

Ameliorative properties of microbially composted Azolla in improving drought resilience in *Zea mays*

Noorafizah Dzahir¹, Zaheda Mohamad Azam¹, Mohamad Azzuan Rosli¹,
Abd Rahman Jabir Mohd Din^{1*} , Nor Zalina Othman^{1,2} 

¹ Innovation Centre in Agritechnology for Advanced Bioprocessing (ICA), Universiti Teknologi Malaysia (UTM), Pagoh Education Hub, 84600 Muar, Johor, Malaysia

² Department of Bioscience, Faculty of Sciences, Universiti Teknologi Malaysia (UTM) 81310 Skudai, Johor Bahru, Malaysia

* Corresponding author's e-mail: arahmanj@utm.my

ABSTRACT

This study investigated the ameliorative role of composted Azolla (CA) as a microbial biostimulant to improve maize growth, soil fertility, and resilience under water-limited conditions. A composted Azolla was developed, which is mainly composed of microbial consortium, *Paenibacillus polymyxa* DSM 36 and *Trichoderma asperellum* NBRC 30498. Maize (*Zea mays*) seedlings were grown under two irrigation regimes (normal and deficit) with CA, autoclavable composted Azolla (ACA) and commercial fertilizer (CF), respectively. At the vegetative stage, the deficit irrigation was performed by withholding the water for 14 days before morphophysiological and biochemical parameters were assessed. Results revealed that CA significantly enhanced shoot and root growth, biomass production, and drought recovery compared to CF and ACA. Under deficit irrigation, CA-treated plants achieved greater shoot length (60.33 ± 3.33 cm) and dry weight (0.59 ± 0.31 g), along with thicker cortical roots and larger xylem vessels. Soil amended with CA exhibited higher nitrogen ($2.75 \pm 0.04\%$), carbon ($23.76 \pm 0.39\%$), microbial abundance (9.88×10^8 cfu/mL), and moisture retention. Biochemical assays showed improved chlorophyll content ($188.69 \mu\text{g g}^{-1}$ FW) and elevated proline levels, signifying enhanced photosynthetic stability and osmotic adjustment. This result demonstrated an ameliorative role of composted Azolla in mitigating drought-induced stress while improving soil health and physiological performance of maize production.

Keywords: *Azolla pinnata*, biostimulant, compost, deficit irrigation, drought stress mitigation, *Zea mays*.

INTRODUCTION

Globally, 80% of arable land is under rain-fed agriculture while, in Malaysia, the depletion of stream flow and El-Nino Southern Oscillation (ENSO) has contributed to the risk of drought stress events [Mahmud and Osawa, 2025]. Hence, facing this climate uncertainty by applying water-saving technologies is critical to sustain food productivity to support the global population in the next 30 years.

Many approaches have been suggested in reducing the risk of growing food crops under drought-stress conditions such as the restricted water-saving irrigation and the high-cost setup of

remote sensing applications [Balota et al., 2024; Zgallai et al., 2024]. Unfortunately, such strategies as developing drought-tolerant transgenic plants through genetic engineering are very laborious and time-consuming. Historically, the application of organic waste materials with minimal environmental impact has been advocated alternatively by farmers as the means of composting, which helps to reduce plant stress while increasing the yield. In this regard, the incorporation of composted-crop residues has been demonstrated to decrease soil evaporation and increase soil water retention. Noteworthy examples include the use of assemblages of mycorrhizal fungi with green waste compost to enhance drought stress

in carob, biochar-compost mixture for rice resilience to drought, compost for *Nigella sativa* and microbial compost for pistachio [Boutasknit et al., 2021; Hazman et al., 2023; Abdou et al., 2023; Paymaneh et al., 2023]. All these composts exhibit beneficial features linked to their ability to prevent ion leakage, promote membrane fluidity, reduce stress-related oxidative damages, and accelerate the accumulation of osmoprotectants such as proline and trehalose. Additionally, the application of compost has been proven to increase water retention of amended soils and relieve the suppressant effect of irrigation deficit on plant productivity [Kranz et al., 2020]. To a certain extent, efforts have been made to compost green waste biomass, owing to the greater nutrients in the composted materials and the presence of water-holding capacity [Nguyen et al., 2012; Alotaibi et al., 2023]. In particular, this water-holding capacity is more pronounced in improving soil aggregation during the onset of drought periods [El-Mageed et al., 2019].

In general, maize (*Zea mays*) is an economically important crop cultivated extensively worldwide and is more often prone to drought stress due to being known as a rain-fed crop [Sah et al., 2020]. Maize has been of particular interest in terms of a source of starch and protein, rich in essential vitamins, and abundant in antioxidant properties and phytochemicals of pharmacological significance [Revilla et al., 2022; Swati et al., 2024]. While Malaysia is heavily reliant on food imports, efforts are underway to aggressively promote maize cultivation to fulfill the increasing domestic demand, particularly in animal feed industries [Yazid et al., 2021]. Drought is the greatest abiotic stress, not only reducing maize yield but also degrading the physiological and nutritional qualities. Not only yield reduction, the availability, absorption and transport of nutrients are disrupted under water-deficient conditions, resulting in delayed flowering. Studies have demonstrated that maize showed drought tolerance mechanisms, possibly by having various assimilations such as altering their deeper morphological root architecture, photosynthetic rate change, and increasing cellular reactive oxygen species (ROS) at the molecular metabolism [Han et al., 2024]. Interestingly, studies have potentially exhibited that maize plants possess memory behaviour following prolonged periodic episodes of drought, enabling them to recover their physiological functions [Walter et al., 2011; Qi et al., 2021]. Even though maize crops

systemically respond through a series of events after perceiving those drought-stress signals, crop resilience for its survival following rehydration is still not discussed adequately. In light of maize worldwide annual loss as a result of drought, the development of cost-effective agronomical practices through composting together with alternate wetting-drying techniques by enabling plants to cope with deficit irrigation conditions is the most viable practical [Schmitt et al., 2010]. Drying-rewetting cycles under compost application have been proven to help in reducing water use up to 30% while increasing the soil mineralization rate by 78% [Novair et al., 2020]. However, the acceptability of these approaches relies on their success in terms of maximizing the yield with minimal water use.

Back in 1960, *Azolla* had been used for centuries by Chinese and Vietnamese farmers and was further expanding the use of *Azolla* under government-funded projects for crop cultivation research (Prabakaran et al., 2021). Given to symbiotic association with blue-green algae (*Anabaena azollae*) in providing both resource complementarities, *Azolla* potentially can fix up to 1.1 tonnes of nitrogen/ha/year [Kollah et al., 2016]. For this reason, conversion of *Azolla* biomass into a spectrum of bio-based products is currently gaining interest in the context of its vital essentiality in chemical-based fertilizer substitution. Jumadi et al. (2014) has also studied the composted *Azolla* application extensively due to its high nitrogen content. Accordingly, Bharali et al. [2021] observed an application of *Azolla* compost minimize the detrimental effect of CH₄ transformation by 36% through the enhancement of porosity and recalcitrant carbon fractions in soils. In this concern, Brouwer et al. [2018] reported that CO₂ sequestration by *Azolla* accounts for 97.2 kg dry weight/ha/day, which was close to the ‘maximum theoretical algal productivity’ value. In fact, *Azolla* is considered one of the fastest aquatic plants ever recorded and it was reported that an initial spreading 500 kg of *Azolla* can lead to up to 20,000 kg/ha within 2 weeks, indicating their rapid ability to sequester CO₂ in the form of biomass [Hamdan and Hourri, 2022]. Considering these facts, treating crops with composted *Azolla* that is enhanced by microbial inoculants could be a sustainable and cost-effective effort, providing immediate economic and environmental benefits while controlling the *Azolla*-dense mat formation [Parmanoan et al., 2025]. While numerous studies

have investigated the rationale of composted materials derived from green waste on agronomic properties, little is known about the actions of composted *Azolla* on maize drought tolerance.

Therefore, keeping the above in view, we aimed to assess the response of maize seedlings when compost was present in the soil mixture under drought stress. Owing to considerable evidence of the influences of compost on maintaining soil moisture, it was hypothesized that the addition of microbially composted *Azolla* to the soil mixtures may practically increase the resilience of maize seedlings toward drought stress. Nevertheless, most of them largely depended on the properties of composted materials as well as cultivation conditions specific to each region of study. On these grounds, the model plant *Zea mays* was grown under the presence of composted *Azolla*, treated as a microbial biostimulant, and chemical fertilizer, to evaluate these treatments on plant drought tolerance response. With this objective, we examined various morphophysiological and biochemical parameters including (i) vegetative-reviving features after drying-wetting conditions, (ii) root anatomical traits observation, and (iii) photosynthetic pigment and several biochemical changes including osmoprotectants, to deduce microbially compost-mediated drought tolerance mechanisms in maize.

METHODOLOGY

Plant materials and experimental conditions

Zea mays seeds were used for drought experiments in this study. The seeds were surface-sterilized with 10% ethanol for 10 min, then with 50% commercial bleaching agent (2.5% NaOCl) for 30 min, then washed 3 times thoroughly with distilled water. Surface-sterilized seeds were pre-germinated on solid Murashige-Skoog (MS) salts medium with 0.8% agar.

Paenibacillus polymyxa DSM 36 and *Trichoderma asperellum* NBRC 30498 were provided by German Collection of Microorganisms and Cell Culture (DSMZ, Germany) and NITE Biological Resource Center (NBRC, Japan). The original strains were kept at -80 °C with 50% glycerol. For consortia inoculant preparation, these two strains were streaked on Nutrient Agar (NA) and Potato Dextrose Agar (PDA), respectively. A 48-h DSM 36 suspension culture was prepared to 1×10^8

cfu mL⁻¹, whereas spore suspension from 7-day-old NBRC 30498 was adjusted to 1×10^6 spores mL⁻¹. This Pesta granules formulation based on kaolin-wheat flour mixture was formulated according to Wong et al. [2021]. An equal volume of DSM 36 and NBRC 30498 approximately 200 mL of suspension each was mixed aseptically into the dough-formed formulations. The mixture was air-dried then ground to pass through a sieve to obtain a uniform granule size.

The pots were 20 cm in height, 18 cm in diameter, and were filled with 700 g of sandy loam soil (which comprised % clay, % silt, and % sand). The soil was autoclaved before use in order to reduce the initial microbial load and avoid soil physicochemical effect variation. The physicochemical properties of the soil were characterized as follows with pH, electrical conductivity, organic content and available phosphorus content of the weight of soil.

Microbially composted *Azolla* preparation

Azolla plant (*Azolla pinnata*) was collected from the catchment tanks located at Universiti Teknologi Malaysia (UTM) Pagoh. The plants were grown in a 360 L container covered with insect-proof net cloth, with natural photoperiod and at 28–32 °C. Collected *Azolla* plants were rinsed with distilled water before being dried at 50 °C to constant weight. The dried *Azolla* plants were used as the main nitrogenous component in the compost preparation, together with rice bran and sawdust. To compost *Azolla*, a mixture of all these components and molasses was used at a 2:1 of total weight, respectively. The aforementioned granular microbial formulation was added at a level of 2.5% on a dry weight basis of the composting mix. The compost mixtures were packed into a 45 L custom-made composting barrels with dimensions of 40 × 123 × 47 cm (length x height x width). Water was added regularly to maintain the initial moisture content in the compost mixtures at 60% respectively. The composting process lasted around 45 days. Once matured with dark brownish and constant temperature, total N, P, K, Mg of the microbially composted *Azolla* were measured before using as a soil amendment. The pH, EC, total N and total P&K were determined as described previously [Mohd Din et al., 2017]. Total carbon (C) was determined by measuring the loss on ignition at 550 °C until a constant weight was achieved in a furnace. The C:N

ratio was calculated using the total C and total N obtained from the analyses. Some properties of composted material are shown in Table 1.

Pot-grown experiments with drought

Three 10-day-old germinated seedlings were transplanted in a drained hole pot (3 seeds each pot), containing approximately 100 g of sterile soil before placing in the growth chamber with 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 65% humidity, 12/12 h light dark photoperiod and 28 °C temperature. The experiment was laid out in a randomized complete block design with factorial arrangement with five replicate pots for three treatments (60 pots altogether). The treatments included three amendments with two kinds of drought stress. The treatments were: amendment with composted *Azolla* (CA), chemical fertilizer

(CF), and autoclavable composted *Azolla* (ACA) as a negative control. Each of these treatments was equally added about 100 g of amendment materials to each respective pot. Two irrigation regimes were employed for each treatment: normal irrigation (150 mL daily) corresponding to the full water requirement and deficit irrigation (50% of normal irrigation volume). Each pot was watered regularly about 150 mL daily. The irrigation regime was withheld to initiate the water deficit condition for After two-week intervals. All plants then exposed to drought were resumed to normal watering/rewetting for another 7 days. After 7 days, the percentage of plant revival from drought treatment was recorded to assess the plant drought tolerance (Figure 1). At the end of the experiment, all plants were harvested for morphophysiological and biochemical changes.

Biochemical assays

Leaf samples were ground in 80% acetone with sand to extract the chlorophyll. The extracts were centrifuged at $10\,000 \times g$ at 4 °C for 10 min to remove any residual materials, and the absorbance of the supernatant was measured at wavelengths 645 and 663 nm using UV-VIS spectrophotometers. The total chlorophyll concentration was determined according to Arnon [1949] using Equation 1:

$$\text{Total chlorophyll content } (\mu\text{g g}^{-1} \text{FW}) = (20.2 \times \text{Abs}_{645}) + (8.02 \times \text{Abs}_{663}) \times (v/1000 \times w) \quad (1)$$

where: *Abs* – absorbance; *v* – final volume of solution and *w* – weight of sample.

Table 1. Chemical properties of the fresh *Azolla* and composted *Azolla*

Parameter ^a	Fresh <i>Azolla</i> biomass	Microbially composted <i>Azolla</i>
pH	6.76	7.43
EC ($\mu\text{S/cm}$)	54.89	14.39
N (%)	3.91	4.31
P (%)	1.53	6.08
K (%)	8.19	4.05
Mg (%)	0.59	1.25
C:N ratio	20.78	13.35

Note: ^aEC – electrical conductivity; N – nitrogen; P – phosphorus; K – potassium; Mg – magnesium.

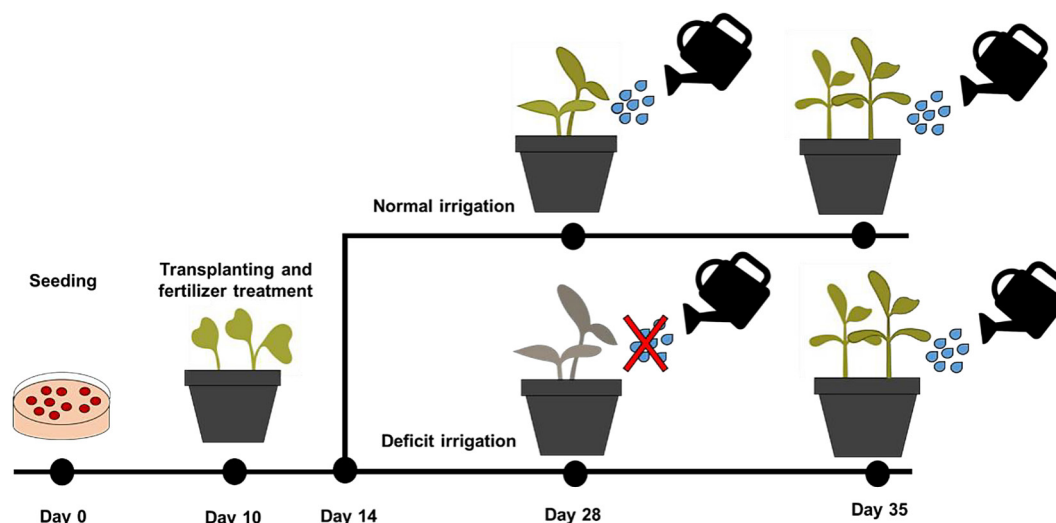


Figure 1. Schematic diagram of the experimental timeline on maize seedlings response to normal and deficit irrigation regimes after treated with microbially composted *Azolla* together with chemical fertilizer

Proline content in the root samples was measured according to Bates [1973]. A 100 mg dried-ground root sample was homogenised in 3% (w/v) of sulfosalicylic acid and centrifuged at 10 000 xg for 15 min at 4 °C. A volume of 2 mL supernatant was added to 2 mL acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 mL 6M phosphoric acid) and 2 ml of glacial acetic acid, followed by incubation at boiling water bath for 1 h and further cooled for 2 min. A volume of 4 mL toluene was added and the mixture was vigorously agitated for 1 min. The extract was measured at 520 nm using toluene as a blank.

Root and agronomic growth assessment

Agronomic attributes like shoot and root length were measured. Fresh weight was weighed using analytical balance. Samples were oven-dried at 65 °C for 72 h to achieve constant dry weight.

Root anatomical traits observation

To view the root anatomical in response to drought treatment, the root systems were washed using tap water to remove any possible particles of soil before storing at 4 °C in 70% ethanol. Root segments were hand-sectioned and stained with 1.5% Toluidin Blue O for 5 min before washing with distilled water. The cross-sectioned roots were observed by compound microscope [Hazman and Brown, 2018].

Statistical analysis

All the biological measurements were performed in triplicate. The data obtained from biological replicates was statistically analyzed by one-way analysis of variance (ANOVA and

presented as means $\pm$ standard deviation (SD). The means were calculated and compared using the least significant difference at $p < 0.05$. All the statistical analyses were conducted using SPSS. Graphs were fitted using Microsoft Office (Excel 2013).

RESULTS AND DISCUSSION

Agronomic growth, biomass production and yield attributes of maize

Results regarding effects of microbially composted *Azolla*, autoclavable composted *Azolla* and chemical fertilizer on agronomic traits under varying irrigation conditions are depicted in Table 2 and Figure 3. It was noticeable that deficit irrigation had a significant effect on agronomic attributes, particularly on the shoot. Table 2 showed that the irrigation regime and treatment type significantly influenced the shoot growth of maize seedlings. Under deficit irrigation, the maize seedlings treated with composted *Azolla* (CA) produced the highest shoot fresh weight, dry weight and shoot length with 5.47 ± 2.40 g and 0.59 ± 0.31 g and 60.33 ± 3.33 cm, respectively. These values were significantly higher ($p < 0.05$) than those other treatments. A similar pattern of agronomic traits for seedlings fertilized with composted *Azolla* was observed under normal irrigation. The shoot dry weight became heavier under deficit irrigation conditions. The comparative morphology of maize seedlings, as shown in Figure 2, further confirmed these quantitative findings. Plants amended with CA appeared to be more vigorous with greener shoots and dense fibrous roots, whereas ACA and CF seedlings showed reduced shoot growth

Table 2. Effect of different irrigation regimes on shoot agronomic traits of maize seedlings

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot length (cm)
Deficit irrigation			
CA	5.47 ± 2.40^a	0.59 ± 0.31^a	60.33 ± 3.33^a
ACA	2.94 ± 0.79^b	0.26 ± 0.10^b	49.10 ± 7.28^b
CF	2.73 ± 1.11^b	0.17 ± 0.04^b	42.17 ± 6.45^b
Normal irrigation			
CA	5.63 ± 2.72^a	0.43 ± 0.24^a	57.33 ± 2.08^a
ACA	1.88 ± 0.47^b	0.13 ± 0.02^b	45.20 ± 3.54^b
CF	2.66 ± 0.27^b	0.22 ± 0.01^b	42.77 ± 4.37^b

Note: data are means $\pm$ SD. The mean values in the same column followed by letters are not significantly differed at 0.05 probability level.



Figure 2. Morphophysiological differences of total maize seedlings in response to drought stress

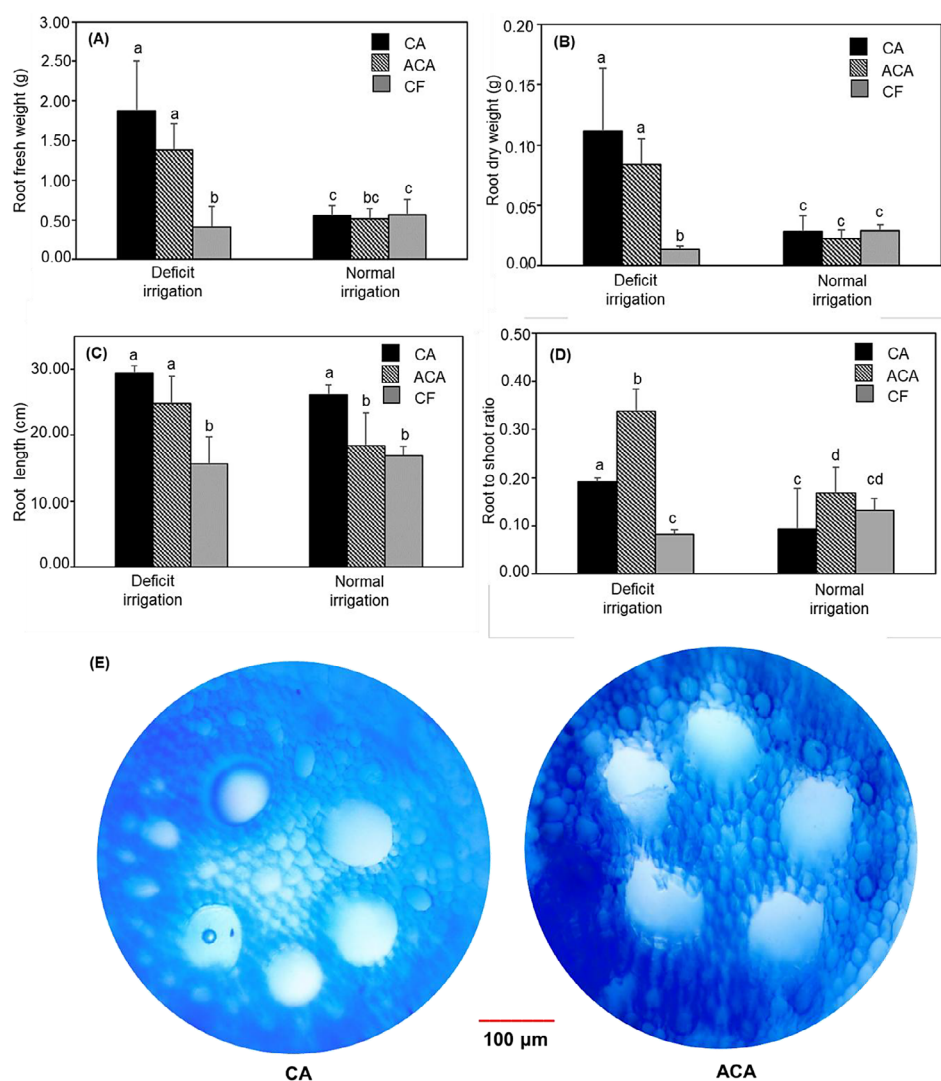


Figure 3. Effect of different irrigation conditions on (a) root fresh weight (b) root dry weight (c) root length and (d) root to shoot ratio in maize seedlings amended with microbially composted *Azolla* and chemical fertilizer.

Vertical bars represent the mean of each treatment ($n = 3$) and the error bars indicate standard deviation. Different letters in each bars indicate significantly different treatments. (e) Digitally stained images of the cross-section of the lateral root under deficit irrigation conditions. Horizontal bars represent 100 μ m

and sparse root systems. This clearly illustrated the revival potential of CA-treated seedlings after drying-wetting cycles, suggesting superior drought resilience mediated by compost and possession of recovery memory behaviour following prolonged periodic episodes of drought. These results were in line with Zarei et al. [2016] findings that recorded the ameliorative effect of organic fertilizer on plant productivity, leading to the proliferation of root hairs. In addition, this might be due to *Trichoderma* and *Paenibacillus* inoculations on *Azolla* that contributed to the compost formulation [Tchameni et al., 2011]. The positive effect of CA might be attributed to improved soil moisture retention and enhanced overall crop physiological development. Our results were in concurrence with those reported by Li et al. [2019], who noticed that organic amendment was responsible for enhancing root activity, which directly led to elongation and whole vegetative growth under water-deficit conditions.

The root fresh and dry weight increased more in response to CA as compared to others. In this case, there was a significant difference between CA and CF seedlings with respect to drought stress conditions (Figure 3A and B). The root-to-shoot ratio was higher in ACA-inoculated maize plants in both normal and deficit irrigation conditions, indicating an adaptive resource allocation strategy to optimize water uptake. Interestingly, the unfavorable effect of drought on these agronomic traits was declined by the application of microbially composted *Azolla*. The present study examined several root anatomical differences among treatments under deficit irrigation conditions as shown in Figure 3E. The CA-treated roots exhibited thicker corticol layers and larger xylem vessels, demonstrating improved hydraulic conductivity. On the other hand, thicker roots have been associated with better soil penetration ability, which was important for root growth under drought conditions [Hazman and Brown, 2018]. In contrast, the ACA root showed reduced xylem development and looser tissue organization, characteristics commonly associated with impaired water transport efficiency. Moreover, Li et al. [2020] also observed a similar anatomical adaptation pattern with xylem expansion in maize roots under water deficit, improving drought adaptation. Kondo et al. [2000] suggested that thickening of the corticol layer could play an imperative role in root structural support when exposed to drying soil.

Soil physicochemical properties status

CA applied to the soil showed noticeable impacts on its physicochemical properties in both conditions. Results in Table 3 showed an overall increase ($2.75 \pm 0.04\%$) in soil N concentration due to the application of CA-based amendments and deficit irrigation regimes. This could be attributed to the mineralization of N in the soil coming from composted material. The general tendency of decreased P contents during water deficit was more pronounced in the case of chemically-treated soils. Interestingly, K levels did not differ significantly among treatments, suggesting that potassium availability was less affected by irrigation regime and microbial activity, possibly due to its mineral-bound nature in the soil matrix. However, higher carbon (C) content observed in CA-treated soils ($23.76 \pm 0.39\%$) indicates enhanced organic carbon sequestration, which can improve soil structure, cation exchange capacity, and water retention. On the other hand, results showed that the variation of soil moisture was influenced by the applied treatment and irrigation supply conditions. Throughout the experiment, a higher soil moisture was consistently observed when ACA was applied with $18.59 \pm 0.13\%$. Nevertheless, non-significant drops in soil moisture content were noticed in deficit-irrigated conditions under CA treatment as compared to commercial CF-treated soils. The CF treatment, despite providing initial mineral nutrients, failed to maintain microbial viability and nutrient stability under stress, highlighting the limitations of purely chemical approaches in drought-prone systems. The elevated soil moisture in CA-treated soil accelerated the recovery of stressed seedlings rapidly. Accordingly, soil amendment with microbially composted material improved soil water retention. Also, upon rewetting after drought, the total bacterial population (9.88×10^8 cfu/mL) was spotted higher in enumeration abundantly in CA-treated soil, followed by ACA (6.66×10^7 cfu/mL) and CF (1.01×10^6 cfu/mL). It has been demonstrated that compost could induce soil physical properties, therefore making more water available for rhizospheric microorganism proliferation [Shin et al., 2022]. Moreover, it was recorded that applying *Azolla* compost could increase soil microbe activity, leading to nutrient recycling in the soil [Razavipour et al., 2018]. Overall, the results indicate that CA treatment effectively enhances both soil nutrient status and microbial activity

Table 3. Result of different irrigation conditions on the mineral content and microbiological analysis

Treatments	N (%)	P (%)	K (%)	C (%)	Moisture (%)	Total bacteria (cfu/mL)
Deficit irrigation						
CA	2.75 ± 0.04 ^a	3.69 ± 0.03 ^a	1.31 ± 0.08 ^a	23.76 ± 0.39 ^a	15.83 ± 0.10 ^a	9.88 × 10 ⁸
ACA	2.68 ± 0.04 ^a	4.45 ± 0.13 ^a	1.13 ± 0.10 ^a	22.56 ± 0.53 ^a	16.66 ± 0.18 ^a	6.66 × 10 ⁷
CF	1.48 ± 0.26 ^b	1.76 ± 0.08 ^b	1.73 ± 0.05 ^a	19.11 ± 0.29 ^a	15.71 ± 0.31 ^a	1.01 × 10 ⁶
Normal irrigation						
CA	2.48 ± 0.04 ^a	4.26 ± 0.13 ^a	1.19 ± 0.05 ^a	21.95 ± 0.31 ^a	16.67 ± 0.15 ^a	6.64 × 10 ⁸
ACA	2.51 ± 0.07 ^a	4.33 ± 0.18 ^a	0.89 ± 0.14 ^a	22.40 ± 0.30 ^a	18.59 ± 0.13 ^b	1.01 × 10 ⁷
CF	1.28 ± 0.05 ^b	2.24 ± 0.29 ^b	1.58 ± 0.06 ^a	18.62 ± 0.26 ^a	14.86 ± 0.16 ^c	8.04 × 10 ⁶

Note: data are means ± SD. The mean values in the same column followed by letters are not significantly differed at 0.05 probability level.

across irrigation regimes. By sustaining higher N, P, and C levels along with dense bacterial populations, CA contributes to better nutrient uptake, root development and physiological resilience of maize under drought.

Biochemical responses under different irrigation regimes

Chlorophyll content of maize seedlings was significantly influenced by both irrigation regime and soil treatment (Figure 4A). Under drought conditions, chlorophyll content was highest in plants treated with chemical fertilizer (CF, 198.95 µg g⁻¹ FW), followed by composted *Azolla* (CA, 188.69 µg g⁻¹ FW), while the ACA recorded the lowest value (124.12 µg g⁻¹ FW). Under normal irrigation, chlorophyll content increased markedly across all treatments, reaching 282.22 µg g⁻¹ FW in CF, 235.95 µg g⁻¹ FW in CA, and 151.35 µg g⁻¹ FW in ACA. The pattern observed in line with findings where *Azolla*-based compost

improved photosynthetic activity and chlorophyll stability under semi-arid rice cultivation [Seleiman et al., 2022]. Proline accumulation, a hallmark of osmotic adjustment in drought physiology, also showed significant variation among treatments (Figure 4B). Under deficit irrigation, CA and CF treatments recorded markedly higher proline levels than ACA, suggesting enhanced osmotic regulation and stress adaptation. Under normal irrigation, proline content decreased across treatments, yet CA and CF maintained slightly elevated levels compared with ACA. The increased proline content in CA-treated seedlings implies that composted *Azolla* stimulated the activation of osmoprotective pathways and compatible solute biosynthesis to preserve cellular hydration and membrane integrity [Eswaran et al., 2024]. Overall, the findings reinforce that *Azolla*-based compost can act as an eco-friendly strategy to enhance photosynthetic and osmoprotective capacity in maize, supporting climate-resilient crop production systems.

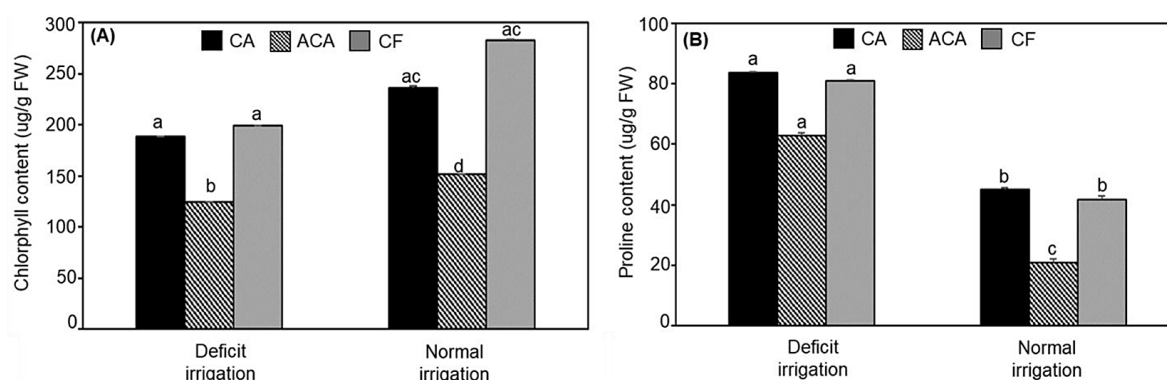


Figure 4. Biochemical changes of (a) chlorophyll and (b) proline in drought and normal affected irrigation of maize seedlings. Vertical bars represent the mean of each treatment (n = 3) and the error bars indicate standard deviation. Different letters in each bars indicate significantly different treatments

CONCLUSIONS

The application of microbially composted *Azolla* under drought conditions enhanced maize growth, physiological performance, and soil health without substantial decreases in agronomic traits of maize plants. CA treatment attenuated water stress effect by retaining soil moisture and improving root anatomy. In this regard, the compost derived from *Azolla* appeared to be a viable substitute to increase soil nitrogen, carbon content and microbial abundance, indicating active nutrient mineralization. Biochemically, CA-treated seedlings maintained higher chlorophyll levels and accumulated more proline under drought, reflecting stronger photosynthetic capacity and osmotic regulation. Collectively, these results confirm that composted *Azolla* functions effectively as a microbial biostimulant, promoting ameliorative effects in the maize-deficient irrigated condition while offering a sustainable alternative to chemical fertilizers.

Acknowledgments

We wish to thank the Universiti Teknologi Malaysia for providing funding and the necessary facilities for this study. The authors also thank Farish Imran Khairul Nizam from Tun Syed Nasir Polytechnic (PTSN), Malaysia for his technical assistance. This study was financially funded by UTM Translational Research Grant (R.J130000.7346.4J688) and Contract Research DTD Grant (R.J130000.7654.4C782).

REFERENCES

- Abdou, N.M., Roby, M.H.H., Al-Huqail, A.A., Elkelish, A., Sayed, A.A.S., Alharbi, B.M., Mhady, H.A.A., Abou-Sreaa, A.I.B. (2023). Compost improving morphophysiological and biochemical traits, seed yield and oil quality of *Nigella sativa* under drought stress. *Agronomy*, 13, 1147. <https://doi.org/10.3390/agronomy13041147>
- Alotaibi, N.M., El-Darder, A.M., Alfurayji, M.A., Abou-Elwafa, S.F. (2023). Green waste-derived compost (GWC) alleviates drought stress and promotes sugar beet productivity and biofortification. *Plant Production Science*, 26, 391–401. <https://doi.org/10.1080/1343943X.2023.2283942>
- Arnon, D.I. (1949). Copper enzymes in isolated chloroplast polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1–15. <https://doi.org/10.1104/pp.24.1.1>
- Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, 39, 205–207. <https://doi.org/10.1007/BF00018060>
- Bharali, A., Baruah, K.K., Bhattacharya, S.S., Kim, K.-H. (2021). The use of *Azolla caroliniana* compost as organic input to irrigated and rainfed rice ecosystems: comparison of its effects in relation to CH₄ emission pattern, soil carbon storage, and grain C interactions. *Journal of Cleaner Production*, 313, 127931. <https://doi.org/10.1016/j.jclepro.2021.127931>
- Boutasknit, A., Baslam, M., Ait-El-Moktar, M., Anli, M., Ben-Laouane, R., Ait-Rahou, Y., Mitsui, T., Douira, A., Modafar, C.E., Wahbi, S., Meddich, A. (2021). Assemblage of indigenous arbuscular mycorrhizal fungi and green waste compost enhance drought stress tolerance in carob (*Ceratonia siliqua* L.) trees. *Scientific Reports*, 11, 22835. <https://doi.org/10.1038/s41598-021-02018-3>
- Brouwer, P., Schluepmann, H., Nierop, K.G., Elderson, J., Bijl, P.K., van der Meer, I., de Visser, W., Reichart, G.-J., Smeekens, S., van der Werf, A. (2018). Growing *Azolla* to produce sustainable protein feed: the effect of differing species and CO₂ concentrations on biomass productivity and chemical composition. *Journal of the Science of Food and Agriculture*, 98, 4759–4768. <https://doi.org/10.1002/jsfa.9016>
- El-Mageed, T.A.A., El-Sherif, A.M.A., El-Mageed, S.A.A., Abdou, N.M. (2019). A novel compost alleviate drought stress for sugar beet production grown in Cd-contaminated saline soil. *Agricultural Water Management*, 226, 105831. <https://doi.org/10.1016/j.agwat.2019.105831>
- Eswaran, S.U.D., Sundaram, L., Perveen, K., Bukhari, N.A., Sayyed, R.Z. (2024). Osmolyte-producing microbial biostimulants regulate the growth of *Arachis hypogaea* L. under drought stress. *BMC Microbiology*, 24, 165. <https://doi.org/10.1186/s12866-024-03320-6>
- Hamdan, H.Z., Hour, A.F. (2022). CO₂ sequestration by propagation of the fast-growing *Azolla* spp. *Environmental Science and Pollution Research*, 29, 16912–16924. <https://doi.org/10.1007/s11356-021-16986-6>
- Han, M., Kasim, S., Yang, Z., Deng, X., Abdullah, H.S., Shuib, E.M., Uddin, M.K. (2024). Evaluation of foliar application of *Elusine indica* extract on growth, photosynthesis, and osmoprotectant contents in maize under drought stress. *Pertanika Journal of Tropical Agricultural Science*, 47, 721–732.
- Hazman, M., Brown, K.M. (2018). Progressive drought alters architectural and anatomical traits of rice roots. *Rice*, 11, 62. <https://doi.org/10.1186/s12284-018-0252-z>

13. Hazman, M., Fawzy, S., Hamdy, A.M., Khaled, A., Mahmoud, A., Khalid, E., Ibrahim, H.M., Gamal, M., Elyazeed, N.A., Saber, N., Ehab, M., Kabil, F. (2023). Enhancing rice resilience to drought by applying bio-char-compost mixture in low-fertile sandy soil. *Beni-Suef University Journal of Basic and Applied Sciences*, 12, 74. <https://doi.org/10.1186/s43088-023-00411-7>
14. Jumadi, O., Hiola, S.F., Hala, Y., Norton, J., Inubushi, K. (2014). Influence of *Azolla* (*Azolla microphylla* Kaulf.) compost on biogenic gas production, inorganic nitrogen and growth of upland kangkong (*Ipomoea aquatica* Forsk.) in a silt loam soil. *Soil Science and Plant Nutrition*, 60, 772–730. <https://doi.org/10.1080/00380768.2014.942879>
15. Kollah, B., Patra, A.K., Mohanty, S.R. (2016). Aquatic microphylla *Azolla*: a perspective paradigm for sustainable agriculture, environment and global climate change. *Environmental Science and Pollution Research*, 23, 4358–4369. <https://doi.org/10.1007/s11356-015-5857-9>
16. Kondo, M., Aguilar, A., Abe, J., Morita, S. (2000). Anatomy of nodal roots in tropical upland and lowland rice varieties. *Plant Production Science*, 3, 437–445. <https://doi.org/10.1626/pps.3.437>
17. Kranz, C.N., McLaughlin, R.A., Johnson, A., Miller, G., Heitman, J.L. (2020). The effects of compost incorporation on soil physical properties in urban soils – a concise review. *Journal of Environmental Management*, 261, 110209. <https://doi.org/10.1016/j.jenvman.2020.110209>
18. Li, X., Zhang, Y., Zhang, W., Zhao, X., Shen, Q. (2019). Organic amendments improve maize root architecture and drought resistance by altering soil microbial communities and root anatomical traits. *Plant and Soil*, 440, 525–541. <https://doi.org/10.1007/s11104-019-04098-4>
19. Li, Z., Gao, Y., Luo, Y., Xu, J. (2020). Compost enriched with beneficial microbes enhances vascular development and drought tolerance in maize roots. *Applied Soil Ecology*, 153, 103592. <https://doi.org/10.1016/j.apsoil.2020.103592>
20. Mahmud, H.B., Osawa, T. (2025). Analysis of the social impact of meteorological drought on Peninsular Malaysia based on the integration of the precipitation satellites and sentiment analysis. *International Journal of Disaster Risk Reduction*, 119, 105314. <https://doi.org/10.1016/j.ijdr.2025.105314>
21. Mohd Din, A.R.J., Cheng, K.K., Sarmidi, M.S. (2017). Assessment of compost extract on yield and phytochemical contents of pak choi (*Brassica rapa* cv *chinensis*) grown under different fertilizer strategies. *Communications in Soil Science and Plant Analysis*, 48, 274–284. <https://doi.org/10.1080/00103624.2016.1269793>
22. Nguyen, T., Fuentes, S., Marschner, P. (2012). Effect of compost on water availability and gas exchange in tomato during drought and recovery. *Plant Soil Environment*, 58, 495–502. <https://doi.org/10.17221/403/2012-PSE>
23. Novair, S.B., Hosseini, H.M., Etesami, H., Razza-vipour, T. (2020). Rice straw and composted *Azolla* alter carbon and nitrogen mineralization and microbial activity of a paddy soil under drying-rewetting cycles. *Applied Soil Ecology*, 154, 103638. <https://doi.org/10.1016/j.apsoil.2020.103638>
24. Parmanoan, D., Faridah, A., Trisakti, B., Sidabutar, R., Irvan, Alexander, V., Fath, M.T.A., Dalimunthe, N.F., Daimon, H., Takriff, M.S. (2025). The effect of turning frequency on the compost dynamics and quality derived from empty fruit bunches, *Azolla microphylla*, and active organic fertilizer. *Journal of Ecological Engineering*, 26, 350–362. <https://doi.org/10.12911/22998993/198745>
25. Paymaneh, Z., Sarcheshmehpour, M., Mohammadi, H., Hesni, M.A. (2023). Vermicompost and/or compost and arbuscular mycorrhizal fungi are conducive to improving the growth of pistachio seedlings to drought stress. *Applied Soil Ecology*, 182, 104717. <https://doi.org/10.1016/j.apsoil.2022.104717>
26. Prabakaran, S., Mohanraj, T., Arumugam, S., Sudalai, S. (2022). A state-of-the-art review on the environmental benefits and prospects of *Azolla* in bio-fuel, bioremediation and biofertilizer applications. *Industrial Crops & Products*, 183, 114942. <https://doi.org/10.1016/j.indcrop.2022.114942>
27. Qi, M., Liu, X., Li, Y., Song, H., Yin, Z., Zhang, F., He Q., Xu, Z., Zhou G. (2021). Photosynthetic resistance and resilience under drought, flooding and rewetting in maize plants. *Photosynthesis Research*, 148, 1–15. <https://doi.org/10.1007/s11120-021-00825-3>
28. Razavipour, T., Moghaddam, S.S., Doaei, S., Noorhosseini, S.A., Damalas, C.A. (2018). *Azolla* (*Azolla filiculoides*) compost improves grain yield of rice (*Oryza sativa* L.) under different irrigation regimes. *Agricultural Water Management*, 209, 1–10. <https://doi.org/10.1016/j.agwat.2018.05.020>
29. Revilla, P., Alves, M.L., Andelkovic, V., Balconi, C., Dinis, I., Mendes-Moreira, P., Redaelli, R., de Galarreta, J.I., Patto, M.C.V., Zilic, S., Malvar, R.A. (2022). Traditional foods from maize (*Zea mays* L.) in Europe. *Frontiers in Nutrition*, 8, 683399. <https://doi.org/10.3389/fnut.2021.683399>
30. Sah, R.P., Chakraborty, M., Prasad, K., Pandit, M., Tudu, V.K., Chakravarty, M.K., Narayan, S.C., Rana, M., Moharana, D. (2020). Impact of water deficit stress in maize: phenology and yield components. *Scientific Reports*, 10, 2944. <https://doi.org/10.1038/s41598-020-59689-7>
31. Seleiman, M.F., Elshayb, O.M., Nada, A.M., El-Leithy, S.A., Baz, L., Alhammad, B.A., Mahdi, A.H.A. (2022). *Azolla* compost as an approach for enhancing growth, productivity and nutrient uptake

- of *Oryza sativa* L. *Agronomy*, 12(2), 416. <https://doi.org/10.3390/agronomy12020416>
32. Schmitt, A., Glaser, B., Borken, W., Matzner, E. (2010). Organic matter quality of a forest soil subjected to repeated drying and different rewetting intensities. *European Journal of Soil Science*, 61, 243–254. <https://doi.org/10.1111/j.1365-2389.2010.01230.x>
33. Shin, S., Aziz, D., El-Sayed, M.E.A., Hazman, M., Almas, L., McFarland, M., El Din, A.S., Burian, S.J. (2022). Systems thinking for planning sustainable desert agriculture systems with saline groundwater irrigation: a review. *Water (Switzerland)*, 14, 1–25. <https://doi.org/10.3390/w14203343>
34. Swati, P., Rasane, P., Kaur, J., Kaur, S., Ercisli, S., Assouguem, A., Ullah, R., Alqahtani, A.S., Singh, J. (2024). The nutritional, phytochemical composition, and utilisation of different parts of maize: a comparative analysis. *Open Agriculture*, 9, 20220358. <https://doi.org/10.1515/opag-2022-0358>
35. Tchameni, S.N., Ngonkeu, M.E.L., Begoude, B.A.D., Wakam Nana, L., Fokom, R., Owona, A.D., Mbarga, J.B., Tchana, T., Tondje, P.R., Etoa, F.X., Kuate, J. (2011). Effect of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on cacao growth and resistance against black pod disease. *Crop Protection*, 30, 1321–1327. <https://doi.org/10.1016/j.cropro.2011.05.003>
36. Wong, C.K.F., Zulperi, D., Saidi, N.B., Vadamalai, G. (2021). A consortium of *Pseudomonas aeruginosa* and *Trichoderma harzianum* for improving growth and induced biochemical changes in *Fusarium* wilt infected bananas. *Tropical Life Sciences Research*, 32, 23–45. <https://doi.org/10.21315/tlsr2021.32.1.2>
37. Walter, J., Nagy, L., Hein, R., Rascher, U., Beierkuhnlein, C., Willner, E., Jentsch, A. (2011). Do plants remember drought? Hints towards a drought-memory in grasses. *Environmental and Experimental Botany*, 71, 34–40. <https://doi.org/10.1016/j.envexpbot.2010.10.020>
38. Yazid, S.N.E., Ng, W.J., Selamat, J., Ismail, S.I., Samsudin, N.I.P. (2021). Diversity and toxigenicity of mycobiota in grain corn: a case study at pioneer grain corn plantations in Terengganu, Malaysia. *Agriculture*, 11, 237. <https://doi.org/10.3390/agriculture11030237>
39. Zarei, M., Paymaneh, Z., Ronaghi, A. (2016). The effects of arbuscular mycorrhizal fungus and water stress on some antioxidant enzymes activities and nutrients uptake of two citrus rootstocks. *Iran Agricultural Research*, 35, 19–26. <https://doi.org/10.22099/iar.2016.3750>
40. Zgallai, H., Zoghalmi, R.I., Annabi, M., Zarrouk, O., Jellali, S., Hamdi, H. (2024). Mitigating soil water deficit using organic waste compost and commercial water retainer: a comparative study under semiarid condition. *Euro-Mediterranean Journal for Environmental Integration*, 9, 377–391. <https://doi.org/10.1007/s41207-023-00437-4>