

## Maize leaf greenness and yield differential response to the biostimulating effect of *Bacillus simplex* under irrigation

### *Bacillus simplex* biostimuláns hatása a kukorica levélzöldességére és termésére öntözött körülmények között

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#### ABSTRACT

The use of bioinoculants continues to gain research attention due to the need to ensure sustainable soil and crop productivity under climate change. Soil and Plant Analysis Development (SPAD) and Normalized Difference Vegetation Index (NDVI) are useful indicators of leaf greenness that have a positive nexus with yield. These parameters are proper indicators of treatment effects. Therefore, this study examined the biostimulating effect of *Bacillus simplex* seed inoculation on leaf greenness and yield of maize under irrigated conditions. A field experiment with treatments consisting of maize seeds inoculated with *Bacillus simplex* and a control under irrigation was conducted. The SPAD and NDVI data were collected at the 12-leaf stage (V12), tasselling stage (VT) and Kernel blister stage (R2), while yield component and yield data were collected at harvest time. Analysed data revealed that *Bacillus simplex* seed inoculation significantly improved NDVI by 16.42% only at the R2 growth stage. Yield and yield components were significantly optimised by *Bacillus simplex* seed inoculation. Cob length, cob weight, weight of seeds per cob, number of seeds per cob and weight of 1000 seeds improved by 18.45%, 24.99%, 24.62%, 18.62% and 6.34%, respectively. The overall grain yield was optimised by 26.97% (3.12 t/ha). Therefore, *Bacillus simplex* can be used as a seed bioinoculant with irrigation to enhance sustainable maize production under climate change.

**Keywords:** maize, NDVI, SPAD, productivity, yield parameters

#### ÖSSZEFOGLALÁS

A biostimulátor készítmények használata egyre nagyobb figyelmet kap a mezőgazdaságban, mivel a változó éghajlat mellett is biztosítani kell a fenntartható talajhasználatot és a növénytermesztés eredményességét. A relatív klorofilltartalom (SPAD érték) és a normalizált differenciált vegetációs index (NDVI érték) a levelek zöldességének jó indikátorai, amelyek egyúttal pozitív kapcsolatban állnak a terméssel. Ezen mutatók a növénytermesztési kutatások gyakori mérőszámaiként szolgálnak. A tanulmányban a kukorica vetőmagok *Bacillus simplex* kezelésének biostimuláló hatását vizsgáltuk a kukorica levelek zöldességére és termésére öntözött körülmények között. Ennek a hatásnak az értékelésére 2022-ben egy szántóföldi kísérlet került beállításra *Bacillus simplex* vetőmagkezeléssel, öntözött körülmények között. A SPAD és NDVI adatokat 12 levél (V12), hímvirágzás (VT) és szemtelítődés (R2) fenofázisokban, míg a terméskomponens és a termésmennyiség adatokat a betakarítás időpontjában gyűjtöttük. Az adatok elemzése alapján megállapítottuk, hogy

a *Bacillus simplex* kezelés az R2 növekedési szakaszban jelentősen, 16,42%-kal javította a kukorica NDVI értékét. A termést és a terméskomponenseket a *Bacillus simplex* vetőmagkezelés egyaránt szignifikánsan javította. A csőhossz, csőtömeg, egy csövön lévő szemek tömege, egy csövön lévő szemek száma és az ezerszemtömeg 18,45%-kal, 24,99%-kal, 24,62%-kal, 18,62%-kal és 6,34%-kal volt magasabb a kontrollhoz képest, emellett pedig a termésmennyiség is 26,97%-kal (3,12 t/ha) növekedett. Az eredményeink alapján megállapítottuk, hogy a *Bacillus simplex* öntözéssel együtt vetőmag biostimulátor készítményként alkalmazható a fenntartható kukoricatermesztésben, segítve a változó éghajlati adottságokhoz való alkalmazkodást.

**Kulcsszavak:** kukorica, NDVI, SPAD, termésmennyiség, terméskomponens

## INTRODUCTION

For the current agricultural production to sustain an adequate food supply, production needs to be increased by 70% by 2050 (Hunter et al., 2017; Etesami et al., 2023). Maize is one of the cereal crops that contributes to this production because of its high global economic and nutritional importance (Jarecki, 2024), making it a crop of high research interest (Ssemugenze et al., 2024). Maize grows in different agro-ecologies with varying soil types, temperatures and other agroclimatic factors (Rehman et al., 2020; Ghazanfar et al., 2024), hence varying productivity. For this reason, achieving sustainable production under climate change requires sustainable methods. Climate change precipitates uncertainty in the hydrological cycle (Li et al., 2017), increasing in the case of water scarcity (drought), which negatively affects crop production (Shirinbayan et al., 2019). Therefore, a synchronised allocation of agricultural irrigation water is necessary (Li et al., 2021). Furthermore, the productivity of soil, a fundamental production resource, is decreasing (Ocwa et al., 2023). Moreover, to meet nutritional requirements for sustainable maize production, optimal nutrient supply is critical (Blanco-Valdes et al., 2022; Jarecki, 2024). Biostimulants from symbiotic microorganisms are emerging as effective, sustainable methods of enhancing agricultural productivity (Ouhaddou et al., 2024).

Beneficial microorganism preparations affect crop growth (El-Zemrany et al., 2007; Coelho et al., 2020) depending on the soil type, microbe species in question, agricultural system (Coelho et al., 2020), as well as crop management (Buzo et al., 2020). *Bacillus* species have versatile functionality, hence commercially utilised in the agro-biotech industry as biopesticides, biofertilisers and

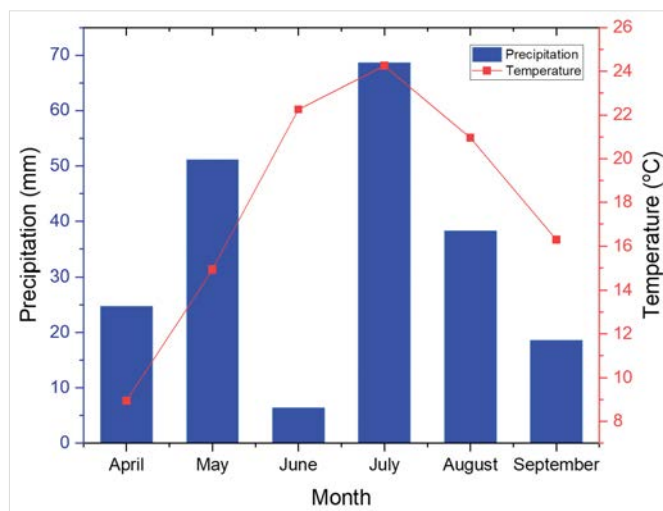
phytostimulants (Etesami et al., 2023). *Bacillus* species such as *Bacillus pumilus*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* are commonly used to treat seeds to enhance plant growth (Ocwa et al., 2024a). *Bacillus* bacteria improve crop growth through siderophore production, solubilisation of phosphorous and potassium, and production of growth hormones (Etesami et al., 2023; Ouhaddou et al., 2024). Etesami et al. (2023) echoed that understanding how growth traits of plants respond to varying *Bacillus* spp. under field conditions is vital because field conditions vary by agroecosystems which affects successful *Bacillus* spp. application. Under field conditions, the ability of the *Bacillus* spp. to enhance greenness is important.

“Leaf greenness is a general term that characterises the color of the leaf in reference to the intensity of the green color associated with chlorophyll concentration” (Rolando et al., 2015). As such, SPAD leaf greenness index (Szulc et al., 2021) and NDVI are useful indicators of leaf greenness. In agriculture, NDVI indicates greenness of the vegetation and vigour (Ouhaddou et al., 2024), and SPAD value indicates the chlorophyll content of the plant, i.e. higher SPAD values show better plant nutrition (Mumtaz et al., 2020). Leaf greenness is reported to have a positive correlation with yield and yield components (Jagła et al., 2019). However, leaf greenness and yield are affected by several factors, including moisture and nutrient supply, soil microbial ecology, climatic conditions of a given season and biological seed treatments. Therefore, this study investigated the biostimulating effect of *Bacillus simplex* seed inoculation on the leaf greenness and yield of maize under irrigated conditions.

## MATERIALS AND METHODS

### Study area

The experiment was conducted at the demonstration garden of the Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen (47°83, 030" N, 21°82, 060" E, 111 m asl) in the 2022 season. The highest temperature was experienced in June, July and August, and peak precipitation in September (Figure 1). The site had leached chernozem soil, which is inadequately supplied with nitrogen and organic matter but rich in phosphorus and magnesium. Details are contained in a published report of Ocwa et al. (2024b).



**Figure 1.** Temperature and rainfall at the experimental area (Source: The University of Debrecen Agrometeorological Station, 2022)

### Experimental design, treatments, and sowing

The experiment was set up with a randomised complete block design (RCBD) with two treatments: seeds inoculated with *Bacillus simplex* bacteria and the control (uninoculated control) with four replications. The seed inoculation treatment was applied before sowing at a rate of 10 g per hectare. The *Bacillus simplex*, containing biological products in the form of powder, was mixed carefully with the seeds to enhance electrostatic adherence to the surface of the seeds. A Kongskilde Vibro Master SGC/SQ 25 mounted seedbed cultivator was used

to prepare the seedbed. Blanket fertiliser application at the rate of 101.25 kg/ha N, 18.75 kg/ha MgO and 26.25 kg/ha CaO was performed. FAO 420 maize hybrid was sown on 2 May 2022 at a plant population of 72100 per hectare. Irrigation was installed and started on 14 June 2022, before the onset of drought. The drip irrigation system was remotely controlled via a mobile phone application (Hydrawise application). Irrigation was started at the minimum value of the maximum field water capacity value in all the phenophases. The irrigation system was removed at physiological maturity, exhibited by the appearance of the black layer in the maize kernels. The total irrigation water applied during the experimental period was 413.6 mm.

### Data collection and analysis

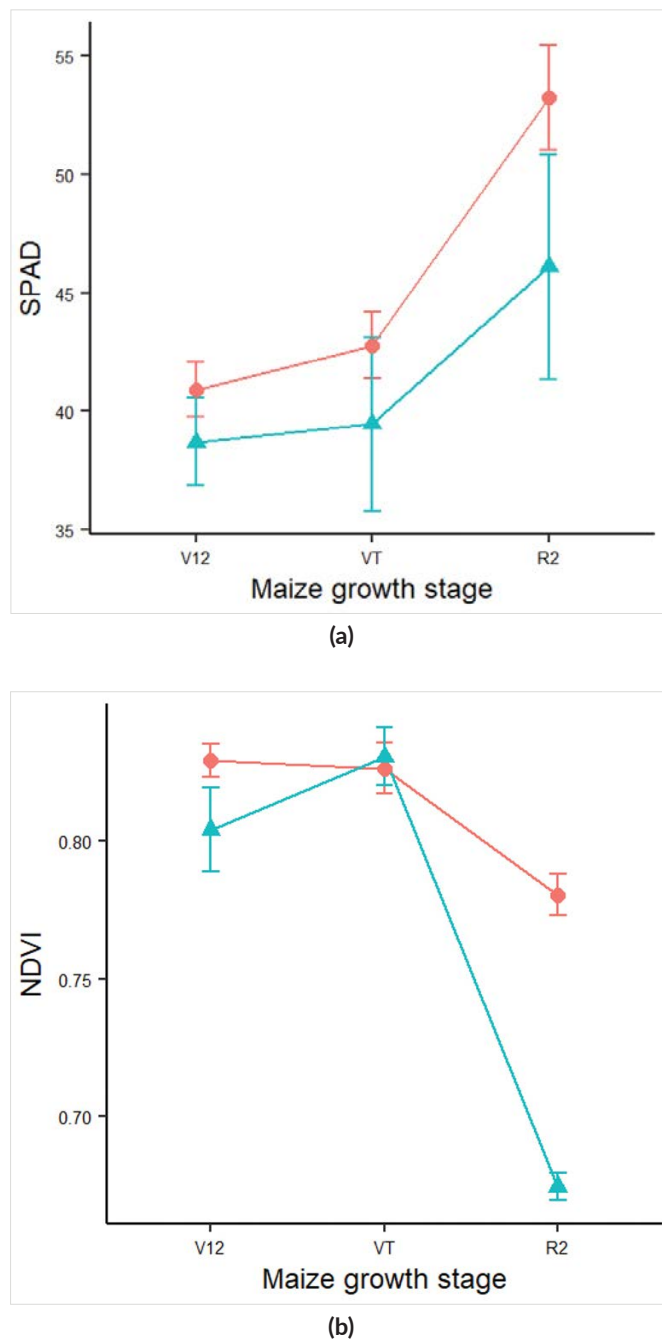
Data was collected on leaf greenness traits; The Soil Plant Analysis Development (SPAD) and Normalized Difference Vegetation Index (NDVI), at 12 leaf stage (V12), tasseling stage (VT) and kernel blister stage (R2), and yield and yield components after harvest. SPAD was measured using a chlorophyll meter on ten randomly selected maize plants. Similarly, NDVI was measured using the Greenseeker on ten randomly selected maize plants. At the same time, yield data were collected on cob length (cm), cob weight (g), cob diameter (mm), number of seeds per cob, weight of seeds per cob, 1000 seed weight, and yield per hectare. Ten cobs per treatment in each replication were randomly collected and processed using a HALDRUP LT-35 laboratory thresher (HALDRUP GmbH, Ilshofen, Germany). Cob length was measured using a ruler calibrated in cm. Cob diameter was determined using a digital Vanier calliper. Cob weight was determined using an electronic weighing balance. Number of seeds per cob, weight of seeds per cob and 1000 seed weight were determined using VSC-201 Vibrating Seed Counter (PLC Tuning Ltd - Hungary), and grain yield (t/ha) was calculated at a moisture content of 14.5%. The t-test was used to determine the effect of the treatments using Minitab 20 Statistical Software.

## RESULTS AND DISCUSSION

### *Effect of Bacillus simplex seed inoculation on SPAD and NDVI of maize*

There were no significant differences in SPAD and NDVI between the two treatments except at the R2 growth stage. At the V12 growth stage, *Bacillus simplex* had a SPAD value of 40.92 compared to the 38.70 in the control. Similarly, at the VT growth stage, *Bacillus simplex* treatment had a SPAD value of 42.78 compared to the 39.46 in the control. At the R2 growth stage, SPAD was 53.23 compared to 46.11 in the control. Although a significant effect was only evident at the R2 growth stage, the result of this study generally suggests a positive impact of *Bacillus simplex*. Earlier, Jarecki (2023) reported no significant difference in SPAD between *Bacillus* species inoculated seeds and the control. In other words, the author reported SPAD of 47.5 in seeds inoculated with *Bacillus* compared to 47.3 in the control. However, Ghazanfar et al. (2024) reported that *Bacillus* species improved SPAD by 67%. Similarly, a study by Hussain et al. (2022) showed that *Bacillus* sp. AZ6 bioactivated zinc results in maximum improvement of SPAD by 66%. According to Mumtaz et al. (2020), inoculation with some *Bacillus* species significantly had higher SPAD content of up to 55.9 under sole application, and between 55.6-65.3 when combined with each other, except a combination of *Bacillus* spp. ZM20 × *B. aryabhattai* S10 which had SPAD (53.1) not significantly different from the control (50.7). As such, because of high SPAD value, authors emphasised that *Bacillus* species promoted better plant nutrition due to improved nutrient uptake.

For NDVI, at growth stages V12 and VT, *Bacillus simplex* had an NDVI close to 0.83. However, at the R2 growth stage, *Bacillus simplex* treatment recorded significantly higher NDVI of 0.78 compared to 0.67 in the control. This denotes a 16.42% improvement of NDVI by *Bacillus simplex* treatment at R2 growth stage (Figure 2). Generally, it is vital to note that, although there were no significant differences at the V12 and VT growth stages, *Bacillus simplex* treatment maintained a positive effect on the NDVI, particularly at the R2 growth stage. Overall, in



**Figure 2.** SPAD and NDVI progression at different stages of maize growth under *Bacillus simplex* treatment: a) SPAD, b) NDVI (vertical bars show the standard error)

all growth stages, *Bacillus simplex* treatment maintained a minimum NDVI of over 0.78, which is within the healthy vegetation range. Earlier, a study by Senger et al. (2022) showed an increase in NDVI due to seed inoculation by *Bacillus simplex*. According to Ouhaddou et al. (2024), under irrigation, biostimulants, including *Bacillus* sp., improved NDVI values from 0.54 to 0.69. Since NDVI indicates

greenness of the vegetation and vigour (Ouhaddou et al., 2024), it means that the *Bacillus simplex* treatment effect was efficacious at the R2 stage. It is worth noting that the R2 growth stage coincided with the period with the highest temperature and low precipitation. Therefore, it is possible that *Bacillus simplex* improved the water use efficiency and nutrient use efficiency of maize, resulting in higher leaf greenness traits.

### Effect of *Bacillus simplex* seed inoculation on the yield and yield components of maize

#### Cob length, cob diameter and cob weight

*Bacillus simplex* treatment significantly ( $P < 0.05$ ) improved cob length, cob diameter and cob weight (Table 1). As a result of the *Bacillus simplex* treatment, the cob length was 20.67 cm compared to 17.45 cm in the control, indicating 18.45% improvement. Similarly, the *Bacillus simplex* treatment resulted in a cob diameter of 49.02 mm compared to 46.27 mm in the control, indicating a 5.94% improvement. In terms of cob weight, an improvement of 24.99% was recorded by *Bacillus simplex* treatment. This result compares with Mumtaz et al. (2020), who showed that several *Bacillus* species resulted in a cob length of 16.0 cm compared to 15.3 cm in the control, resulting in an improvement of 4.6%. The same report showed that *Bacillus* sp. ZM20 had a significantly higher cob weight of 138.2 g as compared to 131.4 g in the uninoculated control. In other words, the result of this study and the published literature provide evidence that the efficacy of *Bacillus* species in improving yield components is promising.

**Table 1.** Cob length, cob diameter and cob weight of maize under *Bacillus simplex* treatment

Treatment	Cob length (cm)	Cob diameter (mm)	Cob weight (g)
<i>Bacillus simplex</i>	20.67±0.37	49.02±0.57	242.6±13.60
Control	17.45±1.20	46.27±0.27	194.1±18.60
P-value	*	**	**

± to the means represents SE of 4 replications. \* means significant at  $P < 0.05$ , \*\* significant at  $P < 0.01$

#### Weight of seeds per cob, number of seeds per cob, and weight of 1000 seeds

There were significant ( $P < 0.05$ ) differences in the weight of seeds per cob, number of seeds per cob, and weight of 1000 seeds between the two treatments (Table 2). The weight of seeds per cob was 213.6 g under *Bacillus simplex* treatment compared to 171.4 g in the control. The number of seeds per cob was 566.4 under *Bacillus simplex* treatment compared to 477.5 in the control. Similarly, the weight of 1000 seeds was 378.29 g under *Bacillus simplex* treatment compared to 355.73 g in the control (Table 2). In other words, *Bacillus simplex* treatment optimised seeds per cob, number of seeds per cob and weight of 1000 seeds. Specifically, *Bacillus simplex* treatment improved the weight of seeds per cob, number of seeds per cob and weight of 1000 seeds by 24.62%, 18.62% and 6.34%, respectively. This compares with Ahmad et al. (2023), who indicated that inoculation with a consortium of bacteria, including *Bacillus* spp. improved 1000-grain weight by 17%. Also, Jarecki (2023) reported significantly higher grain number per cob (457.2) and weight of 1000 seeds (257.7 g) under *Bacillus* species inoculated seeds compared to 453.6 and 252.6 in the control, respectively. According to Ouhaddou et al. (2024), *Bacillus* sp. combination with compost and mycorrhizal fungi, enhanced 1000-grain weight by 43%. From the results of this study and previous literature, it is clear that the level of effect elicited by *Bacillus simplex* depends on other nutrient sources and agro-inputs.

**Table 2.** Weight of seeds per cob, number of seeds per cob and weight of 1000 seeds of maize under *Bacillus simplex* treatment

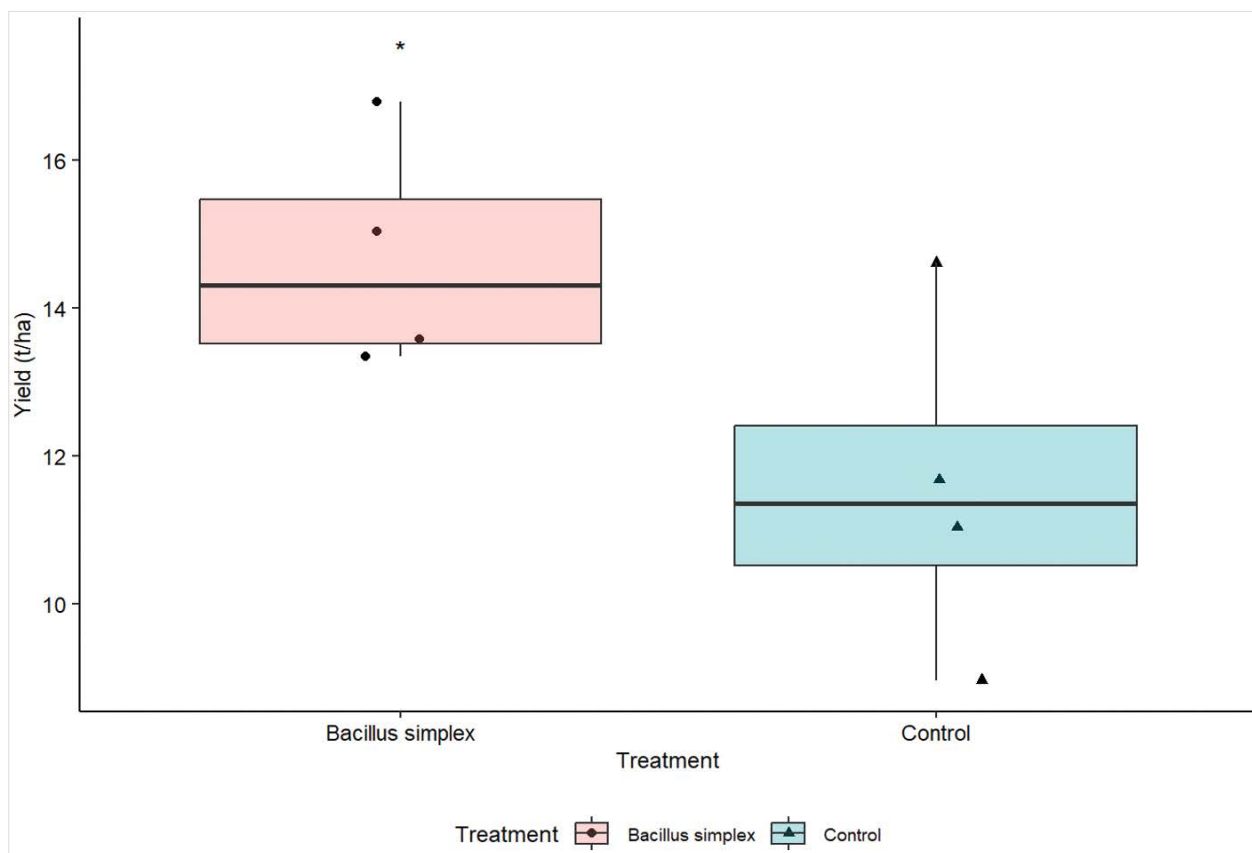
Treatment	Weight of seeds per cob (g)	Number of seeds per cob	Weight of 1000 seeds (g)
<i>Bacillus simplex</i>	213.6±12.1	566.4±23.1	378.29±7.75
Control	171.4±16.3	477.5±30.8	355.73±8.99
P-value	**	*	**

± to the means represents SE of 4 replications. \* means significant at  $P < 0.05$ , \*\* significant at  $P < 0.01$

### Grain yield

There was a significant ( $P < 0.05$ ) difference in the grain yield between the two treatments. The grain yield was 14.69 t/ha under *Bacillus simplex* treatment compared to 11.57 t/ha in the control. This implies that the *Bacillus simplex* treatment enhanced grain yield by 26.97% (3.12 t/ha) (Figure 3). This finding corroborates the results of Senger et al. (2022), who reported 26% (2.1 t/ha) yield increment by *Bacillus simplex* seed inoculation. Besides, *Bacillus* sp. combination with compost and mycorrhizal fungi also enhanced grain yield by 175% (Ouhaddou et al., 2024). Also, the bacterial consortium including *Bacillus* spp. improved grain yield 12% (Ahmad et al., 2023). On the contrary, Jarecki (2023) reported no significant difference in yield between *Bacillus* species inoculated seeds and the control. In other words, the author reported a yield of 9.78 t/ha in the case of seeds inoculated with *Bacillus* compared to 9.7 t/ha in the control. *Bacillus*

species can mobilise nutrients in the soil (Nawaz et al., 2024), and increase nutrient absorption (Rezakhani et al., 2019; Nawaz et al., 2024), and consequently improve plant growth (Sandhya et al., 2011) and yield. In this study, since the field was irrigated, it appears that *Bacillus simplex* treatment improved water use efficiency. Li et al. (2024) noted that *Bacillus* spp. improves water use efficiency. Additionally, *Bacillus* species promote plant growth through the production of indole acetic acid, ammonia production, and catalase activity (Ghazanfar et al., 2024). Therefore, the application of *Bacillus* as bioinoculants could provide a sustainable solution to crop production amidst the climate change crisis and soil quality deterioration. According to Singh and Shyu (2024), *Bacillus* species not only promote plant growth, but also maintain plant health by fostering increased resistance to pathogens, hence contributing to sustainable agriculture under global change.



\* means significant at  $P < 0.05$

Figure 3. Effect of *Bacillus simplex* treatment on the grain yield of maize

### SPAD, NDVI and yield nexus

In Figure 4, the increase in SPAD and NDVI corresponds to the increase in grain yield. Since SPAD shows chlorophyll content that directly affects photosynthetic activity, it also affects yield. In fact, improved chlorophyll content results in improved photosynthetic activity (Djaenuddin et al., 2021) and, consequently, yield. Similarly, Senger et al. (2022) explained that NDVI is used to indicate plant vigour that can be related to higher photosynthetic activity and, consequently, yield. Therefore, the fact that *Bacillus simplex* improved NDVI in this study confirms its efficacy in optimising yield. This result echoes the earlier report that biostimulants from symbiotic microorganisms are emerging as effective, sustainable methods of enhancing agricultural productivity (Ouhaddou et al., 2024).

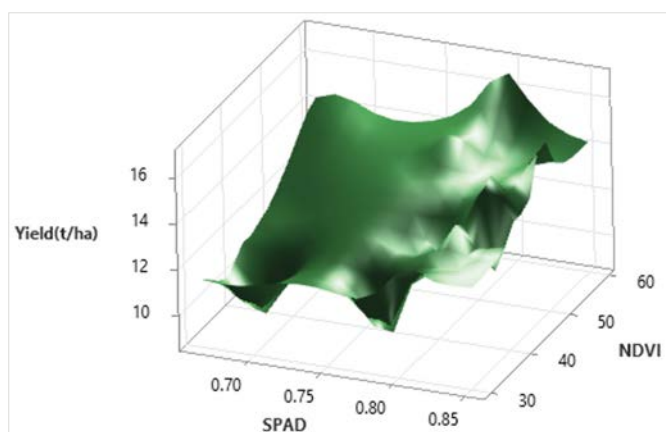


Figure 4. Surface plot of SPAD, NDVI and yield under *Bacillus simplex* treatment

### CONCLUSION

This study investigated the biostimulating effect of *Bacillus simplex* seed inoculation on the leaf greenness based on SPAD and NDVI, and yield of maize under irrigated conditions. The effect was positive for both SPAD and NDVI, although a significant difference was only evident for NDVI at the R2 growth stage. Cob length, cob weight, weight of seeds per cob, number of seeds per cob and weight of 1000 seeds were significantly improved by 18.45%, 24.99%, 24.62%, 18.62% and 6.34%, respectively. The overall grain yield was optimised by 26.97%.

Therefore, *Bacillus simplex* can be used as a seed bioinoculant with irrigation to enhance sustainable maize production under climate change. However, long-term studies under irrigation, and studies under rainfed conditions and nutrient stress are needed to widen the scope and applicability of the results of this study.

### ACKNOWLEDGEMENTS

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