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Plant Growth-Promoting Rhizobacteria (PGPR) and Humic Acid Amendment Improves N-use Efficiency in Sweet Potato

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ABSTRACT

This study aimed to verify the effect of Plant Growth-Promoting Rhizobacteria (PGPR) and humic acid amendment with different N-fertilizer rates on sweet potato. The results showed that inoculation of UPMRB9 in combination with 50% fertilizer-N produced significantly higher dry matter (DM) in leaves, storage, and fibrous roots, and in the whole plant. Similar trends were observed for nitrogen use efficiency (NUE), in which 75% N+UPMB10 and 50% N+UPMRB9 treatments significantly produced higher efficiency by 44.89% and 40%, respectively. In addition, the highest OD of β-carotene, 0.71 and 0.68 mg g^{-1}, were observed in 50% N+UPMRB9 and 75% N+UPMB10, respectively. Sweet potato plants obtained greater NUE and N uptake when lower rates of N were used with the microbial inoculations. These findings show the ability of PGPR-HA to fix nitrogen and thereby increase N availability of soils, reducing the need to provide mineral nitrogen to crops. Thus, applying biofertilizer containing PGPR amended with humic acid could be a sustainable approach to improving the NUE and total N concentration of sweet potato plants. Higher N use efficiency will lead to savings in the amount of N fertilizer needed, thus reducing costs and promoting an eco-friendly approach at the same time.

Key words: PGPR, humic acid, NUE, sweet potato, and β-carotene

INTRODUCTION

The sweet potato [*Ipomoea batatas* (*L.*) Lam] is an essential tuberous crop grown in Central and South America. Aside from cassava being a primary source of starch, the sweet potato plant has a long legacy of being farmed as a starch food in Malaysia (Karim *et al.* 2022). Sweet potato output in Malaysia has decreased owing to land scarcity and conversion of agricultural land to industrial purposes, higher labour expenses, marketing challenges, disease outbreaks, and pricey inputs such as fertilizer (Yasmin *et al.* 2020). The sweet potato plant is a short-season crop that demands inorganic fertilizers to promote quicker nitrogen release into the soil (Singh *et al.* 2022). An increase in world food supply depends on a massive use of chemical fertilizers with the unfortunate consequence of environmental degradation (Zago *et al.* 2019). Excess nitrogen is inefficient and may result in several problems: environmental degradation; increased agricultural production costs; loss of vital soil microorganisms; and water eutrophication. A strategy for increasing output is to use friendly microbes such as plant growth-promoting rhizobacteria (PGPR) (Zhao *et al.* 2022). The application of beneficial bacteria as biofertilizers is becoming crucial in agricultural production because of their

potential to enhance consumer safety and sustainable crop production (Ammar *et al.* 2022). These PGPRs are collected due to their beneficial biochemical and morphological characteristics like N_2-fixing ability and solubilising phosphate and potassium, siderophores, and pectinase. Additionally, it has been noted that these PGPR strains increase the levels of N, P, and K in shoots and storage roots (Ali-Tan *et al.* 2017; Shultana *et al.* 2021).

Humic acid (HA) is a naturally occurring chemical in soil. It is a bioproduct of organic matter breakdown that has been effectively used in crop cultivation (Tang *et al.* 2022). Ekin's study (2020) reported on several direct effects of HA on plant growth. These are: (i) increased macronutrient and micronutrient uptake and root expansion; (ii) improved microbial growth by providing a carbon source that serves as food for microbes; and(iii) increased water retention. A study by Chen *et al*. (2017) showed that applying humic acid nitrogen fertilizer (HA-N) increases the number of storage roots per plant, total fresh weight per storage root, and the yield by 30%. The inclusion of HA-PGPRs might potentially aid in the colonisation of roots by local mycorrhizal fungi. Sweet potatoes are one of the most important root crops worldwide, as they provide food and feed for people and animals and various raw materials for the agricultural sector (Neela and Fanta 2019).

Nitrogen usage efficiency (NUE), describes how well a plant uses applied or fixed nitrogen to produce biomass. It further describes the proportion of crop output to the quantity of nitrogen received through soil roots or from the atmosphere through bacterial fixation (Ullah *et al.* 2019). There are several ways to improve nitrogen use efficiency in plants. One way is to use nitrogen fertilizers with bacteria that can fix nitrogen from the atmosphere and make it available to plants. Another is to use slow-release fertilizers that release nitrogen over a longer period, reducing the amount of fertilizer needed and reducing the environmental impact of nitrogen fertilizer use (Sharma and Bali 2017). Crop rotation and intercropping can also help improve nitrogen use efficiency by reducing the amount of nitrogen lost from the soil and increasing the amount of nitrogen available to plants (Zhu *et al.* 2023). Tolessa (2019) states that NUE is principally dependent on the presence of soil nitrogen, its uptake and integration, photosynthetic and nutrient supply, carbon-nitrogen flow, nitrate transmission, and regulation by light and hormones. It is possible to calculate NUE by using either the nitrogen taken in or the nitrogen used to build tubers. NUE may additionally be determined by physiological and agronomic criteria based on apparent nitrogen recovery. The crop, harvest product, and techniques influence the best approach to determining NUE (Di Gioia *et al.* 2017).

It is well known that sweet potato plants, especially the tubers, produce the pigment carotenoids. One of the carotenoids is the β-carotene, which has antioxidant capabilities, crucial for human nutrition and health (Atmini *et al.* 2022). β-carotene, known as provitamin A, is the most common carotenoid in fruits with the highest vitamin A activity. UV-Vis spectroscopy has been found to be the best method to determine the content of β-carotene, in terms of cost-effectiveness, availability, versatility, simplicity, and speed (Safdarian *et al.* 2021). In this method, determining the analyte concentration in the unknown sample is very simple and fast. However, measuring the analytes in complex samples is difficult, and sample preparation, extraction, and pre-concentration steps are required before measurement (Drapal and Fraser 2019). Although the amount of β-carotene in commercial fruit juices may be higher than the detection limit of a UV-Vis spectroscopy method, the complexity of the matrix in these samples does not allow for its direct measurement (Biswas *et al.* 2011). Methods of extraction used before the final spectrophotometric determination of analytes include solvent extraction and partitioning. In the present study, beneficial bacteria addition with humic acid (HA-PGPR) and N-fertilizer aimed to support N concentration in soil and plant tissue, N uptake, NUE, and the

content of b-carotene in sweet potato plants. This method can potentially increase food security, particularly in developing countries. This study describes a pot trial conducted with two locally isolated bacteria (*Bacillus tequilensis* and *Bacillus subtilis*) with 0.1 % humic acid amendment and three different rates of N-fertilizer (0, 50, and 75 %) under glasshouse conditions.

MATERIALS AND METHODS

Experimental Site

This glasshouse pot trial was conducted at an experimental site, located at field 15 (03° 00′ 12.6″ N; 101° 47′ 22.4″ E and 56.8m above sea level), in the Agriculture Faculty, UPM, on the West coast of Peninsular Malaysia. Throughout the experiment, the site experienced a humid and hot climate, with an average lowest temperature of about 23.5°C, average high temperature of 37°C and a relative humidity of 76.67 %.

PGPR and Humic Acid Collection and Preparation

Bacillus tequilensis (UPMRB9) and *Bacillus subtilis* (UPMB10) are two locally isolated plant growth-promoting rhizobacteria (PGPR) collected from the Microbiology Laboratory, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia. Technical humic acid was purchased from the Sigma-Aldrich Swiss brand products (code 102098564 and 53680-50G). Humic acid, which is black in colour, solubilises slowly in water, and has a pH of less than 6; it is soluble in alkali but insoluble in acid and can be adjusted to pH 7 by using tryptic soy broth media (TSB) which has a pH of 7.23. The characterisation of the physical and chemical properties of humic acid (HA) are as shown in Table 1. Approximately 0.1 g of HA was weighed and transferred into 500-mL Erlenmeyer flasks. TSB media was used to prepare the liquid formulation. About 100 ml of TSB was transferred and autoclaved for 15 min at 121°C. One full loop (approximately 1×10^6 CFU ml^{-1}) was taken from *Bacillus tequilensis* and *Bacillus subtilis* strains and dipped into broth media, then incubated under a constant shaker at 150 rpm for 48 h at 30°C. Each sample was replicated three times.

Soil Collection and Plant Preparation

Mineral soil was collected from Ladang Kongsi, Taman Pertanian Universiti, Universiti Putra Malaysia, Seri Kembangan, Selangor, Malaysia, located at 30 02′ N latitude, 1010 42′ E longitude, and 31 m above sea level. The soil was classified as sandy-clay-textured. Ten grams of air-dried soil samples were weighed and ground to pass through a 2-mm sieve. Physical and chemical soil properties were characterised as shown in Table 1. Thirty-six plastic containers (23 cm high and 26 cm in inner diameter) were filled with 7 kg of soil. Four holes were drilled at the bottom of each container to permit flow-out of excess water. Before planting, Sepang Oren cuttings of the sweet potato variety (30 cm long with 8 nodes) were thoroughly cleaned using sterilized distilled water and soaked in 48-h-old PGPRs-HA formulations for 6-7 h. At planting and every 2-week intervals, 20 mL (approximately 10^9 CFUmL^{-1}) of PGPRs-HA formulations were inoculated onto each plant. The control plants received the same volume of sterile media but without bacteria. NPK fertilizers were applied following the recommendation of Pedram (Kashiani 2012). Urea for nitrogen fertilizer with three levels (0, 50, and 75%), triple superphosphate (TSP), and muriate of potash (MOP) were used based on the recommended rates; urea was applied at 2.16g (50%), and 3.24g (75%). Weeding of pots was handled manually as soon as the weeds appeared, and Mapa Malathon 57 was applied twice during the cultivation period to control the insects on sweet potato plants. The experiment was conducted from March 2022 to June 2022. The sweet potato plants were harvested after 110 days.

TABLE 1
Selected physical and chemical properties of experimental soil and humic acid.

Properties	Soil	Properties	Humic acid
Textural Class	Sandy clay	Color	Black
% Sand	67.7 %	Solubility	Slow
% Silt	5.5%	Molecular Formula	$C_9H_9NO_6$
% Clay	27.6%	Molecular Weight	227.17
pH	5.17	pH	3.5- 6.0
EC (mS/cm)	17.78	Moisture	15 %
CEC (cmolc kg^{-1})	5.82	CEC (cmolc kg^{-1})	70-166
Total C (%)	1.75%	Organic C	30-50%
Total N (%)	0.05%	Nitrogen	3%
Total S (%)	0.04%	Hydrogen	5%

Experimental Layout and Treatments

An incubation experiment was arranged in a randomized complete block design (RCBD) with twelve treatments and three replicates. The detailed treatments are as follows:

T1 = 0% Nitrogen fertilizer (uninoculated)
T2 = 0% Nitrogen fertilizer + UPMB10
T3 = 0% Nitrogen fertilizer + UPMRB9
T4= 50% Nitrogen fertilizer (uninoculated)
T5 = 50% Nitrogen fertilizer + UPMB10
T6= 50% Nitrogen fertilizer + UPMRB9
T7 = 75% Nitrogen fertilizer (uninoculated)
T8= 75% Nitrogen fertilizer + UPMB10
T9 = 75% Nitrogen fertilizer + UPMRB9

Plant Measurements and Sampling

After harvest, the plants were washed and cleaned under running tap water and separated into shoot, fibrous, and storage roots. The plant parts were separately dried in an oven with forced-air circulation at 70°C for 3 days until constant weight was reached. After drying, the samples were weighed using a digital weighing machine to determine the amount of DM accumulated in each plant part and the whole plant. Plant storage roots were harvested, brushed, counted, and classified according to the Brazilian classification proposed by Da Silva *et al*. (1995) Storage roots were then weighed to determine storage root yield.

Nitrogen Determination of the Post-Harvest Soil and Plant Tissues

The micro-Kjeldahl method was used to determine total N content in sweet potato soil, leaves and storage roots. This was followed by adding concentrated H_2SO_4, K_2SO_4, and catalyst, followed by digestion for 1 h at 360°C using block digestion following Bowman *et al.* (1988).

N Uptake Efficiency by Plant and N Removal

N uptake efficiency of the storage root yield was calculated for each cover crop treatment by dividing the storage root yield with the total amount of N taken up by the plants. Nitrogen removal was determined by multiplying N concentration of storage roots by the amount of DM in the storage roots (Fernandes *et al.* 2018).

N-fertilizer use efficiency (NUE)

N-fertilizer use efficiency of the sweet potato plant was calculated after obtaining data on productivity and N uptake for each treatment, according to the methodology of Oliver and Almeida (2018) based on the following formulae:

Nitrogen use efficiency (NUE) of the applied nutrient is the relation between total N uptake by the whole plant from fertilized plots and the amount of applied N (g kg-^{1}).

$$NUE = (N_{FP} - N_C) / R$$

where N_{FP} is the total N uptake by whole plant from N-fertilized plots, N_C is the uptake of N from control pots, and R is N-fertilizer dose applied in the treated pots.

Partial factor of productivity (PFP) of the applied nutrient is the relation between productivity (g kg-^{1}) and the amount of applied N (g kg-^{1}).

$$PFP = P/R$$

where P is productivity of treated pots and R is N-fertilizer rates applied on treated pots.

Efficiency of recovery (ER) of the applied nutrient is the relation between N uptake in tubers (g kg-^{1}) and the amount of applied N (g kg-^{1}).

$$ER = (N_{FT} - N_C) / R$$

where N_{FT} is N uptake of tubers in the treated pots, N_C is uptake of N from control pots, and R is N fertilizer dose applied in the treated pots.

N-Utilisation Efficiency (UtE) of the applied nutrient is the relation between the yield (g kg-^{1}) and the amount of N uptake by the whole plant from fertilized plots (g kg-^{1}).

$$UtE = Y/N_{FP}$$

where Y is fresh yield of root tubers in treated pots and N_{FP} is amount of N uptake by whole plant in treated pots.

Physiological efficiency (PE) of the applied N is the relation between the increase in productivity (g kg-^{1}) and the increase in N uptake by the whole plant (g kg-^{1}).

$$PE = (P_F - P_C) / (N_{FP} - N_C)$$

where P_F is productivity of treated pots, P_C is productivity of control pots, N_{FP} is N uptake of treated pots, and N_C is N uptake of control pots.

Harvest index (HI) is the relation between the amount of N uptake by the tuber (g kg-1) and the amount of N uptake by the whole plant (g kg-^{1}).

$$HI = (N_{FT} / N_{FP}) \times 100$$

where N_{FT} is amount of N uptake by tuber in treated pots and N_{FP} is amount of N uptake by whole plant in treated pots.

β-Carotene Content Detection

β-carotene content was determined using a UV-Vis spectrophotometer to measure the optical density, according to Biswas *et al.* (2011). Acetone solution was used to prepare the β-carotene sample concentration. Working standard solutions were prepared daily in the same solvent and used to spike sweet potato samples. Frozen samples were thawed overnight in a refrigerator (4 ± 1°C). The outer, thinner layer (skin or cuticle) was removed, and the remaining portions were sliced. The components obtained were then ground separately in a spice grinder until they became a fine paste. For extraction, a representative portion of this sample, 1 g, was accurately weighed in a glass test tube. Then 5 ml of chilled acetone was added, and the tube was held for 15 min with occasional shaking at 4 ± 1°C, vortexed at high speed for 10 min, and centrifuged at 1370 x g for 10 min. The supernatant was collected into a separate test tube, and the compound was re-extracted with 5 ml of acetone, followed by centrifugation as above. Both supernatants were pooled and then passed through Whatman filter paper No. 42. The absorbance of the extract was determined at a 449 nm wavelength. The pure solvent (acetone)-based calibration curve of β-carotene was generated by plotting the O.D. value versus the analyte concentration, and linear regression analysis was performed using Microsoft Excel.

Statistical Analysis

SAS 9.4 Statistical Analysis System was used to analyse the data using the Least Significant Difference (LSD) comparison technique at p = 0.05. Differences between treatment means were identified using the analysis of variance procedure (ANOVA).

RESULTS AND DISCUSSION

Effect of Treatments on Sweet Potato Dry Matter and Yield-Contributing Parameters

Inoculation of PGPRs-HA formulations and application of differing nitrogen fertilization rates resulted in significant differences ($P \leq 0.05$) in sweet potato contributing parameters (Table 2). Dry matter (DM) amounts of the leaves, fibrous roots, storage roots, and the whole sweet potato plant were significantly affected. The highest amounts of DM in shoots, fibrous roots, and the whole plant were observed in T6 at 30.57 g kg-^{1}, 9.5 g kg-^{1}, and 83.63 g kg-^{1}, respectively, while the lowest amount was found in T1 (control) compared with all other treatments. Statistically, significant differences were not found in T6 and T8 treatments with regard to DM of storage roots, which saw a significant increase in both treatments at 43.58 g kg-^{1} and 40.34 g kg-^{1}, respectively. Treatment T1 (control) showed the lowest values of DM in storage roots. The highest significant increase was observed in T6 at (542 g kg-^{1}), followed by T8 at (455 g kg-^{1}), in storage root yield compared to the lowest treatment, T1 (control)(Table 2).

Effect of Treatments on Nitrogen Nutritional Status, N Uptake, and Removal

Treatments had a significant ($P \leq 0.05$) impact on total N concentration in post-harvest soil and plant tissue after the inoculation of PGPR-HA formulations (Figure 1). Results of UPMRB9 +50% N-treated sweet potato plants showed a higher concentration of N, followed by treatment of UPMB10 +75% N at 0.28% and 0.26% for soil and at 1.86% and 1.85% for storage roots, respectively. Control 0% N which had the lowest treatment had no tubers growing at 0% and 0.05%. Comparing UPMRB9 + 50% N to the control treatments, total N in soil and storage roots was found to be highest, with an increase of 82.14% and 100%, respectively, Leaf N content was significantly higher with inoculation of UPMB10 (75% N) at 3.1% compared to the lowest control treatments (0% N) at 0.19%. Highest total N content in the leaf increased by 93.87% from UPMRB9 + 50% N compared to the control treatments. There was a significant difference in the plant's nitrogen uptake of sweet potatoes, as represented in Figure 2. The addition of PGPR-HA and N-rate fertilization influenced the uptake. The highest uptake of N

(2.0027 g plant^{-1}) was obtained from UPMRB9 +50% N followed by 2.0012 g plant^{-1} from UPMB10 +75% N, respectively.

TABLE 2

Effects of treatments on dry matter of sweet potato

Treatments	Shoot DM yield	Fibrous root DM yield	Storage root DM yield	Whole plant DM yield	Storage root yield
T1	17.62 ± 0.57 e	5.1 ± 0.16 d	0 e	22.79 ± 0.5 h	0 g
T2	19.89 ± 0.57 de	7.8 ± 0.12 c	4.33 ± 0.6 de	32.02 ± 0.2 g	82 ± 11.8 f
T3	21.30 ± 0.48 cd	7.7 ± 0.15 c	8.93 ± 0.8 d	37.90 ± 1.1 f	117 ± 0.8 ef
T4	20.17 ± 0.94 de	7.8 ± 0.20 c	18.14 ± 0.5 c	46.07 ± 1.1 e	123 ± 1.8 ef
T5	24.04 ± 2.40 bc	8.1 ± 0.12 c	18.98 ± 0.6 c	51.14 ± 2.6 d	251 ± 3.7 d
T6	30.57 ± 2.40 a	9.5 ± 0.28 a	43.58 ± 1.1 a	83.63 ± 0.6 a	542 ± 22.9 a
T7	21.45 ± 0.30 cd	7.8 ± 0.03 c	20.35 ± 0.09 c	49.61 ± 0.2 de	156 ± 3.8 e
T8	26.80 ± 0.33 b	8.9 ± 0.05 b	40.34 ± 0.3 a	76.00 ± 0.6 b	455 ± 3.1 b
T9	25.75 ± 0.58 b	8.2 ± 0.17 c	34.27 ± 34.27 b	68.15 ± 2.9 c	332 ± 1.5 c

Notes: Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ Least Significant Difference (LSD test). The columns represent the mean values ± standard error. T1 = 0% Nitrogen fertilizer (uninoculated), T2 = 0% Nitrogen fertilizer + UPMB10, T3 = 0% Nitrogen fertilizer + UPMRB9, T4= 50% Nitrogen fertilizer (uninoculated), T5 = 50% Nitrogen fertilizer + UPMB10, T6= 50% Nitrogen fertilizer + UPMRB9, T7 = 75% Nitrogen fertilizer (uninoculated), T8= 75% Nitrogen fertilizer + UPMB10, T9 = 75% Nitrogen fertilizer + UPMRB9.

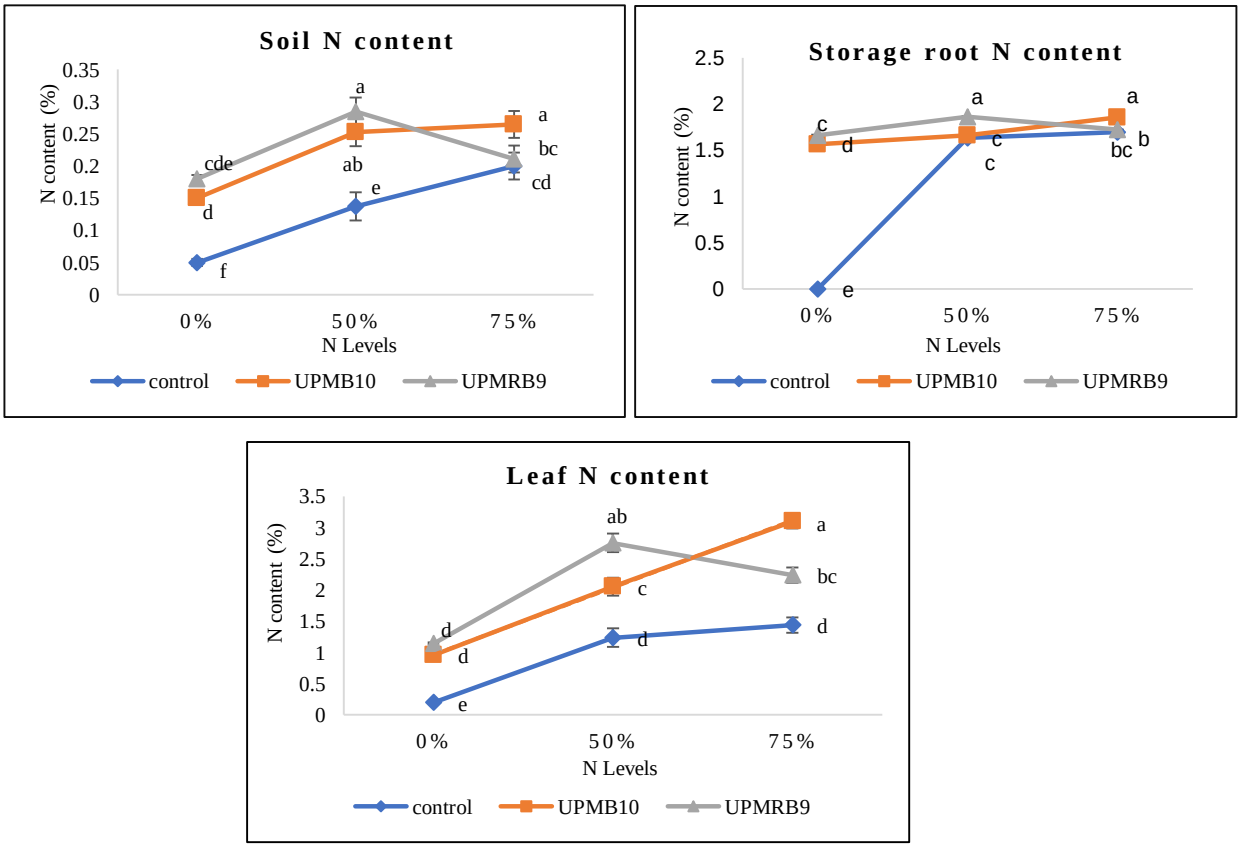

Figure 1: Effect of treatments on soil, storage root and leaf N content. Means with the same letters are not significantly different based on LSD (0.05)

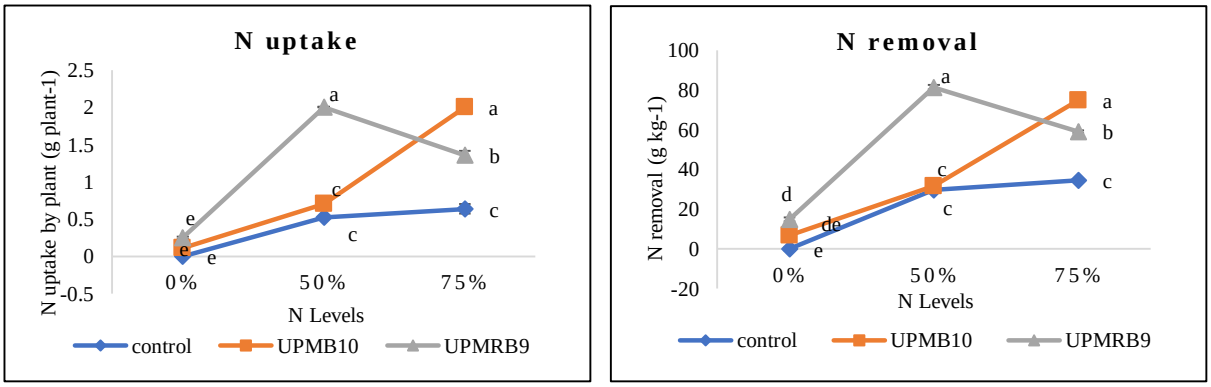

Figure 2: Effect of treatments on N uptake by plant and N removal. Means with the same letters are not significant different based on LSD (0.05)

Effect of Treatments on Nitrogen Use Efficiency (NUE)

For NUE, the best results were found for the levels of 50% nitrogen associated with UPMRB9 inoculant and 75% nitrogen associated with UPMB10. These treatments resulted in significantly higher NUE, by 44.89% and 40%, respectively, compared to controls and other treatments (Table 3). Based on the PFP index, a greater amount of productivity was found in UPMRB9 inoculation with 50% N with the partial factor of productivity being significantly higher by 58.5 g kg-^{1} PFP in relation to the treatment that received 50% N without inoculation. The best outcomes in terms of ER were achieved from UPMRB9 inoculation with 50% N and UPMB10 inoculation with 75% N at 1.86 and 1.85 g kg-^{1}, respectively. The increments were 12.36% and 8.64%, respectively, compared to the uninoculated treatments. In relation to UtE, the best findings, shown in Table 3, were obtained from the same inoculation with different N-fertilization rates. Both UPMRB9 with 50% N and 75% N treatments gave the highest results of UtE at 9.6 and 8.7 g kg^{-1}, respectively, compared to the controls and other treatments. In contrast, the lowest effect was observed from UPMB10 with 0% N at 1.7 g kg^{-1}. For the PE (the relation between the increase in productivity and the increase in N uptake by the whole plant), the highest value was observed from UPMRB9 with 50% N (23.3 g kg^{-1}) with, the lowest value observed from UPMB10 with 0% N (2.7 g kg^{-1}). In relation to the HI (the relation between the amount of N uptake by the tuber and the amount of N uptake by the whole plant), the maximum value obtained was from 0% of N associated with UPMB10 inoculation at 62%, while the minimum value was from 75% of N associated with UPMB10 inoculation at 37.4%.

Effect of Treatments on the Concentration of β-Carotene

The concentration of β-carotene in the sweet potato plant was determined using solvent acetone and checked at 449 nm through a UV-Vis spectrophotometer. The concentration was found to be significantly affected by PGPRs-HA addition. Results (Figure 3) show that the inoculation with treatments of UPMRB9 and 50% N and UPMB10 with 75% N gave the highest optical density (OD) value at 0.71 and 0.68 µg g^{-1}, respectively, compared to the controls and other treatments. There were no significant differences among treatments (UPMRB9 + 0% N, UPMB10 + 0% N, and the control + 50% N) at 0.36, 0.33, and 0.35 µg g^{-1}, respectively, with the lowest OD value being observed in control (+0 N%). The standard curves of the math equations were as follows: y = 0.2071x + 1.2107 (R2 = 0.99)

PGPR combined with humic acid as an amendment formulation had a greater effect on the growth and content of N in the leaves of sweet potato plants than in the storage roots because DM and N uptake by the plant's leaves increased with increasing N, more than they did in the storage roots (Table 2). Fernandes *et al.* (2018) found that nitrogen fertilization had a bigger

influence on sweet potato crop development and N accumulation in shoots than in tubers because DM and N accumulation in shoots grew linearly with increasing nitrogen levels than in tubers. Other studies on the sweet potato have found that higher mineral N rates encourage the development of vegetative plant parts rather than storage roots (Motsa *et al.* 2015; Ellong *et al.* 2014). Okpara *et al. (*2009) state that a high yield is associated with restricted leaf development of the sweet potato crop during the storage root bulking stage. Treatments with low N fertilizer rates and PGPRs-HA formulation resulted in uptake of greater amounts of N by sweet potato plants than in treatments with non-PGPRs-HA formulation. These results show that the ability of the PGPRs-HA formulation to fix atmospheric N increases the availability of N in the soil, reducing the need to supply mineral N to plants grown in succession. Previous studies demonstrated that sugarcane treated with beneficial microbes and half the required amount of N (50 kg ha-^{1}) achieved production levels comparable to plants with a maximum amount of N and no inoculation. Previously, the same investigators detected a higher population of PGPR under 75 kg N ha-^{1} compared to treatments without fertilization and under 150 kg N ha-^{1} (Oliver and Almeida 2018).

TABLE 3

Effect of treatments on NUE and productivity data

N rates	Bacterial treatments	NUE	PFP	ER	UtE	PE	HI
0 %	Control	____	____	____	____	____	____
	UPMB10	____	____	____	1.7 de	2.7 de	62 a
	UPMRB9	____	____	____	3.1 d	5.3 d	59.3 ab
50 %	Control	2.7 c	8.3 d	1.63 d	6.2 c	13.5 c	57 ab
	UPMB10	3.6 b	8.7 d	1.66 cd	5.1 c	11.3 c	44.9 c
	UPMRB9	4.5 a	20 a	1.86 a	9.6 a	23.3 a	41.2 cd
75 %	Control	3.08 c	6.2 e	1.69 bc	6.4 bc	11.9 c	54.1 b
	UPMB10	4.9 a	12.4 b	1.85 a	8.1 ab	21.7 ab	37.4 d
	UPMRB9	3.9 b	10.5 c	1.72 b	8.7 a	19.8 b	43.7 c

Notes: Nitrogen use efficiency (NUE), Partial factor of productivity (PFP), Efficiency of recovery (ER), N-utilization efficiency (UtE), physiological efficiency (PE) and Harvest index (HI) of the sweet potato, under UPMRB9 and UPMB10 strains and different N doses. Means with the same letters are not significantly different based on LSD test ($p \leq 0.05$).

The application of PGPRs-HA could increase the N content of the soil. In this study, N content in the soil was influenced by applying 50% N+UPMRB9 and UPMB10 +75% N, which showed the highest significant measurements at 0.28% and 0.26%, respectively. Ding *et al.* (2020) showed that high urea levels in the soil reduced sweet potato yield and could raise soil acidity, preventing plants from converting ammonia to nitrates. At lower N rates, the treatments with PGPRs-HA increased sweet potato storage roots and the uptake of N in relation to treatments with no PGPRs-HA because PGPR contributed more to nitrogen supply. Ahmad *et al.* (2016) suggest that combining HA and PGPR is a better technique for increasing canola nutrition and production. The same researchers found that HA and PGPR increased N uptake in canola seed, improved plant nutrition, enhanced nutrient availability, and improved root growth. By chelating, humic acid increases the availability of nutrients and the amount absorbed (Moradzadeh *et al.* 2021).

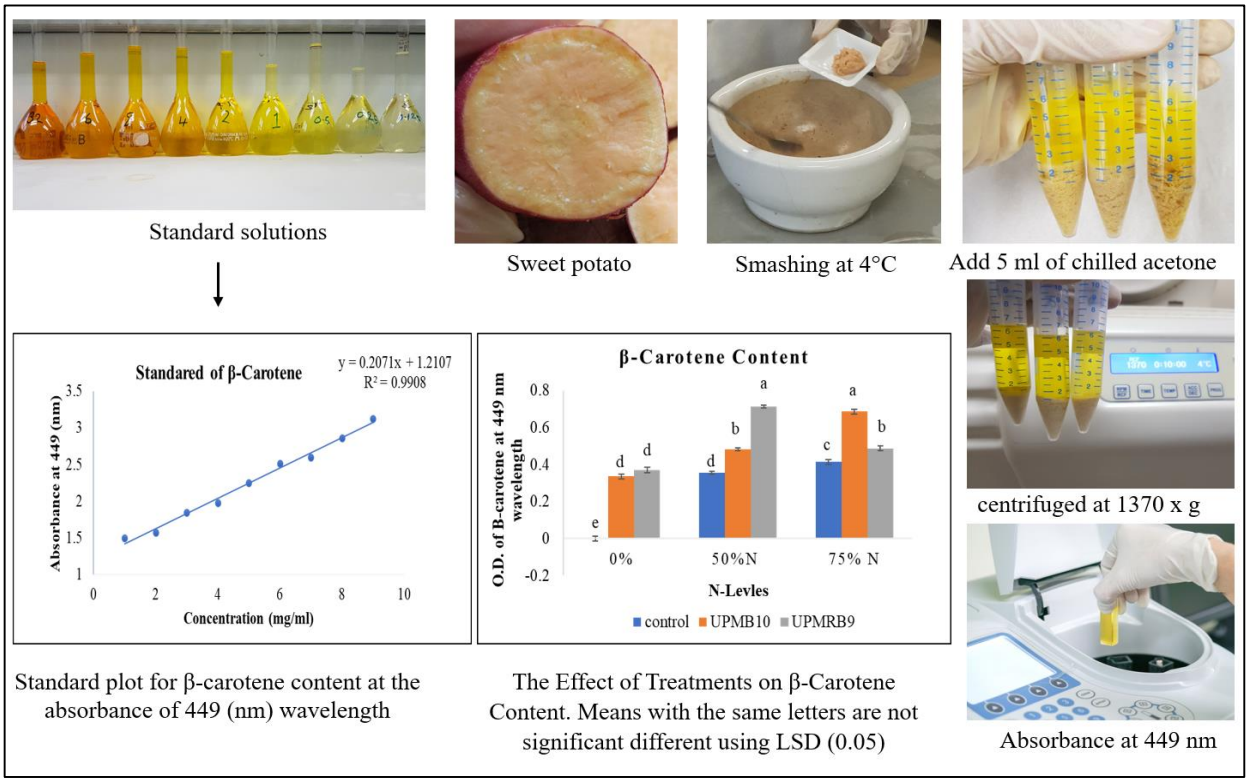

Figure 3: Preparation process of β-carotene extraction and analysis by using acetone solvent

An improved N content is positively connected with improved storage root development because it allows plants to absorb nutrients from the soil. This study found noticeable variations in the nitrogen content of the soil, sweet potato leaves, and storage roots with and without HA and PGPR (Figure 1). PGPR is an environmentally acceptable alternative technology to promote crop nutrient uptake, growth, and agricultural productivity. PGPR application improved onion mineral nutrition, with these plants having the maximum mineral content in the leaves and bulbs (Laftah *et al.* 2022). PGPR also increases nutritional availability by releasing nutrients from organic matter through the production of enzymes. Moreover, the combination of humic acid and bio-inoculants (PGPR) demonstrated a considerable rise in leaf and storage of root N contents (Ashwini *et al*. 2023). Similarly, Ahmad *et al*. (2016) and Zahid *et al*. (2015) reported improved N content and uptake in several crops following PGPR treatment. Additionally, the structure and function of the microbial population in the soil, particularly in the rhizosphere area, are positively influenced by humic compounds (Yuan *et al.* 2022). This could be the cause of our study finding that the interaction between humic acid and PGPR is more effective for nutrient absorption.

PGPR can help increase N- use efficiency in plants by fixing nitrogen from the atmosphere and making it available to plants. Some bacteria form symbiotic relationships with plants, living in nodules on the roots and providing the plant with nitrogen in exchange for carbohydrates. Other bacteria can fix nitrogen in the soil and make it available to plants. In this study, N-use efficiency increased with the addition of PGPRs-HA formulations with lower N fertilizer rates of 50% and 75%. These improvements will make it possible to optimise and lower the amount of nitrogen fertilizer required. Previous studies have shown that plant growth-promoting bacteria (PGPB) play a role in improving nitrogen-use efficiency in plants. Di Benedetto *et al.* (2017) focused on the interaction between PGPB and wheat to improve nitrogen-use efficiency.

Pseudomonas and *Bacillus*, which could oxidise ammonia to NO_2 and ultimately to NO_3, were found to be the probable PGPB. These PGPBs can promote beneficial mycorrhizal-plant interactions, affect biological nitrogen fixation, solubilise phosphate, create phytohormones and other compounds, and protect plants from harmful bacteria (Mataranyika *et al.* 2022; Di Benedetto *et al.* 2017). Leite *et al.* (2020) demonstrated that humic acids enhance plant ability to absorb nutrients and thrive. An alternate method to improve NUE is to apply urea together with humic compounds and humic acids. The findings were similar to those of Kong *et al.* (2022) who found that utilising humic acid and urea greatly boosted the yield and NUE of wheat and maize. Plants that received 50% N-fertilizer associated with UPMRB9 inoculation promoted a higher partial factor of productivity (PFP) than controls and other treatments. Suman *et al.* (2008) showed that the sugarcane plant associated with PGPR inoculation and with the required N dose (50 kg ha-1) achieved production at levels comparable to those achieved by those receiving the full N recommendation without inoculation.

The straightforward and speedy estimation of β-carotene content was effectively accomplished using acetone solvent as the extraction medium and UV-Vis spectrophotometry detection. Results showed that the highest significant optical density (OD) measurements were observed from the UPMRB9+50%N and UPMB10+75%N treatments (0.71 mg g^{-1} and 0.68 mg g^{-1}, respectively). These results showed that PGPRs-HA inoculation significantly increased the β-carotene content of sweet potato storage roots compared to the control. With regard to this effect, Abd-Alrahman *et al.* (2021) observed that applying humic acid consistently enhanced antioxidants such as tocopherol, β-carotene, superoxide dismutase, and ascorbic acid concentrations in crops. Moreover, Aremu *et al.* (2022) found that the application of PGPR significantly promoted the β-carotene content in *Abelmoschus esculentus* genotypes. The same researchers demonstrated that after adding PGPR inoculations, the chemical composition of β-carotene varied with the genotypes; for instance, the *Cannabis sativa* genotype had significantly higher b-carotene content than the Tygra genotype. According to Biswas *et al.* (2011), acetone is a suitable solvent for extracting the β-carotene compound in the samples. Since β-carotene is practically a non-polar molecule acetone, a moderately polar solvent enhanced beta-carotene extraction. It was discovered that this solvent has benefits over a number of organic solvents. The proposed UV-Vis spectrophotometry with the acetone solvent method is environment-friendly, inexpensive, and easily performed (Yilmaz and Soylak 2018).

CONCLUSION

Based on the findings of the current investigation, the inoculation of PGPR-HA with an optimum nitrogen fertilization rate significantly increased N-use efficiency, N uptake, and β-carotene content in sweet potatoes. Humic acid amendments could support the microbial environment, promoting beneficial bacteria and health of crops. Plant development functions, including cell division, hormone management, and energy generation, depend on a number of enzymes that humic acid boosts in plants. Therefore, N-use efficiency can be improved by using nitrogen fertilizers with bacteria that can fix nitrogen from the atmosphere and make it available to plants. This can help reduce the amount of nitrogen fertilizer needed and the environmental impact of excessive nitrogen fertilizer use.

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REFERENCES

Abd-Alrahman, H. A., N.M. Marzouk, S.M. El-Sawy and S.D. Abou-Hussein. 2021. Improving growth, yield and quality of onion plants by amino and humic acids under sandy soil conditions. *Middle East Journal of Applied Science*s 11 (3): 637-648.

Ahmad, S., I. Daur, S.G. Al-Solaimani, S. Mahmood, A.A. Bakhashwain, M.H. Madkour and M. Yasir. 2016. Effect of rhizobacteria inoculation and humic acid application on canola (*Brassica napus L.*) crop. *Pak. J. Bot*. 48(5): 2109-2120.

Ali-Tan, O. M.S. Radziah, K.B. Halimi, M.Z. Abdul Rahim, Abdullah and Z.H. Shamsuddin 2017. Growth and yield responses of rice Cv. MR219 to rhizobia and plant growth- promoting rhizobacteria inoculation under different fertilizer-N rates. *Bangladesh J. Bot.* 46(1): 481-488.

Ammar, Esraa E., Ahmed AA Aioub, Ahmed E. Elesawy, Ali M. Karkour, Moustafa S. Mouhamed, Aliaa A. Amer, and Nouran A. El-Shershaby. 2022. Algae as biofertilizers: between current situation and future prospective. *Saudi Journal of Biological Sciences*. 29(5):3083-96.

Aremu, A. O., G. Makhaye, S.Z. Tesfay, A.S. Gerrano, C.P. Du Plooy and S.O. Amoo. 2022. Influence of commercial seaweed extract and microbial bio-stimulant on growth, yield, phytochemical content, and nutritional quality of five *Abelmoschus esculentus* genotypes. *Agronomy* 12(2): 428.

Ashwini, N., Kumar, P., Joshi, A. K., Sharma, N. C., Sharma, N., & Sharma, N. 2023. Synergistic action of humic acid substances and bio-inoculants in guava (Psidium guajava L.): impact on growth traits, fruiting, nutrient profiling and rhizosphere stochiometry in meadow rainy season plant-soil interface. *Journal of Plant Nutrition*, 46(4), 574-588.

Atmini, K. D., S. Diantina, M. Sabda and D. Koswanudin. 2022. Screening of beta carotene and its correlation with tuber flesh color in sweet potato. In *AIP Conference Proceedings* 2462 (1): 020031.

Biswas, A. K., J. Sahoo and M.K. Chatli. 2011. A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets. *LWT-Food Science and Technology* 44(8): 1809-1813.

Bowman, D. C., J.L.Paul and R.M. Carlson. 1988. A method to exclude nitrate from Kjeldahl digestion of plant tissues. *Communications in Soil Science and Plant Analysis* 19(2): 205-213.

Chen, X., M. Kou, Z. Tang, A. Zhang and H. Li. 2017. The use of humic acid urea fertilizer for increasing yield and utilization of nitrogen in sweet potato. *Plant, Soil and Environment* 63(5): 201-206.

Da Silva, JA Lopes., Gonçalves, M. P., & Rao, M. A. 1995. Kinetics and thermal behaviour of the structure formation process in HMP/sucrose gelation. *International Journal of Biological Macromolecules*, *17*(1), 25-32.

Di Benedetto, N. A., M.R. Corbo, D. Campaniello, M.P. Cataldi, A. Bevilacqua, M. Sinigaglia and Z. Flagella. 2017. The role of plant growth promoting bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. *AIMS Microbiology* 3(3): 413.

Di Gioia, F., M. Gonnella, V. Buono, O. Ayala and P. Santamaria. 2017. Agronomic, physiological and quality response of romaine and red oak-leaf lettuce to nitrogen input. *Italian Journal of Agronomy* 12(1): 47–58.

Ding, Yanqiang, Yanling Jin, Kaize He, Zhuolin Yi, Li Tan, Lisha Liu, Mingshuang Tang, Anping Du, Yang Fang, and Hai Zhao. 2020. Low nitrogen fertilization alters rhizosphere microorganism community and improves sweet potato yield in a nitrogen-deficient rocky soil. *Frontiers in Microbiology* 11: 678.

Drapal, M. and P.D. Fraser. 2019. Determination of carotenoids in sweet potato (*Ipomoea batatas L.*, Lam) tubers: Implications for accurate provitamin A determination in staple sturdy tuber crops. *Phytochemistry* 167: 112102.

Ekin, Z. 2020. Co-application of humic acid and bacillus strains enhances seed and oil yields by mediating nutrient acquisition of safflower (*Carthamus tinctorius L.*) plants in a semi-arid region. *Appl. Ecol. Environ. Res*. 18: 1883-1900.

Ellong, E. N., C. Billard and S. Adenet. 2014. Comparison of physicochemical, organoleptic and nutritional abilities of eight sweet potato (*Ipomoea batatas*) varieties. *Food and Nutrition Sciences* 5(2):6.

Fernandes, A.M., L.G. Campos, M.S. Senna, C.L. da Silva and N.S. Assunção. 2018. Yield and nitrogen use efficiency of sweet potato in response to cover crop and nitrogen management. *Agronomy Journal* 110(5): 2004-2015.

Karim, N. A., T. Devarajan and A. Ahmad. 2022. Principal component analysis for phenotypic characterization of sweet potato (*Ipomoea batatas (L.)* Lam.) genotypes in Malaysia. *Trends in Sciences* 19(12): 4612-4612.

Kashiani, P. 2012. Genetic potential of selected sweet corn inbred lines and analysis of their combining ability assisted by microsatellite DNA markers. Doctoral dissertation, Universiti Putra Malaysia.

Kong, Baishu, Qicong Wu, Yongqiang Li, Taochuan Zhu, Yufei Ming, Chuanfu Li, Chuanrong Li et al. 2022. The application of humic acid urea improves nitrogen use efficiency and crop yield by reducing the nitrogen loss compared with urea. *Agriculture* 12(12): 1996.

Laftah, Sahar Kalaf, and Sundus A. Alabdulla. 2022. Response of Some Growth Traits of Safflower (*Carthamus tinctorius L.*) to Spray with Humic Acid Under Levels of Phosphorus Fertilizer. In *IOP Conference Series: Earth and Environmental Science* 1060 (1): 012115).

Leite, Jose M., Pavithra S. Pitumpe Arachchige, Ignacio A. Ciampitti, Ganga M. Hettiarachchi, Leila Maurmann, Paulo CO Trivelin, PV Vara Prasad, and SV John Sunoj. 2020. Co-addition of humic substances and humic acids with urea enhances foliar nitrogen use efficiency in sugarcane (*Saccharum officinarum L.*). *Heliyon* 6 (10): e05100

Mataranyika, P., P.M. Chimwamurombe, V. Venturi and J.D. Uzabakiriho. 2022. Bacterial bioinoculants adapted for sustainable plant health and soil fertility enhancement in Namibia. *Frontiers in Sustainable Food Systems* 9 (6):1002797 .

Moradzadeh, S., Siavash Moghaddam, S., Rahimi, A., Pourakbar, L., & Sayyed, R. Z. 2021. Combined bio-chemical fertilizers ameliorate agro-biochemical attributes of black cumin (Nigella sativa L.). *Scientific Reports*, 11(1): 11399.

Motsa, N. M., A.T. Modi and T. Mabhaudhi. 2015. Sweet potato (*Ipomoea batatas L.*) as a drought tolerant and food security crop. *South African Journal of Science* 111(11-12): 1-8.

Neela, S. and S.W. Fanta. 2019. Review on nutritional composition of orange- fleshed sweet potato and its role in management of vitamin A deficiency. *Food Science & Nutrition* 7(6): 1920-1945.

Okpara, D. A., O.E. Okon and F. Ekeleme. 2009. Optimizing nitrogen fertilization for production of white and orange-fleshed sweet potato in Southeast Nigeria. *Journal of Plant nutrition* 32(5): 878-891.

Oliver, R. and M. de Almeida Silva. 2018. Interaction between diazotrophic bacteria and N-fertilizer doses on sugarcane crop. *Journal of Plant Nutrition* 41(6): 722-736.

Safdarian, M., P. Hashemi and A. Ghiasvand. 2021. A fast and simple method for determination of β-carotene in commercial fruit juice by cloud point extraction-cold column trapping combined with UV–Vis spectrophotometry. *Food Chemistry* 343: 128481.

Sharma, L. K. and S.K. Bali. 2017. A review of methods to improve nitrogen use efficiency in agriculture. *Sustainability* 10(1): 51.

Shultana, R., A.T. Kee Zuan, M.R. Yusop, H.M. Saud and A.M. El-Shehawi. 2021. *Bacillus tequilensis* strain 'UPMRB9'improves biochemical attributes and nutrient accumulation in different rice varieties under salinity stress. *Plos one* 16(12): e0260869.

Singh J, Devi J, Sagar V. 2022. Vegetable biofortification: an underexploited silver lining for malnutrition management. In: *Biofortification of Staple Crops*. *Springer* 10:379–416.

Suman, A., A. K. Shrivastava, A. Gaur, P. Singh, J. Singh and R. L. Yadav. 2008. Nitrogen use efficiency of sugarcane in relation to its BNF potential and population of *endophytic diazotrophs* at different N levels. *Plant Growth Regulation* 54:1–11.

Tang, C., K. Cheng, B, Liu, M. Antonietti and F. Yang. 2022. Artificial humic acid facilitates biological carbon sequestration under freezing-thawing conditions. *Science of the Total Environment* 25;849:157841.

Tolessa, E. S. 2019. A review on water and nitrogen use efficiency of potato (*Solanum tuberosum L.*) in relation to its yield and yield components. *Archives of Agriculture and Environmental Science* 4(2): 119-132.

Ullah, H., R. Santiago-Arenas, Z. Ferdous, A. Attia and A. Datta. 2019. Improving water use efficiency, nitrogen use efficiency, and radiation use efficiency in field crops under drought stress: A review. *Advances in agronomy* 156: 109-157.

Yasmin, F., R. Othman and M.N.H. Maziz. 2020. Yield and nutrient content of sweet potato in response to plant growth-promoting rhizobacteria (PGPR) inoculation and N fertilization. *Jordan J. Biol. Sci*. 13(1): 117-122.

Yilmaz, E. and M. Soylak. 2018. A novel and simple deep eutectic solvent based liquid phase microextraction method for rhodamine B in cosmetic products and water samples prior to its spectrophotometric determination. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 202: 81-86.

Yuan, Y., S. Gai, C. Tang, Y. Jin, K. Cheng, M. Antonietti and F.Yang. 2022. Artificial humic acid improves maize growth and soil phosphorus utilization efficiency. *Applied Soil Ecology* 179: 104587.

Zago, S. L., M.F. dos Santos, D. Konrad, A.Fiorini, F.R. Rosado, R.F. Missio and E.C.G.Vendruscolo. 2019. Shelf life of *Azospirillum brasilense* in alginate beads enriched with trehalose and humic acid. *J Agric Sci*. 11(6):269.

Zahid, M., M.K. Abbasi, S. Hameed and N. Rahim. 2015. Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays L*.). *Front. Microbial* 17(6):207.

Zhao, C., G. Liu, Y. Chen, Y. Jiang, Y. Shi, L. Zhao and Z. Huo. 2022. Excessive nitrogen application leads to lower rice yield and grain quality by inhibiting the grain filling of inferior grains. *Agriculture* 12(7): 962.

Zhu, X., G.H. Ros, M. Xu, Z. Cai, N. Sun, Y. Duan and W. de Vries. 2023. Long-term impacts of mineral and organic fertilizer inputs on nitrogen use efficiency for different cropping systems and site conditions in Southern China. *European Journal of Agronomy* 1;146:126797.