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Enhancing *Moringa Stenopetala* L. Establishment and Adaptation with Rhizobacteria at High Altitude Hadiya Zone, Central Ethiopia

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Abstract

Moringa stenopetala, vital for nutrition in East Africa, faces climate-driven habitat shifts. This necessitating ecofriendly rhizobacterial inoculation to enhance its highland establishment and stress tolerance. This study evaluated the role of rhizobacteria in the adaptation and establishment of *M. stenopetala* to highland conditions. Potential rhizobacteria previously isolated and tested under greenhouse and field trial by the researcher were used for this study. Moringa seedlings inoculated with single, consortia, and without inoculation was assessed. The survival rate of moringa at the end of the field trial was calculated. All the treatments were done in triplicate. The highest seed germination percentage (92.86%) was observed in a combined treatment (T11). Microbial inoculation showed a seed germination bioassay with a mean difference of 2.5–6.5. The highest records of root and shoot lengths and shoot and root dry weights were obtained with soil inoculation with mixed cultures, giving 19.75 and 88.15 cm/plant, respectively, and 10.32 and 19.78 g/plant, respectively. An integrated treatment displayed the best growth effect on the stem height (91.42 cm) and the stem diameter (0.97 cm). Moringa had a 73% survival rate in mixed microbial inoculation at the sixth month, compared to 57% in FB-49. Greenhouse and field trials have proved that phytobeneficial microbes can be used as inoculum with specific benefits and can also be successful in many environmental soil conditions. Therefore, compared to the single and un-inoculated control, using eco-friendly rhizobacteria inoculums is the optimum method for boosting the *M. stenopetala* survival rate.

Keywords: Adaptation, High altitude, Moringa, Rhizobacteria, Seed germination

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1.Introduction

Moringa is one of the world's most useful tropical trees and often nick-named a 'multi-purpose tree' (Kumsa *et al.*, 2017; Raghavendra *et al.*, 2016). It is a resilient and highly drought-tolerant tree growing on marginalized land and almost all parts of the plant are useful (Nibret *et al.*, 2010). The leaves, seeds, pods, flowers, and roots are excellent sources of nutrients and bioactive compounds (Jain *et al.*, 2019; Vats *et al.*, 2017), especially dietary fiber, proteins, minerals, vitamins, and phytochemicals that offer great nutritional and therapeutic benefits (Aderinola *et al.*, 2018; 2019; 2020; 2022; Gopalakrishnan *et al.*, 2016; Jahn, 1991).

Today, one of the primary global challenges is a food crisis to ensure food and nutrition security for a growing population. Moreover, food security faces a threat due to the arable land base is shrinking, climate change, agrochemicals, and also the reliance on depleting fossil fuel reserves for their production (Velivelli *et al.*, 2015). The global population is projected to grow to over 8 billion people by 2030. This alarming rate demanding major issues for the agricultural sector concerning food production and security. Moreover, there is tremendous vulnerability to climate change in middle and high latitudes (Smol, 2012). Climate change is likely to shrink the land suitable for moringa, and other cash crops, thereby hurting the livelihoods of many people. Thus, it is urgent to develop crop plants and trees with improved biotic and abiotic stresses tolerance (Ngumbi and Kloepper, 2016).

Different tree species responds differently to the different environments and their distribution are influenced by altitude and other environmental conditions (Chauhan *et al.*, 2021). The African moringa (*M. stenopetala*) was domesticated in the Ethiopian highlands and can grow at altitudes of 2,000 meters or higher. *M. stenopetala* has gained attention recently in Ethiopia due to its multiple uses to humans and environmental health. It is a drought-tolerant fast-growing indigenous tree mainly planted and maintained for its nutritional values (Abay *et al.*, 2015).

Rhizobacteria are beneficial microbes that live in the plant microbiome and are capable of increasing plant growth, development, and resistance under abiotic stress (Dimkpa *et al.*, 2009; Marulanda *et al.*, 2009; Ngumbi and Kloepper, 2016). The possible explanations regarding the mechanism(s) associated with bacteria-induced stress alleviations are via the production of different plant hormones (Yang *et al.*, 2009). Rhizobacteria are capable of secreting bacterial biofilm, which can provide the plant with substantial macromolecules such as sugars and oligo-polysaccharides, contributing to numerous functions for plant growth and development (Dimkpa *et al.*, 2009). Indeed, this naturally humectant extracellular biofilm matrix can directly improve water availability in the root medium through increased water retention, even in small concentrations (Chakraborty *et al.*, 2013).

Rhizobacterial genera of *Bacillus* sp. and *Pseudomonas* sp., are important in the soil-plant relationship (Widnyana and Javandira, 2016). Strains of *Bacillus thuringiensis* AZP2 and *Paenibacillus polymyxa* B showed water tolerance in wheat (Timmusk *et al.*, 2014). Thus, microorganisms are essential to increase root growth and improve nutrient and water uptake increase crop yields, and improve quality (Lim and Kim, 2013). The integration of rhizosphere bacteria opens new dimensions to fit in the agroforestry cropping system. The goal of this study was to evaluate the contribution of microbial application on *M. stenopetala* adaptation and establishment in the vicinity of Hossana and other sites with comparable agro-ecology.

2. Materials and Methods

2.1. Description of the Study Area

The study was carried out from December 2021 to December 2022 in Hadiya Zone, Central Ethiopia at Wachemo University experimental site. Its coordinates are 7° 34' 60" N and 37° 52' 60" E and it is located at an elevation of 2,279 meters above sea level (Figure 1). The soil has a sandy loam texture and a pH of 5.5, along with 0.30% total nitrogen and 46.3 mg/kg phosphorus (Daniel and Birhanu, 2018, unpublished).

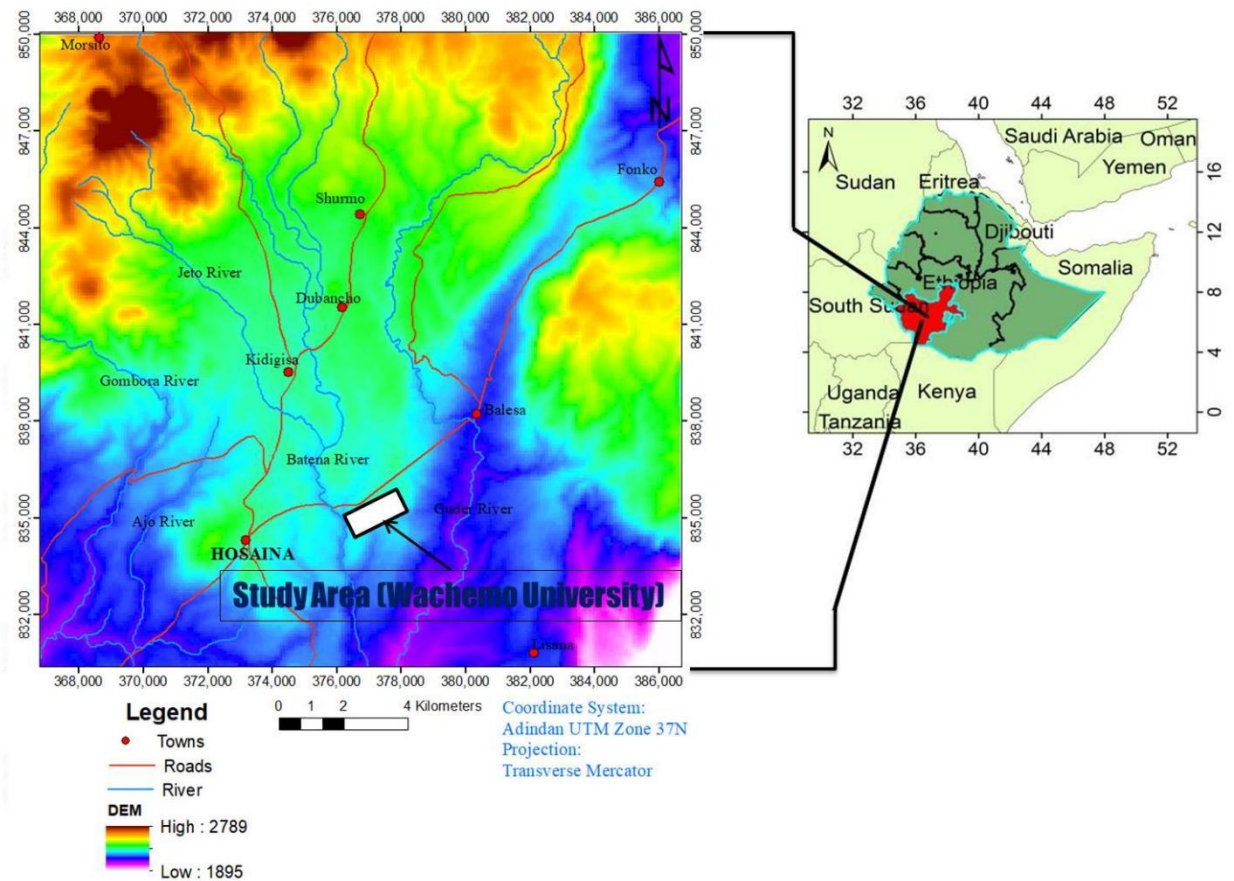


Figure 1. Map of the study area (the arrow ← indicate specific study location)

2.2. Microorganisms and Plant used

Bacterial strains used in this experiment were *Pseudomonas fluorescent* FB-49 and *Acinetobacter calcoaceticus* BS-27 which were previously isolated from the rhizosphere of acacia (*Acacia abyssinica*) and *Juniperus* (Alemayehu *et al.*, 2020) and belong to the microbial collection of Addis Ababa University. Seeds of *M. stenopetala* was collected from Gamo Zone Arbaminch Zuria District, Southern Ethiopia, with 6° 03' 55" N, 37° 33' 36" E GPS Coordinates. The plant specimen was deposited at National Herbarium, Addis Ababa University with ETH-2021-0537 and identified by Dr. Negalign Awoke specialized in Plant Biology and Biodiversity Management.

2.3. Greenhouse Trials

Moringa seeds were surface-sterilized with 2% sodium hypochlorite for 5 min and thoroughly washed with distilled water. Eight seeds were kept in equidistance position in sterilized Petri plates containing sterile moist filter paper and cotton for 7 days for germination (Mia *et al.*, 2012). After seeds germinated, four seedlings were transplanted

into sterile plastic pots. A pot experiment was designed in a completely randomized indoor experiment design. The experiment was conducted in a plastic house at Addis Ababa University. Cultivation was in 20 × 15 cm (in diameter) plastic pots filled with 3 kg soil autoclaved for 1 h. Seeds of *M. stenopetal* were planted at the rate of two/pot and then watered when necessary. Twelve microbial inoculants with greater germination potentials were used for greenhouse trial. Plants were kept in well-watered conditions and fertilized with half-strength Hoagland solution each week to obtain nutrients at a free access rate for 30 days (Magnani *et al.*, 1996). Plants were inoculated during and 7 days' post-transplantation with test strains (10^8 CFU/mL) for 30 days. After 30 days of growth, plants were either suppressed of water or were regularly watered to maintain 20% moisture. The experiment was performed with a completely randomized design and replicated three times. Plants were harvested after 2 weeks of water suppression and data on root length, shoot length, root and shoot dry biomass were recorded (Kumar *et al.*, 2016).

2.4. Field experimental design

Field experiments was conducted at the experimental farm site of Wachemo University. A fairly-level portion of land (44 m x 21 m = 924m²) was plowed, harrowed, and marked into 3.0 x 3.0 m plots separated by 1.0 m paths. The study was conducted using a completely randomized design consisting of 3 factors (3x1). The first factor was corresponded to the bacterial strains (*Pseudomonas* FB-49, *Acinetobacter* BS-27, and the mixture of both, FB-49 + BS-27), while the second factor was corresponded to control, respectively. Triplicates tests was made for each treatment as follows:

- (1) Control (without microbial inoculants) = CTRL.
- (2) Seedling inoculated with *Pseudomonas* FB-49
- (3) Seedling inoculated with *Acinetobacter* BS-27
- (4) Seedling inoculated with FB-49 +BS-27.

2.5. Method of inoculation

The Nutrient Broth (NB) broth was inoculated with fresh (24 h old) bacterial culture. The inoculated NB broth was incubated in a shaker for 48 h at 28°C followed by centrifugation at 10, 000 rpm for 10 min. The supernatant was removed and the pellet

was suspended in distilled water and the optical density (at 600 nm) was adjusted to 1. Moringa seeds were soaked for 3 h in the broth culture thus prepared and were sown in the field. A 100 mL per plant was applied as a soil drench to moringa seedlings in 3 months' interval for 12 months. Surface-sterilized non-inoculated seeds were served as control (Khan *et al.*, 2018).

2.6. Measurement of plant parameters

To avoid destructive harvesting of the plants, the plant parameters measured were plant height, trunk (stem) diameter (0.5 to 5 cm above soil level; Bowers and Turner (2001), was determined at 3rd month after transplanting (Ohsowski *et al.*, 2016). Finally, the survival rate of trees on each treatment was assessed by recording dead and alive plants at the end of the trial and the percentage of survived plants were calculated using the formula:

$$\text{The survival rate of trees} = \frac{\text{Number of survived plants}}{\text{Total number of transplanted trees}} \times 100$$

2.7. Statistical analysis

Moringa growth parameters were subjected to analysis of variance (Duncan's test) to test the significant differences between the study plots. All the results were reported as the mean $\pm$ standard deviation. Percentage analysis was done to determine the tree survival rate of each treatment. The discriminant analysis was used to classify several observations in these groups (Johnson and Wichern, 2002). Mean separations was calculated based on the mean rankings at $p \leq 0.05$ using Duncan's Multiple Range Test. All statistical analyses were performed using SAS 9.0 software package.

3. Results

3.1. In vitro Plant growth promoting traits

The experimental isolates demonstrated in vitro drought stress tolerance and enhanced growth in acacia under greenhouse conditions in previous assessments. The highest drought stress (0.64OD at 600nm) tolerance is exhibited by *Pseudomonas fluorescens* (FB-49) (Table 1).

Table 1. Some potential drought stress tolerant PGPR

Isolate code	The closest relatives	Mean $\pm$ SD 600 nm OD	Tolerance levels
FB-50	<i>Paenibacillus polymyxa</i>	0.51 $\pm$ 0.16	HT
BS-27	<i>Acinetobacter calcoaceticus</i>	0.59 $\pm$ 0.19	HT
BS-19	<i>Pseudomonas putida</i>	0.60 $\pm$ 0.12	HT
FB-49	<i>Pseudomonas fluorescens</i>	0.64 $\pm$ 0.15	HT

HT = highly tolerant to drought

All rhizobacterial isolates produced plant growth promoting (PGP) traits between 52% and 98%. The highest trait production was ammonia production (98%), while the lowest was phosphate solubilization (52%) (Figure 2). These features are important for increasing moringa seed germination and enhancing growth parameters.

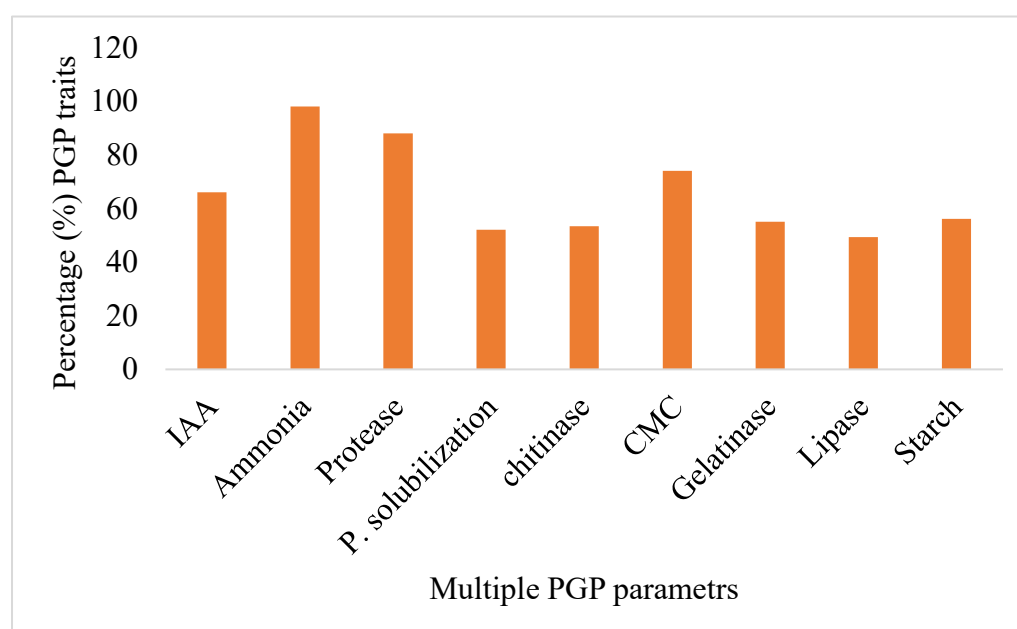


Figure 2. In vitro multiple plant growth promoting traits of potential rhizobacteria PGPR isolates also showed significant results in in vitro adaptation to salinity, acidity, temperature, and heavy metals. *P. fluorescens* showed 100% utilization of all nutritional sources tested (data not shown).

3.2. Moringa Seeds Germination Bioassay

This study resulted a remarkably significant variation in moringa seeds germination in rhizobacterial inoculated seeds as compared to the control. The highest germination percentage (92.86%) was observed in T11 which means a combined treatment (Figure 3). Moreover, T1 and T9 each equally enhance germination rate by 71.43%.

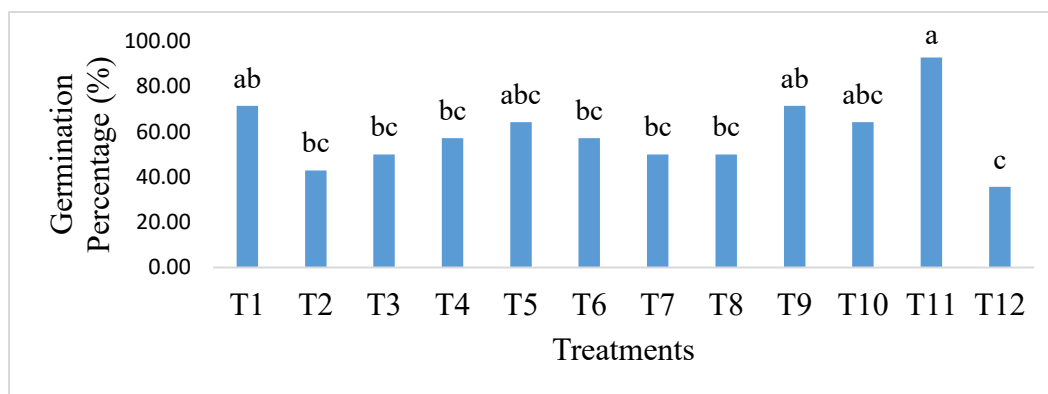


Figure 3. The effect of rhizobacteria inoculations in moringa seeds germination.

The bacterial isolates showed a potential role in moringa seeds germination bioassay with the mean difference between (2.5 – 6.5). The combined treatment exhibited better mean seed germination i.e., 6.5 followed by *P. flourencens* FB-49 i.e., 5.00 (Table 2). As indicated in Figure 4, there is a visible variation in moringa seeds germination enhancement was observed in rhizobacterial treated plates as compared to the control. In this study, *P. putida* and *P. fluorescent* were considered as potential strains in increasing acacia seed germination and vigor index.

Table 2. The mean difference in moringa seed germination assay following bacterial inoculations.

S.No.	Treatments	Treatments description	Mean difference
1	T1	<i>Pseudomonas fluorescent</i> (<i>P. fluorescent</i>)	5.00 ^{ab}
2	T2	<i>Pseudomonas putida</i> (<i>P. putida</i>)	3.00 ^{bc}
3	T3	<i>Paenibacillus polymyxa</i> (<i>P. polymyxa</i>)	3.50 ^{bc}
4	T4	<i>Acinetobacter calcoaceticus</i> (<i>A. calcoaceticus</i>)	4.00 ^{bc}
5	T5	<i>P. fluorescent</i> + <i>P. putida</i>	4.50 ^{abc}
6	T6	<i>P. fluorescent</i> + <i>P. polymyxa</i>	4.00 ^{bc}
7	T7	<i>P. fluorescent</i> + <i>A. calcoaceticus</i>	3.50 ^{bc}
8	T8	<i>P. putida</i> + <i>P. polymyxa</i>	3.50 ^{bc}
9	T9	<i>P. putida</i> + <i>A. calcoaceticus</i>	5.00 ^{ab}
10	T10	<i>P. polymyxa</i> + <i>A. calcoaceticus</i>	4.500 ^{abc}
11	T11	<i>P. fluorescent</i> + <i>P. putida</i> + <i>P. polymyxa</i> + <i>A. calcoaceticus</i>	6.50 ^a
12	T12	Control (without inoculation)	2.50 ^c



Figure 4. Moringa seeds germination potential following rhizobacterial inoculations (left) and control or non-inoculated treatments (right).

3.3. Greenhouse Trials

The greenhouse assay also showed that, inoculation of drought stress rhizobacterial isolates greatly improve the growth of moringa as compared to the control treatment. This indicates that, inoculation of potential bacteria could help moringa adaptation to higher altitude as it fasten its growth. Because the application of bacteria boosts different plant growth promoting traits and augment in moringa farming. The growth of moringa in rhizobial inoculated and control (non-inoculated) treatments were presented in Figures 5. The PGPR application enhanced shoot and root growth of the moringa plants. Therefore, the PGPR maintain the shootand root growth rates under drought stress, which leads to enhanced agricultural productivity.



Figure 5. Growth performance of moringa under greenhouse after rhizobacteria inoculations (left) and non-inoculated or control (right).

The effect of microbial inoculation treatment on growth characters of *M. stenopetala* was recorded in Table 3. Treatments of moringa with microorganisms individually or in combination exhibited highest values for both fresh and dry weights as compared control (untreated plants). The present result clearly indicated that application with mixture of inoculants (T11) gave the best results in increasing shoot height 88.1 cm/plant) followed by T2 and T4 after 60 days of planting. Similar trend is observed in root length where the highest value is in the mixed treatment (T11) 19.75 cm and followed by T4 and T7, 18.5 cm and 17.1 cm respectively.

Table 3. The microbial performance on the fresh and dry biomass in *M. stenopetala* growth under greenhouse condition after 60 days (n = 3)

Treatments	RL (cm)	SH (cm)	RFW (g)	SFW (g)	RDW (g)	SDW (g)
T1	12.35±2.62	58.45±5.52	39.25±3.13	20.05±0.64	4.09±1.38	8.95±1.91
T2	15.25±1.06	84.5±6.32	83.15±5.55	47.5±4.72	5.96±1.82	17.55±4.45
T3	13.1±0.71	78.7±5.83	94.4±6.73	46.75±5.43	7.91±1.61	18.9±0.85
T4	18.5±0.71	79.0±5.61	77.7±5.68	45.5±7.08	6.75±2.23	17.25±0.64
T5	16.25±2.47	46.1±7.17	73.6±6.22	66.65±5.42	5.57±2.49	17.9±8.91
T6	12.8±1.41	62.5±6.67	29.8±6.86	29.45±7.75	5.93±1.37	10.05±6.99
T7	17±1.41	66.0±2.12	74.95±4.12	38.4±0.84	7.0±1.27	17.85±0.49
T8	15.05±2.05	69.1±4.09	64.2±7.7	17.65±5.72	5.66±1.19	17.05±1.90
T9	9.1±4.11	27.3±8.23	25.95±7.83	43.45±7.33	3.92±0.94	3.25±4.59
T10	14.9±1.27	66.75±4.24	87.05±2.24	47.15±3.24	8.09±0.92	16.4±3.25
T11	19.75±0.49	88.15±3.89	95.95±4.19	73.09±5.29	10.32±1.73	19.78±2.35
T12	6.48±5.02	25.11±6.16	23.66±5.46	14.36±6.44	3.26±0.59	6.16±1.34

RL = Root length, SH = Shoot height, RFW = Root fresh weight, SFW = Shoot fresh weight (mean ± SD, n= 3).

3.4. Field Experimental Trials

The response of moringa seedlings to microbial inoculation is presented in Table 4. The growth of *M. stenopetala* inoculated with bacteria and their combinations were measured every two months and the result showed a significant increase in height, branch numbers, and girth of the plant compared to the control (Table 4). Change in height was one of the clear indicators of the plants in the field and showed significantly ($p < 0.05$) higher stem height on plots treated with mixed inoculation compared to the control. An integrated treatment displayed the best growth effect on the stem height 91.42 cm and 0.97 cm in stem diameter (Table 4). The maximum increase in stem height was observed from mixed treatment.

Table 4. Measurements of stem height (SH) and stem diameter (SD) (mean $\pm$ standard deviation) taken at the end of 3rd month under different treatment conditions.

Parameters	Treatments			
	FB-49	BS-27	FB-49 + BS-27	CTRL
SH (cm)	72.33 $\pm$ 1.23	85.01 $\pm$ 2.11	91.42 $\pm$ 0.87	42.53 $\pm$ 2.65
SD (cm)	0.68 $\pm$ 1.22	0.73 $\pm$ 1.58	0.97 $\pm$ 0.89	0.45 $\pm$ 2.05

At the end of third month after planting, *M. stenopetala* indicated the highest survival rate in mixed inoculation (73%) followed by FB-49 (57%) (Figure 6 and Plate 1) as compared to the control treatment (41%). The survival rate showed a pattern of mixed inoculation > FB-49 > BS-27 > CTRL, respectively for all the tree species studied.

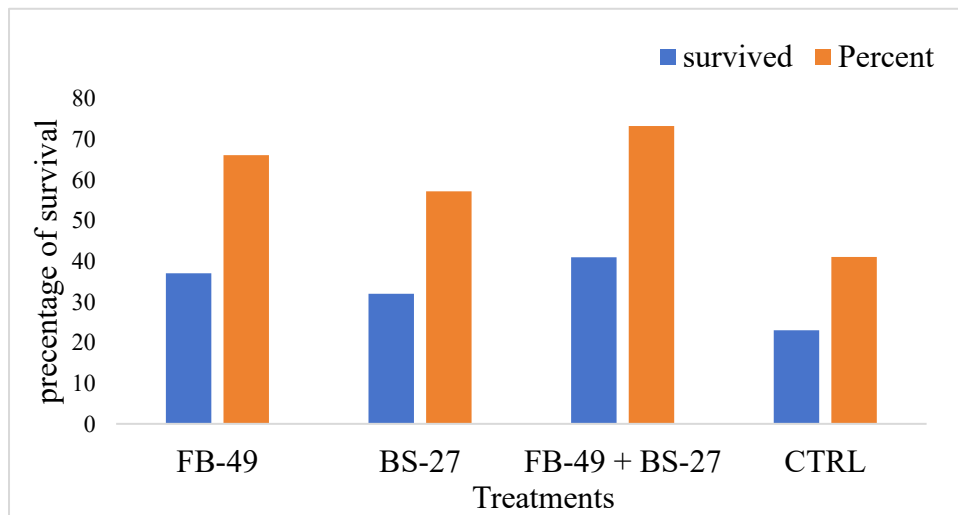


Figure 6. The total survival of transplanted *M. stenopetala* in microbial treated soil after three months growing seasons at Wachemo University experimental site.



Plate 1. Status of moringa adaptation and establishment at Wachemo University experimental site

4. Discussion

4.1. In vitro Plant Growth Promoting Traits

Since, drought disturbs the water potential and turgor of the plants sufficiently, to inhibit with normal functioning of the plant. In another experiment, *Pseudomonas* spp and *Bacillus* spp. with good plant growth promoting ability survived 40.5% PEG concentration (Praveen Kumar *et al.*, 2014). The mechanisms by which PGPR can survive and adapt to extreme drought conditions are associated with secretion of EPS (Putrie *et al.*, 2013; Susilowati *et al.*, 2018; Vurukonda *et al.*, 2016), biofilm formation (Kavamura *et al.*, 2013; Sandhya *et al.*, 2009).

Multiple growth promotion parameters played a great role in moringa adaptation at higher altitude condition and helps to maintain sustainable moringa farming and its diversification. A single rhizobacteria has been found to show multiple modes of action (Dinesh *et al.*, 2015). These features are supposed to be a crucial reason action required for the growth improvement in plants may be as a result of synergistic effect (Chaiharn and Lumyong, 2009; Dinesh *et al.*, 2015).

PGPR isolates also showed a significant result in in vitro adaptation to salinity, acidity, temperature, and heavy metals. It was observed that the extent to which growth was suppressed was directly proportional to the increasing concentration of NaCl. It seems that this high osmotic strength is due to the production of proline, glutamate, glycine, betaine, and trehalose in the cells. Na^+ accumulation declines soil porosity, soil aeration and water conductance (Diby and Harshad, 2015). Soil bacteria inhabiting salty and arid ecosystems have a potential to promote plant growth under salinity and drought conditions (Mapelli *et al.*, 2013). Drought conditions are accompanied by increase in temperature, changes in soil pH, heavy metals and salinity. Therefore, successful deployment of PGPR in stressed ecosystems depends on their ability to withstand and proliferate under adverse environments (Gopalakrishnan *et al.*, 2015). These is very important for the adaptation and utilization of many organic carbon sources available in the nature environment particularly under stressed environment. These may play a significant role in improving plant growth and yield even in hardy environments where other rhizobacteria fail to flourish (Braga *et al.*, 2018; Shazia and Asghari, 2008). Nutritional metabolism by microbial species is essential in determining the functionality of that particular environment (Deng *et al.*, 2011).

4.2. Moringa Seed Germination Bioassay

The drought stress tolerant bacteria could improve the germination speed and play a role in breaking seeds dormancy of moringa. These isolates would have triggered the activity of specific enzymes that promoted early germination such as amylase, which has brought an increase in the availability of starch assimilation (Nezarat and Gholami, 2009). In another study, the highest seed germination percentage of *Eucalyptus grandis* was caused when inoculated with *P. fulva* compared to the control treatment (Mafia *et al.*, 2009). Higher germination rate of 62.5% is reported in mycorrhiza treated moringa tree (Knopfl *et al.*, 2013).

4.3. Greenhouse and Field Performance

PGPR plays an important role in providing resistance and adaptation of plants to drought stresses and has the significant role in deciphering the future food security problems. The application of *P. fluorescens* and *P. putida* results in the better growth, and alleviation of drought stress. Whereas co-inoculations results the maximum performance in moringa growth under greenhouse trails. PGPR increase crop yields, soil fertility and have the potential to contribute to sustainable agriculture because they increase the systemic tolerance to stress (García-Fraile *et al.*, 2015). Microorganisms are essential to increase the exploration potential of roots, and improve nutrient and water uptake, and as physiological parameters that enable plants to tolerate adverse environmental stresses, increase crop yields, and improve quality (Lim and Kim, 2013).

Salah *et al.* (2020) reported a comparable result (79 cm) increment in *M. oleifera* upon mixed microbial application. Also, mixture treatment gave the highest values of fresh root and shoot weight (g/plant) recorded 95.95 g and 73.09 g respectively. The present finding indicated higher fresh weight as compared to 18.2 g/plant in *M. oleifera* reported by salah *et al.* (2020). The biomass increases in microbial treated moringa is possibly a result of the production of growth-promoting substances and N₂ fixation. It is clear that Table 3 indicated the effect of microbial treatments on dry weight showed that the mixture treatment gave the highest SDW and RDW values. A 19.78 g shoot dry weight is observed in combined treated moringa followed by 18.9 g in treatment (T3). The lowest shoot dry weight is found in treatment (T9) even which is lower than the control 6.16 g. Similarly, Salah *et al.* (2020) presented a 5.03 g of dry weight in *M. oleifera* as compared to the control (1.29 g).

The maximum increase in stem height could be due to the inoculation of bacteria that serve mainly as a booster for plant growth during its initial life stages and a synergistic effect may occur. Moreover, other soil microbes such as native rhizobia may interact with the legume trees. Also, inoculants can play an essential role in nutrient uptake and therefore enhance the biomass promotion in plants (Souza *et al.*, 2015). Knopfl *et al.* (2013) indicated that *M. stenopetala* growth characteristics in soil and mycorrhiza inoculum had influence on plant height (18-32 cm) and basal stem diameter 6 - 9 mm diameter.

4.4. Survival Rate and Adaptation

The enhanced survival rates observed in this study suggest that rhizobacterial inoculation is a viable strategy for establishing moringa in highland conditions where climate change is causing habitat shifts. The use of eco-friendly rhizobacteria inoculums is the optimum method for boosting *M. stenopetala* survival rate compared to single and un-inoculated controls. Similarly, a survival rate of 60-90%, was reported on degraded desert soil after microbial inoculation and compost amendment (Bashan *et al.*, 2012). Moreover, the average survival rate of all inoculated three cactus species after 3.5 years was 76% as reported in desert soil compared to the control (2%) (Bashan *et al.*, 2000). Similarly, Bashan *et al.* (2000) reported mentioned the survival rates of mesquite amargo (*Prosopis articulata*) and yellow palo verde (*Parkinsonia microphylla*) were over 80% and the survival rate of mesquite was almost 100% after 10 months of cultivation with inoculations of *Azospirillum brasilense* and *Bacillus pumilus*, *Glomus* sp. and supplementation with compost (Bashan *et al.*, 2009).

5. Conclusion

According to the current study, rhizobacteria that encourage plant growth enhance Moringa establishment and adaption at higher altitudes. Compared to single inoculations and controls, mixed bacterial treatments improve moringa growth characteristics, biomass, and survival rates. When microorganisms are added, Moringa adapts better than the control group. As a result of this discovery, it is advised to use rhizobacteria in moringa production to diversify moringa seedlings under different agro-climatic conditions.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statement

This study did not involve human participants or animal subjects. Experimental procedures were conducted in accordance with institutional and national guidelines for research on plant materials. No ethical approval was required for this work. Neither the CITES Appendices nor the IUCN Red List of Threatened Species include *M. stenopetala*. However, the authors attest that all fieldwork and experimental research on plants, including the gathering of plant material, were carried out in accordance with applicable institutional, national, and international regulations and laws. The study was conducted in compliance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the IUCN Policy Statement on Research Involving Species at Risk of Extinction.

Data availability statement

Data on bacterial isolates used in this study are deposited in Gene Bank database (NCBI)

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