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Stimulation Effect of Rhizospheric Microbes' Plant Growth Promoting Rhizobacteria, Glycoprotein Producing AM Fungi and Synthetic Fertilizers Application on Growth, Yield, Nutrient's acquisition and Alliin Content of Garlic Cultivation Under Field Conditions in Southeast Region of Rajasthan, India

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Abstract

The rhizosphere is the thin region of soil directly influenced by root secretions and microbes, known as the root microbiome. The rhizosphere associated with the roots of a plant contains numerous beneficial bacteria, fungi, and other microorganisms. Microbial constitution plays a vital role in a plant's growth cycle by stimulating its morphology, physiology, and development. Several species in the soil rhizosphere are supportive of plant growth, development, and productivity. The beneficial plant-microbe relationship within the rhizosphere is the key determinant of soil health and plant growth. Plant growth-promoting rhizobacteria support the colonization of AM fungi within plant roots. In the current study, garlic plants were treated with six different biological strains treatment, a combination of specific PGPR (Azotobacter and Azospirillum), PSB, KSB, Bacillus, and AM fungal inoculums. The output from these treatments was considered in different parameters determining the quality and productivity of garlic crops. These microbes help the plants directly or indirectly through the acquisition of nutrients, overall improvement in growth by production of phytohormones, protection from pathogens and other abiotic stressors. Results showed a significant increase in several factors such as nutrient translocation, bulb size, bulb diameter, biological biomass, marketable yield, and AM fungi colonization in root systems in contrast with standard treatment (Control (100% RRF and 50% RRF). Treatment T7 Absolute consortium PGPR + AM fungi (Azotobacter, Azospirillum, Pseudomonas, Frateuria, Bacillus, and AM fungi) performed better than control and other combinations of biological ingredient's utilization in different treatments and combinations. After harvest, garlic bulbs treated with Absolute consortium PGPR + AM fungi increased the Alliin content as well as the primary element responsible for garlic's medicinal properties and its distinctive native taste. Maximum yield (137.15±1.45q ha^{-1}) was recorded in treatment T7, along with maximum values of dry matter (34.45±0.24), TSS (13.354%), starch (5.65%), reducing sugar (1.98%) and Alliin content (0.11 µg) as compared with control and other biological treatments. The best treatment in respect of projected yield was Absolute consortium PGPR + AM fungi) followed by control (50% RRF).

Keywords : PGPR, AM fungi, Chemical fertilizers, Growth, Alliin Content, Yield.

Introduction

The microbial world encompasses a wide array of global biodiversity and acts as a major resource for agricultural and industrial applications. Microbes happen to be the most dominant species among present natural diversity which exists in diverse ecological niches, including extreme environments present in both lithosphere and hydrosphere, where their metabolic abilities play a critical role in geochemical nutrient cycling. The rhizosphere is a succinctly distinguished ecological niche consisting of layers of soil with a dense bacterial population. Their activities are largely influenced by the surrounding plant roots. The bacterial population in the rhizosphere is 100-1000 times higher than in bulk soil, and about 15% of the root surface is covered by microcolonies of

a variety of bacterial and fungal species. A considerably large amount of carbon is fixed by the plant as photosynthates are secreted into the rhizosphere mainly as root exudates that can be used as nutrients by bacterial populations. In reciprocation, the metabolic activities of these bacteria in the rhizosphere stimulate mineral nutrient distribution and uptake by plant roots and provide to plants for growth, development, and productivity. These beneficial bacterial populations of the rhizosphere are commonly known as plant growth-promoting rhizobacteria (PGPR). They promise plant growth (PG) promotion by secreting a variety of metabolites and employing various mechanisms.

Garlic (A*llium sativum*), a perennial plant of the amaryllis family (Amaryllidaceae), is known for its flavourful bulbs. The plant is endemic to central Asia but is cultivated in Italy and southern France and is a classic ingredient in many national and international cuisines. Garlic is the second most widely cultivated bulb crop, after onion, and has long been recognized as a valuable spice and condiment throughout the world. In India, garlic is grown an area of 0.274 million hectares with a production of 1.271 million tons. Garlic possesses highly nutritive value and has been considered a rich source of carbohydrates, proteins, and phosphorus. Ascorbic acid content was also reported to be very high in green garlic. The uninjured bulb contains a colorless, odorless water-soluble amino acid called Alliin (Figure 1) which, after crushing converts into Allicin whose principal ingredient is odoriferous diallyldisulphide. Garlic contains 0-1% volatile oil, whose chief constituents are diallyldisulphide (60%), allyl alcohol (5.4%), dimethyl trisulphide (2-4%), methyl allyltrisulphide (1.5%), methyl allyldisulphide (1.2%) and diallyltrisulphide (1%). Alliin is the most noticeable biomarker in garlic, consisting of about 10 - 30 mg/g in fresh and dry garlic, respectively (Lawson, 1998). The daily intake of garlic in various forms recommended by WHO is 2 - 5 mg of oil, 2 - 5 g of fresh, 0.4 - 1.2 g of dry powdered, and 0.3 - 1 g of the dry extract (Torrey, 1992). As a dietary supplement, 600 - 900 mg of garlic per day is suggested by the American Dietetic Association (ADA). Allicin is the therapeutically useful compound of garlic. However, it is difficult to directly determine it due to its high reactivity and unstable nature. The range of the amount of allicin in garlic samples is reported by several scientists from 3.37 to 8.99 mg/g (Lawson, 1998;Ribak et al., 2004; Silva et al., 2010). The quantity of alliin indicates the quality of garlic. Upon crushing of garlic cloves the enzyme alliinase gets access to alliin and consequently, alliin is converted into allicin with a release of distinctive aroma (Holub et al., 2002). The reported ratio of conversion from alliin to allicin is 3:1 along with pyruvic acid, ammonia, and carbon dioxide as by-products (William & Pant, 2007).

Garlic contains antifungal, antimicrobial, insecticidal, medicinal as well as hypoglycemic properties. Experts also suggest garlic therapy in flatulence, constipation, faulty digestion, inadequate food intake, leprosy, chronic coughs, and many other diseases. It is grown widely in India and particularly in Rajasthan as well on large scale to boost up its per hectare yield and the farmers resort to using inorganic fertilizers containing N, P, and K in sufficiently abundant quantities. The use of inorganic fertilizers to obtain a higher yield with a quality product is not only costly but also a precursor of health hazards by polluting the environment, soil, and water. This anxiety has now led them to devise ways and means to switch over the use of eco-friendly biofertilizers in crop production. Biological organisms such as nitrogen fixer, phosphate solubilizer bacteria (PSB) Potassium mobilizing bacteria (KMB) fix atmospheric nitrogen, solubilize phosphorous, and mobilize potassium to increase soil fertility and biological activities. Biofertilizers are products having living cells of different types of microorganisms, which can convert nutritionally important elements and play a principal role in expanding the availability of macro and micronutrients to the plants, besides these improving building hormones and antimetabolites properties as well. Availability of nitrogen is important for growing plants. It remains the main constituent of protein and nucleic acid molecules and also a part of chlorophyll molecules. Phosphorus (p) is a vital constituent of phospholipids, nucleic acids, and several enzymes. It is desired for the transfer of energy within the plant system and is involved in several metabolic activities. P has a positive effect on early root development (RD), PG, yield, and quality. Potassium (K) plays an indispensable role in plant metabolisms such as photosynthesis, translocation of food, regulation of plant pores, activation of plant catalyst, and resistance against pests and diseases. P improves colour, glossiness, and dry matter accumulation besides improving keeping quality of the crop. Some microbes suppress plant pathogens and insect pests by producing volatile antibiotic metabolites, while others stimulate plant host defense before pathogen infection which indirectly contributes to increased crop productivity. Scientific reports on endophytic colonization and biofilm formation by *Bacillus* spp. have suggested that endophytic colonization and biofilm formation improve the bacterium's ability to act as a biocontrol agent (BCA) against the plant pathogens. In recent years, Bacillus and Paenibacillus spp. has procured global attention because of their utility advantages over other PGPR strains in inoculant formulations, stable maintenance in rhizosphere soil, and greater potential in sustainable agriculture.

Garlic production in India reflects one of the stark challenges faced by global food systems today. Even after allotting the second largest area of land for garlic cultivation in the world, India ranks second in terms of productivity. AM Fungi facilitates macro and micronutrients uptake, especially phosphorus for the host plant, thereby enabling enhanced nutrition (Siquiera et al., 1998; Torrey, 1992; Valdes et al., 1993; Prasad, 2015 Prasad, 2017; Prasad, 2020; Prasad, 2021; Prasad, 2021; Prasad, 2021; Prasad, 2021; Prasad, 2021; Bolandnazar et al., 2007; Prasad, 2022). Due to the absence of AM fungi and native microbial communities in the soil rhizosphere, garlic crops suffer stunted growth. Microbial communities (MC) in the soil influence a PG and productivity by affecting its morphological and physiological development (Prasad, 2017; Prasad, 2020). They are also effective in restoring the former fertility of the soil before it was exposed to Chemical fertilizers

(CF). A wide range of valuable bacterial strains colonizes the plant roots (PR) to form a symbiotic relationship and improve PG and Productivity. The role of the rhizosphere microbiome (RM) for PG and productivity has been well established with an ever-increasing need and demand for biofertilizers. The population of PGPR, however, depends extensively on crop species and soil health (SH) (Tilak et al., 2005). Sustainable production of garlic can be achieved by using PGPR and AM Fungi as biofertilizer inoculants. Among a community of growth-promoting rhizobacteria (GPR), specific strains of Azospirillum show enormous potential in enhancing the growth and productivity of garlic crops. Genus Azospirillum is studied extensively for its role in fixing atmospheric nitrogen and its capacity to produce phytohormones. Garlic plants that were treated with Azospirillum have previously been reported to induce a significant increase in bulb weight, bulb diameter, and root weight (Prasad, 2021; Domenico et al., 2019). Garlic has a fibrous root system, which makes it relatively efficient in absorbing nutrients. As a result, this crop has a relatively low dependency on AM Fungi symbiosis for nutrient acquisition. Nevertheless, AM Fungi have shown significant scope for improving PG and supplying nutrients, especially P (Prasad, 2021; Prasad, 2017; Prasad, 2020; Bolandnazar et al., 2007; Sander & Tinker, 1973; Sharma & Adholeya, 2000). Additionally, plants treated with AM fungi have greater resilience against adverse environmental conditions, thus producing a higher yield per hectare (Prasad, 2021; Prasad, 2021; Sharma & Adholeya, 2000). Specific AM fungal strains, which garlic plants are highly responsive to, associate with the roots of garlic plants to optimize water use and increase tolerance for soil salinity (Sander & Tinker, 1973; Prasad, 2017; Prasad, 2020; Prasad, 2021; Prasad, 2021; Prasad, 2021). By establishing a symbiotic relationship with the plant, AM Fungi can increase plant tolerance to several biotic and abiotic stresses (Prasad, 2017; Prasad, 2020; Patharajan & Raman, 2006). Among global bio-scientific communities, mycorrhizal fungi have the ability for gained increasing attention attributed to several factors for improved nutrition supply, improved crop quality, productivity, and resilience. With the above-mentioned facts in view, a field experiment was conducted to find out an optimal fertilizer input along with microbial consortium PGPR and AM fungi during the winter season for garlic cultivation. Therefore, keeping in view the above facts in mind, an attempt has been made in the present investigation to study the effect of biofertilizers on the growth and quality yield of garlic.

Objectives

The objectives of this study were as follows:

- To study the impact of CFs on the growth, yield, and quality of garlic
- To analyze the effect of different biological consortium single and combined inoculations on the growth, quality parameters, Alliin content, yield of garlic, improve the soil and human health (HH) in addition to saving the environmental pollutions.

Material & Methods

Location, Climate, and Soil of Experimental Site

A field experiment was conducted at the farmer's field of Antah village of Baran district, Rajasthan, India during winter cultivation. The site Antah is positioned among 24.7998°N 76.637°E and its elevation from sea level is 262 meters (859 ft). The climate is dry except in the monsoon season with mean temperature ranging from 10.6 - 24.3 °C in winter. The mean annual rainfall is around 895.2 mm. The soil is clay to sandy loam in texture, well-drained, and of excessive fertility. The major portion of this location is underneath the cultivation of different agricultural, horticultural, medicinal, and aromatic crops and accommodates the growth of several crops including wheat, rice, cotton, tobacco, etc.

Soil Sampling and Analysis

Soil samples (0-30cm) were collected randomly from each site using the standard conning and quartering method before (Initial time) and at harvest. Air-dried soil samples were used for different Physico-chemical analyses. pH was determined in 1.25 (w/v) solutions of dried samples in water and the same was used for the determination of electrical conductivity (EC). Air-dried samples were processed (addition of 40% NaOH and distillation) in a Kel Plus Nitrogen estimation system (Class DX, Pelican Equipment's) followed by determination of available nitrogen by titration with 0.02N H2SO4 (Subbiah & Asija, 1956). The contents of soil organic carbon (SOC) were determined using Sims' method (42). 1.0 g of soil was combined with 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 in this manner. This suspension was carefully mixed and diluted to 200 ml of distilled water, then 10 ml of H_3PO_4 and sodium chloride were added. Estimates of total organic carbon (OC expressed as C) are used to assess the amount of organic matter (OM) in soils. Available phosphorus was determined by the Olsen method using samples with high pH sodium bi-carbonate as extracting agent (Singh et al., 2007). Available potassium was determined in a 1N ammonium extract using a flame photometer (Singh et al., 2007). Extractable micronutrient Cu, Zn, and Fe in soil were analyzed using DTPA extraction standard procedures.

Microorganisms Applications

AM fungi Inoculant

The concentration of consortium AM fungi that were mixed with triple sterile talc powder adjusted with 3000 infected propagules (IP) per gram of inoculants containing growing to subtract, infected roots bits and hyphal and mycelial biomass. Consortium AM fungi inoculum contained *Aculospora logula*-15%, *Glomus fasciculatum*-20%, *Glomus intraradices*-40%, *Gigaspora margarita*-15%, and *Scutellospora heterogama*-10% infective propagules (IP). Consortium AM fungi @1 million IP/ha were applied as a clove treatment method at the time of planting.

PGPR Inoculant

PGPR microbial inoculants (Azotobacter, Azospirillum, Pseudomonas (PSB), Frateuria (KSB), and Bacillus) have been proliferated in a nutrient broth medium. Then every

single PGPR becomes eliminated on the top of the logarithmic growth phase and becomes aseptically transferred to plastic containers, which include triple sterile talc powder, and then were mixed well. PGPR concentration was adjusted to 1×10^8 CFU/g in all inoculants. PGPR mixture was prepared and contained Azotobacter, Azospirillum, PSB, KSB, and Bacillus as per treatment. According to the treatment combination, altogether PGPR @ 2.5 kg ha^{-1} was treated across the garlic claves during the time of sowing. PGPR (Nitrogen fixers, PSB, KSB, and Bacillus) has been additionally applied on garlic growth stages in four equal splits dose.

Farmyard Manure (FYM)

All experimental plots received a uniform dose of well-decomposed and sieved FYM at the rate of 20 t ha^{-1}.

Chemical Fertilizers (CF) Applications

Various level of CF was supplied according to the recommended rate of fertilizers (RRF). Nitrogen 1.2 q N ha^{-1}, Diammonium phosphate (DAP) 1.08 q ha^{-1}, and potash (MOP) 0.45 q ha^{-1} were applied. The sources of the CFs were urea (46% N), DAP (18% N and 46% P_2O_5). All the required amount of DAP was applied in the band just before planting at the depth of 10 cm below the soil level of positioning the roots of the seedlings. Urea was applied thrice, where 50% of the total amount of the CFs was applied as basal dose before sowing and the remaining 50% was applied in three split-dose at the active stage of vegetative growth of the plants. Garlic plants were treated with Dimethoate (40% EC) and Diazol (60%)) at the rate of 1.5-liter active ingredient per 200-liter water per hectare for controlling thrips. Furthermore, other necessary cultural practices such as weeding, and watering were carried out accordingly on all plots using a sprinkler irrigation system.

Field Preparation and Experimental Design

The site of the experiment was opened using a power tiller and kept unveiled to the sun before the next ploughing. Afterward, it was ploughing and cross-ploughing, followed by laddering. The cropping pattern of the land was Paddy–wheat/ maize–vegetables. Identical sizes of garlic cloves (Yamuna Safed-3, Variety: G-282) were selected and picked for treatment with and without consortium PGPR and AM fungi. The treated and untreated cloves were sowed in the experimental plots in the first fortnight of November in a bed size of 10 x 10 meters with a spacing of 15 x 10 cm with four replicates in each treatment in a randomized complete block design (RCDB). PGPR and AM fungi treatments were considered as Azotobacter + Bacillus + AM fungi, Azospirillum + Bacillus + AM fungi, Azotobacter + Azospirillum+ Bacillus, Azotobacter + Azospirillum+ Bacillus + AM fungi, and Absolute consortium PGPR and AM fungi containing Azotobacter + Azospirillum+ Pseudomonas+ Frateuria + Bacillus + consortium AM fungi and compared to control treatment without microbial inoculant (100% RRF and 50% RRF). The plantation was finished in the first week of December with four replicates in each treatment. 100% and 50% RRF was added to control treatments only without microbial treatments. Recommended cultural operations (RCO) were carried out during the entire cropping period to ensure a thriving and healthy crop. Field soil analysis was estimated at an initial time and harvest. The starch was estimations through the stander method and reducing sugar was followed. The bulbs were harvested at the mature stage. The loss of weight of different treatments was recorded at fortnight intervals up to 60 days. For this purpose, randomly selected bulbs of known weight were kept open in perforated trays by taking 50 from each treatment and kept at room temperature. Nutrient uptake in the shoot was determined. Biomass (q ha^{-1}), and gross yield (q ha^{-1}) were recorded at harvest of the crops.

Experimental Data Collection and Analysis

Morphological and Yield Attributes Traits

Data Collected and Measurements

Plant Height (cm): Measured from the ground level to the top of a matured plant at 45 days after sowing (DAS) and at maturity.

The Number Of Leaves Per Plant: The total number of leaves was counted and recorded 45 DAS and at maturity.

Leaf Length (cm): The average length of leaf blades of the plants measured at 45 DAS and maturity and expressed in centimetre's

Shoot Fresh Weight (kg): The above-ground biomass of the plant was harvested by cutting at the crown of the plant at harvest.

Shoot Dry Weight (kg): The shoot fresh mass was oven-dried at the temperature of 65 0C for 72 hours and its dry matter yield was determined and expressed in kg.

Days To Maturity: The total number of days from emergence until 70 percent of the plants have attained physiological maturity.

Total bulb yield (q ha^{-1}): The marketable garlic bulb yields were expressed in q ha^{-1}.

Number And Weight Of Marketable Bulbs: Recorded as the number and weight of healthy and marketable bulbs at harvest.

Biological Fresh Matter Yield: The total yield of the plant including bulbs, shoots, and roots after curing for one week.

Biological dry matter yield: This was determined by oven-drying the biological fresh matter yield at the temperature of 65 ^{0}C for 72 hours and weighed using a scale balance.

Neck Diameter (cm): The average neck width was measured using a veneer caliper and expressed in centimetre's after harvest.

Bulb Diameter (cm): The average radial width was measured by using a veneer caliper and expressed in centimetres after harvest.

Bulb Fresh Weight (g): The average fresh weights of fifteen mature bulbs were measured using digital sensitive balance after harvesting and expressed in grams.

Bulb Dry Weight (g): Bulb fresh weight was recorded, and then the bulbs were chopped into small pieces and oven-dried at 70 0C until to a constant weight. After the weight

was measured using digital balance, percent dry matter was calculated using the formula:

DW (%) = {[DW + CW]-CW}/ {[FW+CW]-CW} X 100
Where: DW = dry weight CW = container weight FW = fresh weight.

Harvest Index: This was recorded as the ratio of bulb dry yield to the biological dry yield and expressed in percentage.

Total Soluble Solids (TSS): The TSS was determined from fifteen randomly selected bulbs using the methodologies (William & Pant, 2007). Aliquot juice was extracted using a juice extractor and 50 ml of the slurry was centrifuged for 15 minutes. The TSS was determined by hand refractometer (ATAGO TC-1E) with a range of 0 to 32° Brix and resolutions of 0.2° Brix by placing 1 to 2 drops of clear juice on the prism, washed with distilled water, and dried with tissue paper before use. The refractometer was standardized against distilled water (0% TSS). Amount of total soluble solids present in the bulb expressed in percentage. The observations were made at harvest. Twenty plants were randomly selected in each replicate for all the characteristics, including plant height, the number of leaves/plants, leaf moisture content, and neck thickness. The analysis of bulb diameter, bulb size index, gross & marketable yield, nutrient uptake, total soluble sugar (TSS), dry matter, and alliin content was done after harvesting the crops.

Assessment of Shoot Nutrients (ASN)

Randomly four places were selected for plants samplings in all garlic plots. For nutrient analysis of the shoot systems, the oven-dried samples were finely ground. N in the shoots was determined using an elemental analyzer (EA 3000, Eurovector, Italy). To estimate the P and K level in the shoots, 1g of the finely ground sample was subjected to a wet oxidation treatment using tri-acid (HNO_3: H_2SO_4: $HClO_4$; 10:1:4) digestion in a digestion block (KELPUS, KES121; Pelican Equipment, Chennai, India) at 200°C. Following acid digestion, the samples were diluted and filtered for further nutrient analysis. Shoots P was determined by the vanadomolybdophoshoric acid colorimetric method Guo & Zhang Christie Li (2006) using a spectrophotometer (Specord 200; Analytik Jena, Germany). K was measured by the ammonium acetate method of Hanway and Heidel (1952) by using a flame photometer (Model FP114; Thermo Scientific, USA). To determine iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) content in shoots samples were digested in a microwave (Mars 5, CEM). Following the US EPA 3051A method (US EPA 2007), the metal concentration in the acid digestive was determined using atomic absorption spectrophotometer (AAS) (SOLAAR, TJA Solution, UK).

Estimation of Alliin Content in Garlic

The garlic cloves of each treatment were collected randomly for analysis of Alliin and Alliinase activity for better quality.

HPLC Analysis

Apparatus and Reagent

Standard alliin (HPLC grade) was obtained from Sigma-Aldrich (CAS Number 17795 - 26- 5). Reagents and solvents of analytical grade (Ethyl acetate, n-hexane, ninhydrin, etc.) were purchased from Fluka Chemicals (Busch, Switzerland) and Across Organics (Hamilton, NJ), respectively. Pre-coated HPTLC glass plates of silica gel 60 F254 were purchased (E. Merck, Darmstadt, Germany). The application of standard and the extracts were made on HPTLC plate band wise with the help of CAMAG automatic TLC sampler-4 and developed in automatic development chamber-II (CAMAG, Muttenz, Switzerland). Scanning and documentation of developed HPLTLC plates were done by CATS 4 and CAMAG TLC Reprostar 3, respectively. A standard stock solution of alliin (1 mg/mL) was prepared by dissolving 10 mg of standard in 10 mL dichloromethane. One millilitre of the stock solutions was again diluted to 9 mL of dichloromethane to get the concentration of 100 μgmL^{-1}. For making a calibration graph, 2 - 16 µL of the standard solution was applied to the HPTLC plate to provide a concentration range of 200 - 1600 $ngband^{-1}$.

Sample Preparation

Fresh garlic samples were weighed and dried in the sun. Dried garlic samples were powdered, and extraction was made with 10 ml of dichloromethane by vigorous stirring for 30 s. The extraction procedure was repeated twice. Similarly, one tablet from each brand was weighed [Average Wt. 603 mg (US) and 550 mg (UK)], crushed and extracted twice with a similar technique. All the extracts were dried by vacuum evaporation of the solvent and percentage yield was calculated. Dried extracts from each sample were dissolved in 10 ml of dichloromethane and filtered through a 0.45 µm filter membrane to make a stock solution for HPTLC analysis. One ml of this stock solution was mixed with 9 ml of dichloromethane to perform the analysis. After optimizing the method, 2 µl from all four samples were applied for analyses. The samples were quantified against the standard alliin used for the calibration curve.

Instrumentation and Chromatographic Conditions

The analytical procedure was performed on HPTLC glass plates of size 20 × 10 cm coated with silica gel 60F254 as the stationary phase. TLC Sampler 4 (Automatic) fitted with a Hamilton Gastight Syringe (1700 Series) of volume 25 µL was used to apply the samples and standard on the HPTLC plate at an application speed of 160 nl/s. The development of the spotted plate was done in a previously saturated automatic developing chamber with mobile phase n-hexane: ethyl acetate (29:1) moving in linear ascending mode. After the development of the HPTLC plate, it was air-dried and sprayed with ninhydrin reagent. For further analyses, Camag TLC scanner IV was used to scan the developed and sprayed plate at 205 nm wavelength in absorbance mode by using the deuterium lamp. The slit dimensions were 4.00 × 0.45 mm, and the scanning speed was 20 mm/s.

Assay of Alliin

Standard alliin and test samples (2 µl each) were spotted on HPTLC plates. The percentage of alliin present in test samples (S1 to S4) was determined by measuring the area for the standard and test samples. Thereby the percentage of alliin was calculated for all samples were reported in Figure 11.

Assessment of Mycorrhizal Root Colonization (MRC) Percentage in the Root System

Roughly 1-2 g freshly collected fine roots were used for staining and the assessment of MRC percentage. Roots were washed in freshwater, cleared with 10% KOH, acidified with 1N HCl, and stained with 0.05% Trypan blue (Phillips & Hayman, 1970). Quantification of root colonization for AM fungi was conducted using the gridline intersection method (Giovanetti & Mosse, 1980). 100 fine stained root segments of each sample were observed under a compound microscope (Leica DM750). The presence or absence of AM fungal structure in the root system such as vesicles, arbuscules, and hyphae at particular fixed points was recorded, and the results were expressed as a percentage MRC of observations.

Statistical Analysis

Observations were made on growth & alteration in physicochemical properties of soil in the experiment land, nutrient uptake, PG, and yield were analyzed using SPSS (SPSS Inc. Version 17.0). Results were expressed as a one-way analysis of variance and the difference in output from 6 different treatments were determined according to Duncan's Multiple Range Test at a significant level of P<0.05.

Results and Discussion

Physio-Chemical Properties of Soil (Day Initial and at Harvest)

Associating the physio-chemical possessions of the soil before and at harvest of garlic were presented in Figures 2-10.

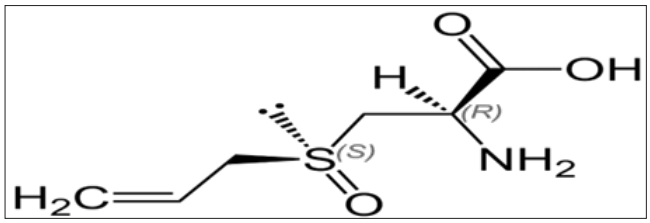
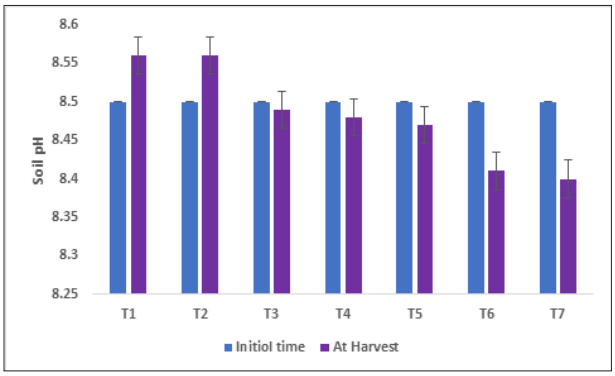


Figure 1: Available Alliin structure in garlic

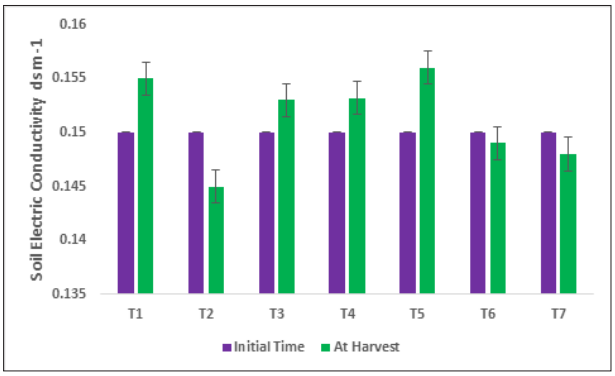
A slight alteration of pH was recorded between the treatments (Figure 2).



T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

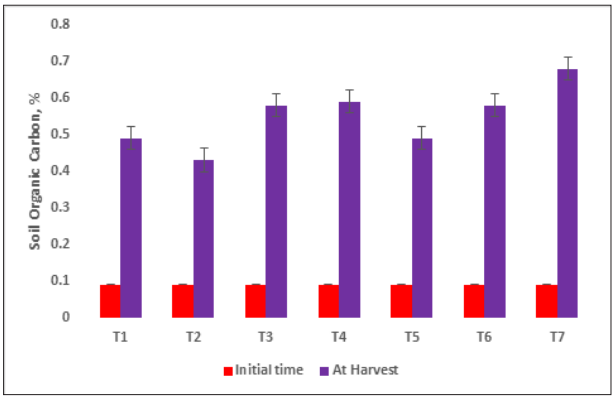
Figure 2: Soil pH at Initial time and after crop harvest (R-4)

Increase and decrease the response of nutrients concentration was observed with an increase in fertilizer treated, with and without PGPR and mycorrhiza inoculation. Microbial-mediated garlic showed a significant (P< 0.05) decrease in electrical conductivity (Figure 3).



T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 3: Soil Electric Conductivity at Initial time and after crop harvest (R-4)

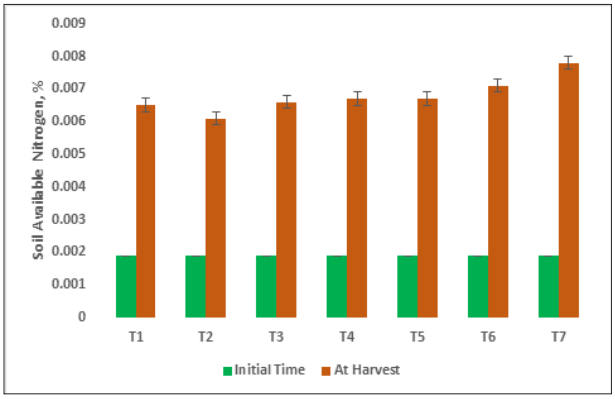


T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 4: Soil organic carbon at the initial time and after crop harvest (R-4)

The incessant variations of equilibrium between cations and anions present in the soil. Plant uptake of soluble salts through root systems by plants and or leaching of cations for instance calcium, magnesium can decrease the pH and at the same time chloride accumulation in the surface due to capillary action can be accountable for the decrease in EC (Prasad, 2021; Prasad, 2021; Prasad, 2021; Prasad, 2021; Prasad, 2021; Brinkman, 1980). Available N in the soil is directly associated with SOM. The consistent intensification in N is due to an increase in SOM and the microbial activities (MA) which make N available from organic matter (OM) to microbial inoculants treated plots. A tremendously significant increase (P<05) in available N (0.0078%) was recorded from the Absolute consortium

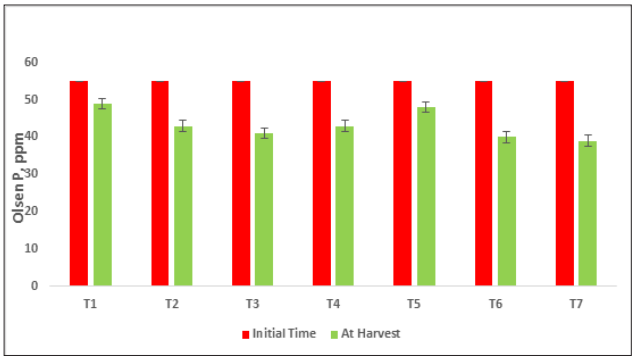
PGPR + AM fungi treated plot (Figure 5) and minimum in 0.0061% control (50% RRF).



T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 5: Soil available nitrogen concentration at an initial time and after crop harvest (R-4)

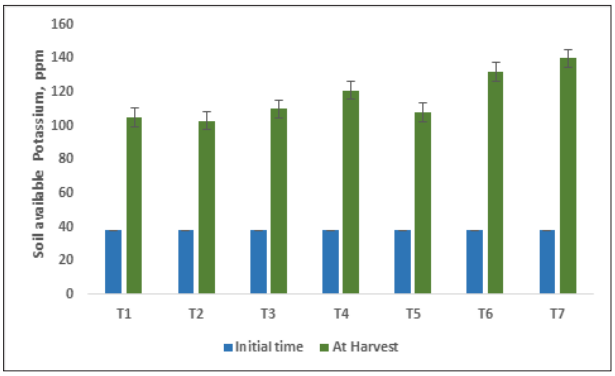
Available P content was observed significantly lower in treatment T7 (39 ppm) as compared to treatment control T2 (43 ppm) soil and it may be due to P mobilizing activity of mycorrhiza, added during plantation activities (Figure 6).



T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

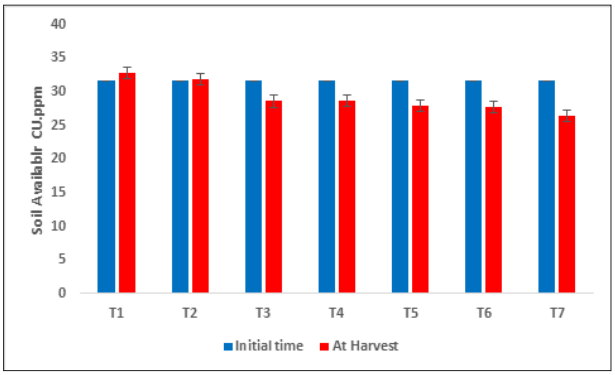
Figure 6: Soil Olsen's P concentration at the initial time and after crop harvest (R-4)

A sharp reduction in K content in soil was recorded in all microbial treated garlic compared to control (Figure 7). Maximum decrease of K has been recorded in Absolute consortium + AM fungi treatment T7 (140 ppm) and minimum in T2 (103 ppm) as compared to initial time (38ppm). A sharp decrease in copper, iron and zinc content in soil was noticed in all microbial treated garlic compared to control where CF was applied (Figure 8-10). Numerous factors counting weathering, upward translocation of soluble ions through capillary action, involvement from the degradation of plants litters can be responsible for such variation in different treatments of garlic crop (Ribak et al., 2004; Prasad, 2021).



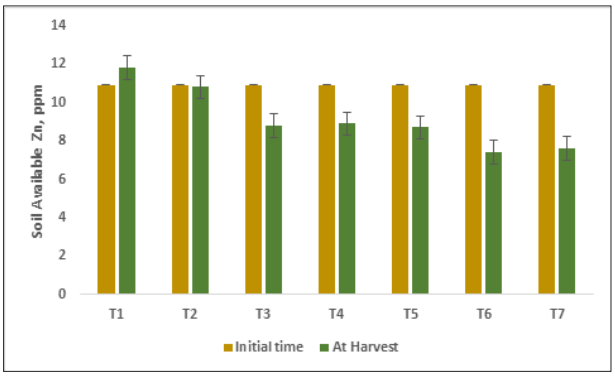
T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 7: Soil available Potassium concentration at an initial time and after crop harvest (R-4)



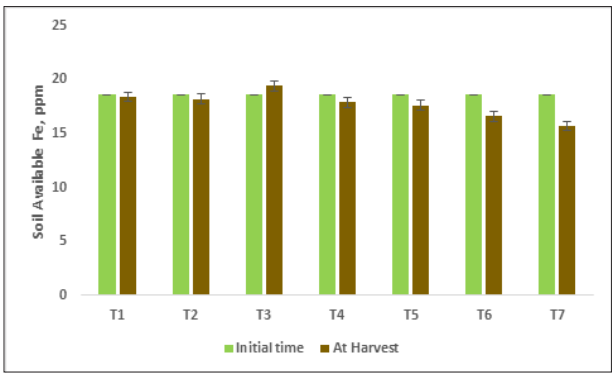
T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 8: Soil available copper concentration at an initial time and after crop harvest (R-4)



T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 9: Soil available Zinc concentration at an initial time and after crop harvest (R-4)



T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 10: Soil available Fe concentration at an initial time and after crop harvest (R-4)

Measurement of Plant Growth Parameter (MPGP)

Observations of Morphological Characters

Garlic plants treated with Absolute consortium PGPR + AM fungi achieved better than the untreated control. There were no records of significant differences at 45 DAS. At the time of harvest, garlic plants treated with Absolute consortium PGPR+ AM Fungi showed significantly increased growth parameters as compared to the other treatments. The supreme PG (71.23±1.68) was recorded in garlic treated with Absolute consortium PGPR + AM fungi (T7) as compared to the control treatment at the time of harvesting (Table 1).

Treatment	Analysis	Plant Height (cm)	No. of Leaves/plant	Leaf Moisture (%)
T1	45 DAS	14.65b±1.59	5.25c±1.69	82.11c±3.29
	At Harvest	69.26b±1.49	8.85b±2.52	28.45b±2.25
T2	45 DAS	13.56a±1.39	4.86c±0.58	81.45c±1.49
	At Harvest	62.18ab±1.69	7.89bc±2.15	28.23b±2.12
T3	45 DAS	14.15ab±1.45	5.56c±4.25	83.14c±4.1
	At Harvest	65.45a±2.51	11.55b±2.53	27.86b±2.23
T4	45 DAS	14.21c±4.12	5.55c±3.91	84.11c±1.34
	At Harvest	65.48b±2.51	11.64bc±2.54	28.45a±2.59
T5	45 DAS	14.15b±2.58	4.96b±0.59	83.11c±3.91
	At Harvest	54.23a±0.52	10.95b±1.21	28.85a±1.54
T6	45 DAS	14.55c±2.45	5.45c±2.25	84.23b±4.45
	At Harvest	69.45b±3.22	8.94ab±0.4	32.01b±4.11
T7	45 DAS	15.23c±2.52	5.95b±1.87	86.43b±2.57
	At Harvest	71.23a±1.45	9.45a±2.22	32.45a±0.59

±SE-Std error; Values in a column followed by the same letter are not significant at p<0.05 according to DMRM. T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum + Bacillus; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Table1: Morphological observation of Garlic crop (Mean ± SE) of 45 DAS and at harvest (N-20)

The association of garlic plants with multiple microbial consortia containing N fixer, PSB, KMB, BCA, and AM Fungi also correlates positively with PG and biomass. It is frequently observed that garlic plants respond positively to AM fungi symbiosis De Melo (2003) without which, a considerably heavy number of fertilizers are needed for optimal growth. Results agreed with (World Health Organization, 2010). The maximum number of leaves (11. 64 ± 2.54) recoded in T7 and the minimum (7.89 ± 2.15) was recorded from T2 (control 50% RRF) at harvest Table 1). Plant height and the number of leaves of inoculated garlic showed a significant (P<0.05) increase in growth parameters as compared to non-inoculated plants (table 1). Absolute consortium PGPR + AM fungi treatment produced the maximum LWP (86.43±2.57%) and the minimum (81.45±1.49%) was noticed in T2 at the time of 75 DAS. Maximum (32.45±0.59%) LWP was recorded from T7 and the minimum of (27.86±2.23%) in T3 at the time of harvest. PGPR and AM fungi association has positively correlated with PG and biomass (Bolandnazar et al., 2007; Prasad, 2021). It is assumed that the allium plant benefits positively from PGPR and AM fungi symbiosis (Prasad, 2017; Prasad, 2020; Prasad, 2021; Bolandnazar et al., 2007), it makes little growth without mycorrhiza unless heavily fertilized (Prasad, 2017; Prasad, 2020; Mosse, 1973; Smith & Read, 1997; Prasad, 2021; Prasad, 2021; Carrow & Dunvan, 2004). The consortium PGPR and AM fungi alone and with a combination (PGPR) treated garlic performed better than the untreated control. Significant differences were recorded between the treatments. Absolute consortium PGPR and AM fungi-mediated garlic showed a significant increase in plant height, the number of leaves/

plant, LWP, and leaf area compared to non-microbial control. Multiple strains of PGPR and AM fungi association have also positively correlated with PG and productivity. It is expected that the garlic plant benefited positively from Pseudomonas, Azotobacter, Azospirillum, Frateuria, Bacillus PGPR microbes, and AM fungi symbiosis in an early application.

Quality attributes characters of garlic recorded at harvest and mentioned in Tables 2 and 3. Maximum bulb neck thickness was noticed in T7 (1.12a±0.25cm) followed by T6 (1.9±0.11cm), T4 (0.97±0.18cm), T5 (0.93±0.17cm), T3 (0.92±0.16cm) and T2 (0.92±0.22cm) whereas the diameter of bulb is concerned, T7 (Absolute consortium PGPR + AM fungi) performed the maximum of 14.45±0.51 cm and minimum of 11.06c±0.79 cm in T5 (Table-2). Highest bulb size index was recorded in T7 (19.45a±0.46 cm^2) followed by T1 (16.56±0.42cm^2), T4 (16.24±0.82 cm^2), T3 (16.23±0.72 cm^2), T6 (15.98±0.52cm^2), T2 (15.45±0.48cm^2) and T5 (15.34±0.49 cm^2). The highest bulb weight of 58.15±2.29gm was observed from T7 (Absolute consortium PGPR+ AM fungi) and the lowest of 38.45c±3.59 gm from T2 (control 50% RRF) (Table 2). The data presented in the Table 2 reveals that the maximum bulb length (6.21a±1.53 cm) of garlic was recorded under treatment T7 (Absolute consortium PGPR + AM fungi) followed by T1 (6.12±1.59cm), T5 (6.03±1.29cm), T2 (5.85±1.39cm), T6 (5.67±1.54cm), T4 (5.18±2.29cm) and T3 (4.89±2.53±1.29cm). These results might be due to the role of mineral fertilizers in the promotion of garlic PG and the protagonist of biofertilizer in increasing the availability of nitrogen, phosphorus, and potassium to garlic plant absorption through consortium PGPR and AM fungi. Significant differences were recorded between microbial consortium-mediated garlic plants as compared to control. Results indicate that consortium PGPR and AM fungi improve almost all morpho- agronomic characters of garlic beneath field environments.

Treatments	Neck Thickness (cm)	Bulb diameter (cm)	Bulb size index (cm^2)	Weight of Bulb (gm)	Bulb length (cm)
T1	0.97b±0.18	13.14a±0.59	16.56b±0.42	56.36a±2.59	6.12ab±1.59
T2	0.92c±0.22	12.12c±0659	15.45c±0.48	38.45c±3.59	5.85b±1.39
T3	0.92b±0.16	12.45b±0.52	16.23bc±0.72	46.45b±2.29	4.89c±2.53
T4	0.95c±0.19	12.11b±0.49	16.24bc±0.82	49.96b±1.51	5.18bc±2.29
T5	0.93c±0.17	11.06c±0.79	15.34c±0.49	48.23c±2.89	6.03ab±1.29
T6	1.9ab±0.11	13.11ab±0.53	15.98±0.52	53.86b±1.45	5.67b±1.54
T7	1.12a±0.25	14.45a±0.51	19.45a±0.46	58.15a±2.29	6.21a±1.53

SE-Std error; Values in a column followed by the same letter are not significant at $p<0.05$ according to DMRM. T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum + Bacillus; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Table 2: Attributes characters of garlic crop (Mean ± SE) at the time of harvest (R-4)

Yield and its Attributes Characters

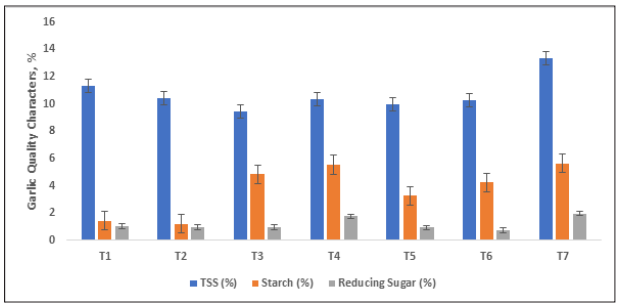
The garlic dry matter, dry biomass, no. of cloves/Bulb, and yield increased significantly in the plants receiving treatment of T7 compared to the other biological treatment and control. (Table 3). Maximum garlic dry matter was recorded in the treatment T7 (Absolute consortium PGPR + AM fungi) followed by T1 (6.12±1.59cm), T5 (6.03±1.29cm), T2 (5.85±1.39cm), T6 (5.67±1.54cm), T4 (5.18±2.29cm), and T3 (4.89±2.53±1.29cm) whereas a maximum number of cloves/bulb were recorded in T7 followed by T1, T6, T4, T3, T5, and T2. Maximum dry biomass was noticed in T7 (6.45±0.56 q ha^{-1}) followed by T5(6.34±0.26 q ha -1), T6 (6.26±0.44 q ha^{-1}), T1 (5.95±0.25 q ha^{-1}), T3 (5.35±0.58 q ha^{-1}), T2 (5.12±0.42 q ha^{-1}) and T4 (4.89±0.38 q ha^{-1}) (Table 3). Significance differences were noticed between the treatments. Absolute consortium PGPR + AM fungi-mediated garlic produced a greater yield than uninoculated control. The highest marketable yield was recorded from T7 (Absolute consortium PGPR + AM fungi) of 167.15±0.44 q h^{-1} and the lowest of 151.56±0.33 q h^{-1} in T2 where 50% RRF was applied (Table 3). The superiority of the treatments T7 (Absolute consortium PGPR+ AM fungi) may be due to the role of nitrogen fertilizers and biofertilizers application are increasing the availability of nitrogen to the garlic plant. The higher bulb yield may be due to greater root proliferation, more uptakes of nutrients and water, more photosynthesis rate, and enhanced food accumulation. Prasad (2021), Bolandnazar (2007) also reported the efficiency of PGPR strains and mycorrhiza as a potential supplement to nitrogenous fertilizer in garlic.

Treatment	Dry matter (%)	No. of Cloves/Bulb	Dry biomass (q h^{-1})	Yield (q h^{-1})
T1	32.45b±1.29	25.12±0.17	5.95c±0.25	159.45b±0.38
T2	29.45c±2.23	22.12±0.17	5.12c±0.42	151.56ab±0.33
T3	30.45b±1.28	24.09±0.17	5.35b±0.58	158.45ab±0.48
T4	31.14cd±1.21	24.15±0.17	4.89b±0.38	155.34b±0.31
T5	30.45bc±1.16	21.18±0.17	6.34bc±0.26	154.25ab±0.11
T6	31.86ab±1.28	24.45±0.17	6.26ab±0.44	158.56ab±0.56
T7	34.45a±1.25	26.45±0.17	6.45a±0.56	167.15a±0.44

±SE-Std error; Values in a column followed by the same letter are not significant at p<0.05 according to DMRM. T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Table 3: Bulb attributes characters of garlic (Mean± SE) at the time of harvest (R4).

The highest TSS% (13.354 %) was recorded from T7 (Absolute consortium PGPR + AM fungi) and the lowest (9.45%) from T3 (Figure 11). Maximum (5.65 %) starch was found in T7 and the minimum (1.23%) in T2 (Control 50% RRF) (Figure11).



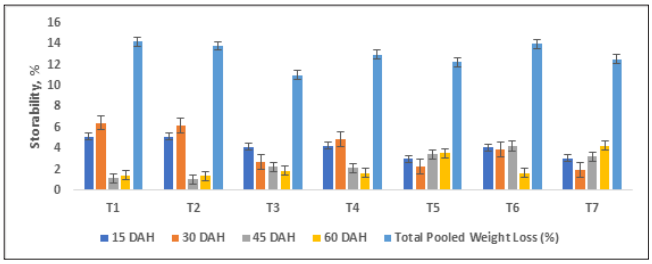
T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Consortium (NPK) + Azospirillum+ Bacillus + AM fungi; DAS= Days after sowing.

Figure 11: Effect of biofertilizers on quality characters of garlic at the time of harvest (R-4)

Percentage reducing sugar was found maximum (1.98%) in T7 and a minimum of 0.74% in T6. The dominance of the different types of consortium bioinoculant such as Absolute consortium PGPR + AM fungi might be because NKP has helped in dynamic vegetative growth and imported deep green color to the greenery which favored photosynthesis activity of the plants resulting in the greater accumulation of food material (Prasad, 2021; Bolandnazar et al., 2007).

Storability of Garlic Crops

Pooled weight loss in garlic was recorded for all seven batches to determine the impact of different treatments on the storability of garlic. It was observed that treatment T7 (Absolute consortium PGPR + AM fungi) accounted for the minimum pooled weight loss (12.53%) after 60 days of harvest and the maximum weight loss (14.20%) was recorded in treatment T1 (Control, 100% RRF) as shown in Figure 12.



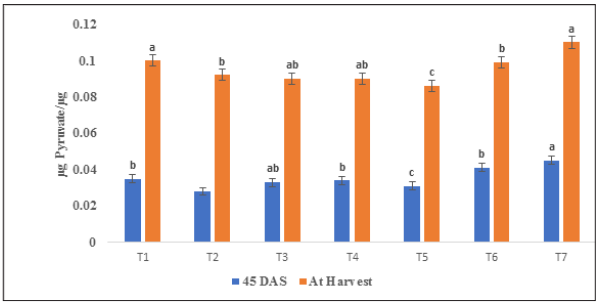
T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 12: Impact of biofertilizers on storability (% of weight loss) of onion after harvest (R-4)

Biochemical Changes in Garlic

Change in Medicinal Properties of Garlic

Alliin content in garlic is responsible for its medicinal properties as a possible cancer preventative. Treatment T7 shows improved Alliin content in garlic crops (0.11 µg Pyruvate/µg) as compared to other treatments as shown in figure 13. The increase in Alliin content also gives garlic its native pungency.



T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

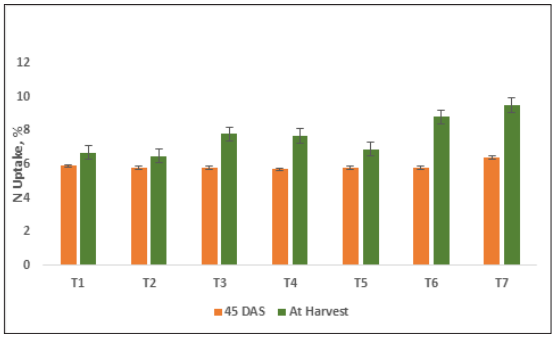
Figure 13: Total Alliin content per kg dried garlic at 45 DAP/S and harvest

Macronutrients Translocation by Shoot system

Nitrogen Translocation

An investigation of data indicates that N uptake (Figure 14)

through garlic plants shows that all the microbial treatments had a significant influence by N uptake as compared to NMC treatment. The maximum N uptake (6.4% at 75DAS and 9.5% at harvest) was obtained under treatment T7 (Absolute consortium PGPR + AM fungi) where the six microbial inoculants were applied. However, the lowest value of N uptake (5.8% at 75 DAS and 6.5% at harvest) by garlic shoot was verified under NMC treatment (50% RRF). The uptake of N by the garlic plants went on increasing with the successive microbial application because the uptake is a resultant of strength and biological yield.

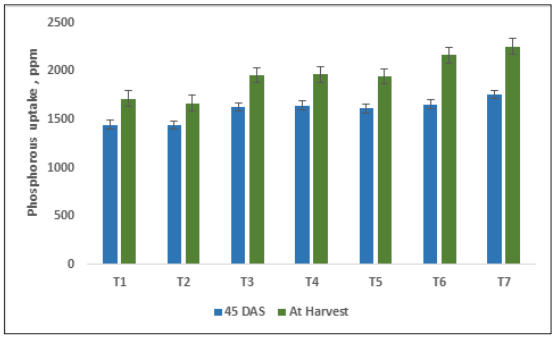


T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 14: Nitrogen uptake in garlic at 45 DAS and harvest (R-4)

Phosphorus Translocation

A glance at data in figure 15 shows the highest translocation of P (1756.71ppm at 45 DAS and 2254.54 ppm at harvest) by the garlic plants under treatment T7 (Absolute consortium PGPR + AM fungi). The minimum P uptake was recorded under T2 (1440.12 ppm at 45 DAS and 1665.34 ppm at harvest). The effect of PGPR + AM fungi application on P uptake was significant (Figure 15). P uptake increased may be due to improved absorption and utilization of available soil P at higher rates.

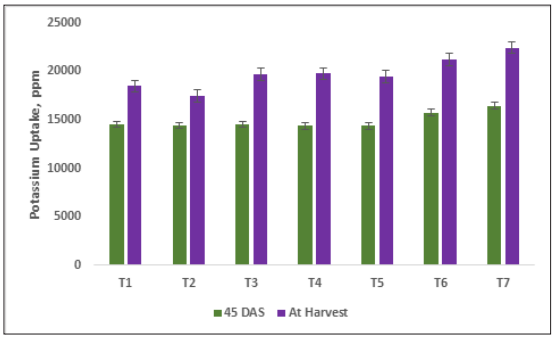


T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 15: Phosphorous uptake in garlic at 45 DAS and harvest (R-4)

Potassium Translocation

Potassium translocation in garlic plants has been presented in figure 16. An inquisition of data indicates that maximum K uptake (16458.45 ppm at 45 DAS and 22430.678 ppm at harvest) by garlic shoot system was recorded in the Absolute consortium PGPR + AM fungi treatment, where the six microbial stimulants were applied followed by T6 (Azotobacter + Azospirillum +Bacillus+ AM fungi), T4 (Azospirillum+ Bacillus+ AM Fungi), T3 (Azotobacter + Bacillus+ AM fungi)), T5 (Azotobacter + Azospirillum + Bacillus), T1 (Control, 100% RRF) and T2 (Control 50% RRF). K uptake was increasing may be due to improved absorption and utilization of potassium at higher rates of available soil potassium.



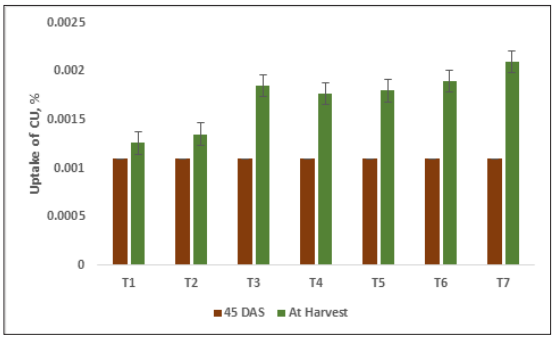
T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 16: Potassium uptake in garlic at 45 DAS and harvest (R-4)

Micronutrient Translocation by shoot

Copper Translocation

The foretaste of data presented in figure 17 shows the highest uptake of copper (0.002%) by the garlic shoot was recorded in treatment Absolute consortium PGPR + AM fungi. The effect of six consortium bio inoculants treatments was noticed to exert a significant effect on the copper removal by garlic shoot. The minimum copper (0.00126%) uptake was recorded under NMC (100% RRF) treatment at harvest.

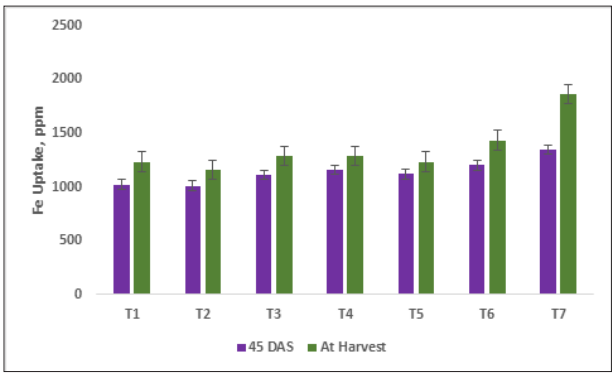


T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 17: Cupper uptake in garlic at 45 DAS and harvest (R-4)

Iron Translocation

The inspection of data presented in figure 18 reveals the uppermost uptake of iron (1345.56ppm at 45 DAS and 1864.67ppm at harvest) by the garlic shoot was recorded in treatment T7. The effect of PGPR and AM fungi in treatments was noticed to exert a significant effect on the iron removal by garlic shoot followed by NMC there 100% and 50% RRF was applied (1023.45 ppm at 45 DAS and 1234.65ppm at harvest, 1011.56 ppm at 45 DAS and 1157.34ppm at harvest respectively).

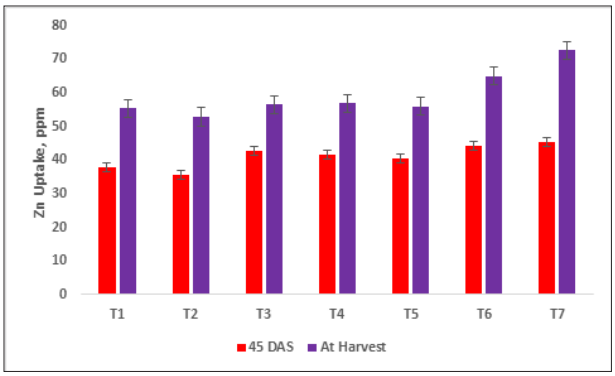


T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 18: Iron uptake in garlic at 45 DAS and harvest (R-4)

Zinc Translocation

The data presented in figure 19 exposes that maximum zinc translocation (45.45 ppm at 45 DAS and 72.67 ppm at harvest) by garlic recorded under treatment T7 (Absolute consortium PGPR + AM fungi) and minimum in T2 (35.56ppm at 45 DAS and 52.86ppm at harvest). The microbial inoculants such as PGPR and AM fungi-mediated garlic plants were found to exert a significant effect on the zinc uptake by garlic shoot systems.

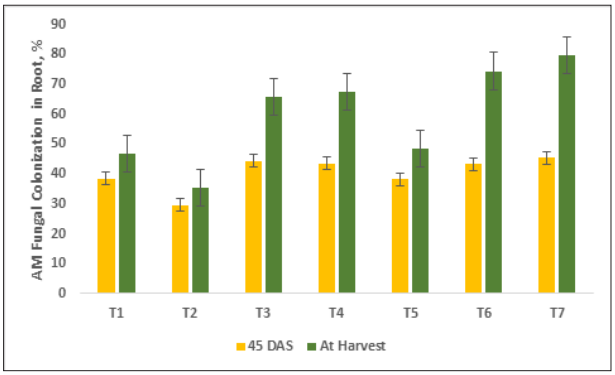


T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 19: Zinc uptake in garlic at 45 DAS and harvest (R-4)

AM fungal Root Colonization

AM fungal root colonization was observed in all treatments (T1 - T7) including uninoculated control plot plants as mentioned in Figure 20. The root colonization level of untreated plants ranges from 29.56- 35.56% in treatment T2 (Control, 50% RRF) and 38.45-46.67% in treatment T1(Control,100% RRF). The maximum root colonization of AM fungi ranges from 45.34-79.56% were recorded in plants treated with Absolute consortium PGPR + AM fungi. AM fungal mediated plants have been stimulated by higher water and mineral nutrients uptake from the soils because they increased the total root surface (Prasad, 2017; Prasad, 2020; Bolandnazar et al., 2007; Carrow & Dunvan, 2004; Giovanetti & Mosse, 1980; El. Desuki et al., 2006; Gerdemann, 1968). The colonization potential of AM fungi decreases with an increase in fertilizer application (18). Root colonization percentage (RCP) was affected with an increase in fertilizer application in all the treatments and crops and values were statistically ($P<0.05$ level) different compared to treatments.



T1 = Control (100% RRF); T2=Control (50% RRF); T3 = Azotobacter + Bacillus + AM fungi; T4 = Azospirillum + Bacillus + AM fungi' T5 = Azotobacter + Azospirillum; T6 = Azotobacter + Azospirillum+ Bacillus + AM fungi; T7 = Absolute consortium PGPR + AM fungi; DAS= Days after sowing.

Figure 20: Mycorrhiza root colonization percentage in garlic at 45 DAS and harvest (R-4)

The outcomes in this study discovered that increased PG parameters, nutrients concentration in soil, plant shoot uptake, and AM fungal RCP when Absolute consortium PGPR and AM fungi inoculated plants, and this was comparable to uninoculated plants treated with high dosages of fertilizer (100% RRF). The influence of PGPR and AM fungi in decreasing fertilizer demand of major crop species was reported by (Bolandnazar et al., 2007; Hanway& Heidel, 1952; Bolandnazar et al., 2007; Prasad, 2021; Singh et al., 2007). It is assumed that PGPR's and AM fungi have the potential to reduce the high application rate of fertilizer needed to produce a high garlic yield (Prasad, 2020; Prasad, 2021; Prasad, 2021). Moreover, the garlic plant benefits positively from AM fungal symbiosis (Prasad, 2021; Steinmetz & Potter, 1996). It creates small growth without mycorrhiza unless severely fertilized (Smith & Read, 1997; Gerdemann, 1968; Krest & Keusgen, 1999; Griffiths et al., 2002; Lawson, 1998; Ortas, 2003; Steinmetz & Potter, 1996). Diminution in PG characteristics, shoot nutrient content, RCP, and yield of control plant with

improvement in fertilizer function. From the results, it appears that garlic should be incorporated with a consortium of Azotobacter, Azospirillum, Pseudomonas, Frateuria, Bacillus, AM fungi with and without combination for better growth, yield, and quality. For increasing storability, the combination of PGPR and AM fungi is effective. Nevertheless, the Absolute consortium PGPR + AM fungi bioinoculant produced the best result compared to different combinations of biofertilizers and recommended rate of CF's. The next may be a particular level with certain considerations of sustainability in production and environmental protection.

Conclusion

Absolute consortium PGPR and AM Fungi utilization prejudiced early growth and nutrient uptake of N, P, K, Cu, Fe, and Zn. Outcomes from treatment T7 in this study prove that consortium PGPR and AM Fungi could eliminate the need for CFs required to produce cash crop garlic by increasing PG parameters, yield, nutrient uptake, and AM Fungi root colonization. Even though Azotobacter and Azospirillum are predominantly associated with biological nitrogen fixation, their applications in the sustainable production of garlic go way beyond fixing atmospheric nitrogen. At harvest, it was observed that soil treated with Absolute consortium PGPR, and AM Fungi had improved levels of pH, SOC, and EC as well. The present study revealed that plants mediated with consortium PGPR, and AM fungi can play a significant role in reducing CFs inputs in sustainable production systems (SPS) of the garlic cash crops. PGPR and AM fungal biofertilizers inoculation influenced growth, productivity, TSS, starch, reducing sugar, Alliin content, and nutrients uptakes (N, P, K, Cu, Fe, and Zn) as compared to the different rates of CFs. From this study, it can be determined that using Absolute consortium PGPR and AM fungi inoculums could reduce the CFs inputs needed to produce vegetables since increased PG parameters, LMP, nutrients concentration, and shoots, and RCP were obtained when Absolute consortium PGPR and AM fungi were applied to garlic plants, and this was comparable to NMC plants treated with 100% and 50% RRF. It can be determined that the usage of Absolute consortium PGPR and AM fungi to economize on fertilizer use in garlic crop production provides a sustainable and environmentally safer substitute and farmers should be encouraged to use biofertilizers for sustainable development.

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Conflict of Interest

No conflict of interest is associated with this work.

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