ISOLATION, CHARACTERIZATION AND EVALUATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM TOMATO RHIZOSPHERE FROM DIFFERENT PARTS OF ETHIOPIA.

PHD DISSERTATION

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

APRIL 2024

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DEREJE HAILE SEMERE

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ADVISORS' APPROVAL SHEET

This is to certify that the dissertation entitled "***Isolation, Characterization and Evaluation of Potential PGPRs from Tomato Rhizosphere from Different Parts of Ethiopia***" submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy (PhD)** with specialization in Plant Biotechnology to the graduate program of the School of Plant and Horticultural Science and has been carried out by **Dereje Haile Semere ID. No. PhD/PlBioT/04/10**, under our supervision. Therefore, we recommend that the student has fulfilled the requirement and hence herby can submit the dissertation to the department.

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   Name of Major advisor

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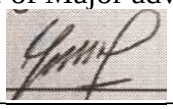
   Name of Major advisor

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BIOGRAPHICAL SKETCH

The author has born on August 25/1986 in Ginager, North Shewa Zone, Amhara regional state, Ethiopia. He attended elementary and secondary school at Ginager primary and secondary schools respectively from 1994 to 2003. He then went to Hailemariam Mamo Comprehensive Secondary and Preparatory School at Debire Birhan from 2004 to 2005 and attended his Secondary and Preparatory School. He took the Ethiopian Higher Education Entrance Examination in 2005 and joined Jimma University in 2006. He obtained BEd. degree in 2009. Then he joined Hawassa University Biology Department as Graduating Assistant (GA-I) and worked for one year. Then he joined Haramaya University to study his Master of Science in Biotechnology from 2010 to 2012. After he completed his study from Haramaya University, he rejoined Hawassa University and served as lecturer. He started his PhD study in Plant Biotechnology under Plant and Horticultural Science School, Hawassa University in 2018 as an end result of which this dissertation has been submitted.

List of Acronyms and abbreviations

AlP	$Al(PO_4)$ or aluminium phosphate
AmP	$(NH_4)_3PO_4$ or ammonium phosphate
ANOVA	analysis of variance
BLAST	basic local alignment search tool
BM	bone meal
CSA	Central Statistics Agency
DI	diseases incidence
FeP	$Fe(PO_4)$ or Ferrous or iron phosphate
GHG	greenhouse gas
HD	halo zone diameter
HGIC	Home and Garden Information Centre
HMWC	high molecular weight carbon
IGS	intergenic spacer
IPDM	Inoculated Plant Dry Matter
IPM	Integrated pest management
K	Koka
LMWC	low molecular weight carbon
LSD	least significant difference
Mk	Meki
N_2	Dinitrogen
NBWRA	Nile Basin Water Resource Atlas
NCBI	national centre for biotechnology information
NPK	nitrogen, phosphorous and potassium mixed fertilizer
NPS	nitrogen, phosphorous and sulfur mixed fertilizer
NPSB	blended fertilizer containing nitrogen, phosphorous, sulfur and boron

P	phosphorous
PBSE	phosphate based symbiotic effectiveness
PCR	polymerase chain reaction
PGPR	plant growth promoting rhizobacteria
PSB	phosphate solubilizing bacteria
PSM	phosphate solubilizing microorganisms
PVK	Pikovskiya's medium
RCBD	randomised complete block design
RDW	root dry weight
SE	symbiotic effectiveness
SI	solubilization index
TCP	$Ca_3(PO_4)_2$ or Tricalcium phosphate
YEMA	yeast extract mannitol agar
Z	Ziway

ABSTRACT

Rhizobacteria inhabit and colonize the plant root-zone and play a significant role in soil quality, health, biodiversity, and productivity. Rhizobacteria that possess beneficial traits in plant growth-promotion and disease protection are called plant growth-promoting rhizobacteria (PGPR). Phosphate solubilizing bacteria (PSB) are the known plant growth-promoters with their multidimensional benefits in plant nutrient access, stress alleviation, phytohormone production, and broad host-range interaction, among others. They improve plants' physiological processes to grow well and produce quality yield. Therefore, exploring and developing potential or competent plant growth-promoter is required to improve soil fertility, farm production, and yield quality with a sustainable and eco-friendly approach. A huge diversity of soil microbes capable of P-solubilization and other plant growth-promoting attributes have been reported so far and remain to be explored. However, local isolation, strain identification, and host-specific evaluation are helpful for better farm production, indigenous competition, and sustainable environmental health. Multistage screening procedures (lab screening, greenhouse trials, and field evaluation) help to select efficient and competent strains. Each stage of evaluation possibly confers the different dimensional attributes of the candidate strain and then empirically strengthens the screening technique. This project aimed to screen efficient PSB strains and develop competent biofertilizers and plant growth-promotors with multidimensional benefits possibly applicable for tomato and other crop production at Koka, Meki, and Ziway Zuria. The project started by assessing the overall tomato production system, agrochemicals, and biofertilizer practices in these specific sites using randomly selected smallholder farmers. Accordingly, smallholder farmers in the study sites produced different crops, including tomatoes, in both seasons under repeated agrochemical practices. Sample soils were collected from different sites, including the tomato rhizosphere at Koka, Meki, and Ziway Zuria, as well as the natural forest soil from Wendogenet and Yirgalem Zuria. Then the experiment proceeded in the laboratory by screening potential PSB strains from collected soil samples using selective media and other preliminary screening techniques. The top 10 PSB isolates were selected and then further evaluated under different conditions. Their P dissolving ability was evaluated in liquid medium from different sources and the amount of dissolved P concentration quantified, plant growth-promoting traits verified, representative biochemical tests conducted, molecular characters (sequence of the 16S rDNA and IGS region between 16S and 23S) analysed and taxonomy identified, antagonistic effects, N_2-fixing ability, symbiotic interactions, and host

range were evaluated accordingly. PSB and possible P-substrates dual inoculation impact on tomato growth and yield were evaluated both at a greenhouse and open field levels, while a host range symbiotic interaction trial was conducted at field conditions using 5 different crops (maize, wheat, faba bean, kidney bean, and onion). From each experimental unit, various parameters were analysed against each PSB strain. During screening in the lab for instance, upon 8 days of incubation on PVK agar plate, Mk-1-25 and K-10-41 strains recorded a higher solubilization index (3.1 and 3.0, respectively), while 5 days of incubation in PVK broth resulted in a growing medium pH change where K-10-41 and K-10-27 significantly lowered the pH to 4.02 and 4.12, respectively. Inoculation in modified PVK broth with different P-substrates (iron phosphate, aluminium phosphate, and bone meal) and incubation for 10 days resulted in a substantial medium pH change and dissolved P. Accordingly, strains from the Koka site dissolved the highest overall mean P concentrations (260.83, 260.38, and 241.91 µg/ml by K-1-29, K-10-41, and K-10-27, respectively) and lower medium pH (4.93 and 4.95 by K-10-27 and K-10-41, respectively). Likewise, Z-12-20 was isolated from Ziway Zuria and found to be a competent strain in dissolving an average of 223.52 µg/ml P and reducing the pH to 4.98 upon 10 days of incubation. Among P-substrates, the highest dissolved P (253.46 µg/ml) was obtained from TCP; nevertheless, the rest of the substrates recorded comparable concentrations. Furthermore, these potential 10 PSB isolates were characterized (morpho-biochemically and molecularly) and taxonomically identified. As a result, they placed to Bacillus, Priestia and Burkholderia species (i.e., K-1-29, Mk-20-7, and Mk-20-20 belong to Priestia megaterium, while K-10-27 and Z-12-20 belong to Bacillus subtilis; Mk-1-25 and Z-13-4 Bacillus halotolerance; K-10-41 Bacillus velezensis, Mk-13-16 belongs to Bacillus amyloliquefaciens, and Z-1-16 belongs to Burkholderia cenocepacia). These strains are highly studied and repeatedly cited by different scholars for their plant growth-promoting and biocontrol roles. To evaluate their symbiotic effectiveness and plant assay responses, the current PSB strains were transferred and inoculated to tomato under greenhouse conditions. So as to improve their interaction and responses, all of them were co-inoculated with six possible P sources (TCP, BM, FeP, AlP, DAP fertilizer, and compost) independently. Accordingly, strains inoculation significantly improved plant growth parameters: shortened tomato germination, shoot length (highest 108.19, 106.43, and 101.81 cm by Z-12-20, Mk-1-25, and K-10-41), leaf parameters (substantially improved by Mk-1-25 and K-1-29), branch and node number, floral parameters (number, cluster, bud), fruit parameters (number, weight, marketability) (on average 6.1 and 5.95 fruits per plant were harvested from K-1-29 and K-10-27, respectively,

158.7 and 149.2 g fruits collected from Mk-20-20 and Z-1-16 inoculation, respectively), root length, root fresh and dry weight substantially were promoted by Z-12-20, shoot fresh and dry weight were significantly enhanced by Mk-1-25 and Z-12-20. Moreover, the overall highest phosphate-based symbiotic effectiveness was recorded by Mk-1-25 (PBSE%=176), followed by Z-12-20 and K-1-29 (PBSE%=144). Among the added P substrates, compost induced the symbiotic interaction, which then resulted in enhanced tomato vegetative growth, fruit number (6.03), and biomass, while AlP and AmP promoted fruit weight (collected on average of 156.73 and 149.61 g, respectively). Having these encouraging and positive responses/results from laboratory and greenhouse trials, the strains were further evaluated at open field conditions using tomato and other host crops. This is because, the in vitro solubilization and beneficial response of the strains might not be appropriately reflected and related to their effects at field level. Similar to greenhouse experiment, tomato-field trial was conducted under dual-inoculation of PSB and possible external P substrates (BM, compost, DAP fertilizer, and a 50% rate mixture of DAP and compost). This synergetic inoculation significantly improved the tomato's growth, development, and yield parameters over uninoculated (control) group. For instance, elevated average shoot length (67.2 cm recorded by Mk-20-7), branch and leaf development (Z-12-20 and Mk-1-25), floral development (K-1-29, Mk-1-25, Mk-20-20, and K-10-41), and fruit parameters (average highest total fruit number: 21.87, 21.82, 21.31, and 20.69 by K-10-41, K-10-27, K-1-29, and Mk-20-20, respectively) and highest fruit weight: 2821.6, 2793.3, and 2780.53 g harvested by K-10-41, K-1-29, and Mk-20-7, inoculation respectively). From the collected total fruits, a greater average number of marketable fruits were obtained from the inoculation of Mk-20-7 (10.44), K-10-41 (10.42), and K-1-29 (10.0 fruits per plant). However, because of strong biological competitors (birds, porcupines, and hyena), blossom end rot and early harvesting, a substantial number of unmarketable fruits were collected from the inoculation of K-10-27 (12.64) and Mk-13-16 (11.44). Compost application improved tomato-bacterial symbiotic interaction and significantly promoted tomato early vegetative growth, while a 50% rate mixture of DAP and compost demonstrated substantial responses at late growth period and resulted in highest tomato shoot length (67.39 cm) and fruit yield (on average 63.06 fruits with a gross weight of 2617.39 g). Similarly, the addition of bone meal enhanced fruit yield (total fruit number (62.82), quality (larger fruit with 10 cm length), and marketability (9.67 healthy fruits per plant)). To strengthen the screening processes and to promote farm practices, the candidate strains were inoculated to different crops to check their symbiotic interaction/response and growth promotion efficiencies at open field. Accordingly, maize,

wheat, onion, faba, and kidney bean were inoculated, and agronomy parameters were evaluated. Because of biological competitors' attacks, grain yield was excluded from the analysis for most of these crops. Among the 10 PSB inoculants, Mk-20-20, Z-12-20, and Z-13-4 significantly induced maize shoot length, whereas leaf number, cob number, and shoot fresh and dry weight were enhanced by inoculation of Z-12-20 and Z-13-4. Similarly, wheat inoculation with Z-1-16 promoted shoot length, tiller, and leaf development; Z-12-20 enhanced wheat flower development; and Mk-20-7 improved wheat biomass yield. Onion inoculation with Mk-20-20 increased shoot length (53.45 cm), shoot fresh weight (44.11 g), Mk-1-25 improved shoot length, leaf number, shoot fresh weight, bulb length, and Z-12-20 induced bulb diameter and bulb weight, while K-10-41 (which was one of the top promotors to tomato and legumes) showed the poorest interaction and limited response against onion. Inoculation of Mk-1-25, Z-1-16, Z-12-20, K-10-41, and K-1-29 promoted faba bean and kidney bean growth and biomass yield. Generally, hierarchical procedures (laboratory-to-field work) were conducted to screen these potential biological growth-promotors. In conclusion, 10 competent PSB stains were selected and evaluated under various conditions including plant beneficial traits, substrate, and host crop preference. Consequently, the current strains have shown positive and encouraging plant-benefiting traits and responses on tomato and other crops especially, K-1-29, K-10-27, K-10-41, Z-12-20, Mk-20-7, and Mk-1-25, can be developed and recommended as biotechnological farm-inputs as potential biofertilizers and PGPR. Besides screening efficient strains, it is critical to apply other supplementals (possible P-substrates) for fruitful symbiotic effectiveness and improved production. Moreover, the future perspective should focus on conducting site-specific field trials, exploring more potential strains, and studying the detailed molecular and physiological symbiotic interactions and responses of the strains to develop competent bioinoculants that are cheap, affordable, ecofriendly, and sustain production especially for smallholder farmers.

Key words: *Biofertilizer, PGPR, PSB, sustainable, smallholder, tomato*

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DECLARATION

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Dereje Haile Semere

April 2024

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LIMITATIONS AND ENCOUNTERED CHALLENGES

As a first batch in the Plant Biotechnology PhD program and the nature of the study program as well as the need for functional high-tech laboratory facilities, we faced different challenges. In brief, the courses in the curriculum were not properly delivered in the regular schedule, the sponsorship and financial allocation of NORAD-IV project was not well defined and terminated at the early stage of our research progress without allocating any budget reservoir or without fulfilling the required laboratory resources and facilities, the program was poorly mentored and manged by the school and CoA, the CoA and HU higher administrators took extended time and chain to give poor responses and frequently they failed to give definite decisions and responses in time, COVID-19 outbreak, and we the students were not prepared plan-B to face such kind of constraints. Boldly, pure two academic years lost because of these submissive administrators and poor managements. Beyond the financial and administrative challenges, laboratory facilities, consumables, market unavailability of chemicals and detergents, the exponential price and market inflation, security issue, Covid-19 outbreak, unpredictable environmental and weather conditions, strong biological competitors (birds, porcupine, hyena, ape and rat) and attack were the major challenges imped the study and research on time completion.

CHAPTER ONE

1. BACKGROUND INFORMATION

1.1 GENERAL INTRODUCTION

Population is increasing at the maximum rate, and sufficient food production is at risk (Shah *et al*., 2021; Verma, 2019). This indicates the increasing challenge scale of agricultural production (Abebe *et al*., 2022), which is the core sector of the world food and economy. Smallholder agroecosystems play a significant role in food security, providing more than 50% of global food production, but nowadays, the sector is challenged with shrinking arable lands, dwindling world economies, and unpredictable climate change (Koskey *et al*., 2021). Agricultural production is usually low under traditional farming systems that follow a similar cultivation system from season to season, dependent on rainfall, and compounded with low soil fertility management and maintenance (Regassa and Elias, 2022), a lack of proper data, analysis, and forecasting experience of the possible climate challenges, among others. The problems deepened in smallholder farmers who are bound by economic limitations (Kidane *et al*., 2022), low or no exposure to farm technologies, agricultural inputs (Abebe *et al*., 2022), poor motivation, and a lack of awareness (Kifle *et al*., 2022).

In Ethiopia, agricultural sector is the main livelihood shareholder for more than 80% of the people as a source of food and income generation (CSA, 2020; Tamru *et al*., 2016). Most Ethiopian farmers are smallholders who own small, fragmented land and practice a traditional farming system (Abera *et al*., 2020). Ethiopian agriculture is broadly dominated by the traditional production system (Simtowe, 2015), resulting in low production, poor resource management/conservation, and food insufficiency. It is manifested by poor adoption, management, and application of low-cost technologies to overcome financial limitations and limited technical skills (Kifle *et al.*, 2022). Almost all smallholder farmers plough their lands in the same manner, grow similar seasonal crops from year to year, and cultivate once a year, which is below its potential (Alemayehu *et al*., 2010). They face climate challenges, soil (high P-fixing, erosion, moisture stress and salinity) and environmental challenges in line with weak institutional support, lack of adequate trainings on proper use of agrochemicals, irrigation and diseases management (Gemechis, 2017). Most research findings, new technologies, government policies and appropriate technical supports are not properly and timely introduced and delivered to farmers. For instance, NGOs, Research Centre and media

(TV/Radio) were found poor source of information (5.66, 1.89 and 0.63%, respectively) in Ziway Dugda area about vegetables production management and inputs (Mersha and Sime, 2022).

Vegetable production is low in Ethiopia, which covers about 1.3% of cropland and accounts for about 5.7% of production in the meher (main rainy) season (CSA, 2021). Tomato (*Lycopersicon esculentum* Mill.) is one of the top-grown, marketable, and nutrient-rich (Sachdev *et al*., 2023) vegetables around the world, with a substantial production increment. Asia took the maximum share (62%), and Africa took the least (12%) portion of world tomato production (Sotelo-Cardona *et al.*, 2021). In Ethiopia, tomato production has no extended history like other fruits and vegetables. It was introduced in the 1940's (Samuel *et al*., 2009 *as cited in* Gemechis, 2017), and now it is one of the most widely grown and nutritious vegetables (Abera *et al*., 2020; Bahilu *et al*., 2020) in major vegetable-producing areas (Gemechu and Beyene, 2019). It gains popularity among smallholder farmers because of extra income generation (Gemechis, 2017) and local consumption. Tomato production requires optimum growing conditions, and it is known for intensive agrochemical consumption (for nutrient supplements, pest and disease control, fruit preservation). It is one of the strategic commodities prioritized by the Ethiopian government to play an important role in poverty mitigation as well as food insecurity programs (Asfaw, 2021). Its production expanded (CSA, 2020; CSA, 2021) along with national agricultural provisions as a potential cash crop (Weldegiorgis *et al*., 2018). For example, 28,365 ton tomato was produced from 6,299 hectares land in 2016 and 35,344.53 ton from 8,995 hectares (FAOSTAT, 2024).

The majority of Ethiopian arable land needs fertility maintenance and fertilizer supplements. The soil lost its fertility due to erosion, leaching, frequent cultivation (Regassa and Elias, 2022), contamination, pollution, etc. Therefore, applying the necessary agricultural inputs and nutrients, along with proper farm management, is strongly recommended to improve agricultural production. Nitrogen and phosphorous are the most essential nutrients for plants, subsequently fertilizer application is increasing (Abebe *et al*., 2022). However, most of the farmers applied fertilizers below the required rate (Simtowe, 2015). The main reasons fall under the economic barrier and lack of sufficient supply. Farmers unable to purchase fundamental agricultural inputs, including fertilizers (Fikadu, 2022; Muleta, 2018), high-quality varieties, pesticides, herbicides, and other agricultural inputs. Moreover, fertilizer importation, transportation, and distribution delays affect application timing and rate.

It is necessary to promote the agricultural sector with up-to-date agricultural technologies and inputs for production intervention. These include the application of improved varieties, affordable agri-inputs, knowledge-based farming systems, agricultural innovations, remote sensing, specific soil and crop-based agrochemical input, crop rotation, inter-cropping, mitigating climate change (Kifle *et al*., 2022), enhancing irrigation, harvesting, transporting, and storage mechanisms. Kidane *et al.* (2022) demonstrated that climatic factors, soil fertility issues, and crop disease infestations are the major driving forces for smallholder farmers to adopt improved agricultural technologies. There are different types of agrochemicals in Ethiopia that are legally registered to be distributed or permitted for market access, including fertilizers, pesticides, herbicides, and improved seeds. Fertilizer is one of the most significant inputs for the agricultural sector (Regassa and Elias, 2022; Simtowe, 2015). On top of the environmental impacts, chemical fertilizer is expensive, with intermittent price increases along with the rise in production costs and the depletion of natural resources (Abebe *et al*., 2022). For example, the declining natural phosphate reservoir (ore) leads to a shift in manufacturers to the most energy- and cost-demanding phosphate sources; consequently, the fertilizer price will be elevated (Sharon *et al.,* 2016). Fertilizer needs special care, control, and follow-up to confirm the standard during manufacturing, import, handling, and storage (Federal Negarit Gazeta, 1998). Tracking is essential for importing, supply timing, quality, marketing, distribution, and application of fertilizers to the required quantity and rate.

Not only synthetic fertilizers but also biofertilizers (Ammar *et al.,* 2023) like N_2-fixing microbes (Fikadu, 2022), PSB, and *Mycorrhiza* can be satisfactorily recommended for specific crops to improve soil nutrients and sustainable agriculture (Nosheen *et al.,* 2021). Biofertilizer is technically living, symbiotically associative, or free-living microorganisms that readily and safely convert complex organic and inorganic compounds into bioavailable nutrients so that plants uptake nutrients easily (Jehangir *et al*., 2017). The environmentally friendly approaches of biofertilizers, none toxic, easy to apply nature, as well as their great potential in sustainable agriculture, have emphasized the need to reduce, if not replace, the use of agrochemical inputs (Abebe *et al*., 2022; Raimi *et al*., 2017). They are important components of integrated soil nutrient management (Jehangir *et al*., 2017) with an advantage of pollution free and cheap price (Ammar *et al.,* 2023; FNCA, 2006). Currently, development of effective biofertilizers for different crops showed substantial progress, and bacterial-based biofertilizers are expanding (Ammar *et al.,* 2023) where nitrogen-fixers are the leading global biofertilizer market followed by P-solubilizing bacteria (Abebe *et al*., 2022). Among

biofertilizers, rhizobium constitute about 79% and PSB about 15% of global market demand (Nosheen *et al.*, 2021) and other inoculants like *Mycorrhizal* fungi make up about 7% (Mącik *et al*., 2023). The production process of biofertilizer technology is simple and requires less capital, technology, and workforce, unlike inorganic fertilizer production, which requires huge energy, a high capital base, and a significant amount of manpower. Therefore, the global market is forecasted to increase from 2.3 bill. USD to 3.9 bill. USD in 2025 with annual growth rate of 11.6% (Kumar *et al.*, 2022).

Phosphorous (P) is the second most essential nutrient for living organisms required for basic molecular synthesis, including nucleic acids and cellular and structural activities (Sharma *et al*., 2013; Hariprasad and Niranjana, 2009; Zaidi *et al*., 2009). It is essential for most life processes; consequently, no life can be sustained without phosphorous (Mitra *et al*., 2020). Plants need phosphate anions (Mohamed *et al*., 2018) for their basic physiological and cellular functions, including photosynthesis, metabolism, signal transduction, cell division, growth, and development (Kour *et al*., 2021; Mitra *et al*., 2020). No atmospheric reservoir is available for P to replenish it; therefore, soil and rock are the main stocks. Both organic and inorganic forms of phosphorus are found in soil (Chen and Liu, 2019; Zhalnina *et al*., 2018; Zaidi *et al.*, 2009), though plant availability of phosphorous is limited (Gómez-Merino and Trejo-Téllez, 2016) due to fixation and complex formation with different metallic ions (Al^{3+}, Ca^{2+}, Fe^{3+} and Zn^{2+}) depending on the type of soil (Amaresan *et al.*, 2022). Most of the worldwide soils are deficient in plant-available P (Kour *et al*., 2021). Substrate scarcity is one factor that causes plants to disrupt their metabolic activities and growth.

Fertilizer application (Nesme *et al.*, 2018) is a very good option for direct plant assimilation (Yadav and Verma, 2012); however, due to the nature of highly reactive phosphate anions, most (75-90%) of the fertilizer will be immobilized to form insoluble residues (Mitra *et al*., 2020) and switched immediately to unavailable forms for plant absorption (Mahanty *et al*., 2017; Baliah *et al*., 2016; Sharma *et al*., 2013; Zaidi *et al.*, 2009). To overcome this problem, farmers applied fertilizer in manyfold excess (Mohamed *et al*., 2018). P-fertilizer has become one of the largest markets around the globe, and it is estimated to reach maximum production by 2040, when production will decline while agricultural demand rises (Sharon *et al.*, 2016). The current global agricultural practice concentrated on non-renewable chemical fertilizers (Deepika and MubarakAli, 2020) while several researches suggested the formulation of integrated soil nutrient management. Therefore, Ethiopia, from many (economic,

environmental and sustainability) perspectives, should better focus on integrated soil fertility amendment (formulation of inorganic, organic and biological fertilizers) (Abebe *et al*., 2022).

Solitary or synergetic application of organic matter and potential microbes, along with appropriate chemical supplements, may enrich the physicochemical properties of soil, improve plant macro- and micronutrient availability, and enhance the overall plant growth and development. Microorganisms are important components of soil health, contributing directly or indirectly through beneficial or harmful activities (Giannelli *et al.,* 2023; Hariprasad and Niranjana, 2009). Broad microbial diversity and abundance are found in the fertile and functional soil zone called the rhizosphere (Kour *et al*., 2021; Planchamp *et al*., 2015). The region is rich and dynamic in root exudates (Zhalnina *et al*., 2018; Tsegaye *et al*., 2017) that attract or repel the microbiome. For example, P starvation guides plants to modify their internal (physiological) and external (basically the root-zone or environment, root architecture, and release of root exudates) arrangements to overcome the deficiency (De Zutter *et al*., 2022; Egamberdieva *et al*., 2015). Thus, as the competition escalated, the niche would be dominated by plant-benefiting microbes; in this case, plant available P accessing microbes. Willey *et al*. (2017) showed that the natural microbial environments are complex and expose microbes to many overlapping gradients of nutrients and other factors, including inhibitory substances. Moreover, agricultural microbiology would possibly indicate and analyse the comprehensive interaction of microorganisms with agriculture, where agriculture is the backbone of the world economy (Verma, 2019).

Broad-based bioinoculants could be applied as biofertilizers, growth-promoters (de Andrade *et al.,* 2023; Kirui *et al.,* 2022), and biocontrol agents in plant root ecology (Naik *et al*., 2008). In addition, they can convert toxic heavy metal residues into non-toxic complexes (bioremediation) and are used as biological reservoirs for organic molecules and nutrients (Yang *et al.*, 2023; Wan *et al*., 2020; Wei *et al.,* 2018). They improve soil fertility and plant growth with minimum cost requirements, which is an essential attribute for small-scale farm production. Phosphate solubilizing microbes (PSM), including bacteria (PSB), are known beneficial microbes (Chen and Liu, 2019) that play considerable roles in the plant rhizosphere to improve the overall plant growth and development (Figure 1.1) (Mekonennen and Kibret, 2021; Mitra *et al.,* 2020; Naik *et al*., 2008), maintain soil fertility (Mohamed *et al.,* 2018; Sharon *et al*., 2016), induce plant responses to pathogens (Yadav *et al*., 2017), reduce chemical consumption, and promote sustainable agriculture (Kour *et al*., 2021).

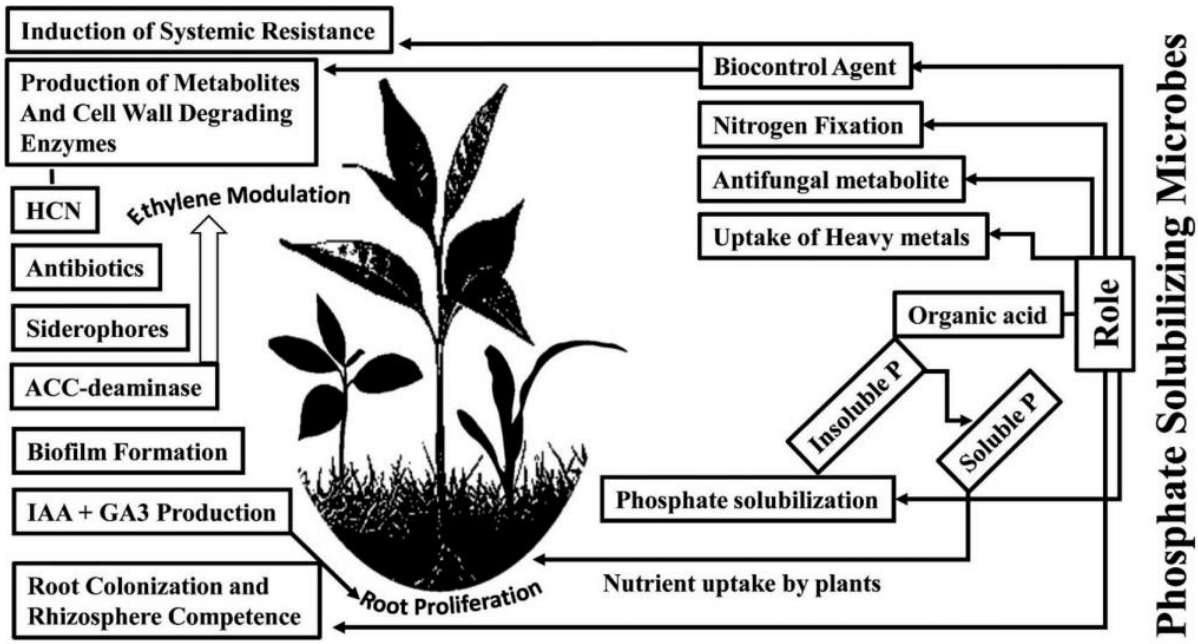


Figure 1.1: Mechanism of phosphate solubilizing microorganisms (PSM) benefit to plants *(adopted from Mitra et al. (2020*)).

Multidimensional PSB are called plant growth-promoting rhizobacteria (PGPR) (Sachdev *et al*., 2023; Zhalnina *et al*., 2018; du Jardin, 2015). Similarly, Kirui *et al.* (2022) mentioned that PSB are the known PGPR where their inoculation significantly encouraged the plant characters. PGPR are root and root-zone colonizing bacteria that induce plant growth and yield (Ammar *et al.*, 2023; Tsegaye *et al*., 2017) through P-solubilization (Amaresan *et al.*, 2022), biological N_2-fixation (Mahanty *et al.,* 2017), quorum sensing, the release of phytohormones (Verma, 2019; Vaikuntapu *et al.*, 2014; Kurabachew and Wydra, 2013), promote plant physiological growth and development, among others. Plants also benefited from them in stress alleviation, stimulation of phytohormone production (IAA, ABA, JA, ET, and secondary metabolites), induction of systemic resistance (ISR), or defence (de Andrade *et al*., 2023; Widnyana, 2018). PGPR could be able to enhance plant defence to exclude or overcome completely or in some degree by using various mechanisms such as pathogens' developmental escape, inducing physiological tolerance, antagonists, cell wall reinforcement and biochemical resistance (siderophore, antibiotics, volatile organic compounds production), lytic enzymes (cellulases, proteases, and chitinase) production (Rabbee *et al*., 2019; Verma, 2019), computing for niche, food, and root exudates. Therefore, these attractive features of PSB make it sound to isolate, characterize, and evaluate their efficiency and agroecology adaptation to develop biological inputs (inoculants) for sustainable farm production.

A huge diversity of soil microbes capable of P-solubilization and other plant growth promotion attributes (Estrada-Bonilla *et al.,* 2021; Mahanty *et al.,* 2017; Yadav *et al*., 2017) have been reported so far and remain to be explored. Numerous microorganisms (bacteria, fungi, and algae) are known for their efficient P solubilization (Amaresan *et al.,* 2022; Kour *et al*., 2021; Baliah *et al*., 2016; Sharma *et al*., 2013) and soil maintenance (Naik *et al*., 2008). They are rich in the rhizosphere, though they are found inside the root of the host plant (Yadav *et al*., 2017; Ahemad and Kibret, 2014). They are ubiquitous in number and differ from soil to soil; however, bacteria are the most distributed organisms in the biosphere (Giannelli *et al*., 2023). The most familiar efficient P-solubilizer genera are *Bacillus, Pseudomonas, Rhizobium, Agrobacterium, Flavobacterium, Penicillium, Aspergillus, Acinetobacter*, *Actinomycetes*, and *Arbuscular mycorrhiza* (Kirui *et al*., 2022; Kour *et al*., 2021; Kalayu, 2019; Alori *et al.,* 2017; Sharon *et al*., 2016; Singh and Jha, 2015). To solubilize insoluble phosphate compounds, microbes used different mechanisms (Figure 1.2) (Sharon *et al*., 2016; Sharma *et al*., 2013; Yadav and Verma, 2012; Zaidi *et al.,* 2009). such as the synthesis of phosphatase enzymes (alkaline and/or acidic phosphatase) (Khudhur, 2017), producing organic acids (Mitra *et al*., 2020), chelation, ion exchange, lowering the medium pH (Amaresan *et al.,* 2022; Estrada-Bonilla *et al.,* 2021), and release phosphate during substrate degradation (Mącik *et al*., 2020). Organic acids, including acetate, malate, oxalate, succinate, citrate, gluconate, ketogluconate, etc., can form complexes with metallic group, thus releasing plant-available phosphate into the soil (Zhang *et al*., 2023; Pande *et al*., 2017; Pathak *et al*., 2017).

Currently, in Ethiopia, well organized and summarized reports lack concerning PGPR associated with vegetable crops and research status (Mekonnen and Kibret, 2021). Very few strains for solitary or combined practice, either imported or locally isolated, were conducted in site-specific field trials for leguminous crops in different parts of Ethiopia (Abeje *et al.,* 2024; Alemayehu, 2020; Muleta, 2018; Argaw, 2012). Similarly, Fikadu (2022) have shown that in Ethiopia, rhizobium biofertilizers are progressively increased for pulse crops production though, there is a limitation in addressing all agro-ecologies. In addition, limited information and research reported on screening and pot trial of rhizobacterial isolates for tomato bacterial wilt biocontrol from Jimma (Lemessa and Zeller, 2007), Ambo, Awassa, Sodo and Wendogenet (Kurabachew and Wydra, 2013). Fenta and Fassil (2017) also isolated PSB from tomato rhizosphere and evaluated under greenhouse conditions. However, there has been no published work thus far in Ethiopia to develop and field-evaluate multifunctional

PSB or PGPR inoculant for tomato production, especially in the study areas. As a result, this study made an effort to screen competent strains that possibly be recommended as bioinoculants (biofertilizers and/or PGPR) for tomato and other crops at Koka, Meki, and Ziway Zuria which are known for tomato and other vegetable production as well as aggressive/repetitive agrochemical consumption. In this paper, ten potential PSB were isolated from tomato rhizosphere soils, morpho-biochemical and molecular (16S rRNA and IGS region between 16S and 23S rRNA sequence) characteristics were determined, and their strain types/taxa were identified. Finally, their plant growth-promoting effects on tomato were verified both at greenhouse and field conditions. The results from different stages (lab scale, greenhouse, and field trials) showed that these strains can dissolve phosphorus effectively and promote the growth of tomato and other plants.

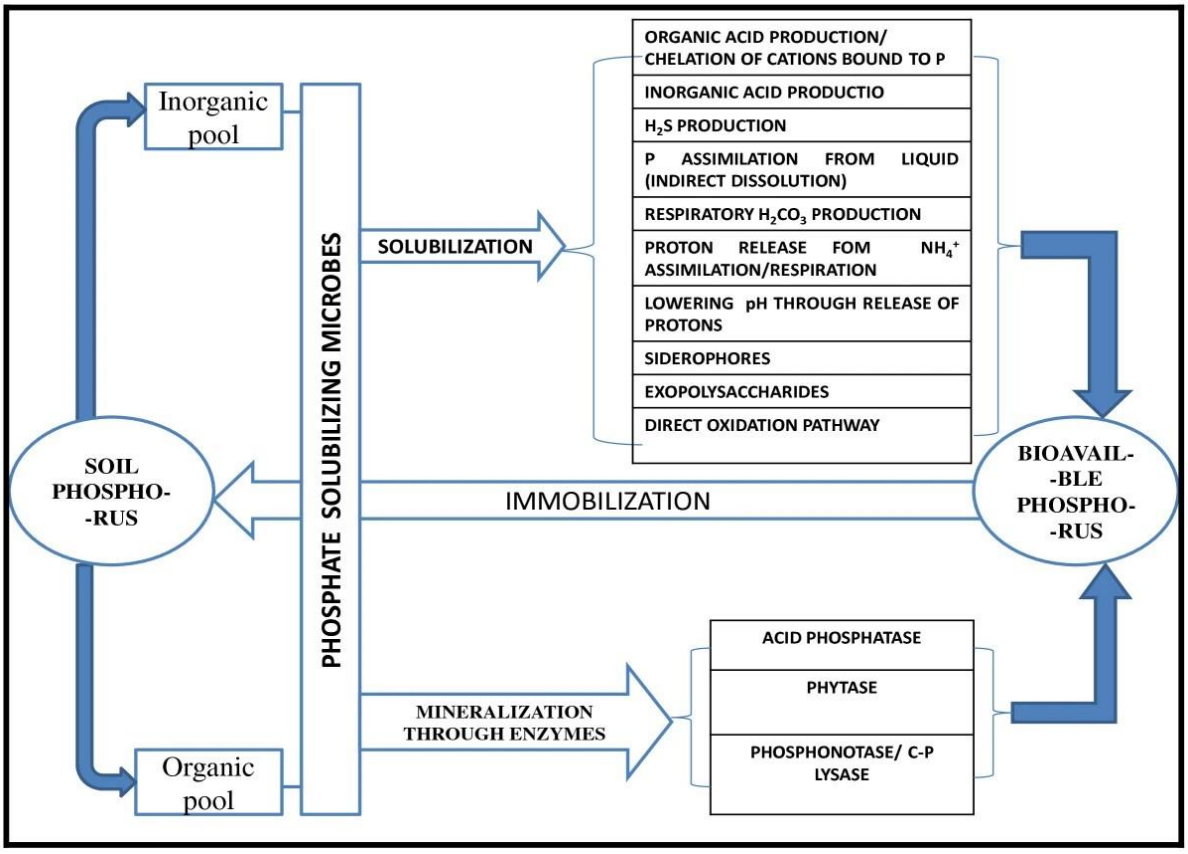


Figure 1.2: Mechanism of soil P solubilization/mineralization and immobilization by PSM (*adopted from Sharma et al., 2013*).

Compared to the economic, environmental, and social impacts, practicing bioinoculants (biofertilizers, biocontrol, and growth-promoters) has compelling advantages over chemical inputs. For instance, Dela *et al.* (2023) indicated the inevitable and reasonable cost of biofertilizers (200–240 ETB/hectare) in Ethiopia for legumes. Similarly, Alemayehu (2020)

reported that rhizobium inoculated faba bean producing smallholder farmers in Arsi zone gained extra yield benefits of 79%, 66% and 42% for faba bean, wheat and barley, respectively over un-inoculated faba bean cultivation. As indicated by Argaw (2012), co-inoculation of soya bean with *Bradyrhizobium japonicum* and *Pseudomonas spp.* resulted higher seed yield/ha (2226) and seed weight (48.3 g) over chemical fertilizer application that resulted 1912 seeds/ha and 46.7 g, respectively. Since P substrates are not easily replenished for chemical fertilizer production, it is highly recommended to dissolve and solubilize phosphate compounds in the soil. PSB serves as an eco-friendly farm input as a supplement or alternative to synthetic fertilizer (Kalayu, 2019). Ammar *et al.* (2023) and Jehangir *et al.* (2017) also indicated that biofertilizers can replace chemical fertilizers since they are easily renewable, less expensive, and help farmers to practice organic farming and fostering harmful chemicals free environment. Similarly, Nosheen *et al.* (2021) indicated that at optimum conditions PSB have a potential to access 30-50 kg P_2O_5/ha which improve production 10-20%. Lemessa and Zeller (2007) also reported that *Pseudomonas* strain APF1 and *Bacillus subtilis* strain B2G reduced tomato bacterial wilt incidence at 45 days of transplantation by 73% and 63% and at 57 days of transplantation by 53% and 52%, respectively under greenhouse trial. Tomato inoculation with *B. cereus* BC1AW and *P. putida* PP3WT significantly reduced bacterial wilt incidence 46.8% and 44.7%, respectively at greenhouse trial (Kurabachew and Wydra, 2013).

It should be clear that not only replacing or preeminent percentage contribution but also 1-5% agrochemical practice lessening through bioinoculant practice would have enormous benefits. In agreement, Ahemad and Kibret (2014) reported that 2-5% of rhizobacterial re-inoculation exert beneficial effects on plants growth. Consequently, screening competent strains and inoculating them to enrich them in these target areas' farm is strongly advisable and productive in many aspects. In line with production advantages, the final inoculant products are affordable, easy to handle, transport, and apply, especially for smallholder farmers. Besides screening efficient strains (active, competent and non-toxic) (Raimi *et al.*, 2021), it is crucial to apply other supplementals (possible P sources) for fruitful symbiotic effectiveness and improved production.

1.2 General Objective:

- To contribute to the development of biofertilizer technology and knowledge in Ethiopia and enhance productivity and fertility of tomato.

1.2.1 Specific objectives:

- Assessing farmers tomato production trends and agrochemical application experience,
- Screening potential P-solubilizing bacteria from rhizosphere soils,
- Characterize the 10 selected PSB isolates morpho-biochemically, and molecularly (DNA Sequencing) and taxonomy identification,
- Evaluate the efficiency of selected PSB strains using tomato plants at greenhouse and open field conditions,
- Crop host range test for the selected PSB strains at field level,
- Antagonistic effect evaluation against tomato late blight (*Phytophthora infestans*) using dual-inoculation on agar plate and tomato plant at greenhouse conditions.

1.3 Thesis Structure

This thesis is structured in accordance with Figure 1.3, and the overall procedural lab-to-field efforts (hierarchical activities) to screen and develop the current potential biofertilizers and plant-growth promoters are best illustrated in Figure 1.4 except for step six in the figure (product development for consumers). The thesis consists of seven chapters. **Chapter 1** (this chapter) presents the general introduction, objectives, and structure of the thesis. **Chapter 2** describes the results of the survey and onsite farm visits about the production system at Koka, Meki and Ziway Zuria, types of frequently cultivated crops, sources of land and water, agrochemical and biofertilizer utilization experiences, tomato production, and farm management. **Chapter 3** demonstrates the laboratory techniques and results in the isolation, screening, and characterization of the selected potential PSB strains. **Chapter 4** discusses a greenhouse pot experiment on tomatoes that are treated with synergetic applications of 10 selected PSB strains and possible P-sources (TCP, AlP, AmP, BM, compost, and fertilizer). **Chapter 5** illustrates the experiment conducted at open field conditions on tomatoes under co-inoculation of PSB strains and possible P substrates (compost, BM, fertilizer, and a mixture of 50% compost and fertilizer recommended rate). **Chapter 6** presents the host range trial confirming the strains symbiotic interaction/effectiveness against different crops (maize, wheat, onion, faba bean, and kidney bean) at field conditions. **Chapter 7** portrays the overall summary and conclusion (i.e., highlights the main issues and findings) of the thesis as well as future perspectives and recommendations for similar research works.

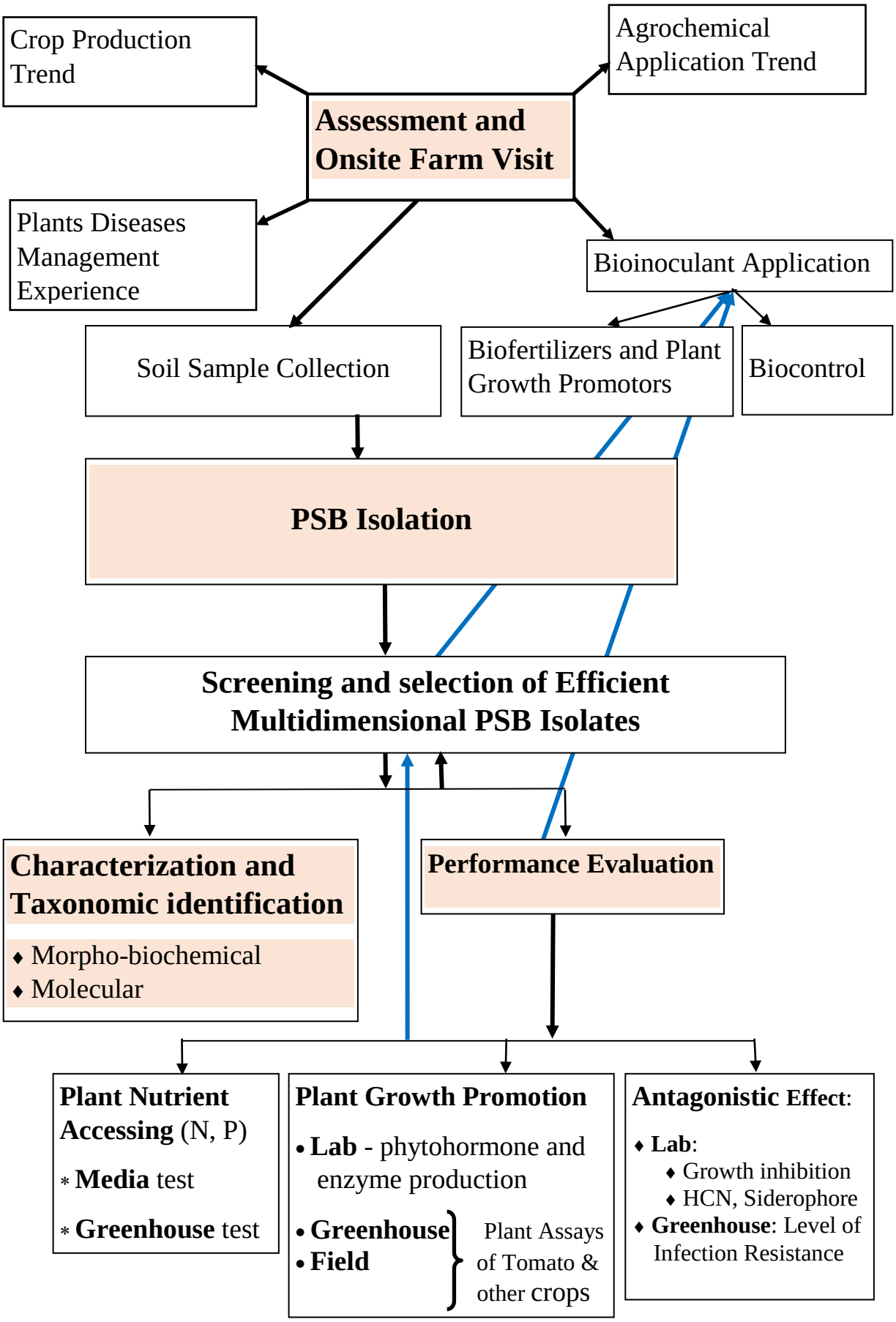


Figure 1.3: Research work and thesis flow diagram.

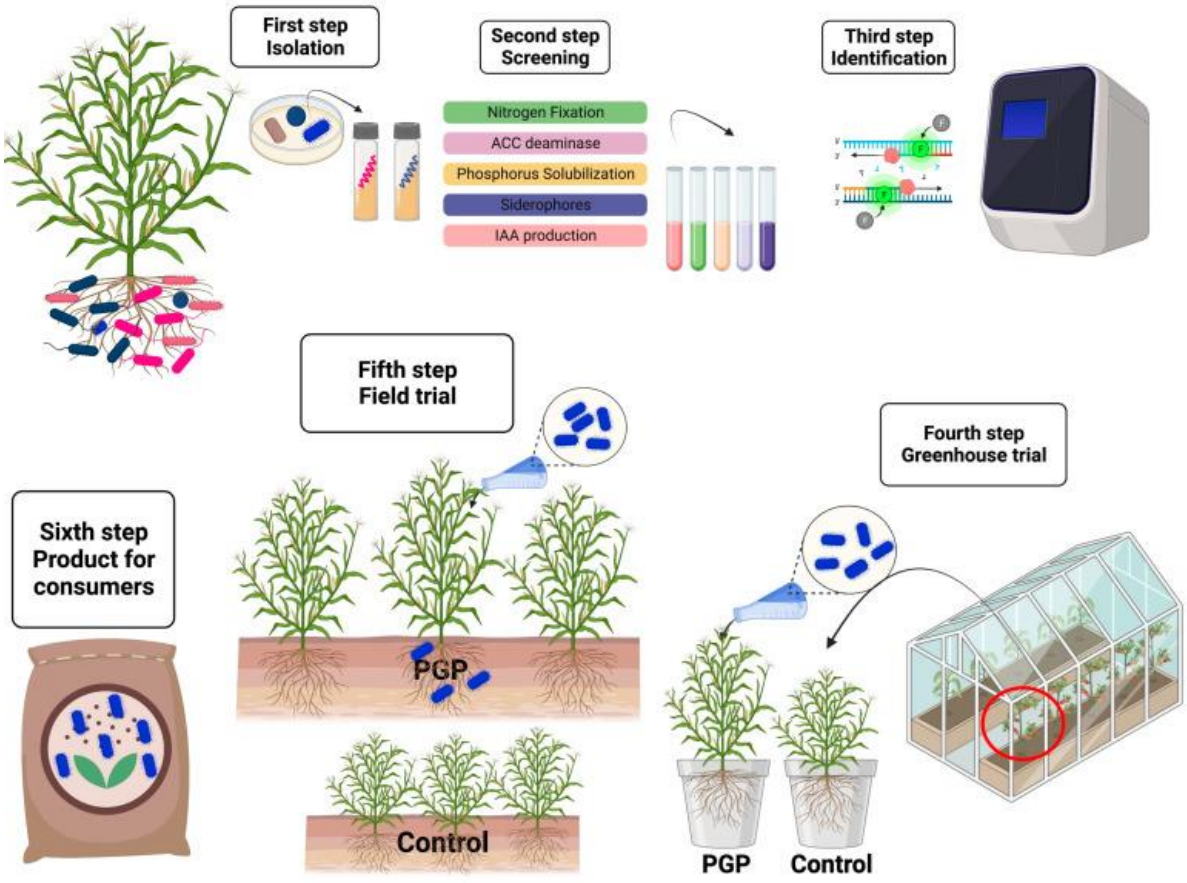


Figure 1. 4: Schematic representation of the steps required to isolate and characterize bacteria that promote plant growth (*adopted from de Andrade et al., 2023*).

Publication And Manuscripts Under Revision

Published:

- Overview of Agrochemicals Application Practices on Tomato Farm by Smallholders at Koka, Meki and Ziway, Ethiopia. Turkish Journal of Agriculture – Food Science and Technology, 10(4). Pp 781-786. https://doi.org/10.24925/turjaf.v10i4.781-786.4672.
- Tomato Production under Synergistic Application of Phosphate Solubilizing Bacteria and Phosphate Amendments. *Hindawi, Advances in Agriculture* 2023, Pp 1-14. https://doi.org/10.1155/2023/4717693.
- Phosphate Solubilizing Bacteria (PSB) Isolation from Tomato Rhizospheres at Koka Dist rict, Ethiopia. *Turkish J. of Agriculture-Food Science and Technology* 2022, 10, sp2. Pp 2892-2898. https://doi.org/10.24925/turjaf.v10isp2.2892-2898.5598.

Under review;

- Plant Growth-Promoting Rhizobacteria Improved Tomato Production (BMC Microbiology: *Submission ID:* **c247ed12-e685-4a27-ba69-e2966c44eadd**).
- Competent Phosphate solubilizing Bacteria Screened from Tomato Rhizosphere and verified under greenhouse condition (Wiley: *Submission ID*: **ID4178404**)

CHAPTER TWO

2. ASSESSMENT AND ONSITE-FARM VISIT REPORT ON TOMATO PRODUCTION TRENDS AT KOKA, MEKI, AND ZIWAY ZURIA

ABSTRACT

Tomato is one of the top-grown nutritious vegetables in Ethiopia; nevertheless, production is low compared to other tomato-growing regions. Lack of improved varieties, soil fertility maintenance, irrigation systems, farming systems, diseases, and pest management are some of the limiting factors. Smallholder farmers produce tomatoes as cash crops because of their rapid production, multiple harvests, and profitability. This survey was conducted in the Rift Valley scheme, specifically around Koka, Meki, and Ziway Zuria, which are known for vegetable production and agrochemical consumption. The survey aimed to assess small-scale farmers' experiences with tomato production, disease and infestation control, agrochemical application, and soil fertility maintenance. According to the respondents, 0.5-8-hectare farms were used for tomato production, and most (83%) of them rent the land on short-term contracts from landowners. Different parts of the tomato were infected by various pathogens; thus, leaves and fruit fungal infections caused severe damage. Since tomatoes are sensitive to stress, they require continuous follow-up, supplements, protection, proper harvesting, and handling. Farmers applied different agrochemicals; however, most of them practiced inaccurate doses, rates, and timing, which encouraged repeated sprays. Poor agricultural practices were observed at the three sites, with the poorest farm management practices observed at the Meki site. It is important to practice proper agricultural inputs, reduce hazardous chemical residues, and protect humans and the environment. Moreover, developing IPM technology is recommended for better agricultural production and sustainability. Future work should practice correct treatment with accurate dose and rate based on proper soil analysis and specific pathogen identification, incorporate competent bioinoculants to improve production, soil fertility, environmental health, and to minimize production costs. Efficient strains would be screened based on their multi-dimensional benefits in nutrient access, plant growth promotion, resistance induction, antagonistic to phytopathogens, and local environment adaptation. For fruitful outcomes, screening from the local environment and target crop rhizosphere is highly recommended.

Keywords*: agrochemical, environment, infestation, smallholder, tomato*

2.1 INTRODUCTION

In Ethiopia, two major tomato production systems are practiced: open field production (the main production approach for local market and industries either using rainfed or irrigation) and limited greenhouse production (Tsehaye *et al*., 2020) targeted for the export market. The majority of fresh tomatoes are produced by smallholder farmers (Abera *et al*., 2020). Limited garden production (Mersha and Sime, 2022; Gemechu and Beyene, 2019) using small-sized and low-yield local varieties is also practiced in different parts of the country for household consumption with a minimum commercial contribution. The average daily vegetable consumption experience in Ethiopia is low; therefore, tomato production enhancement has a positive contribution to health, nutritional content, and a balanced diet (Depenbusch *et al*., 2021) for the community. Its production and commercialization are increased from time to time (Tsehaye *et al*., 2020; Gemechis *et al*., 2012; Lemma, 2002) because of potential income generation, especially for smallholders (Bahilu *et al*., 2020). It is produced both in wet and dry seasons; however, maximal production is reported in dry seasons using irrigation (Begna, 2018). It is also stated that tomato production was positively influenced (increased berries and fruit weight per plant) by irrigation (Gemechu and Beyene, 2019).

Due to many factors, tomato production in Ethiopia is very low as compared to other tomato-growing regions (Asfaw, 2021; Bahilu *et al*., 2020; Tsehaye *et al*., 2020; Gemechis *et al*., 2012). Low agricultural productivity results in food shortages and poverty, minimizes farmers' income, and affects the country's economy (Abera *et al*., 2020). Low access to improved varieties (Gemechu and Beyene, 2019), lack of agricultural technologies (Bahilu *et al*., 2020), lack of knowledge and skill, diseases and pests, environmental-related risks, poor production management, capital limitations, fruit perishability, and market and transport constraints are some of the limiting factors (Mersha and Sime, 2022). Despite these constraints, smallholder farmers have poor efficiency in managing their resources (Weldegiorgis *et al*., 2018), including water, land, fertilizers, labour, and time. Production intervention is needed for the introduction and adoption of proper agronomic practices as well as water consumption techniques (Alemayehu *et al*., 2010). The Ethiopian agricultural minister and research institutions have a vision to benefit people engaged in agriculture and agri-related businesses with improved and appropriate technologies. Improved and appropriate agricultural technologies increase productivity and quality and sustain food security, economic development, and natural resource conservation (Tsedeke, 2007). Plenty

of agrochemicals have been applied on farms to enhance soil fertility (Simtowe, 2015), reduce pathogens (Mergia *et al*., 2021), and promote plant growth. These agrochemicals include fertilizers, pesticides (Mars and Ballantyne, 2004), fungicides, bactericides, herbicides, nutrient supplements, and growth-promoting substances.

Crop production is limited at low available nutrients in soil as a result, balanced fertilizers application is important to increase plant available nutrients, plant uptake and yield (Bhattacharyya *et al*., 2015). Similarly, application of pesticide (chemical or biological agent) helps to deters, incapacitates, kills, or otherwise discourages pests to protect plants or plant products from harmful organisms (Singh and Singh, 2019; Mars and Ballantyne, 2004; Federal Negarit Gazeta, 2010). Pesticides are safe to control pests and diseases, but most smallholder farmers are accompanied by misuse (Mergia *et al*., 2021; Mengistie, 2016), which results in health as well as environmental effects (Alemayehu *et al*., 2010). Similarly, fungicides have vast applications in agriculture to kill or prevent (Worku and Sahela, 2018) the growth of fungi and fungal spores. They are important to protect young plant parts, mature vegetables, and fruits, and to enhance seed storage nonetheless, they are dangerous to humans and the environment. Almost all agricultural inputs are imported from abroad, both by private and public/government companies. Under the current constitutional arrangements, the Ministry of Agriculture (MoA) and its counterparts in the Agriculture Bureau play a pivotal role in regulating, implementing, and monitoring pesticide policies, registration, importation, distribution, and use (Mengistie, 2016). It is necessary to assess, control, and take proper measures to minimize the negative outcomes of these products.

Currently, irrigation farming shows a progressive increment, especially in the Rift Valley scheme (NBWRA, 2021), which includes Koka, Meki, and Ziway districts. Vegetable production excelled in the area because of the conducive environment, resources, and relative availability of agricultural inputs. Tomato is one of the main vegetables known for its production and intensive agrochemical (fertilizers, pesticides, and fungicides) consumption. The relative productivity, irrigation suitability, manageability for farming, market outlet, and agroecological adaptation promote smallholder farmers to produce tomatoes in these areas. In Ethiopia, especially in the Rift Valley and Awash River basin, tomato production (Gemechis, 2017) is exponentially grown because of its high income generation (Worku and Sahela, 2018), fast growth, presence of fertile soil, water resource, conducive environment (Abera *et al*., 2020; Lemma, 2002), irrigation practice, suitable landscape, transportation access, and

geographical proximity to the main road line as well as to the capital Addis Ababa are some of the countable good opportunities for smallholder farmers to produce tomato and other vegetables in these areas.

Since tomatoes are sensitive to different stresses, vulnerable to attack, easily perishable, and fragile, they require considerable follow-up and management, starting from land preparation until final fruit handling and farm waste disposal. Therefore, this survey and on-farm visit was conducted to assess open-field tomato production trends, farmland sources, disease management practices, fertilizers, and other agricultural inputs and application experiences around Koka, Meki, and Ziway Zuria. Hitherto, investigations underlined that smallholder vegetable-producing farmers in Ziway and Meki districts are the most pesticide and fungicide users in Ethiopia (Mengistie, 2016; De Putter *et al*., 2012; Alemayehu *et al.*, 2010). Mergia *et al*. (2021) also elaborated on the intensive spraying and the related human health impacts of pesticides by small-scale vegetable producers. Having up-to-date information on the practical production system and cultivation practices on the ground is helpful in improving production, revising technology and regulation, implementing interventions, and minimizing limitations and gaps. This survey gave good feedback and responses about smallholder farmers' tomato production experience and appeal to applying bioinoculants.

2.2 METHOD OF APPROACH

2.2.1 **Study Area**

This on-farm survey was conducted in the Rift Valley scheme, specifically around Koka, Meki, and Ziway, Ethiopia (Figure 2.1). The areas are located near Koka Dam, Meki River, and Ziway Lake, with midland (Weyna dega) agroecology, a semi-arid climate with biannual rainfall, alongside mixed agriculture production (Girma and Awulachew, 2007). The main soil type is sandy loam, which experiences salinity and alkalinity problems. Sites were selected because of significant tomato production experience and geographical proximity for transportation (Mersha and Sime, 2022). Due to its cultivation expansion, nutritive value, sustainability, potential cash crop (Worku and Sahela, 2018), employment potential, market outlet, and adaptability for diverse agroecology zones (Tsedeke, 2007), tomato was selected for this research project. Tomatoes are one of the most intensive agrochemical-demanding cash crops. Most commercially productive varieties are highly sensitive to disease and vulnerable to nutrient deficiency and other abiotic stresses. Therefore, in this specific report,

the information and elaboration were limited to tomato production and agrochemical consumption, including fertilizers, fungicides, and pesticides at the three sites.

2.2.2 Data Collection Method and Tools

Primary data were collected using formal and informal survey/on-farm visit (i.e. semi-structured questionnaires, direct field observation, interview, and informal discussions). In this assessment, both cropping seasons (Meher/wet season and Belge/dry season) were included in 2012 E.C., and almost all tomato field growing processes were observed. These include land ploughing, preparation, tomato transplantation, weeding, tying or stalking, agrochemical practices, fruit harvesting, transportation, as well as agro-waste and waste/leftover disposal. Totally, 70 purposively random selected tomato producers and active tomato-cultivating farmlands at Koka, Meki and Ziway Zuria were targeted for data and sample collection. Multistage purposive random sampling procedures including target crop cultivation, area, production status, presence of farmer/s in the farm by the time of data collection, and the likes. The collected data were presented in tables, figures, and pictures, then explained and discussed in conjunction with other related works whereas the collected rhizosphere soils were subjected for PSB isolation.

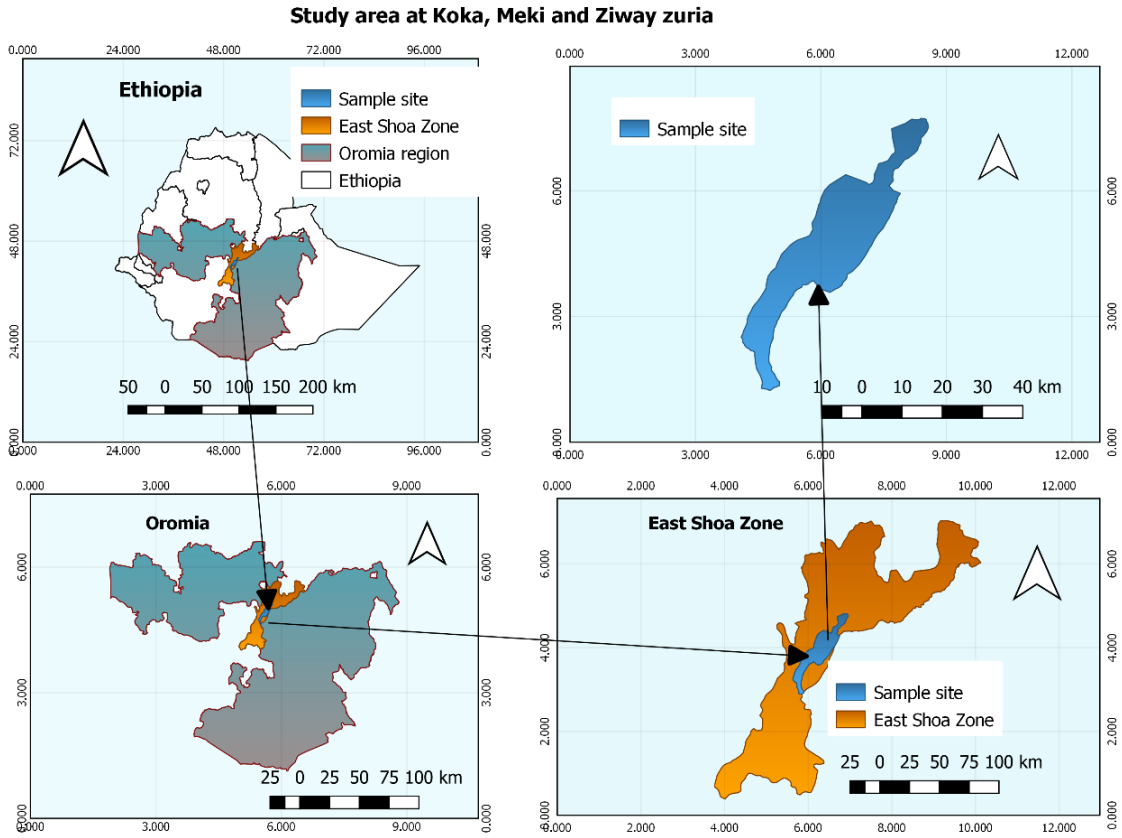


Figure 2.1: Study Area location.

2.3 RESULTS AND DISCUSSION

In this survey, 70 active tomato-producing respondents participated from the three sites (12, 43, and 15 respondents from Koka, Meki, and Ziway Zuria, respectively). Beside these formal respondents, the information was improved by informal interview and discussion with farmers, daily labourers, storekeepers and retailers, car drivers, traders, and agricultural extension experts. Most of the respondents were in the age category of 25–45, but a few participants were below 25 and above 45 years old. In agreement, Mersha and Sime (2022) indicated that younger age groups (on average 37.33 years old) engaged in tomato production while the elder groups of farmers involved in less risky production like onion, cabbage and annual crops in Ziway Dugda district. More than half (53%) of the current respondents were married; the remaining were single and divorced. The marital status of the current study figure less than the report of Asfaw (2021) that 66% of tomato producers in Asaita district were married.

Farmlands in the study sites are fundamentally used for cereal production in the major cropping (wet/meher) season and vegetable production in the dry/belge season using irrigation (De Putter *et al*., 2012). Similarly, different authors have reported that smallholders in the Central Rift Valley produced different crops and vegetables (like tomato, onion, pepper, potato, cabbage, and others) (Abera *et al*., 2020; Mengistie *et al*., 2015) in the wet (meher) season and used irrigation in the dry season (Mersha and Sime, 2022). Most of the time, smallholder farmers (basically landowners) produce crops in the rainy season (CSA, 2020) and rent the land during the dry season (Alemayehu *et al*., 2010). Generally, major crop production chronologies are listed as maize, tomato, onion, teff, wheat, and cabbage. However, these three study sites were similar in the primary production of maize; nevertheless, certain variations were observed in the list of other cereals. For example, teff and wheat were produced more in Meki and Koka; however, kidney beans, green beans, and barley were more prioritized in Ziway Zuria.

Previous reports indicated that most of smallholder farmers in Meki and Ziway districts used their land for vegetable production (Mersha and Sime, 2022; Abera *et al*., 2020; Mengistie *et al*., 2015). It was found that tomato production was practiced on different farmland sizes (0.5-8 hectares); thus, the majority of the respondents produced on $\leq$ 3 hectares (Table 2.1). Mergia *et al*. (2021) reported supportive information that small-scale farmers practiced

vegetable production up to 10 hectares in Ziway Zuria. The current finding shows that smallholder farmers practiced on wider farmlands as compared to other smallholder tomato-producing districts in Ethiopia. For example, Bahilu *et al.* (2020) reported that farmers allocate 0.2-0.25 hectares for tomato production in Silte zone; Asfaw (2021), reported smallholder farmers used 0.23–3 hectares at Asaita district; and Weldegiorgis *et al*. (2018) reported 0.13–4.75 hectares at Hintalo-Wajerat district. Most (83%) of the respondents rent farmlands in the short-term contract agreement for dry-season production. Landowners rent the land for different reasons, including lack of capital to purchase agricultural inputs (De Putter *et al*., 2012), lack of technical skills, being preoccupied by land rent extra costs, poor motivation, failure to avoid traditional agricultural practices, to avoid production risks or challenges, irrigation, pest management, and market finding (Mersha and Sime, 2022). They used a simple traditional rainfed cropping system and were satisfied with the extra income from rent in the dry season. They didn't want to invest their energy and money in irrigation farming instead, they preferred the safe rent of the land. Almost all land rent was subject to contract until the rainy season comes, most probably ending at the end of May or early June. However, Mergia *et al*. (2021) stated that 61.9% of small-scale farmers in Ziway Zuria rented for 3-5-year contracts. They can produce tomatoes and other vegetables once and/or twice per season using irrigation.

Furthermore, landowners benefited from the tenant's elevated rate of fertilizer application residuals with a plus of deep soil ploughing with tractors (Alemayehu *et al*., 2010). Since agriculture is the main sector for their livelihood, they prepared to rain-fed season crops that were basically used for household consumption and somehow for commercial crops. Once they complete harvesting, they immediately rent the land to interested producers until the beginning of next summer. With this common understanding, the two parties will sign the agreement with no boundary for the types of crops to be planted. However, tenants who were subjected to producing tomatoes inquired at least a year back history of the land, such as what kind of crop was harvested, the status of farm productivity or fertility and access to water. Tenants explained the reason that if the land continuously planted tomatoes, green peppers, and potatoes, the productivity of the new tomato planting and fertility of the land deteriorated, as well as enhanced pest infestation. This indicates a good understanding of spores and cysts hidden in the soil from the previous plants and aggressively invading the new plant, which required extra expense for treatments. In addition, if the land repeatedly produced tomatoes, which is the most exhaustive agrochemical consumer, severely, the

previous applied dosage of chemical residues would affect the new young tomato plant (phytotoxicity), and as a result, production would be lessened. HGIC (2021) also explained that tomatoes and related vegetables like potatoes and pepper should not be cultivated on the same land more than once in three years. Therefore, previous cultivation history, access to water and productivity play a pivotal role in the determination of the rent price.

Table 2.1: Land and water resource access for tomato production.

Items	Response	No. of respondents (%)
Tomato producing land in hectare	0.5 - 1.5	34.3
	1.5 - 3	40
	>3	25.7
Source of land	Rent	82.9
	Own	17.1
Tomato production per year	Once	42.9
	Twice	57.1
Source of seed and agricultural inputs	Private Sector	80
	Gov't/(Associations)	20
Access to water for irrigation	Underground	38.6
	River	17.1
	Lake and Dam	35.7
	Use only rainfall	8.57

Small-scale farmers are interested in tomato production more than other vegetables (Lemma, 2002) because of rapid growth, multiple harvests, profitability, and market access (Abera *et al*., 2020; Tsehaye *et al*., 2020). In support of this, Bahilu *et al*. (2020) stated that smallholder farmers produce tomatoes as a cash crop. Similarly, Depenbusch *et al*. (2021) demonstrated that vegetable production is considered a good source of income generation in sub-Saharan countries. In contrast to this, Mersha and Sime (2022) have found that elder tomato and onion producers forced to shift to other vegetables and annual crops production because of high-cost requirement for inputs and being risky business. In agreement to Tsehaye *et al*. (2020), tomatoes were produced both in wet and dry seasons; however, significant production was held in the dry season. Likewise, Gemechu and Beyene (2019) demonstrated a positive correlation between irrigation and tomato productivity. Similarly, Gemechis *et al*. (2012) reported that an elevated tomato production and productivity was recorded in the late February–June cycle. Several contributing factors mentioned by the farmers during the wet/rainy season including: 1) the land used for cereals (maize, teff, wheat, beans, and others) production primarily for household consumption; 2) disease infestation (excessive moisture in the wet season affects vegetable production, including tomato, due to strong diseases and insects' outbreaks that required exhaustive and repeated chemical application); 3) difficulty

in continuous supervision and follow-up of tomato plants; and, in some cases, a lack of transportation access. In support of this, Gemechis (2017), indicated that wet season tomato production affected by fungal diseases, weeds and nutrient stresses. Irrigation is basically done manually with surface water (accessed from rivers, dam or lake), or underground (Table 2.1) using generators and gravitation. Koka Dam, Lake Ziway, Meki, and Dambal Rivers were the main surface water resources. Similarly, Mersha and Sime (2022) indicated that Meki River, Ziway lake, Awash River and their catchments are the main water sources for irrigation. On the other hand, it was observed that farmers dug 8–20 m holes to access underground water. However, Weldegiorgis *et al*. (2018) reported that tomato growers using irrigation systems were found to be inefficient in resource management, including water and economic achievements.

2.3.1 Soil Fertility Maintenance

Adoption of modern inputs like improved seeds, fertilizers, insecticides, fungicides, herbicides, access to irrigation, and other resources, along with proper management, played significant roles in agricultural transformation and productivity (Tamru *et al*., 2016). Soil nutrients can be enhanced for better crop yield by applying well-composted farmyard manure, synthetic fertilizers (Lemma, 2002) and bioinoculants. Fertilizers are the main farm inputs. As indicated by Mersha and Sime (2022), excluding other costs, urea and DAP fertilizers required 5303.18 and 7291.95 ETB/ha, respectively for tomato production. Fertilizer dose and type recommendations vary (Simtowe, 2015) based on soil chemistry, crop type, method of farm practice, stage of the plant, and cropping season. Tomato production requires optimal fertilizer application at the different growth stages and phases, starting from early to late growth (flowering and fruiting) (Lemma, 2002), along with an appropriate water supply for maximal yield and profit.

In the study areas, exhaustive synthetic chemical fertilizer application was observed. All tomato-producing farmers supplemented soil fertility with different fertilizers such as urea, DAP, and other mixed nutrients, including NPK (like Agri Care), NPS, Eco Green, nutrient feeds (Table 2.2) and other supplements along with biocontrol like Sniper (water-soluble fertilizers containing humic acids for mite biocontrol and rich in P, Mg, and Br). Similarly, Girshe *et al.* (2018) indicated that chemical fertilizer is one of the main production factors yet, unwise and excess rate application practiced for tomato production in Central Rift valley,

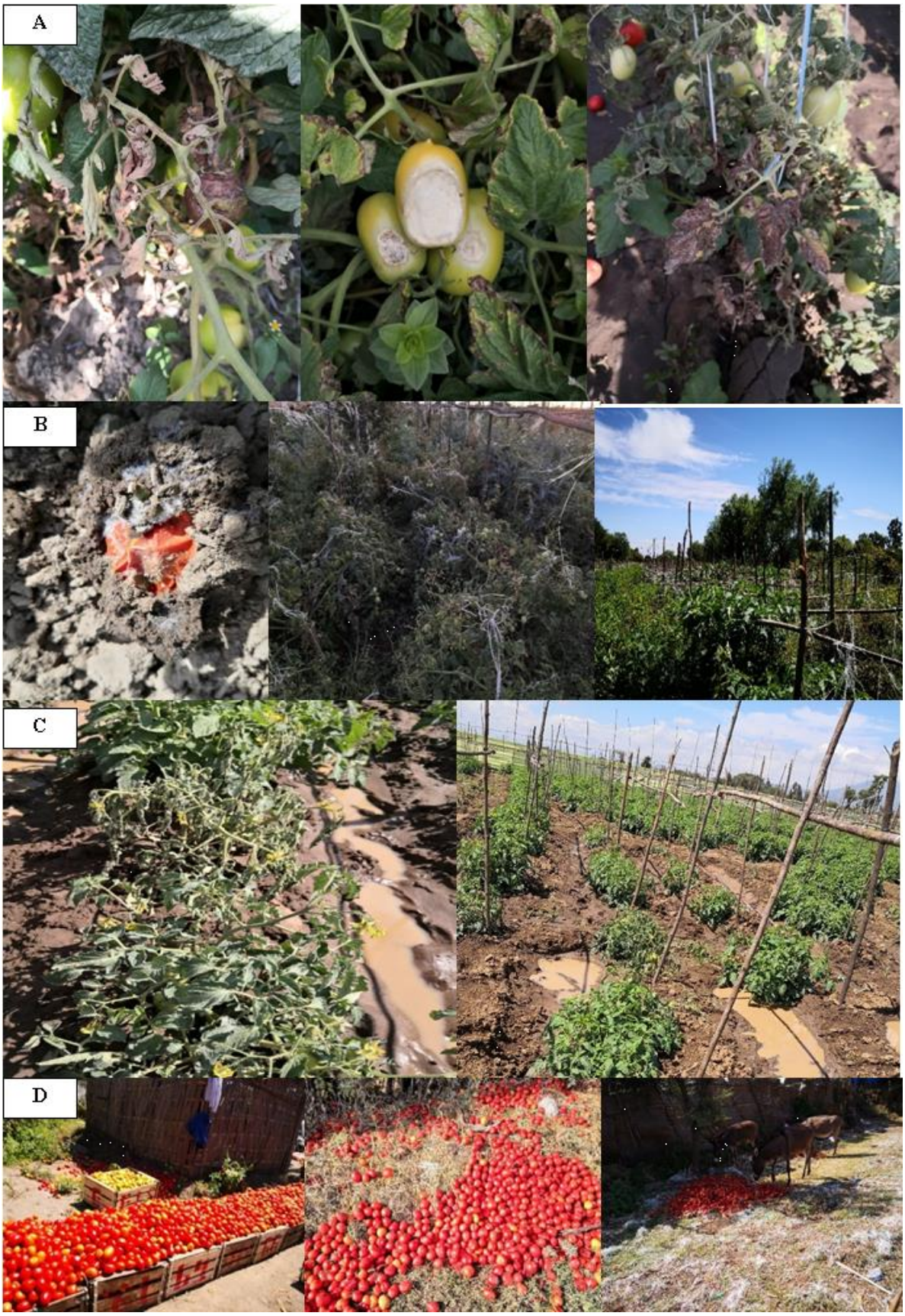
Ethiopia. A survey from CSA (2021) showed that commercial tomato farmlands were applied 1.8 quintals per hectare. However, most of our respondents observed incorrect fertilizer timing, dose/rate, and application procedure. In some farms, fertilizers were applied below the recommended rate, whereas in most farmlands, they were applied at an elevated dose. This is because most tomato growers were tenants; as a result, they assumed that maximizing fertilizer application would enhance their production and productivity. Thus, field and farm observation indicated that young tomato plants showed deformed and depleted growth due to fertilizer doses (usually urea) and poor irrigation management. For example, very high urea was applied in one of the observed Meki farms that strongly challenged the plant (Picture 2.1 C). In line with this, a preliminary survey conducted in the Rift Valley Region by the Adami Tulu Research Centre in 2010/11 cropping season confirmed that farmers applied 400 kg ha^{-1} urea. A supportive statement by Begna (2018) also demonstrated that high fertilizers and overuse application were observed in the Rift Valley Region for vegetable production, yet most fertilizers possibly lost due to runoff water and leaching. Similarly, Abebe *et al.* (2022) reported that 50% of N-fertilizer goes to water bodies, evaporation, and reaction with organic compounds in soil. In addition, farmers applied fertilizers with no comprehensive soil fertility information (Lemma, 2002). Nevertheless, none of them applied compost or biofertilizers by the time of data collection and farm visit. This might be due to a lack of awareness, limited/no access, infestation fear, uncertainty about the practical potential, and short-term land rent and contract agreements. Furthermore, all of them showed their willingness and gave positive responses to apply bioinoculants (biofertilizer, plant growth-promotor and biocontrol) if they offered competent, well-evaluated, and effective strains (Table 2.2).

Table 2.2: Fertilizers application experience.

Item		Response	No of respondents (%)
Fertilizers application experience on tomato farm		Yes	100
		No	-
Types of fertilizers used by smallholder farmers		Urea	100
		DAP	100
		Others	25.7 (NPK, NPS, feed, Ecogreen, nutrients)
Bioinoculants	Biofertilizers and PGPR application experience	Yes	-
		No	100
	Biocontrols application experience	Yes	4.3
		No	95.7
	Willingness to apply Bioinoculants	Yes	100
		No	-

Among the three sites of on-farm observation, Meki site had the poorest farm management practices (plantation, weeding, diseases, and pest management), poor agrochemical application (type, dose, timing, and method of implementation), poor tomato production, and harvesting, poor market exploration and agro-waste handling (Picture 2.1). For example, in one observation, overdose urea and inappropriate watering were applied, and plants were challenged as well as withered (Picture 2.1C). In other observations: 1) liquid fertilizers were diluted beyond the recommended rate; in some cases, improper mixing resulted in solid precipitation and residues; 2) fertilizers were applied by daily labourers manually by hand therefore, uneven distribution to plants and rows was common, 3) application time was random; they didn't consider the plant age; and 4) following fertilizer application, labourers used poor watering and irrigation system and released excess water, which could wash the nutrients before plants absorb them, and the like.

Improved seeds play substantial roles in elevated production and productivity (CSA 2021). Similarly, Yeshiwas *et al.* (2016) indicated that tomato yield and fruit attributes are affected by seed varieties. Most smallholders accessed tomato seeds primarily from private sectors and a few farmers used to buy from the government or farmers cooperatives/associations agricultural input supply agencies. Most (80%) of the farmers preferred private sectors (Table 2.1) for ease access, sufficient supply, advantage of efficient varieties, business competency, cost management, on-time delivery, and nursery capacity. Similarly, Mersha and Sime (2022) reported that 72.61% of smallholder farmers purchased tomato seed from private sectors, 7.64% from government while the rest of them accessed from local market and own source. Contradicting result reported by Tamru *et al*. (2016) that smallholder farmers purchased about 68% of herbicides, 64% of insecticides, and 51% of fungicides from the private sector, whereas 84% of chemical fertilizers and 87% of improved seeds came from the government sector.



Picture 2.1: Tomato on-farm observation (A: leaves and fruits diseases infestation; B: poorly managed farm; C: overdose fertilizer (urea) and water application; D: post-harvest management).

2.3.2 Seedlings and Transplantation

In the study areas, two familiar tomato varieties (*Galilee* (Mersha and Sime, 2022) and *Hawassa*) were widely grown due to their potential productivity and relative resistance. These varieties were chosen based on their previous production experience and/or neighbour tomato growers' recommendations. Frequent tomato seed items of preference include resistance, fruit shape, size, and marketable fruit yield. The *Galilee* variety was considered even more advanced in diseases, drought, and insect resistance, more productive in the dry season, and has high market demand due to its fruit appearance over the *Hawassa* variety. Local fresh tomato market acceptance depends on shape, colour, size, quality, and storability (Abera *et al*., 2020; Lemma, 2002). According to the respondents, *Hawassa* variety was more productive, especially in the wet/rainy season, but strongly susceptible to diseases, requiring long days of maturation and poor market demand due to fruit shape and appearance. However, research conducted in 2015 in different districts, including Koka and Ziway, by Gemechu and Beyene (2019) showed that the *Hawassa* variety was preferred over other hybrid varieties because of its good marketable yield, fruit size, and extended shelf life, whereas *Galilee* had an equivalent fruit size and rational fruit number.

Most of our respondents used nursery-grown (2–3 true leaves and 12–15 cm in length) young tomato plants from delivery agents or suppliers. Farmers preferred transplantation for two basic reasons. The first reason is that, compared to direct seed sowing, nursery plants are easy to manage, enhance viability, growth, and maturation success, as well as shorten production time. Our observation and the practical farmers' experience confirmed that more than 80% of transplanted tomatoes succeeded in plant regeneration together with the successful establishment of plants, except for a few transplantation shocks. The second reason is that direct seed sowing has a germination risk or challenge, vulnerable to bird and disease attack, requires long maturation days, subjected to possible seed loss, etc. However, Lemma (2002) reported that direct sowing revealed some important features, like better fruit production and 15–20 days earlier maturity than transplanted tomatoes, but then required continuous irrigation and weeding. Producers have sufficient understanding and experience of windbreaks, staking with cheap ropes, and trying to maintain plant and row spacing.

2.3.3 Disease Outbreaks and Management

Different parts of tomato were attacked by various organisms. Tomato root, stem, leaf, and fruits can be infected by different organisms (HGIC, 2021), including fungi, bacteria, viruses, nematodes, flies, insects, and higher animals. Even though the time of incidence and duration of infections varied, all the respondents were challenged by repeated tomato disease occurrences (Figure 2.3). In fact, open-field farm production showed higher disease incidence and damage than controlled systems (Sotelo-Cardona *et al.*, 2021). Diseases spread accelerated by temperature, humidity, wind, and rain (HGIC, 2021). All parts of tomato (root, stem, leaf, and fruit) were infected by several pathogens; however, fungal infestation (Figure 2.2) took the lion share in production loss. According to our respondent's tomato leaves and fruit infestation (Figure 2.3 and Picture 2.1A) caused severe damage to production and the economy. In consistence, Gemechis (2017), indicated that late blight (*Phytophthora infestans*), Fusarium wilt (*Fusarium oxysporum*), pests and weeds were the main factors for tomato production in Ziway area.

Farmers sprayed different pesticides and fungicides repeatedly to respond to the pathogens and pests, starting from the early plant stage to the final harvesting. Similarly, Mergia *et al.* (2021) reported that small-scale vegetable farms consuming intensive fungicides and insecticides in Ziway area. Pesticides are considered a good, economical, and safe plant protector, coupled with enhancing farm products (Singh and Singh, 2019). They are used massively around the globe (Mars and Ballantyne, 2004). In Ethiopia, pesticides imported by private and public companies (Mengistie, 2016) and their applications increased exponentially. Despite the fact that prices are high, farmers frequently use broad-spectrum pesticides and fungicides. Farmers increased pesticide application and imposed a negative impact on the environment and human health (Mergia *et al*., 2021; Singh and Singh, 2019). For instance, CSA (2021) described commercial farmlands in the meher season as having applied different amounts of chemicals (herbicides (32.2%), pesticides (31.8%), and fungicides (35%), respectively, to mitigate and control pests. Since farmers couldn't tolerate plant infestations, they heavily relied on pesticides. Smallholders' application patterns are more complicated as compared to those of commercial farmers (Mengistie *et al*., 2015). It is crucial to take strict measures as well as develop a proper diagnosis, rate, and schedule to reduce residual toxicity.

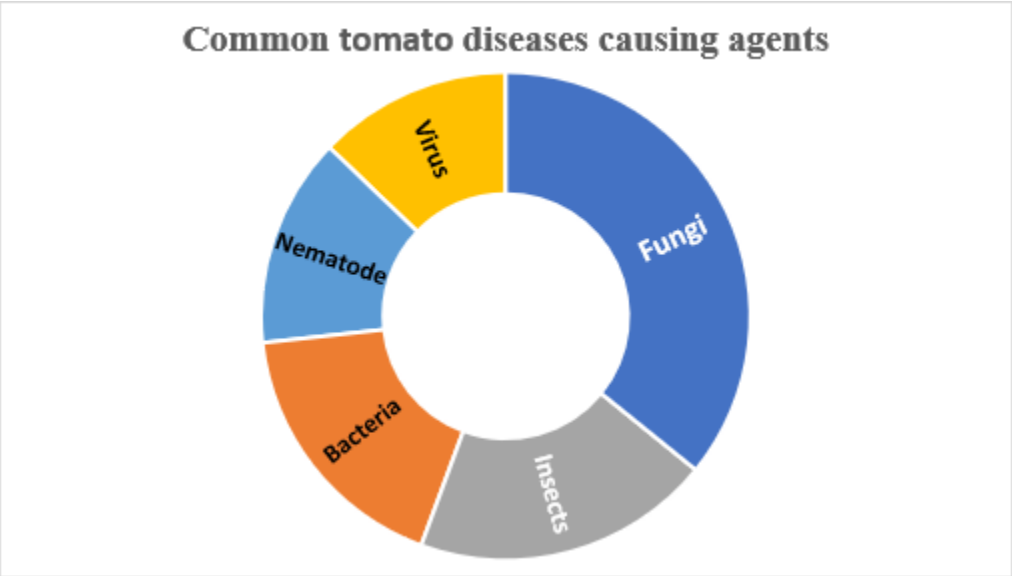


Figure 2.2: Common pathogens observed at Koka, Meki and Ziway Zuria tomato farms.

The current on-farm observation confirmed that different pesticides and fungicides were heavily applied. Some of them include BOSS 72% (fungicide recommended for potato), Cuppercide, Mancozeb, More 720 WP (fungicide recommended for potato late blight), Omaxim (fungicide recommended for potato late blight), Mancolaxyl 72% WP (fungicide recommended for variety crops including tomato), SNIPER LFR (liquid fertilizer and control pests), Fungicide Famoxadone 22.5% + Cymoxalin 30% + WDG Dolar 52.5% (recommended for late and early blight of potato and tomato), and Revolution 325SC (fungicide recommended for onion). Farmers used their previous knowledge, tentative guesses, and other farmers recommendations to identify infected plants and plant parts, determine which type of pesticide could be applied, and determine the duration of the spray. A supportive report by Mergia *et al*. (2021), demonstrated that 34% of small-scale farmers followed their previous experience, and 47.5% communicated with other farmers to apply pesticides. However, a contradictory report from Weldegiorgis *et al*. (2018) demonstrated that 69% of tomato-producing smallholder farmers in Hintalo-Wajirat district didn't exercise pesticides.

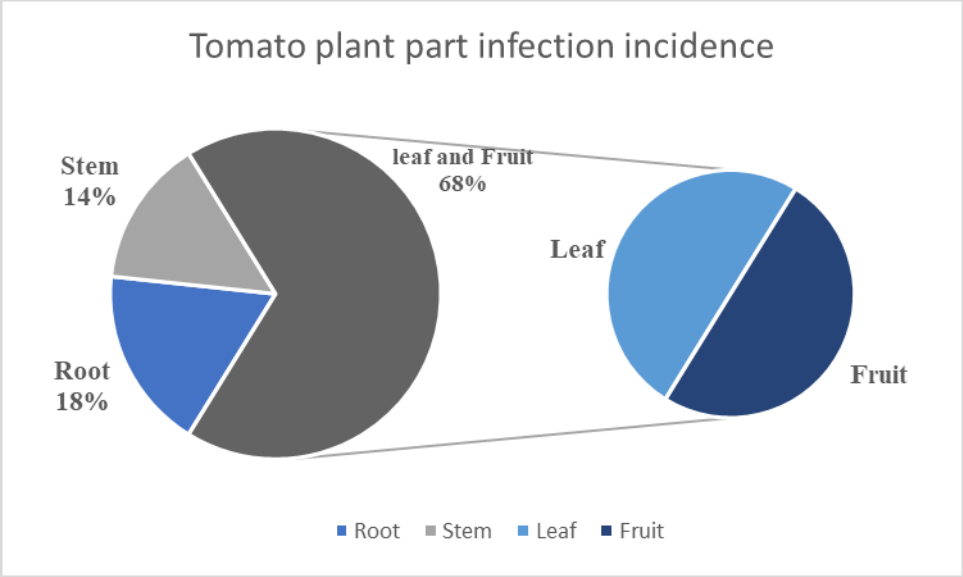


Figure 2.3: Commonly infected tomato plant parts at Koka, Meki and Ziway Zuria farmlands.

Furthermore, smallholders consulted and contacted agrochemical retailers, traders, and other tomato producers, but received limited consultation from agricultural experts. This might be due to inaccessibility, unapproachability, unavailability, lack of motivation, poor farm visit experiences, poor consultation experiences, and follow-up by experts. However, in other parts of Ethiopia, such as in Silte Zone, tomato producers showed better (4–7) contact and consultation experience with experts per production season (Bahilu *et al*., 2020). Respondents in the current study thought that underestimated contact and consultation experiences with agricultural experts on improved farming systems, agricultural technologies, and policy and strategy opportunities hinder their production and profit. Supportively, 32.7% of tomato producers at Ziway Dugda area have no contact with extension agent (DAs or agricultural expertise) (Mersha and Sime, 2022). It is known that most Ethiopian farmers are not academically well educated, lack self-help in gathering information from readings, and are far from updated agricultural technologies. The majority of them are forced to rely on their previous experiences, follow similar farming systems, struggle to adopt new technologies, stick to the traditional farming system, and are dependent on natural rainfed production. That is why the government made available resources for farmers like development agents, extension packages, and agricultural inputs to modify farm styles and improve productivity (CSA, 2021). Frequent extension contacts and visits improve farmers' capacity and understanding of new technologies, practices, and agricultural inputs to enhance their production efficiency (Asfaw, 2021). This is because education empowers existing opportunities and more advanced technologies (Weldegiorgis *et al*., 2018; Bahilu *et al*.,

2020). Farmers who have extension service access have better farm practices, including smart agriculture (resource management, integrated farming, adopting low-cost technologies, irrigation, post-harvest technology, mitigating climatic changes, and adopting an environmentally friendly approach) (Kifle *et al.,* 2022). For better production and productivity, the adoption of improved technologies is in contexts such as changing crop type, adopting improved varieties, reframing planting time and season, improving conservation, and irrigation (Kidane *et al.,* 2022).

Pesticide spraying is done by daily labourers with very limited or no special technical trainings. No farmers were found to follow the correct scientific infestation diagnosis techniques and the respective pesticide rate application. In line with this, Mergia *et al*. (2021) reported that most (64%) of small-scale farmers at Ziway Zuria didn't follow the standard dosage. Farmers follow traditional diagnosis and trial-and-error treatment, with a plus or minus indefinite (either below or above the recommended) rate application. It was observed that some of them sprayed very diluted chemicals (to minimize cost and cover a wider farmland area), whereas in some other fields very strong chemicals were sprayed (concentrated doses sprayed in the assumption of urgent recovery of the infected plant). In either of the indeterminate chemical amount, applications succeed, yet they result in economic loss and damage. An underestimated amount of chemical application reduces the efficacy of pesticides, requires spray repetition, and induces strange and resistant strains. On the other hand, overrated chemical application results in cost loss, plant injury, land-productivity loss due to residue deposits in the soil, environmental damage, and health risks.

Like biofertilizers, tomato producers have no exposure to biological controls (inoculants). It is underlined that improved agricultural inputs and resources should be presented to smallholders to achieve optimal production (Weldegiorgis *et al*., 2018) in line with sustainable environmental protection. In the first place, sufficient community awareness was not created; in the second place, there was no efficient biocontrol evaluated under different conditions and promoted very well. The respondents have shown keen interest and willingness to apply effective bioinoculants (Table 2.2) if the inoculants confirmed efficacy and adaptation under local agroecology. One biocontrol field trial on tomato by PAN Ethiopia was undertaken in Ziway, and visited the research progress. The trial was at the initial stage of the experiment, but tomatoes were observed to be in severe damage by inoculated infestation. The objective was to promote natural predators for *Tuta absoluta* by

using brewer's spent grain feed and eco-green fertilizer. Nonetheless, by the time of the visit, the results were not effective. This might be due to an unbalanced population number between the prey and predator, the time of the experiment (dry season is not conducive for most predator insects), moisture and humidity limitations, used substrate (amount and freshness), human and animal interference (the experiment was conducted in a personal garden), poor follow-up, very high infestation inoculation, and a poor experimental scheme.

Moreover, smallholder farmers have a good understanding of using windbreaks and building artificial borders with different materials and/or crops (maize and sorghum) to minimize tomato disease transmission and biological competitors. Since most of the lands in the areas were covered with similar crop species, the fence probably minimizes and helps to protect tomatoes, if not reduces disease transmission via wind, birds, insects, and direct attack.

2.3.4 Harvesting and Agro-waste Management

Mature fruits were harvested and transported immediately to the market. There were no cold rooms or any storing facilities for freshly harvested tomatoes in the study areas. Apparently, harvesting is done basically based on the market and transportation availability (Abera *et al*., 2020). Marketable, healthy, good-looking ripened fruits were selected and sorted by daily labourers. Farmers can harvest 4–8 rounds from a single healthy, properly managed farming system with a potential production increment until the fourth round then fruit yield decline in the consecutive rounds. The current result agrees with Gemechu and Beyene's (2019) finding, depending on the varieties used, farmers can harvest 5–6 rounds from open field farms. According to our respondents, the amount of tomato production varied among the three sites (in Koka, 60–80 boxes; in Meki, 60–90 boxes; and in Ziway Zuria, 50–80 boxes produced per hectare). Suppose one box's (Picture 2.1D) tomato weight ranged between 60 and 80 kg; thus, farmers produced 3–6.4 tons/ha in Ziway Zuria, 3.6-6.4 tons/ha in Koka, and 3.6–7.2 tons/ha in Meki. Boldly mentioned that compared to other tomato-producing regions, Ethiopian production (5.8 tons/ha (CSA, 2020)) is very low (Worku and Sahela, 2018), including the irrigated areas, with an average production of 3.5 tons/ha at the Upper Awash basin and 1.2 tons/ha at the Guder area (Girma and Awulachew, 2007). Many challenges and contributing factors (Worku and Sahela, 2018) can be mentioned for this low fruit yield, but there are also plenty of opportunities deemed to be improving the production. Production improvement is essential to satisfy the ever-increasing market demand and efficient resource

utilization (land, water, and conducive weather). However, unusual tomato yields were reported by De Putter *et al.* (2012), who found that an average of 31.6 ton/ha in Dugda and 16.3 ton/ha in Adami Tulu Jido Kombolcha districts.

First fruit maturation and production varied among the three sites; in Koka, 40–45 days after transplantation were required, whereas in Meki and Ziway Zuria, 45–60 days were required. The result is comparable with the of Tsehaye *et al*.'s (2020) report that fruit ripening was observed after 40 days of transplantation. These maturation variations might be due to soil type and content, water quality, environmental conditions, farming systems, and the like. It was observed that tomato fruits exposed to sun and physical damage during harvesting and transportation consequently, a substantial fruit have been exposed to sunburn, which minimizes market acceptability and then lowers the price/cost (Picture 2.1D). Abera *et al*. (2020) also indicated that improper harvesting, post-harvest handling, storage, and transportation are the key constraints for significant tomato fruit damage. This fruit loss excelled, especially if the market price was very low and different tomato-producing areas in the country flooded the market, especially in Addis Ababa at the same time. If the market price is very cheap, farmers dispose of fruits on the farm or near the farm that will promote infestations (Picture 2.1B) for the rest of the farm and the next phase of production. This kind of forfeit indicates the absence of stores or tomato processing industries, at least near major tomato-producing areas. This strongly affects farmers' economies, motivation, and production efficiency, as well as the state economy.

Most of the respondents disposed of and mismanaged chemical packing materials, leftover chemicals (air-opened or poured on the ground), agro-wastes, infected plants, fruits, etc. In good agreement, Mergia *et al*. (2021) described that more than 90% of small-scale farmers disposed of agrochemical packings dumped on the farm without rinsing. Smallholder farmers became ignorant of weeding in the late harvesting season (Picture 2.1B) then weeds got a chance to produce and spread seeds in addition to soil mineral depletion. It was also observed that most farmers failed to control infestations in the late harvesting period. Apparently, it should be improved farmers practice of rejecting or throwing cracked, infected, and fermented fruits as well as old or infected plant debris on the farm. This might encourage pathogens to spread more, produce and hide cysts and spores in soil, and produce more resilient strains.

2.4 CONCLUSION AND RECOMMENDATIONS

Tomatoes are highly sensitive to both biological (diseases and infestations) and non-biological (soil nutrients, salinity, water, and weather) stresses, requiring continuous follow-up, supplements, protection, proper harvesting, and handling. Therefore, it is better to maintain the soil with appropriate nutrients based on proper soil analysis, follow appropriate diseases and infestation control along with specific pathogen identification, correct treatment with accurate dose and rate application, follow proper agro-wastes disposal, minimize unnecessary agrochemical practice, protect the environment, and improve farmers awareness with technical trainings. In future work, it will be better to incorporate competent bioinoculants into tomato farm practice due to several positive outcomes, including minimizing production costs, improving efficiency, production sustainability, fertility maintenance, and environmental protection. The candidate strains should be screened based on multi-dimensional benefits in nutrient access, plant growth promotion, resistance induction, antagonistic to phytopathogens, and local environment adaptation. For a fruitful outcome, isolation from the local environment and target crop rhizosphere is highly recommended.

CHAPTER THREE

3. ISOLATION, CHARACTERIZATION AND STRAIN IDENTIFICATION OF COMPETENT PSB STRAINS FROM RHIZOSPHERE SOILS

Abstract

Phosphorous (P) is an essential element, though the majority of soil P is unavailable for plants due to fixation with metallic ions (Al^{3+}, Fe^{3+} and Ca^{2+}). Phosphate solubilizing bacteria (PSB) convert insoluble phosphorous to plant-available forms. Following the plate screening technique, 10 competent PSB isolates were selected from the tomato rhizosphere. Isolate Mk-1-25 scored maximum SI (3.22) with significant pH reduction potential (4.25) while Mk-13-16 formed the largest colony diameter, followed by the least SI (2.65) and pH reducer (5.99). Though speed and level of efficacy varied among isolates, PSB from the Koka site in general were found to be strong solubilizers (K-1-29, K-10-41, and K-10-27 dissolved about 260.83, 260.38, and 241.91 µg/ml, respectively) in PVK broth with different P-sources. $Ca_3(PO_4)_2$ was one of the most dissolved P complexes, resulting in the highest (253.46 µg/ml) dissolution, while $AlPO_4$, $FePO_4$ and bone meal recorded comparable P concentrations (211.78, 213.23, and 212.69 µg/ml, respectively). Specific substrate-isolate interaction analysis exhibited significant variation in dissolved P concentration. The majority of isolates preferred $Ca_3(PO_4)_2$-added broth. For instance, K-10-27 dissolved the highest P from $Ca_3(PO_4)_2$ and relatively lowest from $FePO_4$-containing broth (343.9 and 215.58 µg/ml, respectively); K-10-41 dissolved 337.9 and 223.38 µg/ml in $Ca_3(PO_4)_2$ and $AlPO_4$ broth, respectively; and Mk-1-25 recorded the lowest dissolved P (201.96 µg/ml) in $Ca_3(PO_4)_2$ and showed good competence in $FePO_4$ and bone meal-supplemented broth. Most strains were found to be efficient P solubilizers from different P complexes, able to utilize broad carbon sources, lower medium pH, possibly due to organic acid production, and produce plant growth-promoting traits. Plate screening is a good technique to isolate potential PSB strains; however, it should be supported by other techniques, including liquid medium evaluation. K-1-29, K-10-41, and Z-12-20 are potential candidates to develop biofertilizers and possibly improve soil fertility in a sustainable manner with an eco-friendly approach. Subsequently, by verifying their performance under greenhouse and field adaptation, these selected strains could be applied for tomato and other crops production at Koka, Meki, and Ziway Zuria. From our findings, we recommend that future work should concentrate on evaluation of these strains at greenhouse and field conditions and investigate more efficient strains supported by molecular techniques to develop competent inoculum.

***Keywords**: Biofertilizer, Phosphorous, PSB, Rhizosphere*

3.1 INTRODUCTION

Phosphorous (P) is an essential nutrient (Nesme *et al.*, 2018; Sharma *et al.*, 2013) for plants to synthesize cellular structures and biomolecules (Baliah *et al.*, 2016), including nucleic acids, enzymes, proteins, and phospholipids. It is required for the growth and development (Kour *et al.*, 2021) of roots, leaves, stalks (Sharon *et al.*, 2016), flowers, seeds, and fruits. P accounts for about 0.2–0.8% of plant dry weight (Sharma *et al.*, 2013). The accumulation of P in plant tissues induced genes responsible for developmental and physiological responses as well as increased floral intensity, yield, and quality (Gómez-Merino and Trejo-Téllez, 2016). P is found adequately in the soil; however, most (95–99%) of phosphate is insoluble and inaccessible for plants due to fixation and interaction with metallic elements (Al, Fe or Ca) (Waday *et al.*, 2022; Wan *et al.*, 2020; Pande *et al.*, 2017; Sharon *et al.*, 2016; Zaidi *et al.*, 2009; Hariprasad and Niranjana, 2009). Both organic and inorganic forms of phosphorus are found in soil (Sharma *et al.*, 2013; Yadav and Verma, 2012; Zaidi *et al.*, 2009). Nonetheless, organophosphorus ranges from 30 - 90% of the total phosphorous, whereas plant-available phosphorous is limited (0.1% of the total available soil P) (Gómez-Merino and Trejo-Téllez, 2016).

Plants absorb inorganic phosphorous (Naik *et al.*, 2008) that is liberated by solubilizing microbes (Khudhur, 2017) as well as from supplemented chemical fertilizers (Figure 3.1.1) (Ahemad and Kibret, 2014). It is strongly precipitated and switched immediately to unavailable forms for plant absorption (Zaidi *et al.*, 2009). Most of the worldwide soils are deficient in plant-available P (Kour *et al.*, 2021). Consequently, farmers frequently apply fertilizers while the soil total P content on the farm is relatively high (Zaidi *et al.*, 2009). P supplement (Nesme *et al.*, 2018) through chemical fertilizer is a very good option for direct plant assimilation (Yadav and Verma, 2012); yet it is costive, spendthrift (about 70–90% is rapidly fixed or highly reactive to precipitate with Al^{3+} and Fe^{3+} in acidic and Ca^{2+} in alkaline soils) (Baliah *et al.*, 2016; Sharma *et al.*, 2013; Zaidi *et al.*, 2009), energy-consuming (Kour *et al.*, 2021), and environmentally unfriendly (production, transportation and application is environmentally deleterious). In brief, the economic and environmental pressure of chemical fertilizers (Fikadu, 2022; Raimi *et al.*, 2017), the deterioration of soil fertility and productivity, the depletion of high-grade phosphatic rock, and the current concern to feed the ever-increasing population are driving forces to search for alternative sustainable approaches and options (Ammar *et al.*, 2023). Therefore, it is necessary to establish a mechanism to

access P for plants with an efficient, cheap, and eco-friendly approach for enhanced plant growth and production.

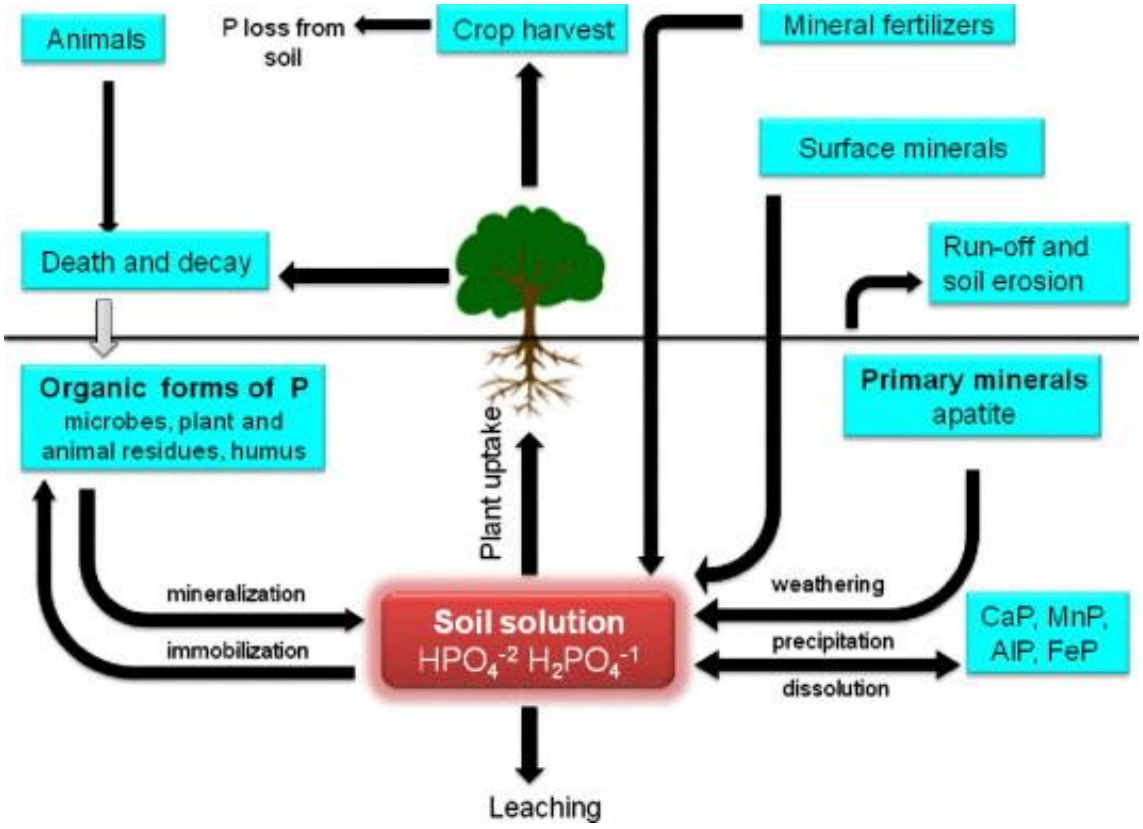


Figure 3.1.1: Movement of phosphorous in the soil (*adopted from Ahemad and Kibret, 2014*)

To resolve plant nutrient deficiencies in a sustainable manner, experts tried to develop biofertilizers by screening beneficial microbes from different niches. Microorganisms play a substantial role (Tsegaye *et al*., 2017) in organic matter decay, the recycling of nutrients (C, N, P, and K), and the correction of plant nutrient deficiencies (Jehangir *et al*., 2017). It is necessary to enrich beneficial microbes in the soil to improve soil fertility and promote plant growth. A wide range of microbial diversity is found in plants' rhizosphere (Kour *et al*., 2021; Bergelson *et al.,* 2019; Baliah *et al.,* 2016), which is the most biologically active soil zone. The rhizosphere (15-20 cm diameter from the root) is a hotspot for biochemical transformation and complex interactions between plants and microbes in the soil (Verma, 2019). The region is rich in root exudates that provide carbon sources (glucose, fructose, arabinose, maltose, galactose, xylose, oligosaccharides etc), amino acids (like alanine, asparagine, tryptophane, cystine, lysine, proline, methionine, glutamine etc), organic acids (citric, oxalic, malic, fumaric, lactic, glutamic, succinic etc), phenolic compounds, secondary metabolites, and communication signals (attract or repel) to the microbiome (Ahemad and

Kibret, 2014). The plant releases plenty of root exudates to respond to root-root, root-bacteria, root-fungi, root-nematode, and root-insect communication (More *et al.*, 2019). Consequently, significant diversity and abundance of beneficial microbes acclimatize this fertile root-soil zone (Rafi *et al.*, 2019; Chandra *et al*., 2018; Karnwal, 2017).

Rhizosphere microbial community structure is the result of interaction and feedback among plant roots, microbes, and the physico-chemical properties of the soil (Quiza *et al.*, 2023; Bergelson *et al.*, 2019; Verma, 2019; Zhalnina *et al*., 2018; Tsegaye *et al*., 2017). The dynamic microbial community exhibits heterogenicity due to metabolic activity differences (Willey *et al*., 2017). Among the beneficial microbes, phosphate solubilizing bacteria (PSB) are known for their multidimensional benefits (Naz *et al*., 2022; Zhang *et al*., 2021; Rodríguez *et al.*, 2006) such as accessing plant available nutrients (Mekonennen and Kibret, 2021), trace elements (Zn and Fe), enhancing phytohormone production (Chandra *et al*., 2018), promoting plant resistance for different stresses, antagonists to phytopathogens (Rabbee *et al*., 2019; Mukhtar *et al*., 2017; Kurabachew and Wydra, 2013), improving the overall growth, development, yield, quality (Chen and Liu, 2019; du Jardin, 2015), and water uptake (Koskey *et al*., 2021). According to Pandey *et al*. (2006), PSB exhibited antifungal activities by producing chitinase, HCN, siderophore, ß-l,3-glucanase, and salicylic acid. They are also considered biological reservoirs for organic molecules and nutrients (Babalola *et al*., 2021). In addition, they are reported to play an important role in immobilization/fixation (Wan *et al.*, 2020) and the conversion of toxic insoluble precipitates of heavy metals like Cd, Cu, Cr, Pb, and Zn to bioavailable forms in the soil (Yang *et al.*, 2023; Zhang *et al*., 2021; Wei *et al.*, 2018; Mahanty *et al*., 2017). Likewise, Mohamed *et al.* (2018) found pesticide-resistant PSB strains that were persistent in the harsh (loaded with aggressive agro-chemicals) environment for integrated farm inputs.

Biological soil fertility maintenance, together with proper agrochemical practices, will provide a fruitful and sustainable production system (Koskey *et al.*, 2021). Several soil microorganisms particularly many bacteria, protozoa, fungi, and actinomycetes, play an important role in correcting plant P deficiency (Liu *et al*., 2023; Quiza *et al.*, 2023; Chandra *et al*., 2018; Behera *et al*., 2014). Bacteria are predominant P-solubilizers and proved to be more effective than fungi (Rafi *et al.*, 2019). They will be able to improve plant-available P nutrients by solubilizing and mineralizing organic and inorganic insoluble sources (Liu *et al.*, 2023; Prabhu *et al*., 2019; Alikhani *et al.*, 2007; Rodríguez *et al.*, 2006) through the

production of various metabolites, organic acids (Sharon *et al*., 2016), and enzymes. Siderophore production is another mechanism of microbial P-solubilization in acidic soil to scavenge Fe (Cui *et al*., 2022). Different researchers have reported various P-solubilizers, namely *Bacillus, Pseudomonas, Rhizobium, Agrobacterium, Enterobacter, Flavobacterium, Penicillium, Bradyrhizobium, Klebsiella, Azospirillum, Serratia, Aspergillus, Acinetobacter, Actinomycetes*, and *Arbuscular mycorrhiza* (Zhang *et al*., 2023; Kirui *et al.,* 2022; Kour *et al*., 2021; Kalayu, 2019; Mohamed *et al*., 2018; Alori *et al.,* 2017; Karnwal, 2017; Sharon *et al*., 2016; Singh and Jha, 2015; Rodríguez *et al*., 2006; Pandey *et al.,* 2006) for their known beneficial effects and plant growth-promoting traits. Similarly, Verma (2019) underlined that microorganisms play a significant role in sustainable agriculture, soil health, and environmental protection.

PSB are abundant in the rhizosphere, promising to improve growth and yield in crop and vegetable production, including tomato (Zhang *et al.,* 2021). Sharon *et al*. (2016) found effective PSB inoculum for tomato production from organic garden tomato and potato rhizosphere soil. The main goal of biofertilizer experts is to discover competent strains and optimize the already-developed inoculants for farm applications. Thus, isolating and screening efficient strains are imperative. Multifunctional phosphate solubilizers play determinant roles as biofertilizers, growth-promotors (Kirui *et al.,* 2022), and biocontrol agents in plant root ecology (Figure 3.1.2) (Ahemad and Kibret, 2014; Zaidi *et al.,* 2009; Naik *et al*., 2008). They improve soil fertility with a minimum cost requirement, which is an essential attribute for small-scale farm production. PSB might form different interactions with the host plant; accordingly, following the respective protocols, they could be isolated from rhizosphere soil, the root surface (epiphytes), or the inside root (endophytes). Sample materials can be collected from various sites, including cultivated farms, rock phosphate, river and wetland areas, forests, and virgin lands. It is comprehensible that isolation of P-solubilizers from different habitats and understanding plant growth-promoting attributes help to screen competent strains (Kour *et al*., 2021). Plant growth-promoting rhizobacteria are increasing in agriculture and have an appeal to replace fertilizers, nutrient supplements, and pesticides (Naz *et al*., 2022; Karnwal, 2017). This is because the application of efficient PSB strains to farm practice (Pande *et al*., 2017) could decrease 50% of fertilizer consumption without affecting crop production (Rafi *et al*., 2019). Similarly, Alemayehu (2020) reported production increment of faba bean, wheat and barley (79, 66 and 42%, respectively) with biofertilizer inoculation over uninoculated cultivation in Arsi zone. As indicated by Argaw

(2012), co-inoculation of soya bean with *Bradyrhizobium japonicum* and *Pseudomonas spp*. resulted a similar growth performance (shoot length (71 cm), days of flowering (82), and days of maturation (150) with chemical fertilization (DAP – urea) in Assossa. Likewise, Abeje *et al.* (2024) demonstrated the combined application of biofertilizer and NPSB (at 19-46-7-0.1 rate) resulted in maximum node per plant (49.33), leaf area index (6.15) and soya bean grain yield (2336.7 kg) while un-inoculated and NPSB fertilized (at 9.5-23-3.5-0.05 rate) soya bean resulted a minimum node per plant (1.67), leaf area index (1.94) and grain yield (1626.7 kg) in Assosa Zone. Similarly, tomato inoculation with rhizobacteria significantly increased plant dry and fresh weight (*Pseudomonas strain* FPF1: 96% and 81%, respectively and *Bacillus subtilis* strain B2G: 75% and 54%, respectively) over the control (Lemessa and Zeller, 2007). In agreement with this, Kurabachew and Wydra (2013) have reported that tomato inoculation with *B. cereus* BC1AW and *P. putida* PP3WT increased shoot dry weight by 75% and 62.5%, respectively over the control under greenhouse pot trial. Likewise, Fenta and Assefa (2017) reported that tomato inoculation with PSB isolate (PSB5) resulted a maximum shoot length (21.16 cm), shoot dry weight (1.3 g), root length (11.16 cm) at 60 days of plantation at greenhouse trial.

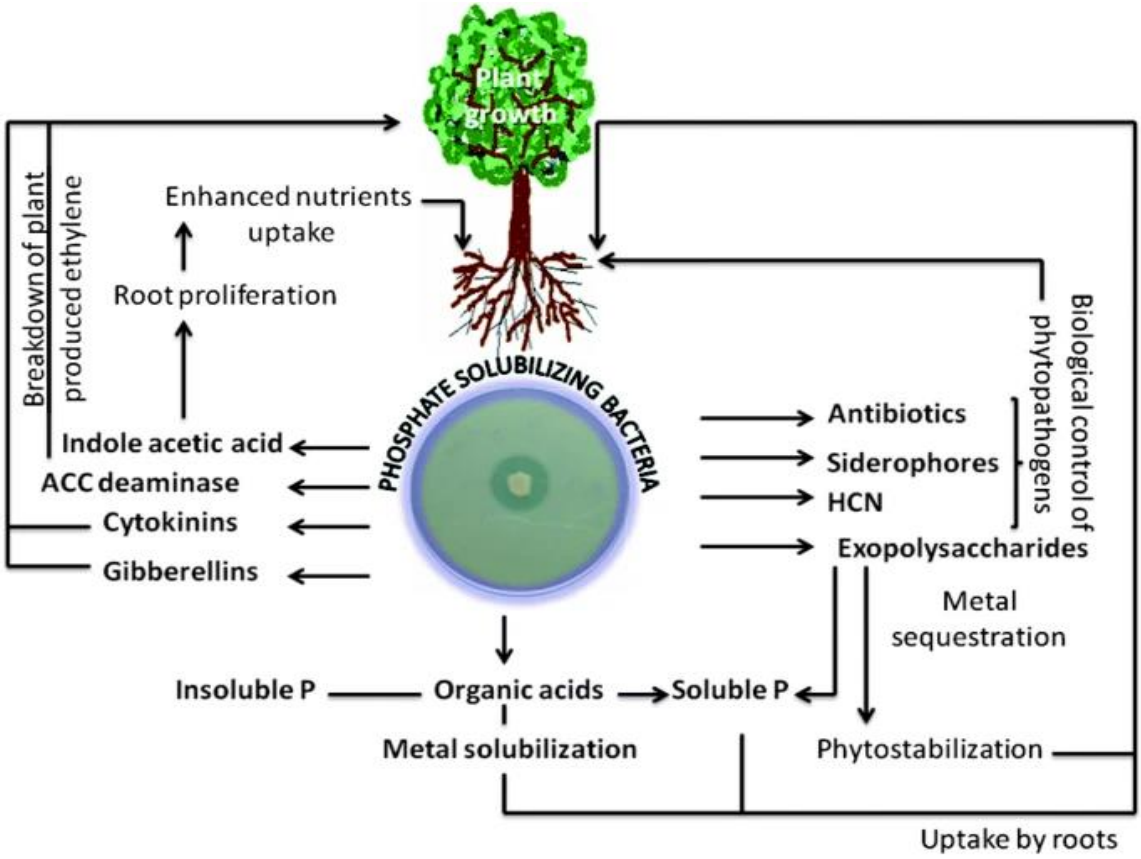


Figure 3.1.2: Mechanism of growth promotion by phosphate solubilizing bacteria (*Source: Zaidi et al., 2009).*

Based on the strong premisses of soil fertility depletion, the need for fertility maintenance, the decline of natural resources for fertilizers production, the environmental concern, the need for heavy agrochemical practice for tomato production, the attractive multidimensional features of PSB, and the available materials and facilities, this research was designed to isolate competent PSB strains with multidimensional benefits from rhizosphere soil that would be able to apply as agricultural inputs or inoculants in the study area for tomato and other crop production. There is untapped microbial potential in biofertilization, biocontrol, and plant growth promotion development for sustainable farm production. Verma (2019) reported that *Bacillus* species isolated from different tomato rhizosphere soils were found to secrete several hydrolytic enzymes such as β-1,3-glucanase, protease, chitinase (Prabhu *et al*., 2019), and cellulase, which have a vital role in plant growth promotion and disease management. Likewise, Rabbee *et al*. (2019) indicated that *Bacillus* species including *B. amyloliquefaciens and B. subtilis* are considered important plant growth-promotors commonly inhabiting plant root microflora. Hariprasad and Niranjana (2009) also demonstrated that PSB isolates from the tomato rhizosphere increased tomato fresh and dry weight, shoot and root length, and seedling P content.

PGPR work in additive hypothesis with multiple mechanisms (biofertilization, rhizoremediation, phytohormone production (du Jardin, 2015) and biopesticide) where their application reduces the global dependence on agro-chemicals (Ahemad and Kibret, 2014). A review by Mekonnen and Kibret (2021) demonstrated that currently, in Ethiopia, there is lack of well-organized and summarized reports about PGPR in relation to vegetable crops and research. In good agreement, Mahanty *et al.* (2017) demonstrated that biofertilizers are considered an integral component of agricultural practices, but limited experience has been reported among developing countries. Despite the opportunity, they are barely used by smallholder farmers because of different limitations efficiency, host-specificity, formulation deficiency, non-commercialization, poor competitive ability of inoculants against native strains and edaphic conditions (Mącik *et al.,* 2020). In comparison to chemical fertilizers, application of biofertilizer may result slower performance, but the beneficial effect is long-lasting (Kumar *et al.,* 2022). Accordingly, this project is imperative due to the aforementioned and many unstated benefits. The selected strains will be recommended as farm inputs (biofertilizers and plant growth-promoters) to promote plant growth and development, minimize production costs, and enhance sustainable production. The inoculant development and production required relatively simple installation and fermentation

technology, low cost, and renewable resources over synthetic chemicals. Plenty of developed strains might be available on the global market; however, local strain screening is essential for indigenous competition and local agro-ecology adaptation. Similarly, Raimi *et al*. (2021) have shown that most crops have high affinity to local/indigenous strains compared to exotic strains. Thus, tomato rhizosphere soils were collected from three vegetable-producing areas (Koka, Meki, and Ziway Zuria) and forest soil from Wendogenet and Yirgalem Zuria then PSB strains were screened and characterized. In addition, isolates were evaluated for their dissolving capability using various P complexes ($Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$ and bone meal (BM)) that possibly found in acidic, basic, or neutral soil ecology and organic matters. Bone meal is concentrated form of phosphorous that decomposes slowly and released P gradually (Abebe *et al*., 2022).

3.2 MATERIAL AND METHODS

3.2.1 **Soil Sample**

To broaden the chance of getting competent PSB isolates, 83 tomato rhizosphere soil samples were collected from three different tomato-producing areas (Koka (13), Meki (45), and Ziway Zuria (15)), and from two forest soil (Wendogenet Forestry (5), and Yirgalem Zuria Forestry (5)). Koka, Meki, and Ziway are found in the Central Rift Valley Scheme, Oromia Region, Ethiopia, known for tomato and other vegetable production using both rainfed as well as irrigation (Mesfin, 2020; Beshir *et al*., 2015; Negash and Mohammed, 2014). Sampling sites and number of samples were determined following multistage purposive random sampling procedure. Soil samples were collected from an actively growing tomato plant rhizosphere at a 30 cm radius and 5–20 cm depth forest soil (Waday *et al.*, 2022) from three locations per sample site, thoroughly mixed, and half a kilogram of soil packed independently in a surface sterilized polyethylene bag, then transported to the Hawassa University Soil Microbiology Laboratory for PSB isolation. Samples were preserved in a refrigerator until PSB isolation activity was completed.

3.2.2 PSB Isolation

All PSB isolation and screening methods were done using Pikovskaya's (1948) procedures with minor modifications. Collected bulk soil samples were ground, thoroughly mixed, sieved, and then 10 gm added to 90 ml of sterilized distilled water, shaken, and settled for 10

mn (Wan *et al*., 2020). From the suspension, one ml was transferred to 9 ml of sterilized distilled water, and serial dilution proceeded to 10^{-6} (Pande *et al*., 2017). Then, 100 µl of aliquot solution from the 5th was dilution spread on Pikovskaya's (PVK) agar medium (composition: 0.5 g yeast extract, 10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.0001 g $MnSO_4.H_2O$, 0.0001 g $FeSO_4.7H_2O$ and 15 g agar per litre of water, pH adjusted to 7) and incubated at 30 ^{0}C for 8 days. Colony and halo zone diameters were measured every two days to evaluate the solubilization index (SI) of each colony.

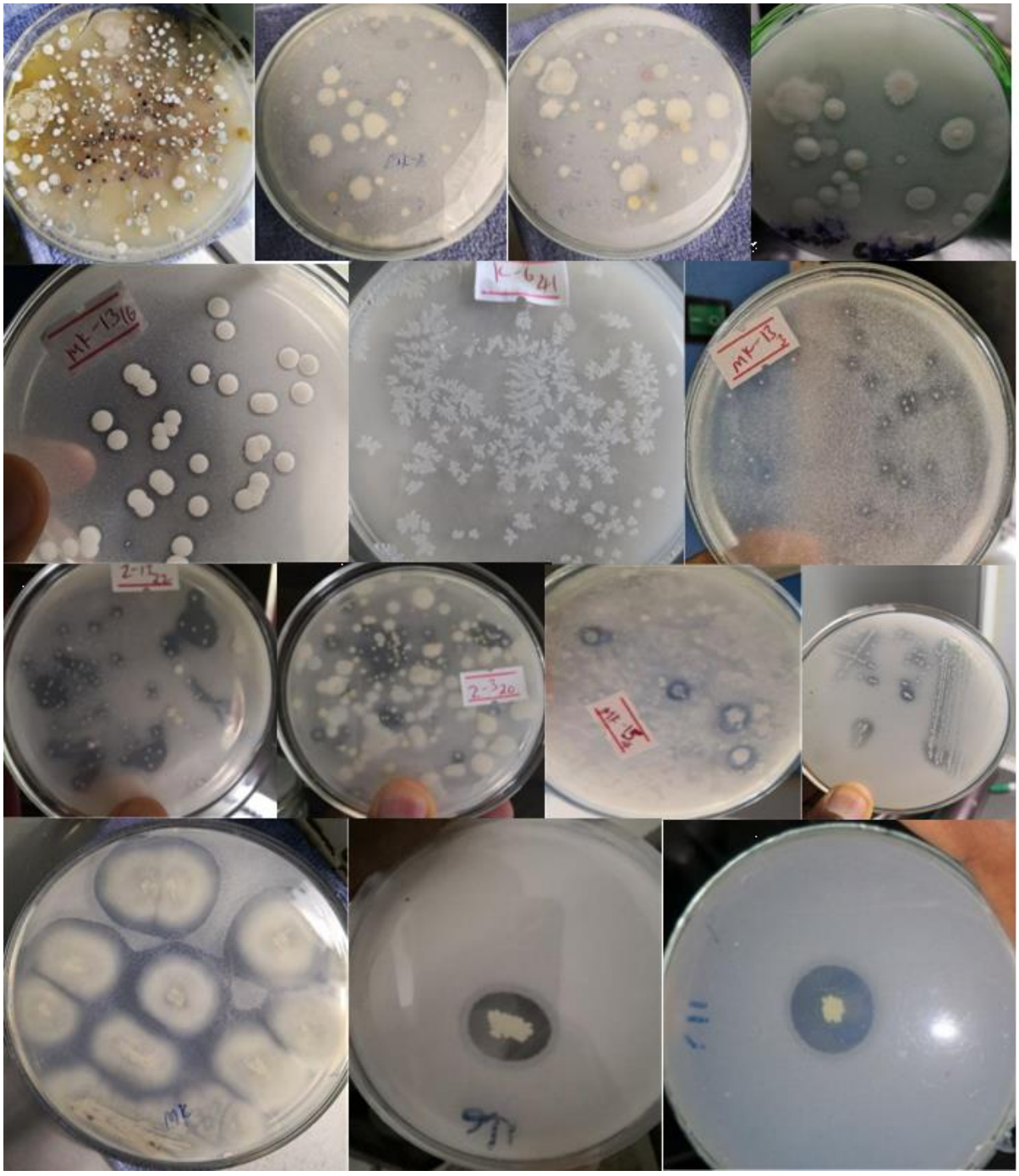
SI was calculated as (Alikhani *et al*., 2007):

$$SI = \frac{\text{Halo zone diameter + Colony diameter}}{\text{Colony diameter}}$$

Bacterial isolates with better clear zone formation (Picture 3.1 and Figure 3.3) were selected and stored in the refrigerator (-20 °C) until isolation was completed from all soil samples. Then each preserved isolate was sub-cultured on fresh PVK agar (Pande *et al*., 2017) to confirm solubilization efficiency and culture purity (Chandra *et al.*, 2018). This culture-re-culture process was repeated until pure culture was observed and solubilization efficiency was maintained. Finally, a total of 100 pure isolates with better halo zone formation were selected for the all locations and further incubated in a shaker incubator (110 rpm) at 30 ^{0}C for five days in liquid PVK medium to evaluate their growth performance and medium pH reduction efficacy. Then the medium pH and colony number were recorded. By combining the SI, colony structure, and pH reduction and resource limitation, the final 10 PSB isolates (9 isolates with a higher average halo zone and one (K-10-41) with the lowest halo zone) were selected and characterized (morphological, G-stain, biochemical, and molecular) for taxonomic identification. These 10 PSB isolates were further evaluated in liquid PVK medium supplemented with different P sources to quantify the concentration of solubilized P in the suspension.

3.2.3 Isolate Naming and Designation

Bacterial isolates were designated by including the name of the place where the soil samples were collected (site), followed by the number of soil samples and the number of isolates from that specific soil sample. Briefly, a prefix "K", "Mk," and "Z" (indicating sampling sites Koka, Meki, and Ziway Zuria, respectively) followed by two serial numbers separated by "-"; the first digit stands for the number of soil samples, and the last digit indicates the number of bacterial isolates from that specific soil sample. *E.g.*, K-10-27 represented the 27th isolate from the 10th soil sample that was collected from the Koka site.



Picture 3.1: PSB isolation from bulk rhizosphere soil and purification on PVK agar medium.

3.2.4 Quantification of Solubilized P in PVK Broth

The current top ten selected PSB isolates were examined on different P-sources ($Ca_3(PO_4)_2$, $FePO_4$, $AlPO_4$ (Sharon *et al.*, 2016) and bone meal as a sole P source) using PVK broth medium to quantify the amount of dissolved P. These corresponding chemicals were added independently into a 250-mL conical flask containing 150 ml of PVK broth and incubated for

10 days in a shaker (110 rpm) incubator at 30 ^{0}C. Each isolate was inoculated separately, and an uninoculated medium was used as a control. The experiment was set up in three replications. Ten ml of the suspensions were sampled at 0, 3, 5, 7, and 10 days of incubation. The medium pH change was examined simply by a pH meter, and the dissolved P was quantified following the Molybdenum blue method (Watanabe and Olsen, 1965). Ammonium molybdate and potassium tartrate reacted in an acid medium with diluted solutions of orthophosphate to form an intensely coloured phospho-molybdate complex. This complex is reduced to an intensely blue-coloured complex by ascorbic acid. The colour is proportional to the phosphorus concentration (Mohamed *et al*., 2018). To remove the insoluble residue and precipitate, the suspensions were centrifuged at 4000 rpm for 10 minutes, and cell-free supernatant was taken for P quantification. Stock solution preparation, standard preparation, and result calculation were done according to the protocol. The spectrophotometer absorbance was set at 650 nm.

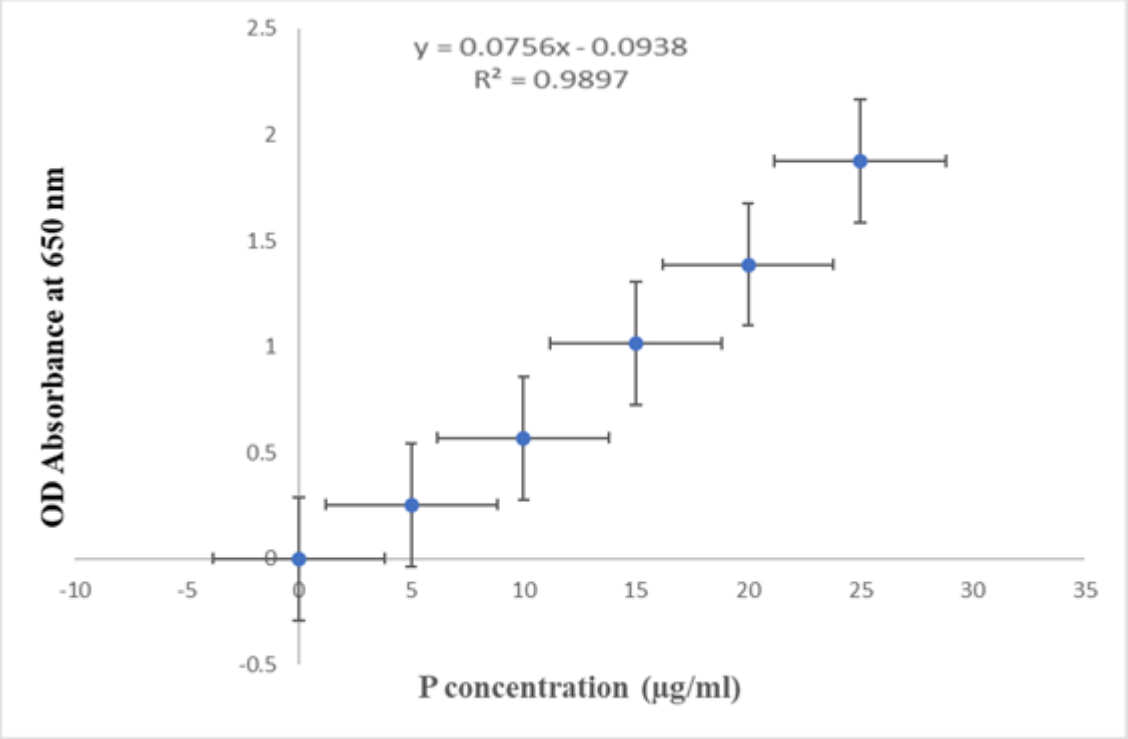


Figure 3.2: Standard curve for quantification of dissolved P.

3.2.5 Characterization and Bacterial Strain Identification

3.2.5.1 Biochemical Characterization (sugar fermentation and growth on different growing conditions)

Sugar fermentation and gas production were tested using 10 sugars (glucose, fructose, maltose, sucrose, raffinose, mannitol, xylose, lactose, arabinose, and starch). Isolates were also tested for resilience and growth capacity at different salt concentrations (1–10%), temperatures (15, 30, 35, 40, and 45 ^{0}C), and pH levels (4.5, 5, 6, 8 and 9). Furthermore, other tests, such as the indole test, TSI test, catalase test, and urease test, were conducted. The plant-promoting traits (HCN, siderophore, IAA production, antagonistic, and nitrogen fixation) were qualitatively evaluated using the corresponding media and reagents.

3.2.5.2 Phytohormone Production, Antagonistic Effect and N_2-fixation Test

Isolates were qualitatively checked for their phytohormone production and plant growth-promoting traits tested using appropriate media and reagents.

1. **Indole test**: Indole production was analysed in nutrient broth with supplementation of 0.1% L-tryptophan (Kurabachew and Wydra, 2013). Isolates were inoculated and incubated at 30 ^{0}C for 24 hours, then 0.5 ml of Kovac's reagent was added, and observed the pink to red colour and ring formation in the reagent layer on top of the medium within seconds of adding the reagent. If a culture is indole-negative, the reagent layer will remain yellow or be slightly cloudy. In addition, qualitative IAA production was determined by using the above-mentioned media and Salkowski's reagent (Amaresan *et al*., 2022).

2. **Hydrogen cyanide (HCN) production**: HCN production was tested on Petri dishes. The bacterial isolates were inoculated on nutrient agar supplemented with glycine (4.4 g/L) (Kurabachew and Wydra, 2013). A Whatman No. 1 filter paper soaked in 2% (w/v) sodium carbonate in a 0.5% (w/v) picric acid solution was placed inside the lid of a Petri dish (Amaresan *et al.*, 2022). The plate was then sealed with parafilm and incubated at 30 ^{0}C for 2 days. A change in filter paper colour from yellow to reddish brown was an indication of HCN production (Kaur and Reddy, 2013).

3. **Siderophore detection**: The Chrome Azurol S (CAS) assay was used for the detection of siderophore production. The CAS assay is a universal chemical test that detects siderophore production (Amaresan *et al.,* 2022). The assay is based on the siderophore's ability to bind to ferric iron with high affinity. The agar contains Chrome Azurol S (CAS) dye, which, when complexed with Fe^{3+}, is blue in colour. If the inoculated organisms secrete siderophores, ferric iron is stripped from the dye, causing the medium to change colours from blue to orange/yellow (Kurabachew and Wydra, 2013; Pérez-Miranda *et al*., 2007). The CAS plates were kept in incubator at 30 ^{0}C for 48 hours. After 48 hours of growth, the CAS plates were examined for growth, which was surrounded by an orange or yellow halo (indicating a positive result for siderophore production) (Kaur and Reddy, 2013).

4. **Antagonistic effect against tomato late blight (*Phytophthora infestans*)** Antagonistic effect of the current 10 PSB strains against tomato late blight (*Phytophthora infestans*) was determined by dual-inoculation technique (Vaikuntapu *et al.,* 2014) on potato dextrose agar (PDA) plate and direct plant infection under greenhouse. The pathogen *P. infestans* was collected from infected tomato plants then cultured on PDA, purified and preserved in refrigerator. From this stock, refreshed active fungal plug of one cm diameter was placed at the centre of PDA, overnight grown bacterial cultures were then spot inoculated at equi-distance about three cm from the central plug then the plates were incubated at 28 °C for 72 h (Vaikuntapu *et al.,* 2014) and halo zone formation or inhibition was observed. Furthermore, pot experiment was held using tomato under greenhouse condition to evaluate the percentage of disease incidence (DI(%)) and to calculate percent of severity index (PSI(%)). Tomatoes were inoculated with the pathogen after 45 days of sowing with fungal cultural suspension ($3x10^5$ cell/ml) by hand sprayer and covered with plastics for 48 h to maintain humidity and contact (Awan *et al.,* 2018). Un-inoculated pots were set as control. *Unfortunately, the greenhouse pot trial was not effective and no data presented in this paper*.

5. **N_2-fixing ability and Nodulation Test**

   The nitrogen fixation potential of bacterial isolates was tested on nitrogen free media for their growth and Congo red response (Somasegaran and Hoben, 1994). They were inoculated on sterilized YEMA media with addition of Congo red and incubated at 30

^{0}C for 7 days then the growth and Congo red absorbance was recorded. Pot trial was also conducted at shade house using sterilized sand. Surface sterilized faba beans (vr. *Chala*) and haricot bean (vr. *G20390*) were used for this test. Seeds with uniform size were selected, surface sterilized, soaked in active strain suspension, let dry for 30 mn, sown on sand pots (3 seeds per pot) and inoculated with one ml of inoculant with the respective strain arrangements. The experiment was laid out with three replications. Uninoculated and fertilized pots used as control. Total nodule count, shoot fresh weight, shoot dry weight, shoot length, total N in shoot and nodule dry weight was evaluated 45 days after sowing (DAS) (Somasegaren and Hoben, 1994).

The effectiveness of isolates in accumulating plant shoot dry matter was calculated as described in Somasegaren and Hoben (1994);

$$SE\ (\%) = \frac{\text{Inoculated plant DM X 100}}{\text{N – Fertilized plant DM}}$$

Where, DM = dry matter, SE= symbiotic effectiveness

The rate of symbiotic effectiveness would be evaluated as: Highly effective > 80%, Effective 50-80%, Lowly effective 35-50% and Infective <35%.

3.2.5.3 Molecular Characterization and Taxonomic Identification

The morpho-biochemical characterization was supported by molecular characterization. Genomic DNA was extracted from bacterial pure cultures using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's instructions. The primer pairs fD1 and rD1 (AGAGTTTGATCCTGGCTCAG) and (AAGGAGGTGATCCAGCC), respectively (Weisburg *et al.,* 1991), were used to generate a 1500-bp PCR product. The IGS region between 16S and 23S rRNA was amplified using FGPS1490 and FGPS132' primers (TGC GGC TGG ATC ACC TCC TT) and (CCG GGT TTC CCC ATT CGG), respectively (Laguerre *et al.*, 1996). FGPS1490 derives from conserved sequences in the 3' part of 16S rDNA genes, and reverse primer FGPL132' corresponds to the 5' part of the 23S rDNA gene right next to the IGS. Sequences of 16S rDNA are known to be highly conserved among eubacteria, and analysis of genetic variations in this region is not appropriate to differentiate strains within species. Greater variability can be obtained by analysing intergenic spacer (IGS) sequences between 16S and 23S rDNA (16S–23S rDNA IGS sequences) in order to examine chromosomally encoded genetic variations at the intraspecies level (Laguerre *et al*.,

1996). The PCR condition was a 95-degree initial denaturation for 5 minutes, followed by 35 cycles of denaturation at 95 degrees for 30 seconds, annealing at 55 degrees for 1 minute, and extension at 72 degrees for 30 seconds, with a final extension at 72 degrees for 5 minutes. The same primers were then used to sequence the PCR products on the ABI 3730XL genetic analyser. The amplified PCR products were quantified on a 1% agarose gel and then sent to the sequencing lab (MRC Holland). The obtained sequence data quality was checked, trimming or filtering low quality reads, forward and reverse DNA sequences were pairwise aligned, and a consensus sequence was executed using BioEdit software, blasted (BLASTn) from NCBI GenBank to execute the respective genera (Table 3.3). Finally, a phylogenetic tree was constructed using neighbour-joining alignment using MEGA X software.

3.2.6 Data Management

Qualitative data were described as present or absent and elaborated with statements, while quantitative data were analysed as the average and ratio in simple statistics, then organized and presented in tables and figures. For dissolved P, a two-way ANOVA with means compared by the Tukey test using R version 4.2.2 and SAS version 9.4 ($p \leq 0.05$). Means of treatments were differentiated using the LSD. Finally, the presented data were explained and discussed in comparison to other related works.

3.3 RESULT

Firstline isolation from the bulk soil using a solid (PVK agar) medium demonstrated the growth of different microorganisms, including bacteria and fungi, with various colony sizes (small to large), colour (white, creamy with a jelly texture, yellow, purple, black, and pink), and structures (circular, and irregular edges) (Picture 3.1). However, this research was targeted to screen only competent PSB, which showed good growth performance with better clear zone formation. A large number of PSB were isolated from sample soils though greater number of isolates (both in total and per sample) were screened from Meki site followed by Koka and Ziway Zuria. Among them, top 100 isolates were screened, but then 10 isolates were selected step-by-step using preliminary screening techniques. PSB isolates showed various growth rate and halo zone formation; hence, some isolates formed detectable colonies on the 2nd day of incubation, while others developed an observable colony after 72 hours (Supplementary Table 3.2). Similarly, some isolates produced a measurable clear zone

around the colony on the 2nd day, while others produced it after 4 days of incubation (K-10-41 and Mk-1-25) (Figure 3.3). On the other hand, Z-12-20 produced a larger colony and halo zone diameter (0.3 and 0.5 cm, respectively) on the second day (Figure 3.3), nevertheless, it was one of the least pH-reducing agents in broth medium (Figure 3.4).

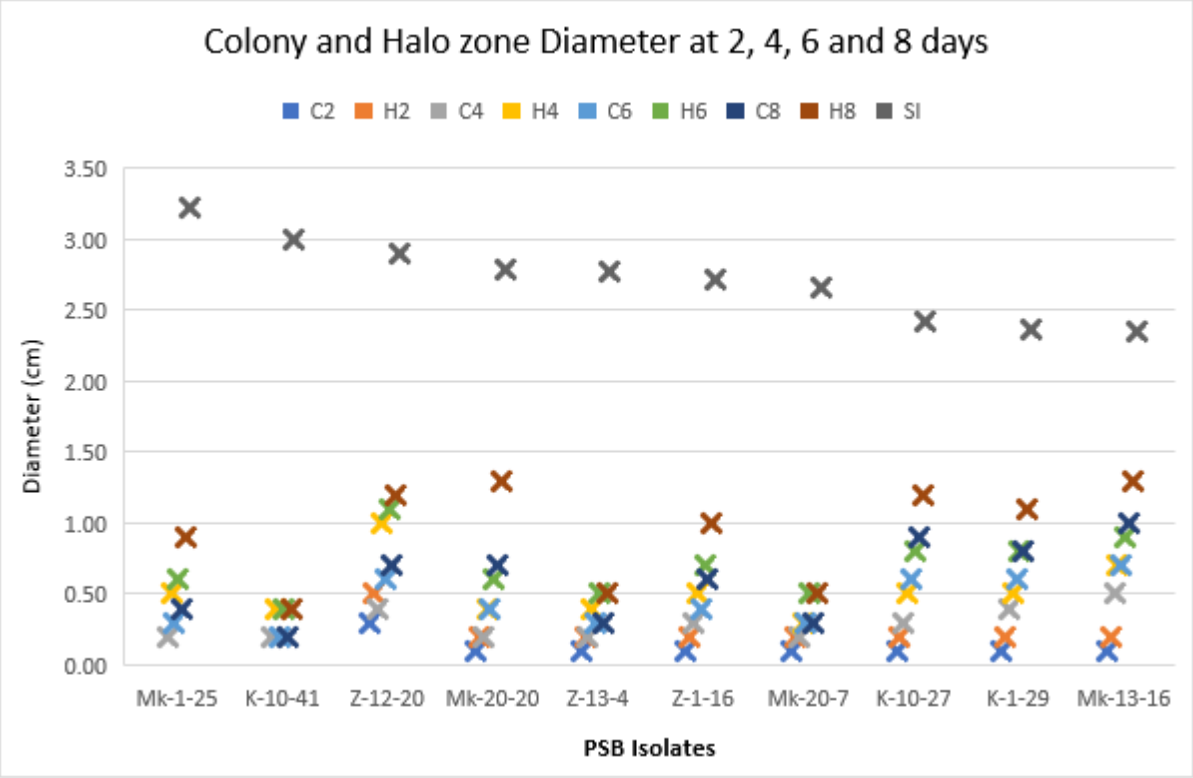
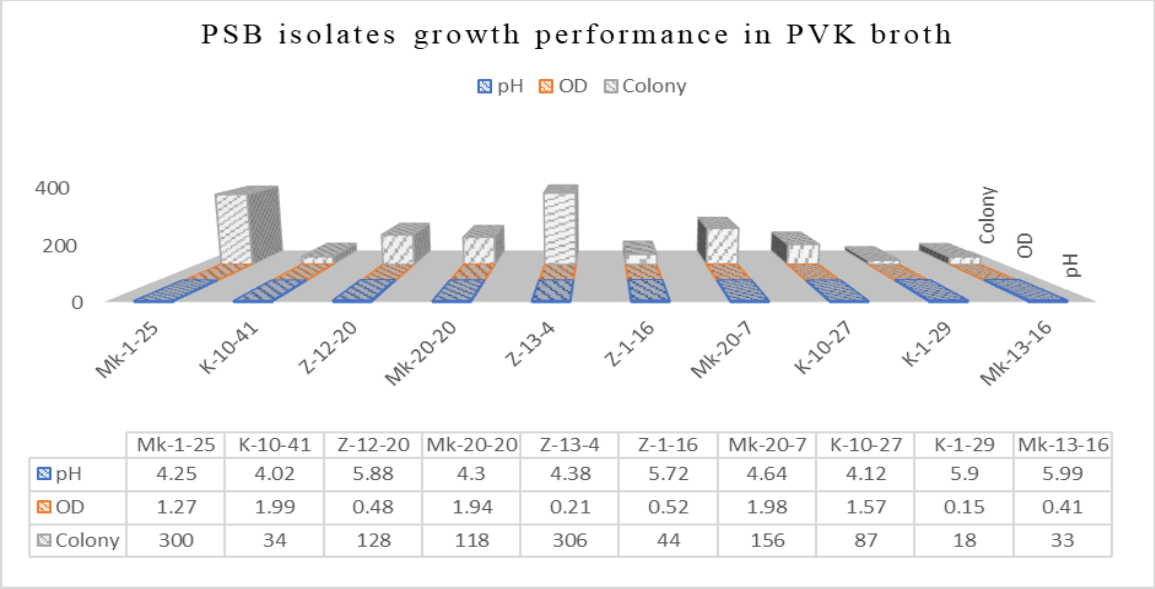


Figure 3.3: PSB isolates colony and halo zone diameter at different incubation periods and Solubilization Index (SI). (C=Colony, H=Halo zone, Mk=Meki. Z=Ziway, K=Koka).

There were more PSB isolates that scored 3 and above SI; due to repeatability, culture impurity, and viability limitations in the consecutive culturing, they were cast off from screening. Among the selected 10 PSB isolates, Mk-1-25 scored the maximum SI (3.22) (Figure 3.3 and Supplementary Table 3.2) with significant pH reduction potential (4.25) (Fig 3.4) while Mk-13-16 recorded the largest colony diameter and the least SI (2.35) (Figure 3.3) as well as the least liquid medium pH reducer (5.99) (Figure 3.4). On the contrary, K-10-41 recorded the lowest colony and halo zone diameters (0.2 and 0.4 cm, respectively) up to 8 days of incubation (Figure 3.3) even though it reduced the liquid medium pH significantly (4.02) (Figure 3.4).



	Mk-1-25	K-10-41	Z-12-20	Mk-20-20	Z-13-4	Z-1-16	Mk-20-7	K-10-27	K-1-29	Mk-13-16
pH	4.25	4.02	5.88	4.3	4.38	5.72	4.64	4.12	5.9	5.99
OD	1.27	1.99	0.48	1.94	0.21	0.52	1.98	1.57	0.15	0.41
Colony	300	34	128	118	306	44	156	87	18	33

Figure 3.4: Effect of PSB isolates on growing medium pH incubated for 5 days.

It is important to verify the growth and P solubilization efficiency of isolates in liquid medium (Figure 3.4, Table 3.5 and Supplementary table 3.1). This is because some isolates showed significant solubilization efficacy on agar plats (higher SI) but then failed to confirm in liquid medium, or the reverse might happen; otherwise, they might show consistent efficacy both on agar as well as in liquid medium. For instance, among the 10 selected PSB isolates, K-10-41 and Mk-1-25 recorded the largest SI (3 and 3.22, respectively (Figure 3.3)) and reduced the medium pH prominently (4.02 and 4.25, respectively) while K-1-29 and Mk-13-16 recorded the lowest SI (2.37 and 2.35, respectively) and the least liquid medium pH change (5.9 and 5.99, respectively) (Figure 3.4).

Table 3.1: Sugar fermentation test.

Isolate	**Sugar type**																					
	Glu		Suc		Fru		Mani		Malt		Xyl		Raf		Sta		Lac		Dex		Ara	
	F	G	F	G	F	G	F	G	F	G	F	G	F	G	F	G	F	G	F	G	F	G
k-1-29	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
k-10-27	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	-	+	+	+	+
k-10-41	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+
Z-1-16	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	+	+	-	+	+	-	-
Z-12-20	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-
Z-13-4	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+
MK-1-25	+	+	+	+	+	-	+	+	+	+	-	-	-	-	+	+	+	-	+	+	+	-
MK-13-16	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-	-
MK-20-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MK-20-20	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-

***NB*:** F= sugar fermentation, G= gas production.

The sugar fermentation ability of the isolates was evaluated on 10 different sugars added to the growing medium independently as carbon sources and incubated for five days. Glucose, sucrose, fructose, dextrose, mannitol, maltose, lactose, and starch were fermented by all isolates and most of them fermented along with gas production; similarly, raffinose except Z-1-16 and Mk-1-25, and arabinose except Z-1-16, Mk-13-16 and Mk-20-20. Xylose was poorly fermented sugar (Table 3.1). Z-1-16 was found to be relatively narrow fermenter (unable to ferment arabinose, raffinose and xylose), whereas K-1-29, Mk-20-7, and Z-12-20 fermented all the given sugars, possibly implicating their capability to utilize broad energy sources.

Table 3.2: Salt and temperature tolerance test.

	Salt concentration (%)										Temperature (^{0}C)				
Isolate	1	2	3	4	5	6	7	8	9	10	15	20	35	40	45
k-1-29	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
k-10-27	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
k-10-41	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
Z-1-16	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
Z-12-20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Z-13-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mk-1-25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mk-13-16	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
Mk-20-7	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
Mk-20-20	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+

All the current PSB strains were able to grow at various temperature (15-45^{0}C) and at low to medium salt concentrations (1-6%), whereas Mk-1-25, Z-12-20 and Z-13-4 were found the most salt resilient strains that grew at 10% (Table 3.2). The colour changes in the triple sugar iron (TSI) test into yellow and red indicating the fermentation of sugars (glucose, lactose, and sucrose), and production of organic acids. The test was conducted on a slant using test tubes. Yellow colour appearance of both the slant and butt indicated the fermentation of the three sugars, while the yellow butt and red or violet slant showed the fermentation of glucose. All of the PSB strains fermented the three sugars as a result the test appeared yellow, confirming a growing medium pH change due to the production of organic acids (Table 3.3). Similarly, all strains detected positive for indole, urease, and catalase tests, whereas all strains except Z-1-16 and Z-13-4 were able to produce HCN (Table 3.3 and Supplementary picture 3.1). Siderophore production was determined using CAS-media, that PSB strains exhibiting an orange hallow zone were detected positive for siderophore production (all PSB isolates detected positive except Z-1-16, Mk-13-16, and Mk-20-7, Table 3.3). K-1-29, Mk-20-7, Mk-

20-20 and Z-13-4 were unable to demonstrate observable antagonistic effect on *Phytophthora infestans* (which was cultured from infected tomato) using dual-inoculation on PDA plate, while Mk-1-25, Z-1-16 and Z-12-20 demonstrated encouraging antagonistic effects and the rest of strains demonstrated limited/no response (Supplementary picture 3.1(B)).

Table 3.3: Ability to grow on different media and pH, enzyme and phytohormone production test and BLAST result with identity percent similarity of DNA sequence.

Isolates	Gram staining	Urase test	Indole test	TSI test	Catalase test	IAA	HCN	Siderophore	N-fixation	Antagonistic	pH						
											4	4.5	5	5.5	6	8	9
k-1-29	+	+	+	y/y	+	+	+	+	+	+	+	+	+	+	+	+	+
k-10-27	+	+	+	y/y	+	+	+	+	+	+	+	+	+	+	+	+	+
k-10-41	+	+	+	y/y	+	+	+	+	+	+	+	+	+	+	+	+	+
Z-1-16	-	+	+	y/y	+	+	-	-	+	+	-	-	+	+	+	-	-
Z-12-20	+	+	+	y/y	+	+	+	+	+	+	+	+	+	+	+	+	-
Z-13-4	+	+	+	y/r	+	+	-	+	+	-	+	+	+	+	+	+	-
MK-1-25	+	+	+	y/y	+	+	+	+	+	+	+	+	+	+	+	+	+
MK-13-16	+	+	+	y/y	+	+	+	-	+	+	+	+	+	+	+	-	-
MK-20-7	+	+	+	y/y	+	+	+	-	-	-	-	-	+	+	+	-	-
MK-20-20	+	+	+	y/r	+	+	+	+	+	-	+	+	+	+	+	+	+

Isolates BLAST identity based on IGS region between16S and 23S rRNA sequence

Isolate	Sample Source	NCBI strain	QC(%)	Identity(%)	E value
k-1-29	T. rhizosphere	*Priestia megaterium*	100	100	2e-120
k-10-27	T. rhizosphere	*Bacillus subtilis*	100	100	0.0
k-10-41	T. rhizosphere	*Bacillus velezensis*	100	100	5e-52
Z-1-16	T. rhizosphere	*Burkholderia cenocepacia*	100	100	0.0
Z-12-20	T. rhizosphere	*Bacillus subtilis*	100	100	0.0
Z-13-4	T. rhizosphere	*Bacillus halotolerance*	100	100	4e-121
MK-1-25	T. rhizosphere	*Bacillus halotolerance*	100	100	5e-126
MK-13-16	T. rhizosphere	*Bacillus amyloliquefaciens*	100	100	0.0
MK-20-7	T. rhizosphere	*Priestia megaterium*	100	100	1e-126
MK-20-20	T. rhizosphere	*Bacillus megaterium*	100	100	8e-170

***NB*:** "+" = positive response, "-" = negative response, r= red, y= yellow, T=tomato, QC=Query Coverage.

Nitrogen-fixing ability of the strains was evaluated both on agar plate and pot trial under shade house. Accordingly, all strains except Mk-20-7 (Table 3.3) were able to grow on N-free agar (YEMA) and absorbed Congo red to the centre of the colony (Supplementary picture 3.1 (C)) while K-10-27, Mk-1-25 and Z-12-20 performed better. Among the strains, 80% of them (except K-10-41 and Mk-20-7) were unable to nodulate with faba bean whereas

all of them have been nodulated with haricot bean (Supplementary table 3.4). Nodule formation might fail because of the nature of the strain unable to nodulate, or host crop preference, non-endophytic or endophytic non-nodulating, etc. Inoculation of faba bean with Z-12-20 resulted in a maximum shoot and root length (54.33 and 22.67 cm, respectively) at 45 days of sowing and the second highest symbiotic effectiveness (126.8%) while Mk-1-25 resulted in shortest shoot length (35 cm) and minimum shoot fresh weight (11.53 g); K-1-29 resulted in a minimum shoot dry weight, root length and SE(%) (2.73 g, 12.67 cm and 99.97%, respectively). Similarly, haricot bean inoculation with Mk-20-20 improved shoot length (81 cm), shoot fresh and dry weight as well as SE(%) (14.27 g, 3.6 g and 120%, respectively), while Mk-1-25 improved shoot dry weight (3.73 g), root length (18.3 cm) and SE(%) (124.47%), Z-1-16 recorded the highest nodule number (13.67) and nodule fresh weight (0.57 g), Z-12-20 recorded the highest nodule dry weight (0.1 g). K-1-29 demonstrated limited response and resulted in shortest root length (13 cm), lower shoot dry weight (2.87 g), and relatively lower SE(%) (95.57%) against haricot bean at 45 days of sowing (Supplementary table 3.4). Moreover, all of them showed effective symbiotic effectiveness (>85%) with both faba bean and haricot bean while Z-12-20, Mk-20-20, Mk-1-25 and Z-1-16 demonstrated an encouraging result for further evaluation and inoculant development.

Similarly, antagonistic effect of the strains against late blight (*Phytophthora infestans*) was evaluated using dual-inoculation technique both on agar plate and direct plant infection. Dual-inoculation response on PDA plate agar indicated that Mk-1-25, Z-1-16 and Z-12-20 demonstrated encouraging antagonistic effects while the rest of the strains demonstrated limited/no observable response. Positive response in this study indicates halo zone formation by PSB strains, mycelial growth inhibition, proximity avoidance or directional growth of *P. infestans*, and unknown colourful metabolite or chemical(s) production (Table 3.3 and Supplementary picture 3.1 (B). Nevertheless, the greenhouse pot trail (the response from direct tomato plant inoculation) was not effective and was not evaluated, no detectable data recorded. This is because the pathogen was not able to attack/invade tomato successfully. Repeated fungal suspension inoculation and even direct contact by infected tomato plants that collected from field was made, yet none of the plant was able to show the infection incidence. The possible reason might be the pathogen couldn't resist moisture and temperature stresses in the greenhouse especially starting from morning 9:00 until 4:00 afternoon, it was too hot. Since, the greenhouse (found in the main campus) controlling system was not functional and

there was no regulatory method for temperature, moisture and air circulation, we tried to control manually, as a result this might not help and create a conducive condition or environment for the pathogen. Antagonistic response is one of the important traits of plant growth-promoting bacteria and one of the essential cost-related alternatives demanding for tomato production. Therefore, by considering the encouraging response from agar plate, this experiment is recommended for future perspective to be repeated and evaluated under optimized conditions.

3.3.1 P-solubilization Efficiency in Liquid PVK Medium

PSB performance in PVK broth supplemented with different P sources was quantified by measuring the rate of pH change and dissolved P. Dissolved P was quantified using a spectrophotometer calibrated with a diluted stock solution; hence, the standard curve developed (Figure 3.2). Strains showed significant differences ($p<0.05$) in pH and dissolved P concentration (Table 3.4). As the incubation period extended until 10 days, the medium pH decreased while the dissolved P amount rose, particularly after the 3rd day of incubation. All inoculated flasks achieved greater dissolved P over uninoculated (control) broth in all sampling days (Table 3.5). Even though the speed and level of efficacy varied among strains, PSB screened from the Koka site in general were found to be strong solubilizers (K-1-29, K-10-41, and K-10-27 dissolved about 260.83, 260.38, and 241.91 µg/ml, respectively). Similarly, TCP was one of the most dissolved P complexes, which resulted in the highest (253.46 µg/ml) phosphorous concentration at 10 days of incubation, while the addition of aluminium phosphate, iron phosphate, and bone meal resulted in comparable P concentrations (211.78, 213.23, and 212.69 µg/ml, respectively) (Table 3.5 and Supplementary Figure 3.1(A)).

Table 3.4: Two-way ANOVA result for pH, absorbance and dissolved P in PVK broth.

Parameter	Source	DF	Day zero	Day 3	Day 5	Day 7	Day 10
Medium pH change	Isolate	10	0.0483*	0.568*	1.6***	1.45***	0.87***
	Treatment	3	0.66**	0.95*	2.21**	1.48***	2.66***
	Residual	118	0.016	0.128	0.2	0.21	0.26
Absorbance	Isolate	10	0.006*	0.078**	0.4***	0.45***	0.359***
	Treatment	3	0.09**	0.756**	0.11ns	0.214***	0.09**
	Residual	118	0.002	0.0273	0.042	0.03	0.016
Dissolved P Concentration	Isolate	10	32.7*	10.14**	4289***	4509***	7621***
	Treatment	3	49.8**	98.49**	1140ns	2133***	13809***
	Residual	118	12.1	3.57	440	310	651

NB: P=0.05 set for LSD. ns: no significance difference at p 5%, * has significance, ** has highly significance difference, *** has very strong difference.

Table 3.5: Mean value of dissolved P (µg/ml) at different incubation days from PVK broth modified with various P substrate and PSB isolates inoculation.

Isolate	Day zero	Day 3	Day 5	Day 7	Day 10
Control	2.08±2.45^{c}	12.16+2.86^{d}	111.97+27.71^{g}	123.32+23.83^{d}	159.27+37.3^{c}
K-1-29	6.9±5.47^{ab}	14.1+2.06^{abc}	176.15+16.97^{a}	190.7+37.32^{ab}	260.8+53.77^{a}
K-10-27	6.9±5.99^{ab}	14.1+3.33^{abc}	166.75+8.5^{ab}	181.19+2.15^{bc}	241.9+52.05^{a}
K-10-41	8.32±6.19^{a}	13.7+1.12^{bc}	153.12+30.77^{bcde}	199.17+36.11^{a}	260.4+54.44^{a}
Mk-1-25	4.4±3.92^{bc}	13.25+2.23^{cd}	162.54+9.95^{abc}	182.47+1.27^{bc}	220.47+9.59^{b}
Mk-13-16	5.16±5.13^{b}	14.79+1.71^{ab}	148.70+16.3^{cde}	182.44+2.91^{bc}	222.42+4.23^{b}
Mk-20-20	5.37±3.98^{b}	13.3+0.98^{bcd}	143.39+14.21^{def}	182.45+3.22^{bc}	221.54+4.51^{b}
Mk-20-7	4.79±4.21^{bc}	15.55+5.07^{a}	126.62+27.35^{fg}	173.4+23.83^{c}	219.39+24.18^{b}
Z-1-16	5.6±5.86^{ab}	14.77+0.01^{abc}	165.16+21.18^{abc}	184.31+5.24^{bc}	221.02+6.10^{b}
Z-12-20	4.77±5.04^{bc}	13.74+0.66^{bc}	157.97+23.24^{bcd}	181.26+2.72^{bc}	223.52+3.43^{b}
Z-13-4	4.27±4.36^{bc}	14.49+0.94^{abc}	139.45+25.57^{ef}	182.29+3.26^{bc}	221.84+5.17^{b}
CV	65.3	13.5	13.97	9.86	11.45
LSD	2.81	1.53	16.96	14.22	20.62
P Treatments					
AlP	2.19±1.22^{b}	14.37+1.69^{ab}	146.36+21.72^{b}	174.18+19.29^{b}	211.78+20.24^{b}
BM	10.53±2.48^{a}	12.19+2.0^{b}	144.49+28.84^{b}	175.57+24.33^{b}	212.69+27.65^{b}
FeP	2.61±2.68^{b}	13.21+1.18^{b}	157.34+25.0^{a}	173.62+22.62^{b}	213.23+28.42^{b}
TCP	5.98±6.33^{ab}	16.22+2.84^{a}	152.48+32.17^{ab}	190.45+32.79^{a}	253.46+52.91^{a}
CV	65.3	13.5	13.97	9.86	11.45
LSD	1.7	0.92	10.23	8.58	12.44

NB: Means with similar letters have no significant difference at p=0.05.

Microbial growth increased the production of various metabolites and/or compounds, including organic acids. These organic acid accumulations lower the medium pH, which indirectly advocates the concentration of dissolved P (Table 3.4 and 3.5). The solubilization capacity analysis of individual isolates in relation to a particular P-treatment exhibited significant response variation along with substrate preference. The majority of isolates preferred TCP-added broth. For instance, K-10-41, Mk-20-20, Mk-1-25, and Z-13-4 substantially lowered the pH of TCP-containing broth, whereas Z-1-16 exceptionally reduced AlP-containing broth at the 10th day of incubation (Figure 3.7 and Supplementary Table 3.3). This substrate-based response of isolates to demonstrate their utmost efficacy might suggest the superlative environment and favourable habitat/ecology to be recommended as inoculum. K-10-27 dissolved the highest (343.9 µg/ml) and relatively the lowest (215.58 µg/ml) P in TCP and FeP-containing broth, respectively; K-10-41 dissolved 337.9 and 223.38 µg/ml in TCP and AlP broth, respectively; while Mk-1-25 recorded the lowest dissolved (201.96 µg/ml) P in TCP and showed good competence in FeP and BM-added broth (Supplementary Figure 3.2).

3.3.2 PCR and DNA Sequence Data

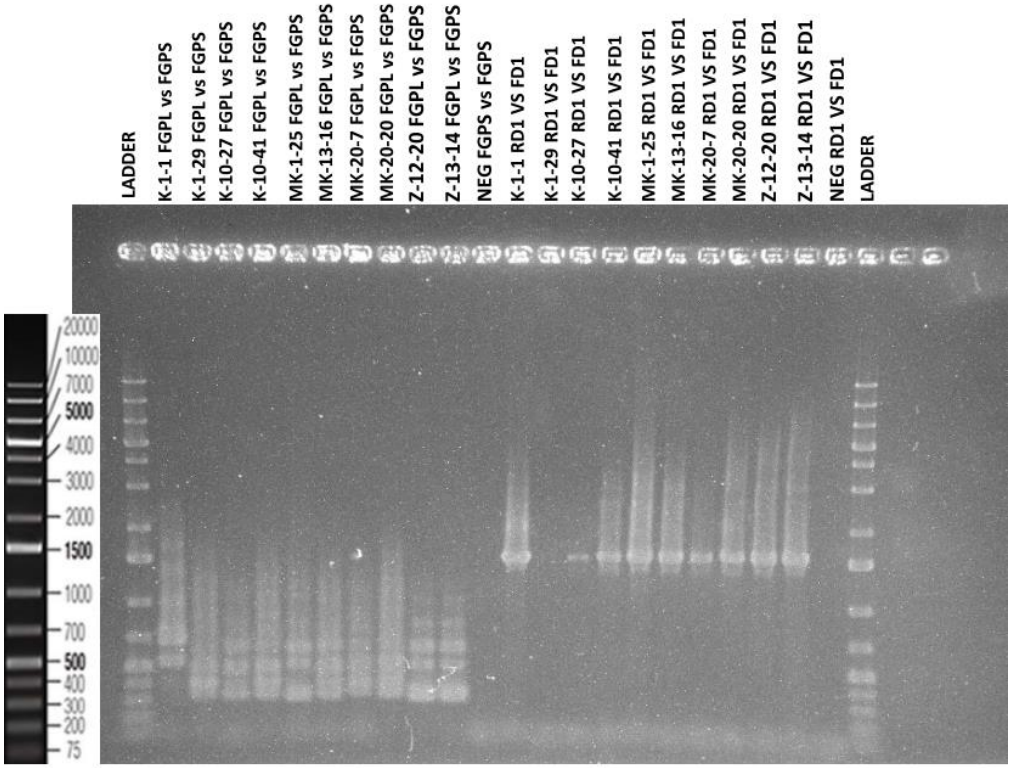


Figure 3.5: Agarose gel image for amplified 16S rDNA and IGS region between 16S and 23S rRNA.

Extracted DNA from each candidate bacteria was amplified with PCR using two primers (targeting the 16S region and IGS between the 16S and 23S rDNA regions) and quantified using a 1% agarose gel. Accordingly, DNA with 1500 bp base length was amplified from each sample, but a better PCR product was obtained with IGS region-based primers (Figure 3.5). The sequence data also confirmed that most of the bacterial 16S DNA sequence data were poor in quality, while all IGS between 16S and 23S region sequence data were of good quality. Therefore, because of the poor PCR product at 1500bp, quality of the current 16S DNA sequence data and poor distinguishing ability among related species, it was excluded, whereas IGS was used to infer taxa and evolutionary history (Figure 3.6). All the current strains except Z-1-16 were placed to *Bacillus* genus; however, due to the availability of technology and resources, *Priestia* species which were previously placed under *Bacillus*, now a days, they stand as a separate taxon. Accordingly, these 10 PSB isolates were placed into three genera, namely, *Bacillus*, *Priestia* and *Burkholdoria* (Table 3.3 and Figure 3.6). Among the ten PSB strains, three (K-1-29, Mk-20-7, and Mk-20-20) fall under the *Priestia megaterium* subspecies, while K-10-27 and Z-12-20 were grouped under *Bacillus subtilis*, Mk-1-25 and Z-13-4 grouped under *Bacillus halotolerance*, K-10-41 are grouped under

Bacillus velezensis, Mk-13-16 is grouped under *Bacillus amyloliquefaciens*, and Z-1-16 is grouped under *Burkholderia cenocepacia* (Table 3.3 and Figure 3.6). Furthermore, whole genome sequencing is required to place the strain's accession number precisely.

Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length =2.18961171 *is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). (next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 19 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1106 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).*

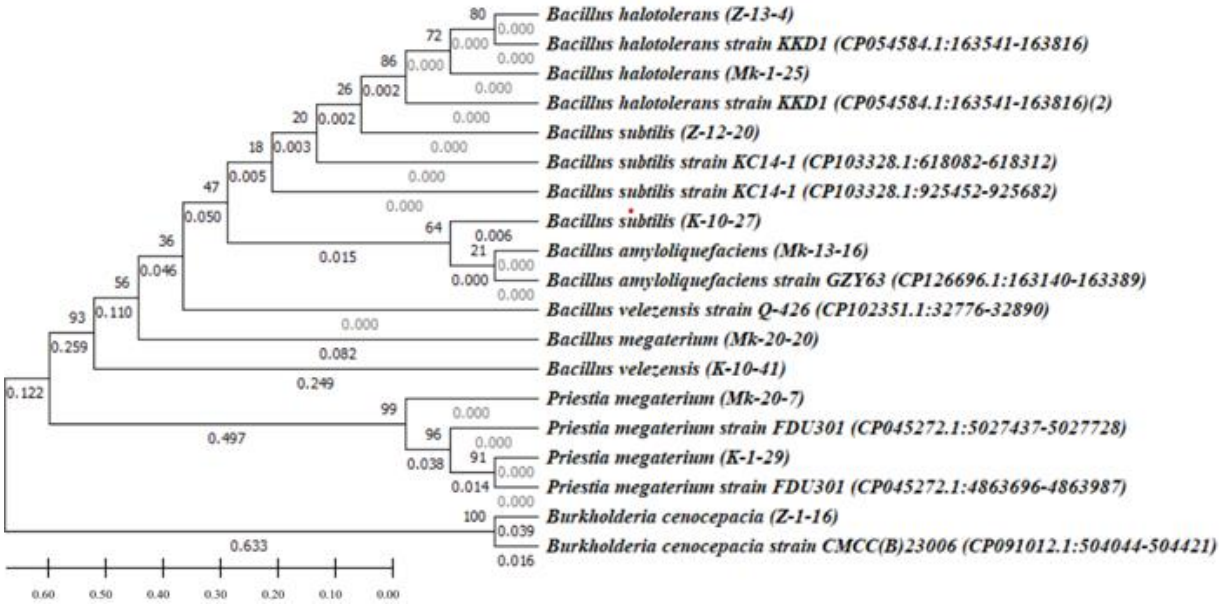


Figure 3.6: Phylogenetic tree for IGS between 16S and 23S rDNA sequence for the 10 PSB strains using Neighbour-joining method with MEGA X.

3.4 DISCUSSION

The reactive nature of phosphate ions makes them unavailable for biological utility (Wan *et al.*, 2020; Prabhu *et al.*, 2019). As a result, transition and transformation mechanisms of physical, chemical (like pH change), and biological (such as microbial) activities influence the release of plant-available P (Qian *et al.*, 2010). As demonstrated by Behera *et al*. (2014), microorganisms play a significant role in the natural P-cycle (solubilization and mineralization) process via phosphatase enzymes, chelating, and metabolites (organic acids).

Understanding the role of microbes in P-transformation leads to screen efficient strains that are then applied as inoculant to improve plant-available P and agricultural production. PSB are usually found in soil, water bodies, and sediments (Wan *et al*., 2020). However, Verma (2019) showed that microbial diversity in soil is much greater than in other environments because soil offers various microhabitats and root exudates (rich sources of energy and nutrients). Zhang *et al.* (2021) also stated that PSB are widely distributed in different soils, which can benefit plants in various ways. Moreover, different reports demonstrated that the rhizosphere soil contains more PSB population than non-rhizosphere soil (Waday *et al.,* 2022; Pande *et al.,* 2017; Ahemad and Kibret, 2014; Hariprasad and Niranjana, 2009).

Using PVK agar medium, a large number of PSB were isolated from tomato rhizosphere soil collected from the three study sites (Koka, Meki, and Ziway Zuria). Farmlands in the study area are used for crop production in both seasons and promote aggressive agrochemical consumption. Even if it is expected to decline beneficial microbes in such farming systems (Zaidi *et al.,* 2009), it was found that all tomato rhizosphere soil samples comprised bundles of P-solubilizers. In a similar way, Baliah *et al.* (2016) reported that PSB populations were found to be rich in tomato rhizosphere. The availability of enriched root exudates synchronizes plants and the microbial community in the rhizosphere (More *et al.,* 2019). Plants control the rhizomicrobial community composition and abundance by regulating root exudate molecules (timing, amount and constituent) and signal molecules which can favour specific species and regulate their genetic and biochemical activities (Rachel *et al*., 2018). It is also stated that phosphate-solubilizing microbes are abundant (20–40% of the total population) in the rhizosphere to compete for root exudates (Kour *et al*., 2021). Likewise, Yadav and Verma (2012) described that among soil P-solubilizing microbes, bacteria constitute 1–50% of the total P-solubilizers with great fold solubilization efficiency. Sharma *et al.* (2011) also stated that high population and metabolically active PSB concentrated in the rhizosphere.

Halo zone formation on agar PVK medium was used as the first line detection of P-solubilization from TCP ($Ca_3(PO_4)_2$) by the candidate strains (Naik *et al*., 2008; Alikhani *et al.,* 2007). Among the three sites, a relatively large number of PSB isolates (both per sample as well as in total) were screened from Meki, followed by Koka, then Ziway Zuria. Different contributing factors can be mentioned, including the environmental conditions, soil profile (Supplementary Table 3.1) (Mesfin, 2020; Beshir *et al.,* 2015; Negash and Mohammed,

2014), farm production and cultivation trends, as well as the natural population of the microbial community, among others. In support of this, Rachel *et al.* (2018) and Zhalnina *et al.* (2018) described that microbial communities and their activities in the rhizosphere are governed by plant species, geographical locations, climate, and farm management. Soil characteristics, pH, moisture, and temperature are also deemed to be the limiting factors (Zhang *et al.*, 2023; Nosheen *et al.*, 2021). Undeniably, a large number of soils taken from Meki sites due to the long tomato and other vegetable production history, wide range of farmlands, and many smallholders participation in tomato production. It was observed that most farmlands engaged in vegetable cultivation in line with other crop production both in the dry and wet/rainy seasons (Chapter two) due to the availability of conducive environments and resources.

Kirui *et al.* (2022) and Aliyat *et al*. (2020) described SI and colony morphology as primary standards for the selection of PSB isolates. During isolation, different microbes (Picture 3.1) appeared in various colony shapes (round, smooth, rough, and branched), sizes (small, medium, and large), and colours (white, yellow, creamy-white, pink, purple, green, blue-green, and black). Similarly, Sharon *et al*. (2016) demonstrated the variation of PSB colony structures in size, shape, and colour. It was also observed that a few PSB isolates (e.g., K-10-41) showed antibiotic effects on neighbouring colonies that made a clear zone around the colony, possibly indicating self-defence and niche or nutrient competition. Supportively, Wang *et al.* (2017) have shown the impact of antibiotic production by soil microbes to control colonization dynamics in the rhizosphere.

Following the preliminary screening method, the final 10 PSB isolates were selected with a SI range of 2.35–3.22 (Figure 3.4) which is comparable with Fenta and Assefa's (2017) report who isolated PSB from tomato rhizosphere (SI = 1.8-3.5), Mohamed *et al*.'s (2018) report who screened 3 PSB strains with 2.8–3.2 SI from tomato, and Muleta (2018) screened 5 strains (SI = 1.5–3.3) from soyabean rhizospheres. The current maximum SI (3.22) by Mk-1-25 was higher than Paul and Sinha's (2017) finding that the KUPSB12 strain isolated from a river sample formed 2.85; and lower than the findings of Haile *et al.* (2016), who reported 4.5 SI by HUPSB-35 and HUPSB-45 that were screened from lupine rhizosphere soil from Gojam, Ethiopia. Similarly, Wan *et al.* (2020) published PSB strains grown on solid NBRIP media with different P sources that produced a higher halo zone to colony ratio of 4.62 (TCP), 6.75 (phytate), and 8.5 (AlP). In addition, different reports have shown that PSB

strains with SI $\geq$ 4 were screened from various rhizosphere samples, including PSB_1 from the coffee rhizosphere with 5.09 (Waday *et al.*, 2022), from maize with 4.88 (Pande *et al.*, 2017), from phosphate mines with 4.79$\pm$0.57 (Aliyat *et al*., 2020) and from natural coffee 2.05-5.82 (Muleta *et al.*, 2013). However, agar plate quantification is a rough P solubilization potential measurement (Sharon *et al.*, 2016); therefore, it should be supported with additional liquid medium quantification (Alikhani *et al.*, 2007). In a good agreement, Kour *et al*. (2021) stated that clear zone formation is not a sole selection criterion, but rather should be strengthened by other supplementary screening methods.

3.4.1 Quantification of Dissolved P

PSB exhibited a distinctive ability to utilize specific nutrient sources (Wan *et al.*, 2020; Chandra *et al.*, 2018; Pathak *et al.*, 2017). The type of carbon source and P-substrates added to the growing media would determine the strain's P-solubilization efficiency. Davis *et al.* (2005) also have shown that the type of growing medium significantly determined the size and colony number of the soil bacterial community. Similarly, Behera *et al.* (2014) underlined that microbial P solubilization efficacy depends on different factors, such as the nutritional, physiological, and growth conditions of the inoculant. In the current experiment, the majority of PSB strains demonstrated broad sugar fermentation (Table 3.1). This might suggest that these isolates are capable of utilizing most of the sugars from the plant root exudates in the rhizosphere. On the other hand, they have shown a significant amount of dissolved P (57.5–101 µg/ml) differences compared to the un-inoculated (control) group (Table 3.5). TCP was typically and preferably solubilized by the majority of the strains, though there was no overstated difference among the rest of the P-complexes (Supplementary Figure 3.1(A)). In agreement with this, Wan *et al.* (2020) reported that TCP was the optimal P-source for PSB in liquid media because TCP is more pH-sensitive than other inorganic P compounds (Zhang *et al.*, 2023). Sharon *et al.* (2016) also strengthens this finding that PSB strains isolated from tomato and potato rhizospheres dissolved much greater P from TCP than FeP-containing medium. In addition, Muleta (2018) reported that a PSB strain, EPS1, released more dissolved P from TCP than FeP-containing broth. However, the current result contradicts with Mukhtar *et al.*'s (2017) finding that 7-days incubation of PSB strains in RP-modified PVK broth composited with sucrose and maltose as a carbon source resulted in 75.9 and 55.92 µg/ml dissolved P, respectively, whereas the usual glucose-based PVK broth resulted in a low (49.92 µg/ml) amount of dissolved P.

Determination of dissolved phosphorous from various solutions containing different phosphorous forms using phosphate ion reactions against the reagents is a common colorimetry method (O'Dell, 1993). It is known that several microbes would show different solubilization efficiencies in solid and/or liquid media. PSB strains in this experiment demonstrated significant competence on solid as well as liquid PVK media. Among the 10 tested PSB, K-10-41 verified the overall solubilization efficiency and recorded a higher SI (3) on the agar plate (Figure 3.3) and elevated dissolved P in the broth (260.38 µg/ml (Table 3.5: Supplementary Figure 3.1 (B) and Supplementary Figure 3.2)). An extraordinary finding by Pande *et al.* (2017) have stated that PSB strain (C1) showed a maximum SI (4.88), dissolved P (305.49 µg/ml), and low medium pH (3.08). Likewise, Waday *et al.* (2022) reported an isolate from the coffee rhizosphere that demonstrated a maximum SI of 5.09 and 270 µg/ml dissolved P. On the other hand, K-1-29 showed a lower SI (2.37) on the agar plate; nevertheless, it was recorded the highest overall dissolved P (260.83 µg/ml (Table 3.5 and Supplementary Figure 3.1(B))) in PVK broth. A supportive finding reported by Sharon *et al.* (2016), an *Enterobacter sp*. from potato showed a small index on an agar plate and dissolved a significant amount of P in liquid medium. In contrast, Mk-1-25 showed the highest solubilization clear zone surrounding the colony (SI = 3.22) then failed to repeat in liquid media and recorded the lowest (216.77 µg/ml) overall mean dissolved P.

The specific interaction analysis of each isolate against individual P-sources showed a strong variation in the concentration of dissolved P. For instance, Mk-1-25 was among the top solubilizers in BM and FeP-added broth (ranked 1st and 2nd with corresponding 228 and 225.64 µg/ml P, respectively), whereas it dissolved relatively low P in AlP and TCP broth at the 10th day of incubation (Supplementary Figure 3.2). This result was much greater than Rodríguez *et al.'s* (2006) report that 7 days of incubation of *Serratia sp.* S119 in modified NBRIP broth with FeP and AlP resulted in 17.2 and 141.6 µg/ml of dissolved P, respectively. Then again, K-10-41 was one of the top inoculants in overall dissolved P (260.83 µg/ml (Table 3.5)) and ranked 3rd in TCP, FeP and AlP (with corresponding 337.89, 225.14, and 223.38 µg/ml solubilized P, respectively) and 10th in BM-containing broth (with 212.84 µg/ml P). Similarly, K-10-27, the 3rd solubilizer in overall solubilized P concentration (Table 3.5), nonetheless, it dissolved prominent P in TCP and AlP comprising broth (1st and 2nd with 343.9 and 224.48 µg/ml, respectively), while it was one of the least P dissolvent in FeP (9th with 215.58 µg/ml) (Supplementary Figure 3.2). In general, PSB strains were found to be

promising P-solubilizers from different P-complexes; hence, the current result was higher than Qian *et al.'s* (2010) findings on the 10-days of incubation of the most efficient bacterial strain isolated from shallow lake and wetlands, which recorded about 170 µg/ml of accumulated P. Pandey *et al.* (2006) also stated a maximum solubilized P (247 µg/ml) by *Pseudomonas putida* at 15 days of incubation. It should be clear that quantifiable P in the supernatant is either dissolved or assimilated from microbial cell tissues. Comparatively, a specific substrate-based response will give an indirect clue about the background of enzymes they possibly produced, the possible environmental preconditions (pH, organic matter, type of P-supplements), and the pace or rate of inoculum.

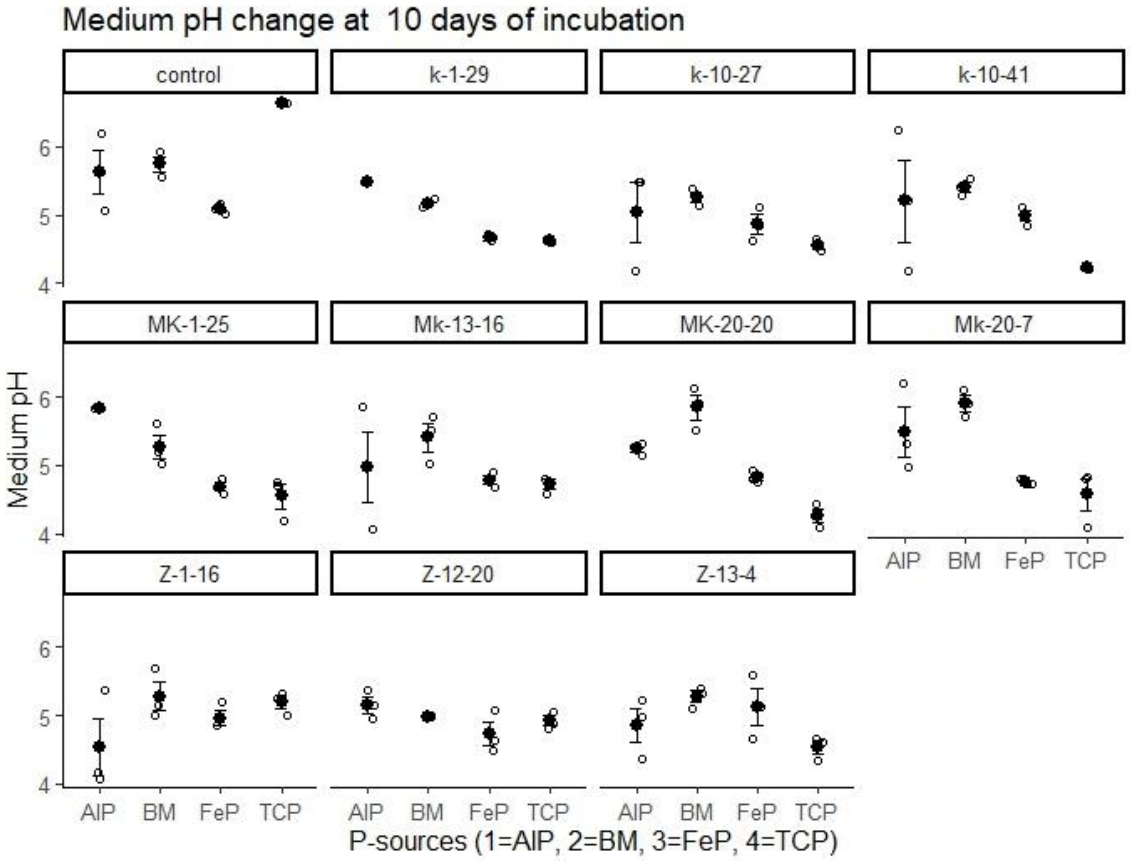


Figure 3.7: The effect of PSB strains on the pH of PVK broth modified with P-complexes.

3.4.2 pH Change

Medium pH change is considered one method for indirect estimation of P-solubilization (Kour *et al.*, 2021; Baliah *et al.*, 2016; Sharon *et al.*, 2016; Zaidi *et al.*, 2009). Almost all the PSB isolates lowered the broth medium pH to an observable level over the control (uninoculated flasks) (Figure 3.4 and Supplementary Table 3.3). Similarly, Chen and Liu

(2019) found a PSB strain (S32) significantly solubilized P from TCP by lowering the medium pH to 3.51. Kaur and Reddy (2013) also showed that P solubilization is accompanied by a pH reduction of up to 3.7 upon 5 days of incubation. In concurrence, Estrada-Bonilla *et al*. (2021) and Naik *et al*. (2008) demonstrated that PSB strains inoculated in PVK medium reduced pH to 4.8–3.41 at different incubation periods. Microbial activities induce pH change because of different metabolites (Babalola *et al*., 2021), including siderophores and organic acids, which in turn create a reactive medium to promote the solubilization of water sparingly soluble phosphate compounds, dissociate the metallic ions (Al^{3+}, Fe^{3+} and Ca^{2+}) then release phosphate ions. Yadav and Verma (2012) also showed that PSMs increased TCP ($Ca_3(PO_4)_2$ solubilization by secreting organic acid and reducing pH, which led to a high affinity for calcium and a higher solubilization of phosphorous.

Bacterial organic acid production, metabolism, and genetic regulation are correlated with the level of P-solubilization (Yang *et al.,* 2023). Medium acidification from the initial pH range (7) clearly indicated the production of organic acids (Paul and Sinha, 2017). As indicated by Rachel *et al.* (2018), PSM excrete organic acids and H^+ to solubilize from Ca, Fe and Al-phosphate. The most common low-molecular-weight organic acids linked to microbial P dissolution are oxalic, lactic, citric, malic, succinic, acetic, gluconic, and formic acids (Zhang *et al.*, 2023; Pathak *et al.,* 2017; Sharon *et al.*, 2016; Hariprasad and Niranjana, 2009). Similarly, Rodríguez *et al*. (2006) showed that PSB strain inoculation in NBRIP broth (modified with $FePO_4$ and $AlPO_4$) significantly reduced the pH to 2.84 and 3.15, respectively, which strongly deviated from the current findings of 4.8 in TCP, 4.86 in FeP, 5.22 in AlP, and 5.41 in BM broth (Figure 3.4 and Supplementary Table 3.3). On the contrary, Fenta and Assefa (2017) reported lower pH valve (4.33) and dissolved P from BM composited broth than TCP (pH=6.25 at 10 days of incubation). As demonstrated by Chen and Liu (2019), 7-days incubation of the S32 strain on different P sources showed that type of substrate and solubilization efficiency were strongly linked; priority was given to TCP to solubilize larger P, while the least preferred substrate was ferrous phosphate (i.e. TCP > $AlPO_4$ > FePO4), and the medium pH was reduced to 3.51, 3.46, and 4.03, respectively. In agreement, Zhang *et al.* (2023) revealed that TCP dissolved at an acidic pH while iron and aluminium phosphates required a stronger acidic (pH 2.5 or below) medium to dissolve. Likewise, Cui *et al*. (2022) reported that FeP has a lower solubilization rate than TCP and requires a pH of 2–2.5; thus, the dissolution rate in soil is limited because of buffering capacity.

As bacterial incubation days increased, their population and metabolites exponentially increased, especially in the first 5 days; consequently, rapid medium pH change and dissolved P concentration were recorded (Supplementary Table 3.3, and Supplementary Figure 3.1). In agreement with this, Muleta *et al.* (2013) reported that liquid medium pH was strongly reduced from 7 to 4.83-3.93 by PSB strains at day 3 of incubation while in the remaining days no further pH change was observed. Supportively, Qian *et al.* (2010) and Mohamed *et al.* (2018) stated that bacterial growth patterns would suggest the accumulated P concentration. Wan *et al.* (2020) also indicated that the expression of P-cycling-related genes exponentially increased along with the extracellular phosphorous concentration, then declined at longer incubation. Correspondingly, Mukhtar *et al.* (2017) reported that six PSB strains screened from sugarcane, wheat, and the *Atriplex amnicola* rhizosphere reduced the PVK broth pH and increased P-solubilization up to 14 days. Moreover, many reports (Behera *et al.*, 2019; Pande *et al.,* 2017; Rodríguez *et al.,* 2006; Pandey *et al.,* 2006) have indicated that lowering the medium pH negatively correlated with dissolved P in the medium. In agreement to this, incubation day has a positive correlation while medium pH change has negative correlation with dissolved P (Muleta *et al*., 2013).

The natural holobiont (plant-microbiomes association) is complex phyto-microbiome interaction; relatively a constant partner relationship; between a plant and microbiomes that are ancient and long co-evolution (Rachel *et al.,* 2018). The information concerning their relationship is minor so, the detail understanding and knowledge still remains. However, it is expected to discover various additional and surprising relationships that are beneficial to plants. Phyto-microbiome relation is regulated and governed by the plant as well as microbial community (Rachel *et al.,* 2018) on top of non-biological factors. For instance, the plant control over rhizo-microbiome by root exudates of various composition (sugars, amino acids, organic acids, and complex secondary metabolites) and signal molecules which can be more suitable and recruit specific species (hub-species), regulate their genetic and biochemical activities while the microbiome in-turn produce quorum sensing compounds when warrant conditions and physiological shift plants to respond, produce and release phytohormones, secondary metabolites and volatile organic molecules to boost plant growth, root architecture, biomass, response to stresses and elevate photosynthetic capacity (Rachel *et al.,* 2018). Moreover, the root-microbiome (abundance, diversity and bacterial community structure) influenced and shaped by host genetic variation and environmental conditions (Quiza *et al.,*

2023) as a result, beneficial microbial communities currently are gaining attention in crop breeding programs (Bergelson *et al.,* 2019). Supportively, a rhizosphere metagenomic study of different wheat genotypes were resulted in a significant variation of rhizomicrobes (both in diversity and microbial activities), mostly visible in rhizobacteria, among wheat genotypes, plant growth stage and plant compartment (Quiza *et al.*, 2023). GWAS study on Arabidopsis root-microbes indicated that plant genes associated with rhizobacteria phenotype include genes that involve in immunity (falvin monooxygenase), cell wall modification, sugar processing, cellulase activities, root development (radial pattern formation, root morphogenesis, pectate activities, vascular and aging) (Bergelson *et al.*, 2019),

Furthermore, the molecular basis of plant-rhizobacteria interaction mechanisms responsible for physiological changes have been recognised with the help of omics (Rachel *et al.,* 2018). A complete genome sequence of PGPR *Bacillus* sp. strain OA1 indicated plant growth promotion mechanisms and related bacterial gene ontology (i.e., N-fixation (*nif* gene and nitrogen regulatory protein), phosphate solubilization (phosphate ABC transporter, substrate binding protein, chemotaxis response phosphatase CheZ, ribose 5-phosphate isomerase A, glucose-6-phosphate-1-dehydrogenase, pyrophosphokinase and nucleotide 5-triphosphatase Rdg8), siderophore (ferrichrom transport ATP-binding protein FhuC, ferrichrome transport system permease protein FhuG, ferric uptake regulation protein FUR), IAA production (IAA acetyl transferase, auxin efflux carrier family protein) and exopolysaccharide production (ExoD protein and exopolyphosphatase) (Babalola *et al*., 2021).

3.4.3 The Taxonomy of the PSB

Based on their characters (morpho-biochemical and DNA sequence), the current 10 PSB isolates are taxonomically grouped under three genera *Priestia* (basonym: *Bacillus* (Shwed *et al*., 2021), *Bacillus, and Burkholdoria* and six species (i.e., K-1-29, Mk-20-7, and Mk-20-20 belong to *Priestia megaterium*, while K-10-27 and Z-12-20 belong to *Bacillus subtilis*, Mk-1-25 and Z-13-4 belong to *Bacillus halotolerance*, K-10-41 belongs to *Bacillus velezensis*, Mk-13-16 goes to *Bacillus amyloliquefaciens*, and Z-1-16 was grouped under *Burkholderia cenocepacia* (Figure 3.6). These beneficial bacterial strains are repeatedly reported in different publications by demonstrating comparable results and characteristics obtained from this experiment and other plant growth-promoting traits. For instance, Rabbee *et al*. (2019), Verma (2019) and Karnwal (2017) described that P-solubilization, HCN, IAA, and

siderophore production are frequent and known plant growth-promoting traits of the dominant and most important PGPR species of *Bacillus* and *Pseudomonas*. In good agreement, Kumar *et al.* (2022) indicated that bacterial strains from *Bacillus* and *Burkholderia* showed P-solubilizing ability along with HCN, cytokinin and IAA production. Ahemad and Kibret (2014) stated that 80% of rhizobacteria from various crops synthesized and released IAA as secondary metabolite which plays an important role in rhizobacteria-plant interaction as well as act as a reciprocal signalling molecule affecting gene expression in several microorganisms. Similarly, Sharon *et al.* (2016) indicated that soil bacterial genera such as *Bacillus, Pseudomonas,* and *Enterobacter* were found to be the most powerful phosphate solubilizers. Nosheen *et al.* (2021) indicated that bacterial species from *Bacillus, Pseudomonas* and *Rhizobium* are the dominant biofertilizers applied as P-solubilizers and plant growth-promotors. Rafi *et al.* (2019) also strengthen the current findings that PSB isolates from the rhizosphere of wheat, alfalfa, cotton, and tomato-grown fields in semiarid regions are predominantly identified as *Bacillus and Pseudomonas species*. Muleta *et al*. (2013) also reported that 51 *Burkholderia,* 147 *Bacillus*, 134 *Pseudomonas* and other PSB strains were screened from Bonga and Yayu natural coffee forest. Moreover, a meta-analysis on performance of beneficial bacterial species indicated that application of *Burkholderia, Bacillus* and *Pseudomonas* spp. displayed positive effects on plant P-uptake, shoot- and root-biomass though, *Burkholderia* spp. outperforms more followed by *Bacillus* spp. (De Zutter *et al*., 2022). As indicated by Mącik *et al*. (2023), *Burkholderia* spp. benefit plants more by solubilizing potassium in addition to P.

3.5 CONCLUSION AND RECOMMENDATIONS

Tomatoes' rhizosphere was found to be rich in PSM, and the upmost 10 competent PSB strains were selected and evaluated under different conditions. Most of the selected strains were found to be efficient P solubilizers from different sources, able to utilize broad carbon sources, lower the medium pH possibly due to organic acid production, and produce plant growth-promoting traits. Plate screening is a first-line technique to isolate potential PSB; however, it should be supported with other techniques, including liquid medium evaluation, to screen efficient strains. This is because isolates might demonstrate elevated potential on the plate, then fail to repeat in liquid media (as Mk-1-25) or (as K-1-29) show limited solubilization on the plate and find excellent solubilizer in liquid media; if not, they might

verify their competence both on the plate as well as in liquid media (as K-10-41 and Z-12-20). Therefore, K-1-29, K-10-41, and Z-12-20 are potential and multifunctional candidates to develop biofertilizers that may exhibit positive outcomes in the plant growth-promotion, agro-ecosystem, improving soil fertility in a sustainable manner and using an eco-friendly approach. Subsequently, by verifying their performance under greenhouse and field conditions, these selected strains could be applied for tomato and other crops production at Koka, Meki, and Ziway Zuria. Whole genome sequence is recommended to assign their exact strain accession number. Furthermore, future work should concentrate on evaluation of these strains at greenhouse and field conditions and investigate more efficient strains supported by molecular techniques. Biofertilizer and plant growth-promotors development optimization with multifunctional PSB strains will improve soil fertility, organic farming, crop production, and environmental health.

CHAPTER FOUR

4. DUAL-INOCULATION EFFECT OF PSB STRAINS AND POSSIBLE P-SOURCES ON TOMATO GROWTH PERFORMANCE UNDER GREENHOUSE CONDITIONS.

Abstract

A pot experiment was conducted at a greenhouse using 10 pre-screened PSB strains to evaluate tomato growth performance and symbiotic effectiveness. Tomato requires intensive application of agrochemicals, and its production in Ethiopia is low compared to other tomato-growing regions. PSB strains have tremendous benefits in maintaining soil fertility, producing phytohormones, alleviating plant stresses, and promoting beneficial microbial activities. For this experiment, each isolate was co-inoculated separately with different external possible P sources ($AlPO_4$, $Ca_3(PO_4)_2$, $(NH_4)_3PO_4$, bone meal, DAP fertilizer, and compost) and set in three replications. The inoculated tomatoes showed significant differences from the controls, and most plant parameters showed significant differences among P treatments. Especially, four strains (K-1-29, Mk-1-25, Mk-20-20, and Z-12-20) showed substantial improvement in tomato growth performance. For instance, Mk-20-20 promoted seed germination and fruit yield; K-1-29 induced the number of leaves and nodes at the early growth stage, flower clusters, buds, number of fruits, and stem thickness; Mk-1-25 promoted shoot length, leaf count, late-stage branch development, and phosphate-based symbiotic effectiveness (PBSE); Z-12-20 induced shoot length, shoot and root fresh weight, and root dry weight. Synergetic applications, particularly PSB strains together with compost and aluminium phosphate, were found to be remarkable complements to achieve fruitful tomato-PSB symbiotic interaction and growth performance. Co-inoculation of strains and compost significantly increased plant height, number of leaves and branches, floral parameters (clusters, buds, and open flowers), fruit number, fresh weight of shoots and roots, root dry weight, and overall PBSE whereas, aluminium phosphate greatly improved germination, early flower cluster development, flower opening, and most importantly, fruit weight. It is strongly recommended that these four selected strains be characterized to further confirm their symbiotic effectiveness in a site-specific field trial on tomatoes as well as other crops for use as biofertilizers and growth-promoting bioinoculants.

Keywords: *Compost, Phosphorous, PSB, Symbiotic Effectiveness*

4.1 INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most nutritionally desirable commercial crops (Widnyana, 2018). Due to its sensitive nature (Baba *et al*., 2017), tomato production is challenged by different factors; thus, production requires aggressive agrochemical consumption. In Ethiopia, tomato production increased from time to time due to attractive markets, relative production profit (Mersha and Sime, 2022), repeated fruit harvesting, suitability for off-season production, improvements in irrigation practice, and farmers' awareness. Almost all Ethiopian farmers follow a traditional farming system (Kifle *et al.,* 2022), which is why its production and productivity are very low compared to other regions (Asfaw, 2021; CSA, 2021) (Chapter two). It is demanding to excel in agricultural production and productivity to feed the ever-increasing population and minimize climate issues. Enhanced crop production requires optimum soil fertility, a conducive growing environment, improved agro-technologies, pest/infestation management, proper land preparation, and improved harvest-post-harvest practices (Kidane *et al.,* 2022; Kifle *et al.,* 2022). Application of fertilizer (the main agricultural input), pesticides, and herbicides (safeguard crops from pests and weed invasion) improve productivity and minimizes production loss (CSA, 2021). However, repeated and excess application of synthetic fertilizers and pesticides leads to several environmental, agricultural, and health issues (Abebe *et al*., 2022; Sayyed *et al*., 2019; Egamberdieva *et al.,* 2015).

Production cost and economic barriers are the main reasons for smallholder farmers to implement improved agricultural technologies (quality seeds, proper soil-based treatments, high-performance environmentally friendly agrochemicals, proper farm follow-up, etc. Supportively, Kifle *et al*. (2022) indicated that adoption of climate smart agriculture practice affected by financial, natural, and technological accesses. Therefore, it is better to design technologies that improve productivity and minimize production costs with an eco-friendly approach. The development of biofertilizers and bio-inoculants (Baba *et al*., 2017) plays significant roles in production improvements, soil and environmental health maintenance, and minimizing production costs (Nosheen *et al.,* 2021; Egamberdieva *et al.,* 2015). There are millions, if not billions, of beneficial microorganisms found in the soil (Sayyed *et al*., 2019). The soil microbial community influences soil fertility through decomposition, mineralization, nutrient release, and/or storage, promotes other beneficial microbial activity (Chen and Liu, 2019; Poonia and Dhaka, 2012), and reduces plant pathogens (Planchamp *et al*., 2015; Shavit

et al., 2013). Furthermore, fruitful outputs will be obtained if they are applied together with appropriate agrochemicals (De Zutter *et al*., 2022). This is because the natural soil biological response and organic farming provide slow nutrient accessibility with gradual responses that impede elevated production. Therefore, integrated fertilization (fertilizer, compost and biofertilizer) practice has an economical, environmental and sustainability benefits (Abebe *et al*., 2022).

Phosphorous is the second most important element for growth and development (Yasmeen *et al.,* 2022; Mitra *et al.,* 2020; Chen and Liu, 2019; Baba *et al*., 2017; Poonia and Dhaka, 2012) that is involved in many metabolic pathways and is essential to produce biomolecules (De Zutter *et al*., 2022). Most (>95%) of soil phosphorous is found in insoluble form (Pande *et al*., 2017), and plants assimilate a very tiny amount of phosphate ions from soil (Sharma *et al*., 2013). Whenever plants face P deficiency, they try to optimize their internal physiology and rhizosphere environment (De Zutter *et al*., 2022). Soil can be supplemented by the addition of fertilizers, inorganic minerals, and dead organic residues (Kifle *et al.,* 2022; Wan *et al*., 2020); however, only 5-25% P fertilizer is utilized by plants (Chen and Liu, 2019). Plant availability of phosphorous is limited due to soil desorption and fixation with Fe^{3+}, Al^{3+}, and Ca^{2+} to form insoluble composites or salts (De Zutter *et al*., 2022; Liu *et al*., 2020; Chen and Liu, 2019; Bhattacharyya *et al*., 2015; Chen *et al*., 2014; Poonia and Dhaka, 2012). To resolve the phosphorous deficiency, farmers applied intensive fertilizers into the soil, which resulted in stable plant inaccessible P accumulation. Due to the rise in fertilizers cost, environmental health issues, and wise resource utilization, the application of phosphate solubilizing bacteria (PSB) has many advantages to improve soil fertility and productivity (Yasmeen *et al.,* 2022). Many kinds of literature have reported that multidimensional PSB strains maintain soil fertility and soil biota to achieve sustainable agriculture (Wan *et al*., 2020; Chen and Liu, 2019; Pande *et al*., 2017; Sharma *et al*., 2013; Collavino *et al.*, 2010). Apart from solubilization and mineralization, they influence plants' phytohormones and metabolic pathways to mitigate P-deficiency (De Zutter *et al*., 2022; Rilling *et al.,* 2019). Furthermore, PSB play a significant role in tomato growth-promoting traits like the production of IAA, siderophore, HCN, ammonia, and ACC deaminase activity (Egamberdieva *et al.,* 2015; Vaikuntapu *et al*., 2014; Wlapola and Yoon, 2013). Their rhizosphere interaction and microbial activities are influenced by nutrient availability, composition of root exudates, indigenous competition, soil management, and climatic conditions (Pellegrini *et al.,* 2021; Nosheen *et al.,* 2021; Rilling *et al.,* 2019). Plants attract

beneficial microbes by releasing various compounds, including volatile compounds (CO_2, ethanol, acetone, ethylene, and the likes), low molecular weight compounds (LMWCs like organic acids, sugars, amino acids, and vitamins), and high molecular weight compounds (HMWCs, namely enzymes and polysaccharides) (Verma, 2019).

In Ethiopia, the application of PSB inoculants (Muleta *et al*., 2021) remains very limited due to a lack of well-developed efficient strains, technology, awareness, practical exposure, poor adoption skills, greenhouse and field trials and evaluations, inconsistent results or environmental factors for imported strains, and poor motivation among the agri-sectors. Numerous studies (Muleta, 2018; Muleta *et al*., 2013; Woyessa and Assefa, 2011; Lemessa and Zeller, 2007; Fenta and Assefa, 2017) have been conducted so far to explore potential PSB isolates from different crops and agroecologies; nevertheless, very limited experiments have been done on symbiotic effectiveness evaluation and authentication along with co-inoculation of extra P sources at the greenhouse level. Therefore, this research was conducted to evaluate tomato growth performance under PSB and extra P-substrate co-inoculation, symbiotic effectiveness, and assess their suitable P-substrate for dual-inoculation.

4.2 MATERIAL AND METHODs

4.2.1 Study Area

The experiment was conducted in a greenhouse condition at the Hawassa University's main campus (located at $7^0$3’13” N and $38^0$30’2” E), Hawassa, Ethiopia. The research was conducted from December 2021 to March 2022.

4.2.2 PSB Isolates Preparation

Selected ten PSB strains were taken from the current project isolation at Hawassa University Soil Microbiology Lab and were isolated from tomato rhizosphere soils collected from three main tomato-producing areas, namely Koka, Meki, and Ziway Zuria. These pre-screened potential PSB strains were selected based on P-solubilization efficiency on Pikovskaya’s medium. Their efficiency was evaluated both in liquid and solid media in line with other primitive screening methods using morphological and biochemical tests (Chapter 3). These isolates were prepared for inoculation by refreshing them in TCP-broth at 30 ^{0}C for 48 h in a shaker incubator (121 rpm). Each PSB isolate was inoculated separately.

4.2.3 Soil Preparation, Treatment Mixing, and Pot Arrangements

The experiment was conducted in surface-sterilized, plastic pots filled with 3 kg of soil in a greenhouse. Seeds and plastic pots were surface sterilized according to Somasegaran and Hoben (1994). Soil was filled in each pot alongside the respective supplementary P substrates (TCP, BM, AlP, AmP, compost, and DAP) and one control (soil without any external P substrate). These treatments were selected because of the possibilities found in natural soil, such as rock phosphates, alkaline soil, acidic soil (AlP and FeP), organic matter (debris from dead animals and plants), or intentional application. In soil, whatever the concentration, one or more of these substrates would be found naturally or incorporated intentionally during farm practices; thus, verifying the strains' response/capacity on these possible P substrates will give a good picture to recommend them for which soil type and which substrate can be co-inoculated for fruitful response. Depending on the phosphate composition and the recommendation rate, the added P sources were measured in their respective amount (Table 4.1). In these measurements, the concentration of phosphate in each substrate and the recommendation rate of DAP fertilizer and compost per hectare were used to determine the amount of substrates per pot accordingly.

Table 4.1: Type and amount of added treatments.

No	Treatment	Amount per pot	Inoculum
1	TCP ($Ca_2(PO_4)_2$)	0.23gm	Control +10 Isolates
2	Aluminium phosphate (AlP)	0.14gm	Control +10 Isolates
3	Bone Meal (BM)	0.33gm	Control +10 Isolates
4	Compost	1:3 ratio	Control +10 Isolates
5	Fertilizer (DAP)	0.17gm	Control +10 Isolates
6	Soil only (control)	-	Control +10 Isolates
7	Ammonium phosphate (AmP)	0.14gm	Control +10 Isolates

***NB**. Each P-substrate was co-inoculated with individual strain separately and each treatment has a control (i.e., uninoculated or isolate-free) pot.*

4.2.4 Experimental Set up and Strain Inoculation

Healthy tomato (a familiar variety used by farmers called *Galilee* (Mersha and Sime, 2022)) seeds were selected, surface sterilized, and soaked in refreshed active inoculum suspension overnight (Pande *et al*., 2017) to ensure all sides of the seeds made proper contact. Seed

inoculation with PGPR have demonstrated the induction of tomato germination, length, fruit yield (Widnyana, 2018), wilt resistance both in field and at greenhouse trials (Egamberdieva *et al.,* 2015). Seed inoculation helps the bacteria to colonize the root, improve indigenous competition, and exploit the root rhizosphere niche at the early stage of the plant. Then the extra suspension was removed from the seed and the seeds were allowed to dry (Yasmeen *et al.,* 2022) for 30 mn to enhance isolate-seed fixation. Four seeds were sown at 2 cm depth in each pot per the respective P sources with three replications and arranged in CRBD. One millilitre of PSB suspension was inoculated before the seeds were covered by soil. After 20 days of sowing, plants were thinned down to one per pot and irrigated every day. In order to maximize root colonization, one millilitere of active culture was re-inoculated with their respective arrangements in each pot at 2 cm depth. For the nitrogen source, urea was added at the recommended rate. Data were collected starting from germination until harvesting. Tomato was harvested after 110 days of sowing, and measurements were taken on shoot height, biomass, fruit number, fruit weight, root length, and the estimated total P in the shoot. Plant height and biomass are the important traits frequently monitored to assess the impact of selected PSB strains on the target host plant and plant P-uptake (De Zutter *et al*., 2022). Based on tomato shoot dry weight, the effect of isolates on the amount of P in the shoot and the level of symbiotic effectiveness were calculated according to the formula provided by Somasegaren and Hoben (1994) as follows:

PBSE (%) = (IPDM)*100/ PFPDM

Where, PBSE= Phosphate Based Symbiotic Effectiveness, IPDM = Inoculated Plant Dry Matter, PFPDM= Phosphate Fertilized Plant Dry Matter

4.2.5 Data Organization and Analysis

Quantitative data that has been collected, tabulated, and then analysed using R 4.2.1 and SAS 9.4 versions. The variables were subjected to ANOVA (two-way analysis using Fisher's and Tukey's tests) (significance set at p=0.05). The means of the treatments were differentiated using the LSD. Finally, the analysed data was explained and discussed in reference to other related works.

Picture 4.1: Greenhouse tomato growth phases treated by different PSB strains and possible external P-substrates.

4.3 RESULT

This study was focused on the effect of the combined application of selected PSB strains and external possible P-substrate on tomato growth and development under greenhouse conditions. Based on the recorded data at various tomato growth stages, considerable responses were observed. Throughout the tomato growth, PSB strains showed significant ($p<0.05$) differences against the control, and almost all parameters showed significant differences among P treatments. For instance, certain isolates significantly improved seed germination (shorten the date (Table 4.2)), others promoted tomato shoot length (Table 4.3 and Figure 4.1), development of leaf (Table 4.4 and 4.8), flower clusters, buds, early flowering (Table 4.5 and Figure 4.2), early fruit maturation, fruit yield and biomass (Table 4.7, Figure 4.3, Picture 4.1 and 4.2 and supplementary Figure 4.1 and 4.2).

Table 4.2: Mean value for tomato seed germination and true leaf development dates.

Isolate	Germination	True leaf dev't	Treatments	Germination	True leaf dev't
Control	7.43 ± 0.6^{a}	12.95 ± 0.74^{a}	AlP	5.49 ± 0.76^{c}	11.0 ± 0.9^{bcd}
K-1-29	6.05 ± 1.12^{bcd}	11.38 ± 1.43^{b}	AmP	6.12 ± 1.43^{ab}	11.27 ± 1.13^{bc}
K-10-27	5.43 ± 0.93^{de}	10.43 ± 0.75^{d}	BM	6.58 ± 1.3^{a}	11.39 ± 0.93^{b}
K-10-41	6.05 ± 1.36^{bcd}	11.43 ± 1.33^{b}	Com	5.64 ± 1.27^{bc}	10.36 ± 1.11^{e}
Mk-1-25	6.43 ± 1.4^{b}	11.24 ± 1.22^{bc}	Fert	5.7 ± 1.29^{bc}	10.85 ± 1.03^{d}
Mk-13-16	5.67 ± 1.11^{cde}	11.0 ± 0.9^{bc}	Soil	6.42 ± 1.3^{a}	12.79 ± 1.11^{a}
Mk-20-20	5.38 ± 0.87^{e}	11.0 ± 0.95^{bc}	TCP	5.79 ± 0.99^{bc}	10.88 ± 0.89^{cd}
Mk-20-7	5.67 ± 1.24^{cde}	11.01 ± 1.18^{bc}	CV	18.37	7.4
Z-1-16	5.57 ± 1.21^{cde}	11.19 ± 1.17^{bc}	LSD	0.67	0.4
Z-12-20	5.76 ± 1.26^{cde}	10.95 ± 1.43^{bc}			
Z-13-4	6.14 ± 1.31^{bc}	10.76 ± 0.54^{cd}			
CV	18.37	7.4			
LSD	0.53	0.51			

NB: Means with similar letters have no significant difference at p=0.05.

Synergetic application of PSB strains and compost strongly promoted tomato plant height and recorded the largest shoot length in all growth periods (Table 4.3). Co-inoculation partiality and P-substrate prioritization of strains (Figure 4.1) might suggest two implications: 1) habitat or soil condition, especially in case of soil acidity and alkalinity; and 2) predominant type of phosphatase enzyme possibly produced. If isolates significantly respond to aluminium and iron phosphate, it might give a sign for their habitat preference; then primarily they are convenient to be applied in acidic soil (mostly synthesize acid phosphatase).

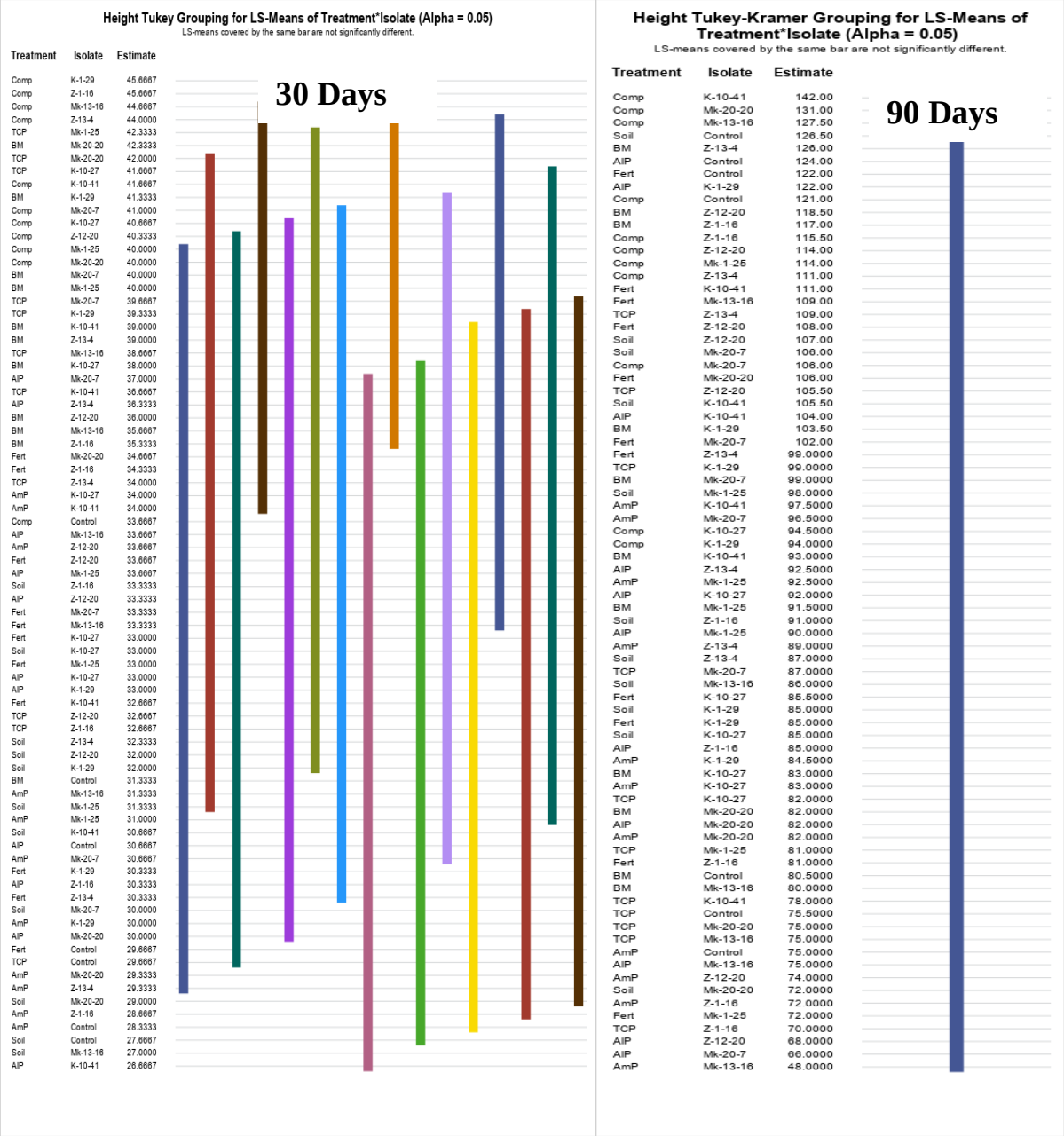


Figure 4.1: The specific interaction response of PSB inoculation and P-substrate on tomato height development at 30 and 90 days of plantation.

4.3.1 Tomato Germination and Growth Performance

Tomato seed germination and growth were induced by the inoculation of PSB isolates. All isolates stimulated germination compared to the uninoculated (control) seeds. Strain Mk-20-20 recorded the fastest germination date (on average 5.38) among the rest of the strains, while uninoculated seeds took longer (on average 7.4) days to germinate (Table 4.2). Among the external P sources, aluminium phosphate (AlP) amendment considerably encouraged tomato seed germination. Similarly, application of K-10-27 and compost shortens the true leaf

development while uninoculated and untreated soil required longer (on average 12.79) days to develop true leaves (Table 4.2).

Table 4.3: Mean value of tomato plant shoot length at different growing periods.

Tomato plant height (cm)					
Isolate	30 days	45 days	60 days	90 days	Harvest
Control	30.14±2.1^{b}	41.05±6.18^{b}	75.33±5.28^{c}	87.91±5.68^{b}	92.76±5.9^{b}
K-1-29	35.95±6.5^{a}	50.33±6.74^{a}	80.67±12.83^{abc}	96.29±19.92^{ab}	100.38±1922^{ab}
K-10-27	36.19±4.32^{a}	49.76±4.96^{a}	76.95±10.32^{bc}	89.1±20.42^{b}	93.86±20.59^{b}
K-10-41	34.48±5.65^{a}	49.95±9.55^{a}	81.43±14.03^{abc}	98.48±19.1^{ab}	101.81±21.18^{ab}
Mk-1-25	35.9±4.95^{a}	48.52±7.4^{a}	80.81±13.46^{abc}	96.29±22.75^{ab}	106.43±26.11^{a}
Mk-13-16	34.9±5.73^{a}	48.81±6.01^{a}	82.05±13.16^{abc}	92.71±19.33^{ab}	98.71±25.24^{ab}
Mk-20-20	35.33±6.4^{a}	50.48±6.95^{a}	82.91±15.46^{ab}	94.57±25.57^{ab}	98.00±25.24^{ab}
Mk-20-7	35.95±5.5^{a}	49.33±6.23^{a}	79.1±12.51^{bc}	95.1±18.13^{ab}	101.76±22.23^{ab}
Z-1-16	34.33±5.78^{a}	49.29±11.7^{a}	79.52±18.28^{bc}	91.62±20.25^{b}	101.43±24.25^{ab}
Z-12-20	34.52±3.95^{a}	48.38±7.01^{a}	86.52±9.78^{a}	102.52±19.7^{a}	108.19±22.08^{a}
Z-13-4	35.05±5.64^{a}	49.52±6^{a}	81.86±11.74^{abc}	97.71±24.96^{ab}	100.76±25.78^{ab}
CV	10.21	12.02	14.06	18.64	19.66
LSD	2.16	2.84	6.9	10.75	12.00
P-treatments					
AlP	32.52±3.95^{c}	43.24±5.59^{e}	79.18±11.83^{bc}	94.16±22.78^{b}	101.46±26.83^{b}
AmP	30.94±3.08^{cd}	48.85±5.14^{cd}	75.85±10.31^{cd}	84.39±15.42^{c}	88.94±18.04^{d}
BM	38.0±4.03^{b}	51.09±6.98^{bc}	82.06±13.42^{b}	94.58±21.48^{b}	99.09±20.59^{bc}
Comp	41.58±4.35^{a}	58.18±7.43^{a}	95.27±11.1^{a}	118.39±17.75^{a}	123.15±17.76^{a}
Fert	32.58±2.53^{c}	51.7±4.82^{b}	80.67±10.03^{bc}	95.00±16.15^{b}	98.79±17.91^{bcd}
Soil	30.58±3.10^{d}	42.49±6.76^{e}	83.03±15.59^{b}	102.46±19.95^{b}	113.39±25.5^{a}
TCP	37.21±5.16^{b}	47.18±6.58^{d}	72.88±7.3^{d}	82.85±13.43^{c}	91.24±14.94^{cd}
CV	10.21	12.02	14.06	18.64	19.66
LSD	1.72	3.56	5.5	8.57	9.58

**Means ± Std with similar letters have no significant difference at P=0.05. PSB isolates named as letter/s followed by numbers which indicate soil sampling site name, soil sample number and number of isolates from that specific sample respectively. K=Koka, Mk=Meki, Z=Ziway. Treatments used: AlP=Aluminium phosphate, AmP=Ammonium phosphate, BM=bone meal, Comp=compost, Fert=fertilizer (DAP), and TCP=tricalcium phosphate.*

The overall tomato height means comparison among PSB isolates seemed to be no significant difference at 30 and 45 days though, the tallest tomato shoot length (36.19 and 50.48 cm) were recorded from K-10-27 and Mk-20-20 inoculation at 30 and 45 days, respectively (Table 4.3). The control (inoculum-free) tomatoes recorded the minimum plant height in all growth periods (an average of 30.19, 41.05, 75.33, 87.91, and 92.76 cm at 30, 45, 60, 90, and 110 days, respectively). While inoculation of Z-12-20 promoted tomatoes to record the longest shoot length starting from 60 days until harvesting (86.52, 102.52, and 108.19 cm at

60, 90, and harvesting (110) days, respectively). Among all inoculated isolates, K-10-27 was positively promoted at early growth phase but then relatively recorded the lowest plant height as the plantation days increased (Table 4.3). The addition of compost showed significant ($p<0.05$) promotion of tomato-isolate collaboration and recorded the highest plant height throughout the growing periods, while untreated soil with external P supplements showed a progressive shoot length increment starting from 60 days until harvesting. However, analysis of each isolate interaction against the specific P-complexes indicated a significant ($p<0.05$) plant height difference (Figure 4.1). For example, K-1-29 and Z-1-16 recorded a maximum plant height (on average 45.67 cm) at 30 days when they were co-inoculated with compost; however, co-inoculation with AmP relatively resulted lower plant height record (30.0 and 28.67 cm, respectively) on a similar growth period (Figure 4.1). Even though Z-1-16 was deemed to be one of the least tomato height inducers; when analysed across all treatments at 30 days of plantation (Table 4.3); it responded differently to each P supplement (Figure 4.1). Similarly, the performance of Mk-20-20 and Mk-1-25 strengthens the substrate effect on isolate-tomato interaction and response variability. They showed the least interaction with compost (which was primarily selected by the rest of isolates) but then substantially responded with TCP. Likewise, K-10-41 recorded a minimum plant height (26.67cm) when applied together with AlP, whereas substantial tomato height (41.67 cm) was recorded with compost at 30 days of sowing. This clearly demonstrated that each isolate has a specific substrate requirement in order to attain fruitful and successful symbiotic interaction.

Table 4.4: Mean value of tomato leaf number at different plantation dates.

Isolates effect on tomato leaf number					
Isolate	30 days	45 days	60 days	90 days	Harvest
Control	10.24 ± 0.54^{b}	10.71 ± 0.78^{c}	14.43 ± 1.25^{b}	18.38 ± 2.16^{a}	12.71 ± 2.45^{ab}
K-1-29	11.24 ± 1.34^{a}	12.76 ± 1.26^{a}	15.43 ± 2.71^{ab}	18.33 ± 2.65^{a}	11.76 ± 2.47^{ab}
K-10-27	10.61 ± 1.02^{b}	$12.38\pm1.6a^{b}$	15.1 ± 1.45^{ab}	17.76 ± 2.98^{a}	11.19 ± 2.93^{b}
K-10-41	10.67 ± 0.8^{b}	12 ± 1.23^{b}	15.29 ± 1.65^{ab}	18.29 ± 2.57^{a}	11.57 ± 2.42^{ab}
Mk-1-25	10.71 ± 1.10^{b}	12.29 ± 2^{ab}	15.57 ± 1.65^{a}	18.1 ± 3.48^{a}	12.38 ± 2.75^{ab}
Mk-13-16	10.38 ± 0.81^{b}	12.05 ± 1.12^{b}	14.81 ± 1.94^{ab}	18.29 ± 2.26^{a}	12.43 ± 2.98^{ab}
Mk-20-20	10.33 ± 1.11^{b}	12.2 ± 1.17^{ab}	15.33 ± 1.84^{ab}	18.52 ± 2.38^{a}	11.75 ± 2.23^{ab}
Mk-20-7	10.52 ± 0.60^{b}	12.1 ± 0.77^{ab}	15 ± 1.84^{ab}	18.38 ± 2.42^{a}	12.86 ± 3.12^{a}
Z-1-16	10.48 ± 0.87^{b}	12.14 ± 1.31^{ab}	15.24 ± 2.07^{ab}	18.19 ± 1.86^{a}	11.81 ± 2.93^{ab}
Z-12-20	10.67 ± 0.86^{b}	11.81 ± 0.87^{b}	15.33 ± 1.11^{ab}	18.33 ± 1.71^{a}	12.43 ± 2.71^{ab}
Z-13-4	10.48 ± 0.98^{b}	11.86 ± 0.91^{b}	15 ± 1.34^{ab}	17.67 ± 2.52^{a}	12.00 ± 3.18^{ab}
CV	7.97	9.61	11.76	13.11	21.47
LSD	0.51	0.56	1.08	1.16	1.26
Treatments effect on tomato leaf number					
Treatment	30 days	45 days	60 days	90days	Harvest

AlP	10.79±1.29^{b}	11.73±1.55^{c}	15.18±2.34^{bc}	18.52±3.18^{bc}	11.91±2.83^{bc}
AmP	10.21±0.96^{cd}	11.58±1.03^{c}	14.15±1.46^{d}	17.24±2.18^{d}	11.09±2.38^{c}
BM	10.82±0.77^{b}	12.03±0.95^{bc}	14.58±1.5^{cd}	17.76±2.74^{bcd}	11.85±2.67^{bc}
Comp	11.24±0.79^{a}	13.00±1.32^{a}	16.97±1.47^{a}	19.82±1.83^{a}	12.55±2.37^{b}
Fert	10.64±0.78^{b}	12.36±0.86^{b}	15.46±1.12^{b}	18.09±2.3^{bcd}	11.67±2.38^{bc}
Soil	10.52±0.58^{bc}	11.7±1.21^{c}	14.91±1.97^{bcd}	18.61±1.90^{b}	14.27±2.76^{a}
TCP	9.82±0.58^{d}	11.79±1.52^{c}	14.73±2.17^{bcd}	17.39±1.98^{cd}	11.12±2.64^{c}
CV	7.97	9.61	11.76	13.11	21.47
LSD	0.41	0.70	0.86	1.45	1.58

NB: Means with similar letters have no significant difference at p=0.05.

At the early growing stages, except for isolate K-1-29, all isolates showed no significant difference in the development of total tomato leaf number. K-1-29 induced tomatoes to produce maximal mean leaf number at 30 and 45 days (11.24 and 12.76, respectively), whereas Mk-1-25 at 60 days (15.27) and Mk-20-7 recorded the highest active leaf numbers (an average of 12.86) at harvesting time (Table 4.4). Likewise, the addition of compost significantly ($p<0.05$) improved tomato leaf numbers until 90 days. However, uninoculated tomatoes recorded more leaf numbers in the subsequent growth periods. Furthermore, three mid-leaves were selected from each tomato to measure leaf length and the number of leaflets at the 45th and 60th days of plantation. These sampling days were chosen because of vigorous tomato growth conditions and performance (active height development, emerging flowers, start fruit set, and optimum growth period). Sampled leaves were counted and labelled from the bottom of the plant to the upper part. At 45 days of sowing, there was no significant difference in the lower leaf length among isolates, although there was a difference in the number of leaflets (Table 4.8). Yet, K-1-29, Z-13-4, and Mk-20-7 recorded maximum leaf length, whereas leaflets were significantly ($p<0.05$) improved by Mk-1-25 and K-1-29. Similarly, on the 60th day of the plantation, Mk-1-25 meaningfully improved leaf length and Z-12-20 encouraged the development of leaflets. Compost co-inoculation significantly ($p<0.05$) promoted leaf length in all sampled leaves both at 45 and 60 plantation days, whereas fertilizer and BM improved leaflet development (Table 4.8). Having more leaf number and leaf size enhances photosynthetic activities, promotes maturation, increases plant biomass, and increases yield.

At the early growth stage, the uninoculated tomatoes developed a better branch number, and at later plantation days, PSB-inoculated tomatoes progressively increased the number of branches. Z-12-20 was induced to develop more (an average of 2.33) branches at 60 days and Mk-1-25 at the subsequent development stages (Table 4.6). The application of compost

effectively encouraged branching throughout tomato growth phases. The number of tomato node development counted at 30 and 110 (harvesting) days in that case, Mk-1-25 inoculation found one of the least inducers at the beginning however, it showed progressive promotion to development more nodes (on average 21.38) at the final growth stages (Table 4.6).

4.3.2 Flower Parameters

Tomato inoculation with Mk-1-25 was encouraged to set more flower clusters (on average 1.38 clusters at 30 days and meaningfully increased throughout the remaining periods) (Table 4.5), Mk-20-20 enhanced early flower openings (Figure 4.2), while inoculation with Z-1-16, K-1-29, and Mk-20-7 improved flower bud development at 30, 60, and 90 days, respectively (Table 4.5). AlP induced flower cluster development and flower openings at early tomato growth stages, while compost improved tomatoes to set more flower clusters, bud number, and number of opened flowers at the late plantation stages (on average, 6.12 clusters (Table 4.5), 27.21 buds, and about 23 opened flowers per plant recorded at 90 days (Figure 4.2)). These external possible P-sources amendment practices demonstrated substantial improvements in the tomato-strain interaction and responses. Moreover, the synergetic application of strains with compost, AlP, and BM encouraged tomato flower openings (Figure 4.2).

Table 4.5: Total number of tomato flower cluster and buds at different plantation days.

Inoculation effect on total flower Cluster number					Inoculation effect on flower bud number		
Inoculant	**30 days**	**45days**	**60days**	**90days**	**30 days**	**60days**	**90days**
Control	0.86±0.34^{e}	1.24±0.54^{d}	2.81±0.81^{d}	4.38±0.97^{a}	3.1±1.76^{d}	12.67±3.34^{b}	19.95±5.94
K-1-29	1.19±0.4^{abcd}	2.62±1.6^{a}	4.48±1.37^{a}	5.24±2.3^{a}	4.43±0.75^{abc}	18.81±7.47^{a}	23.19±9.16
K-10-27	1.29±0.46^{abc}	2.19±0.4^{abc}	3.71±1.31^{bc}	4.76±1.73^{a}	4.52±1.12^{abc}	16.57±5.92^{a}	21.48±6.28
K-10-41	1.14±0.36^{abcd}	2.14±0.48^{bc}	3.86±1.01^{abc}	4.81±1.5^{a}	4.67±0.48^{ab}	16.81±4334^{a}	*21.19*±5.93
Mk-1-25	1.38±1.02^{a}	2.19±0.81^{abc}	3.71±1.31^{bc}	5.24±249^{a}	4.0±1.52^{c}	16.29±6.94^{a}	21.38±6.98
Mk-13-16	1.24±0.44^{abcd}	2.19±0.75^{abc}	3.62±1.12^{bc}	4.95±1.12^{a}	4.67±0.48^{ab}	15.95±4.63^{a}	22.00±5.84
Mk-20-20	1.33±0.48^{ab}	2.29±0.72^{ab}	4.14±1.11^{ab}	4.71±1.52^{a}	4.29±0.56^{abc}	17.86±4.93^{a}	22.19±6.78
Mk-20-7	1.1±0.3b^{cde}	2.29±0.56^{ab}	3.67±1.24^{bc}	5.14±1.65^{a}	4.57±0.68^{ab}	16.1±5.92^{a}	23.33±6.88
Z-1-16	1.24±0.44^{abcd}	2.14±0.48^{bc}	3.86±1.2^{abc}	4.48±1.54^{a}	4.76±0.44^{a}	17.38±6.17^{a}	21.24±6.73
Z-12-20	1.05±0.22^{cde}	2.14±0.66^{bc}	3.48±1.17^{c}	4.91±1.58^{a}	4.38±0.59^{abc}	16.43±5.44^{a}	21.76±6.86
Z-13-4	1.0±0.00d^{e}	1.81±0.60^{c}	3.76±1.09^{bc}	4.52±1.83^{a}	4.19±0.93^{bc}	16.52±5^{a}	20.81±5.97
CV	36.88	34.18	27.78	33.74	21.17	29.46	29.15
LSD	0.26	0.35	0.63	0.79	0.56	2.96	3.07
Possible P-Sources							
AlP	1.61±0.79^{a}	2.06±0.61^{bc}	3.42±0.87^{b}	4.94±2.14^{b}	4.42±0.71^{ab}	14.76±4.05^{b}	21.61±6.78^{b}

AmP	1.1±0.29^{bc}	2.12±0.6^{bc}	3.46±1.18^{b}	4.76±1.75^{b}	4.33±0.6^{ab}	15.49±4.63^{b}	21.52±6.11^{b}
BM	1.24±0.44^{b}	2.21±0.96^{b}	3.61±1.03^{b}	4.73±1.76^{b}	4.03±1.29^{bc}	15.42±4.57^{b}	21.55±7.65^{b}
Comp	0.94±0.43^{c}	2.06±0.66^{bc}	5.03±1.57^{a}	6.12±1.8^{a}	3.88±1.75^{c}	23.21±5.58^{a}	27.21±5.75^{a}
Fert	1.06±0.35^{bc}	1.79±0.49^{c}	3.42±0.79^{b}	4.21±1.24^{b}	4.27±0.98^{abc}	15.03±3.62^{b}	19.27±5.99^{b}
Soil	1.15±0.34^{b}	1.91±0.52^{bc}	3.39±0.83^{b}	4.24±1.25^{b}	4.64±0.49^{a}	15.12±3.7^{b}	19.18±5.67^{b}
TCP	1.06±0.24^{bc}	2.64±1.3^{a}	3.82±1.16^{b}	4.64±1.14^{b}	4.7±0.53^{a}	16.39±5.49^{b}	21.46±5.54^{b}
CV	36.88	34.18	27.78	33.74	21.17	29.46	29.15
LSD	0.21	0.44	0.05	0.99	0.44	2.36	3.85

NB: Means with similar letters have no significant difference at p=0.05.

Table 4.6: Mean value of tomato branch and node development at various growth periods.

Isolates effect on branch number					**Node number**	
Inoculant	45 days	60 days	90 days	Harvest	30 days	Harvest
Control	1.76±3.29^{a}	1.76±0.51^{ab}	1.82±0.68^{b}	2.76±1.38^{ab}	9.71±0.90^{b}	21.48±3.78
K-1-29	1.48±2.18^{abc}	2±2.59^{ab}	2.22±0.87^{b}	2.52±1.12^{b}	10.62±1.4^{a}	20.57±2.66
K-10-27	0.86±0.36^{abc}	1.29±0.56^{b}	1.76±0.9^{b}	2.57±1.21^{b}	10.0±1.1^{b}	19.91±4.05
K-10-41	0.62±0.5^{c}	1.38±0.59^{ab}	1.86±0.85^{b}	2.62±1.28^{b}	10.05±1.07^{ab}	19.71±3.02
Mk-1-25	1.67±2.44^{ab}	1.95±2.58^{ab}	2.62±2.99^{a}	3.43±2.79^{a}	9.91±2^{b}	21.38±3.53
Mk-13-16	0.52±0.51^{c}	1.38±0.74^{ab}	1.67±0.58^{b}	2.48±1.08^{b}	10.1±0.94^{ab}	20.19±3.54
Mk-20-20	1.05±0.59^{abc}	1.67±0.58^{ab}	1.91±0.9^{b}	2.86±1.24^{ab}	10.1±1.04^{ab}	20.08±2.94
Mk-20-7	0.95±0.38^{abc}	1.52±0.81^{ab}	2.1±0.83^{ab}	2.81±1.12^{ab}	10.24±0.9^{ab}	20.67±3.77
Z-1-16	0.76±0.44^{bc}	1.48±0.6^{ab}	1.81±0.6^{b}	2.29±1^{b}	9.95±1.07^{b}	20.52±3.71
Z-12-20	0.86±0.36^{abc}	2.33±3.32^{a}	2.46±0.7^{b}	2.52±1.72^{b}	9.95±0.87^{b}	20.81±3.28
Z-13-4	1.24±2.28^{abc}	1.52±0.75^{ab}	1.67±0.66^{b}	2.67±1.2^{ab}	10.14±0.85^{ab}	20.14±3.32
CV	47.74	69.88	62.74	48.69	10.09	14.51
LSD	0.77	0.96	0.56	0.8	0.62	1.81
Treatments effect on branch number					**Node number**	
Treatment	45 days	60 days	90 days	Harvest	30 days	Harvest
AlP	1.03±2.07^{b}	1.79±2.07^{ab}	2.3±2.37^{a}	3.09±2.17^{a}	9.82±1.86^{b}	21.00±5.24^{b}
AmP	0.91±0.29^{b}	1.7±2.6^{ab}	1.85±0.97^{ab}	1.94±0.83^{b}	8.97±1.10^{c}	18.58±2.05^{cd}
BM	0.91±0.29^{b}	1.27±0.91^{b}	1.79±0.78^{ab}	1.97±1.16^{b}	10.42±0.75^{a}	19.91±2.53^{bc}
Comp	1.91±3.05^{a}	2.18±0.58^{a}	2.37±0.74^{a}	3.43±0.85^{a}	10.76±0.87^{a}	23.18±2.1^{a}
Fert	0.91±0.46^{b}	1.58±0.71^{ab}	1.65±0.67^{b}	1.94±1.06^{b}	10.3±0.73^{ab}	19.85±2^{bc}
Soil	0.79±0.65^{b}	1.24±0.56^{b}	1.73±0.76^{b}	3.21±1.47^{a}	10.39±0.61^{a}	22.67±2.45^{a}
TCP	1.03±1.86^{b}	1.67±2.09^{ab}	1.97±0.88^{ab}	3.61±1.12^{a}	9.82±0.58^{b}	18.27±2.98^{d}
CV	47.74	69.88	62.74	48.69	10.09	14.51
LSD	0.96	0.77	0.7	0.63	0.49	1.44

NB: Means with similar letters have no significant difference at p=0.05.

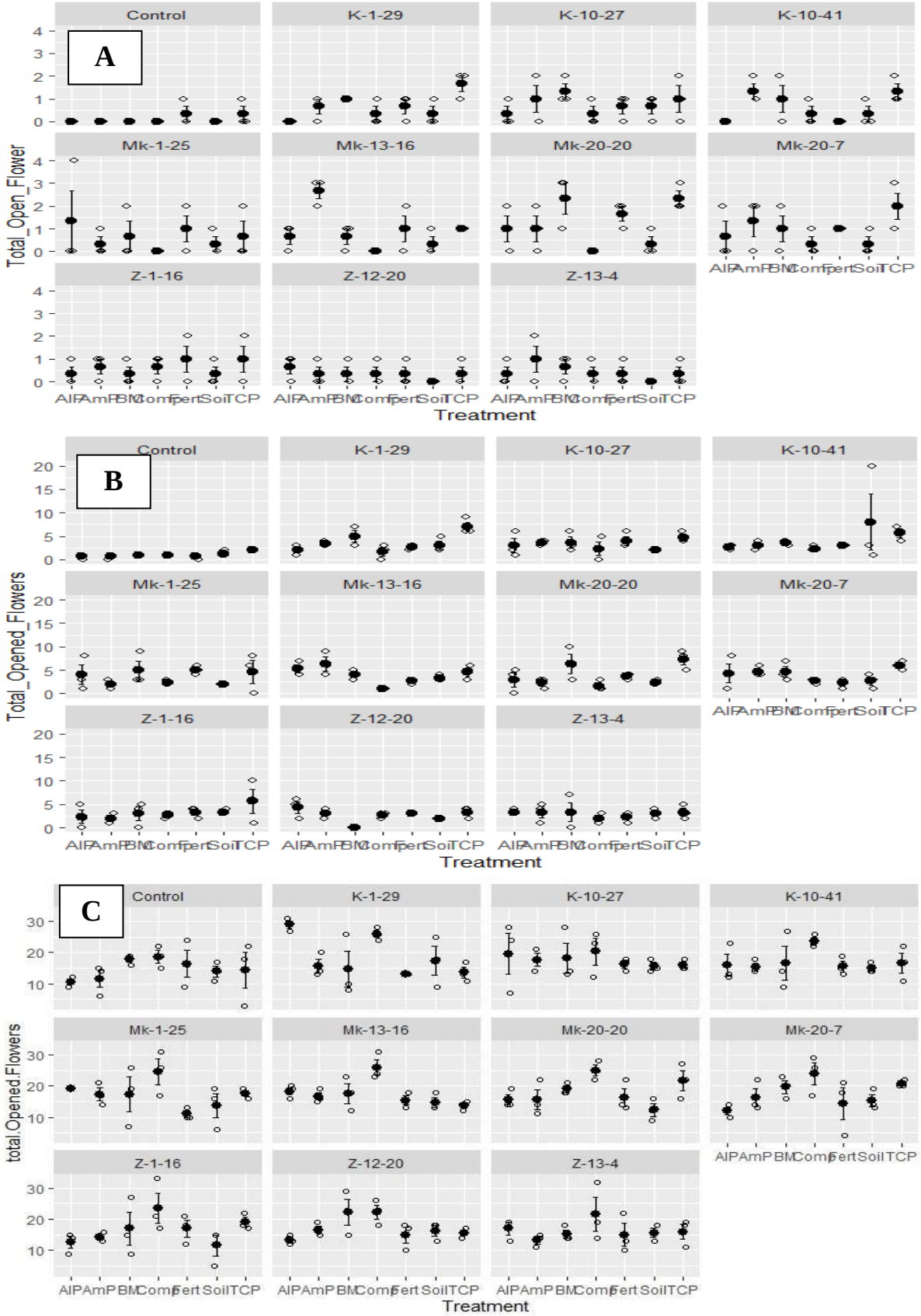


Figure 4.2: Number of total opened flowers (A= 30, B= 60 and C= 90 days of tomato plantation in greenhouse under different P-substrate and PSB strain co-inoculation).

4.3.3 Tomato Yield and Biomass

The highest average fruit number (6.1) and total fruit weight (158.71 g) per plant were harvested from the inoculation of K-1-29 and Mk-20-20, respectively (Figure 4.3 and Supplementary Figure 4.1). The interaction of individual strains and added P substrates confirmed that each strain responded differently to the respective supplemental P sources. For instance, Mk-20-20 produced the top-ranked average fruit weight while co-inoculated with AmP, with BM, and control (soil without external P-source), 3rd with DAP fertilizer, 4th with compost and AlP, and 9th with TCP. Similarly, K-1-29 ranked 1st in fruit numbers when combined with TCP, 2nd with compost, AmP, and fertilizer, 4th with AlP, 5th with untreated soil, and 8th with BM (Figure 4.3). Generally, co-inoculation with compost promotes fruit numbers, whereas AlP and AmP improve fruit size and fruit weight (Figure 4.3 and Supplementary Figure 4.1). In this experiment, fertilizer-isolates interaction resulted in a minimum fruit yield and recorded unusual performance in most tomato growth parameters. Hence, further investigation and field trial is recommended.

By the time of harvesting (110 days after sowing), some tomato plants had started to dry and had lost most of their leaves (Picture 4.1 and 4.2). Tomatoes inoculated with K-1-29 improved stem thickness (3.27 cm), whereas Z-12-20 was induced to improve shoot fresh weight (102.57 g), root fresh (40.42 g) and dry weight (10.39 g) (Table 4.7). The longest root length (36.95 cm) was recorded by control (uninoculated) and Mk-20-7-inoculated tomatoes. This might indicate that Mk-20-7 induced root growth and stretch the root system to explore and absorb more nutrients and water. The addition of aluminium phosphate showed a positive interaction to improve the stem thickness (3.29 cm), although it was recorded as having one of the lowest fresh and dry weights for both shoot and root (Table 4.7). On the other hand, compost application significantly ($p<0.05$) improved tomato shoots fresh and dry weight as well as root fresh weight. Possibly, this might confirm that plant height and shoot weight are positively correlated, as compost substantially improved tomato height (Table 4.3 and Figure 4.1) and indirectly suggested the accumulation of P in the shoot system. Untreated soil (control group) resulted in the longest (38.21 cm) root development, which confirmed that the plant extends the root system as much as possible to navigate the available nutrients, indirectly causing delayed maturation time and harvesting.

Picture 4.2: Tomato harvesting from greenhouse experiment and biomass measurements.

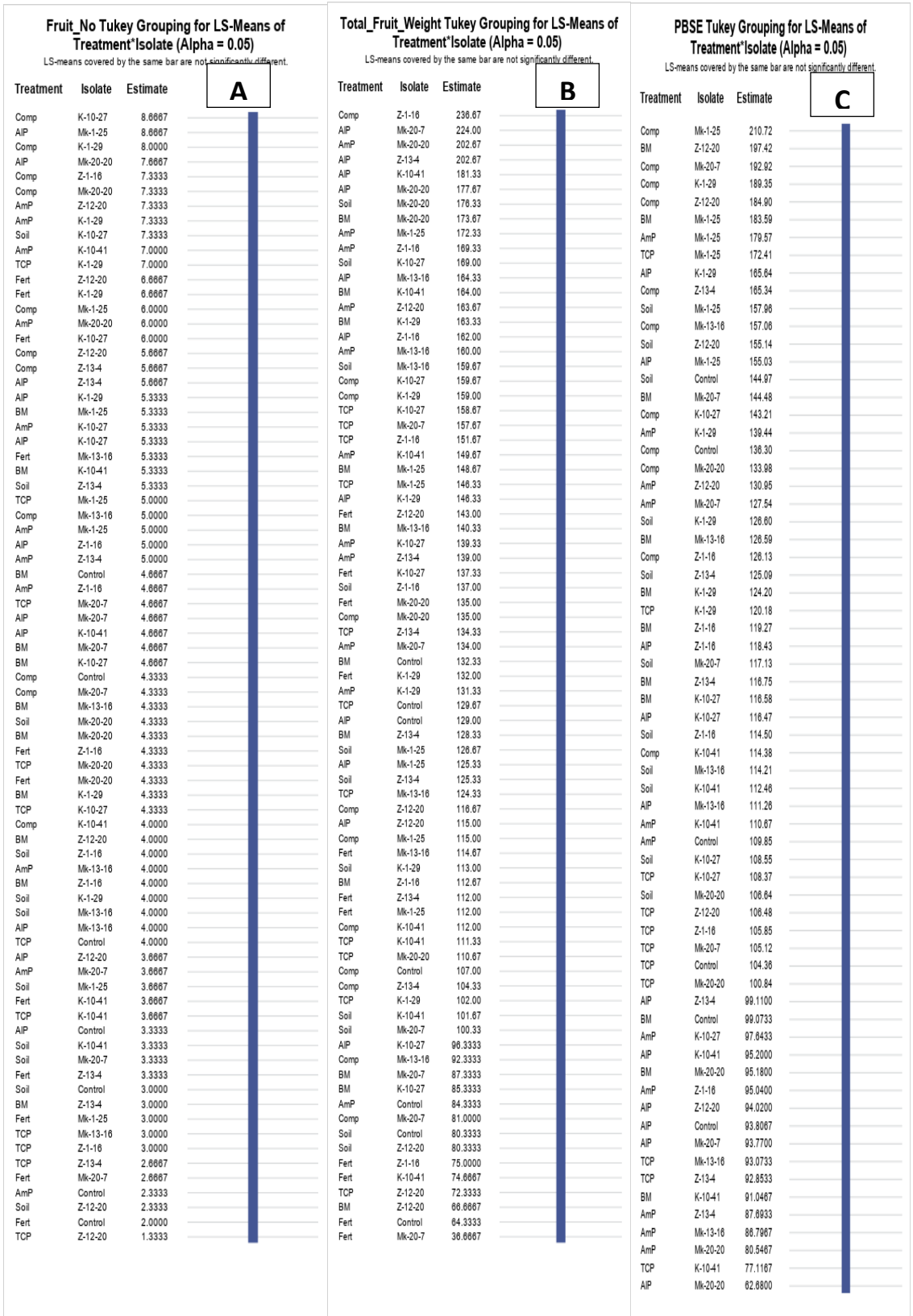


Figure 4.3: Typical response of PSB strains and P-complexes on tomato fruit number (A), weight (B) and overall symbiotic effectiveness (C).

4.3.4 Symbiotic Effectiveness and Plant Phosphorus Estimation

The difference between strain-inoculated and fertilized plants shoot dry weight is used to estimate the level of plant-inoculum phosphate-based symbiotic effectiveness and shoot accumulated P. To this effect, the higher the phosphate-based symbiotic effectiveness, the more shoot dry matter, which indirectly confirmed the higher P uptake by plants and accumulation in the shoot. Nevertheless, it should be clear that since the symbiotic interaction is between biological organisms, the strains' symbiotic effectiveness may not be pronounced precisely in terms of shoot dry weight and rough estimation of shoot P content; somewhat, their overall response and contributions in macro- and micronutrient accession, phytohormone production, antagonistic effects, stress mitigation and induction of yield should be considered. Almost all inoculated PSB strains showed encouraging symbiotic effectiveness with tomatoes. Mk-1-25 formed strong symbiosis (Figure 4.3) which resulted in a maximum PBSE(%) (176.55), whereas Mk-20-20 recorded one of the lowest estimations (96.64) (Table 4.7). Simultaneously, the addition of extra P sources improved tomato-strain symbiotic effectiveness. For instance, compost was found to be the top (159.48%), inducer while TCP was relatively one of the least (100.42%) symbiotic inducers.

Table 4.7: Mean value of tomato biomass yield.

Isolates effect on tomato fresh and dry weight							
Inoculant	Stem thick.	Shoot-FW	Shoot DW	Root length	Root FW	Root DW	PBSE (%)
Control	3.16±0.24^{ab}	98.14±31.82^{ab}	17.32±4.52^{a}	36.95±8^{a}	40.17±18.41^{a}	9.37±3.57^{ab}	114.72±39.87^{cd}
K-1-29	3.27±0.22^{a}	84.29±34.0^{bc}	17.71±4.83^{a}	35.19±7.66^{abc}	29.42±11.81^{bc}	8.48±3.13^{bc}	144.24±58.47^{b}
K-10-27	3.21±0.22^{ab}	81.00±22.11^{bc}	16.5±4.6^{a}	33.14±5.51^{abc}	28.71±7.54^{bc}	8.21±2.37^{bc}	115.14±41.32^{cd}
K-10-41	3.11±0.27^{b}	85.86±33.63^{abc}	15.74±4.58^{a}	35.29±7.24^{abc}	32.47±18.17^{abc}	8.42±2.75^{bc}	100.15±27.8^{d}
Mk-1-25	3.18±0.21^{ab}	93.48±35.34^{abc}	17.87±4.54^{a}	32.62±7^{bc}	30.49±12.08^{bc}	8.09±2.51^{bc}	176.55±65.7^{a}
Mk-13-16	3.11±0.31^{b}	79.48±34.9^{c}	16.98±4.54^{a}	33.62±5.66^{abc}	28.43±18.52^{bc}	8.04±2.42^{bc}	114.83±36.71^{cd}
Mk-20-20	3.11±0.23^{b}	78.91±39.88^{c}	17.47±5.25^{a}	34.05±6.10^{abc}	35.27±18.82^{abc}	8.12±3.4^{bc}	96.64±31.11^{d}
Mk-20-7	3.2±0.25^{ab}	91.33±31.85^{abc}	16.71±5.67^{a}	36.91±7.15^{a}	40.3±16.08^{a}	8.72±2.38^{ab}	130.16±50.46^{bc}
Z-1-16	3.12±0.23^{b}	81.33±31.61^{bc}	16.37±5.97^{a}	32.0±7.06^{c}	27.41±9.14^{c}	6.83±1.91^{c}	113.20±35.47^{cd}
Z-12-20	3.21±0.3^{ab}	102.57±40.25^{a}	17.78±5.85^{a}	36.33±7.22^{ab}	40.42±20.01^{a}	10.39±4.8^{a}	144.82±45.10^{b}
Z-13-4	3.08±0.28^{b}	89.76±24.84^{abc}	16.54±4.51^{a}	34.86±7.14^{abc}	36.51±23.76^{ab}	9.23±4^{ab}	114.47±48.15^{cd}
CV	7.67	33.23	25.118	18.71	44.18	8.54	33.64
LSD	0.15	17.75	2.6	3.94	9.03	1.81	27.45
Treatments effect on tomato fresh and dry weight							
Treatment	Stem thick	Shoot FW	Shoot DW	Root length	Root FW	Root DW	PBSE (%)
AlP	3.29±0.22^{a}	77.61±32.29^{c}	15.12±4.6^{c}	36.36±7.47^{ab}	26.38±12d^{e}	7.82±2.63^{b}	109.58±49.04^{bc}
AmP	3.12±0.24^{bc}	74.21±21.86^{c}	15.8±3.66^{c}	33.49±6.79^{bc}	33.93±15.2^{bc}	8.27±2.65^{ab}	113.25±45.93^{bc}
BM	3.03±0.24^{c}	84.91±32.48^{bc}	18.06±4.95^{b}	30.61±5.05^{c}	23.84±13^{e}	6.35±2.94^{c}	128.56±47.47^{b}
Com	3.22±0.22^{ab}	122.46±33.91^{a}	22.21±4.89^{a}	36.24±7.19^{ab}	42.61±18^{a}	9.0±3.03^{ab}	159.48±56.69^{a}

Fert	3.11±0.23^{bc}	77.58±24.39^{c}	14.93±3.83^{c}	36.0±5.79^{ab}	35.92±13.5^{abc}	9.34±2.85^{a}	125.75±35.48^{bc}
Soil	3.14±0.27^{bc}	99.06±28.49^{b}	17.89±3.92^{b}	38.21±6.75^{a}	40.61±18.36^{ab}	9.41±3.69^{a}	107.88±41.42^{c}
TCP	3.22±0.28^{ab}	79±31.24^{c}	14.98±3.56^{c}	31.52±6.43^{c}	31.91±16.09^{cd}	9.57±3.33^{a}	100.42±38.22^{c}
CV	7.67	33.23	25.118	18.71	44.18	8.54	32.6
LSD	0.12	14.16	2.08	3.14	7.2	1.44	20.28

NB: Means with similar letters have no significant difference at p=0.05. PBSE= phosphate based symbiotic effectiveness.

4.4 DISCUSSION

The agricultural sector needs feasible intervention to improve resource utilization, increase sustainable production, and ensure food security for the rapidly growing population by reducing climate change risks (Kifle *et al.,* 2022; Nosheen *et al.,* 2021). As indicated by Kidane *et al.* (2022) soil fertility maintenance and crop disease management are the main challenges for Ethiopian agricultural production. Tomato is one of the top commercial crops demanding aggressive agrochemical consumption. Synthetic fertilizers and chemical applications are the usual tomato farming practices; nevertheless, cost constraints, health impacts, fixation, and residual accumulation are a matter of concern. Application of P-solubilizers (Zhang *et al.*, 2021) alongside organic or inorganic phosphate supplements to tomato farms improved P solubility and plant availability, reduced P-fixation, stimulated tomato growth, disease resistance, yield, and biomass (Liu *et al*., 2020; Baba *et al*., 2017). Similarly, Fenta and Assefa (2017) reported that application of PSB isolate together with TCP increased tomato shoot biomass and P uptake under greenhouse experiment.

Earlier research reports indicated that microbial activities in the root-zone revealed improvements in plant available nutrients (Zhang *et al.*, 2021; Pande *et al*., 2017), water and nutrients uptake (De Zutter *et al*., 2022; Nosheen *et al.,* 2021), plant development, stimulation of growth hormones (Widnyana, 2018; Egamberdieva *et al.,* 2015), increased soil pathogen mitigation (Planchamp *et al*., 2015), activate induced systemic resistance (Shavit *et al*., 2013), stress alleviation, yield, and biomass (Zaidi *et al.,* 2009). In the current study, ten selected PSB strains were evaluated for their symbiotic interaction and response efficacy towards biofertilizer and PGPR development for tomato farms. As indicated in Raimi *et al.* (2021), competent strains will be selected based on plant growth-promoting ability at *in vitro* (molecular, genomics, metabolomics along with cultured-based), greenhouse and field trials. Sharma *et al*. (2013) also indicated that *in vitro* solubilization competence helps to screen candidate PSB strains for crop production prosecution. De Zutter *et al*. (2022) also described

the top-down selection strategy in which many isolates are preliminarily screened on growing media, then the best-performing isolates are screened *in planta* assays under a greenhouse, and finally top-class isolates are selected based on open field evaluation. This is because, the effect of rhizobacteria in crops productivity/response varies under lab, greenhouse and field trials (Ahemad and Kibret, 2014). Therefore, these ten PSB isolates with maximal solubilization efficiency in the laboratory were selected and tested for tomato plant assays in a controlled environment.

As demonstrated by Poonia and Dhaka (2012), basic agronomic parameters like plant height, leaf area index, and yield-related traits are useful signposts to judge PSB efficacy. In agreement with this, the current study showed that tomato seed germination, plant height, floral parameter development, biomass, and fruit yield were the major traits induced by the inoculation of PSB. It is important to note that plant development stages guide strain patterns of activity to demonstrate their significant effects. Their degree of response varied depending on the growth stage of the tomato and the nature of the strains. Similarly, Collavino *et al*. (2010) described that different strains colonize plant root zones at different speeds and respond differently to plant parameters, including height, root, and biomass. For instance, inoculation of Mk-20-20 significantly ($p<0.05$) improved tomato seed germination and fruit weight (Table 4.8 and Figure 4.4 and supplementary Figure 4.1); K-1-29 significantly ($p<0.05$) promoted leaf and node number at the early growth stage, flower clusters, buds, fruit numbers, and stem thickness. A comparable publication by Egamberdieva *et al*. (2015) demonstrated that PGPR stimulated tomato and pepper seed germination and growth under greenhouse production. Likewise, Vaikuntapu *et al*. (2014) indicated that tomato inoculation with growth-promoting bacteria positively influenced the growth and resistance to *Fusarium* species. Pellegrini *et al.* (2021) also reported a supportive result that PGPR inoculation of onion seeds improved germination, growth, yield, quality, and soil fertility.

Besides the overall symbiotic effectiveness, Mk-1-25 substantially improved tomato plant height, leaf numbers, and branch development at the late growth stages. Strain Z-12-20-induced plant height at the 60^{th} and succeeding growing days (Table 4.3), shoot and root fresh weight, as well as root dry weight (Table 4.7). In agreement with this, Wlapola and Yoon (2013) reported that PSB strain inoculation improved tomato height and biomass. Likewise, Fenta and Assefa (2017), indicated that PSB isolates inoculation of tomato increased shoot length, dry matter, and phosphorous uptake at greenhouse trial. These two strains (Mk-1-25

and Z-12-20) recorded the maximum shoot dry weight (17.87 and 17.78 g, respectively (Table 4.7)) and percentage of phosphate-based symbiotic effectiveness (176.55 and 144.82%, respectively (Figure 4.3)), which indicated successful tomato-strain interaction and collaboration. Muleta (2018) demonstrated that >80% SE is highly effective, whereas <35% SE is ineffective. Accordingly, the current strains showed strong symbiotic effectiveness (>80%) with tomato. Likewise, it is estimated that phosphorous contributes 0.2-0.8% of plant shoot dry weight (Nosheen *et al.*, 2021; Chen and Liu, 2019), hence, the shoot dry mass ratio of inoculated tomatoes over fertilized tomatoes is used to estimate the efficacy of the strain's symbiotic relation and the amount of shoot P (%) (Somasegaren and Hoben, 1994). Supportively, De Zutter *et al.* (2022) reported that inoculation of *Burkholderia* spp. improved plant P-uptake, and *Enterobacter* spp. significantly increased plant shoot biomass. Fenta and Assefa (2017) also indicated PSB application increased tomato shoot dry matter and P uptake. Moreover, it should be underlined that, as biological entities, the success of PSB inoculation might not be solely related to shoot dry weight and shoot P content; rather, their collective induction in the host crop germination, vegetative growth and yield could reflect their symbiotic effect. Their contribution in phytohormone production, stress alleviation and other important attributes broadly benefit the plant including total fruit yield improvement. As a result, the maximum average fruit number (6.1) was harvested from K-1-29 inoculated tomatoes, and the largest overall tomato fruit weight (158.71 g) was harvested from Mk-20-20 inoculation. The current fruit yield was lower in fruit number but much greater in fruit weight than Poonia and Dhaka's (2012) finding that they harvested 16.34 fruits per plant with a total of 77.75 g. Furthermore, various PGPR strains such as *Bacillus subtilis* (Errington and Aart, 2020; Martinez, 2013), *Bacillus halotolerance* (Wu *et al*., 2022), *Priestia megaterium* (Hwang *et al*., 2022), *Bacillus amyloliquefaciens* (Naz *et al*., 2022), *Bacillus velezensis* (Rabbee *et al*., 2019), and *Burkholderia cenocepacia* (Ryall *et al*., 2008) have been exhibited different plant growth-promoting activities and stresses alleviation on plants. Similarly, Kumar *et al.* (2022) indicated that bacterial species from *B. amyloliquefaciens, B. halotolerance and B. velezensis* sp. exhibited about 71% inhibition on *Fusarium sp., Marcophomina* and *Alternaria* sp. on common bean.

In addition, this study corroborates with other studies that inoculation of PSB observed a considerable impact on plant assays and yield on different crops (i.e., tomato (Zhang *et al.*, 2021; Baba *et al*., 2017; Wlapola and Yoon, 2013; Poonia and Dhaka, 2012), onion (Pellegrini *et al., 2021),* bean (Collavino *et al.*, 2010), maize (Yasmeen *et al.*, 2022; Manzoor

et al., 2017; Pande *et al*., 2017), rice (Chen and Liu, 2019), wheat and sugarcane (De Zutter *et al*., 2022)). Furthermore, Egamberdieva *et al.* (2015) indicated that PGPR increased soil structure, rhizosphere nutrient availability, and root volume (root length, dry weight, and branching) for maximum absorption. On the other side, Z-13-4, Mk-13-16, and Z-1-16 relatively showed limited observable impacts on tomato growth and development under greenhouse condition. Similarly, Collavino *et al.* (2010) reported a supportive result that R4M-F strain inoculation showed no positive effects on the bean plant. In line with this, Sharma *et al.* (2013) also stated that while PSB isolates were subjected to further evaluation and verification of their symbiotic effectiveness and direct plant-available P contribution, only a very few isolates showed realistic symbiotic competence.

To maximize tomato-isolate symbiotic interaction and plant availability of phosphorous, six possible P sources (aluminium phosphate, ammonium phosphate, tricalcium phosphate, bone meal, compost, and DAP fertilizer) were amended to the respective pots. Accordingly, all strains showed higher symbiotic effectiveness (Table 4.7). As shown by Wan *et al*. (2020), PSB strains have different abilities to solubilize various insoluble phosphate groups, including TCP, AlP, and others. They have substrate, nutritional, and environmental specificities for their optimum performance. Similarly, Chen and Liu (2019) reported that strain S32 solubilizes TCP at a greater speed than lecithin or rock phosphate. The present result strongly demonstrated that the combined application of PSB strain and external P substrates resulted in significant ($p<0.05$) improvements in plant assays (plant height, leaf and floral development, biomass, and fruit yield) compared to the control groups. In agreement with this, Yasmeen *et al.* (2022) have found that the application of rock phosphate and PSB strain (*Bacillus cereus* GS6) significantly increased maize growth and productivity.

Combined application of PSB and compost significantly ($p<0.05$) increased tomato height, leaf number, branch, flower parameters (clusters, bud number, open flowers), fruit number, shoot and root fresh weight, root dry weight, and overall symbiotic effectiveness. Among the six added external possible P substrates, compost significantly ($p<0.05$) induced tomato-PSB interaction and elevated plant height development at all growth stages (Table 4.3 and Figure 4.1) and recorded the highest plant height (123.15 cm), which was lower than the report of Liu *et al.* (2020), who found the highest tomato height (192.5 cm) with significant improvement of root activities, photosynthetic rate, and fruit yield from the combined application of compost and PSB. Likewise, comparable research conducted in Burusa district,

South-West Ethiopia, by Girshe *et al.* (2018) confirmed that optimum compost tea application significantly ($p<0.05$) improved tomato height (157.64 cm), number of branches, root length (28.81 cm), date of flowering, fruit maturation, and fruit yield. This might suggest that tomatoes obtained multidimensional benefits from compost in terms of nutrient absorption, improved water holding capacity (boost soil moisture), better water and air circulation, as well as induced microbial activities to stimulate tomato growth hormones. In a similar fashion, AlP co-inoculation with PSB strains significantly ($p<0.05$) enhanced germination, early flower cluster development, flower openings, and most importantly, fruit weight.

As demonstrated by Poonia and Dhaka (2012), co-inoculation of fertilizer and PSB significantly improved tomato height, leaf area index, and fruit yield. Other related publications by Baba *et al.* (2017) and Zaidi *et al.* (2009) also recommended PSB strains inoculation together with an appropriate fertilizer rate. However, for unclear reasons, the current result indicated that PSB strains preferred more other phosphate substrates over inorganic fertilizer. In most cases, strains showed limited efficiency to induce tomato vegetative growth and fruit yield while they were synergistically applied with DAP fertilizer. In line to this, De Zutter *et al.* (2022) published a supportive document stating that the addition of fertilizer has no significant effect on PSB effectiveness at field or greenhouse experiments. On the contrary, untreated pots (soil without external P supplements) showed minimal plant height at the initial growth stages but it improved through time and recorded the second highest tomato shoot height at the late growth periods and then the longest (38.21 cm) root proliferation. This might be due to the plant being reframed and slowly adjusted in the root system to maximize the clutching of water and nutrients from the surrounding area at the later growth stages to keep persistent growth progress while other treated plants stopped growing and began drying. In agreement with this, Zhang *et al.* (2021) have found that tomato root parameters significantly increased by the control groups. In support of this, De Zutter *et al.* (2022) have justified that plants increased the root architecture and root-to-shoot ratio to overcome nutritional starvation. Similarly, Collavino *et al.* (2010) reported that uninoculated and unfertilized plants recorded lower leaf area and shoot dry weight but higher root/height ratio than treated plants. Even though it needs repeated verification, including field trials, this result might suggest that co-inoculation of potential PSB strains at the side of extra P sources would improve tomato production with the easiest, cheapest, and most eco-friendly approach.

4.5 CONCLUSION AND RECOMMENDATIONS

Tomato production requires optimum growing conditions and efficient disease control. Among the ten PSB strains, four of them (K-1-29, Mk-1-25, Mk-20-20, and Z-12-20) achieved encouraging results in different growth parameters at various growing stages of tomato. These four strains improved substantially tomato shoot length, leaf numbers, flower development, maturation, shoot, and root biomass. Fruit number and fruit weight were significantly improved by K-1-29 and Mk-20-20, respectively. Synergistic application, especially with compost, AlP, and TCP, encouraged symbiotic collaboration, tomato growth, development, and fruit yield. This finding might serve as a starting point to consider these PSB to combat plant-available P deficiency and agro-biotechnological applications. These four sequentially selected competitive strains are strongly recommended to be verified at open field level then, can be applied as cheap, affordable, and ecofriendly farm input for tomato as well as other crops. Moreover, the experiment has been showed a new horizon: efficient PSB strain inoculation together with available possible P substrate to improve tomato growth and fruit yield.

Table 4.8: Mean value of three middle leaf lengths and number of leaflets from 45 and 60 days grown tomato.

Isolates effect on tomato leaves length and leaflet number at 45 days of sowing

Isolate	Leaf Length-a	Leaflet-a	Leaf length-b	Leaflet-b	Leaf length-c	Leaflet-c
Control	28.71±3.54	9.29±2.33^{c}	28.95±3.07^{b}	11.43±1.29^{c}	27.43±5.24^{b}	13.24±3^{b}
K-1-29	30.05±4.02	12.14±2.9^{ab}	31.0±3.87^{ab}	14.38±2.73^{ab}	30.0±4.01^{ab}	15.4±2.48^{a}
K-10-27	28.38±4.3	11.8±2.23^{ab}	30.57±3.09^{ab}	13.19±2.16^{ab}	30.1±3.94^{ab}	14.5±2.48^{ab}
K-10-41	28.14±4.46	11.81±2.4^{ab}	30.48±4.42^{ab}	13.38±2.11^{ab}	28.0±7.56^{ab}	13.67±2.4^{b}
Mk-1-25	28.17±4.98	13.2±5.79^{a}	30.0±6.57^{ab}	14.81±5.83^{a}	28.24±6^{ab}	13.3±2.39^{b}
Mk-13-16	28.91±3.86	12.1±1.83^{ab}	30.71±3.48^{ab}	13.76±2.1^{ab}	29.8±3.65^{ab}	14.1±2.31^{ab}
Mk-20-20	29.05±3.88	11.2±2.29^{b}	30.81±3.68^{ab}	13.52±2.99^{ab}	29.29±4.3^{ab}	13.76±2.5^{b}
Mk-20-7	28.43±6.85	12.4±2.75^{ab}	30.86±3.14^{ab}	13.57±2.44^{ab}	30.52±3.78^{a}	14.5±1.40^{ab}
Z-1-16	28.24±5.67	11.33±2^{b}	30.33±5.62^{ab}	12.0±3^{bc}	29.6±6.82^{ab}	14.14±2.2^{ab}
Z-12-20	29.38±3.22	11.5±2.73^{b}	30.52±3.57^{ab}	12.33±2.46^{ab}	29.3±4.58^{ab}	14.6±2.77^{ab}
Z-13-4	30.00±2.74	12.1±1.3^{ab}	31.76±2.81^{a}	13.29±2.15^{ab}	29.4±4.79^{ab}	13.5±1.72^{b}
CV	13.81	23.88	12.41	20.94	16.69	16.57
LSD	2.43	1.7	2.31	1.71	2.97	1.42

Treatments effect on tomato leaves length and leaflet number at 45 days of sowing

Treatment	Leaf Length-a	Leaflet-a	Leaf length-b	Leaflet-b	Leaf length-c	Leaflet-c
AlP	26.36±5.27^{d}	10.3±2.59^{b}	28.79±3.35^{d}	12.18±2.21^{c}	27.67±3.23^{c}	13.3±1.96^{c}
AmP	27.33±3.15^{cd}	12.±1.98^{a}	28.97±3.49^{d}	12.76±2.21^{bc}	27.46±4.63^{c}	14.0±2.7^{abc}
BM	30.46±2.75^{b}	12.1±2.27^{a}	32.39±2.52^{ab}	14.18±2.26^{a}	30.2±4.53^{ab}	14.97±2.2^{a}
Com	32.97±2.79^{a}	11.7±1.71^{a}	33.27±3.24^{a}	13.64±2.28^{ab}	32.52±6.62^{a}	14.5±2.46^{ab}
Fert	28.91±4.03^{bc}	12.3±2.85^{a}	30.91±5.25^{bc}	13.42±2.81^{abc}	30.4±3.69^{ab}	14.4±2.4^{abc}
Soil	27.82±5.32^{cd}	11.9±4.87^{a}	29.15±5.25^{cd}	14.58±4.74^{a}	28.0±5.91^{bc}	13.9±2.0^{abc}
TCP	28.52±3.45^{c}	11.6±2.83^{ab}	30.33±4.17^{cd}	13.21±2.68^{abc}	28.4±4.54^{bc}	13.4±2.69^{bc}
CV	13.81	23.88	12.41	20.94	16.69	16.57
LSD	1.94	1.36	1.84	1.36	2.37	1.13

Isolates effect on tomato leaves length and leaflet number at 60 days of sowing

Isolate	Leaf Length-a	Leaflet-a	Leaf length-b	Leaflet-b	Leaf length-c	Leaflet-c
Control	34.13.67^{ab}	13.91±2.21^{c}	34.3±3.15^{ab}	15.1±1.93^{c}	34.2±3.36^{ab}	14.67±1.88^{b}
K-1-29	31.76±4.54^{bc}	15.67±2.22^{abc}	33.57±4.7^{ab}	16.8±2.04^{b}	33.1±3.94^{abc}	16.52±1.54^{a}
K-10-27	31.76±4.15^{bc}	14.48±2.18^{bc}	32.71±3.29^{b}	15.8±2.2^{bc}	31.9±3.02^{bc}	15.5±2.16^{ab}
K-10-41	32.24±4.2^{abc}	15.71±2.85^{ab}	32.91±4.7^{b}	16.2±2.14^{bc}	31.14±7.64^{c}	15.95±2.0^{ab}
Mk-1-25	34.38±5.88^{a}	16.0±2.12^{ab}	35.52±4.61^{a}	17.0±1.55^{ab}	34.71±4.51^{a}	16.71±1.82^{a}
Mk-13-16	33.2±4.16^{abc}	15.76±2.57^{ab}	33.5±3.31^{ab}	16.5±1.54^{bc}	33.95±3.2^{ab}	16.29±2.15^{a}
Mk-20-20	33.4±3.25^{abc}	15.67±2.22^{abc}	33.9±3.61^{ab}	15.8±1.76^{bc}	32.95±3.3^{abc}	15.6±2.42^{ab}
Mk-20-7	31.29±4.33^{c}	15.76±2.21^{ab}	32.71±4.28^{b}	17.2±2.21^{ab}	32.48±4.2^{abc}	16.67±1.85^{a}
Z-1-16	32.3±5.06^{abc}	15.43±2.6^{bc}	33.3±5.16^{ab}	16.1±2^{bc}	32.81±4.7^{abc}	16.24±2.41^{a}
Z-12-20	32.52±6.3^{abc}	17.24±6.55^{a}	32.67±7.16^{b}	18.43±5.7^{a}	30.86±7.4^{c}	16.38±4.03^{a}
Z-13-4	33.0±3.48^{abc}	15.67±2.22^{abc}	34.86±3.3^{ab}	16.67±2.2^{b}	33.48±3.6^{abc}	15.9±2.36^{ab}
CV	12.74	18.86	11.91	15.16	13.35	14.17
LSD	2.54	1.79	2.44	1.52	2.67	1.38

P-sources effect on tomato leaves length and leaflet number at the 60 days of sowing

Treatment	Leaf Length-a	Leaflet-a	Leaf length-b	Leaflet-b	Leaf length-c	Leaflet-c
AlP	31.55±3.53^{cd}	14.97±2.14^{c}	31.67±4.04^{c}	15.85±2.1^{bc}	31.7±3.38^{bc}	15.49±2.35^{c}

AmP	30.24±4.44^{d}	14.58±3.6^{c}	31.64±4.51^{c}	16.6±3.4^{abc}	31.12±3.85^{c}	16.0±3.41^{bc}
BM	33.18±5.38^{bc}	15.85±4.72^{abc}	34.46±4.8^{b}	17±3.98^{ab}	32.9±7.08^{bc}	16.1±2.02^{abc}
Com	36.18±2.62^{a}	16.52±2.99^{ab}	37.27±2.43^{a}	17.2±2.43^{a}	36.7±2.31^{a}	17.15±2.51^{a}
Fert	33.64±4.06^{b}	16.58±1.77^{a}	34.42±3.69^{b}	17.2±1.67^{a}	33.2±4.56^{bc}	16.6±1.73^{ab}
Soil	33.52±5.15^{bc}	15.09±2.44^{bc}	34.42±4.72^{b}	16.1±1.8^{abc}	33.42±4.87^{b}	15.51±1.86^{c}
TCP	30.79±3.4^{d}	15.42±2.12^{abc}	31.49±3.41^{c}	15.7±1.9^{c}	31.12±3.54^{c}	15.36±1.73^{c}
CV	12.74	18.86	11.91	15.16	13.35	14.17
LSD	2.02	1.43	1.94	1.21	2.12	1.1

NB: Means with similar letters have no significant difference at p=0.05.

CHAPTER FIVE

5. TOMATO PRODUCTION UNDER SYNERGISTIC APPLICATION OF PHOSPHATE SOLUBILIZING BACTERIA AND PHOSPHATE AMENDMENTS

Abstract

PSB has multidimensional benefits in broad host range interaction, nutrient access, phytohormone induction, stress alleviation, biocontrol activity, resistance, and eco-friend approach. This study aimed to evaluate the efficacy of PSB isolates co-inoculated with compost, bone meal, and DAP fertilizer on tomato growth response. Tomato seeds were treated with 10 selected PSB isolates separately and grown for 20 days on a seedbed, then transplanted to a field that was treated with external P-sources and enriched by PSB inoculum. PSB isolates showed positive interaction and achieved significant plant assays, including plant height, leaves, branches, flowers, and fruit development. Strain K-10-41 significantly promoted tomato plant height, floral development, and fruit yield; Mk-20-7 enhanced height and fruit weight; whereas K-10-27 induced tomato fruit numbers. Compost application promoted tomato-PSB interaction and induced tomato vegetative growth, whereas bone meal was the least promotor for most tomato plant assays. Bone meal was however, one of the top fruit development inducers (harvested 20.94 fruits/plant weighing 881.97 g). Mixing 50% mixture of recommended compost and DAP fertilizer application enhanced tomato vegetative growth and fruit yield (21 fruits per plant harvested that weighed 872.46 g). Based on the overwhelming performance, K-10-41 and Mk-20-7 applications together with compost and fertilizer mixture were found effective. Therefore, the result of this study implies that application of competent PSB strains together with nutrient supplements improved symbiotic effectiveness, sustainable production, and environmental health. Consequently, these promising strains would be recommended for tomato production of higher yield and sustainability after verifying their efficacy at different agroecology. Screening potential strains and evaluating their competence under different conditions would be the future perspectives to develop efficient inoculants. Moreover, synergetic application of organic supplements (compost, farmyard, bone meal, or other biowastes), bioinoculants, and proper agrochemicals would maximize production, environmental health, and is feasible for the economic, social, and ecological sense of balance.

Keywords: *bone meal, compost, fertilizer, isolate, PGPR, PSB, tomato*

5.1 INTRODUCTION

Phosphorous (P) is one of the macro elements (Alori *et al.,* 2017) essential to plant molecular, physiological, and structural activities (Kumar *et al.,* 2022; Yasmeen *et al.,* 2022; Egamberdieva *et al.,* 2015). The soil might be rich in phosphorous (400–1200 mg/kg soil; Verma *et al.,* 2019), although plant-available phosphorus is very limited. Since soil P deficiency is the limiting factor for crop production, application of organic matter and chemical fertilizers are common to overcome nutrient deficiency (Alori *et al.,* 2017; Etissa *et al.,* 2014). Due to the rapid fixation nature of phosphate ions, most of the applied fertilizer (Kumar *et al.,* 2022) converted to unavailable residues (Dash *et al.,* 2017). Different complications with the deficiency of plant available nutrients (Amanullah and Khan, 2015), the efficacy of applied chemical fertilizers, and other constraints including production cost, environmental issue, and production demand calls organic matters and biological inputs for sustainable crop production (Kirui *et al*., 2022; Estrada-Bonilla *et al.,* 2021; Santos-Villalobos and Parra-Cota, 2021; Mitra *et al.,* 2020). As indicated in Rilling *et al*. (2019), diverse microbial groups, including beneficial microbes are rich in the root zone computing for root exudates. Plant growth-promoting rhizobacteria (PGPR) are considered as efficient biological inputs (Zhang *et al.,* 2021; Amaya-Gómez *et al.,* 2020) that enrich soil fertility, induce plant stress resistance, promote growth and development (Kumar *et al.*, 2021), reduce agrochemicals consumption, reduce production cost, improve crop yield, maintain environmental health (Kumar *et al.,* 2022), etc. Generally, Verma *et al.,* (2019) summarized that PGPR benefits plants in four major processes, namely, plant growth enhancement, biocontrol, stress management, and soil renovation by rhizoremediation. PSB are known PGPR (Kirui *et al*., 2022; Yasmeen *et al.,* 2022) with multidimensional benefits (Khan *et al.*, 2022; Sharma *et al.,* 2013), including broad host range interaction, accessing nutrients, phytohormone induction, stress alleviation, biocontrol activity, resistance enhancement (Figure 5.1), and eco-friendly approach (Amanullah and Khan, 2015).

Application of efficient PSB strains (Liu *et al*., 2020) effectively colonizes the root zone (Amaya-Gómez *et al.,* 2020; Verma *et al.,* 2019) to solubilize phosphate composites and make them accessible for plants which perchance supplement or replace synthetic fertilizer (Naz *et al*., 2022; Kalayu, 2019; Sharma *et al*., 2013). Phosphatase enzymes and organic acid production are employed to facilitate the solubilization of insoluble phosphate groups (Estrada-Bonilla *et al.,* 2021). Repeated inoculation of efficient PSB strains eventually leads

to successful colonization and the richness of beneficial microbes (Nosheen *et al.*, 2021; Liu *et al.*, 2020). Efficient strains can be applied as a supplement or alternative to synthetic fertilizer, yet, practicing together with proper agricultural inputs drives fruitful production Kalayu (2019) and Sharma *et al*. (2013). Currently, potential strains are amended with organic matter, cell protectants, and nanoparticles to increase their survival and efficacy (Kumar *et al.*, 2022).

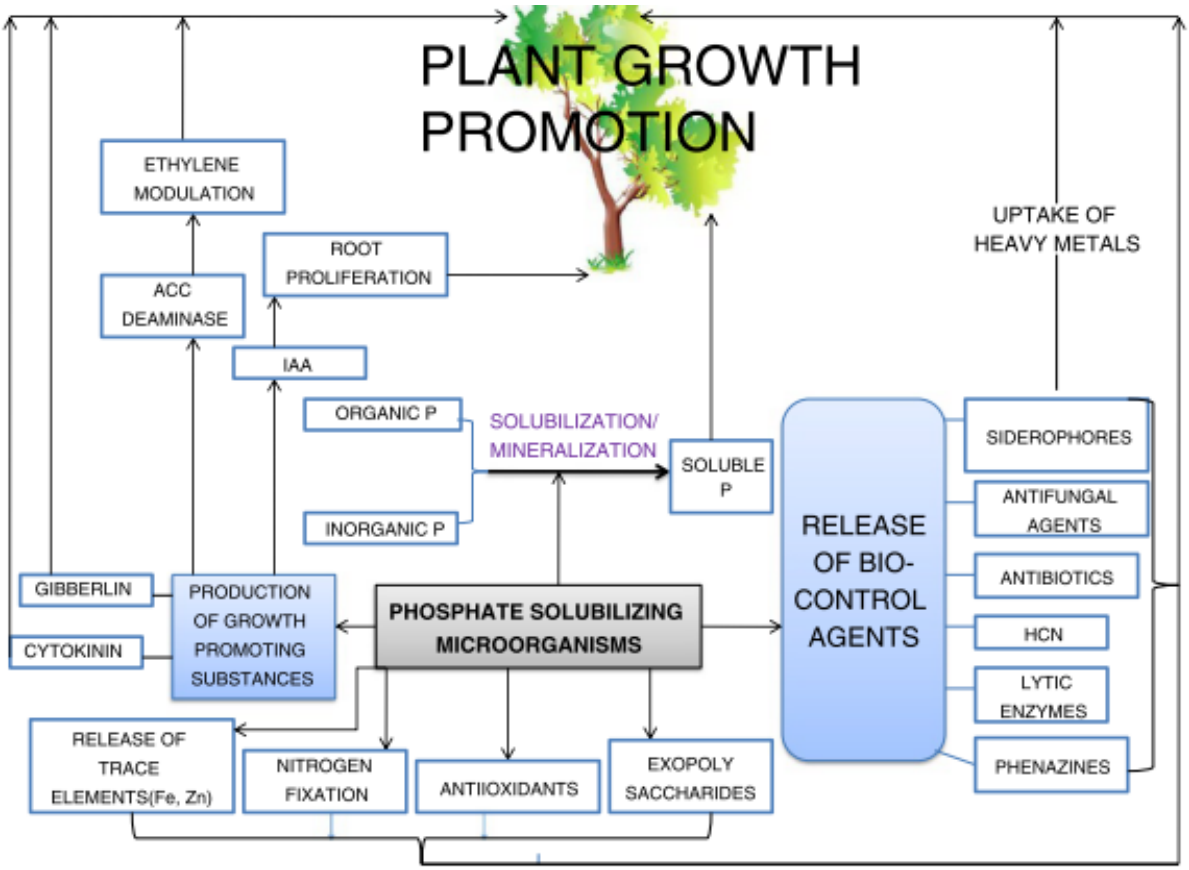


Figure 5.1: Schematic diagram for plant growth promoting mechanisms of PSM (*Figure adopted from Sharma et al., 2013*).

Most arable lands in Ethiopia are poorly managed and have lost soil fertility (Kidane *et al.*, 2022). Farmers follow traditional farm practices that might have contributed to strong soil fertility depletion and low productivity. As a result, smallholder farmers increased fertilizer application season-to-season (Tamene *et al.*, 2017). Ammar *et al.* (2023) and Abebe *et al.* (2022) indicated that in modern agri-practices chemical fertilizer application increased even if their repetitive use contributes for destruction of land fertility and environmental health. Though, the production cost is heightened (Kirui *et al.*, 2022), soil fertility maintenance (nutrient amendment) and proper farm practice (ploughing, irrigation, conservation, and weeding) are essential to increase production. Despite the increasing trends overtime, the proper chemical fertilizers application rate is relatively low (Chapter two). For instance,

Etissa *et al.* (2014) reported that 51.5% of vegetable farms in the Central Rift Valley (which is one of the top vegetable producing areas in the country) needs immediate amendments. Accordingly, farmers applied a maximum fertilizer dose (200–800 kg/ha DAP) for tomato and onion production. The impact of this overrated and unwise fertilizer application was narrated by Girshe *et al.* (2018) in the way of economic loss, environmental pollution, soil fertility deterioration, and residue accumulation. The defective application might be attributable to a lack of proper fertilizer recommendation, climatic shocks, environmental risks, economic barriers, and the awareness of smallholder farmers (Chapter two). Moreover, most smallholder farmers practiced poor organic matter application trends due to competition for feed, cooking fuel, construction, and bedding purposes alongside substandard chemical fertilizers uses irrespective of the crops, soil nutrients composition, landscape, and rainfall gradients, which result in poor crop response and production (Tamene *et al.*, 2017). Tomato is one of the most agrochemicals consuming crops. Tomato production in Ethiopia is increased from time to time. The production and yield are low compared to other tomato-producing regions due to low fertility maintenance (Girshe *et al.*, 2018), inputs, poor infestation, and farm management (Chapter two). Since tomato is vulnerable to various stresses (Zhang *et al.*, 2021), it showed minimal tolerance for different challenges. As a result, they require continuous supervision and monitoring, proper infestation control (Bai *et al*., 2018), fertility maintenance (nutrient amendment), optimum growing conditions, and a conducive environment, among others.

This study focused on cheap, affordable, and easily available tomato fertilization to replace or supplement traditional farm practices. Efficient PSM can supplement about 30-35 kg of P_2O_5/ha (Dash *et al*., 2017). As shown by Amanullah and Khan (2015), most soils have less organic matter, as a result complementing organic matters (compost, farmyard, or other organic wastes) and bioinoculants (biofertilizers and growth promoters) alongside recommended fertilizer rate can help to achieve the expected crop production in a sustainable manner. Previous reports on tomato inoculation with PSB (Zhang *et al.*, 2021; Amanullah and Khan, 2015) indicated production increment, disease resistance, stress resistance, and a reduced production cost. Similarly, Poonia and Dhaka (2012) have reported encouraging result after the application of PSB inoculum on tomato together with an appropriate fertilizer rate. In Ethiopia, different studies have been conducted so far to screen PSB isolates from different crops and agroecology (Abeje *et al.*, 2024; Muleta *et al*., 2018; Kurabachew and Wydra, 2013; Muleta *et al*., 2013; Woyessa and Assefa, 2011). However, the efficacy of a

very limited number of isolates is attested at greenhouse and field levels. As indicated by Mekonennen and Kibret (2021), in Ethiopia, though there is a shortage of well-organized report and research status about PGPR and vegetables association, there was no reports on field trials. Similarly, limited or no organic supplements like bone meal and compost application have been evaluated on tomato production. Therefore, this study aimed to evaluate the efficacy of PSB strains co-inoculated with compost, bone meal, and DAP fertilizer on tomato growth and yield. It is designed to reduce the agrochemicals consumption, to minimize farm production cost, and to improve sustainable production by presenting competent bioinoculants along with nutrient supplements.

5.2 MATERIAL AND METHODS

5.2.1 Study Farm Preparation

The study was conducted from December 2021-March 2022 using irrigation (it was dry season) at Hawassa University main campus research site, Hawassa, Ethiopia. The land was twice ploughed, well prepared then blocks arranged in RCBD design with three replications. The blocks assigned for the group of external P-source treatments (i.e., each block was partitioned in to 5 plots for the respective P-treatment) and the plots assigned for the group of rows (i.e., each plot partitioned in to 11 rows for the 10 isolates and one control). Each experimental unit was arranged 1.5 m x 60 cm with 2 m space between blocks and 30 cm distance among each tomato plant. Each block was arranged with five plots (for external P-sources: 1) compost, 2) DAP fertilizer, 3) bone meal, 4) a mixture of 50% compost and 50% of the recommended rate of fertilizer added separately as per the recommended rate and one plot set control (no external P-source)). Mixed P-sources (50% compost and 50% DAP fertilizer mixture) reflected as an integrated means to enrich the soil and improve the response (Estrada-Bonilla *et al.*, 2021). Then the plots were placed into 11 rows to inoculate the 10 pre-screened PSB strains (K-1-29, K-10-27, K-10-41, Mk-1-25, Mk-13-16, Mk-20-7, Mk-20-20, Z-1-16, Z-12-20, and Z-13-4) per row separately and one row set control (inoculum-free).

5.2.2 Inoculum Preparation and Seed Inoculation

Pre-screened (Chapter three) from the tomato rhizosphere and characterized, 10 PSB strains were taken from the Hawassa University Soil Microbiology Lab and refreshed in PVK broth

for 48 h in a shaker (121 rpm) incubator. To provide a uniform coating the refreshed active culture used to soak tomato seeds (Kumar *et al.,* 2022). Familiar tomato seeds called *Galilee* variety was soaked overnight in the respective PSB isolates separately. This tomato variety was chosen for the reason that it was strongly preferred by smallholder farmers and widely cultivated due to its good fruit quality, yield, and marketability. Materials and seeds surface sterilization was conducted following the method given by Somasegaran and Hoben (1994). After overnight soaking (Yasmeen *et al.,* 2022), the extra inoculum was poured and seeds had left to dry for 30 minutes, then one seed per cell/hole was sown at 2 cm depth on a seedling tray containing compost and soil mixture. Before seeds were covered by the soil, one ml of inoculum was added (Estrada-Bonilla *et al.,* 2021) to enrich colonization and cover the seeds. The conventional seed coating (De Zutter *et al*., 2022) and seed inoculation strategies help to improve colonization and interaction (Pellegrini *et al.,* 2021). Each tray labelled as per the respected PSB inoculum and let to grow in a shade for 20 days. Trays were watered every day until transplantation was held. Transplantation is common practice for tomato (Etissa *et al.,* 2014) production than direct sowing because of germination issues, weeding and infestation control.

5.2.3 Tomato Transplantation and Growth Performance Recording

Twenty days grown healthy tomato plants were selected from each tray and transplanted to the farm. In doing so, the selected healthy tomato seedlings were transplanted to the respective rows then one ml of the corresponding active culture was inoculated before covering the root zone with soil as described by Estrada-Bonilla *et al.* (2021). This extra inoculation would maximize root and rhizosphere colonization as well as improve indigenous competition (Pellegrini *et al.,* 2021). Five tomatoes were planted per row then one week after transplantation, missing tomatoes or seedlings that failed to regenerate were replaced. Afterward, regular watering, weeding, staking, and monitoring were conducted until harvesting. Twenty days after transplanting, all treatments were re-inoculated with one ml culture of the corresponding PSB strains to increase the population and to maximize the root-zone colonization (i.e., to overcome indigenous competition and improve dominance). Urea was added to all treatments, including control, at the first date and on the 45^{th} day of transplantation as per the recommended rate. Maize was planted at the corner of the farm to serve as biological control or bordering. Data were taken from three tomato plants per row at 30, 45, and 60 days of transplantation and harvesting (90 days). Plant assays like tomato

height, leaf development, branch number, flower parameters, and fruit yield (number and weight) were recorded. The final total fruits were harvested after three months of maintenance of transplantation.

5.2.4 Data Analysis

The collected quantitative data were organized and analysed by R 4.2.1 and SAS 9.4 version software. ANOVA was conducted using Fisher's and Tukey's tests (significance set at p=0.05). the means of the treatments were differentiated using the LSD. Finally, the analysed data were explained and discussed in reference to other related works.

Picture 5.1: PSB and P-substrate co-inoculated tomato growth progress and fruiting at open field trial.

5.3 RESULT

PSB strains showed positive interactions with tomatoes to achieve significant plant assays against the control. For instance, the mean-variance of tomato plant height indicates significant ($p<0.05$) differences among strains and controls at different growth periods (Table 5.1 and Figure 5.2(A, B, C and D)). The highest plant height was recorded by Mk-20-7 at 30 and harvesting (90) days (15.12 and 67.2 cm, respectively), K-10-41 at 45 days (41.25 cm), and Mk-1-25 recorded 56.57 cm at 60 days, whereas inoculum-free (control) tomatoes recorded the least average height at 30, 45, and 60 days of transplantation (Table 5.1). Similarly, supplemented P-sources showed significant ($p<0.05$) differences among the treatments where the prominent tomato height was recorded from compost application at early growth stage whereas compost and fertilizer mixture improved at later growth periods (Table 5.1).

Table 5.1: Tomato plant height at different growth periods.

Isolate	30 days	45 days	60 days	Harvest
Control	12.3 ± 2.43^{c}	35.23 ± 6.77^{c}	47.45 ± 6.02^{c}	65.07 ± 9.45
K-1-29	14.32 ± 3.36^{ab}	39.74 ± 6.98^{ab}	54.89 ± 5.7^{ab}	65.27 ± 8.95
K-10-27	14.82 ± 2.86^{ab}	39.4 ± 8.76^{ab}	52.48 ± 6.04^{b}	63.73 ± 6.89
K-10-41	14.47 ± 3.21^{ab}	41.25 ± 6.22^{a}	53.12 ± 5.66^{ab}	61.73 ± 6.38
Mk-1-25	14.65 ± 2.52^{ab}	39.88 ± 6.84^{ab}	56.57 ± 6.35^{a}	65.53 ± 6.41
Mk-13-16	14.22 ± 3.84^{ab}	40.1 ± 8.2^{ab}	54.02 ± 6.83^{ab}	64.4 ± 8.06
Mk-20-20	14.23 ± 3.51^{ab}	38.57 ± 8.61^{ab}	53.55 ± 7.2^{ab}	65.2 ± 6.67
Mk-20-7	15.12 ± 3.71^{a}	39.45 ± 9.28^{ab}	54.2 ± 7.48^{ab}	67.2 ± 14.67
Z-1-16	14.48 ± 3.16^{ab}	39.04 ± 8.99^{ab}	53.5 ± 7.1^{ab}	64.4 ± 7.47
Z-12-20	15.15 ± 2.73^{a}	40.0 ± 6.96^{ab}	55.5 ± 5.05^{b}	63.67 ± 7.82
Z-13-4	13.62 ± 3.31^{bc}	37.79 ± 9.64^{bc}	52.64 ± 7.83^{b}	65.27 ± 6.57
CV	13.13	10.45	9.62	13.0
LSD	1.36	2.95	3.71	6.07
P-supplements				
BM	14.16 ± 2.83^{b}	38.67 ± 6.54^{ab}	52.56 ± 6.48	64.79 ± 10.52^{ab}
Compost	15.24 ± 3.94^{a}	40.27 ± 8.42^{a}	53.02 ± 6.41	63.12 ± 6.17^{b}
Fert	13.68 ± 2.65^{b}	39.83 ± 6.53^{a}	54.34 ± 5.99	64.09 ± 5.70^{ab}
Fert & Comp	14.1 ± 3.1^{b}	37.57 ± 9.63^{b}	52.49 ± 8.25	67.39 ± 9.47^{a}
Soil	14.36 ± 3.17^{ab}	39.33 ± 8.17^{ab}	54.83 ± 6.24	64.27 ± 8.39^{ab}
CV	12.99	10.45	9.62	13.0
LSD	0.92	1.99	2.5	4.1

**Means with similar letters has no significant difference at P=0.05.*

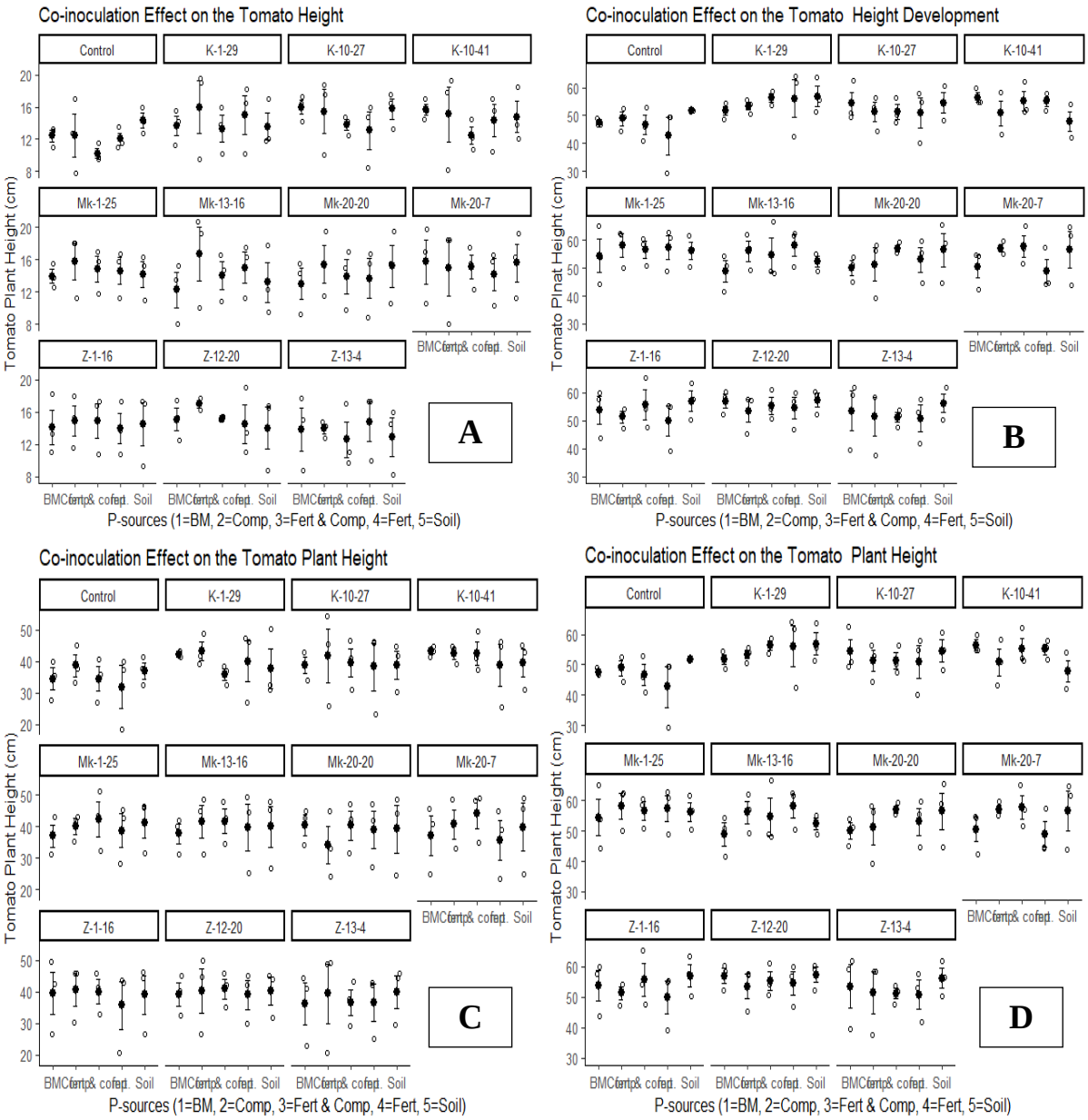


Figure 5.2: Tomato plant height at different growth periods (A= 30, B= 45, C= 60 and D= 90 days) of transplantation. The plant was co-inoculated with PSB isolate and P-supplement.

5.3.1 Tomatoes Leaves, Branches and Floral Development

Application of PSB strains together with external P-supplements showed positive results on tomato leaves and floral development. Analysing the leaf number mean-variance indicated that strains significantly improved tomato leaf development over the control (Figure 5.3). According to the three (30, 45, and 60) days of collected data (Table 5.2), the control (uninoculated tomatoes) recorded a minimum average number of leaves (5.4 and 11.05 at 30 and 60 days, respectively), whereas Z-12-20 inoculated tomatoes developed more leaves (7.43 and 11.37) at 30 and 45 days, respectively, and Mk-1-25 generated the highest leaves

(12.6) at 60 days after transplantation, which were contemplated as the active growth periods. Likewise, the addition of compost promoted early-stage leaf development (7.24 at 30 days), although fertilizer was induced at the mid-growth stages (45 days) and mixed application of 50% rate of the recommended fertilizer and compost induced more tomato leaf (12.32) development at 60 days (Table 5.2).

Table 5.2: Tomato Leaf and number of opened flowers at different growth periods.

	Leaf number			**Opened flowers number**		
Isolate	30 days	45days	60days	30 days	45days	60days
Control	5.4±0.98^{b}	10.82+0.71^{abc}	11.05+1.26^{c}	1.53+0.91^{c}	0.13±0.23^{ab}	8.18+3.78
K-1-29	7.1±0.92^{a}	10.77+1.14^{abc}	12.14+1.43^{ab}	2.52+1.49^{a}	0.21±0.38^{a}	9.88+3.62
K-10-27	7.25±0.92^{a}	10.48+1.26^{bc}	11.96+1.24^{ab}	1.78+1.24^{bc}	0.07±0.18^{ab}	8.39+4.36
K-10-41	7.23±0.77^{a}	10.85+1.46^{abc}	11.78+1.24^{bc}	2.61+1.54^{a}	0.00^{b}	9.48+3.89
Mk-1-25	7.3±0.66^{a}	11.2+2.24^{ab}	12.6+1.76^{a}	2.45+1.71^{ab}	0.13±0.3^{ab}	9.33+3.84
Mk-13-16	7.13±1.3^{a}	11.23+1.02^{ab}	12.25+1.32^{ab}	2.02+1.06^{abc}	0.07±0.18^{ab}	8.93+4.86
Mk-20-20	7.1±0.99^{a}	11.02+1.5^{abc}	11.98+1.21^{ab}	1.98+1.22^{abc}	0.2±0.37^{a}	8.77+5.01
Mk-20-7	7.18±1.66^{a}	10.87+0.93^{abc}	11.98+1.63^{ab}	2.22+1.6^{abc}	0.1±0.28^{ab}	8.1+3.9
Z-1-16	7.28±0.77^{a}	10.22+1.18^{c}	12.5+1.23^{ab}	2.0+1.84^{abc}	0.1±0.28^{ab}	8.64+3.64
Z-12-20	7.43±0.69^{a}	11.37+1.24^{a}	12.45+1.2^{ab}	2.38+1.49^{ab}	0.1±0.28^{ab}	8.83+3.0
Z-13-4	6.98±0.69^{a}	11.25+1.63^{ab}	11.81+11.25^{abc}	2.5+1.66^{a}	0.07±0.18^{ab}	8.0+4.3
CV	9.91	10.27	9.18	45.0	66.73	39.05
LSD	0.50	0.81	0.8	0.71	0.18	2.24
Possible P-sources						
BM	6.92±0.94	10.98+1.37^{ab}	11.74+1.23^{b}	0.00^{c}	2.23+1.45^{ab}	8.69+4.46^{ab}
Compost	7.24±1.12	10.68+1.56^{b}	12.17+1.17^{ab}	0.31±0.42^{a}	2.4+1.69^{a}	9.72+3.64^{a}
Fert	6.93±1.03	11.17+1.86^{a}	11.88+1.26^{ab}	0.06±0.17^{bc}	1.82+1.32^{b}	9.12+3.39^{ab}
Fert & Comp	7.01±1.23	10.86+1.15^{ab}	12.32+1.87^{a}	0.09±0.2^{bc}	2.2+1.47^{ab}	7.71+4.08^{b}
Soil	7.08±0.85	10.88+1.13^{ab}	12.13+1.3^{ab}	0.14±0.29^{b}	2.27+1.32^{ab}	9.47+4.29^{a}
CV	9.91	10.27	9.18	66.73	45.0	38.52
LSD	0.34	0.55	0.54	0.12	0.48	1.68

***NB**: Means with similar letters has no significant difference at P=0.05.*

Tomato branch, flower bud development, and flower openings were significantly ($p<0.05$) improved by the joint application of PSB strains and P-treatments. Inoculation stimulated tomato development at distinct levels at different growth periods, relatively the maximum number of tomato branches (4.9, 5.55, and 6.57) developed by inoculation of Mk-1-25, Z-13-4, and K-10-41 at 30, 45, and 60 days, respectively (Table 5.3). Flower bud number substantially improved by compost supplementation aside from inoculation of Mk-1-25 and Mk-20-20 at 30 and 45 days, respectively (Table 5.3). Similarly, early flower opening and the total number of opened flowers were significantly induced by compost application and inoculation of K-1-29 (Table 5.2).

Table 5.3: Tomato branch and flower bud development at various growth periods

	Branch development			Flower bud development		
Isolate	**30 days**	**45days**	**60days**	**30 days**	**45 days**	**60 days**
Control	3.5±1.22^{b}	3.67+1.01^{f}	5.43±1.12^{ab}	5.98±1.69^{ab}	0.75±0.62^{ab}	10.82±2.45^{a}
K-1-29	4.32±1.11^{a}	4.66+1.15^{bcde}	5.75±0.94^{ab}	5.37±1.83^{abc}	0.77±0.47^{ab}	10.4±1.91^{ab}
K-10-27	4.4±1.38^{a}	4.53+076^{cde}	5.77±2.45^{ab}	5.07±1.89^{abc}	0.67±0.41^{ab}	10.51±2.28^{ab}
K-10-41	4.77±0.92^{a}	4.84+1.24^{bcd}	6.57±3.94^{a}	5.58±1.35^{abc}	0.77±0.53^{ab}	10.83±1.49^{a}
Mk-1-25	4.9±1.11^{a}	4.96+1.08^{bc}	6.27±2.12^{ab}	5.23±1.54^{abc}	0.83±0.42^{a}	10.82±2.32^{a}
Mk-13-16	4.87±1.22^{a}	4.97+0.81^{bc}	5.42±0.74^{ab}	5.17±1.91^{abc}	0.62±0.52^{ab}	10.06±2.42^{ab}
Mk-20-20	4.84±1.58^{a}	4.32+1.33^{de}	5.42±0.97^{ab}	6.29±5.33^{a}	0.73±0.45^{ab}	10.23±2.61^{ab}
Mk-20-7	4.42±1.02^{a}	4.52+1.22^{cde}	5.12±0.71^{b}	4.83±2.21^{bc}	0.73±0.57^{ab}	9.8±1.88^{ab}
Z-1-16	4.31±1.30^{a}	4.27±1.0^{ef}	6.15±2.29^{ab}	5.25±1.98^{abc}	0.62±0.49^{ab}	10.1±2.42^{ab}
Z-12-20	4.88±0.97^{a}	5.12±1.08^{ab}	5.28±1.03^{b}	5.28±1.51^{abc}	0.77±0.4^{ab}	10.78±1.89^{a}
Z-13-4	4.63±0.98^{a}	5.55±1.54^{a}	5.6±1.09^{ab}	4.53±1.74^{c}	0.57±0.43^{b}	9.25±1.22^{b}
CV	17.04	16.63	30.83	32.22	51.62	18.77
LSD	0.57	0.56	1.27	1.24	0.27	1.39
P-Treatment						
BM	4.45±0.91^{bc}	4.71±1.11^{ab}	5.09+0.88^{b}	0.65±0.42^{b}	4.81±1.58^{bc}	9.4±1.9^{c}
Compost	4.67±1.3^{b}	4.47±1.44^{b}	5.35+0.89^{b}	0.89±0.58^{a}	6.11±3.6^{a}	10.30±1.69^{abc}
Fert	4.66±1.14^{b}	4.49±1.45^{b}	5.73+1.04^{b}	0.65±0.43^{b}	5.49±1.47^{ab}	10.67±1.74^{ab}
Fert&Comp	4.12±1.12^{c}	4.85±1.15^{a}	5.41+1.58^{b}	0.64±0.46^{b}	4.17±1.58^{c}	9.88±2.57^{bc}
Soil	5.15±1.35^{a}	4.78±1.36^{ab}	6.87+3.21^{a}	0.73±0.47^{ab}	6.05±2.12^{a}	11.13±2.2^{a}
CV	16.55	16.63	30.83	51.62	32.22	18.77
LSD	0.382	0.38	0.85	0.18	0.84	0.938

NB: *Means with similar letters has no significant difference at P=0.05.*

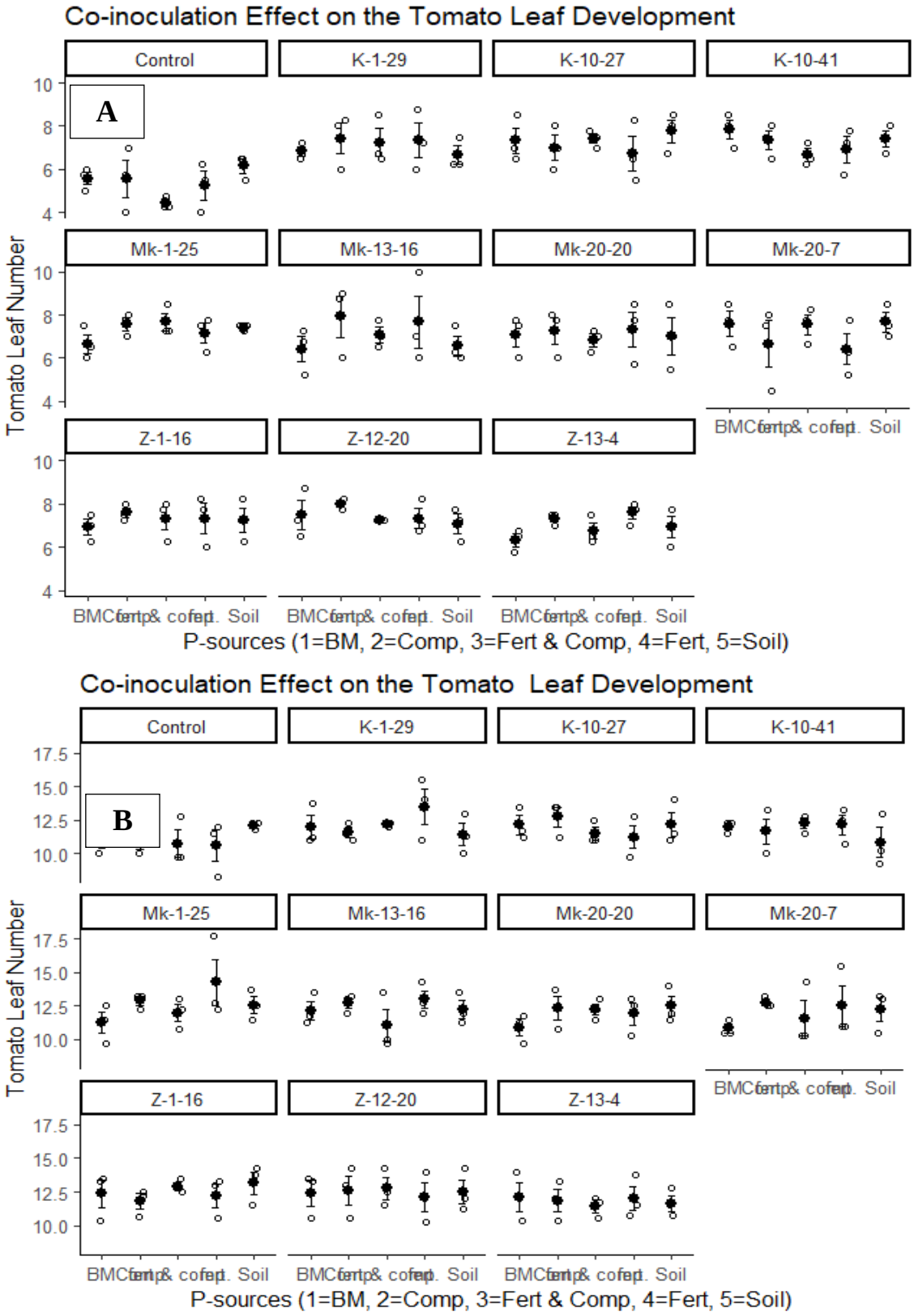


Figure 5.3: Tomato leaves development at different growth periods (A= 30 days, B= 60 days) of transplantation.

5.3.2 Fruit Yield

Starting from 60 days of transplantation, damaged and 50% mature fruits were harvested from three tomato plants per row with frequent farm monitoring (Picture 5.1). At the time of final harvesting (90 days after transplantation), fruit number, size, and weight variation were realised (Table 5.4 and Supplementary Table 5.1). Even if tomatoes were not faced observable impairment by any infestation, a considerable number of fruits were damaged by blossom end rot. This physiological disorder might possibly be due to the water shortage and limitations (irregular watering time and amount because of supply fluctuation). It was observed that the blossom end rot diminished the external fruit protective layer; as a result, the fruit flesh part was exposed and created a conducive circumstance for various biological visitors (insects, worms, birds, fungal infestations, and other microbial infestations) (Picture 5.2). This irrigation instability resulted in a significant ($p<0.05$) number of unmarketable tomato fruits and loss (Table 5.4). The total amount of mean fruit variance indicated that K-10-41 inoculated tomatoes induced to produce the highest tomato fruit number (65.6) with significant ($p<0.05$) marketable fruits (31.27). On the other hand, inoculation of K-10-27 produced the second highest (65.47) average fruit number with a considerable amount (37.47) of unmarketable fruits, whereas Mk-20-7 produced the maximum (31.33) marketable fruits and the least (28.07) number of unmarketable fruits (Table 5.4 and Figure 5.4). Similarly, K-1-29 ranked the 3rd inoculum in terms of total fruit number (63.93) and number of marketable (30) fruits. Though fruit weight was not exacerbated among isolates, inoculation showed considerable improvement against the control. K-10-41 recorded the maximum overall average fruit weight (2821.6 g), followed by K-1-29 (2793.3 g) (Table 5.4).

Picture 5.2: Blossom end rot and subsequent fruit infection**.**

Table 5.4: Tomato fruit yield.

Isolate	Marketable Fruit	Unmarketable Fruit	Total fruit No.	Total Fruit weight (g)
Control	19.0±11.77[b]	32.0±9.58[ab]	51.0±17.36[b]	1700.0±828.0[b]
K-1-29	30.0±9.78[a]	33.93±11.68[ab]	63.93±11.27[a]	2793.3±740.36[a]
K-10-27	28.0±14.72[a]	37.47±15.57[a]	65.47±20.31[a]	2563.4±900.09[a]
K-10-41	31.27±10.12[a]	34.33±13.77[ab]	65.6±19.52[a]	2821.6±765.85[a]
Mk-1-25	24.6±11.21[ab]	34.13±8.65[ab]	58.73±11.3[ab]	2268.07±825.79[ab]
Mk-13-16	28.4±16.32[a]	31.4±9.4[ab]	59.8±22.95[ab]	2781.3±1377.7[a]
Mk-20-20	27.27±12.2[ab]	34.8±15.09[ab]	62.07±19.31[ab]	2583.47±691.21[a]
Mk-20-7	31.33±14.68[a]	28.07±9.36[b]	59.4±12.66[ab]	2780.53±964.66[a]
Z-1-16	25.93±13.51[ab]	32.27±9.58[ab]	58.2±12.35[ab]	2507.2±768.78[a]
Z-12-20	28.73±15.01[a]	31.53±9.73[ab]	60.27±15.18[ab]	2558.4±1004.15[a]
Z-13-4	28.8±12.94[a]	30.93±8.29[ab]	59.73±12.13[ab]	2508.93±838.61[a]
CV	41.87	33.69	26.74	34.23
LSD	8.33	7.97	11.65	62.79
P-Treatment				
BM	29.0±17.06	33.82±9.35	62.82±20.61	2645.91±1257.65
Compost	27.42±15.39	34.46±11.37	57.88±16.33	2456.0±1072.65
Fert	27.91±11.59	31.79±8.05	59.7±13.41	2474.58±739.31
Fert & Comp	27.61±9.64	34.46±14.54	63.06±15.67	2617.39±660.3
Soil	25.94±11.04	32.52±11.42	58.46±14.92	2472.61±802.17
CV	44.89	33.69	26.74	34.23
LSD	6.02	5.38	7.85	42.9

NB: *Means with similar letters has no significant difference at P=0.05.*

Application of external P-sources promoted tomato-PSB interaction, response and increased fruit yield (Figure 5.4). For instance, compost application promoted most tomato growth parameters (plant height, flower buds, and flower opening), nevertheless, it resulted in the least fruit number (57.88) and the minimum fruit weight (2456 g). Quite the reverse, the bone meal was the least promotor for most tomato plant assays, but it was one of the top fruit development inducers (on average, 20.94 fruits per plant were harvested which recorded the highest fruit weight of 2645.91 g) (Table 5.4 and Figure 5.4 and 5.5). Generally, bone meal and the mixture of 50% rate of the recommended compost and fertilizer showed a significant ($p<0.05$) effect on overall tomato fruit yield, while fertilizer application recorded the least number of unmarketable fruits, which might suggest that fertilizer promotes healthy and marketable fruit production next to bone meal.

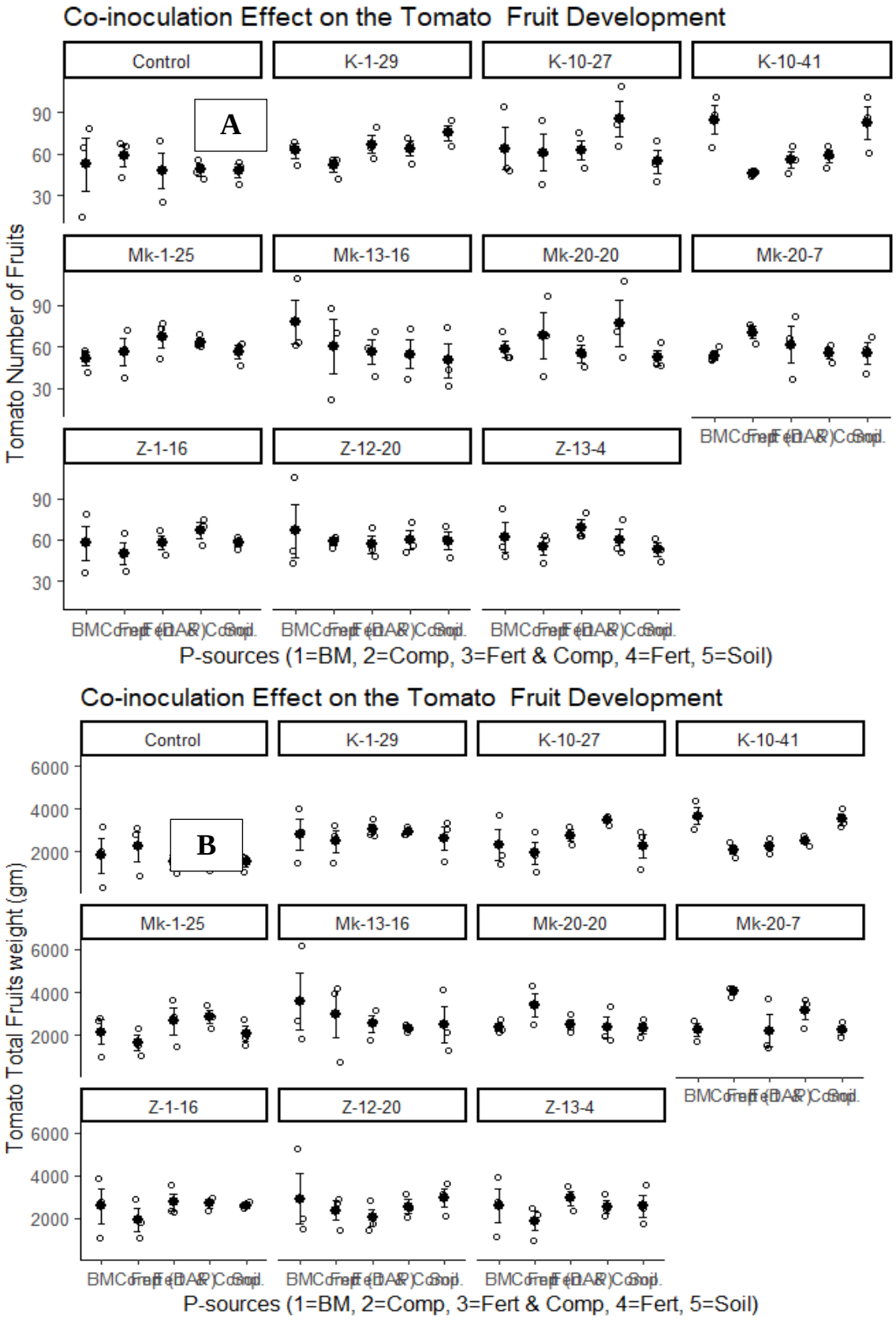


Figure 5.4: Tomato fruit yield (A= total fruit number and B= total fruit weight (g))

5.4 DISCUSSION

According to Manzoor *et al*. (2017) the application of PSB, rock phosphate, compost, manure, or fertilizers increased the amount of P that is accessible to plants. In this field trial, PSB and external possible P-sources used in conjunction to tomato growth and yield performance. Based on their prior laboratory (Chapter 3) and greenhouse (Chapter 4) performance, a total of 10 PSB strains were chosen and they were co-inoculated with three different supplementary P-sources (compost, bone meal (BM), and synthetic fertilizer). Four growth periods (30, 45, 60 and 90 days) were used after tomato transplantation to evaluate the overall growth and development as well as tomato fruit yield.

As shown by Verma *et al*. (2019) and Mącik *et al*. (2023), chemical fertilizer controls about 95% of the global fertilizer market share, with biofertilizers accounting for the remaining 5%. Despite the annual biofertilizers market increment, rhizobium constitute about 79% and PSB about 15% global market demand as indicated in Nosheen *et al.*'s (2021) review. Plant growth-promoting characteristics were used to filter potential biofertilizer strains from rhizosphere (Kumar *et al.,* 2022). Hierarchical screening procedures (lab screening, greenhouse and field evaluation) help to select efficient PSB inoculum. Despite *in vitro* solubilization and greenhouse promotions, PSB application under field conditions is very limited. Laboratory screening, greenhouse and field trials used to analyse the effectiveness of PGPR (Amaya-Gómez *et al*., 2020). For a strain to become effective, it should possess good competence, persistence, and stabilization under the provided environmental conditions. Open field evaluation and efficacy confirmation experiments give a good picture for maximal exploitation of efficient strains. As shown by Kirui *et al*. (2022) and Amanullah and Khan (2015), once potential isolates are screened, they will be inoculated into the soil, then stabilize sustainable production, and minimize production cost. Given the fact that open field cultivation is exposed to a stressful environment, as demonstrated by Bai *et al.* (2018), the true potential evaluation and efficiency verification provides a practical figure of whether the selected strain is competent or incompetent before distributing to the farmers. Likewise, De Zutter *et al.* (2022) underlined that field trials are mandatory because most (>90%) of the PSB symbiotic effectiveness studies conducted under greenhouse conditions become unsatisfactory and less effective when tested on field trials. Similarly, Nosheen *et al.* (2021) have shown that biofertilizers could be hero or villain at field trial depending on the soil, host plant and environmental conditions. Liu *et al.* (2020) also stated that the inoculation of

competent PSB strains successfully colonized the root zone and enriched the rhizosphere, while some strains failed to succeed. Moreover, it is essential to prove their efficiency and symbiotic effectiveness under various field conditions. This is due to the fact that the environment and soil characteristics have significantly impacted the success of PSM establishment and performance (Mitra *et al.*, (2020); Sharma *et al*., (2013)). The interaction and response of inoculants are influenced by soil factors, distance from roots, plant species type, growth stage, root exudate composition, hormone signalling, native microbiota, and climatic conditions (Santos-Villalobos and Parra-Cota, 2021; Rachel *et al.*, 2018). Similarly, Rilling *et al*. (2019) indicated that PGPR performance should be demonstrated in different soil, crop, and agro-ecological circumstances.

In this study, PSB strains were inoculated to tomato seeds and root zone to promote inoculum interaction and colonization intended for better stimulation of tomato growth and fruit yield. A supportive publication from De Zutter *et al*. (2022) described that soil drench, root dip, spray, and seed coating mechanisms were used to apply PSB strains; nevertheless, seed coating and root dip predominantly increased P-uptake and plant biomass. A sympathetic study by Pellegrini *et al*. (2021) on *Allium cepa* L. showed that seed inoculation approach is considered the proven strategy to stabilize the inoculum and improve cultivation. In a similar fashion, Egamberdieva (2015) reported that tomato seed inoculation with PGPR has demonstrated the induction of wilt resistance both in field and greenhouse trials. Poonia and Dhaka (2012) also found PSB inoculation of soil and tomato root (seedling) dip, together with the recommended fertilizer rate improved tomato growth and yield. Similarly, Amaya-Gómez *et al*. (2020) indicated that successful colonization of the root surface and vicinity (rhizosphere) by the inoculum leads to the release of different compounds that promote plant-bacteria interaction, synchronization, confront indigenous microbes, and enhance plant growth.

In the present study, PSB inoculation of tomatoes showed a significant ($p<0.05$) difference over the control and among the added P-substrates. For instance, tomato plant shoot length was strongly promoted by Z-12-20, Mk-1-25, and Mk-20-7 (Tale 5.1), the number of leaves, branches, and flower buds' development were encouraged by Mk-20-20, Mk-1-25, and K-10-41 (Table 5.2 and 5.3) and fruit development was enhanced by K-10-41, Mk-20-7, and K-10-27 (Table 5.4, Supplementary Table 5.1 and Figure 5.5). Comparable studies demonstrated that PSB strains inoculation such as N3 (Zhang *et al.,* 2021) and MBP 2.1 (Khan *et al.,* 2016)

improved tomato growth parameters (plant height, root length, chlorophyll content, and biomass). The possible reason is that perhaps the PSB stimulated tomato by enhancing P-accessing, growth hormone production, biocontrol activity, and reducing toxic chemicals absorption and accumulation in the shoot as well as roots. In line with this, Khan *et al*. (2016) have reported that PGPR promotes plant growth through a wide range of mechanisms (phytohormones production, alleviating environmental stresses, and production of secondary metabolites). A review by Alori *et al*. (2017), strengthens the assumption that soil microorganisms enhance plant nutrient acquisition. In the current study, tomato inoculated with Mk-20-7 recorded the highest tomato plant height mean (67.2 cm), which was greater than Etissa *et al.'s* (2014) finding (59.2 cm) but lower than Poonia and Dhaka's (2012) report that found maximum tomato plant height 86.3 cm from the complemented application of PSB and fertilizer. The Mk-20-7 strain was also one of the top tomato inducers to develop more marketable (31.33) fruits inferring that this efficient strain positively interacted with tomato to increase the height (67.2 cm (Table 5.1)) thus led to the development of more healthy fruits. In the same way, K-1-29 effectively interacted with tomatoes and promoted plant height (Table 5.1), the overall fruit yield, and gross fruit weight (2793.3 g, (Table 5.4)). Similarly, Alori *et al. (*2017) reviewed the overall benefit of adopting rhizosphere microbes in enhancing plant nutrients, stimulating root and shoot growth, increased height, biomass, and yield. A supportive finding published by Dash *et al.* (2017) indicated that PSB inoculation increased rice root growth, P-uptake, biomass, and grain yield.

Sharon *et al.* (2016) demonstrated that tomato inoculation with *Pantoea sp.* recorded elevated P incorporation and biomass. In this study, the three PSB strains stimulated tomato growth parameters, fruit yield and response to stress. K-10-27 encouraged the development of a greater number of fruits (65.47), (though the majority of fruits (37.47) were unmarketable due to blossom end rot), while Mk-20-7 improved directly or indirectly the physiological response to resist stresses and develop more healthy and marketable fruits (31.33), whereas K-10-41 encouraged tomato to resist water stress as well as increased fruit yield (2821.3 g fruit weight recorded from a total of 65.6 fruits, among them 31.27 were marketable) (Table 5.4). It was indicated that water shortage, limited irrigation access and moisture stress can cause blossom end rot and other infections (Gemechis, 2017). In agreement with this, Rabbee *et al*. (2019) and Verma *et al*. (2019) reported that the application of PGPR promote plants directly (nutrient accessing, phytohormone production) and indirectly (antibiotic response, competition for niche, and stress tolerance) to increase plant growth, biomass, and yield. The

three PSB strains (K-10-27, K-10-41, and Mk-20-7) demonstrated substantial improvement in fruit number and weight, which was a prominent recognition which can possibly be recommended for tomato farm application under repeated efficacy attest and conformation. In line with this, Kumar *et al*. (2022) reported that the application of biofertilizers improved seed germination, seedling growth, yield, and quality. This might perhaps suggest PSB inoculation induces functional tomato gene expressions, as indicated by Zhang *et al*. (2021), which regulates physiological parameters of chlorophyll synthesis, metabolism, transportation, and resistance, in the reverse tomato modified and released metabolites to promote PSB colonization.

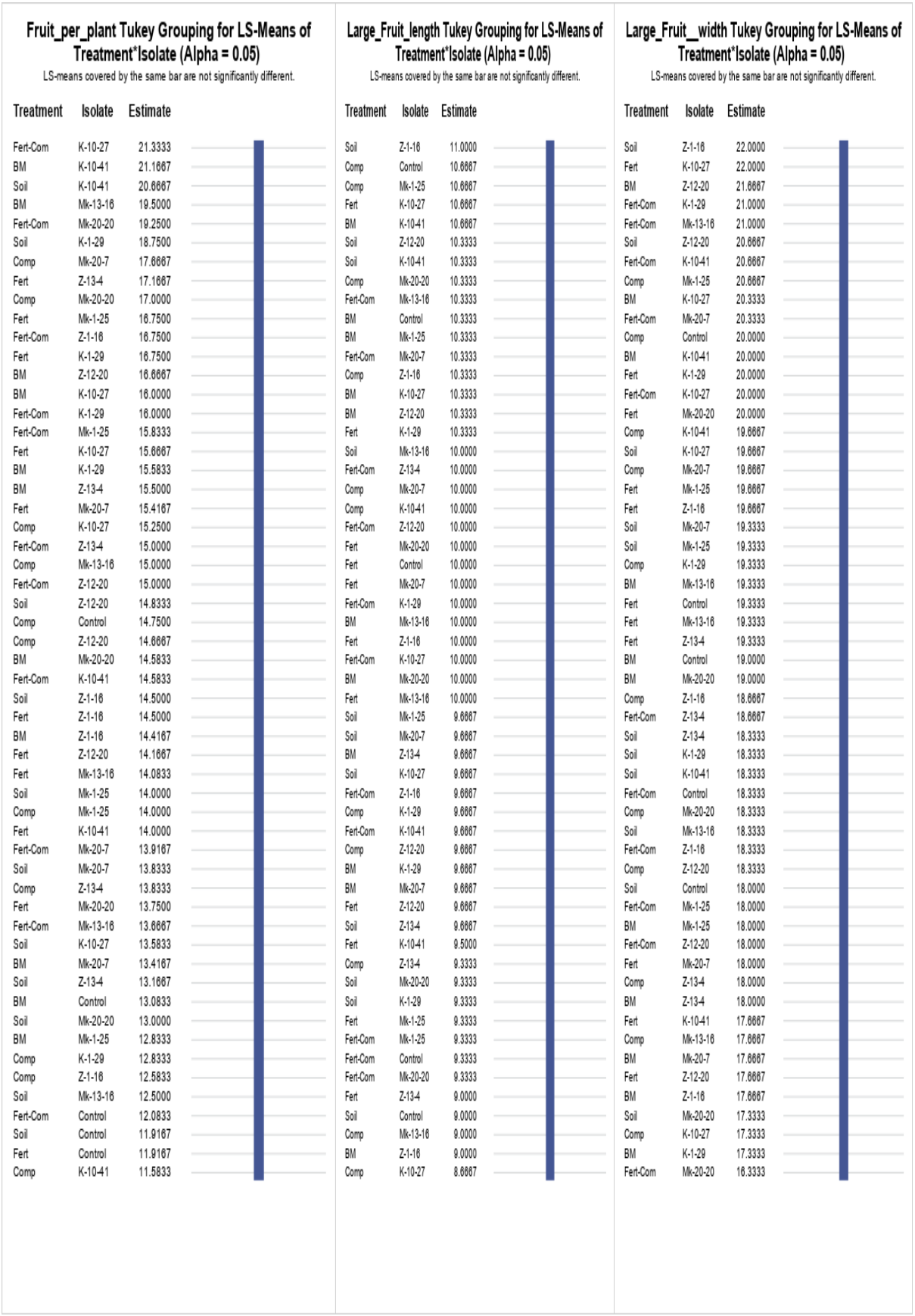


Figure 5.5: Tomato fruit yield per plant and larger fruit size difference among treatments.

As shown by Alori *et al.* (2017), PSM increased plant availability of phosphorous through the solubilization and mineralization of insoluble P-compounds (i.e., both inorganic and organic phosphorous). Combined application of organic matters, biological inputs, and synthetic chemicals consents integrated soil fertility maintenance that improve soil properties and crop yield (Abebe *et al*., 2022). Application of external P-supplements in conjunction with PSB strains improved interaction and response between strains and tomato. Particularly, compost and PSB boosted tomato growth indices (height, number of leaves, flower buds, and flower openings). These were consistent with the findings of Etissa *et al.* (2014) and Girshe *et al*. (2018), which revealed that locally available organic fertilizers such as farmyard and compost application to tomato enhanced growth and development that fortified economic, social, and environmental feasibility. A review by Tamene *et al.* (2017) also elaborated organic matter amendment (green manures, animal dung, and crop residues) enhanced soil nutrient availability, moisture, microbial activities, and crop production. A study on sugarcane indicated that PSB co-inoculation with compost showed significant shoot nutrients (P, N, and K) composition over control and other supplements, including rock-phosphate and TSP (Estrada-Bonilla *et al.*, 2021). Similarly, Amanullah and Khan (2015) found that the interaction of compost and PSB significantly increased maize yield and yield components (grain number and weight). Likewise, Yasmeen *et al.* (2022) found that maize grain yield has been significantly affected by the interaction of a potent PSB strain (*Bacillus cereus* GS6) and rock-phosphate.

In the current study, PSB co-inoculation with a mixture of 50% compost and 50% of the recommended DAP fertilizer significantly ($p<0.05$) improved both tomato vegetative growth (plant height 67.39 cm) and fruit yield (the highest (63.06) total fruit number and the 2nd highest (2617.39 g) total fruit weight) (Table 5.4). This assorted co-inoculation promoted tomato height, number of leaves, flower buds, fruit number, fruit size, and weight, whereas bone meal strongly encouraged fruit values (recorded a total of 62.82 fruit numbers, among them 29 were marketable, the highest fruit weight (2645.91 g) and the larger fruit size (Table 5.4 and Supplementary Table 5.1)). These results are in consistent with the findings of Tamene *et al*. (2017), indicated that combined application of half of the recommended fertilizer rate and compost improved soil pH and micronutrients and strongly increased wheat and teff productivity. In the current experiment, fertilizer was found to be an intermediate inducer compared to other treatments, except for leaf development and fruit width. The result is consistent with the findings of Estrada-Bonilla *et al.* (2021), which indicated that in

contrast to fertilizer amendment, simultaneous application of PSB strains and compost amendment showed significant improvement in physical, chemical, and biological soil properties, which improved shoot nutrition and growth. In contrast, many reports have indicated that fertilizer and PSB inoculation, among other treatments, has shown notable success in different crops production and productivity, such as maize (Yasmeen *et al.,* 2022; Amanullah and Khan, 2015), *Allium cepa* L. (Pellegrini *et al.,* 2021), tomato (Sharon *et al.,* 2016; Poonia and Dhaka, 2012), Mungbam (Khan *et al.,* 2022), rice (Chen and Liu, 2019; Dash *et al.,* 2017*),* and wheat (Kumar *et al.,* 2021).

Generally, as shown by Santos-Villalobos and Parra-Cota (2021), the current agricultural production requires intensive agricultural practices, including improved agro-inputs, plantations, irrigation, and Phyto-infestation control. In line to this, Abebe *et al*. (2022) and Deepika and MubarakAli (2020) have indicated that massive chemicals practiced in modern agriculture. Likewise, tomato production needs optimum growing conditions (nutrient-rich soil or growing medium), proper water supply, proper infestation control, mentoring, proper harvest, and post-harvest management. Soil nutrients could be supplemented as biowastes, organic matter, fertilizers, and/or biofertilizers. Biofertilizers such as nitrogen fixers, PSB, and *Mycorrhizal* fungi application alone or together (Khan *et al.,* 2022; Kumar *et al.,* 2022; Nosheen *et al.,* 2021) improve soil nutrients, root and shoot growth, as well as maintain plant and soil health.

5.5 CONCLUSION AND RECCOMENDATIONS

Application of competent PSB strains together with nutrient supplements improved symbiotic effectiveness, sustainable production, and environmental health. Among the 10 PSB isolates K-10-41 strongly stimulated tomato height, branch and flower development besides fruit yield (number, size and weight (2821.6 g). Similarly, Mk-20-7 enhanced plant height as well as fruit yield (a total of 59.4 fruits weighing 2780.53 g with a substantial number of marketable (31.33) fruits were harvested). Likewise, the addition of compost enhanced more of tomato vegetative growth whereas bone meal induced fruit yield (a total of 62.82 fruits with a gross weight of 2645.91 g were harvested wherein 29 of the fruits were marketable), while the mixture (50% of the recommended rate of compost and fertilizer) improved the overall tomato growth performance. Hence, K-10-41 and Mk-20-7 applications together with bone meal and the mixture of compost and fertilizer were found effective. Consequently, they

are recommended for fruitful and sustainable tomato production especially for smallholder farmers. To distribute these promising PSB isolates as inoculants, it is better to confirm their competence under different agroecologies, conduct whole genome sequence to identify the specific strain accession number, and evaluate their response to other crops (host range test). Synergetic application of organic supplements (compost, farmyard, bone meal or other biowastes), bioinoculants and proper agrochemicals maximize production and environmental health. This kind of farm practice is feasible for the economic, social and ecological sense of balance in line with soil and environmental health maintenance. Therefore, exploring more efficient/competent strains as well as cheap, easily available and eco-friendly supplements for combined application are the future prospects to improve soil fertility, plant growth, development and production.

CHAPTER SIX

6. CROP HOST RANGE OF SELECTED PSB STRAINS AT OPEN FIELD CONDITION

Abstract

The current PSB strains were eventually tethered to tomatoes, and their efficiency was evaluated under laboratory, greenhouse, and field conditions. Thus, it is important to broaden the host symbiotic interaction response under different crops to check their persistence. This experiment will provide a supportive and valid scientific or empirical background to recommend them as biofertilizers and plant growth-promotors as an alternative or in combination with chemical fertilizers. Five different crops (faba bean, kidney bean, maize, onion, and wheat) were taken for this field trial and inoculated with PSB strains. Plant assays like shoot length, leaf development, tiller/branch, flower, pod, corncob number, and biomass yield parameters were recorded accordingly. Even though the degree of response varied among PSB inoculants, most of them demonstrated promising achievements and encouraging results in most plant parameters (seed germination, shoot length, flowering, yield, and biomass) over the control (uninoculated group). The overall host range evaluation of inoculants against the test crops indicated that Z-12-20 demonstrated broad host-range (positively interacted and encouraged growth and biomass of four among five crops (faba bean, maize, wheat, and onion)). Similarly, Mk-1-25 was found to be the second strain with a broader host range, which positively influenced and promoted faba bean, kidney bean, and onion growth and biomass. Yet, K-1-29 (one of the top promoters of tomato) has shown the least interaction among the currently tested crops. Wheat and faba bean have been found to be symbiotically interactive with multiple strains to enhance various plant attributes, whereas maize and onion have shown limited performance with most of the candidate strains. On the other hand, legume crops (faba bean and kidney bean) have demonstrated variable responses against each strain in consort with their developmental stages. It's been concluded that each strain has its own host crop preference; therefore, this research implicates the need for repeated site- and crop-specific field trials to verify strains' efficacy as well as further study to deeply understand the metabolic and molecular interactions between the candidate inoculant and target host crop.

Keywords: *Biofertilizer, Biomass, Cereal, PSB, Onion, Symbiotic interaction*

6.1 INTRODUCTION

Conventional crop production relies on heavy fertilizer applications, which pose different consequences, such as social, economic, and environmental (Tinna *et al*., 2020). Regardless of the benefit in agriculture, Mahanty *et al.* (2017) briefly described the harmful impacts of chemical fertilizers on the environment and living beings. To feed the dramatically growing world population, repeated and over application of fertilizers (Nosheen *et al.*, 2021), GHG emissions, and aggressive exploitation of arable lands lead to complex climate changes and minimize (for example, 3.8% wheat and 5.5% maize) productivity (Shah *et al*., 2021). Repeated and injurious application of fertilizer leads to a decline in soil fertility, soil microbial diversity, and crop production (Sharma *et al.*, 2013). Unless plants absorb the applied fertilizers, they would accumulate in soil and waterbodies, where the residues would deplete soil nutrients, water holding capacity, and possibly increase salinity. Considering the hazardous effects of chemical fertilizers, biofertilizer and plant growth-promotor application (Mitra *et al*., 2020) are inevitable and supposed to be a safe alternative to minimize agroecological disorders. Various soil microorganisms, including bacteria, can maintain soil fertility and improve plant nutrients (Nosheen *et al.*, 2021; Liu *et al*., 2020). PSB are one of the fundamental bacterial groups in accessing nutrient, improving soil fertility, increasing uptake of minerals, enhancing plant growth, regulating phytopathogens, and improving stress tolerance (Pellegrini *et al*., 2021). They have comprehensive benefits and may require less resource, cost, and technical skills to implement. These multidimensional and wide-range PSB (also called PGPR) promote the plant directly (through biofertilizer activity) or indirectly (through biopesticide activity) (Figure 6.1) (de Andrade *et al.*, 2023; Verma, 2019; Mahanty *et al.*, 2017).

Like nitrogen fixers, PSB are not crop-specific for leguminous species. Comparatively, PSB have quite an extended host range and can form satisfactory interactions with different crops, including vegetables, cereals, legumes, tubers, and different other plant species. Even though their interaction is not classified by certain species, somehow, they have host preference for their elevated and fruitful symbiotic interaction and response (De Zutter *et al*., 2022). This is because the plant root architecture, physiological, metabolic, and molecular approaches will determine the level and population of the microbial community in the root zone (Giannelli *et al.*, 2023; Planchamp *et al.*, 2015). Not only the type of plant but also the developmental stages and physiological status of the plant significantly determine the rhizosphere

community through their root exudates and metabolites, either to repel or attract (Ahemad and Kibret, 2014). In the root zone, bacterial colonization and survival are influenced by plant growth factors and plant residues (Verma, 2019). A review by de Andrade *et al. (*2023) indicated that, depending on the plant species, 10–40% of plant photosynthetic metabolites are released in the rhizodeposition. A supportive statement by Pathak *et al*. (2017) indicated that PSB and their mechanisms depend on nutritional, physiological, and growth conditions. Once the root zone stabilized and dominated by certain microbial groups, the nutritional, physiological, and physical properties of the rhizospheric soil would be altered by them (Verma, 2019).

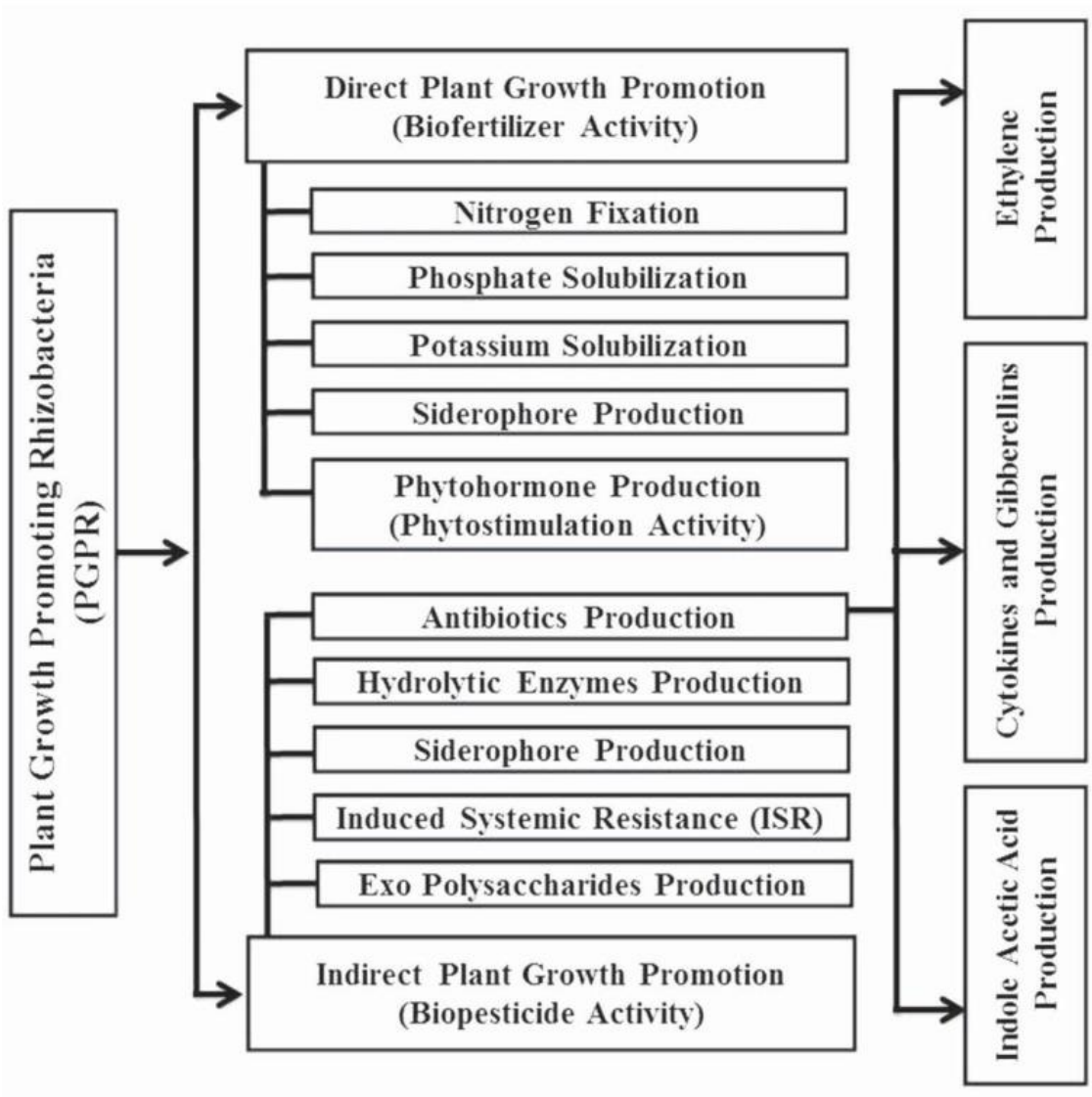


Figure 6.1: Diagram for direct and indirect beneficial effects of PGPR on agricultural crop (*Source: Verma, 2019*).

Therefore, this study was designed to examine the range of host crops for the selected 10 PSB strains that were isolated from the tomato rhizosphere. The host plant and the associated phyto-microbiome (holobiont) reside in plant-associated microbes' synergetic relations and signal exchanges (Shah *et al.,* 2021). The current PSB strains were eventually tethered to tomatoes, and their efficiency was evaluated under laboratory, greenhouse, and field conditions. They showed plenty of positive and encouraging responses, even though their degree of efficacy was quite different at each experimental level. Thus, it is important to broaden the host-symbiotic interaction and response under different crops to check their persistence. This is because most PSB strains have a broader host range; on the other hand, farmers used their lands (including the sampled tomato farms in this study) to cultivate various crops so that the strains might interact prominently with other crop(s) even though they were screened from a certain crop, in this case tomato. Inoculation of other plant species other than the primary host revealed the effect or productivity of the inoculants (Pellegrini *et al*., 2021). Moreover, it is necessary to create a valid scientific or empirical background that can support the development, practice, and alternative to chemical fertilizers.

Multistep screening and evaluation processes, as well as optimization of viability states, considerably improve bioinoculant formulation and farmer acceptance (Mahanty *et al*., 2017). Lack of practical evidence is one of the limiting factors for farmers to adopt and practice microbe-based technologies (Shah *et al.,* 2021). Any positive feedback from inoculation of a test crop would improve the acceptance and feasibility of the candidate strains. Thus, the current host range field trial probably would create enthusiasm to develop, produce, optimize, and practice biofertilizers and plant growth-promotors in Ethiopia, especially in the study areas. Competence and positive response of the candidate strains in any of the test crops will motivate and gain farmers' attention to apply thus inoculants, at least in one of the crops productions, or in the future practices. This is because the price of biofertilizers/PGPR is cheap and much lower than that of chemical fertilizers. In one way or another, repeated application of a single or various host crops tends to enrich the population and increase the survival, colonization and competence of the candidate PSB strains in the farm. Moreover, Alemayehu (2020), found that one-time biofertilizer application to faba bean increased the yield not only the legume but also the consecutive cultivated cereals (wheat and barley) in Arsi zone. In line to this, Nosheen *et al.* (2021) indicated that 3-4 years continuous application of biofertilizers improve root colonization and mother inoculant duplication hence, no need of further application.

6.2 MATERIAL AND METHODS

6.2.1 Study Farm Preparation

The study was conducted from February to July 2023 using irrigation at the Hawassa University main campus research site. The land was twice ploughed and well prepared, then plots and rows were arranged in RCBD design with three replications. Each experimental unit was arranged at 2 m x 1.5 m with one meter of space between plots and 10-30 cm of distance among each plant depending on the type of crop (Table 6.1 and Picture 6.1). Then the plots were placed into 11 rows to inoculate independently the 10 pre-screened PSB strains (Chapter 3) (K-1-29, K-10-27, K-10-41, Mk-1-25, Mk-13-16, Mk-20-7, Mk-20-20, Z-1-16, Z-12-20, and Z-13-4) per row and one row set control (inoculum-free). Onion assigned plot was arranged in 8 rows (7 rows for selected PSB isolates: K-1-29, K-10-27, K-10-41, Mk-1-25, Mk-20-7, Mk-20-20, and Z-12-20 inoculation, and one row for the control group). These seven strains were selected based on their advanced responses at various levels (lab-field evaluation (Chapter 3-5)) and because of the experimental farm space limitation.

6.2.2 Inoculum Preparation and Seed Inoculation

Pre-screened and characterized 10 PSB strains were taken (Chapter 3), and refreshed with PVK broth for 48 hrs in a shaker (121 rpm) incubator. To provide a uniform coating (Kumar *et al.,* 2022), the refreshed active culture was used to soak seeds. Five different crops (faba bean, kidney bean, maize, onion, and wheat) were taken for this host range field trial. Selected varieties of each crop and recently harvested seeds (except onion) from the Hawassa University research site (main campus) were collected from different researchers. Onion seed (*Bomby Red* variety) was purchased from the market. The seeds were soaked overnight (Pande *et al*., 2017) in the respective PSB isolates separately. Materials and seed surface sterilization were conducted according to Somasegaran and Hoben (1994). After overnight soaking (Yasmeen *et al.,* 2022), the extra inoculum was poured, and seeds were left dried for 30 minutes (Picture 6.1), then seeds were sown at 2 cm depth according to the respective proposed plot arrangements (Table 6.1). Before seeds were covered by soil, one millilitre of inoculum was added (Estrada-Bonilla *et al.,* 2021) to enrich colonization and then cover the seeds. Conventional seed coating (De Zutter *et al*., 2022) and seed inoculation strategies help to improve colonization and interaction (Pellegrini *et al.,* 2021). Each plot and row were arranged in three replications as per the respected PSB inoculum and the assigned host crop.

Watering was done twice a day until 20 days of sowing, then once a day in the consecutive plantation periods.

Table 6.1: Plots and rows arrangements and spacing (between rows and between plants).

Crop type	PSB inoculant per plot	Plant spacing (inter vs intra)
Faba bean	10 PSB strains + control	60 cm and 10 cm
Kidney bean	10 PSB strains + control	60 cm and 10 cm
Maize	10 PSB strains + control	60 cm and 30 cm
Wheat	10 PSB strains + control	60 cm and 10 cm
Onion	7 PSB strains + control	60 cm and 10 cm

NB*: inter and intra spacing sources*: faba bean: Gezahegn and Tesfaye (2017); kidney bean: Bekele *et al*. (2019); maize: Getaneh *et al*. (2016); wheat: Mekonnen (2017) (with some modifications).

Thirty days after sowing, all the seedling root zone soil was re-inoculated with one millilitre of the corresponding active culture, as shown by Estrada-Bonilla *et al*. (2021). This extra inoculation would maximize root and rhizosphere colonization (Pellegrini *et al., 2021)* as well as improve indigenous competition. Afterwards, regular watering, weeding, and monitoring were conducted until harvesting. Urea was added to all crops, including the control groups, at the first date and after 45 days of sowing as per the recommended rate for the corresponding crops. Maize was planted at the corner of the experimental farm as a biological control and border. Data were taken from three plants per row at 30, 60, and 90 days of sowing and harvesting, except for the onion and maize. Onion data were taken at the harvesting date (120 days of sowing), maize was harvested, and biomass data were taken at 170 days of sowing. Plant assays like shoot length, leaf development, tiller/branch, flower, pod, corncob number, and yield parameters (number and weight) were recorded.

6.2.3 Data Analysis

Collected quantitative data were organized and analysed by R.4.3.1 software. One way ANOVA was conducted using Fisher's and Tukey's tests (significance set at 5%). Finally, the analysed data were explained and discussed in reference to other related works.

Picture 6.1: Land preparation, seed inoculation with the respected inoculant (row) and plant growth progress of onion, wheat, maize, kidney bean and faba bean (crop per plot).

6.3 RESULT

A host range test for selected PSB strains was conducted at field level on five different crops, including cereals, legumes, and tubers (i.e., maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), onion (*Allium cepa* L.), faba bean (*Vicia faba* L.), and kidney bean (*Phaseolus vulgaris*)). Accordingly, inoculants showed various responses against each crop. Even if the degree of efficiency variation among PSB isolates was observed, most of the PSB inoculants demonstrated greater achievements and encouraging results in most plant parameters over the control (uninoculated group). Moreover, PSB inoculation significantly promoted seed germination in all tested crops (Table 6.2). Thus, Mk-1-25 was found to be an influential germination inducer and meaningfully shorten the germination date for the test crops (in average, wheat germinated at 5.33 days, faba bean at 6, kidney bean at 5.67 days, and onion at 6 days of sowing). Inoculum-free seeds took longer to break their dormancy than inoculated groups. Among the ten PSB strains, in general, isolates from Ziway Zuria have shown a limited response to germination for most of the tested crops, especially Z-1-16, demonstrated the poorest effect on wheat and kidney bean germination (Table 6.3). Among the tested crops, faba bean took a longer time followed by maize, while onion seeds needed shorter (6.75) days to germinate at direct field sowing (Table 6.2).

Table 6.2: ANOVA result for seed germination date of different crops treated with PSB.

Crop type	Items	**DF**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Faba bean	Isolate	10	23.58	2.36	3.11	0.0126*
	Residuals	22	16.67	0.76		
Kidney bean	Isolate	10	13.52	1.35	2.478	0.0365*
	Residuals	22	12.0	0.55		
Maize	Isolate	10	28.3	2.83	4.92	0.0009***
	Residuals	22	12.67	0.58		
Wheat	Isolate	10	21.21	2.12	5.385	0.0005***
	Residuals	22	8.667	0.39		
Onion	Isolate	7	9.83	1.41	2.593	0.049*
	Residuals	16	8.667	0.54		
	MSerror	**DF**	**Mean**	**CV**	**t-value**	**LSD**
Faba bean	0.76	22	7.5	11.63	2.07	1.47
Kidney bean	0.55	22	6.88	10.74	2.07	1.25
Maize	0.58	22	7.03	10.79	2.07	1.29
Wheat	0.39	22	6.94	9.05	2.07	1.06
Onion	0.54	16	6.75	10.9	2.12	1.27

NB: * significant at 0.05, ** significant at 0.01, *** significant at 0.001.

Table 6.3: Mean value of germination date of different crops treated with various strains.

Isolate	Faba bean	Kidney bean	Maize	Wheat	Onion
Control	8.67+0.58^{a}	7.67+0.67^{a}	8.4+1.0^{ab}	8.0+1.0^{a}	8.0+1.0^{a}
K-1-29	8.0+1.0^{ab}	6.0+1.0^{cd}	6.0+1.0^{d}	7.33+0.58^{abc}	7.33+0.58^{ab}
K-10-27	6.0+1.0^{c}	7.33+0.58^{ab}	6.67+0.58^{cd}	6.67+0.58^{bcd}	6.67+0.58^{bc}
K-10-41	6.67+1.15^{bc}	6.67+0.58^{abcd}	6.0+1.0^{d}	7.33+0.58^{abc}	7.0+1.0^{abc}
Mk-1-25	6.0+1.0^{c}	5.67+1.55^{d}	6.67+0.58^{cd}	5.33+0.58^{e}	6.0+0.0^{c}
Mk-13-16	8.0+1.0^{ab}	6.33+0.58^{bcd}	7.67+0.58^{abc}	6.33+0.58^{cde}	-
Mk-20-20	7.67+1.16^{ab}	7.0+0.0^{abc}	8.0+1.0^{ab}	7.33+0.58^{abc}	6.67+1.1^{6bc}
Mk-20-7	7.67+0.58^{ab}	6.67+0.58^{abcd}	7.67+0.58^{d}	5.67+0.58^{de}	6.33+0.58^{bc}
Z-1-16	7.67+0.58^{ab}	7.67+0.58^{a}	8.67+0.58^{a}	7.33+0.58^{abc}	-
Z-12-20	8.33+0.58^{a}	7.33+1.55^{ab}	7.33+0.58^{bc}	7.33+0.58^{abc}	6.0+0.0^{c}
Z-13-4	7.67+0.58^{ab}	7.33+0.58^{ab}	6.67+0.58^{cd}	7.67+0.58^{ab}	-
CV	11.63	10.74	10.79	9.05	10.9
LSD	1.47	1.25	1.29	1.06	1.27

NB: Means with similar letters have no significant difference at p = 0.05.

PSB inoculation of faba bean induced growth and development, starting from germination to the following growing periods, which resulted in enhanced biomass. Compared to inoculated groups, the inoculum free (control) group showed shorter shoot length, minimum branch, leaf, flower, pod development (Table 6.4) and the least overall biomass (Table 6.6). Inoculation of K-10-41 meaningfully promoted faba bean shoot length (14.22 and 66 cm at 30 and 90 days of sowing, respectively), more leaf development (7.78 at 30 days of plantation), and elevated branch/tiller (on average, 8.33 per plant) development at late growing periods (Table 6.4). Likewise, Mk-13-16 encouraged leaf and pod development (12.22 and 14.67, respectively) then resulted the second highest shoot dry weight (35.77 g (Table 6.6)). Moreover, each inoculant demonstrated various interactions and responses at different growth stages, such as Z-1-16 promoted shoot length at 30 and 60 days, seed development (on average 3.33 seed per pod), Mk-20-7 promoted shoot length and leaf development at 60 days, Mk-1-25 promoted more branch (8.45) and pod (1.22) development at 60 days and resulted a maximum shoot fresh weight (268 g), Z-12-20 encouraged early flower development (5.22 at 30 days), and the highest shoot dry weight (37.73 g) (Table 6.4, and 6.6). Thus, K-10-41, Mk-1-25, Mk-13-16, Z-1-16, and Z-12-20 could be recommended as faba bean farm inoculum by conducting further site-specific field trials.

Table 6.4: Mean value of shoot length and shoot parameters of faba bean at different growing periods that treated with 10 different PSB strains.

	Shoot length (cm)			Branch Number		Leaf		Flower		Pod per plant	
Isolate	30 days	60 days	90 days	60 days	90 days	30 days	60 days	30 days	60 days	60 days	90 days
Control	11.56±3.75	30.22+10.8	54.0+8.66	3.78+2.01^{b}	6.0+2.0^{abc}	6.33+0.67	9.33+3.93	3.44+5.96	1.67+0.34	0.0	7.33+7.5
K-1-29	10.89±1.58	35.56+5.3	61.7+11.6	3.55+2.12^{b}	3.83+0.58^{c}	6.78+0.84	10.67+5.2	1.56+2.7	7.33+7.22	0.56+0.51	13.0+5.0
K-10-27	13.0+3.22	40.55+9.01	60.0+9.85	6.22+1.89^{ab}	6.7+2.9^{abc}	6.78+0.69	9.67+4.84	3.56+3.1	6.78+6.27	0.56+0.96	7.4+6.93
K-10-41	14.22+3.72	38.78+3.53	66.0+7.55	5.67+1.0^{ab}	8.33+0.58^{a}	7.78+1.26	11.11+5.37	2.4+3.41	6.11+5.09	1.0+1.73	7.67+5.51
Mk-1-25	11.57+2.7	41.11+7.73	65.0+4.36	7.45+6.62^{a}	7.9+4.58^{ab}	6.33+0.88	10.0+4.7	3.44+5.96	4.44+4.29	1.22+2.12	8.33+3.22
Mk-13-16	12.44+2.52	37.11+2.27	58.0+6.0	5.33+2.31^{ab}	7.0+1.0^{abc}	7.22+0.69	12.22+6.26	3.33+5.77	5.44+4.00	0.56+96	14.67+4.2
Mk-20-20	12.11+4.86	34.44+16.6	63.0+8.7	4.22+2.12^{ab}	4.43+1.7^{bc}	6.34+1.53	10.22+4.55	1.67+2.89	4.22+5.18	1.0+1.73	14.0+4.36
Mk-20-7	13.78+2.77	43.0+5.55	66.3+5.7	4.56+2.22^{ab}	6.0+2.0^{abc}	7.33+0.88	11.33+5.51	4.89+0.19	5.67+2.73	0.78+0.84	8.67+4.16
Z-1-16	14.0+1.53	41.45+6.84	61.0+5.2	4.89+1.54^{ab}	7.0+3.0^{abc}	7.0+0.67	11.11+5.42	5.11+5.35	4.45+3.67	1.11+1.92	6.0+2.65
Z-12-20	12.55+3.34	38.89+8.81	59.7+3.5	5.0+2.18^{ab}	7.3+0.58^{ab}	7.0+0.58	11.22+5.64	5.22+9.1	2.89+1.34	0.0	12.67+8.1
Z-13-4	11.67+0.88	35.0+4.1	59.0+12.1	4.22+1.83^{ab}	7.67+2.1^{ab}	6.67+0.34	10.78+5.18	0.86+2.72	1.55+1.68	0.11+0.19	10.0+8.72
CV	24.1	21.84	13.16	53.78	35.18	12.79	48.40	59.49	63.74	21.93	58.51
LSD	5.1	14	13.64	4.63	3.81	1.49	8.77	7.91	7.3	1.91	9.85

NB: Means with similar letters have no significant difference at p= 0.05.

Kidney bean inoculation with selected PSB strains improved seed germination (Table 6.2 and 6.3), vegetative growth, and biomass yield. For instance, Mk-1-25 induced shoot length, branch, leaf development, early flower, and pod development. Similarly, K-10-41 encouraged plant height (66.67 cm (Table 6.5)) at late growth periods and yield (6 seeds per pod and the second highest shoot fresh weight (287 g) (Table 6.6). K-10-27 inoculation improved shoot length (67.33 cm), to develop more leaf number (27.22), seeds (6 per pod), and shoot dry weight (61.4 g). K-1-29 also encouraged the development of pods per plant (34.0 (Table 6.5)), seed per pod (6.0), and the highest shoot dry weight (61.97 g) (Table 6.6). Likewise, Mk-1-25 enhanced early shoot development with a greater number of leaves (7.22 and 27.22 at 30 and 60 days, respectively), a greater number of flowers (7.33) at 30 days, and more pods (Table 6.5) at 60 days of plantation. In general, K-10-27, K-10-41 Mk-1-25, and Z-12-20 strains encouraged germination, vegetative growth, development, and yield. As a result, they could be recommended for kidney bean farm inputs by conducting further field tests at different sites.

Table 6.5: Mean value of shoot parameters and pod development at different growing periods of kidney bean that treated with 10 PSB strains.

	Shoot length			**Branch**		**Leaf number**		**Flower**		**Pod per plant**	
Isolate	30 days	60 days	90 days	60 days	90 days	30 days	60 days	30 days	60 days	60 days	90 days
Control	9.78+1.0	34.11+10.8	62.33+12.66	11.44+4.55^{b}	17.67+3.51^{b}	6.2+0.39	21.4+5.68	0.00	16.6+9.18^{ab}	7.78+6.84	25.7+4.93^{ab}
K-1-29	9.9+1.95	33.33+3.72	55.67+4.04	14.56+4.07^{ab}	24.00+2.0^{ab}	6.9+0.77	26.44+1.9	5.7+5.7	15.8+6.54^{ab}	4.56+3.47	34.00+7.21^{a}
K-10-27	9.56+2.1	37.55+3.53	67.33+5.69	14.78+1.26^{ab}	21.00+5.0^{ab}	6.67+1.2	27.2+3.67	0.00	16.8+1.02^{ab}	8.22+3.67	24.00+6.0^{ab}
K-10-41	9.0+0.56	36.56+5.36	66.67+12.86	17.45+1.95^{ab}	19.00+8.89^{b}	6.78+0.2	24.89+5.0	5.7+4.7	11.44+1.71^{b}	5.45+6.31	29.3+12.7^{ab}
Mk-1-25	10.4+1.0	39.56+4.48	59.00+6.24	17.55+3.67^{ab}	24.67+2.08^{ab}	7.2+0.19	27.2+1.17	7.3+8.3	22.7+8.54^{ab}	9.56+6.35	26.7+3.79^{ab}
Mk-13-16	9.89+0.7	37.1+3.37	57.67+5.77	17.56+3.17^{ab}	19.33+3.21^{b}	6.9+0.77	25.8+1.71	0.00	25.00+8.74^{a}	8.33+3.76	22.00+7.55^{b}
Mk-20-20	8.7+1.45	37.11+6.83	62.33+1.69	15.89+2.11^{ab}	18.33+0.58^{b}	6.67+0.7	25.00+2.3	3.5+3.0	19.89+4.7^{ab}	6.89+2.04	28.7+3.06^{ab}
Mk-20-7	9.2+2.01	35.44+1.26	60.33+10.69	16.56+5.06^{ab}	21.67+3.06^{ab}	6.4+1.17	25.0+4.16	1.9+3.3	21.0+5.37^{ab}	6.89+5.59	27.7+6.03^{ab}
Z-1-16	9.67+1.3	39.67+4.73	61.67+10.02	19.56+4.44^{a}	25.33+4.16^{ab}	7.2+0.39	26.8+4.86	0.00	24.45+7.13^{a}	9.55+3.97	30.7+6.43^{ab}
Z-12-20	9.7+1.45	38.00+6.11	56.67+6.11	18.33+6.74^{ab}	24.00+6.93^{ab}	7.0+0.33	26.2+4.17	3.6+6.2	22.0+9.95^{ab}	9.44+6.53	30.0+8.72^{ab}
Z-13-4	10.8+3.4	38.56+8.83	59.33+7.57	15.55+6.01^{ab}	27.33+4.16^{a}	6.2+0.69	23.89+4.9	4.6+4.1	21.9+5.99^{ab}	7.89+6.36	35.67+4.73^{a}
CV	17.86	15.87	14.82	26.91	20.64	10.39	15.27	48.82	34.67	28.03	24.3
LSD	2.93	10.07	5.06	7.18	7.7	1.19	6.58	7.36	11.61	8.86	11.76

NB: Means with similar letters have no significant difference at p = 0.05.

Table 6.6: Seed and biomass yield of faba bean and kidney bean under PSB treatment.

	Faba bean			Kidney bean		
Isolate	Seed per pod	Shoot fresh Weight (g)	Shoot Dry Weight (g)	Seed per pod	Shoot fresh Weight (g)	Shoot Dry Weight (g)
Control	2.7+0.58^{ab}	127.33+71.23^{c}	19.2+15.84	5.7+0.58^{ab}	232.67+45.35	52.87+6.48
K-1-29	2.3+0.58^{b}	159.67+71.1^{abc}	27.6+10.83	6.00+1.0^{a}	242.67+38.42	50.33+1.01
K-10-27	2.33+.68^{b}	129.7+90.16^{bc}	25.1+23.13	6.00+0.0^{a}	263.67+87.66	61.4+15.17
K-10-41	2.3+0.58^{b}	249.3+97.16^{ab}	23.9+14.48	6.00+0.0^{a}	287.0+162.75	61.97+39.8
Mk-1-25	2.7+0.58^{ab}	268.0+54.51^{a}	33.27+2.83	5.00+0.0^{ab}	212.33+28.92	51.13+8.9
Mk-13-16	3.0+0.0^{ab}	174.67+10.6^{abc}	35.77+18.4	4.3+0.58^{b}	197.33+33.3	54.33+3.5
Mk-20-20	2.7+0.58^{ab}	155.7+51.01^{abc}	20.83+17.8	6.00+0.0^{a}	221.00+2.65	50.1+13.21
Mk-20-7	2.3+0.58^{b}	204.0+74.05^{abc}	21.7+5.29	5.3+0.58^{ab}	230.33+49.34	53.3+16.07
Z-1-16	3.33+0.0^{a}	213.7+34.39^{abc}	18.97+4.31	4.33+2.08^{b}	226.33+60.14	57.20+6.75
Z-12-20	3.0+0.0^{ab}	233.7+98.42^{abc}	37.7+13.41	5.7+0.58^{ab}	276.3+138.35	59.7+28.76
Z-13-4	2.7+0.58^{ab}	165.33+84.2^{abc}	22.6+14.92	5.3+0.58^{ab}	307.33+16.92	57.4+12.04
CV	19.58	37.99	24.57	14.71	31.39	31.72
LSD	0.88	12.71	2.08	1.35	10.3	9.77

NB: Means with similar letters have no significant difference at p = 0.05.

Maize was one of the major crops cultivated in the Rift Valley, and as a result, it was one of the test crops for this study to evaluate the interaction effects with current inoculants. Even though PSB inoculation didn't show a significant difference in all plant parameters over the control group, they showed encouraging results on germination, growth, and biomass yield. Some of the strains demonstrated prominent interaction and response, such as Z-13-4-induced shoot length, a greater leaf number (12.33), and a greater average corncob (2.33) development with the highest shoot fresh weight (588 g (Table 6.7)); Mk-20-20 enhanced shoot length (156 cm at 90 days), Z-12-20-induced shoot length at vegetative state (30.22 and 104.44 cm at 30 and 60 days, respectively), promoted to develop a greater number of leaves (8.11 and 12.33 at 30 and 60 days, respectively), and resulted in the highest shoot dry weight (236.3 g) (Table 6.7). Based on the current results of the symbiotic interaction of maize and PSB strains, the three (Z-13-4, Mk-20-20, and Z-12-20) strains could be recommended as maize inoculants by verifying their interaction response at different sites. On the other hand, PSB isolates screened from Koka exhibited promising results with other test crops yet, they demonstrated a very limited response with maize. This clearly demonstrated that the type of host crop is one of the limiting factors for strains to show fruitful interaction and response, even if the crops are frequently cultivated on the same farmland.

Table 6.7: Maize mean values for shoot development at various growing period and biomass yield.

	Plant Height (cm)			Leaf Number		Yield parameters		
Isolate	30 DOS	60 DOS	90 DOS	30 DOS	60 DOS	Cob No.	SFW (g)	SDW (g)
Control	27.55+3.72	79.00+8.84^{c}	135.33+15.01^{b}	7.89+1.17	11.22+0.51^{ab}	1.67+0.34^{bc}	423.67+180.14	146.93+39.06^{ab}
K-1-29	28.00+5.05	87.45+4.6^{abc}	135.22+9.13^{b}	7.78+0.69	11.33+1.2^{ab}	1.78+0.19^{abc}	532.67+309.98	115.63+21.15^{b}
K-10-27	27.22+4.85	88.78+15.98^{abc}	139.67+6.36^{ab}	7.78+0.51	11.34+0.58^{ab}	1.89+0.51^{abc}	458.33+22.01	136.10+17.51^{ab}
K-10-41	22.89+4.55	84.55+8.55^{c}	135.89+13.24^{ab}	7.22+0.19	11.11+0.38^{b}	1.89+0.19^{abc}	467.0+31.05	175.19+120.35^{ab}
Mk-1-25	25.22+3.87	84.11+15.02^{c}	132.66+12.34^{b}	7.56+0.51	11.67+1.21^{ab}	1.78+0.39^{bc}	412.33+83.34	144.23+14.57^{ab}
Mk-13-16	28.44+2.72	88.78+3.1^{abc}	151.00+11.53^{ab}	7.67+0.34	11.56+0.51^{ab}	1.78+0.19^{abc}	455.0+22.82	188.30+21.08^{ab}
Mk-20-20	25.00+8.72	79.89+8.69^{c}	156.00+6.66^{a}	7.67+1.73	11.22+0.51^{ab}	2.22+0.19^{ab}	532.67+22.37	136.60+40.61^{ab}
Mk-20-7	29.00+4.7	86.67+11.46^{bc}	149.89+18.53^{ab}	7.89+0.38	11.89+0.51^{ab}	1.56+0.51^{c}	336.33+48.42	169.97+32.24^{ab}
Z-1-16	25.00+4.49	89.55+13.68^{abc}	151.33+4.91^{ab}	7.67+0.88	11.89+0.51^{ab}	1.89+0.19^{abc}	502.33+70.03	148.87+58.26^{ab}
Z-12-20	30.22+6.17	104.44+8.23^{a}	148.11+13.01^{ab}	8.11+1.26	11.89+0.19^{ab}	2.00+0.33^{abc}	383.0+96.99	236.30+26.62^{a}
Z-13-4	29.78+5.42	102.33+7.88^{ab}	146.56+13.67^{ab}	8.45+0.69	12.33+0.58^{a}	2.33+0.34^{a}	588.0+28.87	169.57+72.6^{ab}
CV	18.73	11.72	8.33	11.29	5.84	17.37	32.91	39.72
LSD	8.72	7.59	0.28	1.49	1.15	0.56	7.94	8.07

NB: Means with similar letters have no significant difference at p = 0.05, SFW = Shoot Fresh Weight, SDW = Shoot Dry Weight, DOS = Days of Sowing

Wheat was another test crop to evaluate the response of strains' interaction and host symbiotic effectiveness for this field trial. PSB inoculation to wheat improved germination (Table 6.2 and 6.3), vegetative growth, and shoot biomass (Table 6.8). Due to the strong biological competitors, the grain yield was not included in the result analysis (completely destroyed by birds (Picture 6.1). Wheat shoot growth was significantly encouraged by Mk-13-16 at 60 days (58.67 cm) and Z-1-16 at 90 days (65.67 cm), leaf development by Z-1-16 (12.33), tiller development by K-10-41 and Z-1-16 (12.67 and 12.33, respectively) (Table 6.8) and biomass (the highest shoot fresh and dry weight (48.27 and 18.83 g, respectively (Table 6.8) was recorded from Mk-20-7 inoculation. Therefore, wheat inoculation with Mk-20-7, Z-1-16, Mk-13-16, and Z-12-20 might result in fruitful cultivation and production; however, these strains should be verified their efficacy under repeated site-specific field trials.

Table 6.8: Mean of shoot length and number of tillers at different growing periods of wheat treated with 10 PSB strains.

	Plant Height (cm)			Number of Tillers			Leaf number		Flower No	Biomass	
Isolate	30 DOS	60 DOS	90 DOS	30 DOS	60 DOS	90 DOS	30 DOS	60 DOS	60 DOS	SFW (g)	SDW (g)
Control	18.06+3.6	36.78+2.8^{b}	51.33+10.26^{b}	2.78+1.35^{ab}	3.67+1.34^{b}	9.67+5.5	7.89+5.59	9.56+4.0	3.67+3.84	46.1+10.22	9.37+4.9
K-1-29	18.22+2.7	43.44+18.8^{ab}	58.67+4.62^{ab}	2.89+0.7^{ab}	5.78+2.87^{ab}	9.67+3.1	7.11+3.65	9.66+2.3	2.78+2.36	34.80+3.34	10.77+0.9
K-10-27	19.1+4.4	42.11+18.5^{ab}	60.67+6.51^{ab}	3.44+0.84^{ab}	5.22+2.34^{ab}	11.3+2.3	7.00+3.46	11.67+3.5	2.89+1.17	38.30+15.1	14.10+6.1
K-10-41	18.45+1.1	43.33+11.1^{ab}	61.67+4.93^{ab}	2.78+0.19^{ab}	5.00+1.15^{ab}	12.7+1.2	7.22+3.56	9.67+1.2	3.00+0.58	33.37+11.6	10.93+1.8
Mk-1-25	20.33+3.5	45.67+3.2^{ab}	57.67+4.04^{ab}	3.33+1.0^{ab}	4.00+1.33^{ab}	10.7+2.5	9.11+7.12	11.89+4.2	3.11+2.17	32.43+10.9	14.20+4.7
Mk-13-16	19.33+2.2	58.67+23.2^{a}	63.33+5.77^{a}	3.22+0.39^{ab}	7.56+3.5^{a}	11.0+1.7	8.22+8.63	10.00+1.5	4.11+5.74	41.70+11.0	13.27+6.2
Mk-20-20	17.33+3.4	48.11+4.6^{ab}	61.33+7.02^{ab}	2.89+1.26^{ab}	5.22+2.14^{ab}	10.0+3.0	8.67+6.35	10.11+4.1	2.89+2.7	42.43+27.8	13.37+3.95
Mk-20-7	20.78+2.8	46.89+6.6^{ab}	64.00+6.56^{a}	3.11+0.51^{ab}	5.44+2.5^{ab}	11.7+4.9	8.55+5.87	10.33+2.3	3.89+1.4	48.27+24.2	18.83+10.1
Z-1-16	20.00+2.5	46.56+6.7^{ab}	65.67+5.13^{a}	3.78+0.84^{a}	5.22+1.54^{ab}	12.3+3.1	8.67+6.35	12.66+3.2	3.67+1.7	44.47+9.58	15.03+6.85
Z-12-20	20.33+2.3	47.55+9.3^{ab}	61.00+4.58^{ab}	3.44+0.2^{ab}	5.55+2.41^{ab}	11.7+2.5	8.11+5.39	12.11+2.3	4.45+3.0	35.53+21.6	15.13+8.75
Z-13-4	17.33+4.4	42.22+10.5^{ab}	59.67+10^{ab}	2.33+1.0^{b}	5.11+2.79^{ab}	12.0+4.0	8.11+5.69	9.78+4.29	2.11+1.2	37.17+23.0	13.87+7.6
CV	16.54	27.16	11.1	27.22	43.61	29.7	41.73	29.85	22.26	42.84	45.92
LSD	5.31	0.96	11.36	1.42	3.88	5.61	9.79	5.4	4.63	4.66	10.52

NB: Means with similar letters have no significant difference at p = 0.05, SFW = Shoot Fresh Weight, SDW = Shoot Dry Weight, DOS = Days of Sowing

PSB inoculations of onion have shown positive results, demonstrated constructive interactions with it and improved germination (Table 6.2 and 6.3), growth and development. Among the ten PSB strains, three (Mk-1-25, Mk-20-20 and Z-12-20) of them significantly ($p<0.05$) promoted the development of various plant parameters. Accordingly, Mk-20-20 positively influenced onion to develop stretched shoot length (53.45 cm), root length (9.22 cm) and shoot fresh weight (44.11 g); Mk-1-25 improved plant shoot length (50.67 cm), a greater leaf number (9.67), bulb length (6.72 cm) and shoot fresh weight (39.89 g). Likewise, Z-12-20 positively interacted with and promoted the crop to develop a greater bulb size (on average 14.22 cm diameter and 51.33 g weight) (Table 6.9). As a result, these three potential PSB strains (Z-12-20, Mk-1-25 and Mk-20-20) could be recommended for onion farm under repeated site-specific field trials. On the other hand, some isolates formed poor interaction and have shown weak response to onion growth and development, even below the control group. For instance, K-10-41 was one of a good candidate inoculum for most of the crops nevertheless, it has been shown poor interaction and response with onion in most recorded plant parameters. This might clearly suggest that the range and type of host crop limit the symbiotic interaction and the response of the candidates.

Table 6-9: Mean value of onion shoot, bulb and root parameters after 120 days of growth under selected 10 PSB strains treatment.

Isolate	PH (cm)	Leaf Number	Bulb Length (cm)	Bulb Diameter (cm)	SFW (g)	BFW (g)	Root length (cm)	TBWPP (g)
Control	47.33+6.69	8.22+2.17^{ab}	4.5+0.7^{b}	13.33+1.3^{ab}	33.00+13.61	44.33+11.0	8.55+2.6	2621.33+636.72
k-1-29	46.45+3.97	8.44+1.64^{ab}	4.5+0.72^{b}	13.61+0.3^{ab}	32.45+10.12	47.78+9.76	7.67+0.3	2363.33+303.58
k-10-27	44.78+4.35	9.22+1.17^{a}	4.66+0.43^{ab}	12.67+1.2^{ab}	30.78+6.81	44.22+9.02	9.39+2.1	2746.67+103.35
K-10-41	44.00+3.84	6.55+1.35^{b}	4.17+0.44^{b}	12.34+1.5^{b}	24.33+3.18	37.56+7.07	8.11+1.0	2410.0+69.94
Z-12-20	47.33+3.61	8.66+0.58^{ab}	5.05+0.63^{ab}	14.22+0.8^{a}	30.78+5.34	51.33+3.51	9.00+3.5	2881.0+692.0
Mk-1-25	50.67+5.2	9.67+1.76^{a}	6.72+2.9^{a}	12.55+1.1^{ab}	39.89+8.67	39.00+2.6	6.45+3.7	2177.67+322.1
Mk-20-20	53.45+2.6	8.33+1.53^{ab}	4.56+0.92^{b}	13.39+0.4^{ab}	44.11+16.4	49.45+10.2	9.22+2.2	2697.0+934.6
Mk-20-7	46.44	8.66+1.53^{ab}	4.84+0.76^{ab}	13.28+1.4^{ab}	34.67+18.8	45.33+11.8	8.89+2.8	2685.3+1040.5
CV	10.55	18.04	24.81	8.23	34.23	19.48	29.95	24.01
LSD	8.68	2.65	2.09	1.88	20.0	15.13	4.36	9.03

NB: Means with similar letters have no significant difference at p = 0.05. SFW = Shoot Fresh Weight, BFW = Bulb Fresh Weight, TBWPP = Total Bulb Weight per plot

6.4 DISCUSSION

Cost, energy, and environmental concerns are the major challenges related to chemical fertilizer practice in agriculture (de Andrade *et al.,* 2023; Giannelli *et al.*, 2023; Shah *et al*., 2021). Application of biofertilizers and bio-stimulants has shown a progressive increment along the globe due to the escalating environmental issues, positive response of inoculation, rise of food demand, cost feasibility (Nosheen *et al.,* 2021), and the like. They improve soil fertility and plant-assimilated nutrients by fixing atmospheric nitrogen and dissolving insoluble phosphorous (Mahanty *et al.,* 2017). Most of the agricultural soil contains large reservoirs of total phosphorous but it is deficient in plant-available forms (Mitra *et al*., 2020). In this regard, many PSB are known to solubilize and supply available P to plants (Verma, 2019). Their application to seed, soil, or root (Kumar *et al.*, 2022) colonizes the rhizosphere and/or interior part of the plant, then promotes plant growth and available nutrients (Mącik *et al.,* 2020). Similarly, Liu *et al*. (2020) have shown that PSB application improved plant P-uptake and yield both in controlled environments and at field experiments. The application of PSB to both monocots and dicot plants improved the plant P-uptake, root and shoot length as well as biomass (De Zutter *et al*., 2022). Supportively, a review by Nosheen *et al.* (2021) have shown that biofertilizers saves 50% of fertilizers cost in sugarcane production while mixed biofertilizers application to rice significantly increased production and successfully substitute inorganic fertilizer.

In this field trial, five different crops were taken and inoculated with pre-screened PSB isolates from the tomato rhizosphere. These variable types of crops were assumed to be representative of the different groups (cereal, legume, and tuber crops). This is because these different plant species have different nature and biological responses and follow different mechanisms in nutrient absorption, root design, root-microbial interaction, and the management of various challenges. Especially the plant root system and root zone configuration significantly influence the rhizosphere microbial populations. Similarly, Mahanty *et al*. (2017) stated that the composition and diversity of the root exudates (a chemical attractant for heterogenous microbial communities in the root zone) considerably depend on the plant species and the microorganisms. De Zutter *et al*. (2022) also indicated that the type of plant species matter rhizosphere-deposits and rhizosphere microbial crosstalk. Therefore, the choice of these crops is thoughtfully considered as representative namely, maize and wheat from cereal, faba and kidney beans from legumes, and onion from tuber

crops. Most of these crops have a good cultivation history in the study areas, and they are important crops for daily diet, nutrition, cash and foreign exchange. In the current study, the selected competent PSB strains positively influenced these various crops' growth, development, and yield. The inoculants demonstrated encouraging and promising results in all crops, and the analysis of recorded data have shown an encouraging result achieved over the control groups in most plant traits (germination, shoot length, leaf, flower, tiller/branch, pod, and corncob development, as well as biomass (Table 6.2 – 6.9)). In a good agreement, Pellegrini *et al.* (2021) and Tinna *et al*. (2020) have indicated that PGPR stimulated plant germination growth and development, and improved yield and quality. Likewise, Mitra *et al.* (2020), Verma (2019), and Mahanty *et al.* (2017) also demonstrated that PGPR improved agricultural production by enhancing seed germination, growth, leaf area, crop yield, and biomass. It is treasured if the inoculants actively stimulate the plant throughout the growth stage, yet due to many reasons, it is unlikely. Exceptionally, they might stimulate the plant throughout its entire growth; otherwise, they promote at certain growth stages, then decline or have no observable effect in the remaining growth periods. Likewise, Shah *et al.* (2021) reviewed that synergetic (plant and associated microbes) interactions at field conditions were influenced by various factors, including the type and growth of the plant, the strain, other members of the phyto-microbiome community, and soil physico-chemical properties (temperature, pH, fertility, and nutrient contents).

The overall host range evaluation of the current PSB strains against these test crops indicated that Z-12-20 generally demonstrated broad host range (positively affected and encouraged growth and biomass of all the tested crops (faba and kidney bean, maize, wheat, and onion) (Tabel 6.4–6.9). Similarly, Mk-1-25 was found to be the second strain with a broader host range, which positively influenced and promoted faba bean, kidney bean, and onion growth and biomass. Shah *et al.* (2021) supportively indicated that the production of phytohormones by plant growth-promoting bacteria influences seed germination, root system, shoot elongation, flowering, and overall plant development. In addition, Mk-13-16, Mk-20-20, K-10-41, and Z-1-16 have shown relatively a wider host range that induced and promoted vegetative growth and biomass yield in at least two of the test crops, i.e., Mk-13-16 faba bean and wheat, Mk-20-20 maize and onion, and Z-1-16 faba bean and wheat (Tabel 6.4 - 6.9). On the contrary, K-1-29 (was one of the top promoters of tomato) has shown the least interaction among the current tested crops. Likewise, K-10-41 also exceptionally demonstrated poor interaction with onion. Moreover, strains from Meki and Ziway Zuria have relatively

demonstrated a broad host range, while strains from Koka sites were a narrow host range. Likewise, wheat and faba beans have been symbiotically interactive with multiple strains to enhance various plant attributes, whereas maize and onion have shown a limited performance with most of the candidate strains. On the other hand, legume crops (faba bean and kidney bean) have demonstrated variable responses against the strains along the developmental stages of the crop probably, these plants might regulate the rhizo-microbial community and responses by controlling the releasing type and amount of metabolites and root exudates at different growth stages. In this context, Giannelli *et al*. (2023) have clearly shown that depending on the type of plant species and age, plants release root exudates (rhizodeposits) of approximately 11% of net photosynthetic carbon and 10–16% of plant nitrogen. Andrade *et al. (*2023) also reported that, depending on the plant species, 10–40% of plant photosynthetic metabolites released as root exudates. Similarly, Ahemad and Kibret (2014) indicated that 5-21% of photosynthetically fixed carbon released to rhizosphere as root exudates. Moreover, a review by de Andrade *et al.* (2023) strengthens the current study that the agriculture and horticulture contributions of PGPR in plant root systems, growth promotion, and nutrient availability improved yield and plant protection.

Different investigations have reported the successful story of PSB bio-stimulation and their satisfactory interactions with different crops like maize (Yasmeen *et al.,* 2022; Planchamp *et al.,* 2015), tomato (Liu *et al.,* 2020; Mitra *et al.,* 2020; Verma, 2019; Wei *et al.,* 2018; Mahanty *et al*., 2017), onion (Pellegrini *et al*., 2021; Tinna *et al.,* 2020*),* wheat (De Zutter *et al*., 2022; Wu *et al*., 2022; Kumar *et al*., 2021; Mukhtar *et al*., 2017), and kidney bean (Dela *et al*., 2023). PSB use various mechanisms to boost plant interaction and plant traits. They are a promising tool to alleviate plant stresses and increase agricultural productivity. In this context, Giannelli *et al.* (2023) have reported that beneficial PGPR exerts major effects on the host plant by improving nutrient assimilation, phytohormones, and resistance to different biotic and abiotic stresses. On the other hand, it is challenging to find competent strains for various crops because the initial selection is based on certain positive traits for a distinctive crop variety (Mahanty *et al.,* 2017). Beside the solitary inoculation, for fruitful results, scholars recommended integrated inoculation of two or more strains (Kumar *et al.,* 2022) and/or synergetic application together with other supplements, substrates, and agrochemicals (Tinna *et al.,* 2020; Sharma *et al.,* 2013). Likewise, Liu *et al*. (2020) have indicated that PSB co-inoculation with manure and fertilizer improved soil attributes and crop growth. Dela *et al*. (2023) also demonstrated that the combined application of blended NPSB fertilizer and

biofertilizer improved soil nutrients, common bean yield and yield qualities. Abeje *et al.* (2024) also reported that combined application of biofertilizer, lime and NPSB fertilizer significantly improved soya bean nodulation, leaf area index, grain yield and economic benefit in Assosa zone.

For widespread utilization of biofertilizers and PGPR, there are several core issues to be addressed. These include the selection of competent and effective multifunctional strains (active, efficient and non-toxic), elaboration of their practice from laboratory and greenhouse experiments to large-scale commercialization (by improving the growth, storage, formulation, shipping, and application of the strains) (Mahanty *et al*., 2017; Sharma *et al.,* 2013). In addition, government should promote and commercialize biofertilizers and PGPR by designing strategies in input subsidies, market development and financial support, tax incentives, and privatizing the industry (Raimi *et al.,* 2021). Skills, farmers awareness as well as the regulation of quality and standard should be improved.

6.5 CONCLUSION AND RECOMMENDATIONS

This field trial on different crops (maize, wheat, onion, kidney bean, and faba bean) inoculation with selected PSB showed that each strain has its own host crop specificity to form fertile interactions and positively influence or promote the growth and development of the crop. Similarly, it was observed that host crop growth-stage could matter the strains interaction and response. This might be related to the host crop's developmental change possibly alter and regulate the amount and type of released root exudates and plant metabolites which indirectly regulate the rhizo-microbiome. Therefore, this research gives a good sign for future work: the need for repeated field trials to verify strains' efficacy as well as to deeply understand the metabolic and molecular responses between the candidate inoculants and the target host crop. Future works therefore should focus on the effectiveness and competence of inoculants in different agro-ecologies with various crops and the molecular and metabolic responses of the interactions, explore more competent strains, then optimize efficient strains for farm application.

CHAPTER SEVEN

7. SUMMARY, CONCLUSION AND RECOMMENDATIONS

7.1 General Summary (Highlights)

This research project was aimed to screen potential biofertilizers and plant growth-promotors primarily for tomato production at Koka, Meki, and Ziway Zuria. The experiment was started by assessing the overall crop production system, agrochemical practice and biofertilizer application in these areas. Accordingly, it was found that smallholder farmers produced various crops, including vegetables, cereals, and legumes, for household consumption and commercial purposes (income generation). Cabbage, tomato, potato, and onion are the leading vegetables, while maize is vastly produced cereal crop in the wet season. The presence of a conducive environment along with resource availability (convenient land, accessible water, cheap labour, road facilities, and transportation) promotes vegetable and horticultural production in the region. It was observed that these crops, especially vegetable production, need immense agrochemical practices, though tomatoes required elevated chemical application. Unfortunately, there was no bioinoculants (biofertilizer and growth-promotors) practice in the areas by the time of data collection.

Biofertilizers are the good actors in maintaining soil fertility through their single or multiple beneficial effects, and they are the main contributors to soil health, quality, and biodiversity. They are undetachable components of organic farming because of their eco-friendly approach and positive interactions with the host plant to sustain agricultural production. Potential biofertilizers and plant growth-promotors were isolated from the local farm and related environments to minimize endogenous competition and improve colonization and adaptation. The sample soils were collected from different sites, including tomato rhizosphere soil samples from Koka, Meki, and Ziway Zuria, as well as the natural forest soil from Wendogenet and Yirgalem Zuria. From these collected soil samples, plenty of PSB isolates were screened using the plate screening method on PVK agar. Then, following various preliminary screening techniques (SI, culture-re-culture purity, viability, medium pH change, and P-dissolving ability), the top 10 PSB strains were selected for further characterization and efficiency evaluation. For instance, during plate screening, Mk-13-16, Mk-20-20, and K-10-27 produced a larger colony and halo-zone diameter upon 8 days of incubation, while Mk-1-

25, K-10-41, and Z-12-20 were recorded relatively larger solubilization index (SI). To strengthen the screening procedure, isolates were incubated for 5 days in PVK broth to check their medium pH reduction ability and cellular growth (turbidity). Accordingly, K-10-41 and K-10-27 significantly reduced the pH to 4.02 and 4.12, respectively. In addition, morpho-biochemical and plant growth-promoting (G-staining test, sugar fermentation, salt and temperature resistance, urease, catalase, HCN, siderophore and IAA production, antagonistic and nitrogen fixation) as well as molecular (16S rRNA and IGS region between 16S and 23S rRNA sequence) characteristics were determined and strains were identified.

Once the top 10 PSB strains were screened and characterized, they were subjected to evaluate at different (laboratory, greenhouse, and field) levels to verify their efficacy and plant beneficial attributes. In doing so, in the laboratory, beyond PVK agar media, their solubilization efficiency was evaluated and quantified using modified PVK broth supplemented with four different phosphate substrates (TCP, AlP, FeP, and bone meal). The strains were separately inoculated into each substrate composite broth and incubated for 10 days, then medium pH and dissolved P were quantified at different incubation days. Accordingly, K-1-29 and K-10-41 recorded the highest dissolved P (260.83 and 260.38 µg/ml, respectively) at the 10^{th} day of incubation. Among the added substrates, TCP dissolved considerable P (253.46 µg/ml), while the other substrates dissolved a comparable amount (213.23, 212.69, and 211.78 µg/ml from FeP, BM, and AlP, respectively).

Following laboratory verification, strains were tested their symbiotic interactions with tomatoes under greenhouse conditions. This pot trial was conducted under dual-inoculation of PSB strain along with an external phosphate substrate (TCP, BM, compost, AmP, AlP, and DAP fertilizer) to improve the tomato-bacterial interaction, and two (negative and positive) controls were used. Tomatoes were grown for 110 days, and then different plant parameters were recorded and analysed. It was found that inoculation significantly ($p<0.05$) improved almost all plant parameters. For instance, tomato germination was shortened by the inoculation of Mk-20-20 and K-10-27 together with AlP and compost. Similarly, tomato shoot length was substantially improved by the inoculation of Z-12-20, Mk-1-25, and K-10-41 together with compost. Leaf development improved with the inoculation of compost along with Mk-20-7, Mk-20-20, Mk-1-25, and K-1-29. Floral parameters were significantly improved by inoculating K-1-29 with compost. Likewise, K-1-29 improved fruit yield and the overall phosphate-based symbiotic effectiveness (PBSE (%) = 144). The maximum

average fruit weight per plant was harvested from the inoculation of Mk-20-20 (158.7 g). Z-12-20 recorded the second highest shoot fresh and dry weight, root length (36.33 cm), root fresh weight (40.42 g), root dry weight (10.39 g), and PBSE (%) (144). Mk-1-25 inoculation improved shoot dry weight (17.87 g) and PBSE (%) (176). Generally, the current greenhouse trial confirmed that synergistic application of strain and P-substrate enhanced symbiotic effectiveness and attained positive responses. Summarizing the result of current greenhouse experiment, K-1-29, Z-12-20, Mk-1-25, K-10-27, Mk-20-7, and K-10-41 have revealed encouraging responses. Among the applied P-substrates, compost significantly improved the symbiotic interaction and plant response. On the other hand, Z-13-4 relatively, demonstrated poor symbiotic interaction and limited observable responses in most recorded plant parameters. While analysing the interaction and responses of strains against specific the P-substrate, each strain has its own substrate preferences, and the responses vary accordingly.

After the greenhouse trial, the experiment was extended to open-field evaluation. The field trial helps to verify the strains' symbiotic effectiveness with tomato and other crops (maize, wheat, faba bean, kidney bean, and onion) and their ability to compete against endogenous microbes that colonize and adhered the rhizosphere. Any positive results from field trial will validate the strain to be recommended as biofertilizer and plant growth-promotor in line with farmer's acceptance. The tomato inoculation trial was conducted both in dry and wet seasons, while the host range test was conducted in the dry season only. However, due to some limiting factors, wet season tomato trial results were excluded, and partial data from host range trial was included in this thesis presentation.

A tomato (the primary target of this project) field trial was designed and conducted under dual-inoculation (PSB + possible external P-substrates (compost, BM, and DAP fertilizer and a mixture of 50% compost and DAP)). This synergistic inoculation can improve the success of tomato-strain interaction and the response of tomato. For instance, tomato shoot length was significantly promoted by Mk-20-7, Z-12-20, and Mk-1-25 in all data-recorded growth periods while, branch and floral development were considerably improved by Z-12-20, Mk-1-25, and K-10-41 inoculations. Tomato fruit yield and yield parameters were also significantly ($p<0.05$) improved by the inoculation as a result, elevated total fruit number per plant was harvested from K-10-41 (21.87), K-10-27 (21.82), K-1-29 (21.31), and Mk-20-20 (20.69), while uninoculated tomato (control group) produced 14.67 fruits per plant. Among the collected total fruits, a greater number of marketable fruits were harvested from Mk-20-7

(10.44), K-10-41 (10.42), and K-1-29 (10.0), while the control group produced 4.33 marketable fruits per plant. Apparently, because of different factors and early harvesting, a considerable number of unmarketable fruits were collected from the inoculation of K-10-27 (12.64) and Mk-13-16 (11.44 fruits per plant). The overall fruit weight was significantly improved by inoculation; therefore, the highest total fruit weight recorded from K-10-41 (2821.6 g), K-1-29 (2793.3 g), Mk-13-16 (2781.3 g) and Mk-20-7 (2780.53 g). Moreover, this experiment showed a new horizon that efficient PSB strain inoculation together with available possible P sources could improve tomato growth parameters like the root, leaf, stem, flower development and fruit yield. Among P-substrates, compost and the mixture of 50% rate of compost and fertilizer applications significantly enhanced tomato vegetative growth attributes, while bone meal and the mixture of 50% compost and fertilizer meaningfully improved fruit yield (on average larger fruit number of 62.82 and 63.06 and a total fruit weight of 2645.91 and 2617.39 g, respectively).

Field level host range trial of the candidate strains was another practical efficacy validation which could strengthen the screening technique, provide a good picture about their multidimensional benefits, broad host interaction, and responses. It might also help to catch farmers' attention and encourage them to practice. Five different crops (maize and wheat from cereals, faba bean and kidney bean from legumes, and onion from tubers) were inoculated with each PSB strains. Growth and biomass yield of these crops were analysed; however, because of strong biological competitors (birds, porcupines and hyena), grain yield was not included. In summary of the obtained data from this particular experiment, maize inoculation with Z-12-20, Mk-20-20, and Z-13-4 promoted shoot length, leaf development, corncob number, and shoot biomass (both shoot fresh and dry weight). Similarly, wheat inoculation with Z-1-16 and Mk-13-16 improved shoot length, leaf, tiller, and flower development; Mk-20-7 improved shoot fresh and dry weight. Z-12-20 also considerably promoted wheat leaf and flower development and shoot dry weight. Likewise, onion inoculation with Mk-1-25, Mk-20-20, and Z-12-20 enhanced shoot length, leaf development, bulb parameters, and shoot weight. The inoculation of legumes demonstrated variable responses throughout their growth stages. Mk-13-16 and Mk-1-25 inoculation to faba bean promoted shoot length, branch, leaf, pod, and seed development, while Z-12-20 improved flower, seed development, and biomass (shoot fresh and dry weight). Similarly, Mk-1-25, Z-1-16, and K-10-27 inoculation of kidney beans enhanced vegetative growth, while K-10-27 and K-10-41 induced seed development and biomass. Even though each PSB strain

demonstrated various responses against a specific crop, generally, Z-12-20, Mk-1-25, Mk-13-16, K-10-41, and Mk-20-20 have shown an encouraging response and wider host range.

7.2 Conclusions Remarks

To address the ever-increasing population and food demand, agricultural production is the main target expected to be improved. Agricultural mechanisms in production, harvesting, storage, and transportation improved progressively over time while, agricultural practices aimed at improving crop production. Chemical fertilizers and other agrochemical applications may improve production nonetheless, repeated and long-term practice results in soil acidity, fertility depletion, reduced quality in consistence with environmental and health problems. Despite the soil fertility status, environmental condition, and farming system, fruits and vegetables, including tomatoes, required high-doze agrochemical practices. Therefore, alternative mechanisms and biotechnological approaches are required to maintain soil fertility and improve production sustainability. In this regard, biofertilizers and plant growth-promoting microbes play a significant role. PSB are known plant growth-promoters with their nutrient accessing, phytohormone production, and other multidimensional benefits with a plus of broad host range positive interactions. The current PSB strains belong to *Bacillus*, *Burkholderia* and *Priestia* species, where most of the subspecies placed in these genera have been repeatedly and strongly mentioned in various publications for their substantial role play in plant growth promotion, biocontrol, and agroecological traits. Therefore, among the current strains, top 6 PSB strains (K-10-41, K-10-27, Mk-1-25, Mk-20-7, K-1-29, and Z-12-20) were found competent and potential plant growth-promoters, possibly they can be optimized, developed, and recommended primarily for tomato and other crop farms in the study areas. They demonstrated encouraging and beneficial effects in nutrient access, phytohormone production, antagonistic production, and minimize production cost. They may have ecological, agronomic, and biotechnological impacts by promoting and optimizing integrated soil fertility maintenance, pest management, and sustainable productivity of smallholder farmers. To screen potential strains, hierarchical screening techniques, namely laboratory scale, greenhouse, and field trials (i.e., lab-field efforts), are essential to understand the actual and detail response of the strain at different levels, and that will give a good picture in the selection procedure. As a result, these procedures were employed in the present study.

In conclusion, K-10-41, K-10-27, Mk-1-25, Mk-20-7, K-1-29, and Z-12-20 were found potential inputs for tomato production. Similarly, Z-12-20, Mk-1-25, Mk-13-16, Mk-20-20, Z-1-16, and K-10-41 demonstrated promising symbiotic interactions and responses with different crops. These candidates (especially Z-12-20, K-10-41, K-10-27, Mk-20-7, K-1-29, and Mk-1-25) can be applied as biofertilizers and plant growth-promotors alone or together with other agro-inputs; hence, the application of competent strains in combination with proper agrochemicals will improve their efficacy and productivity of smallholder farmers. Synergetic application of organic supplements (compost, farmyard manure, bonemeal, rock phosphate, or other biowastes), bioinoculants, and appropriate agrochemicals help to maximize production and environmental health. This kind of farm practice is feasible for the economic, social, and ecological sense of balance in line with soil and environmental health maintenance. Therefore, exploring more efficient and competent strains as well as cheap, easily available, and eco-friendly supplements for combined application are the future prospects to improve soil fertility, plant growth, development, and farm production.

7.3 Recommendations

To develop, produce, and commercialize efficient and competent biofertilizers and plant growth-promoters, it would be better if the future perspective focused on the followings:

- Create awareness and improve the knowledge, understanding, and skills of farmers and stakeholders to practice biofertilizers and plant growth-promotors,
- Conduct site-specific field trials for selected potential strains,
- Commercialize and advertise competent strains by Gov’t and Agri-sectors,
- Study the detail molecular and physiological mechanisms of symbiotic interactions and responses between the strain and host plant,
- Explore more potential strains with multidimensional benefits,
- Explore accessible cheap biowastes, and organic matters such as bone meal, farmyard, manure, and compost and then practice synergetic application with competent strains,
- Introduce dual application of potential strains together with organic matters, proper fertilizer rate, and other agrochemical inputs,
- Optimize carriers for storing, packaging and transporting the strains, etc

REFERENCES

Abebe, T.G., Tamtam,M.R., Abebe, A.A., Abtemariam, K.A., Shigut, T.G., Dejen, Y.A., Haile, E.G. 2022. Growing Use and Impacts of Chemical Fertilizers and Assessing Alternative Organic Fertilizer Sources in Ethiopia. *Applied and Environmental Soil Science*. Pp 1-14. https://doi.org/10.1155/2022/4738416.

Abeje, A., Alemayehu, G. and Feyisa, T. 2024. Effects of biofertilizers, lime and inorganic NPSB fertilizers on nodulation, growth and yield of soybean (*Glycine max* L. M.) in Assosa Zone, Western Ethiopia, Journal of Plant Nutrition, 47(4). Pp. 615-627. https://doi.org/10.1080/01904167.2023.2280136.

Abera, G., Ibrahim, A.M., Forsido, S.F., & Kuyu, C.G. 2020. Assessment on post-harvest losses of tomato (*Lycopersicon esculentem* Mill.) in selected districts of East Shewa Zone of Ethiopia using a commodity system analysis methodology. *Heliyon*, 6(4). Pp1-13. https://doi.org/10.1016/j.heliyon.2020.e03749.

Ahemad, M. and Kibret, M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University - Science*, 26(1). 1-20. https://doi.org/10.1016/j.jksus.2013.05.001.

Alemayehu, N., Hoekstra, D., Berhe, K., Jaleta, M. 2010. Irrigated vegetable promotion and expansion: the case of Ada'a Woreda, Oromia Region, Ethiopia. IPMS Case Study. Nairobi (Kenya): ILRI.

Alikhani, H.A., Saleh-Rastin, N., Antoun, H. 2007. Phosphate solubilization activity of rhizobia native to Iranian soils. *In*: Velázquez, E., Rodríguez-Barrueco, C. (*Eds*) First International Meeting on Microbial Phosphate Solubilization. Developments in Plant and Soil Sciences. 102. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5765-6_4.

Aliyat, F.Z., Maldani, M., Guilli, M.E., Nassiri, Ibijbijen, J. 2020. Isolation and characterization of Phosphate Solubilizing Bacteria from Phosphate Solid Sludge of the Moroccan phosphate Mines. *The Open Agriculture Journal*, 14. Pp 16-24.

Amanullah and Khan, A. 2015. Phosphorus and Compost Management Influence Maize (*Zea mays*) Productivity Under Semiarid Condition with and without Phosphate Solubilizing Bacteria. *Frontiers in Plant Science,* 61(1083). https://doi.org/10.3389/fpls.2015.01083.

Amaresan, N., Amin, D. and Patel, P. 2022. Practical Handbook on Agricultural Microbiology. Humana Press. Springer Nature, New York, U.S.A. https://doi.org/10.1007/978-1-0716-1724-3.

Ammar, E.E., Rady, H.A., Khattab, A.M. *et al.* 2023. A comprehensive overview of eco-friendly bio-fertilizers extracted from living organisms. *Environ Sci Pollut Res.,* 30. Pp 113119–113137. https://doi.org/10.1007/s11356-023-30260-x.

Amaya-Gómez, C.V., Porcel, M., Mesa-Garriga, L., Gómez-Álvarez, M.I. 2020. A Framework for the Selection of Plant Growth-Promoting Rhizobacteria Based on Bacterial Competence Mechanisms, *Applied and Environviromental Microbiology*, 86(14). Pp 1-12.

Argaw, A. 2012. Evaluation of Co-inoculation of *Bradyrhizobium japonicum* and Phosphate Solubilizing *Pseudomonas spp*. Effect on Soybean (*Glycine max* L. (Merr.)) in Assossa Area. *J. Agr. Sci. Tech*. 14. Pp 213-224.

Asfaw, D.M. 2021. Analysis of technical efficiency of smallholder tomato producers in Asaita district, Afar National Regional State, Ethiopia. *PLoS ONE,* 16. Pp11-16. https://doi.org/10.1371/journal.pone.0257366.

Awan, Z.A., Shoaib, A. and Khan, K.A., 2018. Variations in total phenolics and antioxidant enzymes cause phenotypic variability and differential resistant response in tomato genotypes against early blight disease. Elsevier, *Scintia Horticulturae*, 239 (2018). Pp 216-223.

Baba, Z.H., Tahir, S., Wani, F.S. and Hamid, B. 2017. Impact of Phosphate Solubilizing Bact eria and Inorganic Fertilizers on Yield Attributes of Tomato. *Int. J. Curr. Microbiol. App . Sci*., 6(6). Pp 3233-3239. https://doi.org/10.20546/ijcmas.2017.606.381.

Babalola, O.O., Fasusi, O.A., Amoo, A.E., Ayangbenro, A.S. 2021. Complete genome sequence of a plant growth-promoting rhizobacterium, Bacillus sp. strain OA1, isolated from soybeans. *Biocatalysis and Agricultural Biotechnology*, 36. https://doi.org/10.1016/j.bcab.2021.102121.

Bai, Y., Kissoudis, C., Yan, Z., Visser, R.F. and van der Linden, G. 2018. Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. *The Plant Journal*, 93(4). Pp 781–793. https://doi.org/10.1111/tpj.13800.

Bahilu, E., Derebe, E., Gosaye, E. 2020. Determinants of Smallholder Commercialization of Tomato Crop in Siltie Zone, Southern Ethiopia. *Agriculture, Forestry and Fisheries*, 9. 163-173.

Baliah, N.T., Pandiarajan, G., Kumar, B.M. 2016. Isolation, identification and characterizatio n of phosphate solubilizing bacteria from different crop soils of Srivilliputtur Taluk, Virudhunagar District, Tamil Nadu. *Tropical Ecology*, 57 (3). Pp 465-474.

Begna, F. 2018. Evaluation of appropriate type and rate of new organic fertilizers with inorganic fertilizers for growth and yield of tomato at east Shewa, Ethiopia. *Int. J. of Unani and Integrative Medicin*., 2. Pp 7-10.

Behera, B.C., Singdevsachan, S.K., Mishra, R.R., Dutta, S.K., Thatoi, H.N. 2014. Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove: A review. *Biocatalysis and Agricultural Biotechnology*, 3(2). Pp 97-110. https://doi.org/10.1016/j.bcab.2013.09.008.

Bekele, S., Mekbib, F., Keneni, G. and Amsalu, B. 2019. Genetic Progress for Yield and Yield Components and Reaction to bean Anthracnos (*Colletotrichum lindemuthianum*) of Large-Seeded Food Type Common Bean (*Phaseolus vulgaris*) Varieties in West Shoa Zone, Ethiopia. *East African Journal of Sciences*, 13 (1). Pp 15-26.

Bergelson, J., Mittelstrass, J. & Horton, M.W. 2019. Characterizing both bacteria and fungi improves understanding of the *Arabidopsis* root microbiome. *Sci. Rep,* 9(24). https://doi.org/10.1038/s41598-018-37208-z

Beshir, H., M., Walley, F.L. Bueckert, R., Tar'an, B. 2015. Response of snap beancultivars to Rhizobium inoculation under dryland agriculture in Ethiopia. *Agronomy*, 5 (3), Pp.291-308. http://dx.doi.org/10.3390/agronomy5030291.

Bhattacharyya, P., Nayak, A. K., Shahid, M., Tripathi, R., Mohanty, S., Kumar, A. 2015. Effects of 42-year long-term fertilizer management on soil phosphorus availability, fractionation, adsorption-desorption isotherm and plant uptake in flooded tropical rice. *Crop J.*, 3. Pp 387–395. https://doi:10.1016/j.cj.2015.03.009.

Central Statistical Agency (CSA), 2020. Crop Production Forecast Sample Survey, Volum III. Report Crops and Livestock Utilization Crops (Private Peasant Holdings, Meher season). Addis Ababa, Ethiopia. 1–157.

Central Statistical Agency (CSA), 2020. Agricultural Sample Survey 2019/20 (2012 E.C.) Volume I Report on Area and Production of Major Crops. Addis Ababa.

Central Statistical Agency (CSA), 2021. Agricultural Sample Survey, Volum III. Report on Crops and Livestock Utilization Crops (Private Peasant Holdings, Meher season). Addis Ababa, Ethiopia. Pp1–154.

Central Statistical Agency (CSA),2021. Agricultural Sample Survey, Volum I. Report on Area and Production Major Crops (Private Peasant Holdings, Meher season). Addis Ababa, Ethiopia. 1–139.

Central Statistical Agency (CSA), 2021. Large and Medium Scale Commercial farms Sample Survey Results at Country and Regional Level Volume VIII. Addis Ababa, Ethiopia. Pp1-67.

Chandra, S., Askari, K., Kumari, M. 2018. Optimization of indole acetic acid production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effects on plant growth. *Journal of Genetic Engineering and Biotechnology*, 16. Pp 581–586. https://doi.org/10.1016/j.jgeb.2018.09.001.

Chen, Q. and Liu, S., 2019. Identification and Characterization of the Phosphate-Solubilizing Bacterium *Pantoea* sp. S32 in Reclamation Soil in Shanxi, China. *Front. Microbiol*. 10(2071). https://doi.org/10.3389/fmicb.2019.02171.

Chen, Y., Fan, J.B, Du, L., Xu, H., Zhang, Q.H. and He, Y.Q. 2014. The application of phosphate solubilizing endophyte *Pantoea dispersa* triggers the microbial community in red acidic soil. *Applied soil Ecology*, 84. Pp235-244. http://dx.doi.org/10.1016/j.apsoil.2014.05.014.

Collavino, M.M., Sansberro, P.A., Mroginski, L.A. and Aguilar, O.M. 2010. Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. *Biol Fertil Soils* 46**.** Pp 727–738. https://doi.org/10.1007/s00374-010-0480-x.

Cui, K., Xu, T., Chen, J., Yang, H., Liu, X., Zhuo, R., Peng, Y., Tang, W., Wang, R., Chen, L., Zhang, X., Zhang, Z., He, Z., Wang, X., Liu, C., Chen, Y., Zhu, Y. 2022. Siderophores, a potential phosphate solubilizer from the endophyte Streptomyces sp. CoT10, improved phosphorus mobilization for host plant growth and rhizosphere

modulation. *Journal of Cleaner Production*, 367. https://doi.org/10.1016/j.jclepro.2022.133110.

Egamberdieva, D., Shrivastava, S., Varma, A. 2015. Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants. *Springer Nature*, Switzerland, 42. Pp 1-16. https://doi.org/10.1007/978-3-319-13401-7.

Dash, N., Pahari, A., Dangar, T.K. 2017. Functionalities of phosphate-solubilizing bacteria of rice rhizosphere: techniques and perspectives. *In* Shukla, P. (Ed.), *Recent Advances in Applied Microbiology*, *Springer Nature*, Singapore. Pp 151–163. https://doi.org/10.1007/978-981-10-5275-0_7.

Davis, K.E.R., Joseph, S.J. and Janssen, P.H. 2005. Effects of Growth Medium, Inoculum Size, and Incubation Time on Culturability and Isolation of Soil Bacteria. *Applied and Environmental Microbiology,* 71(2).Pp 826-834. https://doi.org/10.1128/AEM.71.2.826–834.2005.

Deepika, P. and MubarakAli, D. 2020. Production and assessment of microalgal liquid fertilizer for the enhanced growth of four crop plants. *Biocatalysis and Agricultural Biotechnology*, 28. Pp 1-6. https://doi.org/10.1016/j.bcab.2020.101701.

de Andrade, L.A., Santos, C.H.B., Frezarin, E.T., Sales, L.R., Rigobelo, E.C. 2023. Plant Growth-Promoting Rhizobacteria for Sustainable Agricultural Production. *Microorganisms*. 11(4). https://doi.org/10.3390/microorganisms11041088.

De Putter, H., Hengsdijk, H., Roba, S.T. & Wayu, D.A. 2012. Scope study of Horticulture Smallholder Production in Central Rift valley of Ethiopia. Plant Research International, Business Unit Agrosystems. Report 495.

De Zutter, N., Ameye, M., Bekaert, B., Verwaeren, J., De Gelder L. and Audenaert K. 2022. Uncovering New Insights and Misconceptions on the Effectiveness of Phosphate Solubilizing Rhizobacteria in Plants: A Meta-Analysis. *Front. Plant Sci*. 13(858804). Pp1-14. https://doi.org/10.3389/fpls.2022.858804.

Dela, M., Shanka, D., Dalga, D. 2023. Biofertilizer and NPSB fertilizer application effects on nodulation and productivity of common bean (*Phaseolus vulgaris* L.) at Sodo Zuria, Southern Ethiopia. *Open Life Sci.* 18(1). https://doi.org/10.1515/biol-2022-0537.

Depenbusch, L., Schreinemachers, P., Roothaert, R., Namazzi, S., Onyango, C., Bongole, S., and Mutebi, J. 2021. Impact of home garden interventions in East Africa: Results of thre e randomized controlled trials. *Food Policy*. 104. Pp 1-14. https://doi.org/10.1016/j.foodpol.2021.102140.

du Jardin, P. 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae,* 196. pp. 3–14. http://dx.doi.org/10.1016/j.scienta.2015.09.021,

Etissa, E., Dechassa, N., Alamirew, T., Alemayehu, Y. and Desalegne, L. 2014. Household Fertilizers Use and Soil Fertility Management Practices in Vegetable Crops Production: The Case of Central Rift Valley of Ethiopia. *Science, Technology and Arts Research Journal*, 2(4). Pp 47-55. http://dx.doi.org/10.4314/star.v2i4.9.

Alori, E.T., Glick, B.R. and Babalola, O.O. 2017. Microbial Phosphorus Solubilization & Its Potential for Use in Sustainable Agriculture," *Frontiers in Microbiology.* 8(971). Pp1-8. https://doi.org/10.3389/fmicb.2017.00971.

Errington, J., Aart, L.V. 2020. Microbe Profile: *Bacillus subtilis*: model organism for cellular development, and industrial workhorse. *Microbiology* (*Reading*), 166(5). Pp425-427. https://doi.org/10.1099/mic.0.000922.

Estrada-Bonilla, G.A., Durrer, A., Cardoso, E.N. 2021. Use of compost and phosphate-solubilizing bacteria affect sugarcane mineral nutrition, phosphorus availability, and the soil bacterial community. *Applied Soil Ecology*, 157. Pp 1-9. https://doi.org/10.1016/j.apsoil.2020.103760.

FAOSTAT. (Food and Agricultural Organization of the United Nations). 2024. Food and agriculture data http://www.fao.org/faostat/en/#data/RF.

Federal Negarit Gazeta of the Federal democratic republic of Ethiopia, Proclamation No. 674/2010. Pesticide Registration and Control proclamation. Addis Ababa, Ethiopia. Pp5504-5533.

Federal Negarit Gazeta of the Federal Democratic Republic of Ethiopia, Proclamation No. 137/1998. Fertilizer Manufacturing and Trade. Addis Ababa, Ethiopia. Pp899-907.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Fenta, L. and Assefa, F. 2017. Isolation and Characterization of Phosphate Solubilizing Bacteria from Tomato (Solanum l.) Rhizosphere and Their Effect on Growth and Phosphorus uptake of the Host Plant under Green House Experiment. *Int. J. of Adv. Res*., 3. Pp 2320–5407.

Fikadu, O. 2022. The Response of Faba Bean (*Vicia faba* L.) to Different Strains of Rhizobium Biofertilizer (*Rhizobium leguminosarum*) at Horro District, Western Oromia, Ethiopia. *International Journal of Applied Agricultural Sciences*, 8(4). Pp 150-155. https://doi.org/10.11648/j.ijaas.20220804.12.

Forum for Nuclear Cooperation in Asia (FNCA), 2006. Biofertilizer Manual, published by Japan Atomic Industrial Forum (JAIF), Japan. Pp18-138.

Kalayu, G. 2019. Phosphate Solubilizing Microorganisms: Promising Approach as Biofertiliz ers. *Hindawi, International Journal of Agronomy*. Pp 1-7. https://doi.org/10.1155/2019/4917256.

Gemechis, A. O., 2017. Optimization of productivity and quality of irrigated tomato (Solanum lycopersicum L.) by smallholders in the Central Rift Valley area of Oromia, Ethiopia. PhD Thesis, Wageningen University, Wageningen, the Netherlands. Pp 262.

Gemechis, A.O., Struik, P.C., Emana, B. 2012. Tomato production in Ethiopia: constraints and opportunities. *Tropentag 2012, International Research on Food Security, Natural Resource Management and Rural Development. Resilience of Agricultural Systems against Crises: Book of Abstracts, 373*.

Gemechu, G.E., Beyene, T.M. 2019. Evaluation of Tomato (*Solanum lycopersicum* L.) Varieties for Yield and Fruit Quality in Ethiopia. A Review. *Food Science and Quality Management*. 89. 18-26.

Getaneh, L., Belete, K., Tana, T. 2016. Growth and Productivity of Maize (Zea mays L.) as Influenced by Inter- and Intra-Row Spacing in Kombolcha, Eastern Ethiopia. *Journal of Biology, Agriculture and Healthcare,* 6 (13). Pp 90-101.

Gezahegn, A.M. and Tesfaye, K. 2017. Optimum inter and intra row spacing for faba bean production under Fluvisols. *MAYFEB Journal of Agricultural Science*, 4. Pp 10-19.

Giannelli, G., Potestio, S., Visioli, G. 2023. The Contribution of PGPR in Salt Stress Tolerance in Crops: Unravelling the Molecular Mechanisms of Cross-Talk between Plant and Bacteria. *Plants,* 12 (2197). https://doi.org/10.3390/plants12112197.

Girma, M.M. Awulachew, S.B. 2007. Irrigation practices in Ethiopia: Characteristics of selected irrigation schemes. *Colombo, Sri Lanka: International Water Management Institute. 80p.* (IWMI Working Paper 124).

Girshe, L., Mengistu, H. and Tadesse, T. 2018. The Effects of Rate and Method of Aerated Compost Tea Application on Yield and Yield component of Tomato (Lycopersicon esculentum Mill.) at Burusa, South Western Ethiopia. *Int. J. of Multidisciplinary and Current research*, 6. Pp 52-58. https://doi.org/10.14741/ijmcr.v6i01.10906.

Gómez-Merino, F.C. and Trejo-Téllez, L.I. 2016. Conventional and novel uses of phosphite in horticulture: potentialities and challenges. *Italus Hortus*, 23 (2). Pp 1-13. https://www.soihs.it/public/32/Gomez-Merino%20e%20Trejo%20Tellez_02.pdf.

Khan, A.L., Halo, B.A., Elyassi, A., Ali, S., Al-Hosni, K., Hussain, J. 2016. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum. Electronic Journal of Biotechnology*, 21. Pp. 58–64. https://doi.org/10.1016/j.ejbt.2016.02.001.

Khan, H., Ali Akbar, W., Shah, Z., Ur Rahim, Taj, H.A., Alatalo, J.M. 2022. Coupling phosphate-solubilizing bacteria (PSB) with inorganic phosphorus fertilizer improves mungbean (*Vigna radiata*) phosphorus acquisition, nitrogen fixation, and yield in alkaline-calcareous soil. *Heliyon,* 8(3). https://doi.org/10.1016/j.heliyon.2022.e09081.

Haile, D., Mekbib, F., Assefa, F. 2016. Isolation of Phosphate Solubilizing Bacteria from White Lupin (Lupinus albus L.) Rhizosphere Soils Collected from Gojam, Ethiopia. *J Fertil Pestic,* 7(2). Pp 2471-2478. https://doi.org/10.4172/2471-2728.1000172.

Hariprasad, P. & Niranjana, S.R. 2009. Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. *Plant Soil,* 316. Pp 13-24. http://doi.org/10.1007/s11104-008-9754-6.

Home and Garden Information Centre (HGIC), 2021. Tomato Diseases and Disorders. Factsheet HGIC 2217. https://hgic.clemson.edu/factsheet/tomato-diseases-disorders/

Hwang, H.H., Chien, P.R., Huang, F.C., Yeh, P.H., Hung, S.W., Deng, W.L., Huang, C.C. 2022. A Plant Endophytic Bacterium *Priestia megaterium* StrainBP-R2 Isolated from the

Halophyte *Bolboschoenus planiculmis* Enhances Plant Growth under Salt and Drought Stresses. *Microorganisms*. 10(10). https://doi.org/10.3390/microorganisms10102047.

Jehangir, I.A., Mir, M.A., Bhat, M.A. and Ahangar, M.A., 2017. Biofertilizer an approach to sustainability in agriculture. *Int. J. Pure App. Biosci*. 5(5). Pp327-334.

Kaur, G. and Reddy, M.S. 2013. Role of phosphate-solubilizing bacteria in improving the soil fertility and crop productivity in organic farming. Archives of Agronomy and Soil Science, 60(4). Pp 549-564. DOI: 10.1080/03650340.2013.817667.

Karnwal, A. 2017. Isolation and identification of plant growth promoting rhizobacteria from maize (*Zea mays* L.) rhizosphere and their plant growth promoting effect on rice (*Oryza sativa* L.). Journal of Plant Protection Research, 57(2). Pp 144-151. https://doi.org/10.1515/jppr-2017-0020.

Kidane, R., Wanner, T., Nursey-Bray, M., Masud-All-Kamal, M., Atampugre, G. 2022. The Role of Climatic and Non-Climatic Factors in Smallholder Farmers' Adaptation Responses: Insights from Rural Ethiopia. *Sustainability, MDPI* 14(5715). Pp 1-20. https://doi.org/10.3390/su14095715.

Kifle, T., Ayal, D.Y., Mulugeta, M. 2022. Factors influencing farmers adoption of climate smart agriculture to respond climate variability in Siyadebrina Wayu District, Central highland of Ethiopia. *Climate Services,* 26(100290). Pp1-10. https://doi.org/10.1016/j.cliser.2022.100290.

Kirui, C.K., Njeru, E.M., Runo, S. 2022. Diversity and Phosphate Solubilization Efficiency of Phosphate Solubilizing Bacteria Isolated from Semi-Arid Agroecosystems of Eastern Kenya. *Microbial Insights*, 15. Pp 1-12. http://dx.doi.org/10.1177/11786361221088991.

Khudhur, A.M. 2017. Isolation and characterization of phosphate solubilizing bacteria in Erbil soil and study their effects on Cicer arietinum plant growth and phosphorus uptake. *ZJPAS.* 29 (s4). 66-75. http://dx.doi.org/10.21271/ZJPAS.29.s4.7.

Koskey, G., Mburu, S.W., Awino, R., Njeru, E.M. and Maingi, J.M. 2021. Potential Use of Beneficial Microorganisms for Soil Amelioration, Phytopathogen Biocontrol, and Sustainable Crop Production in Smallholder Agroecosystems. *Front. Sustain. Food Syst.,* 5. https://doi.org/10.3389/fsufs.2021.606308.

Kour, D., Rana, K.L., Kaur, T., Yadav, N., Yadav, A.N., Kumar, M., Kumar, V., Dhaliwal, H.S., Saxena, A.K. 2021. Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and -mobilizing microbes: A review. *Elsevier, Pedosphere,* 31(1). Pp 43–75.

Kumar, A., Maurya, B.M., Raghuwanshi, R. 2021. The microbial consortium of indigenous rhizobacteria improving plant health, yield and nutrient content in wheat (*Triticum aestivum*). *Journal of Plant Nutrition,* 44(13). Pp 1942-956. https://doi.org/10.1080/01904167.2021.1884706.

Kumar, S., Diksha, S.S., Kumar, R. 2022. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Current Research in Microbial Sciences*. 3. Pp 1-26. https://doi.org/10.1016/j.crmicr.2021.100094.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547-1549.

Kurabachew, H. and Wydra, K. 2013. Characterization of plant growth promoting rhizobacteria and their potential as bioprotectant against tomato bacterial wilt caused by *Ralstonia solanacearum*. *Biological Control*, 67. Pp 75–83. http://dx.doi.org/10.1016/j.biocontrol.2013.07.004.

Laguerre, G., Mavingui, P., Allard, M.R., Charnay, M.P., Louvrier, P., Mazurier, S.I., Rigottier-Gois, L., Amarger, N. 1996. Typing of rhizobia by PCR DNA fingerprinting and PCR-restriction fragment length polymorphism analysis of chromosomal and symbiotic gene regions: application to *Rhizobium leguminosarum* and its different biovars. *Appl Environ Microbiol*, 62(6). Pp 2029-36.

Lemma, D. 2002. Tomatoes research experiences and production prospects. Ethiopian Agricultural Research Organization (EARO), Addis Ababa, Ethiopia. Pp 1-28.

Lemessa, F. and Zeller, W. 2007. Screening rhizobacteria for biological control of Ralstonia solanacearum in Ethiopia. *Biological Control,* 42. Pp 336–344. https://doi.org/10.1016/j.biocontrol.2007.05.014.

Liu, J., Qi, W., Li Q., Wang, S.G., Song, C., Yuan, X. 2020. Exogenous phosphorus-solubiliz ing bacteria changed the rhizosphere microbial community indirectly. *3 Biotech, Springer*, 10(164). Pp 1-11. https://doi.org/10.1007/s13205-020-2099-4.

Liu, S., Zhang, X., Pan, J., Ma, Z. 2023. Differences in available phosphorus in temperate and subtropical forest soils. *Applied Soil Ecology*, 187. https://doi.org/10.1016/j.apsoil.2023.104849.

Mącik, M., Gryta, A., Frąc, M. 2020. Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. *In*: Advances in Agronomy, 162. *Editor*(s): Donald L. Sparks, *Academic Press*. https://doi.org/10.1016/bs.agron.2020.02.001.

Mącik, M., Gryta, A., Sas-Paszt, L., Frąc, M. 2023. New insight into the soil bacterial and fungal microbiome after phosphorus biofertilizer application as an important driver of regenerative agriculture including biodiversity loss reversal and soil health restoration. Applied Soil Ecology, 189. https://doi.org/10.1016/j.apsoil.2023.104941.

Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, Das, B., Ghosh, A., Tribedi, P. 2017. Biofertilizers: a potential approach for sustainable agriculture development. *Environ Sci Pollut Res.* 24. Pp 3315–3335. https://doi.org/10.1007/s11356-016-8104-0.

Manzoor, M., Abbasi, M.K. and Sultan, T. 2017. Isolation of phosphate solubilizing bacteria from maize Rhizosphere and their potential for rock phosphate Solubilization–mineralization and plant growth promotion," *Geomicrobiology Journal*, 34(1). Pp 81–95. https://doi.org/10.1080/01490451.2016.1146373.

Mars, T.C. and Ballantyne, B. 2004. Pesticide Toxicology and International Regulations. John Wiley and Sons, Ltd, UK, London.

Martinez, R.M. 2013. *Bacillus subtilis*, In Brenner's Encyclopedia of Genetics (Second Edition), *Editor*(s): Stanley Maloy, Kelly Hughes, Academic Press, Pp 246-248, https://doi.org/10.1016/B978-0-12-374984-0.00125-X.

Mekonnen, A. 2017. Effects of Seeding Rate and Row Spacing on Yield and Yield Components of Bread Wheat (*Triticum Aestivum* L.) in Gozamin District, East Gojam Zone, Ethiopia. *Journal of Biology, Agriculture and Healthcare*, 7 (4). Pp 19-37.

Mekonnen, H. and Kibret, M. 2021. The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia. *Chem. Biol. Technol. Agric*. 8(15). https://doi.org/10.1186/s40538-021-00213-y.

Mengistie, B.T. 2016. Policy-Practice Nexus: Pesticide Registration, OPEN Distribution and use in Ethiopia. *SM J Environ Toxicol.* 2(1):1006. Pp1-13.

Mengistie, B.T., Mol, A.J., Oosterveer, P. 2015. Pesticide use practices among smallholder v egetable farmers in Ethiopian Central Rift Valley. *Env. Dev. Sust.* 19. Pp 301–324. https://doi.org.10.1007/s10668-015-9728-9.

Mergia, M.K., Weldemariam, E.D., Eklo, O.M., Yimer, G.T., 2021. Small-scale Farmer Pesticide Knowledge and Practice and Impacts on the Environment and Human Health in Ethiopia. *Journal of Health & Pollution*. 11(30). Pp1-19. https://doi.org.10.5696/2156-9614-11.30.210607.

Mersha, D. and Sime, M. 2022. Analysis of Tomato Value Chain in Central Rift Valley of Ethiopia. Ethiopian Institute of Agricultural Research, Report No. 135. Addis Ababa, Ethiopia. https://www.eiar.gov.et.

Mesfin, H. 2020. Characterization of Agricultural Soils of Meki and Adamitulu in the Central Rift Valley of Ethiopia. *Acad. Res. J. Agri. Sci. Res*. 8(1). Pp12-23. https://www.academicresearchjournals.org/ARJASR/PDF/2020/January/Mesfin.pdf.

Mitra, D., Anđelković, S., Panneerselvam, P., Senapati, A., Vasić, T., Ganeshamurthy, A.N., Chauhan, M., Uniyal, N., Mahakur, B. & Radha, T.K. 2020. Phosphate-Solubilizing Microbes and Biocontrol Agent for Plant Nutrition and Protection: Current Perspective. *Communications in Soil Science and Plant Analysis*, 51(5). Pp 645-657.

Mohamed, E.H., Farag, A.G. and Youssef, S.A. 2018. Phosphate Solubilization by Bacillus subtilis and Serratia marcescens Isolated from Tomato Plant Rhizosphere. *Journal of Environmental Protection*, 9(3). Pp 266-277. https://doi.org/10.4236/jep.2018.93018.

More, S.S., Shinde, S.E., Kasture, M.C. 2019. Root exudates a key factor for soil and plant: An overview. *The Pharma Innovation Journal*. 8 (6). Pp 449-459.

Mukhtar, S., Shahid, I., Mehnaz, S., Malik, K.A. 2017. Assessment of two carrier materials for phosphate solubilizing biofertilizers and their effect on growth of wheat (*Triticum aestivum* L.). *Microbiological Research*, 205. Pp 107–117. http://dx.doi.org/10.1016/j.micres.2017.08.011.

Muleta, D. 2018. The potential for *Bradyrhizobia* and phosphate solubilising microorganisms to improve soybean (*Glycine max* (L.) Merr.) production in acid soils in Ethiopia. A PhD. Thesis Submitted to The School of Agriculture, Food & Wine, The University of Adelaide, Australia.

Muleta, D., Assefa, F., Börjesson, E., Granhall, U. 2013. Phosphate-solubilising rhizobacteria associated with Coffea arabica L. in natural coffee forests of southwestern Ethiopia, *Journal of the Saudi Society of Agricultural Sciences*, 12(1). Pp 73-84. https://doi.org/10.1016/j.jssas.2012.07.002.

Naik, P.R., Raman, G., Narayanan, K.B., Natarajan, S. 2008. Assessment of genetic and functional diversity of phosphate solubilizing *fluorescent pseudomonads* isolated from rhizospheric soil. *BMC Microbiology* 8 (230). https://doi.org/10.1186/1471-2180-8-230.

Naz, R., Asif, T., Mubeen, S., Khushhal, S. 2022. Seed application with microbial inoculants for enhanced plant growth. *In:* Developments in Applied Microbiology and Biotechnology; *Editor*(s): Musa Seymen, Ertan S Kurtar, Ceknas Erdinc, Ajay Kumar. Sustainable Horticulture, Academic Press. Pages 333-368. https://doi.org/10.1016/B978-0-323-91861-9.00008-2.

Negash, H. and Mohammed, M. 2014. Soil Fertility in Koka Nagawo Area of Lumme District in East Shoa Zone of Oromia Region, Ethiopia. *East African Journal of Sciences*, 8 (1). Pp 1-12. https://www.ajol.info/index.php/eajsci/article/view/119349.

Nesme, T., Metson, G.S., Bennett, E.M. 2018. Global Phosphorous flows through agricultural trade. *Glob. Environ. Change*, 50. Pp 133–141. https://doi.org/10.1016/j.gloenvcha.2018.04.004.

Nile Basin Water Resource Atlas (NBWRA), 2021. Available from: Irrigation areas in Ethiopia – Nile Basin Water Resources Atlas retrieved on June 21/2021.

Nosheen, S., Ajmal, I., Song, Y. 2021. Microbes as Biofertilizers, a Potential Approach for Sustainable Crop Production. *Sustainability*, 13(1868). https://doi.org/10.3390/su13041868.

O'Dell, W. 1993. Method 365.1, Revision 2.0: Determination of Phosphorus by Semi-Automated Colorimetry. Environmental Monitoring Systems Laboratory Office of Research and Development U.S. Environmental Protection Agency. Cincinnati, Ohio. Pp 1-15. http://www.epa.gov/.

Pande, A., Pandey, P., Mehra, S., Singh, M., Kaushik, S. 2017. Phenotypic and genotypic cha racterization of phosphate solubilizing bacteria and their efficiency on the growth of mai ze. *J Genet Eng Biotechnol*. 15(2). Pp 379-391. https://doi.org/10.1016/j.jgeb.2017.06.005.

Pandey, A., Trivedi, P., Kumar, B. and Palni, L.M.S. 2006. Characterization of a Phosphate Solubilizing and Antagonistic Strain of *Pseudomonas putida* (B0) Isolated from a Sub-Alpine Location in the Indian Central Himalaya. *Curr Microbiol*, 53. Pp 102–107. https://doi.org/10.1007/s00284-006-4590-5.

Pathak, R., Paudel, V., Shrestha, A., Lamichhane, J., Gauchan, D. P. 2017. Isolation of Phosphate Solubilizing Bacteria and Their Use for Plant Growth Promotion in Tomato Seedling and Plant. Journal of Science, Engineering and Technology, 13(2). Pp 61-70. https://doi.org/10.3126/kuset.v13i2.21284.

Paul, D., Sinha, S.N. 2017. solation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. *Annals of Agrarian Science*, 15(1). Pp 130-136. https://doi.org/10.1016/j.aasci.2016.10.001.

Pellegrini, M., Spera, D.M., Ercole, C., Del Gallo, M. 2021. *Allium cepa* L. Inoculation with a Consortium of Plant Growth-Promoting Bacteria: Effects on Plants, Soil, and the Autochthonous Microbial Community. *Microorganisms*, 9(639). Pp 1-12. https://doi.org/10.3390/microorganisms9030639.

Pérez-Miranda, S., Cabirol, N., George-Téllez, R., Zamudio-Rivera, L.S., Fernández, F.J. 2007. O-CAS, a fast and universal method for siderophore detection. *Journal of Microbiological Methods*, 70. Pp 127 –131. https://doi.org/10.1016/j.mimet.2007.03.023.

Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species, *Mikrobiologiya*, vol. 17, pp. 362–370.

Planchamp, C., Glauser, G. and Mauch-Mani, B. 2015. Root inoculation with *Pseudomonas putida* KT2440 induces transcriptional and metabolic changes and systemic resistance in maize plants. *Frontiers in Plant Science*, 5(719). Pp 1-10. https://doi.org/10.3389/fpls.2014.00719.

Poonia, M.K. and Dhaka, B.L. 2012. Effect of phosphorus solublizing bacteria (PSB) on growth and yield in tomato. *J. Hortl. Sci.* 7(1). Pp 104-107. https://jhs.iihr.res.in/index.php/jhs/article/view/404.

Prabhu, N., Borkar, S., Garg, S. 2019. Phosphate solubilization by microorganisms: Overvie w, mechanisms, applications and advances, Editor(s): Surya Nandan Meena, Milind Mohan Naik, Advances in Biological Science Research, Academic Press, Pp 161-176, https://doi.org/10.1016/B978-0-12-817497-5.00011-2.

Qian, Y., Shi J., Chen, Y., Lou, L., Cui, X., Cao, R., Li, P. and Tang, J. 2010. Characterization of Phosphate Solubilizing Bacteria in Sediments from a Shallow Eutrophic Lake and a Wetland: Isolation, Molecular Identification and Phosphorus Release Ability Determination. Molecules, 15(11). Pp. 8518–8533. https://doi.org/10.3390/molecules15118518.

Quiza, L., Tremblay, J., Pagé, A.P. 2023. The effect of wheat genotype on the microbiome is more evident in roots and varies through time. *ISME COMMUN.* 3 (32). https://doi.org/10.1038/s43705-023-00238-4.

Rabbee, M.F., Ali, M.S., Choi, J., Hwang, B.S., Jeong, S.C., Baek, K.H. 2019. *Bacillus velezensis:* A Valuable Member of Bioactive Molecules within Plant Microbiomes. *Molecules*, 24(6). https://doi.org/10.3390/molecules24061046.

Rachel, B., Stefan, R.j., Gayathri, I., John, L., Dana, P., Emily, R., Sowmyalakshmi S., Donald S. 2018. Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Frontiers in Plant Science*, 9. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2018.01473.

Rafi, M.M., Krishnaveni, M.S., Charyulu, P.N. 2019. Phosphate-Solubilizing Microorganisms and Their Emerging Role in Sustainable Agriculture: Recent Developments in Applied Microbiology and Biochemistry. Academic Press, Pp 223-233. https://doi.org/10.1016/B978-0-12-816328-3.00017-9.

Raimi, A., Adeleke, R., and Roopnarain, A., 2017. Soil fertility challenges and Biofertiliser as a viable alternative for increasing smallholder farmer crop productivity in sub-Saharan Africa. *Cogent Food & Agri. 3.*pp1-26.

Raimi, A., Roopnarain, A., Adeleke, R. 2021. Biofertilizer production in Africa: Current status, factors impeding adoption and strategies for success. *Scientific African*, 11. https://doi.org/10.1016/j.sciaf.2021.e00694.

Regassa, H. and Elias, E. 2022. Dry matter production, nitrogen yield and estimation of nitrogen fixation of legumes on vertisols of the Ethiopian highlands. *Heliyon*, 8(12). https://doi.org/10.1016/j.heliyon.2022.e12523.

Rilling, J.I., Acuña, J.J., Nannipieri, P., Cassan, F., Maruyama, F., Jorquera, M.A. 2019. Current opinion and perspectives on the methods for tracking and monitoring plant growth–promoting bacteria. *Soil Biology and Biochemistry*, 130. Pp 205-219. https://doi.org/10.1016/j.soilbio.2018.12.012.

Rodríguez, H., Fraga, R., Gonzales, T., Bashan, Y., 2006. Genetics of phosphate solubilization and its applications for improving plant growth-promoting bacteria. *Plant Soil.* 287. Pp 15–21.

Ryall, B., Lee, X., Zlosnik, J.E., Hoshino, S., Williams, H.D. 2008. Bacteria of the *Burkholderia cepacia* complex are cyanogenic under biofilm and colonial growth conditions. BMC Microbiol, 8(108). https://doi.org/10.1186/1471-2180-8-108.

Sachdev, S., Bauddh, K., Singh, R.P. 2023. Native Rhizospheric Microbes Mediated Management of Biotic Stress and Growth Promotion of Tomato. *Sustainability, 15(1).* 593. https://doi.org/10.3390/su15010593.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Santos-Villalobos, S.I. and Parra-Cota, F.I. 2021. Current trends in plant growth-promoting microorganisms research for sustainable food security. *Current Research in Microbial Sciences*, 2. https://doi.org/10.1016/j.crmicr.2020.100016.

Sayyed, R.Z., Reddy, M.S. and Antonius, S. 2019. Plant Growth Promoting Rhizobacteria (PGPR): Prospects for Sustainable Agriculture. Springer Nature Singapore Pte Ltd, Singapore. Pp-335. https://doi.org/10.1007/978-981-13-6790-8.

Shah, A., Nazari, M., Antar, M., Msimbira, L.A., Naamala, J., Lyu, D., Rabileh, M., Zajonc, J. and Smith, D.L. 2021. PGPR in Agriculture: A Sustainable Approach to Increasing Climate Change Resilience. *Front. Sustain. Food Syst.* 5(667546). https://doi.org/10.3389/fsufs.2021.667546.

Sharma, S., Kumar, V., Tripathi, R.B. 2011. Isolation of Phosphate Solubilizing Microbes (PSMs) from Soil. *J. Microbiol. Biotech. Res.*, 1 (2). Pp 90-95. http://scholarsresearchlibrary.com/archive.html.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., and Gobi, T.A. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, 2(587). Pp1-14. https://doi.org/10.1186/2193-1801-2-587.

Sharon, J.A., Hathwaik, L.T., Glenn, G.M., V, S.H and Lee, C.C., 2016. Isolation of efficient phosphate solubilizing bacteria capable of enhancing tomato plant growth. *Journal of Soil Science and Plant Nutrition*, 16 (2). Pp 525-536. http://dx.doi.org/10.4067/S0718-95162016005000043.

Shavit, R., Ofek-Lalzar, M., Burdman, S., Morin, S. 2013. Inoculation of tomato plants with rhizobacteria enhances the performance of the phloem-feeding insect *Bemisia tabaci*. *Frontiers in Plant Science*, 4(336). Pp 1-12. https://www.frontiersin.org/articles/10.3389/fpls.2013.00306.

Shwed, P.S., Crosthwait, J., Weedmark, K., Hoover, E. and Dussault, F. 2021. Complete Genome Sequences of Priestia megaterium Type and Clinical Strains Feature Complex Plasmid Arrays. *Microbiology Resource Announcements*, 10(27). Pp 403-21. https://journals.asm.org/doi/abs/10.1128/mra.00403-21.

Simtowe, F. 2015. Support for the Establishment of a Regional Fertilizer Policy and Regulatory Framework in East and Southern Africa. An Assessment of National Fertilizer Policies, Regulations and Standards for Ethiopia. African Fertilizer and agribusiness Partnership, productivity prosperity partnership. Pp 1-34. https://www.researchgate.net/publication/297161589.

Singh, L. and Jha, S. 2015. Isolation of phosphate solubilizing bacteria from rhizospheric soil and its physiological studies. Eco. Env. & Cons. 21 (2): 1039-1047.

Singh, P.K., and Singh, R.L. 2019. Food Safety and Human Health. Academic Press an in print of Elsevier. UK.

Somasegaran, P. and Hoben, H.J. 1994. Handbook for Rhizobia: Methods in Legumes-Rhizobium Technology. *Springer-Verlag, New York, Inc*., USA. Pp-455.

Sotelo-Cardona, P., Lin, M.Y., Srinivasan, R. 2021. Growing Tomato under Protected Cultivation Conditions: Overall Effects on Productivity, Nutritional Yield, and Pest Incidences. *Crops*. 97–110. https://doi.org/10.3390/.

Tamene, L., Amede, T., Kihara, J., Tibebe, D. and Schulz, S. 2017. A review of soil fertility management and crop response to fertilizer application in Ethiopia: towards development of site- and context-specific fertilizer recommendation. International Centre for Tropical

Agriculture (CIAT), CIAT Publication No. 443, Addis Ababa, Ethiopia. Pp 1-86. http://hdl.handle.net/10568/82996.

Tamru, S., Minten, B., Alemu, D., Bachewe, F. 2016. The rapid expansion of herbicide use in smallholder agriculture in Ethiopia: Patterns, drivers, and implications. Ethiopia Strategy Support program (ESSP working Paper 94). Pp 1-17.

Tamura, K., Nei, M., and Kumar, S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Tinna, D., Garg, N., Sharma, S., Pandove, G., Chawla, N. 2020. Utilization of plant growth promoting rhizobacteria as root dipping of seedlings for improving bulb yield and curtailing mineral fertilizer use in onion under field conditions. *Sci. Hortic.*, 207(2). https://doi.org/10.1016/j.scienta.2020.109432.

Tsedeke, A. 2007. Focusing Agricultural research to Address Development Needs: Direction for Agricultural research in Ethiopia, EIAR, Addis Ababa, Ethiopia. Pp1-63.

Tsegaye, Z., Assefa F. and Beyene D. 2017. Properties and Application of Plant Growth Promoting Rhizobacteria. *Int. J. Curr. Trend. Pharmacobiol. Med. Sci*., 2(1). Pp 30-43

Tsehaye, K., Tekestebrhan, A., Wendwesen, T. 2020. Proportional Enactment of Tomato (*Solanum Lycopersicum* L. Mill) Varieties under Greenhouse Production Systems of Tigray Biotechnology Centre, Ethiopia. *Int. J. R in Agr. and Forestry* **7**. Pp 1-11.

Vaikuntapu, P.R., Dutta, S., Samudrala, R.B. Rao, V.N., Kalam, S. and Podile, A.R. 2014. Preferential Promotion of *Lycopersicon esculentum* (Tomato) Growth by Plant Growth Promoting Bacteria Associated with Tomato. *Indian Journal of Microbiology*, 54(4). Pp 403-412. http://doi.org/10.1007/s12088-014-0470-z.

Verma, D.K. 2019. Microbiology for sustainable agriculture, soil health, and environmental protection. Apple Academic Press Inc., Florida, USA. Pp 1-429.

Verma, M., Mishra, J., Arora, N.K. 2019. Plant growth-promoting rhizobacteria: diversity and applications. in Sobti, R. C. (*Ed*.), Environmental Biotechnology: For Sustainable Future, Springer, Singapore. Pp. 129–173. https://doi.org/10.1007/978-981-10-7284-0_6.

Waday, Y.A., Aklilu, E.G., Bultum, M.S., Ancha, V.R. 2022. Optimization of soluble phosphate and IAA production using response surface methodology and ANN approach. *Heliyon*, 8(12). https://doi.org/10.1016/j.heliyon.2022.e1222.

Wan, W., Qin Y., Wu, H., Zuo, W., He, H., Tan J., Wang, Y. and He, D. 2020. Isolation and Characterization of Phosphorus Solubilizing Bacteria with Multiple Phosphorus Sources Utilizing Capability and Their Potential for Lead Immobilization in Soil. *Front. Microbiol*., 11(752). Pp 1-15. https://doi.org/10.3389/fmicb.2020.00752.

Wang, Z., Xu, G., Ma, P., Lin, Y., Yang, X., Cao, C. 2017. Isolation and Characterization of a Phosphorus-Solubilizing Bacterium from Rhizosphere Soils and Its Colonization of Chinese Cabbage (*Brassica campestris* ssp. *chinensis*). Front. Microbiol. 8 (1270). https://www.frontiersin.org/articles/10.3389/fmicb.2017.01270/full.

Watanabe, F. and Olsen, S. 1965. Test of an ascorbic acid method for determining P in water and $NaHCO_3$ extracts from soil. *Soil Sci.Am.J*, 29. pp 677-678.

Wei, T., Lv, X., Jia, H., Hua, L., Xu, H., Zhou, R., Zhao, J., Ren, X., Guo, J. 2018. Effects of salicylic acid, Fe(II) and plant growth-promoting bacteria on Cd accumulation and toxicity alleviation of Cd tolerant and sensitive tomato genotypes. *Journal of Environmental Management*, 214. Pp 164-171. https://doi.org/10.1016/j.jenvman.2018.02.100.

Weisburg, W.G., Barns, S.M., Pelletier, D.A. and Lane, D.J. 1996. 16S Ribosomal DNA Amplification for Phylogenetic Study. *Journal of Bacteriology*, 173(2). Pp 697-703.

Weldegiorgis, L.G., Mezgebo, G.K., Gebremariam, H.G., Kahsay, Z.A. 2018. Resources Use Efficiency of Irrigated Tomato Production of Small-scale Farmers. *International Journal of Vegetable Science*, 24. Pp 456 - 465.

Widnyana, I.K. 2018. PGPR (Plant Growth Promoting Rizobacteria) Benefits in Spurring Germination, Growth and Increase the Yield of Tomato Plants. In Recent Advances in tomato Breeding and Production. https://doi.org/10.5772/intechopen.78776.

Willey, J.M., Sherwood, L.M. and Woolverton, C.J. 2017. Prescott's microbiology. 10th Edit. McGraw-Hill education publisher, New York.

Wlapola, B.C. and Yoon, H.O. 2013. Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake. *African Journal of Microbiology Research*, 7(3). Pp 266-275.

Worku, M. and Sahela, S. 2018. Review on Disease Management Practice of Tomato Wilt Caused *Fusarium oxysporum* in Case of Ethiopia. *J Plant Pathol Microbiol* 9: 460. https://doi.org.10.4172/2157-7471.1000460.

Woyessa, D., Assefa, F. 2011. Diversity and plant growth promoting properties of rhizobacter ia isolated from tef under greenhouse condition. *Res. J. Microbiol*., 6. Pp 343- 355.

Wu, X., Fan, Y., Wang, R., Zhao, Q., Ali, Q., Wu, H., Gu, Q., Borriss, R., Xie, Y. and Gao, X. 2022. *Bacillus halotolerance* KKD1 induces physiological, metabolic and molecular reprogramming in wheat under saline condition. *Front. Plant Sci.* 13(978066). https://doi.org/10.3389/fpls.2022.978066.

Yadav, A.J., Kumar, R., Kumar, S., Kumar, V., Sugitha, T.K., Singh, B., Chauahan, V.S., Dhaliwal, H.S., Saxena, A.K. 2017. Beneficial microbiomes: Biodiversity and potential biotechnological applications for sustainable agriculture and human health. Journal of Applied Biology & Biotechnology. 5(6). Pp 45-57. http://www.jabonline.in.

Yadav, B.K., Verma, A. 2012. Phosphate Solubilization and Mobilization in Soil Through Microorganisms Under Arid Ecosystems. In (Ed.), The Functioning of Ecosystems. https://doi.org/10.5772/35917.

Yang, Z., Liu, Z., Zhao, F., Yu, L., Yang, W., Si, M., Liao, Q. 2023. Organic acid, phosphate, sulfate and ammonium co-metabolism releasing insoluble phosphate by Klebsiella aerogenes to simultaneously stabilize lead and cadmium. *Journal of Hazardous Materials*, 443. https://doi.org/10.1016/j.jhazmat.2022.130378.

Yasmeen, T., Arif, M.S., Shahzad, S.M., Riaz, M., Tufail, M.A., Mubarik, M.S., Ahmad, A., Ali,S. Albasher, G., Shakoor, A. 2022. Abandoned agriculture soil can be recultivated by promoting biological phosphorus fertility when amended with nano-rock phosphate and suitable bacterial inoculant. *Ecotoxicology and Environmental Safety*, 234. Pp 1-11. https://doi.org/10.1016/j.ecoenv.2022.113385.

Yeshiwas, Y., Belew, D., Tolessa, K. 2016. Tomato (*Solanum lycopersicum* L.) Yield and Fruit Quality Attributes as Affected by Varieties and Growth Conditions. *World Journal of Agricultural Sciences* 12. Pp 404-408.

Zaidi, A., Khan, M.S., Ahemad, M., Oves, M., Wani, P.A. 2009. Recent Advances in Plant Growth Promotion by Phosphate-Solubilizing Microbes. In: Khan, M., Zaidi, A., Musarrat, J. (eds) Microbial Strategies for Crop Improvement. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-01979-1_2.

Zhalnina, K., Louie, K.B., Hao, Hao, Z., Mansoori, N., da Rocha, U.N., Shi, S., Cho, H., Karaoz U., Loqué, D., Bowen, P.B., K. Firestone, M., Northern, R. T. and Brodie, L.E. 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat. Microbiol.* 3. Pp 470–480. https://doi.org/10.1038/s41564-018-0129-3.

Zhang, J., Xiao, Q., Wang, P. 2021. Phosphate-solubilizing bacterium *Burkholderia* sp. strain N3 facilitates the regulation of gene expression and improves tomato seedling growth under cadmium stress. *Ecotoxicology and Environmental Safety*, 217. Pp 2-12. https://doi.org/10.1016/j.ecoenv.2021.112268.

Zhang, X., Rajendran, A., Grimm, S., Sun, X., Lin, H., He, R., Hu, B. 2023. Screening of calcium- and iron-targeted phosphorus solubilizing fungi for agriculture production. *Rhizosphere*, 26. https://doi.org/10.1016/j.rhisph.2023.100689.

Supplementary Materials

Figure from Isolation Experiment

Supplementary Table 3.1: Chemical properties of the Soil profile at Koka, Meki & Ziway Zuria

Profile	Ziway Zuria	Meki (surface1)	Koka (Lume1)
Texture class	sandy loam	Sandy loam	Clay loam
pH	8.2	7.5	7.79
EC ($ms \cdot cm^{-1}$) (1:2.5)	0.26	1.37	0.31
Organic matter (%)	1.15	1.483	1.9
TN (%)	0.07	0.153	0.12
Available P ($mg \cdot P \cdot kg^{-1}$)	36.42	4.37	12.14
Available K ($mg \cdot K \cdot kg^{-1}$)	717.3	2.23	3.56
Cu ($mg \cdot kg^{-1}$ soil)	0.32	0.25	1.32
Fe ($mg \cdot kg^{-1}$ soil)	4.58	7.0	3.37
Mn ($mg \cdot kg^{-1}$ soil)	4.63	10.5	10.66
Zn ($mg \cdot kg^{-1}$ soil)	1.5	2.88	0.59

Source: *Beshir et al. (2015), Negash and Mohammed (2014), Mesfin (2020).*

Supplementary Table -3.2: Colony and Halo zone diameter of selected top 20 PSB isolates.

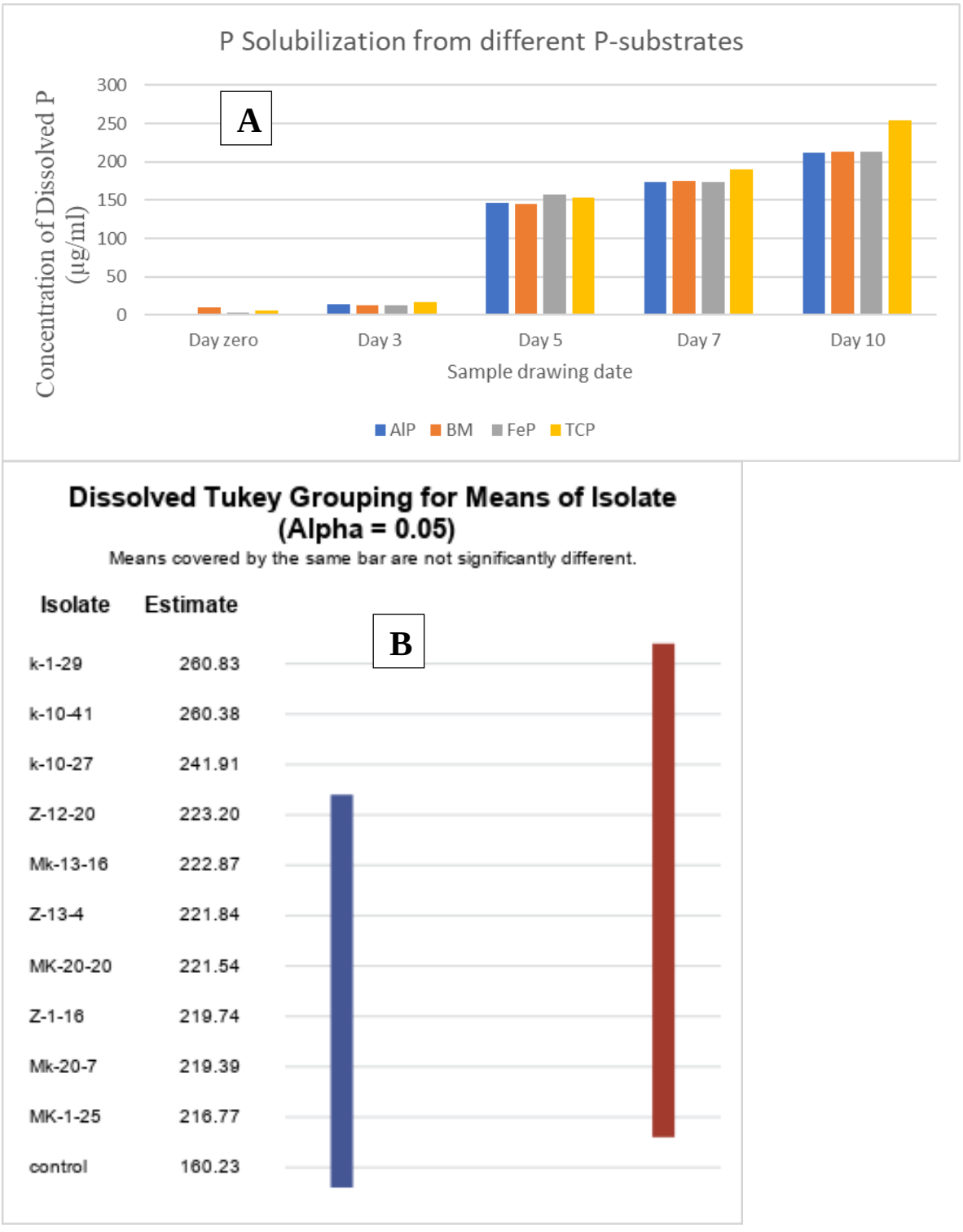
				D2		D4		D6		D8		C	H	
Isolate	pH	Colony	OD	C2	H2	C4	H4	C6	H6	C8	H8	AVG	AVG	SI
k-10-27	4.12	87	1.561	0.1	0.2	0.3	0.5	0.6	0.8	0.9	1.2	0.48	0.68	2.42
k-10-41	4.02	34	1.977			0.2	0.4	0.2	0.4	0.2	0.4	0.15	0.30	3.00
K-1-29	5.9	18	0.144	0.1	0.2	0.4	0.5	0.6	0.8	0.8	1.1	0.48	0.65	2.37
mk-1-25	4.25	300	1.261			0.2	0.5	0.3	0.6	0.4	0.9	0.23	0.50	3.22
mk-13-1	4.07	106	1.993	0.1	0.1	0.3	0.4	0.4	0.6	0.5	0.8	0.33	0.48	2.46
mk-13-16	5.99	33	0.403	0.1	0.2	0.5	0.7	0.7	0.9	1	1.3	0.58	0.78	2.35
mk-13-5	4.41	136	1.993	0.1	0.2	0.1	0.2	0.2	0.4	0.3	0.6	0.18	0.35	3.00
MK-14-1	4.83	23	0.508	0.4	0.6	0.5	0.7	0.5	0.7	0.7	0.9	0.53	0.73	2.38
mk-17-15	4.84	58	1.436	0.2	0.3	0.4	0.5	0.5	0.6	0.5	0.6	0.40	0.50	2.25
mk-20-20	4.3	118	1.931	0.1	0.2	0.2	0.4	0.4	0.6	0.7	1.3	0.35	0.63	2.79
mk-20-7	4.64	156	1.975	0.1	0.2	0.2	0.3	0.3	0.5	0.3	0.5	0.23	0.38	2.67
MK-3-28	3.96	25	1.907	0.1	0.3	0.3	0.5	0.4	0.6	0.5	0.8	0.33	0.55	2.69
z-1-16	5.72	44	0.502	0.1	0.2	0.3	0.5	0.4	0.7	0.6	1	0.35	0.60	2.71
z-12-20	5.88	128	0.473	0.3	0.5	0.4	1	0.6	1.1	0.7	1.2	0.50	0.95	2.90
z-12-24	4.08	15	1.967	0.2	0.3	0.4	0.8	0.5	0.9	0.6	0.9	0.43	0.73	2.71
z-13-11	4.68	200	1.523	0.3	0.5	0.4	0.6	0.6	0.9	0.7	1.1	0.63	0.90	2.44
z-13-4	4.38	306	0.197	0.1	0.2	0.2	0.4	0.3	0.5	0.3	0.5	0.23	0.40	2.78
z-13-5	3.55	662	1.844	0.2	0.3	0.2	0.3	0.3	0.5	0.3	0.5	0.25	0.40	2.60
z-15-6	3.65	640	1.984	0.1	0.2	0.2	0.3	0.2	0.3	0.4	0.6	0.23	0.35	2.56
Z-8-33	6.01	11	1.855	0.4	0.5	0.9	1.2	1.4	1.6	2.1	2.5	1.20	1.45	2.21

Supplementary Table -3.3: mean value of PVK broth pH treated with different P-sources and PSB isolates incubated for 10 days.

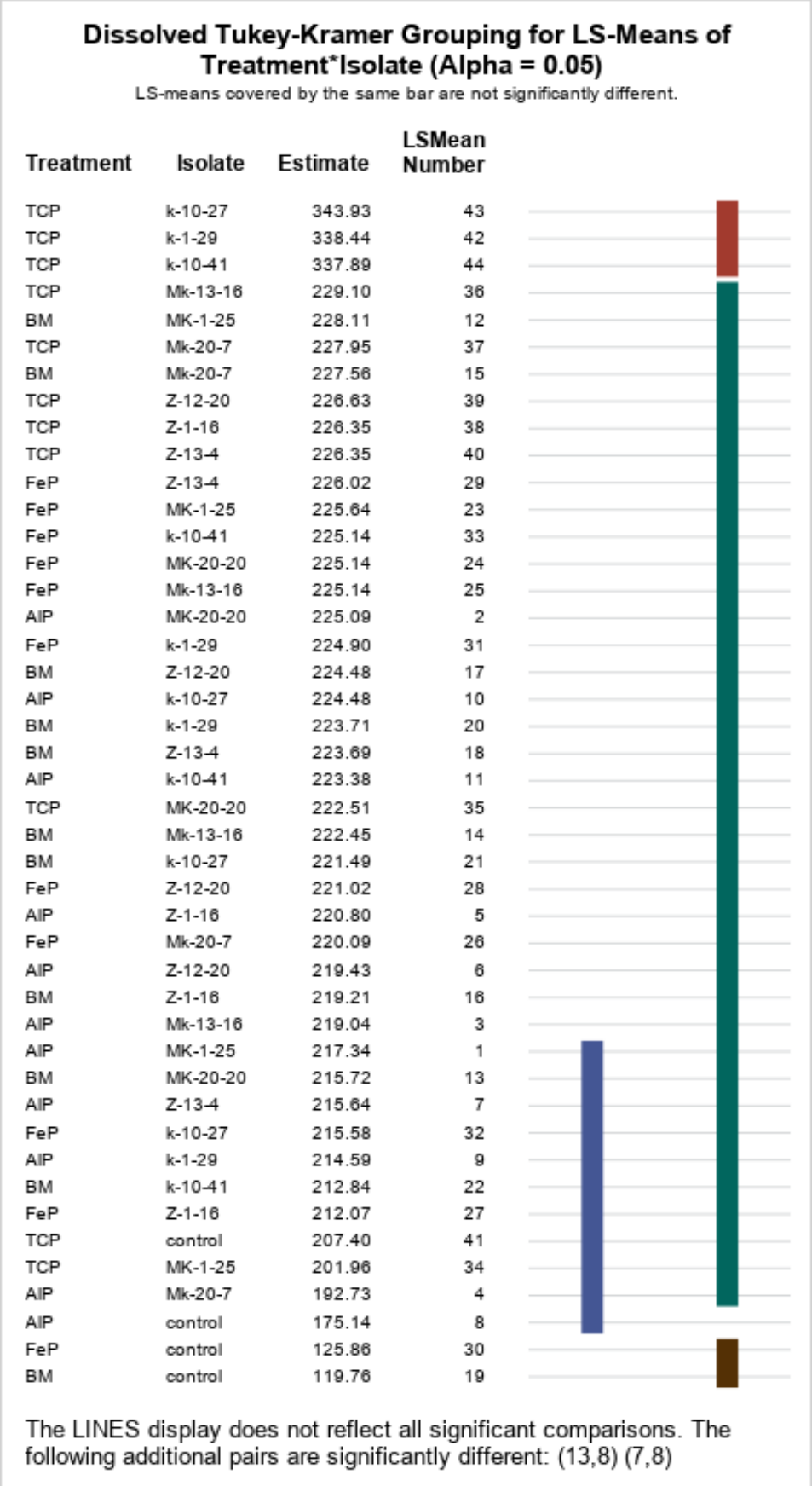
Isolate	Day zero	Day 3	Day 5	Day 7	Day 10
Control	7.16±0.14^{ab}	6.95±0.07a	6.61±0.23a	6.18±0.52a	5.77±0.64a
K-1-29	7.03±0.12^{cd}	6.47±0.27bc	5.76±0.23b	5.34±0.35b	4.98±0.38b
K-10-27	7.0±0.21^{d}	6.2±0.59cd	5.56±0.54b	5.08±0.43bc	4.93±0.44b
K-10-41	7.01±0.15^{bcd}	6.1±0.72bd	5.47±0.69bc	5.08±0.64bc	4.95±0.65b
Mk-1-25	7.00±0.27^{cd}	6.36±0.56bcd	5.49±0.69b	5.25±0.65b	5.09±0.57b
Mk-13-16	7.06±0.15^{bcd}	6.42±0.4bc	5.64±0.49b	5.13±0.47bc	5.05±0.55b
Mk-20-20	7.04±0.28^{cd}	6.48±0.41bc	5.58±0.56b	5.07±0.52bc	5.04±0.62b
Mk-20-7	7.2±0.13^{a}	6.54±0.47b	5.80±0.75b	5.34±0.61b	5.26±0.7b
Z-1-16	7.03±0.13^{cd}	6.4±0.43bc	5.64±0.37b	5.08±0.42bc	5.01±0.49b
Z-12-20	7.05±0.17^{cd}	6.3±0.53bcd	5.76±0.27b	5.21±0.29b	4.98±0.31b
Z-13-4	7.1±0.15^{abc}	6.34±0.43bcd	5.11±0.27c	4.81±0.3c	4.95±0.41b
CV	1.8	5.58	7.86	8.67	10.07
LSD	0.102	0.29	0.36	0.37	0.41
Treatment					
AlP	7.08±0.12^{b}	6.41±0.35b	5.84±0.49ab	5.32±0.58ab	5.22±0.61a
BM	7.1±0.06^{b}	6.27±0.43b	5.9±0.39a	5.48±0.41a	5.41±0.34a
FeP	7.22±0.11^{a}	6.89±0.15a	5.65±0.45b	5.42±0.36c	4.86±0.37b
TCP	6.87±0.21^{c}	6.08±0.57c	5.32±0.79c	5.37±0.76bc	4.8±0.73b
CV	1.8	5.58	7.86	8.67	10.07
LSD	0.062	0.18	0.22	0.22	0.25

Supplementary table 3.4 Authentication and Symbiotic Effectiveness against Soya bean and Faba bean.

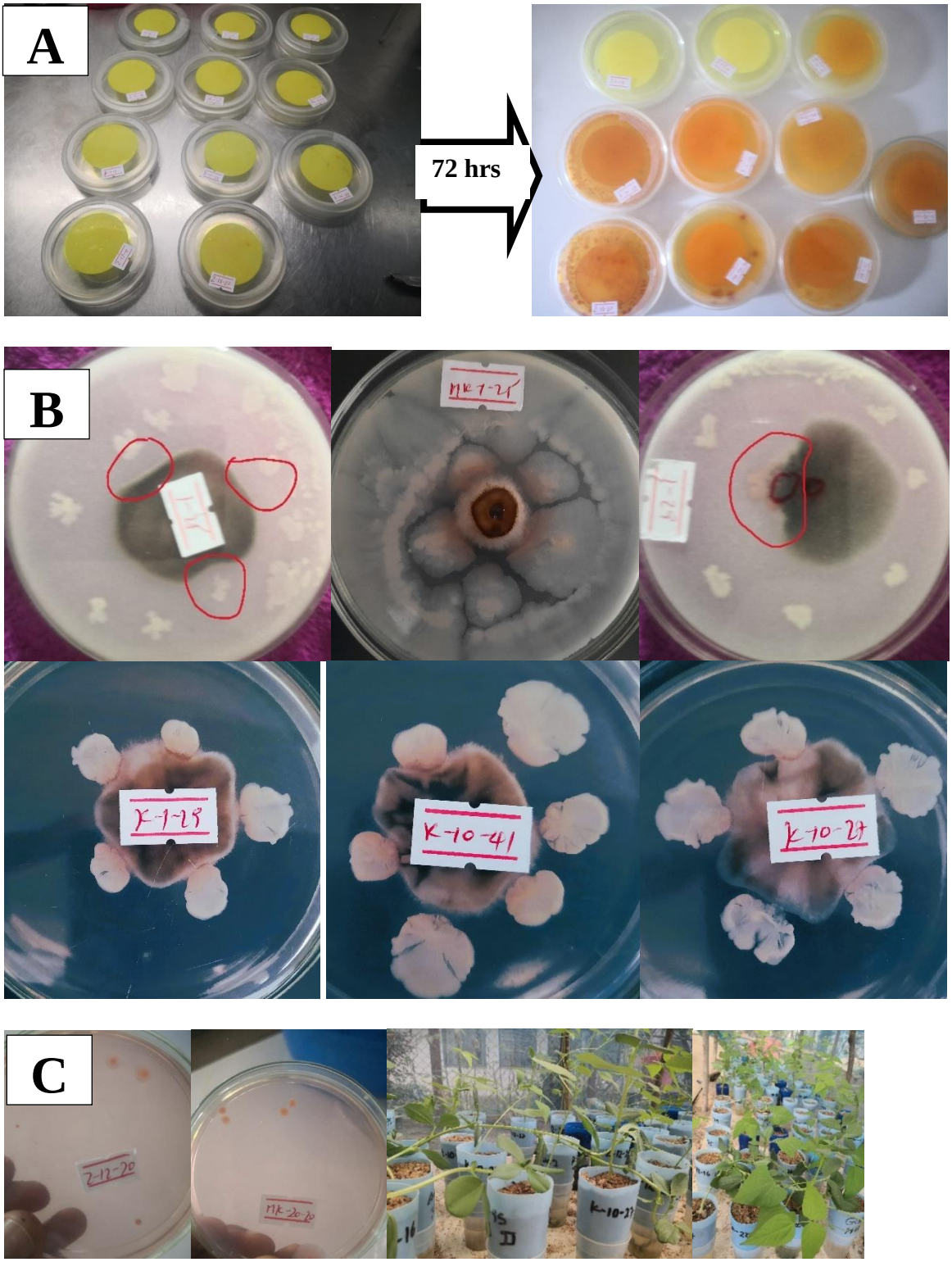
Soya bean								
Strain	PH (cm)	SFW (g)	SDW (g)	SE%	RL (cm)	Node No	NFW (g)	NDW (g)
Control	50.0+4.0^{c}	8.7+0.3^{e}	3.0+0.1^{de}	100.0+3.3^{de}	15.3+1.53^{ab}	0.0^{c}	0.0^{d}	0.0^{e}
k-1-29	61.0+6.6^{bc}	10.2+1.4^{de}	2.87+0.15^{e}	95.57+5.1^{e}	13.0+1.0^{b}	6.67+0.6^{abc}	0.38+0.1^{abc}	0.03+0.01^{cde}
k-10-27	57.3+2.52^{c}	9.8+0.4^{de}	3.1+0.1^{cde}	103.3+3.35^{cde}	18.3+2.52^{a}	13.0+1.0^{a}	0.45+0.1^{ab}	0.08+0.01^{ab}
K-10-41	66.0+15.5^{abc}	11.17+2.16^{cde}	3.57+0.6^{ab}	118.9+19.51^{ab}	14.3+1.53^{b}	7.3+4.2^{abc}	0.3+0.1^{abc}	0.03+0.03^{cde}
Mk-1-25	50.0+7.55^{c}	8.73+1.15^{e}	3.73+0.12^{a}	124.47+3.9^{a}	16.3+0.6^{ab}	10.0+5.3^{ab}	0.09+0.07^{cd}	0.02+0.02^{de}
Mk-13-16	74.67+7.37^{ab}	13.1+1.65^{abc}	3.2+0.4^{bcde}	106.67+13.35^{bcde}	15.67+2.1^{ab}	6.67+1.2^{abc}	0.28+0.14^{bcd}	0.04+0.02^{cd}
Mk-20-7	54.0+3.46^{c}	15.4+0.36^{a}	3.3+0.12^{abcd}	111.1+3.81^{abcd}	15.67+3.51^{ab}	4.67+2.1^{bc}	0.5+0.35^{ab}	0.04+0.01^{cd}
Mk-20-20	81.0+12.77^{a}	14.27+0.82^{ab}	3.6+0.4^{ab}	120.0+13.3^{ab}	14.67+0.6^{ab}	11.67+4.73^{ab}	0.45+0.05^{ab}	0.06+0.01^{bcd}
Z-1-16	60.67+14.57^{bc}	10.53+2.87^{cde}	3.0+0.21^{de}	101.1+3.97^{de}	16.3+4.0^{ab}	13.67+8.6^{a}	0.57+0.2^{a}	0.06+0.02^{bc}
Z-12-20	66.3+4.04^{abc}	12.23+1.4^{bcd}	3.5+0.12^{abc}	117.77+3.87^{abc}	16.3+1.53^{ab}	10.3+4.51^{ab}	0.53+0.05^{ab}	0.1+0.12^{a}
Z-13-4	59.67+16.17^{bc}	10.0+2.3^{de}	3.6+0.15^{ab}	118.87+5.1^{ab}	16.0+2.0^{ab}	14.0+8.9^{a}	0.56+0.31^{ab}	0.12+0.06^{a}
CV	16.03	13.91	8.19	8.18		53.3	46.38	42.08
LSD	16.8	2.66	0.46	15.34		8.05	0.03	0.038
Faba bean								
Control	46.67+4.73^{abcd}	14.3+1.84^{ab}	2.87+0.31^{de}	100.0+0.0^{de}	23.3+5.77^{a}			
k-1-29	40.33+5.03^{cde}	12.0+1.28^{cd}	2.73+0.12^{e}	99.97+4.2^{e}	12.67+0.58^{c}			
k-10-27	40.33+3.79^{cde}	11.87+0.4^{d}	3.17+0.21^{bcd}	115.87+7.6b^{cd}	18.33+4.9^{abc}			
K-10-41	39.67+5.51^{de}	12.63+1.2^{bcd}	3.27+0.21^{bc}	119.5+7.6^{bc}	15.3+1.15^{bc}			
Mk-1-25	35.0+7.0^{e}	11.53+1.2^{d}	3.07+0.15^{cd}	112.2+5.6^{cd}	21.0+4.36^{ab}			
Mk-13-16	48.33+6.81^{abc}	13.53+1.6^{bcd}	3.87+0.21^{a}	141.5+7.6^{a}	19.0+5.0^{ab}			
Mk-20-7	39.33+2.1^{de}	16.33+0.6^{a}	3.43+0.15^{b}	125.6+5.6^{b}	18.67+2.3^{abc}			
Mk-20-20	49.67+1.53^{ab}	13.97+0.6^{bc}	3.43+0.15^{b}	125.6+5.6^{b}	16.3+0.6^{bc}			
Z-1-16	42.33+3.5^{bcde}	11.6+1.8^{d}	3.47+0.12^{b}	126.8+4.2^{b}	18.67+1.2^{abc}			
Z-12-20	54.33+6.81^{a}	14.5+1.1^{ab}	3.43+0.15^{b}	125.6+5.6^{b}	22.67+4.2^{a}			
Z-13-4	43.3+4.16^{bcde}	12.73+0.5^{bcd}	3.03+0.15^{cde}	111.0+5.6^{cde}	18.0+3.6^{abc}			
CV	11.38	9.17	5.62	5.62	19.28			
LSD	8.39	2.05	0.31	11.31	6.06			



Supplementary Figure 3.1: Concentration of dissolved P from PSB inoculated PVK broth treated with 4 different P-sources (A= Quantity of dissolved P corresponding to added P-source, B=total dissolved P at day 10 incubation corresponding to PSB inoculum).



Supplementary Figure -3.2: Total dissolved P from the interaction response of individual PSB isolates against specific P-substrate at 10 days incubation.



Supplementary Picture 3.1: A) HCN production detection after 72 hr incubation, B) antagonistic test using plat dual inoculation, C) Nitrogen fixation test on plate and Nodule formation after 45 days of sowing on sand.

From Greenhouse Experiment

Supplementary Table 4.1: PSB isolates and external P sources application effects on tomato fruit yield.

Isolate	Total Fruit No	Total Fruit Weight
Control	3.38±1.66^{d}	103.86±46.13^{c}
K-1-29	6.1±3.48^{a}	135.29±54.64^{abc}
K-10-27	5.95±2.75^{ab}	135.1±61.52^{abc}
K-10-41	*4.52*±2.96^{abcd}	127.81±66.15^{abc}
Mk-1-25	5.24±3.86^{abc}	135.19±56.19^{abc}
Mk-13-16	4.24±1.9^{cd}	136.52±73.24^{abc}
Mk-20-20	5.48±2.68^{abc}	158.71±58.57^{a}
Mk-20-7	4.0±1.93^{cd}	117.29±74.33^{abc}
Z-1-16	4.62±2.22^{abcd}	149.19±70.79^{ab}
Z-12-20	4.43±3.27^{bcd}	108.24±61.08^{c}
Z-13-4	4.38±2.36^{bcd}	135.14±62.14^{abc}
CV	55.91	46.81
LSD	1.62	37.33

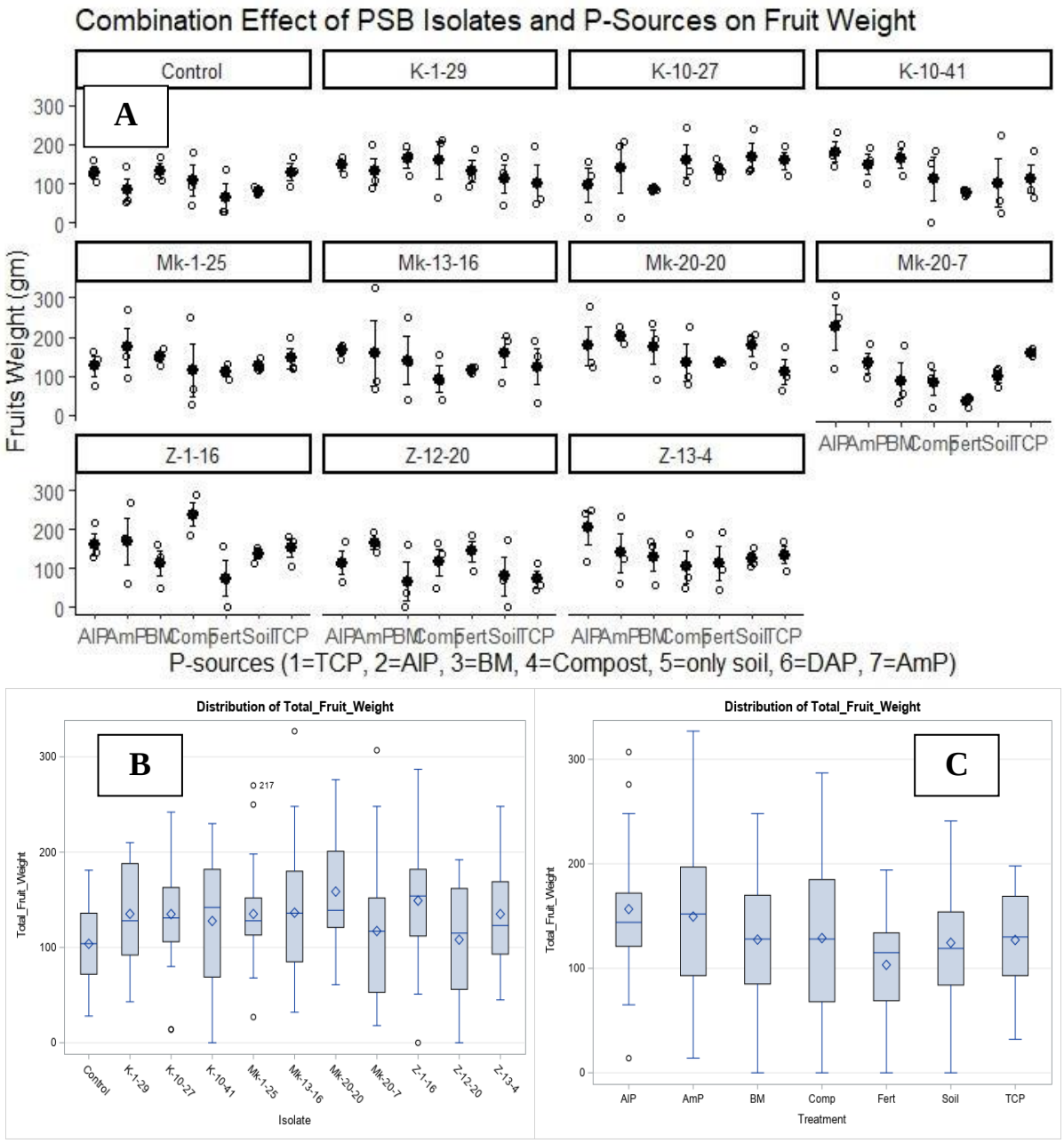
Treatment	Total Fruit No	Total Fruit Weight
AlP	5.27±3.05^{ab}	156.73±61.55^{a}
AmP	5.24±2.69^{ab}	149.61±72.38^{ab}
BM	4.42±2.61^{bc}	127.52±61.50^{abc}
Com	6.03±3.28^{a}	128.97±76.23^{abc}
Fert	4.36±2.37^{bc}	103.33±50.18^{c}
Soil	4.06±2.25^{bc}	124.52±57.04^{bc}
TCP	3.91±2.66^{c}	12718^{abc}
CV	55.91	46.81
LSD	1.29	29.78

NB: Means with similar letters have no significant difference at p=0.05.

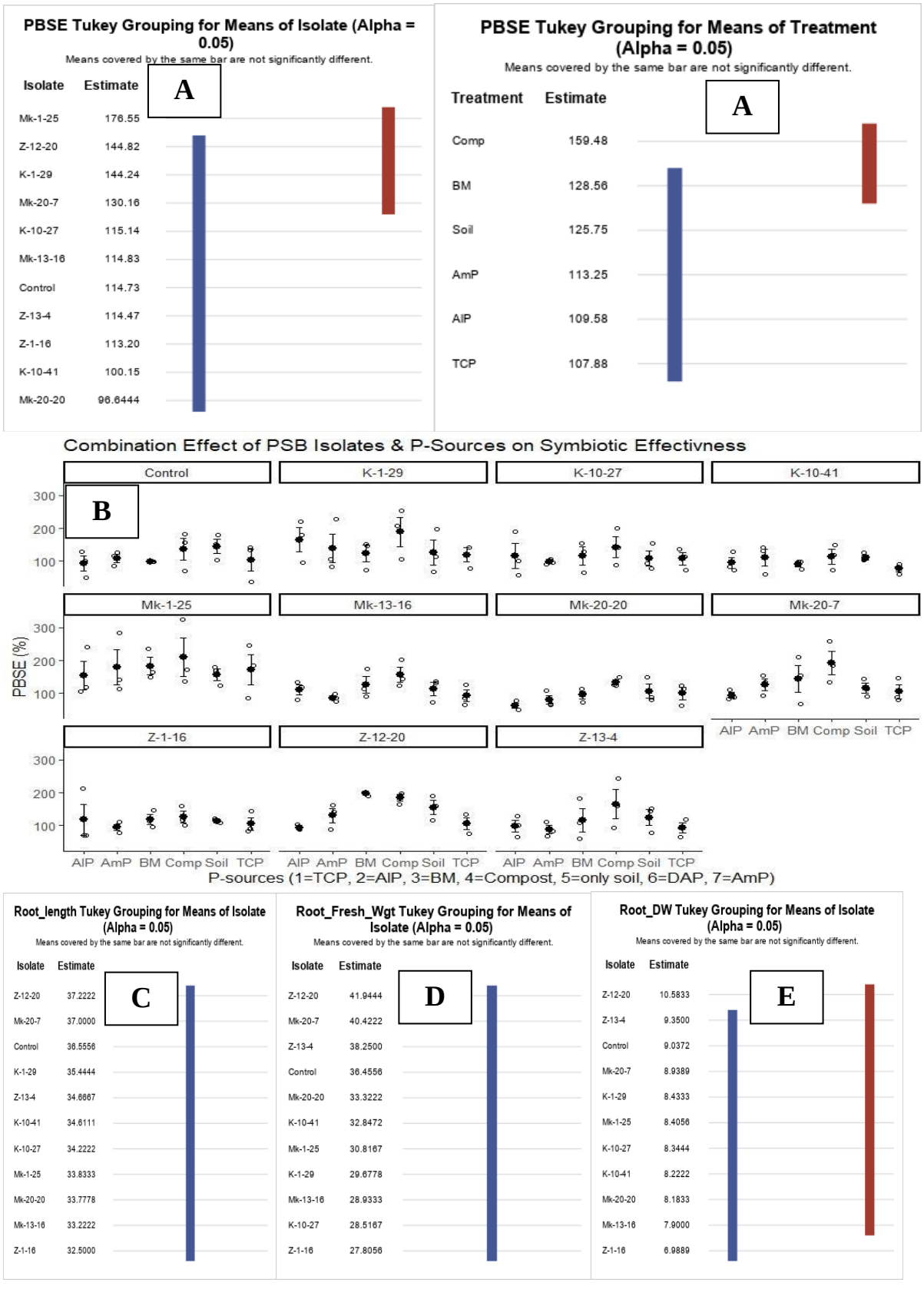
Supplementary Table 4.6: Total number of flower buds and opened flowers at different plantation days.

Isolates effect on tomato total flower bud number				**Isolates effect on total opened flower no.**		
Isolate	30 days	60days	90days	30 days	60days	90days
Control	3.1±1.76^{d}	12.67±3.34^{b}	19.95±5.94^{a}	0.1±0.3^{d}	8.43±4.32^{d}	14.81±5.39^{b}
K-1-29	4.43±0.75^{abc}	18.81±7.47^{a}	23.19±9.16^{a}	0.67±0.66^{bc}	13.81±6.47^{a}	18.48±7.48^{a}
K-10-27	4.52±1.12^{abc}	16.57±5.92^{a}	21.48±6.28^{a}	0.76±0.70^{bc}	12.14±3.9^{abc}	17.71±5.51^{ab}
K-10-41	4.67±0.48^{ab}	16.81±4334^{a}	*21.19*±5.93^{a}	0.62±0.74^{bc}	11.38±3.73^{bc}	17.00±5.07^{ab}
Mk-1-25	4.0±1.52^{c}	16.29±6.94^{a}	21.38±6.98^{a}	0.62±1.07^{bc}	12.33±4.61^{abc}	17.24±6.1^{ab}
Mk-13-16	4.67±0.48^{ab}	15.95±4.63^{a}	22.00±5.84^{a}	0.91±0.94^{ab}	12.29±3.48^{abc}	17.48±4.76^{ab}
Mk-20-20	4.29±0.56^{abc}	17.86±4.93^{a}	22.19±6.78^{a}	1.24±1.09^{a}	13.76±3.56^{ab}	17.95±5.32^{a}
Mk-20-7	4.57±0.68^{ab}	16.1±5.92^{a}	23.33±6.88^{a}	0.95±0.92^{ab}	12.00±3.65^{abc}	17.48±5.73^{ab}
Z-1-16	4.76±0.44^{a}	17.38±6.17^{a}	21.24±6.73^{a}	0.62±0.67^{bc}	12.67±3.32^{abc}	16.48±6.15^{ab}
Z-12-20	4.38±0.59^{abc}	16.43±5.44^{a}	21.76±6.86^{a}	0.33±0.48^{cd}	11.71±4.7^{abc}	17.38±4.64^{ab}
Z-13-4	4.19±0.93^{bc}	16.52±5^{a}	20.81±5.97^{a}	0.43±0.6^{cd}	11.05±3.53^{c}	16.29±4.84^{ab}
CV	21.17	29.46	29.15	109.84	33.01	28.8
LSD	0.56	2.96	3.07	0.44	2.4	2.39
Treatment	**30 days**	**60days**	**90days**	**30 days**	**60days**	**90days**
AlP	4.42±0.71^{ab}	14.76±4.05^{b}	21.61±6.78^{b}	1.09±0.91^{a}	11.64±3.77^{bcd}	16.67±6.09^{bc}
AmP	4.33±0.6^{ab}	15.49±4.63^{b}	21.52±6.11^{b}	0.49±0.87^{cd}	11.18±3.41^{bcd}	15.52±3.44^{bc}
BM	4.03±1.29^{bc}	15.42±4.57^{b}	21.55±7.65^{b}	0.85±0.87^{ab}	12.36±3.25^{bc}	17.82±6.12^{b}
Comp	3.88±1.75^{c}	23.21±5.58^{a}	27.21±5.75^{a}	0.24±0.44^{d}	15.21±6.04^{a}	23.27±5.28^{a}
Fert	4.27±0.98^{abc}	15.03±3.62^{b}	19.27±5.99^{b}	0.27±0.45^{d}	10.21±3.15^{d}	15.03±4.41^{c}
Soil	4.64±0.49^{a}	15.12±3.7^{b}	19.18±5.67^{b}	0.27±0.72^{bc}	10.55±3.09^{cd}	14.7±3.94^{c}
TCP	4.7±0.53^{a}	16.39±5.49^{b}	21.46±5.54^{b}	0.94±0.93^{ab}	12.58±5.08^{b}	16.82±4.45^{bc}
CV	21.17	29.46	29.15	109.84	33.01	28.8
LSD	0.44	2.36	3.85	0.35	1.92	3

NB: Means with similar letters have no significant difference at p=0.05.



Supplementary Figure 4.1: Amount of total tomato fruits weight collected from greenhouse cultivation (A= fruit weight corresponding to specific isolate*P-source interaction, B= corresponding to the respective isolate, C= corresponding to external P sources).



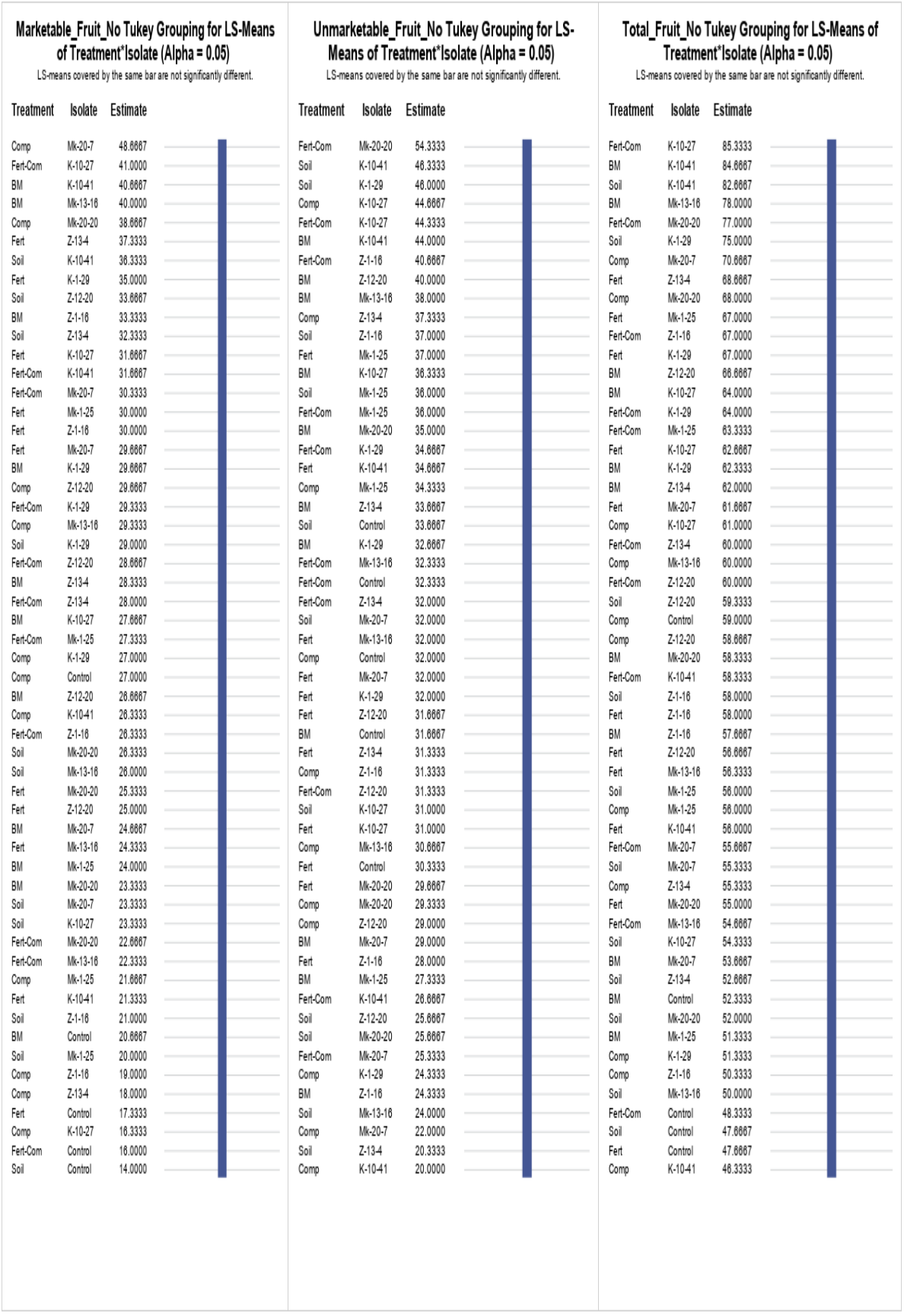
Supplementary Figure 4.2: Percentage of phosphate based symbiotic effectiveness (PSBE%) (A= % of overall isolates response and substrate effect, B= % of specific isolate-substrate interaction effect, C= Isolates effect on root length, D= isolates effect on root fresh weight, E= isolates effect on root fresh weight).

Tables from Field Trial

Supplementary Table 5.1: Synergetic application effect of PSB isolates and external P-supplements on tomato fruit yield and fruit size.

Isolate	Fruit yield per plant	Lager Fruit length(cm)	Lager Fruit Width(cm)	Small Fruit Length(cm)	Small Fruit Width(cm)
Control	17.0+5.79^{b}	9.87+0.99^{a}	18.93+2.12^{ab}	3.43+0.07^{abc}	6.57+1.08^{ab}
K-1-29	21.31+3.76^{a}	9.8+1.01^{a}	19.2+2.27^{ab}	3.17+0.79^{bc}	5.53+1.2^{c}
K-10-27	21.82+6.76^{a}	9.87+1.06^{a}	19.87+2.23^{a}	3.1+0.78^{c}	5.8+1.28^{bc}
K-10-41	21.87+6.51^{a}	10.03+0.86^{a}	19.27+1.67^{ab}	3.07+0.46^{c}	5.7+1.1^{bc}
Mk-1-25	19.58+3.77^{ab}	9.87+1.13^{a}	19.13+2.0^{ab}	3.43+0.75^{abc}	6.3+1.24^{abc}
Mk-13-16	19.93+7.65^{ab}	9.87+1.19^{a}	19.13+2.97^{ab}	3.67+1.08^{ab}	6.9+1.67^{a}
Mk-20-20	20.69+6.44^{ab}	9.8+0.78^{a}	18.2+2.24^{b}	3.73+0.96^{a}	6.93+1.79^{a}
Mk-20-7	19.8+4.22^{ab}	9.93+0.7^{a}	19.0+2.20^{ab}	3.47+0.92^{abc}	6.3+0.92^{abc}
Z-1-16	19.4+4.12^{ab}	10.0+0.85^{a}	19.27+2.34^{ab}	3.5+0.71^{abc}	6.3+1.27^{abc}
Z-12-20	20.09+5.06^{ab}	10.0+0.93^{a}	19.67+2.34^{ab}	3.67+1.03^{ab}	6.53+1.46^{ab}
Z-13-4	19.91+4.05^{ab}	9.53+1.06^{a}	18.47+2.13^{ab}	3.37+0.58^{abc}	6.33+0.96^{abc}
CV	26.74	9.9	11.79	22.92	19.93
LSD	3.88	0.71	1.62	0.57	0.91
P-Treatment					
BM	20.94+6.87^{a}	10.0+0.97^{a}	18.91+2.47^{a}	3.55+0.94b^{a}	6.35+1.53^{ab}
Compost	19.29+5.44^{a}	9.85+0.97^{a}	18.88+1.63^{a}	3.35+0.71^{ab}	6.09+1.31^{ab}
Fert	19.9+4.47^{a}	9.86+1.07^{a}	19.33+2.01^{a}	3.08+0.69^{b}	5.79+1.27^{a}
Fert & Comp	21.02+5.22^{a}	9.82+0.88^{a}	19.15+2.66^{a}	3.58+0.99^{a}	6.61+1.3^{a}
Soil	19.49+4.97^{a}	9.82+0.88^{a}	19.06+2.29^{a}	3.55+0.65^{a}	6.62+1.08^{a}
CV	26.74	0.48	11.79	22.92	19.93
LSD	2.62	9.9	1.09	0.38	0.61

**Means with similar letters has no significant difference at P=0.05.*



Marketable_Fruit_No Tukey Grouping for LS-Means of Treatment*Isolate (Alpha = 0.05)

LS-means covered by the same bar are not significantly different.

Treatment	Isolate	Estimate
Comp	Mk-20-7	48.6667
Fert-Com	K-10-27	41.0000
BM	K-10-41	40.6667
BM	Mk-13-16	40.0000
Comp	Mk-20-20	38.6667
Fert	Z-13-4	37.3333
Soil	K-10-41	36.3333
Fert	K-1-29	35.0000
Soil	Z-12-20	33.6667
BM	Z-1-16	33.3333
Soil	Z-13-4	32.3333
Fert	K-10-27	31.6667
Fert-Com	K-10-41	31.6667
Fert-Com	Mk-20-7	30.3333
Fert	Mk-1-25	30.0000
Fert	Z-1-16	30.0000
Fert	Mk-20-7	29.6667
BM	K-1-29	29.6667
Comp	Z-12-20	29.6667
Fert-Com	K-1-29	29.3333
Comp	Mk-13-16	29.3333
Soil	K-1-29	29.0000
Fert-Com	Z-12-20	28.6667
BM	Z-13-4	28.3333
Fert-Com	Z-13-4	28.0000
BM	K-10-27	27.6667
Fert-Com	Mk-1-25	27.3333
Comp	K-1-29	27.0000
Comp	Control	27.0000
BM	Z-12-20	26.6667
Comp	K-10-41	26.3333
Fert-Com	Z-1-16	26.3333
Soil	Mk-20-20	26.3333
Soil	Mk-13-16	26.0000
Fert	Mk-20-20	25.3333
Fert	Z-12-20	25.0000
BM	Mk-20-7	24.6667
Fert	Mk-13-16	24.3333
BM	Mk-1-25	24.0000
BM	Mk-20-20	23.3333
Soil	Mk-20-7	23.3333
Soil	K-10-27	23.3333
Fert-Com	Mk-20-20	22.6667
Fert-Com	Mk-13-16	22.3333
Comp	Mk-1-25	21.6667
Fert	K-10-41	21.3333
Soil	Z-1-16	21.0000
BM	Control	20.6667
Soil	Mk-1-25	20.0000
Comp	Z-1-16	19.0000
Comp	Z-13-4	18.0000
Fert	Control	17.3333
Comp	K-10-27	16.3333
Fert-Com	Control	16.0000
Soil	Control	14.0000

Unmarketable_Fruit_No Tukey Grouping for LS-Means of Treatment*Isolate (Alpha = 0.05)

LS-means covered by the same bar are not significantly different.

Treatment	Isolate	Estimate
Fert-Com	Mk-20-20	54.3333
Soil	K-10-41	46.3333
Soil	K-1-29	46.0000
Comp	K-10-27	44.6667
Fert-Com	K-10-27	44.3333
BM	K-10-41	44.0000
Fert-Com	Z-1-16	40.6667
BM	Z-12-20	40.0000
BM	Mk-13-16	38.0000
Comp	Z-13-4	37.3333
Soil	Z-1-16	37.0000
Fert	Mk-1-25	37.0000
BM	K-10-27	36.3333
Soil	Mk-1-25	36.0000
Fert-Com	Mk-1-25	36.0000
BM	Mk-20-20	35.0000
Fert-Com	K-1-29	34.6667
Fert	K-10-41	34.6667
Comp	Mk-1-25	34.3333
BM	Z-13-4	33.6667
Soil	Control	33.6667
BM	K-1-29	32.6667
Fert-Com	Mk-13-16	32.3333
Fert-Com	Control	32.3333
Fert-Com	Z-13-4	32.0000
Soil	Mk-20-7	32.0000
Fert	Mk-13-16	32.0000
Comp	Control	32.0000
Fert	Mk-20-7	32.0000
Fert	K-1-29	32.0000
Fert	Z-12-20	31.6667
BM	Control	31.6667
Fert	Z-13-4	31.3333
Comp	Z-1-16	31.3333
Fert-Com	Z-12-20	31.3333
Soil	K-10-27	31.0000
Fert	K-10-27	31.0000
Comp	Mk-13-16	30.6667
Fert	Control	30.3333
Fert	Mk-20-20	29.6667
Comp	Mk-20-20	29.3333
Comp	Z-12-20	29.0000
BM	Mk-20-7	29.0000
Fert	Z-1-16	28.0000
BM	Mk-1-25	27.3333
Fert-Com	K-10-41	26.6667
Soil	Z-12-20	25.6667
Soil	Mk-20-20	25.6667
Fert-Com	Mk-20-7	25.3333
Comp	K-1-29	24.3333
BM	Z-1-16	24.3333
Soil	Mk-13-16	24.0000
Comp	Mk-20-7	22.0000
Soil	Z-13-4	20.3333
Comp	K-10-41	20.0000

Total_Fruit_No Tukey Grouping for LS-Means of Treatment*Isolate (Alpha = 0.05)

LS-means covered by the same bar are not significantly different.

Treatment	Isolate	Estimate
Fert-Com	K-10-27	85.3333
BM	K-10-41	84.6667
Soil	K-10-41	82.6667
BM	Mk-13-16	78.0000
Fert-Com	Mk-20-20	77.0000
Soil	K-1-29	75.0000
Comp	Mk-20-7	70.6667
Fert	Z-13-4	68.6667
Comp	Mk-20-20	68.0000
Fert	Mk-1-25	67.0000
Fert-Com	Z-1-16	67.0000
Fert	K-1-29	67.0000
BM	Z-12-20	66.6667
BM	K-10-27	64.0000
Fert-Com	K-1-29	64.0000
Fert-Com	Mk-1-25	63.3333
Fert	K-10-27	62.6667
BM	K-1-29	62.3333
BM	Z-13-4	62.0000
Fert	Mk-20-7	61.6667
Comp	K-10-27	61.0000
Fert-Com	Z-13-4	60.0000
Comp	Mk-13-16	60.0000
Fert-Com	Z-12-20	60.0000
Soil	Z-12-20	59.3333
Comp	Control	59.0000
Comp	Z-12-20	58.6667
BM	Mk-20-20	58.3333
Fert-Com	K-10-41	58.3333
Soil	Z-1-16	58.0000
Fert	Z-1-16	58.0000
BM	Z-1-16	57.6667
Fert	Z-12-20	56.6667
Fert	Mk-13-16	56.3333
Soil	Mk-1-25	56.0000
Comp	Mk-1-25	56.0000
Fert	K-10-41	56.0000
Fert-Com	Mk-20-7	55.6667
Soil	Mk-20-7	55.3333
Comp	Z-13-4	55.3333
Fert	Mk-20-20	55.0000
Fert-Com	Mk-13-16	54.6667
Soil	K-10-27	54.3333
BM	Mk-20-7	53.6667
Soil	Z-13-4	52.6667
BM	Control	52.3333
Soil	Mk-20-20	52.0000
BM	Mk-1-25	51.3333
Comp	K-1-29	51.3333
Comp	Z-1-16	50.3333
Soil	Mk-13-16	50.0000
Fert-Com	Control	48.3333
Soil	Control	47.6667
Fert	Control	47.6667
Comp	K-10-41	46.3333

Supplementary Figure 5.1: Synergetic application effect of PSB strain and P-sources on tomato fruit yield.

Publications

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Overview of Agrochemicals Application Practices on Tomato Farm by Smallholders at Koka, Meki and Ziway, Ethiopia

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ABSTRACT

Promoting the agricultural sector with up-to-date technologies and inputs is convenient to enhance productivity. Production intervention is needed by introducing and adopting proper agronomic practices. Improved agricultural technologies increase production, quality, sustain food security, economic development and natural resource conservation. Koka, Meki and Ziway are known for their vegetable production as well as intensive agrochemicals consumption. Agrochemicals are applied on farm to enhance soil fertility, reduce pathogens, and induce plant growth. Tomato is one of the main commercial cash crops in these areas. Most commercially productive tomato varieties are highly sensitive to disease, vulnerable to nutrient deficiency, and other abiotic stress that requires rigorous agrochemical inputs. Ethiopian tomato production is very low due to various contributing factors including lack of improved varieties, diseases, pests, poor farming system, soil fertility maintenance as well as poor irrigation system. Farmers in the study areas applied inaccurate agrochemicals dose, rate, and application schedule that foster repeated spray. Intense agrochemical application leads to adverse environmental and health impacts due to deposit of toxic chemicals, residue leakage to water bodies and air pollution. It is important to practice proper agricultural inputs, reduce hazardous chemical residues, protect humans, other beneficial organisms and the environment. Moreover, developing IPM technology is recommended for better healthy agricultural production and sustainability.

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Introduction

In Ethiopia, the agricultural sector is the main livelihood shareholder for more than 80% of the people. Most Ethiopian farmers are smallholders owned small, fragmented land and practice a traditional farming system that resulted in low productivity (Abera et al., 2020). Low productivity may perhaps result from lack of awareness, soil maintenance, poor farming/cropping system, totally dependent on annual rainfall, poor soil and water conservation practice, limited or no agricultural technology input, poor pest and weed management, harvesting methods and the likes. Almost all farmers plough their lands in the same manner, grow similar seasonal crops from year-to-year and cultivate once a year which is below its potential (Alemayehu et al., 2010).

Water resource (surface and underground water) as well as bimodal rainfall is available in most parts of Ethiopia. Nevertheless, farmers do not properly manage and practice this important resource very well. Most important crops including export crops are produced in rainfed agriculture. Low agricultural productivity (Abera *et. al*., 2020) results in food shortage, poverty, lessens farmers' income and affects the state's economy. In Ethiopia, practicing small-scale irrigation in a fragmented and traditional way has a long history. Limited amount of irrigation practices are implemented on government and private farmlands for vegetables, fruits, horticulture and crops production. Most of the irrigation practices are gravity methods (i.e. the water flows through gravitational force from higher levels to lower levels). Irrigation types in Ethiopia include: traditional small-scale operated by local community, modern communal scheme built by government, modern private scheme operated by private sectors and public scheme built in cooperation and operated by public enterprises (NBWRA, 2021).

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Phosphate Solubilizing Bacteria (PSB) Isolation from Tomato Rhizospheres at Koka District, Ethiopia#

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ABSTRACT

Phosphorous is an essential macronutrient for plant growth and development. Most of the soil is deficient in plant available phosphorous and due to economic limitations majority of Ethiopian farmers applied inadequate fertilizers. It is essential to stabilize a mechanism to access P for plants with an efficient, cheap, and eco-friendly approach for enhanced crop growth and production. The main objective of this study was to screen efficient phosphate solubilizing bacteria from tomato rhizosphere soil. Using halo zone formation on PVK agar medium, more than 400 PSB isolates were isolated from 13 rhizosphere soil samples. By evaluating SI, texture in the culture-re-culturing process, liquid medium pH change efficiency and growth rate, upmost three promising PSB isolates (K-1-29, K-10-27, and K-10-41) were selected. Incubation of the isolates in PVK broth for five days showed significant pH reduction. For instance, isolate K-10-41 showed significant pH change (4.02) which indicates the organic acid production. Isolation and evaluation of efficient phosphate solubilizing bacteria maintain soil fertility, promote plant growth, induce plant response to pathogens, reduce agrochemical consumption and promote sustainable agriculture. Therefore, these selected PSB isolates need further detailed study for taxonomic identification, plant growth promotion, host range, and phytopathogen response. Local isolation improves environmental adaptation and indigenous competition.

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Introduction

Phosphorous (P) is an essential nutrient (Nesme et al., 2018; Sharma et al., 2013) for plants to synthesize cellular structures and biomolecules (Baliah et al., 2016) including nucleic acids, enzymes/proteins, and phospholipids. It is required for the growth and development (Kour et al., 2021) of roots, leaves, stalks (Sharon et al., 2016), flowers, seeds, and fruits. P accounts about 0.2-0.8% of plant dry weight (Sharma et al., 2013). Both organic and inorganic forms of phosphorus are found in soil (Tian et al., 2021; Yadav and Verma, 2012, Kkan et al., 2009), however, organophos ranges from 30-90% of the total phosphorous. Nevertheless, plants absorb inorganic phosphorous (Naik et al., 2008) that is accessed by solubilizing microbes (Khudhur, 2017) as well as from supplemented chemical fertilizers. It is strongly precipitated (Kour et al., 2021) and switched immediately to unavailable forms for plant absorption (Naik et al., 2008; Kkan et al., 2009). Most of worldwide soils are deficient in plant-available P (Kour et al., 2021). P supplement (Nesme et al., 2018) through synthetic fertilizers application is a very good option for direct plant assimilation (Yadav and Verma, 2012). Yet, it is reported costive, spendthrift (about 75-90% is rapidly fixed or highly reactive to precipitate with Al^{3+} and Fe^{3+} in acidic and Ca^{2+} in alkaline soils) (Baliah et al., 2016; Sharma et al., 2013; Kkan et al., 2009), energy-consuming (Kour et al., 2021), and environmentally unfriend (production, transportation and application is environmentally deleterious). Therefore, it is necessary to establish a mechanism to access P for plants with an efficient, cheap and eco-friendly approach for enhanced plant growth and production.

The majority of Ethiopian arable land needs fertilizer supplement. However, most of the farmers applied fertilizers below the required rate (Simtowe, 2015). The

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Research Article

Tomato Production under Synergistic Application of Phosphate Solubilizing Bacteria and Phosphate Amendments

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Phosphate solubilizing bacteria have multi-dimensional benefits in broad host range interaction, accessing nutrients, phytohormone induction, stress alleviation, biocontrol activity, and eco-friend approach. This study aimed to evaluate the efficacy of PSB isolates coinoculated with compost, bone meal, and DAP fertilizer on tomato growth response. Tomato seeds were treated with 10 selected PSB isolates separately and grown for 20 days on seedbed, then transplanted to field that was treated with external P-sources and enriched by PSB inoculum. PSB isolates showed positive interaction and achieved significant plant assays including plant height, leaves, branches, flowers, and fruit development. Isolate K-10-41 significantly promoted tomato plant height, floral development, and fruit yield, Mk-20-7 enhanced height and fruit weight whereas K-10-27 induced tomato fruit numbers. Compost application promoted tomato-PSB interaction and induced tomato vegetative growth whereas bone meal was least promotor for most of tomato plant assays. Bone meal was however, one of the top fruit development inducers (harvested 20.94 fruits/plant weighing 881.97 gm). Mixing 50% of recommended compost and DAP fertilizer application enhanced tomato vegetative growth and fruit yield (21 fruits/plant harvested that weighed 872.46 gm). Based on the overwhelming performance, K-10-41 and Mk-20-7 application together with compost and fertilizer mixture were found effective. Therefore, the results of this study imply that application of competent PSB isolates together with nutrient supplements improved symbiotic effectiveness, sustainable production, and environmental health. Consequently, these promising isolates would be recommended for tomato production of higher yield and sustainability after verifying their efficacy at different agroecology and taxonomic identification. Screening potential strains and evaluating their competence under different conditions would be the future perspectives to develop efficient inoculants. Moreover, synergetic application of organic supplements (compost, farmyard, bone meal, or other biowastes), bioinoculants, and proper agrochemicals maximize production and environmental health and is feasible for the economic, social, and ecological sense of balance.

1. Introduction

Phosphorous (P) is one of the macro elements [1] essential to plant molecular, physiological, and structural activities [2–4]. The soil might be rich in phosphorous (400–1200 mg/kg soil) [5] although plant available phosphorus is very limited. Since soil P deficiency is often a limiting factor for crop production, application of organic matter and chemical fertilizers are common to overcome the nutrient deficiency [1, 6]. Due to the rapid fixation nature of phosphate ions, most of the applied fertilizer [2] converted to unavailable residues [7]. Different complications with the deficiency of plant available nutrients [8], the efficacy of applied chemical fertilizers and other constraints including production cost, environmental issue, and production demand calls organic matters and biological inputs for sustainable crop production [9–12]. As indicated in Rilling et al., 2019 [13] diverse microbial groups including beneficial microbes are rich in the root zone competing for root exudates. Plant growth-promoting rhizobacteria (PGPR) are considered as efficient biological inputs [14, 15] that enrich soil fertility, induce plant stress resistance, promote growth and

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