Original Research Article

SYNERGISTIC INFLUENCE OF BACTERIAL AND FUNGAL INOCULUM ON MICROBIAL BIOMASS CARBON, PHOSPHORUS AND NITROGEN

ABSTRACT

Background: Soil microbial biomass plays a critical role in nutrient cycling, storing significant amounts of carbon, nitrogen, and phosphorus essential for soil fertility. Beneficial microorganisms like *Azotobacterchroococcum* and *Serendipitaindica* have demonstrated plant growth-promoting properties by enhancing nutrient uptake, stress resistance, and microbial biomass. Their synergistic use aligns with sustainable agriculture practices, reducing dependency on chemical fertilizers and improving soil health.

Material and Methods: The study utilized cultures of *S. indica* and *A.chroococcum* grown in jaggery-based broth for bulk multiplication were made during the present study. Tests in the field were carried out during May–October 2023, with treatments: Control (C), T1 (*A. chroococcum*), T2 (*S. indica*), and T3 (combination). Microbial biomass carbon (MBC), Phosphorus(MBP), and Nitrogen(MBN) were estimated using fumigation extraction techniques. Statistical analysis was performed using ANOVA and Duncan's Multiple Range Test at a 5% significance level.

Results: The study revealed substantial increase in soil microbial biomass carbon (MBC), phosphorus (MBP), and nitrogen (MBN) in rice varieties PB 1121 and PB 1718 following treatment with *S. indica* and *A. chroococcum*. The combined application of both microbes demonstrated a synergistic effect, resulting in higher increases in MBC, MBP, and MBN compared to single treatments. PB 1718 exhibited the greatest enhancement under the combined treatment.

Conclusion: The co-inoculation of *A. chroococcum* and *S. indica* significantly enhanced soil microbial biomass carbon, phosphorus, and nitrogen compared to individual treatments or control. This synergistic effect supports nutrient cycling and sustainable agricultural practices by reducing chemical fertilizer use and improving soil fertility. The findings highlight the potential of microbial inoculants in developing innovative bio-fertilizers, fostering long-term agricultural sustainability.

Keywords: Soil microbial biomass, Carbon, Phosphorus, Nitrogen*, S. indica, A. chroococcum*

1. INTRODUCTION

Carbon (C), nitrogen (N), and phosphorus (P) are essential elements for plant growth (Chang et al., 2022). Carbon provides the main source of energy in ecosystems (Wang et al., 2021), while nitrogen and phosphorus are vital for processes like electron transfer in respiration and are key factors that

limit primary production (Tang et al., 2018; Zhang et al., 2018). In soil ecosystems, these nutrients support the growth and reproduction of microbes by supplying essential elements. Healthy microbial communities improve nutrient cycling and break down organic matter, making nutrients more accessible to plants. The interaction between these nutrients, soil microbes, and plants creates an environment where plants can grow better, produce higher yields, and handle stress more effectively. Carbon, nitrogen, and phosphorus are more than just nutrients—they are essential for plant health and productivity, powering the complex processes that sustain life both in the soil and above ground.

Soil microbial biomass makes up about 1–3% of the total organic carbon in soil and holds significant amounts of nutrients like nitrogen (N) and phosphorus (P). Microbes have lower C:N and C:P ratios compared to plants, meaning they store proportionally more nutrients (Schmidt et al., 2002, Bar-On et al., 2018). Microbial phosphorus can account for about one-third of the total soil phosphorus in certain ecosystems like arctic heath. On a global scale, the amounts of nitrogen and phosphorus stored in microbes are similar to what plants hold, even though plants store much more carbon (Zechmeister-Boltenstern et al., 2015).Small changes in microbial biomass can significantly affect how nutrients like nitrogen and phosphorus are released into the soil or taken up by microbes, especially in soils where these nutrients are limited and plant growth depends on them (Jonasson et al., 1996, 1999, Whitman et al., 1998). Therefore, microbial biomass plays a critical role in storing carbon and nutrients in an ecosystem. It's also an essential factor to consider when studying the effects of climate change or other global changes on ecosystems (Smith & Paul, 1990).

The integration of beneficial microorganisms into agricultural practices has garnered significant attention for its potential to enhance soil fertility and crop productivity sustainably. Among these microorganisms, the bacterium *Azotobacterchroococcum* and the fungus *Serendipitaindica* (formerly *Piriformosporaindica*) have been extensively studied for their plant growth-promoting properties. *A. chroococcum* is a free-living nitrogen-fixing bacterium known to improve plant growth by fixing atmospheric nitrogen and producing growth-promoting substances such as indole-3-acetic acid (IAA). *S. indica* is an endophytic fungus that forms symbiotic relationships with plant roots, enhancing nutrient uptake and providing resistance against various stresses (Qu et al., 2024, Serazetdinova et al., 2024).

The soil microbial biomass constitutes a significant reservoir of carbon and phosphorus, which are crucial for nutrient cycling and soil fertility. Research has shown that microbial biomass carbon (MBC) and phosphorus (MBP) are vital components of soil organic matter and are essential for sustaining agricultural productivity (Schmidt et al., 2019). Studies indicate that the presence of A. chroococcum and S. indica enhances the availability and storage of these nutrients in the soil.Recent studies have explored the synergistic effects of co-inoculating plants with *A. chroococcum* and *S. indica*. For example, a study on wheat production showed that A. chroococcum, in conjunction with blue-green algae, significantly improved soil microbial biomass carbon and overall soil fertility (El-Sharkawy et al., 2024).

The use of microbial inoculum such as *A. chroococcum*and *S. indica* aligns with sustainable agricultural practices. These microorganisms reduce the need for chemical fertilizers, thereby minimizing environmental pollution and promoting soil health. By enhancing microbial biomass carbon and phosphorus and nitrogen, *A. chroococcum* and *S. indica* contribute to long-term soil fertility and agricultural sustainability. The synergistic influence of *A. chroococcum* and *S. indica*on soil microbial biomass presents exciting opportunities for future research and application. Understanding their combined effects on soil nutrient dynamics and plant health can lead to the development of innovative bio-fertilizers and soil amendments. Hence in this study we aim to discuss the Synergistic effect of *A. Chroococccum* and *S. Indica*inoculum on Microbial Biomass Carbon (MBC), Phosphorus (MBP), and Nitrogen (MBN).

2. MATERIALS AND METHODS

2.1 Study Site

The study site is located at 77° 42' 43.60" N and 29° 4' 16.09" E, representing the geographical coordinates of Shobhit Institute of Engineering and Technology, Meerut. The land type is classified as built-up, with high groundwater prospects, making it favourable for various water-based applications. The geomorphology of the site is identified as alluvial, which is characteristic of fertile and productive landforms.

2.2 Experimental Design

Twenty four fields (each measuring 2 x 2 meter) were prepared using standard agronomical practices for cultivation of two varieties of rice in research farm of Shobhit Institute of Engineering and Technology Meerut. Sowing was done in May 2023 and transplantation in July 2023 when the seedlings were 40-day-old having 8-10 cm in size. The rice varieties PB 1121and PB 1718 were procured from Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut and these are long duration varieties. All recommended standard cultivation practices for both varieties were used.
A Completely Randomized Design (CRD) with 3 replicates for each treatment was used in this study. 14-day-old microbial biomass of *S. indica* and 4-day-old biomass of *A. chroococcum* cultured in 100mL medium were added in 500g sterile saw dust as carrier and spread in 2 x 2 meter field where transplantation was made. Control (C) field was treated similarly but without microbial inoculum. Treatment (T1) was treated with *A. chroococcum* alone, T2 with *S. indica* alone while T3 included combination of both *A. chroococcum*and *S. indica*in equal ratio by mixing half of each inoculum.

2.3 Soil Biomass Estimation

2.3.1 Soil microbial biomass carbon (MBC) estimation

Soil microbial biomass carbon (MBC) was estimated using the fumigation extraction method given by Jenkinson and Ladd, 1981. 10 gm of 2 mm sieved soil was taken in two sets of 50 ml Erlenmeyer flask. One set was fumigated by placing it in a desiccator with ethanol-free chloroform for 24 hours, while the other set was kept in a refrigerator as the non-fumigated control. After fumigation, both sets were extracted with 25 ml of 0.5 M K_2SO_4 solution and shaken for 30 minutes. The extract was filtered using Whatman filter paper no. 42.For organic carbon measurement, 5 ml of the filtered extract was digested with a pinch of potassium persulfate ($K_2S_2O_8$) and 1 ml of 0.025 M H_2SO_4 in a digestion block at 120 °C for 2 hours. The evolved CO_2-carbon was trapped in a 6 ml vial containing 4 ml of 0.1 N NaOH and placed within the digestion tube. A blank was prepared using 5 ml of K_2SO_4 instead of a soil sample. After digestion, the diffusion tube is left undisturbed for 12 hours to allow complete absorption of the evolved CO_2-carbon.

Following this, the shell vial containing the alkali was removed, and the unused or unreacted alkali was titrated against 0.1 N HCl in the presence of 1 M $BaCl_2$ to stabilize the trapped CO_2-carbon. Phenolphthalein was used as the indicator. The efficiency factor (Kc) for extraction is set at 0.45. The MBC was calculated using the formula:

$$MBC = \frac{OCf - OCuf}{Kc}$$

where,OC*f* represents the organic carbon extracted from fumigated soil,
OC*uf* represents the organic carbon extracted from non-fumigated soil, and
K_c is the efficiency of extraction =0.45.

2.3.2 Soil microbial biomass phosphorous (MBP) estimation

Soil microbial biomass phosphorus (MBP) was measured using a modified fumigation and $NaHCO_3$ extraction technique given by Brookes et al., 1982. 10 gm of 2 mm sieved soil were divided into two sets and placed in 50 ml glass beakers. One set was fumigated by placing it in a desiccator with ethanol-free chloroform for 24 hours, while the other set was kept in a refrigerator as the non-fumigated control. Both fumigated and non-fumigated soil samples were extracted with 0.5 N $NaHCO_3$. 5 gm of soil was placed in a 150 ml conical flask, and 50 ml of 0.5 N $NaHCO_3$ was added. The mixture was shaken for 5 minutes on a rotating shaker.

Before filtration, phosphate-free charcoal was added to decolorize the filtrate. From the filtrate, 5 ml was transferred into a flask and mixed with a few drops of p-nitrophenol, causing the solution to turn yellow. To adjust the pH to 5.0, approximately 0.5 ml of 5 N H_2SO_4 was added drop by drop until the yellow colour becomes colourless. The volume was then made up to 20 ml, and 4 ml of ascorbic acid was added, followed by making the final volumeupto 25 ml. The addition of ascorbic acid results in the development of a blue colour, and the optical density (OD) was measured at 730 nm. An efficiency factor (Kp) of 0.40 was used to convert the phosphorus released during fumigation into microbial

biomass phosphorus. The calculated MBP represents the phosphorus contained within the microbial biomass of the soil sample.

2.3.3 Soil microbial biomass Nitrogen (MBN) estimation

Soil microbial biomass nitrogen (MBN) was estimated using the fumigation extraction method given by Brookes et al., 1985. 10 grams of 2 mm sieved soil was taken and divided into two sets, each placed in a 50 ml glass beaker. One set was fumigated with ethanol-free chloroform for 24 hours in a desiccator, while the other set was kept in a refrigerator as the non-fumigated sample. Both the fumigated and non-fumigated samples were then extracted using 40 ml of 0.5 M K_2SO_4 solution at a 1:4 soil-to-extractant ratio, followed by shaking for 30 minutes. The extract was filtered through Whatman filter paper No. 42, and 30 ml of the filtrate was transferred to a digestion tube. To which, 1 ml of 0.165 M $CuSO_4$ and 10 ml of concentrated H_2SO_4 were added, and the mixture was digested at 390 ± 2°C for 3 hours. After digestion, the residue was diluted with distilled water, and total nitrogen is measured using the Kjeldahl method. The MBN is then calculated using an efficiency factor (Kn) of 0.54.

$$MBN = \frac{Nf - Nuf}{Kn}$$

where, N*f* represents the organic carbon extracted from fumigated soil,
N*uf* represents the organic carbon extracted from non-fumigated soil, and
K_n is the efficiency of extraction =0.54.

2.4 Statistical analysis

ANOVA was used to examine experimental data with the IBM SPSS Statistics 20. Analysis of variance (ANOVA) was conducted to compare the significant or insignificant difference in the effect of treatments on microbial biomass carbon, phosphorus and nitrogen using Duncan's Multiple Range test with the least significant difference at a 5% level of significance (α=0.05).

3. RESULTS

3.1 Soil microbial biomass carbon (MBC)

The present study revealed that the microbial biomass Carbon (MBC) content soil in *S. indica*or *A. chroococcum* treated variety PB 1121 increased by 11 and 67% respectively over the control. The combined effect of *S. indica*and *A. chroococcum* further increased the microbial biomass Carbon (MBC) content by 122 % suggesting its synergistic action .Similarly, the microbial biomass Carbon (MBC) content soil in *S. indica*or *A. chroococcum* treated variety PB 1718 increased by 120 and 146% respectively over the control. Their combined application further enhanced Soil microbial biomass carbon (MBC)by 213%.

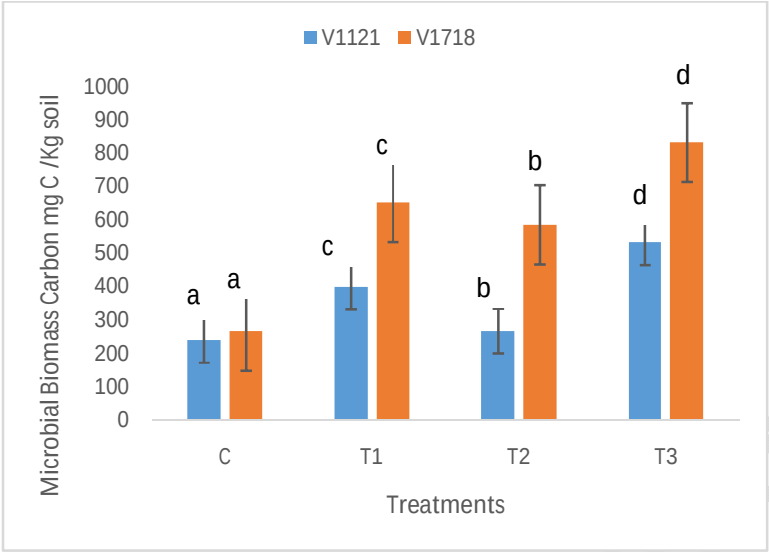

Fig 1: *S indica* and *A. chrococcum* for the improvement Microbial Biomass Carbon (MBC) in rice varieties PB 1121 and PB1718. Data are the means of three replicates (n = 3).

3.2 Soil microbial biomass Phosphorous (MBP)

The present study revealed that the microbial biomass phosphorus (MBP) content soil in *S. indica*or *A. chroococcum* treated variety PB 1121 increased by 197 and 218% respectively over the control. The combined effect of *S. indica*and *A. chroococcum* further increased the microbial biomass phosphorus (MBP) content by 273% suggesting its synergistic action .Similarly, the microbial biomass phosphorus (MBP) content soil in *S. indica*or *A. chroococcum* treated variety PB 1718 increased by 17 and 54% respectively over the control. The combined effect of *S. indica*and *A. chroococcum* further increased the microbial biomass phosphorus (MBP) content by 147%.

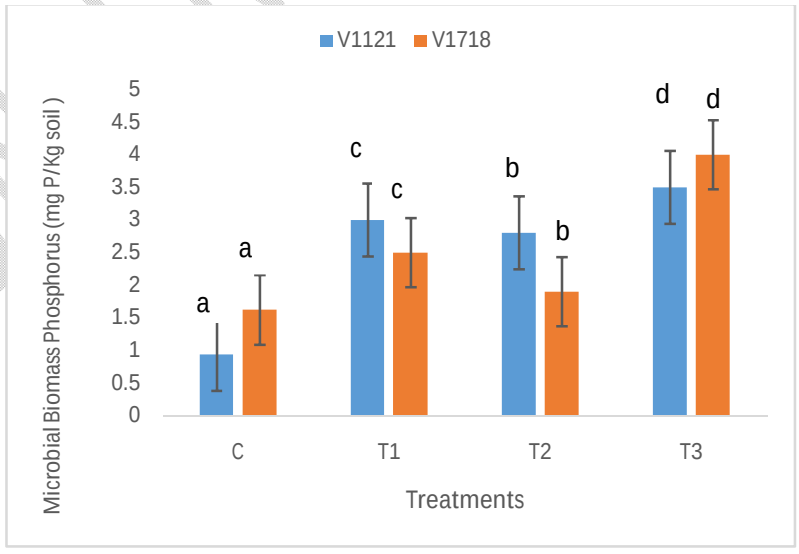

Fig 2: *S indica* and *A. chrococcum* for the improvement Microbial Biomass Phosphorus (MBP) in rice varieties PB 1121 and PB1718. Data are the means of three replicates (n = 3).

3.3 Soil microbial biomass Nitrogen (MBN)

The present study revealed that the microbial biomass Nitrogen (MBN) content soil in *S. indica*or *A. chroococcum* treated variety PB 1121 increased by 12.5 and 67.5% respectively over the control. The combined effect of *S. indica*and *A. chroococcum* further increased the microbial biomass Nitrogen (MBN) content by 123% suggesting its synergistic action .Similarly, the microbial biomass Nitrogen (MBN) content soil in *S. indica*or *A. chroococcum* treated variety PB 1718 increased by 123 and 148% respectively over the control. The combined effect of *S. indica*and *A. chroococcum* further increased the microbial biomass Nitrogen (MBN) content by 216%.

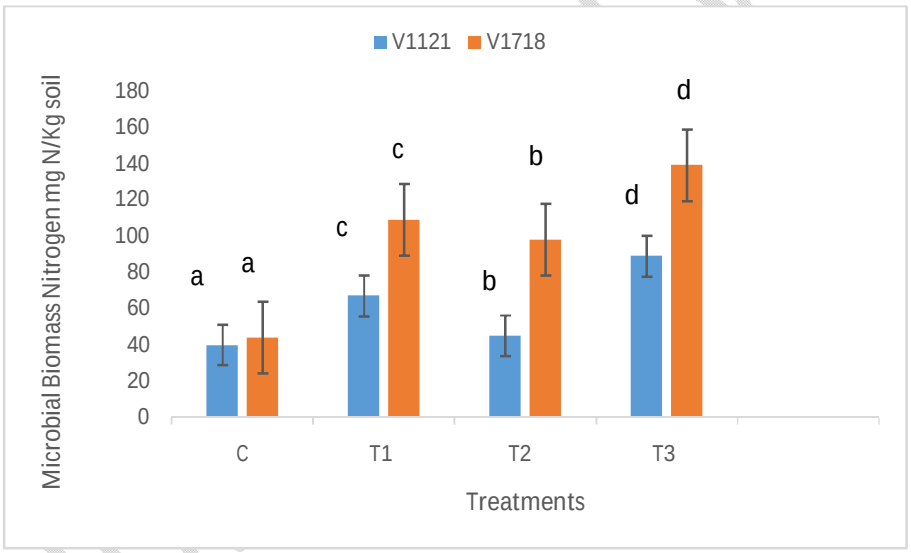

Fig 3: *S indica* and *A. chrococcum* for the improvement Microbial Biomass Nitrogen (MBN) in rice varieties PB 1121 and PB1718. Data are the means of three replicates (n = 3).

4. DISCUSSION

This study shows that adding microbial inoculants significantly improves the levels of microbial biomass carbon (MBC), phosphorus (MBP), and nitrogen (MBN) in the soil of rice varieties PB 1121 and PB 1718. The combination of *S. indica* and *A. chroococcum* had the strongest effect, indicating that these microbes work well together to enhance soil health.

The increase in MBC from using microbial inoculants is consistent with previous studies that highlight the importance of beneficial microbes in boosting soil organic carbon. For example, a study by Philip

et al. (2018) found that combining inorganic fertilizers with biofertilizers, like *A. chroococcum*, greatly increased soil microbial biomass carbon and enzyme activity in wetland rice fields.

The rise in MBP seen in this study matches findings from other research showing that microbial inoculants can improve phosphorus availability and the structure of soil microbes. For instance, a study on mixed cropping systems found that combining organic materials with beneficial microbes improved soil nutrients and increased microbial biomass phosphorus (Wang et al., 2024). Also, managing nano-fertilizers along with microbial inoculants was shown to significantly boost soil health and increase microbial biomass phosphorus levels (Sahoo et al., 2024).

The increase in soil microbial biomass nitrogen (MBN) with microbial inoculation has also been widely reported. Recent studies show that adding rice straw and nitrogen fertilizers can boost microbial activity and nitrogen fixation, improving soil health and the balance of soil microbes (He et al., 2024). Furthermore, using nitrogen fertilizers along with beneficial microbes in rice farming has been shown to improve nitrogen use efficiency and increase microbial biomass nitrogen levels in the soil (Ju et al. 2024).

5. CONCLUSION

The study emphasizes on the use of microbial inoculants in enhancing soil microbial biomass carbon, phosphorus, and nitrogen. The combined effect of *S. indica* and *A. chroococcum* suggests that using both microbes together can be an effective approach to improve soil health and nutrient availability. There was a constant maximum rise from the combination effect, suggesting a synergistic impact between the two microbes. *S. indica* positively impacts rice plant production and general health by developing mutualistic connections with plants. It eventually leads to increased crop production.This study demonstrates the potential of *A. chroococcum* and *S. indica* as biofertilizers, providing a sustainable method of enhancing crop production and soil quality without excessively depending on chemical fertilizers. Future research should investigate the long-term advantages and potential uses of these microbial mixtures in sustainable farming practices.

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