Nig. J. Biotech. Vol. 41(2): 149-157 (Dec. 2024)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: https://dx.doi.org/10.4314/njb.v41i2.14

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Influence of Rhizospheric Bacteria on Disease Suppression and Yield of Tomato under *Sclerotium rolfsii* Stress during Rainy and Dry Planting Season

[1]*Taiwo, M. O., [1]Oyedeji B. A., [2]Abiola-Kuforiji, O. T. and [1]Omeonu, F. C.

[1]Department of Microbiology, Chrisland University, Nigeria
[2]Department of Molecular Biology and Biotechnology, Chrisland University, Nigeria

Abstract

Agrochemicals used in controlling plant diseases are costly and their application is not sustainable, hence, the adoption of biological approaches. Microbial inoculants were screened in cycles under *Sclerotium rolfsii* stress to select host-mediated microbiota (HMM) and isolate rhizospheric bacteria with biocontrol potentials. Microbial isolates were evaluated *in-vitro* and on field under *S. rolfsii* stress. Results showed that microbiota inoculant significantly reduced disease severity till the 6th round. Identified isolates (*Bacillus cereus, B. thuringiensis, B. mycoides* and *B. tropicus*) displayed varying inhibitory effects against the growth of *S. rolfsii in-vitro.* During field trial, disease severity was significantly ($P \leq 0.05$) reduced in all treatments by 71-85% (dry season) and 25-75% (rainy season) than the control (0%). However, the fruit weight of harvested tomatoes from plants treated with HMM (238g, 464g) and *B. cereus* AN14 (209g, 390g) were the most significant in dry and rainy seasons. These inoculants should be considered a biocontrol agent for diseases caused by *S. rolfsii.*

Keywords: Bacillus sp., Biocontrol, Field trial, Plant pathogen, Soil

***Corresponding author email:** taiwomikeo@gmail.com https://orcid.org/0000-0001-9594-9355

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the world's most consumed household staple foods. They are known to be rich in nutrients, low in pH, and high in moisture (FAO, 2020; Ojesola et al., 2024). The intrinsic qualities of Tomatoes also make them vulnerable to infestation by pathogens and render them unsafe for food. The most significant and widespread diseases of vegetables are caused by pathogenic fungi (Osunmuyiwa et al., 2021). They infect a variety of tomato fruit and result in devastating losses that have a significant financial impact throughout production, storage, marketing and transportation (Akintokun and Taiwo, 2016a). *S. rolfsii* Sacc causes rot disease. It forms a small, discrete, light-brown, water-soaked lesion on the plant. The disease frequently goes undetected in the soil until the plant starts to wilt. Often, wilting occurs during the day and plants recoup at night (Rachel et al., 2022). However, under ideal circumstances, the wilting can advance quickly, become irreversible, and result in mortality (Xie and Vallad, 2016). The pre-harvest manifestation of this disease may seriously affect post-harvest yield. To achieve sustainable agriculture, diseases caused by *S. rolfsii* needs to be managed effectively. Several conventional management strategies such as the use of agrochemicals affect environmental and human health, hence, the need for biological approaches. Rhizospheric microorganisms are mostly recruited by tomato plants in association with their surrounding soil making them unique to the plant biome (Chen et al., 2022). Though, different microbial groups have affiliated functions, the most significant actors are the rhizospheric bacteria (Karthika et al., 2020). To survive under southern blight stress, these bacteria prevent or significantly lower the pathogenicity of *S. rolfsii* by producing enzymatic and antimicrobial products (Karthik et al., 2017)**.** Previous studies such as that of Abbas et al. (2019) and Asghari et al. (2020) have isolated rhizospheric bacteria *in-vitro* and reported enhanced plant growth in the greenhouse. However, many of these inoculants have performed inconsistently during field assay, thus, the integration of Host-Mediated Artificial Selection (HMAS), a more novel method which recruits beneficial microorganisms and guarantees the consistency of tomato productivity under the influence of disease stresses. The objective of this study is to select and evaluate host-mediated rhizospheric bacteria for growth and yield of tomatoes on the field under *S. rolfsii* stress.

Materials and Methods

Study area

This study was carried out at the teaching and research farm of the Federal University of Agriculture Abeokuta (FUNAAB) located at latitude 7° 15' N and longitude 3° 28' E. in the savannah agroecological zone of odeda local government area of Ogun State, Nigeria.

Collection of samples

Soil samples were aseptically collected at 15 cm depth from different geographical locations in Abeokuta, Ogun state. Tomato samples of beske varieties, an indigenous variety of tomato locally grown in Nigeria were bought from an agro-outlet in Abeokuta, Ogun state. *S. rolfsii* was collected from the plant pathology laboratory of the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria.

Pathogenicity of S. rolfsii to tomato

The ability of *S. rolfsii* to infect tomato plants was achieved by the method described by Carmona et al. (2020) with little modifications. Disease symptoms were scored qualitatively using a rating scale of 1 – 4 described by Akintokun and Taiwo (2016a) with little modification. The identity of the pathogen was determined by examining the macroscopic and microscopic characteristics, and compared with standard mycological identification keys and taxonomic descriptions (Diaz et al. 2018).

Screening of microbiota in cycles under S. rolfsii stress

Disease-tolerant microbiota were selected using the experimental approach proposed by Mueller and Sachs (2015) with little modification. The experimental setup includes tomato seeds grown in cycles under *S. rolfsii* stress. This study was set up in a conventional screenhouse with treatments replicated 3 times in a completely randomized design. Already sterilized seeds were first grown in sterilized soil for 7 days (Round 0). When approximately 83% (15 of 18) of the planting pots had plants showing severe disease symptoms, the growth was halted (Round 1)**.** The rhizospheric soil from the pots with healthy plants were selected and combined. A portion of the rhizosphere soil was kept for bacterial isolation while 10 g soil of microbiota was suspended in 90 ml sterile distilled water and adjusted to a final density of 1×10^7 CFU/ml to serve as inoculum for another round of the cycle. The screening of HMM continued in cycles of rounds until the disease reduction percentage significantly decreased. Disease expressions were recorded as follows: Disease incidence (DI) = Percentage of tomato plant parts that showed visible signs of disease compared to the total number of parts examined.

$$DRP = 100\, x\, \frac{DI\ control - DI\ treatment}{DI\ control}$$

Where, DRP = Disease reduction percentage and DI = Disease incidence

Isolation and molecular identification of rhizospheric bacteria

In each cycle above, bacteria were isolated from the rhizospheric of healthy plants using standard microbiology techniques (Akintokun and Taiwo, 2016b). Following the incubation, the growth on LB agar medium was monitored and expressed as CFU/g of soil. Isolated bacteria were subjected to 16S rDNA sequencing to identify the strains. Phylogenetic analysis was conducted in MEGA11 and inferred using the neighbor-joining method (Tamura et al., 2021).

Biocontrol potential of rhizospheric bacteria

The percentage inhibitory effect of bacteria was evaluated against *S. rolfsii* using the method described by Akintokun et al. (2016a). After incubating for 4 days at 25°C, the inhibitory effect was examined using the formula,

$$Inhibition\ growth\ (\%)\ =\frac{(b-a)}{b}\ x\ 100$$

Where, a = radial growth of fungi interacting with antagonistic bacteria. b = radial growth of fungi only in control plate.

Field application of rhizospheric bacteria on disease suppression and yield of tomato in two planting seasons

The field trial was conducted at the FUNAAB's Teaching and Research farm in dry and rainy seasons respectively. Physico-chemical properties were done on field soil samples using the method of AOAC (2015). The experiment used a completely randomized design and was duplicated three times on a layout of 15 m × 30 m with a distance of 0.3 m within rows and 0.4 m between rows. Each suspension of rhizospheric bacteria and *S. rolfsii,* or microbiota and *S. rolfsii* were applied simultaneously to 14-day-old tomato seedlings while the control had only *S. rolfsii*. Germination was observed and recorded for DI, DRP, number of fruits and fruit weight.

Analysis of data

Data obtained were analysed by analysis of variance (ANOVA), while the means were separated using Tukey HSD test at α ≤ 0.05

Result and Discussion

Pathogenicity of S. rolfsii to tomato

Early symptoms showed curling of leaves at the lower part of the plant which later wilted and dried-off at the later part. The most severe symptom was the formation of sclerotia mat on the soil's surface at the root of infected plant which later collapsed the stem. Stunted growth was generally observed in treatment plant in comparison to the control. When viewed on plates, the mycelium has a rough, round-shaped hyphal filament during early development. The matured mycelium develops a tiny tuff of whitish sclerotia which later turns brown. Microscopic examination revealed the branched mycelium with septate hyphae, confirming its identity. The re-isolated pathogen from the infected tomato plants showed a similar identity to the original isolates, fulfilling Koch's postulates.

Effect of HMAS on the severity of S. rolfsii stress

Disease incidence caused by *S. rolfsii* was significantly (P ≤ 0.05) higher across the rounds of planting in control than in microbiota. However, microbiota inoculant significantly reduced the disease by 64.54%, 69.57%, 72.56%, 74.12%, 75.31% and 77.12% in rounds 1, 2, 3, 4, 5 and 6, respectively (Figure 1, Plate 1). HMAS has proved by this result to be a reliable approach to select beneficial microorganisms which guarantee the suppression of southern blight. Jochum et al. (2019) used similar method to screen and select drought-tolerant bacteria. Microbiota responsible for this disease suppression compete with pathogens to prevent or limit their growth and survival (Chen et al., 2020).

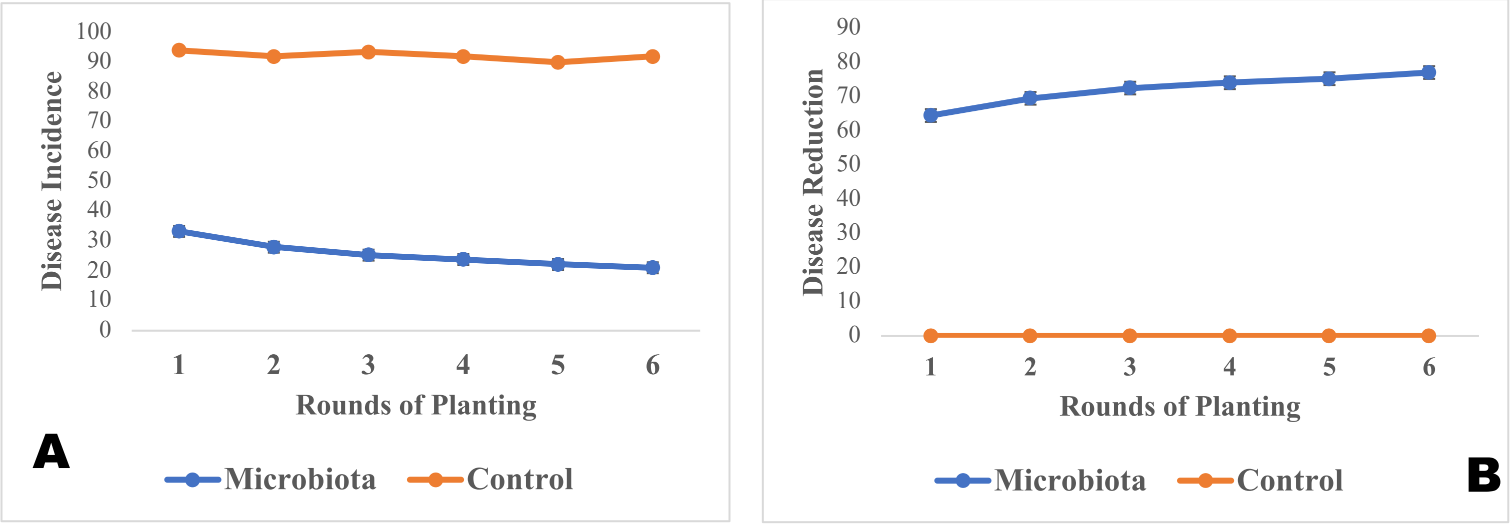

Fig 1: Effect of HMAS on the severity of *S. rolfsii* stress. Treatments between each round were compared ($\alpha \leq 0.05$) using Tukey's HSD test to show significant differences.

Tomato Plants growing in soil without HMM showing stunted growth caused by *S. rolfsii* (Control)

Tomato Plants growing in soil treated with HMM showing tolerance to disease symptoms caused by *S. rolfsii*

Plate 1: Effect of HMAS on *S. rolfsii* stress

Total bacterial counts of soil samples under S. rolfsii stress

Bacterial counts from soil samples under *S. rolfsii* stress were significantly reduced in each round (Figure 2). This may be due to the death of microorganisms that cannot withstand prolonged stress. This is similar to the report of Adandonon et al. (2021) who reported a significant reduction in bacterial populations under *S. rolfsii* in flooded soil*.* The isolated bacteria from this study reflect their ability to survive under stressed conditions without mortality (Oves et al*.,* 2019).

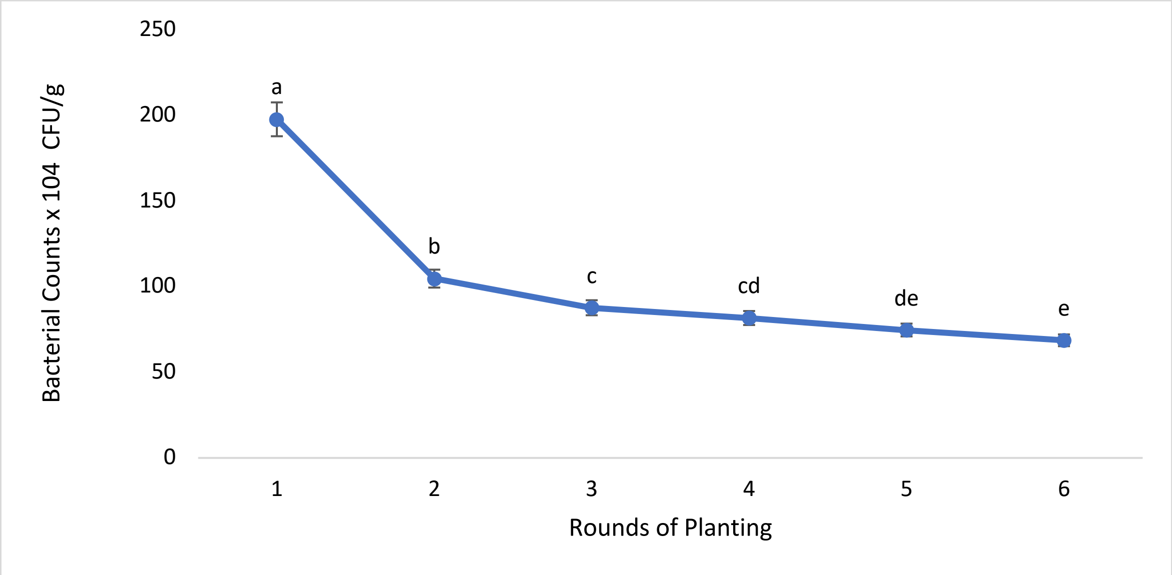

Fig 2: Total bacterial counts of soil samples under *S. rolfsii* stress. Means with different letters along the bars are significantly different (P≤ 0.05) from each other.

Molecular identity and phylogenetic relatedness of rhizospheric bacteria

Results from the molecular analysis of bacterial strains based on 16S rRNA gene sequence analysis confirmed that the identity of the isolates are *B. cereus, B. thuringiensis, B. mycoides* and *B. tropicus* (Table 1). Though all microbial groups such as fungi, archaea, eubacteria and viruses are all found in tomato rhizosphere (Naumova et al. 2022), this study has shown that eubacteria dominate this region and play the most significant role in pathogen suppression. The evolutionary tree has 12 nucleotide sequences with the closest branched strain belonging to similar genealogy (Figure 3).

Table 1: Identity of rhizospheric bacterial strains obtained from NCBI genebank

Identified rhizospheric bacteria from this study	Closest related bacterial strain from NCBI genebank	Percentage identity	Accession number
B. cereus AN14	*B. cereus* NPK1122	98.65%	MN691545.1
B. thuringiensis AN10	*B. thuringiensis* BD120L1-B25	97.41%	FR877754.1
B. sp. ANP	*B. mycoides* N42r	95.73%	MOP133280.1
B. sp. AN22	*B. tropicus* BH-16S	96.89%	OL445008.1

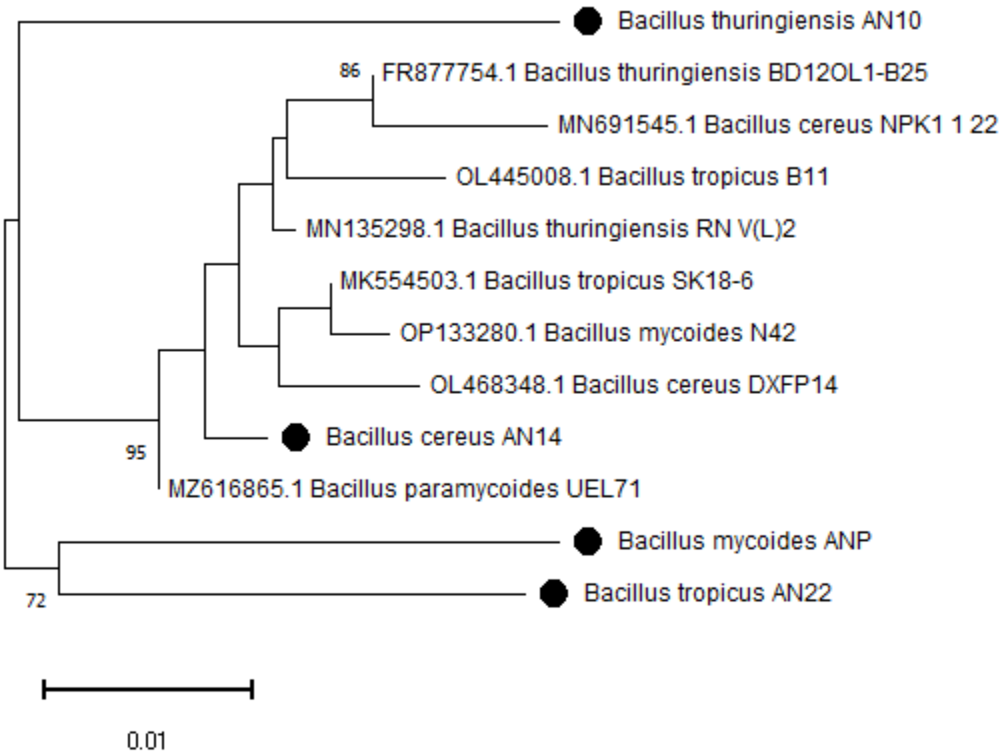

Fig 3: Phylogenetic relationship of rhizospheric bacteria inferred using the neighbour-joining method.

In-vitro inhibitory effect of rhizospheric bacteria against S. rolfsii

Rhizospheric bacteria displayed varying inhibitory effects against the growth of *S. rolfsii in-vitro. B. cereus* AN14 (87.08%), *B.* sp. ANP (83.50%) and *B. cereus* AN14 (87.08%) were more significant than *B.* sp. AN22 (60.58%) and the control (0%) (Figure 2). This is similar to the work of Akintokun and Taiwo (2016a) who reported rhizobacteria to inhibit *Fusarium oxysporum* and *Rhizoctonia* *solani*. Similarly, *Bacillus* genus has been reported by Karthika et al. (2020) to control *Rhizoctonia solani*, *R. rolfsii*, and *Pythium ultimum.* When PGPR reduces or eliminates the negative impacts of one or more plant pathogens, crop development is indirectly promoted (Backman, and Sikora, 2018). This might be due to the synthesis of substances that inhibit or foster pathogen resistance.

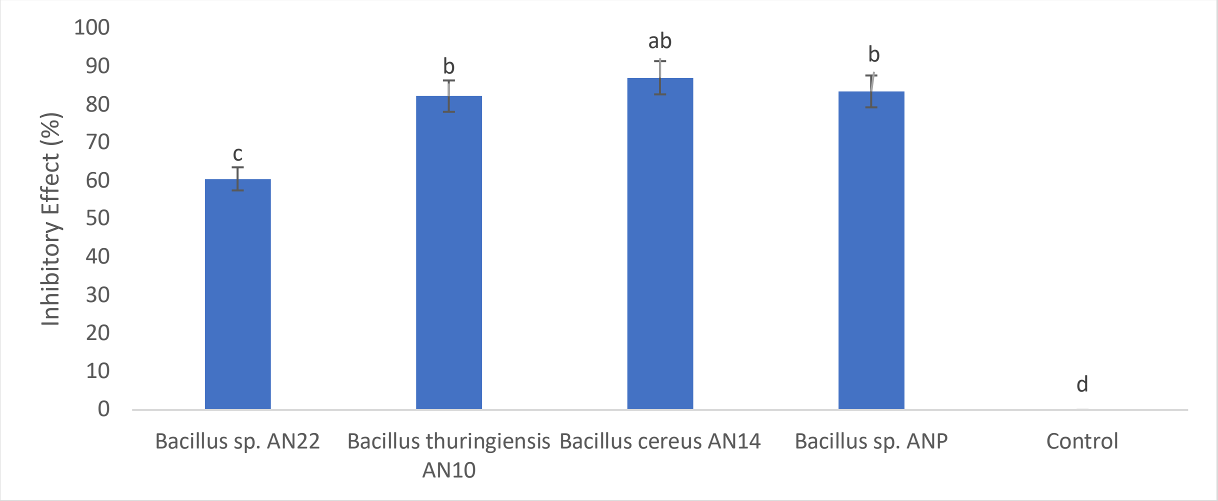

Fig 4: *In-vitro* percentage inhibitory effect of rhizospheric bacteria against *S. rolfsii*. Means with different letters along the bars are significantly different (P≤ 0.05) from each other.

Physico-chemical properties of soil before and after planting

The soil texture before and after planting was sandy. The pH increased from 6.02 to 6.46 while the calcium, organic matter, available phosphorus and moisture content reduced after planting. On contrary, TOC, iron and zinc content of soil increased after planting. The percentage of total nitrogen in soil before planting (0.24%) was similar after planting (0.22%). (Table 2). Nutritional contents of soil are depleted by plants during germination (Akintokun, et al., 2019). However, microbial inoculants from this study improved or minimised the loss of these nutrients through symbiotic activities.

Table 2: Physico-chemical properties of soil before and after planting

Parameters determined	Before planting	After planting
% Sand	91.40	86.65
% Silt	5.40	10.40
% Clay	3.19	2.94
pH	6.02	6.46
% TOC	1.32	1.43
% OM	17.36	10.54
% TN	0.24	0.22
Ca cmol/kg	3.50	2.44
Fe mg/kg	0.14	0.17
Zn mg/kg	0.98	1.14

Av. P mg/kg	6.90	6.70
Moisture content (%)	24.32	26.23

TOC = Total Organic Carbon, OM = Organic Matter, TN = Total Nitrogen
Av. P. = available Phosphorus

Field application of rhizospheric bacteria against S. rolfsii during the dry and rainy seasons

Tomato plants treated with microbial inoculants displayed lower incidence of diseases caused by *S. rolfsii* than in the control. The symptoms of diseases caused by this pathogen were significantly ($P \leq 0.05$) reduced in all treatments than the control. However, the number of fruits and fruit weight of harvested tomatoes from plants treated with *B. cereus* AN14 and HMM in rainy and dry seasons were significantly higher than other treatments and the control (Table 3). Rhizospheric bacteria through indirect mechanisms promote plant growth and yield. The indirect promotion of plant growth occurs when bacteria reduce or inhibit the harmful effects of one or more plant pathogens (Akintokun and Taiwo, 2016a). This was possible through the production of antagonistic substances which inhibited the targeted pathogen, inducing resistance to pathogens, production of lytic enzymes which disrupt and degrade the cell wall of *S. rolfsii*, predation and parasitism and a combination of these mechanisms (Pliego et al., 2017). According to Beattie (2016), any bacterium that reduces the severity of plant disease is known as a biocontrol agent.

Table 3: Field application of rhizospheric bacteria against *S. rolfsii* during the dry and rainy seasons

	Disease incidence	Disease reduction	Number of fruits	Fruit weight	Disease incidence	Disease reduction	Number of fruits	Fruit weight
	DRY SEASON				**RAINY SEASON**			
B. sp. AN22	22.00±1.0^{b}	71.00±2.8^{a}	12±0.3^{bc}	165±10.1^{b}	42.33±2.9^{b}	25.00±2.8^{d}	15±1.7^{b}	279±4.6^{c}
B. thuringiensis AN10	16.00±2.0^{b}	82.67±3.1^{a}	14±0.5^{ab}	194±5.1^{ab}	29.67±2.4^{c}	46.00±3.4^{c}	24±1.4^{a}	387±5.5^{b}
B. cereus AN14	15.33±0.6^{b}	80.33±2.7^{a}	16±0.3^{a}	209±14.8^{ab}	20.67±1.2^{cd}	62.50±1.7^{ab}	24±1.4^{a}	390±6.9^{b}
B. sp. ANP	19.33±2.6^{b}	75.00±4.6^{a}	14±0.8^{ab}	203±12.4^{ab}	25.33±1.4^{c}	55.40±2.8^{bc}	19±1.1^{ab}	246±3.6^{d}
HMM	14.00±2.0^{b}	85.33±3.3^{a}	15±0.8^{a}	238±13.4^{a}	14.33±1.4^{d}	75.00±5.7^{a}	24±1.5^{a}	464±5.3^{a}
Control	76.00±4.9^{a}	0.00±0.0^{b}	10±0.5^{c}	77±5.2^{c}	56.00±2.6^{a}	0.00±0.0^{e}	12±2.0^{b}	169±9.5^{e}

Results are values of mean ± standard error of mean for three replicates. Values in a column that are followed by various letters indicate significant differences according to Tukey's HSD test at a significant level ($P \leq 0.05$).

Conclusion

The application of rhizospheric bacteria and the combination of culturable and unculturable microorganisms is an effective environmentally safe strategy to achieve sustainable plant growth and crop yield. HMAS is a better approach to selecting beneficial soil microorganisms which guarantee the consistency of tomato growth promotion and health. HMM and *B. cereus* AN14 isolated from this study controlled diseases caused by *S. rolfsii*, connoting promising inoculants in tomato cultivation during dry and rainy seasons. It plays a significant role in promoting the yield of tomatoes on the field during rainy and dry seasons.

Acknowledgement

The Authors appreciate the Directorate of Teaching and Research Farm, FUNAAB for granting the request to use their research farm for the study.

References

Abbas, R., Rasul, S., Aslam, K., Baber, M., Shahid, M., Mubeen, F. and Naqqash, T. (2019). Halotolerant PGPR: a hope for cultivation of saline soils. *J. King Saudi Uni. – Sci.* 31: 1195–1201.

Adandonon, A., Momma, N., Hoshino, Y. T., and Makino, T. (2021). Studies on Bacterial Populations Concomitant with *Sclerotium rolfsii* sclerotia in Flooded Soil, as Estimated by 16S rRNA Gene, PCR-DGGE and Sequence Analyses. *Sci. Dom. Int.* 76–89. https://doi.org/10.9734/bpi/nvbs/v4/9163d

Akintokun, A.K and Taiwo, M.O. (2016a). Biocontrol Potentials of Individual Specie of Rhizobacteria and Their Consortium against Phytopathogenic *Fusarium oxysporum* And *Rhizoctonia solani*. *Inter. J. Scient. Res. Env. Sci. 4*, 219-227. https://doi.org/10.12983/ijsres-2016-p0219-0227

Akintokun A.K and Taiwo M.O. (2016b). Comparison of single culture and the consortium of growth-promoting rhizobacteria from three tomato (*Lycopersicon esculentum* Mill) varieties. *Adv. Plants & Agric. Res.* 2016: 5 (1): 448-455. https://doi.org/10.15406/apar.2016.05.00167

Akintokun, A.K., Ojesola, C.O., Akintokun, P.O. and Oloyede, A.R. (2019). Antagonistic effect of *Bacillus thuringiensis* for the control of bacterial wilt of tomato (*Lycopersicon esculentum* Mill) *Nig. J. Biotech.* 36 (1): 94-102. https://dx.doi.org/10.4314/njb.v36i1.13

Akintokun, A.K., Taiwo, M.O., Akintokun, P.O. and Obuotor, T.M. (2016). Screening of indigenous Plant Growth-Promoting Bacterial Strains for enhancing growth of Tomato (*Lycopersicon esculentum* Mill.) in Nigeria. *J. Glob. Agric. & Eco.* 6(2):127-135

Asghari, B., Khademian, R. and Sedaghati, B. (2020). Plant-Growth Promoting Rhizobacteria (PGPR) confer drought resistance and stimulate biosynthesis of secondary metabolites in pennyroyal (*Mentha pulegium* L.) under water shortage condition. *Sci. Hort.* 263:109132.

Association of Official Analytical Chemists (AOAC). (2000). Ash of Animal Feed. Official Methods of Analysis, Association of Official Analytical Chemists, 15th ed. Washington, DC. Pp 1-4.

Backman, P.A. and Sikora, R.A. (2018). Endophytes: An emerging tool for biological control. *Bio. Cont.* 46: 1–3. http://dx.doi.org/10.1016/j.biocontrol.2008.03.009

Carmona, S.L., Burbano-David, D., Gómez, M.R., Lopez, W., Ceballos, N., Castaño-Zapata, J., Simbaqueba, J. and Soto-Suárez, M. (2020). Characterization of Pathogenic and Nonpathogenic *Fusarium oxysporum* Isolates Associated with Commercial Tomato Crops in the Andean Region of Colombia. *Pathogens* 9(70):1-30. https://doi.org/10.3390/pathogens9010070

Chen, M., Wang, J., Liu, B., Zhu, Y., Xiao, R., Yang, W., Ge, C. and Chen, Z. (2020). Biocontrol of tomato bacterial wilt by the new strain *Bacillus velezensis* FJAT-46737 and its lipopeptides. *BMC Micro*. 20:160. https://doi.org/10.1186/s12866-020-01851-2

Chen, S., Sun, Y., Wei, Y., Li, H. and Yang, S. (2022) Different rhizosphere soil microbes are recruited by tomatoes with different fruit colour phenotypes. *BMC Micro.* 22, 210. https://doi.org/10.1186/s12866-022-02620-z

Díaz, F., Castellanos, J. S., Mateo Vargas Hernández, Serna, S. A., Guadalupe, O., Verduzco, C. V., and Ramos, M. A. (2018). Diagnosis and Integrated Management of Fruit Rot in *Cucurbita argyrosperma* caused by *Sclerotium* rolfsii. *The Plant Patho. J.*, *34*(3), 171–181. https://doi.org/10.5423/ppj.oa.08.2017.0185

Food and Agricultural Organisation (FAO). (2020). The Value Chain Approach;

Strengthening Value Chains to Promote Economic Opportunities. Available at http://fao.org/. Retrieved 6th December, 2022.

Jochum, M.D., McWilliams, K.L., Pierson, E.A. and Jo, Y.K. (2019). Host-mediated microbiome engineering (HMME) of drought tolerance in the wheat rhizosphere*. PLoS ONE* 14(12): e0225933. https://doi.org/10.1371/journal.pone.0225933

Karthik, C., Elangovan, N., Kumar, T.S., Govindharaju, S., Barathi, S., Oves M. and Arulselvi, P.I. (2017). Characterization of multifarious plant growth promoting traits of rhizobacterial strain AR6 under Chromium (VI) stress. *Micro. Res.* 204: 65–71. https://doi.org/10.1016/j.micres.2017.07.008

Karthika, S., Midhun, S.J. and Jisha, M. (2020). A potential antifungal and growth-promoting bacterium *Bacillus sp*. KTMA4 from tomato rhizosphere. *Micro. Patho.* 142:104049. https://doi.org/10.1016/j.micpath.2020.104049

Mueller, U.G. and Sachs, J.L., (2015). Engineering Microbiomes to Improve Plant and Animal Health. *Trends in Micro.* 23: 606-617. https://doi.org/10.1016/j.tim.2015.07.009

Naumova, N., Baturina, O., Nechaeva, T., and Kabilov, M. (2022). Root and Rhizosphere Microbiome of Tomato Plants Grown in the Open Field in the South of West Siberia under Mineral Fertilization. *Horticulturae*, *8*(11), 1051. https://doi.org/10.3390/horticulturae8111051

Ojesola, C. O., Akintoku, A.K., Akintokun, P.O., Taiwo, M.O. and S. O. Adebajo, S.O. (2024) Growth Evaluation of Tomato Plant (*Solanum lycopersicum* L.) in Response to Melon Seed Shell Biochar Soil Amendment. *FUOYE J. Pure & Appl. Sci*. 9(1):118-130. https://doi.org/10.55518/fjpas.HZZP3752

Osunmuyiwa, O.O., Sakariyawo, O.S., Adewunmi, I., Kareem, R., Debnath, K.B., Banmeke, T., Jenkins, D.P., Peacock, A.D. (2021). Transforming farming value-chains in sub-Saharan Africa through social and economically inclusive sustainable energy solutions pg. 1-43

Oves, M., Khan, M.S. and Qari, H.A. (2019). Chromium-reducing and phosphate-solubilizing *Achromobacter xylosoxidans* bacteria from the heavy metal-contaminated soil of the Brass city, Moradabad, India. *Int. J. Env. Sci. & Tech.* 16:6967–6984. http://dx.doi.org/10.1007/s13762-019-02300-y

Rachel, R.R., Gauthier, N. and Pandey, S. (2022). Sclerotinia Diseases of Vegetable Crops. *Plant Patho. Fact Sheet*. PPFS-VG-29. Pg 1-5

Tamura, K., Stecher G., and Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Bio. & Evo.* 38(7):3022–3027 https://doi.org/10.1093/molbev/msab120

Xie, C. and Vallad, G. (2016). Integrated Management of Southern Blight in Vegetable Production. Publication. Florida Cooperative Extension Service. Pg. 12-19.