y) | 1 | 0.04 | 0.01 | 43086** | 247.44** | 53.98* | 2.50 |
| Replication (R) | 4 | 0.12 | 0.02 | 1549 | 12.61 | 19.11 | 4.92 |
| Cultivar (C) | 2 | 0.48** | 1.71** | 59802** | 557.06** | 225.48** | 5.41 |
| Y × C | 2 | 0.09 | 0.01 | 48491** | 154.65** | 294.21** | 11.43 |
| $Error_1$ | 8 | 0.06 | 0.06 | 784 | 8.23 | 10.68 | 3.39 |
| Microbial Fertilizer (MF) | 4 | 0.18 | 0.04 | 13037** | 205.35** | 634.09** | 3.73 |
| Y × MF | 4 | 0.03 | 0.02** | 2483* | 31.31 | 45.37 | 3.22 |
| C × MF | 8 | 0.47* | 0.05 | 618 | 38.49 | 94.55 | 13.34 |
| Y × C × MF | 8 | 0.04 | 0.09 | 898 | 11.98 | 51.30 | 4.28 |
| $Error_2$ | 48 | 0.13 | 0.05 | 949 | 26.41 | 50.78 | 5.32 |
| Coefficient of Variation (%) | | 1.95 | 4.39 | 4.86 | 2.69 | 2.05 | 3.15 |
| **Source of variation** | **SD** | **AC** | **PC** | **SC** | **FC** | **ADF** | **NDF** |
| Year (Y) | 1 | 0.42 | 2.22** | 8.84* | 0.17 | 0.29 | 0.64 |
| Replication (R) | 4 | 0.01 | 0.04 | 0.66 | 0.08 | 0.11 | 0.12 |
| Cultivar (C) | 2 | 0.59** | 3.41** | 0.40 | 3.11** | 4.85** | 2.01** |
| Y × C | 2 | 1.00** | 2.08** | 16.63** | 0.13 | 1.55** | 0.54* |
| $Error_1$ | 8 | 0.02 | 0.10 | 1.52 | 0.06 | 0.09 | 0.10 |
| Microbial Fertilizer (MF) | 4 | 0.05 | 1.33** | 1.13 | 0.30** | 1.19** | 1.61** |
| Y × MF | 4 | 0.03 | 0.31* | 1.43 | 0.29** | 2.62** | 0.50* |
| C × MF | 8 | 0.05 | 0.30* | 3.18** | 0.36 | 2.16** | 0.60** |
| Y × C × MF | 8 | 0.33** | 0.42** | 2.69** | 0.39** | 1.76** | 0.58** |
| $Error_2$ | 48 | 0.02 | 0.11 | 0.89 | 0.06 | 0.18 | 0.17 |
| Coefficient of Variation (%) | | 9.22 | 3.79 | 1.27 | 4.96 | 10.17 | 2.47 |

**: Significant at P<0.01, *Significant at P<0.05, C: Cultivars, MF: Microbial fertilizer, EL: Ear length (cm), ED: Ear diameter (cm), NKE: Number of kernels per ear, GY: Grain yield (t $ha^{-1}$), TGW: Thousand-grain weight (g), HW: Hectoliter weight (%), AC: Ash content (%), FC: Fat content, PC: Protein content (%), SC: Starch content (%), ADF: Acid detergent fiber (%), NDF: Neutral detergent fiber (%)

Most of the main factors-year, cultivar, and microbial fertilizer dose-as well as their two-way and three-way interactions, had statistically significant effects on the investigated yield and quality parameters. The traits such as ear length, ear diameter, number of kernels per ear, grain yield, thousand-grain weight, hectoliter weight, ash content, fat content, protein content, starch content, acid detergent fiber (ADF), and neutral detergent fiber (NDF) were significantly influenced by year, cultivar, and microbial fertilizer dose at the 1% or 5% significance levels. Additionally, several interactions, including year × cultivar, year × microbial fertilizer dose, cultivar × microbial fertilizer dose, and year × cultivar × microbial fertilizer dose, also showed significant effects on various traits. These findings suggest that yield and quality in maize are substantially influenced by the combined effects of genetic, environmental, and microbial factors (Table 1, Figures 2-4).

Ear length was significantly affected by cultivar (P<0.01) and the cultivar × microbial fertilizer dose interaction (P<0.05) (Table 1). Based on the mean values, the longest ears were obtained from cultivar P0937 (18.86 cm), followed by Sy Andromeda (18.66 cm), while P0573 had the shortest ears (18.42 cm) (Table 2). Although the mean ear lengths across microbial fertilizer doses ranged between 18.54 and 18.50 cm, the differences were not statistically significant. In the cultivar × dose interaction, the longest ears were observed with $MF_2$ in P0937, $MF_0$ in P0573, and $MF_4$ in Sy Andromeda (Table 4). The average ear lengths for 2021 and 2022 were 18.6 cm and 18.7 cm, respectively (Table 2).

Ear diameter was significantly influenced by cultivar and the year × microbial fertilizer dose interaction (P<0.01) (Table 1). The highest mean ear diameter was obtained from P0937 (5.11 cm), whereas P0573 had the lowest (4.63 cm) (Table 2). According to the cultivar × dose interaction, the widest diameters were observed under $MF_2$ in P0937, $MF_0$ in P0573, and $MF_4$

in Sy Andromeda (Table 4). The yearly averages were 4.9 cm and 4.8 cm (Table 2). In the year × microbial fertilizer dose interaction, the highest ear diameter was recorded under $MF_1$ in 2021, and the lowest under $MF_0$ in the same year (Figure 4).

**Table 2- Mean values of grain yield and yield components for cultivar and microbial fertilizer dose across the combined years**

| | ***EL*** | ***ED*** | ***NKE*** | ***GY*** | ***TGW*** | ***HW*** |
|---|---|---|---|---|---|---|
| Cultivars | | | | | | |
| P0937 | 18.86 a | 5.11 a | 671.00 a | 19.57 a | 344.99 b | 72.93 |
| P0573 | 18.42 c | 4.63 b | 646.14 b | 18.99 b | 346.04 b | 73.70 |
| Sy Andromeda | 18.66 b | 4.81 b | 585.02 c | 18.72 c | 350.17 a | 73.00 |
| Microbial fertilizer | | | | | | |
| $MF_0$ | 18.54 | 4.78 | 590.70 c | 19.01 bc | 343.21cd | 72.72 |
| $MF_1$ | 18.54 | 4.85 | 640.34 b | 19.15 b | 348.36 b | 73.06 |
| $MF_2$ | 18.66 | 4.91 | 663.04 a | 19.62 a | 355.82 a | 73.06 |
| $MF_3$ | 18.75 | 4.83 | 645.64 ab | 18.96 bc | 347.74 bc | 73.94 |
| $MF_4$ | 18.73 | 4.87 | 632.20 b | 18.71 c | 340.19 d | 73.28 |
| Year | | | | | | |
| 2021 | 18.63 | 4.86 | 612.50 b | 18.93 b | 347.84 a | 73.38 |
| 2022 | 18.67 | 4.84 | 656.26 a | 19.26 a | 346.29 b | 73.04 |

The values followed by common letters at each column are not significant at P>0.05 level of probability using the Duncan test

The number of kernels per ear was significantly affected by year, cultivar, and microbial fertilizer dose (P<0.01), as well as by the year × cultivar and year × microbial fertilizer dose interactions (P<0.05) (Table 1). The highest kernel count was recorded in P0937 (671.00), and the lowest in Sy Andromeda (585.02) (Table 2). $MF_2$ and $MF_3$ formed the statistically superior group, whereas $MF_0$ had the lowest kernel number. Yearly means were 612 and 656 (Table 2). In the cultivar × dose interaction, the P0937 × $MF_2$ combination was outstanding (Table 4). According to the year × cultivar interaction, P0937 consistently produced the highest kernel count in both years (Figure 3), while the year × microbial fertilizer interaction indicated that $MF_2$ in 2022 and $MF_0$ in 2021 represented the extremes (Figure 4).

Grain yield was significantly influenced by year, cultivar, microbial fertilizer dose, and the year × cultivar interaction at the 1% level (Table 1). P0937 had the highest yield (19.57 t $ha^{-1}$), while Sy Andromeda had the lowest (18.72 t $ha^{-1}$) (Table 2). $MF_2$ resulted in the highest yield (19.62 t $ha^{-1}$). Mean yields were 18.93 and 19.26 t $ha^{-1}$ for 2021 and 2022, respectively, with P0937 performing best in both years (Table 2; Figure 3).

Thousand-grain weight was significantly affected by cultivar, microbial fertilizer dose, and the year × cultivar interaction (P<0.01), while the difference between years was significant at P<0.05 (Table 1). The highest value was observed in Sy Andromeda (350.17 g) and the lowest in P0937 (344.99 g) (Table 2). $MF_2$ led to the highest thousand-grain weight (355.82 g). Yearly averages were 348.0 g and 347.5 g. In the cultivar × dose interaction, the Sy Andromeda × $MF_2$ combination was most effective (Table 4, Figure 3).

No statistically significant differences were detected for hectoliter weight. Mean values ranged from 72.93 to 73.00 kg among cultivars and from 72.72 to 73.94 kg across MF doses. The annual averages were 73.4 kg in the first year and 73.0 kg in the second year (Table 2).

For ash content, the effects of cultivar, year × cultivar, and year × cultivar × MF dose interactions were significant at the 1% level (Table 1). The highest ash content (1.76%) was recorded in Sy Andromeda, while the lowest (1.48%) was obtained from P0573. Across MF doses, ash content ranged between 1.56% and 1.69%, and annual means were 1.7% and 1.6%, respectively (Table 3). In the cultivar × MF dose interaction, the Sy Andromeda × $MF_2$ combination was the most prominent (Table 4). According to the year × cultivar interaction, the highest ash content was obtained from Sy Andromeda and the lowest from P0573, both in 2021 (Figure 3).

For protein content, the effects of year, cultivar, and MF dose were significant at the 1% level, while year × MF and cultivar × MF interactions were significant at the 5% level (Table 1). The highest protein content was obtained from Sy Andromeda (9.12%) and under the $MF_2$ dose (9.06%) (Table 3). The highest value in the cultivar × MF interaction was also observed in the Sy Andromeda × $MF_2$ combination (Table 4). The annual averages were 8.9% and 8.7% (Table 4). According to the year × cultivar interaction, the highest protein content was recorded in Sy Andromeda in 2021 (Figure 3). Based on the MF × year interaction, the highest protein content was found under $MF_2$ and $MF_3$ in 2021 (Figure 4).

**Table 3- Mean values of quality traits for cultivar and microbial fertilizer dose across the combined years**

| | *AC* | *PC* | *SC* | *FC* | *ADF* | *NDF* |
|---|---|---|---|---|---|---|
| Cultivars | | | | | | |
| P0937 | 1.63 b | 8.61 b | 73.89 | 4.73 b | 3.83 c | 16.53 a |
| P0573 | 1.48 c | 8.48 b | 73.82 | 5.03 a | 4.62 a | 16.17 b |
| Sy Andromeda | 1.76 a | 9.12 a | 74.04 | 4.38 c | 4.11 b | 16.68 a |
| Microbial fertilizer | | | | | | |
| $MF_0$ | 1.62 | 8.43 c | 73.77 | 4.67 bc | 4.18 b | 16.51 a |
| $MF_1$ | 1.64 | 8.89 ab | 73.80 | 4.58 c | 4.33 ab | 16.54 a |
| $MF_2$ | 1.59 | 9.06 a | 73.78 | 4.91 a | 4.48 a | 16.64 a |
| $MF_3$ | 1.69 | 8.83 b | 73.87 | 4.76 ab | 4.16 b | 16.68 a |
| $MF_4$ | 1.56 | 8.47 c | 74.36 | 4.64 bc | 3.79 c | 15.94 b |
| Year | | | | | | |
| 2021 | 1.69 | 8.89 a | 69.60 b | 4.67 | 4.13 | 16.38 |
| 2022 | 1.55 | 8.68 b | 70.23 a | 4.76 | 4.24 | 16.54 |

The values followed by common letters at each column are not significant at P>0.05 level of probability using the Duncan test.

Starch content was significantly affected by year (P<0.05), and by year × cultivar and year × cultivar × MF dose interactions (P<0.01) (Table 1). Starch content ranged between 73.77% and 74.36% on average (Table 3), with the highest value (75.08%) obtained from the Sy Andromeda × $MF_4$ combination (Table 4). According to the year × cultivar interaction, the highest starch content was recorded in Sy Andromeda in 2022 (Figure 3).

Fat content was significantly influenced (P<0.01) by cultivar, MF dose, MF × year, and year × MF × cultivar interactions (Table 1). The highest fat content was measured in P0573 (5.03%) and under $MF_2$ (4.91%) dose. In the cultivar × MF dose interaction, the highest fat content was obtained from the P0573 × $MF_2$ combination (Table 4). Annual averages were 4.7% and 4.8%, respectively (Table 3). According to the MF × year interaction, the highest fat content was obtained from $MF_2$ in 2021 (Figure 4).

ADF and NDF values were significantly affected by all main factors and their interactions (Table 1). The highest ADF value among cultivars was obtained from P0573 (4.62%) and the lowest from P0937 (3.83%). $MF_1$ and $MF_2$ were statistically grouped together with the highest ADF values among the MF doses. In the cultivar × MF dose interaction, the highest ADF value (5.29%) was found in Sy Andromeda under $MF_1$, while the lowest (3.22%) was recorded in P0937 under $MF_4$ (Table 3). According to the year × cultivar interaction, P0573 and Sy Andromeda had the highest ADF values in the first year, while Sy Andromeda continued to rank among the highest in the second year as well (Figure 3). In the year × MF interaction, $MF_1$ and $MF_3$ in the first year and $MF_2$ and $MF_3$ in the second year formed the top statistical group in terms of ADF (Figure 3). Additionally, ADF values were 4.1% in the first year and 4.2% in the second year.

**Table 4- Cultivar × microbial fertilizer dose interaction for the studied traits, based on mean values over years**

| *Cultivar* | *MF* | *EL* | *ED* | *NKE* | *GY* | *TGW* | *HW* | *AC* | *PC* | *SC* | *FC* | *ADF* | *NDF* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P0937** | $MF_0$ | 18.73a-d | 5.00 | 628.47 | 19.59 | 338.58 | 71.17 | 1.69 | 8.60b-e | 73.19cd | 4.60efg | 3.95b-e | 16.80ab |
| | $MF_1$ | 18.93ab | 5.00 | 681.50 | 19.74 | 346.66 | 73.67 | 1.59 | 8.68bcd | 73.14d | 5.00bc | 3.83b-e | 16.88ab |
| | $MF_2$ | 19.07a | 5.24 | 709.07 | 20.43 | 361.39 | 74.17 | 1.61 | 8.82b | 74.09a-d | 4.82cde | 3.67de | 16.62abc |
| | $MF_3$ | 18.90ab | 5.20 | 674.73 | 19.22 | 342.13 | 73.17 | 1.81 | 8.65bcd | 74.18a-d | 4.69def | 4.49a-d | 16.88ab |
| | $MF_4$ | 18.68a-e | 5.14 | 666.07 | 18.84 | 336.18 | 72.50 | 1.47 | 8.30de | 74.85ab | 4.56efg | 3.22e | 15.48e |
| **P0573** | $MF_0$ | 18.66a-e | 4.52 | 600.43 | 18.94 | 342.98 | 73.67 | 1.43 | 8.23e | 74.28abc | 4.81cde | 4.86a | 16.18cd |
| | $MF_1$ | 18.30efg | 4.70 | 658.60 | 18.88 | 346.94 | 73.33 | 1.48 | 8.42cde | 73.99a-d | 4.89bcd | 4.53a-d | 16.10d |
| | $MF_2$ | 18.07g | 4.64 | 676.07 | 19.26 | 350.20 | 74.00 | 1.51 | 8.79bc | 74.13a-d | 5.28a | 4.47a-d | 16.25cd |
| | $MF_3$ | 18.40c-g | 4.63 | 663.27 | 19.06 | 349.07 | 75.33 | 1.48 | 8.61b-e | 73.53cd | 5.12ab | 4.66ab | 16.22cd |
| | $MF_4$ | 18.60b-f | 4.71 | 632.33 | 18.79 | 340.99 | 72.17 | 1.49 | 8.35de | 73.15d | 5.04abc | 4.61abc | 16.12d |
| **Sy Andromeda** | $MF_0$ | 18.23fg | 4.81 | 543.20 | 18.52 | 348.08 | 73.33 | 1.75 | 8.47b-e | 73.84bcd | 4.61ef | 3.73cde | 16.54bcd |
| | $MF_1$ | 18.40d-g | 4.92 | 580.93 | 18.83 | 351.48 | 72.17 | 1.87 | 9.56a | 74.28abc | 3.85h | 4.64b | 16.63abc |
| | $MF_2$ | 18.87abc | 4.84 | 604.00 | 19.18 | 355.87 | 71.00 | 1.66 | 9.56a | 73.13d | 4.64def | 5.29a | 17.05a |
| | $MF_3$ | 18.90ab | 4.71 | 598.93 | 18.60 | 352.04 | 73.33 | 1.78 | 9.22a | 73.88bcd | 4.48eg | 3.33de | 16.94ab |
| | $MF_4$ | 18.92ab | 4.80 | 598.07 | 18.49 | 343.40 | 75.17 | 1.72 | 8.76bc | 75.08a | 4.34g | 3.54cde | 16.23cd |

The values followed by common letters at each column are not significant at P>0.05 level of probability using the Duncan test.

Among cultivars, the highest NDF content (16.67%) was found in Sy Andromeda, followed by P0937, which belonged to the same statistical group. The lowest NDF value (16.17%) was recorded in P0573. Only $MF_4$ among the MF doses showed the lowest NDF value and was statistically distinct (Table 3). In the cultivar × MF dose interaction, the highest NDF value (17.05%) was recorded in Sy Andromeda under $MF_2$, while the lowest (15.48%) was observed in P0937 under $MF_4$ (Table 4). According to the year × cultivar interaction, P0937 and Sy Andromeda consistently had the highest NDF values in both years

(Figure 3). In the year × MF dose interaction, the highest NDF value was observed under $MF_2$ in the second year, while the lowest was under $MF_5$ in the same year. ADF values were 4.1% and 4.2% in the first and second years, respectively (Figure 4).

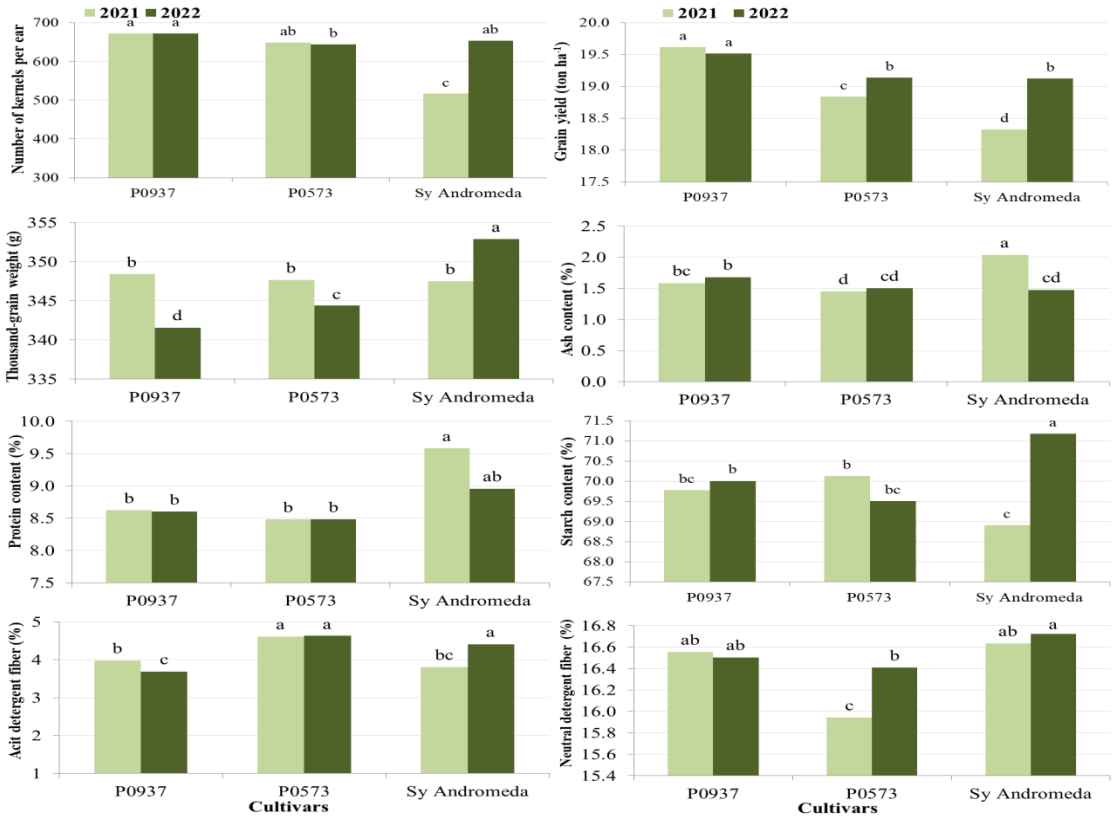



**Figure 3- Average values for the traits with significant cultivar × year interaction effects**

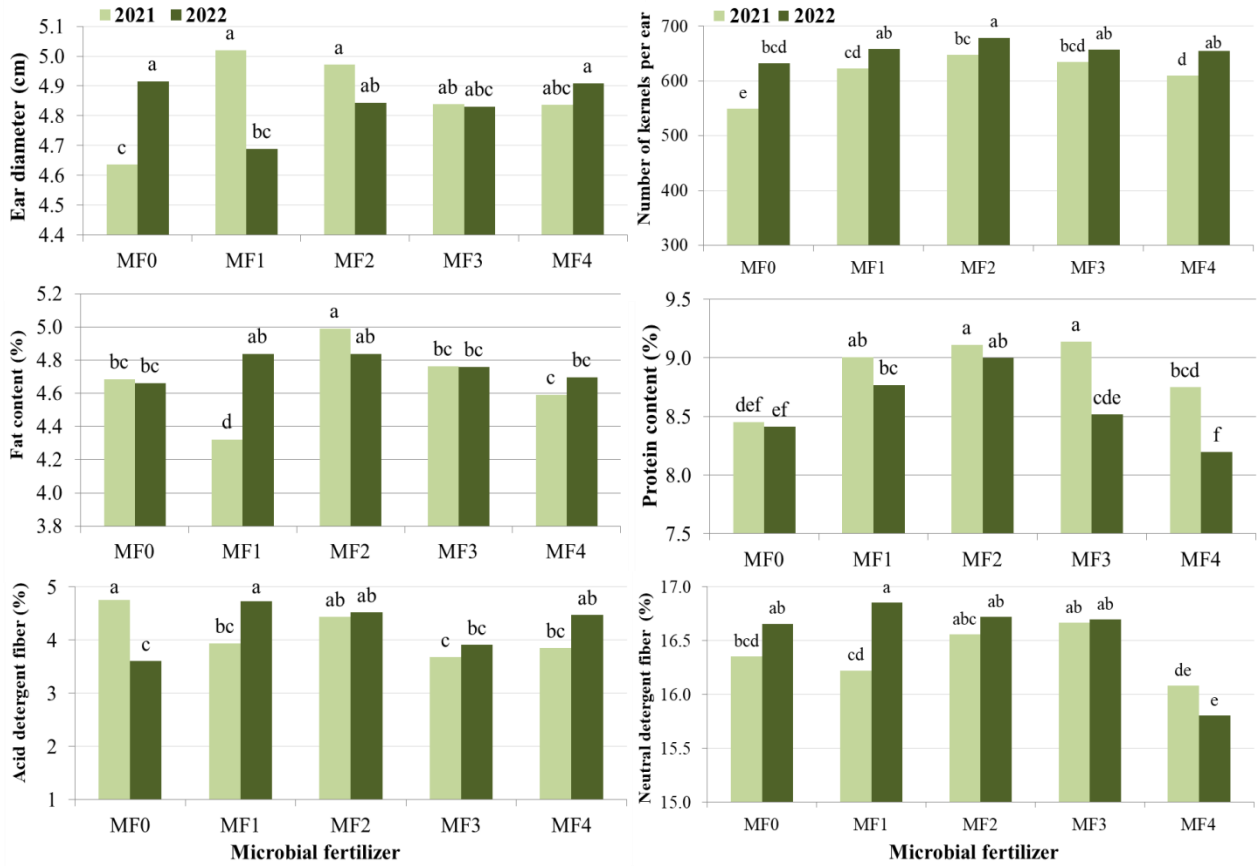



**Figure 4- Average values for the traits with significant microbial fertilizer × year interaction effects**

## 4. Discussion

This study demonstrated that ear morphology, yield components, and grain quality characteristics in maize were significantly influenced by MF applications, cultivar differences, and year-to-year climatic variation. The significant main effects of year, cultivar, and MF dose, as well as their interactions (Table 1), emphasize the multifactorial control of these agronomic traits under field conditions.

Ear morphology, particularly ear length and diameter, responded differentially to cultivars and MF doses. The longest ear (18.86 cm) was recorded in cultivar P0937, especially under $MF_2$ application (Table 2; Table 4), which supports previous studies highlighting genotype × fertilizer interactions in cob development (Shafai et al. 2020; Dusak 2021). Although MF applications did not significantly alter overall ear length means (Table 2), specific cultivar × MF combinations (e.g., P0937 × $MF_2$) were statistically superior, indicating a genotype-specific response. Ear diameter was significantly influenced by the year × MF dose interaction ($P<0.01$), with $MF_1$ producing the widest diameter in 2021 (Figure 3). These findings are in agreement with the results reported by Akgun et al. (2021), who emphasized that ear morphological traits in maize are governed by the interaction between genotype and nutrient management. Their study demonstrated that genotypic differences in ear length, ear diameter, and kernel formation are closely associated with differential responses to nutrient availability, thereby supporting the combined influence of genetic background and nutrient management observed in the present study.

Kernel number per ear was significantly increased by the $MF_2$ and $MF_3$ doses, with the most pronounced response observed in the hybrid P0937 (Table 2, Table 4). These findings are consistent with Dogan et al. (2020), who reported that improved nutrient availability through fertilization enhances reproductive development in maize. The superiority of the P0937 × $MF_2$ interaction suggests that microbial biofertilizers may promote reproductive development indirectly by improving nitrogen availability and stimulating physiological processes related to assimilate supply. Adequate nitrogen availability during the critical period from ear initiation to silking enhances floret survival, reduces kernel abortion, and ultimately increases kernel number per ear (Below 2002; Setter & Flannigan 1986; Ciampitti & Vyn 2013). Moreover, the significant year × MF dose interaction indicates that kernel number was higher under $MF_2$ in 2022 and lower under $MF_0$ in 2021 (Figure 4). Such differences can be attributed to variations in rainfall and humidity between years, which are known to influence nutrient uptake efficiency and reproductive success in maize (Bänziger et al. 1999).

Grain yield was highest in P0937 (19.57 t $ha^{-1}$), under $MF_2$ (19.62 t $ha^{-1}$), with overall higher yields in 2022 (Table 2). The year × cultivar interaction (Figure 3) further reveals the superior and consistent performance of P0937 in both years. The result confirm that both genotypic potential and MF treatments are critical for improving yield, corroborating findings from Cihangir & Oktem (2016). The favorable climatic conditions in 2022-with higher rainfall (209.3 mm) and humidity (53.7%)-likely contributed to improved physiological processes, as warmer and wetter seasons are known to support biomass accumulation and kernel filling. Grain yield in maize is a complex, polygenic trait strongly shaped by genotype–environment–management interactions. In many studies, the primary objective has been to enhance yield per unit area. While the selection of cultivar is crucial for improving yield, environmental factors such as climate and soil, along with applied agricultural practices, also play a significant role. Previous research has shown that grain yield varies depending on the genotypes used, years, locations, and agricultural practices (Aghanejad et al. 2015; Dag et al. 2024a).

Thousand-grain weight (TGW) was significantly affected by cultivar, MF dose, and their interaction (Table 1, Table 4). Sy Andromeda × $MF_2$ produced the highest TGW (355.82 g), suggesting that microbial fertilizers enhanced grain filling through improved nutrient availability (Figure 3). Although the year effect was marginal, the interaction patterns again reflected the importance of genotype-specific responses under variable climatic conditions. The number of ears per unit area, the number of kernels per ear, and the thousand-kernel weight are the main factors determining grain yield in maize. Similarly, Dag et al. (2024b) found that the application of Vitormone Plus (*Azotobacter* spp.) increased the thousand-kernel weight from 484.77 to 506.85 g. Hectoliter weight showed no statistically significant variation (Table 1), and mean values ranged narrowly between 72.72–73.94 kg (Table 2). This result suggests that hectoliter weight may be relatively stable across environmental and fertilizer treatments, consistent with previous reports (Doganlar 2018).

Grain quality parameters such as ash, fat, protein, and starch content revealed complex interactions. Ash content was highest in Sy Andromeda (1.76%), particularly under $MF_2$ (Table 3, Table 4), indicating a cultivar-dependent mineral uptake. The year × cultivar interaction (Figure 3) shows that ash content peaked in Sy Andromeda in 2021, possibly reflecting stress-induced mineral accumulation under relatively drier conditions.

Protein content was influenced by all main factors and most interactions (Table 1). Sy Andromeda × $MF_2$ yielded the highest value (9.12%) (Table 4), while annual variation (8.9% in 2021 vs. 8.7% in 2022) (Table 3). The highest protein content was also found under $MF_2$ and $MF_3$ in 2021 (Figure 3), emphasizing the synergy between MF input and weather stress. Protein content in maize is a trait influenced by genetic makeup, environmental conditions, and agricultural practices (Deliboran et al. 2018). Various studies have reported a wide range of protein content in maize, including values between 7.46% and 8.81% (Alp and Koca 2020), and 11.05% and 14.50% (Ozturk & Buyukgoz 2021). Gao et al. (2020) reported that protein concentrations ranged from 85.8 to 342.6 mg/g in response to various fertilizer combinations applied to maize. However, Dag

et al. (2024b) identified significant differences in protein content among maize cultivars, but noted that microbial fertilizer (*Azotobacter* spp.) doses did not significantly alter protein content, which ranged from 10.13% to 10.22%. These findings suggest that the effects of microbial fertilizers may vary depending on their composition, as well as the maize cultivar and environmental conditions. Several studies have reported that biofertilizer applications can influence protein content in maize (Soleymanifard & Naseri 2014; Fadlalla et al. 2016).

Starch content was highest in Sy Andromeda × $MF_4$ (75.08%) (Table 4), while average values varied between 73.77% and 74.36% across treatments (Table 3). The year × cultivar interaction (Figure 2) again highlights Sy Andromeda as superior in starch accumulation, especially in 2022—likely due to more favorable weather. Starch content in maize varies depending on cultivar and location. Previous studies have reported starch contents ranging from 58.89% to 65.57% (Kilinc et al. 2018; Alp & Koca 2020). In their study investigating the effects of different biofertilizer mixtures (including A. *chroococcum*, AMF, and B. *circulans*) on maize, Gao et al. (2020) reported that starch content ranged from 14.3% to 47.6%, and that the applications led to an increase in starch concentration. However, Dag et al. (2024b) found that microbial fertilizers had no significant effect on starch content in maize kernels, which ranged between 68.54% and 69.04%.

Fat content reached its highest value in the hybrid P0573 under the $MF_2$ application (5.03%) (Table 3, Table 4), and the year × MF dose interaction indicated that $MF_2$ application in 2021 was the most effective for oil accumulation (Figure 4). This result suggests that an intermediate microbial fertilizer dose provided more suitable conditions for oil synthesis than either lower or higher doses. Moderate improvements in nutrient availability, particularly nitrogen, are known to support balanced carbon assimilation and assimilate distribution during the grain-filling period, thereby promoting oil accumulation in maize kernels (Below 2002; Ciampitti & Vyn 2013). While insufficient nutrient supply may restrict metabolic activity, excessive inputs do not necessarily result in additional increases in oil content. The favorable response of P0573 under $MF_2$ indicates that oil accumulation in maize grain is closely related to the genetic structure of the hybrid, as oil content is largely controlled by genotype and typically ranges between 3.5% and 5.5% in maize (Kahrıman et al. 2017). These results suggest that optimized microbial fertilizer doses can support the expression of the genetic potential for oil accumulation in suitable hybrids. Moreover, maize oil holds significant global importance in terms of industrial use and human nutrition. In other studies, Fadlalla et al. (2016) stated that maize oil content varied between 3.05% and 3.90% in the first year, and between 2.52% and 4.25% in the second year.

Fiber fractions, namely ADF and NDF, were affected by all sources of variation (Table 1). The highest ADF (5.29%) was observed in Sy Andromeda × $MF_1$, and the lowest (3.22%) in P0937 × $MF_4$ (Table 3 and Table 4). According to the year × cultivar interaction (Figure 2), ADF was higher in 2021, a drier season, suggesting stress-induced lignification. Similar patterns were observed in year × MF interactions (Figure 3). NDF was highest in Sy Andromeda × $MF_2$ (17.05%), and lowest in P0937 × $MF_4$ (15.48%) (Table 4). The year × cultivar interaction (Figure 2) shows consistent cultivar patterns across years, while $MF_2$ in 2022 had the highest NDF value among year × MF interactions (Figure 3). These findings reflect the dynamic response of fiber composition to both genetic and environmental factors. In previous studies, Radosavljević et al. (2012) determined that the ADF (acid detergent fiber) content in maize ranged between 3.89% and 4.88%, which is consistent with the ADF values of 3.65% to 5.22% reported by Mut et al. (2022). Dag et al. (2024b) reported that NDF content in maize grain ranged from 14.09% to 14.32% and was not affected by microbial fertilizer application. Taken together, the results confirm that microbial fertilizers can improve maize productivity and quality traits, but their effectiveness is closely dependent on cultivar type and climatic conditions. For instance, 2022's higher rainfall (209.3 mm) and relative humidity (53.7%) (compared to 176.3 mm and 51.8% in 2021) likely enhanced water and nutrient uptake, resulting in improved kernel number, starch, and fat content (Table 2 and Table 3; Figures 3 and 4) affected not only yield traits but also some quality attributes like fat and protein content.

## 5. Conclusions

This two-year field study clearly demonstrates that maize yield, yield components, and grain quality traits are jointly shaped by microbial fertilizer dose, cultivar, and interannual climatic variability. Among the evaluated hybrids, P0937 consistently exhibited superior agronomic performance in terms of grain yield, kernel number per ear, and ear morphological traits, whereas Sy Andromeda was distinguished by its higher protein, starch, and fiber contents.

When all traits were considered together, the $MF_2$ dose emerged as the most effective microbial fertilizer level, providing the highest or most favorable values for the majority of yield and quality parameters. The significant main and interaction effects detected for most traits indicate that responses to microbial fertilizer applications are strongly genotype- and environment-dependent. These findings highlight the necessity of developing cultivar-specific and environment-sensitive microbial fertilizer strategies rather than relying on uniform application practices.

Climatic conditions played a decisive role in determining treatment effectiveness. The higher rainfall and relative humidity recorded in 2022 enhanced kernel number, grain yield, and the accumulation of starch and fat, demonstrating that favorable environmental conditions can amplify the positive effects of microbial fertilizers, whereas less favorable conditions may limit their efficiency.

In conclusion, the integration of microbial fertilizers into maize production systems offers substantial potential to improve both productivity and grain quality when appropriate dose–cultivar combinations are applied. Based on the present results, the $MF_2$ dose can be recommended as an effective application level under similar ecological conditions. Future studies should focus on long-term field evaluations to assess impacts on soil health, economic sustainability, and the combined use of microbial fertilizers with conventional nutrient management practices, as well as to elucidate the physiological and microbiological mechanisms underlying genotype-specific responses.

**Author Declarations**

**Authors' contributions:** Conceptualization: M.B., and O.D.E.K., Methodology: M.B., O.D.E.K., and Z.M. Investigation: M.B., and Y.M.K. Data analysis: O.D.E.K., and Z.M. Writing—original draft: S.D. Writing—review and editing: M.B., O.D.E.K., Z.M., and Y.M.K. All authors read and approved the final manuscript.

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