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ARTICLE

Rhizobium Inoculation and Micronutrient Addition Influence the Growth, Yield, Quality and Nutrient Uptake of Garden Peas (*Pisum sativum* L.)

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ABSTRACT

Garden pea productivity and qualities are hampered in zinc (Zn), boron (B), and molybdenum (Mo) deficient soil. Thus, the combination of micronutrients (i.e., Zn, B, and Mo) and rhizobium is necessary to increase the productivity and quality of garden peas, since this management for garden peas is neglected in Bangladesh. Therefore, the present study was made to assess the effectiveness of rhizobium inoculant singly or in combination with the micronutrients (i.e., Zn, B, and Mo) on growth, yield, nutrient uptake, and quality of garden peas. Treatments were: T_1 = Control, T_2 = *Rhizobium* inoculation at 50 g/kg seed, $T_3 = T_2 + Zn_3Mo_1$, $T_4 = T_2 + B_2Mo_1$, $T_5 = T_2 + Zn_3B_2$, $T_6 = T_2 + Zn_3B_2Mo_1$ and $T_7 = Zn_3B_2Mo_1$. All treatments were arranged in a randomized complete block design and repeated all treatments in three times. The application of 3 kg Zn, 2 kg B, and 1 kg Mo ha^{-1} with inoculation of *Rhizobium* at 50 g kg^{-1} seed (T_6) facilitated to increase of 44.8% in the green pod and 29.7% seed yield over control. The same treatment contributed to attaining the maximum nodulation (25.3 plant^{-1}), Vitamin C (43.5 mg 100 g^{-1}), protein content (22.2%), and nutrient uptake as well as accumulation in garden peas. Among all treatment combinations, treatment T_6 was found superior to others based on microbial activities, soil fertility, and profitability. The results of the study found that the application of 3 kg Zn, 2 kg B, and 1 kg Mo ha^{-1} in combination with *Rhizobium* inoculation (50 g kg^{-1} seed) can improve the yield and quality of garden peas. The results of the study have the potential for the areas, where there is no use of *Rhizobium* inoculant or Zn, B, and Mo fertilizer for cultivation of garden pea.

KEYWORDS

Nodulation; nutrient content; *Pisum sativum* L.; profitability; rhizobium; Zn; B; Mo

1 Introduction

The increasing global population is a great challenge for ensuring food and nutritional security in the current era of climate change [1]. Additionally, declining soil health with time is another challenge for achieving higher crop productivity [2]. Therefore, it is inevitable that the food demands of the increasing population will be met by increasing agricultural productivity [3]. Garden pea is a legume crop that can play a key role in attaining food and nutritional security [4]. It is enriched with high protein and carbohydrate contents [5] and is also a rich source of minerals and antioxidants [3]. Garden pea cultivation is convenient for conserving soil fertility through biological nitrogen fixation [6]. Legume crops have their own physiological and morphological features to procure nitrogen via symbiotic bacteria [7], which require an appropriate amount of essential micronutrients (Zn, B, and Mo) and biofertilizers for improved growth and development [6]. Mostly, inequity and excess chemical fertilizer, particularly nitrogenous fertilizer application, increase crop production but affect the ecological balance, deteriorating soil, water, and human health [8,9]. Thus, the excessive use of inorganic fertilizers is desirable in advanced technologies to reduce production costs and save the environment and human health [10]. Consequently, the integration of an appropriate amount of micronutrients (zinc, boron, and molybdenum) with *Rhizobium* inoculant is crucial and has emerged as an innovative technology for ensuring garden pea growth and yield and sustaining soil fertility [11,12].

Rhizobium plays a vital role in improving soil health by influencing the growth and development of plants through the formation of root nodules and N fixation [13–15]. *Rhizobium* inoculants support the ability of NPK and other plant nutrients to be absorbed by crops, triggering the growth and development of plants and resulting in increased crop yields [16,17]. Several researchers have reported that the yield of several crops can increase by approximately 25% due to the influence of *Rhizobium* and that the use of N-and P-based fertilizers can decrease the yield by approximately 25% to 50%, respectively [18,19]. Other studies have shown that the use of effective *Rhizobia* strains can enhance legume productivity [13,20].

Zinc (Zn), boron (B), and molybdenum (Mo) are the three most deficient micronutrients in the acidic and calcareous soils of South Asia, including Bangladesh and India, and their deficiencies in crop productivity have been reported to be approximately 45%, 46%, and 31%, respectively [21]. Earlier findings also reported that Zn, B, and Mo are crucial for enhancing the fixation of N in legumes [22,23]. Among these micronutrients, Zn is also involved in metabolism as well as biological N fixation through the formation of nodules [24]. In addition, Zn influences the water uptake capacity of plants and transports nutrients, leading to increased crop productivity and reducing the adverse effects of various stresses [25,26]. Zn is also required in plants for hormone biosynthesis [27] and physiological processes of protein synthesis [24]. B is involved in plant cell wall formation and stabilizes nodular cell walls and membrane stability [28,29]. B assists in increasing the flowering, pollen germination, and growth of pollen tubes, pod setting, and yield of legumes [30]. It is beneficial for chlorophyll synthesis and carbohydrate metabolism [31]. B stimulates the uptake and acquisition of macronutrients by plants, particularly N, P, and K [32]. On the other hand, Mo has a significant role in proper plant metabolic functioning and is involved in enzymatic activities such as cofactors for the *Rhizobium* nitrogenase enzyme [33]. It helps in N acclimatization and the synthesis of phytohormones [33]. Mo application increases the canopy, nodulation, and yield of crops [34].

The present study aimed to determine whether the application of a single *Rhizobium* inoculant or a combination of *Rhizobium* inoculants with micronutrients (Zn, B, and Mo) enhances the growth, yield, nodulation, nutrient uptake, and quality of garden peas. This study also investigated economic returns and soil microbes.

2 Methods

2.1 Experimental Location, Soil and Climate

The study was carried out during the winter seasons of 2019–20 and 2020–21 at the Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur 1701, Bangladesh. The study site was located in agro-ecological zone 28 (Madhupur tract) (23°98´N latitude and 90°41´E longitude and is 8.4 m above sea level). The soil type of the experimental region was a grey terrace that was chemically acidic and belonged to the *Chhiata* soil series (Texonomy: Aeric Haplaquepts) [35]. Texturally, the soil was clay loam (30.38% sand, 35.20% silt, and 34.42% clay) with a pH of 6.2%, 1.29% organic matter, 0.073% total N, 12.0 ppm P, 0.14 meq./100 g soil K, 15.0 ppm S, 0.78 ppm Zn, and 0.14 ppm B. The particle density, bulk density, and porosity of the soil were 2.55 g/cc, 1.38 g/cc, and 46.0%, respectively. The field capacity (FC) was 26.8% of the gravimetric water content, and the cation exchange capacity (CEC) was 7.4 Cmol kg^{-1} soil. The physicochemical properties of the presowing and postharvest soils were analyzed by standard methods [36].

The climate conditions of the study site are characterized as subtropical and humid. The monthly average temperatures during the study period ranged from 13.0°C to 36.1°C, and the monthly rainfall ranged from 0 to 218 mm. The maximum and minimum monthly average temperature, humidity, and rainfall during the experimental periods (2019–20 and 2020–21) were recorded and are presented in Figs. 1a and 1b.

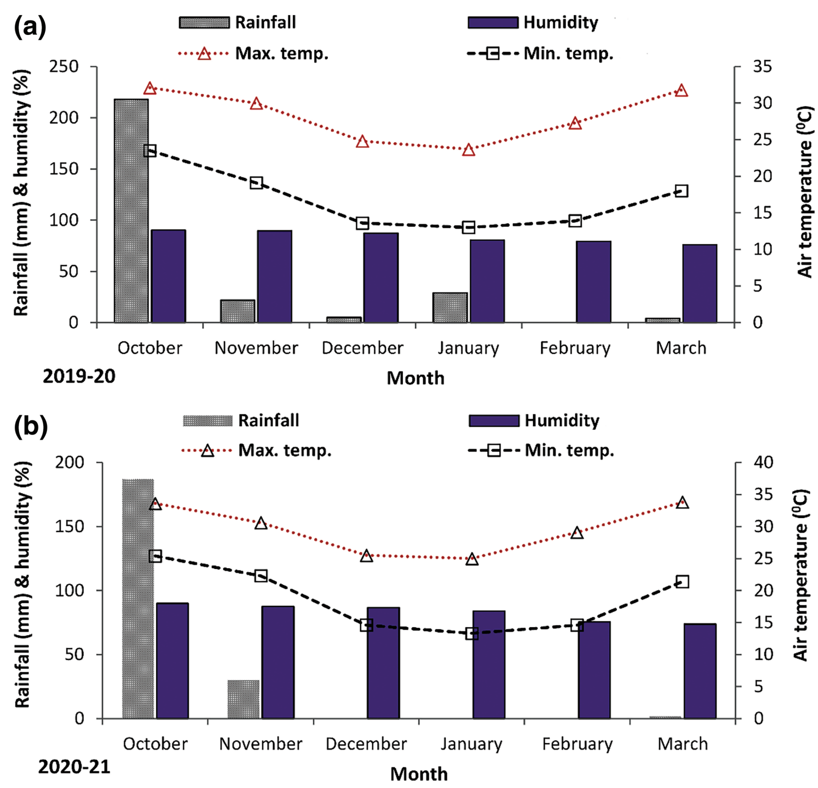

Figure 1: Monthly average minimum and maximum air temperature, rainfall and monthly average humidity during the experimental periods of 2019–20 (a) and 2020–21 (b)

2.2 Land Preparation and Layout

The experimental soils were opened by a tractor-driven plough with 4 passes, and a tractor-driven rotavator was also used for uniform tilling and levelling. The soil was manually cleaned to remove weeds and stubbles. The treatments were T_1 = Control (no Zn, B, Mo or *Rhizobium inoculant*), T_2 = *Rhizobium* inoculation (50 g kg^{-1} seed), T_3 = Zn_3Mo_1 + *Rhizobium* inoculant as applied in T_2, T_4 = B_2Mo_1 + *Rhizobium* inoculant *as* applied in T_2, T_5 = Zn_3B_2 + *Rhizobium* inoculant as applied in T_2, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium* inoculant as applied in T_2 and T_7 = $Zn_3B_2Mo_1$. All treatments were arranged in a randomized complete block design and repeated three times.

The other common fertilizers were applied in all plots in elemental form (i.e., N, P, K and S at 40, 24, 50 and 10 kg ha^{-1}, respectively) along with 5 t ha^{-1} decomposed cow dung. The sources of N, P, K, S, Zn, B and Mo were urea, TSP, MoP, gypsum, zinc sulphate (monohydrate), boric acid, and ammonium molybdate fertilizers, respectively. The peat-based *Rhizobium* inoculum (rhizobium strain BARI RPs 504) was used at a rate of 50 g kg^{-1} seed, which was prepared and supplied by the Soil Science Division of BARI. The experimental plots were divided into three equal blocks consisting of seven-unit plots in each block. The size of each unit plot was 3 m × 2 m. The unit plots were separated by 50 cm gaps, and the replicated blocks were separated from each other by a distance of 1 m.

2.3 Isolation of Rhizobium and Biochemical Tests

The *Rhizobium* inoculum (strain) was isolated from clean and freshly collected healthy root nodules of Garden pea. The selected root nodules were cleaned with distilled water and sterilized several times with sodium hypochlorite (NaOCl) solution. The sterilized nodules were transferred to Petri dishes and then crushed with the help of a sterilized glass rod to obtain a milky suspension, which was subsequently streaked on a yeast extract mannitol agar (YEMA) plate. The isolate was subcultured on a series of YEMA plates for pure cultures of the *Rhizobium* strain. The pure culture was maintained on YEMA slants and incubated at 28 ± 2°C for 48 h. The slants were preserved in a refrigerator at 4°C after sufficient growth [37] for further investigation.

The identified isolate (*Rhizobium*) was confirmed by several tests, including Gram staining and growth on YEMA with congo red. Nodulation ability was also tested on homologous hosts by plant infection tests according to the technique of Somasegaran et al. [38]. A sterilized inoculation loop was used for the entire test. The isolate was inoculated into broth media and kept for 3–5 days in a rotary shaker for good growth. The growth culture (20 ml suspension) was inoculated into 100 g of sterilized peat soil made of poly pack, which was subsequently incubated for 5–7 days at 28 ± 2°C before field application.

2.4 Plant Material, Fertilizer Application and Seed Sowing

Seeds of garden pea (BARI Motorshuti-3) were obtained from the Olericulture Division of HRC, BARI, Gazipur 1701, Bangladesh. Cowdung at 5 t ha^{-1} and other common fertilizers, including half of the urea and half of the MoP, were applied basally during the final plot preparation. Full amounts of zinc sulphate, boric acid and ammonium molybdate were applied plotwise as per prescribed treatments. The applied fertilizers were mixed thoroughly into the soil. The peat soil carrier-based *Rhizobium* inoculant was coated gently with healthy dry seeds at a rate of 50 g kg^{-1} seed, and the seeds were placed under shade for a few minutes for air drying before sowing. The plots with uninoculated seeds were sown first to avoid contamination, and then, the plots with inoculated seeds were sown at 120 kg ha^{-1} with a spacing of 20 cm × 10 cm on 09 December 2019 and 10 December 2020. The remaining half of the urea and MoP was applied between the rows as banding at 25 days after sowing (DAS), and it was mixed properly with the soil.

2.5 Intercultural Management

As the soil was dry, postsowing irrigation was performed immediately after seed sowing to facilitate proper germination. Each plot was irrigated with a hosepipe starting at 7 DAS at regular intervals of

7–10 days based on the soil moisture. The crop was manually weeded twice, at 25 and 40 DAS. The fungicides Provax 200 wp and distance M-45 were sprayed with 2 g/L water at 35 and 45 DAS to control root rot and brown spot disease, respectively. The infestation of insects such as pod borers and aphids was minimized by spraying 0.5 ml/L water with Imitaf 20 SL at 35 and 45 DAS. The test crop was harvested plotwise on two dates at two stages. The first half was harvested at the green pod stage, and the second half was harvested at the dry seed stage. Green pods were harvested at the tender stage on 04 February 2020 and 05 February 2021 from four rows in each plot. The remaining crop in the plot was allowed to grow until maturity, and mature pods were harvested for dry seed yield. The perfect maturity of the garden peas indicated that when the plants and pods turned brown, the seeds became hard.

2.6 Data Collection

Active modulation data were calculated from five randomly selected plants in each plot at 50 DAS. Five plants were uprooted smoothly from each plot with the help of a hand hoe and were carefully washed and dried under normal conditions. The lengths of the roots of five plants were measured, and the number of root nodules on each plant was counted and averaged. Ten nodules were randomly selected from five plants of each treatment to measure the individual nodule length, diameter, and individual nodule weight, and the values were averaged. The detached pods of four rows in each plot were weighed by an electric balance to determine the yield of the green pods, which was converted to kg ha^{-1}. The green seeds were separated from the composite pods of four rows to measure the weight of the 100-green seeds. Fresh green pod samples (250 g) from each treatment were collected and preserved in a refrigerator at −30°C in the postharvest laboratory of the HRC of BARI for quality analysis. Total soluble solids (TSSs), titratable acidity, and vitamin C content were recorded following standard methods. The total soluble solid content was assessed by taking a drop of green seed juice on a hand refractometer glass lens (Atago Ltd., PAL-1, Tokyo, Japan), and the results are expressed in ^{0}Brix as described by Anonymous [39]. The titratable acidity was determined according to the accepted method of Ranganna [40]. The level of vitamin C (ascorbic acid) was estimated from a standard method [39]. After the maturity of the remaining crop in each treatment plot, the growth and yield contributing characteristics, viz., plant height, number of branches $plant^{-1}$, number of pods $plant^{-1}$, pod length, number of seeds pod^{-1}, and 100-seed weight, were recorded for five randomly selected plants. Data on the dry seed and straw yields were recorded from the remaining mature test crops and converted to kg ha^{-1}.

2.7 Plant Sample Analysis

Treatment basis straw and seed samples were oven-dried at 70°C for 48 h and ground with a Cyclotec™ 1093 sample mill (Made in Sweden). The ground straw and seed samples were digested by a diacid mixture (HNO_3-$HClO_4$) (5:1) according to the method described by Piper (1966) [41]. The digested mixtures (straw and seed) were utilized to determine the N content following the Micro-Kjeldahl method [42], the P content via the spectrophotometer method, the K content via the atomic absorption spectrophotometer method and the S content via the turbidity method via $BaCl_2$ via the spectrophotometer. The Zn content of the digest was directly measured by atomic absorption spectroscopy (VARIAN SpectrAA 55B, Australia). The B content in the digest was determined by a spectrophotometer through the azomethine-H method [36]. The Mo concentration data are not shown due to a lack of laboratory facilities.

The seed protein content of garden peas was measured after multiplying the N content by the constant food factor of 6.25 [43].

Dry crop yields and nutrient contents in seeds and dry plants (straw) were used to determine nutrient (N, P, K, S, Zn and B) uptake according to the following formula [44]:

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Nutrient content (\%)} \times \text{Dry matter yield (kg/ha.)}}{100} \tag{1}$$

2.8 Preparation and Isolation of Microbes

Postharvest soil samples were collected by standard procedures at a soil depth of 0–15 cm. Total *Rhizobium*, free-living bacteria, phosphate-solubilizing bacteria (PSB), actinomycetes and fungal colonies were grown in different prepared media. After serial dilution, one drop of solution was poured into a Petri dish with different types of media. The Petri plates were incubated for three days to count total bacteria, *Rhizobium*, free-living bacteria, PSB, actinomycetes and fungal colonies. Bacterial media were prepared as nutrient agar media containing 28 g/L distilled water up to 1.00 L. The ingredients of the media were mixed in a normal glass flask with the required amount of distilled water. The initial pH of the medium was adjusted to 7.0. The medium was dissolved by boiling and autoclaved at 1210°C for 15 min. YEMA (yeast mannitol agar) medium contained the following ingredients: 0.5 g of K_2HPO_4, 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.1 g of NaCl, 0.2 g of $CaCl_2{\cdot}6H_2O$, 0.01 g of $FeCl_3{\cdot}6H_2O$, 10 g of mannitol, 0.5 g of yeast extract, 15.00 g of agar powder, and 1 L of distilled water. The initial pH of the medium was adjusted to 7.0 by adding 0.1 N HCl solution. The agar used in this medium was dissolved by boiling, and the medium was autoclaved at 1210°C for 15 min. PSB media plates were prepared with Pikovaskya's medium. Composition of 1 l of Pikovskaya media: 10 g of glucose, 5 g of $Ca_3(PO_4)_2$, 0.5 g of $(NH_4)_2SO_4$, 0.2 g of NaCl, 0.1 g of $MgSO_4{\cdot}7H_2O$, 0.2 g of KCl, 0.5 g of yeast extract, and 0.5 g of $MnSO_4$. H_2O (0.002 g), $FeSO_4.7H_2O$ (0.002 g) and agar (15 g) were used [45].

Actinomycete agar media: Plates were prepared with Actinomycete agar media. The composition of the Actinomycetes agar medium in 1 l was 21.7 g of Actinomycetes agar with 5 ml of glycerol. The initial pH of the medium was adjusted to 7.0 by adding 0.1 N HCl solution.

PDA (potato dextrose agar) media: The plates were prepared with PDA media. The amount of PDA medium in 1 litre was 39 g. The initial pH of the medium was adjusted to 7.0 by adding 0.1 N HCl solution. The agar used in this medium was dissolved by boiling, and the medium was autoclaved at 1210°C for 15 min. After autoclaving, 10 ml of lactic acid was mixed per litre of media. Nitrogen-free bacteria (NFB) media: Plates were prepared with nitrogen-free bacterial media. The nitrogen-free bacterial media included 5 g of malic acid, 0.5 g of K_2HPO_4, 0.1 g of NaCl, 0.2 g of $MgSO_4.7H_2O$, 0.02 g of $CaCl_2$, 5% BTB in 2 ml of 0.02 N KOH, 8 ml of 1.64% FE-EDTA and 20 g of agar. The initial pH of the media was adjusted to 7.0 by adding 1 N KOH solution. The agar used in this medium was dissolved by boiling, and the medium was autoclaved at 12°C for 15 min.

2.9 Estimation of the Benefit-to-Cost Ratio

The green pod and dry seed yield of garden peas were used to compute the gross return per hectare of land. The gross return was calculated after multiplying the yield by the farmgate unit price of the green pod and dry seed.

$$\text{Gross return } \left(\text{USD ha}^{-1}\right) = \text{Yield } \left(\text{kg ha}^{-1}\right) \times \text{Unit price of green pod or dry seed} \tag{2}$$

The gross margin was calculated by subtracting the total variable cost from the gross return according to the following formula:

$$\text{Gross margin } \left(\text{USD ha}^{-1}\right) = \text{Gross return } \left(\text{USD ha}^{-1}\right) - \text{total variable cost } \left(\text{USD ha}^{-1}\right) \tag{3}$$

Treatment total variable cost was measured by adding the cost incurred for labourers, ploughing and inputs of each treatment. The shadow prices (land rent, straw cost, etc.) were not considered.

The benefit-cost ratio (BCR) was calculated from the gross return divided by the total variable cost of cultivation. The BCR formula is as follows:

$$\text{Benefit} - \text{costatio} = \frac{\text{Gross return (USD per ha.)}}{\text{Total variable cost (USD per ha.)}} \quad (4)$$

2.10 Statistical Analysis

Data on growth, yield and yield attributes, quality characteristics, nutrient content and nutrient uptake were subjected to two-way ANOVA by using SAS software (version 9.4). The microbial population data in postharvest soil were analysed by analysis of variance (ANOVA) using SAS software (version 9.4). The mean separation test for all the data were was performed by using Tukey's HSD test at the 0.05 ($p \leq 0.05$) level of probability.

3 Results

3.1 Yields of Garden Pea Influenced by Rhizobium and Micronutrients

The year 2020–21 was found to be more favourable for the growth and development of garden peas, resulting in significantly greater green pod and seed yields from 2019–20 (Table 1). The use of a single *Rhizobium* inoculant or combined with Zn, B and Mo fertilizers had a positive influence on the green pod, seed, and straw yields of garden peas (Table 1). An increase in the green pod yield (8691 kg ha^{-1}) was recorded in the T_6 treatment group, which was significantly greater than that in the other treatment groups. Similarly, the T_6 treatment resulted in greater seed yield (1869 kg ha^{-1}) than did the T_7, T_5 and T_3 treatments. Both the green pod and seed yields were lowest in the control (T_1) treatment (Table 1). The highest straw yield (1770 kg ha^{-1}) was achieved in the T_6 treatment, followed by the T_7, T_5 and T_3 treatments (Table 1). However, compared with the control treatment, the combined application of the rhizobium inoculant and micronutrients (Zn, B and Mo) resulted in a 44.8% greater percentage of green pods and a 29.7% greater seed yield (Table 1).

Table 1: Green pod yield, seed yield and straw yield of garden pea according to the year of cultivation; the application of Zn, B and Mo with *Rhizobium* inoculant; and their interaction (Y * T) in the 2019–20 and 2020–21 seasons

Cultivation year	Green pods yield (kg ha^{-1})	Seeds yield (kg ha^{-1})	Straw yield (kg ha^{-1})
2019–20	6695 ± 194.1^{b}	1579 ± 34.4^{b}	1595 ± 34.6^{a}
2020–21	8140 ± 179.6^{a}	1815 ± 36.7^{a}	1636 ± 36.3^{a}
Level of significance	**	**	ns
MSD value	206	53.0	52.8
CV (%)	4.37	4.90	5.15

Treatment	Green pods yield (kg ha^{-1})	Yield increment over control (%)	Seeds yield (kg ha^{-1})	Yield increment over control (%)	Straw yield (kg ha^{-1})
T_1	6001 ± 467.3^{d}	–	1442 ± 78.9^{d}	–	1321 ± 24.5^{d}
T_2	7093 ± 263.5^{c}	18.2	1629 ± 48.9^{c}	13.0	1570 ± 40.9^{c}

(Continued)

Table 1 (continued)

Treatment	Green pods yield (kg ha^{-1})	Yield increment over control (%)	Seeds yield (kg ha^{-1})	Yield increment over control (%)	Straw yield (kg ha^{-1})
T_3	7289 ± 309.5^{c}	21.4	$1717 \pm 55.9^{a–c}$	19.1	$1642 \pm 33.3^{a–c}$
T_4	7492 ± 384.6^{bc}	24.8	1702 ± 80.2^{bc}	18.0	1613 ± 39.6^{bc}
T_5	7385 ± 378.9^{bc}	23.1	$1727 \pm 52.4^{a–c}$	19.7	$1652 \pm 31.9^{a–c}$
T_6	8691 ± 398.5^{a}	44.8	1869 ± 93.4^{a}	29.7	1770 ± 46.6^{a}
T_7	7971 ± 251.3^{b}	32.8	1793 ± 58.5^{ab}	24.3	1743 ± 39.6^{ab}
Level of significance	**	–	**	–	**
MSD value	597	–	153	–	153
CV (%)	4.37	–	4.90	–	5.15
Interaction (Y × T)	ns	–	ns	–	ns

Note: T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1 + *Rhizobium*, T_3 = Zn_3Mo_1 + *Rhizobium*, T_4 = B_2Mo_1 + *Rhizobium*, T_5 = Zn_3B_2 + *Rhizobium*, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium* and T_7 = $Zn_3B_2Mo_1$. This means that a column that includes the same letters is not significantly different at the 5% level according to Tukey's HSD test. MSD = minimum significant difference, CV = coefficient of variation, Y year, T treatment. The values are the means ± standard errors (n = 3). ns indicates not significant at $p > 0.05$, * indicates significant at $p \leq 0.05$ and ** indicates significant at $p \leq 0.01$ according to ANOVA.

3.2 Growth and Yield Contributing Characteristics of Garden Pea as Influenced by Rhizobium and Micronutrients

The year of cultivation affected the plant height, number of branches per plant and number of pods per garden pea plant (Table 2). The experimental results indicated that the tallest plant (40.4 cm), the greatest number of branches per plant (5.54) and the greatest number of pods per plant (8.05) were recorded in 2020–21, while the lowest values were recorded in 2019–20. The application of Zn, B and Mo with *Rhizobium* inoculation had a significant influence on the growth and yield of garden peas (Table 2). The tallest plant (42.1 cm) was found in the T_6 treatment, which was comparable to the T_7, T_3 and T_4 treatments, while the greatest number of branches per plant (5.69) was also found in the T_6 treatment, followed by the T_7, T_5, T_4 and T_3 treatments. The lowest values of both parameters were detected in the control treatment (T_1) (Table 2). The increase in pod length (6.39 cm) in the T_6 treatment was comparable to that in the T_5 and T_7 treatments, although the greatest number of pods per plant (8.80) was also recorded after the application of 3 kg Zn, 2 kg B and 1 kg Mo ha^{-1} with Rhizobium inoculation (50 g kg^{-1} seed) (T_6), which was significantly equal to that in the T_7 treatment, and the lowest values of both parameters were observed in the control T_1 treatment. Significantly, the maximum number of seeds per pod (6.11) was obtained in T_6, while the minimum value (4.85) was obtained in the control treatment. The 100 g green seed and dry seed weights reached a maximum of 55.6 and 27.1 g, respectively, in the T_6 treatment, both of which were significantly different from those in the other treatments. However, the performance of T_6 was statistically identical to that of T_7 for 100-green seed weights and that of T_7, T_5, and T_3 for dry seed weights. Both the green pod and dry seed weights were minimal in the control treatment (Table 2).

3.3 Root Nodulation of Garden Pea Plants Influenced by Rhizobium and Micronutrients

The year of cultivation affected only the length of the roots of the garden pea plants. The greatest root length (12.4 cm) was observed in 2020–21, and the lowest (11.7 cm) was in 2019–20 (Table 3). The root length, active nodulation, nodule length and diameter and nodule weight of garden peas significantly responded to the application of Zn, B and Mo combined with *Rhizobium* inoculation (Table 3). The longest root (12.7 cm) was noted in T6, which was comparable to most of the treatments, while the lowest value was observed in the control plot. In the present study, 50 days after sowing, more active nodules per plant (25.3) were recorded in the T_6 treatment than in the other treatments, which was statistically similar to most of the treatments except for the control. However, the nodule length was greater (3.74 mm) in the same T_6 treatment group than in the T_7, T_5 and T_3 treatment groups. The greatest nodule diameter (2.67 mm) was found in the T_6 treatment group. In terms of nodule weight, the heaviest nodule (0.0076 g) was noted in the T_6 treatment group, which was comparable to that in the T_7 and T_5 treatment groups, but the control treatment group had the lightest (0.004 g) nodule (Table 3).

Table 2: Plant height, number of branches/plants, pod length, number of pods/plant, number of seeds/pod, 100-green seed weight and 100-dry seed weight of garden pea as influenced by year of cultivation, the application of Zn, B and Mo with *Rhizobium* inoculant and their interaction in the 2019–20 and 2020–21 seasons

Year of cultivation	Plant height (cm)	Branches plant^{-1}	Pod length (cm)	Pods plant^{-1}	Seeds pod^{-1}	100-green seeds wt. (g)	100-seeds wt. (g)
2019–20	35.6 ± 0.69^{b}	5.21 ± 0.09^{b}	6.07 ± 0.07^{a}	7.71 ± 0.18^{b}	5.48 ± 0.10^{a}	52.3 ± 0.53^{a}	25.8 ± 0.25^{a}
2020–21	40.4 ± 0.81^{a}	5.54 ± 0.14^{a}	6.08 ± 0.07^{a}	8.05 ± 0.25^{a}	5.49 ± 0.10^{a}	52.6 ± 0.58^{a}	26.0 ± 0.39^{a}
Level of significance	**	*	ns	*	ns	ns	ns
MSD value	1.61	0.28	0.12	0.29	0.14	1.03	0.51
CV (%)	6.68	8.10	3.14	5.89	4.11	3.10	3.10
Treatments							
T_1	33.3 ± 1.52^{c}	4.82 ± 0.23^{c}	5.61 ± 0.16^{c}	6.99 ± 0.42^{d}	4.85 ± 0.09^{d}	49.9 ± 1.30^{c}	23.3 ± 0.69^{c}
T_2	37.5 ± 1.19^{bc}	5.06 ± 0.25^{bc}	5.96 ± 0.09^{b}	7.64 ± 0.49^{c}	5.15 ± 0.13^{cd}	52.2 ± 0.45^{bc}	25.9 ± 0.49^{ab}
T_3	38.7 ± 1.19^{ab}	5.40 ± 0.27^{ab}	6.03 ± 0.10^{b}	8.07 ± 0.44^{bc}	5.49 ± 0.13^{bc}	52.4 ± 0.39^{bc}	26.3 ± 0.36^{ab}
T_4	38.0 ± 1.49^{ab}	5.51 ± 0.16^{ab}	6.04 ± 0.03^{b}	7.59 ± 0.16^{cd}	5.58 ± 0.11^{b}	51.8 ± 0.56^{bc}	25.8 ± 0.18^{b}
T_5	37.1 ± 1.00^{bc}	5.53 ± 0.15^{ab}	6.29 ± 0.05^{ab}	7.56 ± 0.27^{cd}	5.51 ± 0.06^{bc}	50.9 ± 0.49^{c}	26.4 ± 0.25^{ab}
T_6	42.1 ± 2.08^{a}	5.69 ± 0.20^{a}	6.39 ± 0.03^{a}	8.80 ± 0.31^{a}	6.11 ± 0.14^{a}	55.6 ± 1.13^{a}	27.1 ± 0.44^{a}
T_7	39.7 ± 1.62^{ab}	5.63 ± 0.18^{a}	6.21 ± 0.06^{ab}	8.55 ± 0.33^{ab}	5.71 ± 0.11^{b}	54.5 ± 0.23^{ab}	26.6 ± 0.22^{ab}
Level of significance	**	*	**	**	**	**	**
MSD value	4.68	0.80	0.35	0.86	0.42	2.99	1.48
CV (%)	6.68	8.10	3.14	5.89	4.11	3.10	3.10
Interaction (Y × T)	ns	ns	ns	ns	ns	ns	ns

Note: T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1 + *Rhizobium*, T_3 = Zn_3Mo_1+ *Rhizobium*, T_4 = B_2Mo_1 + *Rhizobium*, T_5 = Zn_3B_2 + *Rhizobium*, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium* and T_7 = $Zn_3B_2Mo_1$. This means that a column that includes the same letters is not significantly different at the 5% level according to Tukey's HSD test. MSD = minimum significant difference, CV (%) = coefficient of variation, Y year, T = treatment. The values are the means ± standard errors (n = 3). ns indicates not significant at $p > 0.05$, * indicates significant at $p \le 0.05$ and ** indicates significant at $p \le 0.01$ according to ANOVA.

Table 3: Effect of cultivation year; the application of Zn, B and Mo with *Rhizobium* inoculant; and their interaction (Y × T) on the root length, number of active nodules per plant, nodule length, nodule diameter and nodule weight of garden pea

Year of cultivation	Root length (cm)	Number of active nodules plant^{-1} after 50 days	Nodule length (mm)	Nodule diameter (mm)	Nodule wt. (g)
2019-20	11.7 ± 0.15^{b}	23.6 ± 0.42^{a}	3.46 ± 0.05^{a}	2.47 ± 0.03^{a}	0.0057 ± 0.0003^{a}
2020-21	12.4 ± 0.20^{a}	24.1 ± 0.40^{a}	3.47 ± 0.06^{a}	2.48 ± 0.03^{a}	0.0058 ± 0.0003^{a}
Level of significance	**	ns	ns	ns	ns
MSD value	0.44	0.82	0.10	0.04	0.0005
CV (%)	5.82	5.44	4.36	2.50	14.1
Treatment					
T_1	11.4 ± 0.33^{b}	20.9 ± 0.39^{b}	3.13 ± 0.03^{c}	2.21 ± 0.02^{c}	0.0040 ± 0.0002^{c}
T_2	12.3 ± 0.32^{ab}	23.5 ± 0.47^{a}	3.41 ± 0.10^{b}	2.45 ± 0.02^{b}	0.0049 ± 0.0002^{c}
T_3	11.8 ± 0.30^{ab}	24.3 ± 0.57^{a}	3.47 ± 0.13^{ab}	2.48 ± 0.02^{b}	0.0053 ± 0.0002^{bc}
T_4	11.9 ± 0.37^{ab}	24.1 ± 0.90^{a}	3.42 ± 0.04^{b}	2.46 ± 0.03^{b}	0.0052 ± 0.0003^{bc}
T_5	11.6 ± 0.29^{ab}	24.6 ± 0.40^{a}	3.51 ± 0.07^{ab}	2.52 ± 0.04^{b}	0.0064 ± 0.0004^{ab}
T_6	12.7 ± 0.33^{a}	25.3 ± 0.59^{a}	3.74 ± 0.10^{a}	2.67 ± 0.05^{a}	0.0076 ± 0.0007^{a}
T_7	12.5 ± 0.32^{ab}	24.4 ± 0.57^{a}	3.56 ± 0.04^{ab}	2.53 ± 0.05^{b}	0.0071 ± 0.0005^{a}
Level of significance	**	**	**	**	**
MSD value	1.29	2.39	0.28	0.11	0.002
CV (%)	5.82	5.44	4.36	2.50	14.1
Interaction (Y × T)	ns	ns	ns	ns	ns

Note: T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1 + *Rhizobium*, T_3 = Zn_3Mo_1 + *Rhizobium*, T_4 = B_2Mo_1 + *Rhizobium*, T_5 = Zn_3B_2 + *Rhizobium*, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium* and T_7 = $Zn_3B_2Mo_1$. This means that a column that includes the same letters is not significantly different at the 5% level according to Tukey's HSD test. MSD = minimum significant difference, CV = coefficient of variation, Y year, T treatment. The values are the means ± standard errors (n = 3). ns indicates not significant at $p > 0.05$, * indicates significant at $p \leq 0.05$ and ** indicates significant at $p \leq 0.01$ according to ANOVA.

3.4 Effect of Rhizobium and Micronutrients on the Quality of Garden Pea

The vitamin C and protein contents were significantly different between the two years. The highest content of vitamin C (42.7 mg 100 g^{-1}) and maximum protein content (21.7%) of garden peas were attained in 2020–21, and both were lower in 2019–20 (data not shown). The quality traits, viz., TSS, titratable acidity, vitamin C content and protein content, of garden peas, responded positively to the application of Zn, B and Mo combined with *Rhizobium* inoculation (Fig. 2).

The highest total soluble solid content (^{0}Brix 14.3) was detected in $T_{6,\text{ which was}}$ comparable to most of the treatments, and the minimum value (^{0}Brix 12.3) was detected in the control treatment (Fig. 2). The value of titratable acidity was highest (1.31%) in the treatments with 3 kg Zn and 2 kg B ha^{-1} *Rhizobium* inoculation (T_5), which was statistically similar to that in the T_6, T_4 and T_2 treatments (Fig. 2). The highest amount of vitamin C (43.5 mg 100 g^{-1}) was found in the T_6 treatment, which was statistically similar to most of the treatments, and the lowest amount (39.7 mg 100 g^{-1}) was found in the T_1 treatment (Fig. 2). The same T_6 treatment resulted in greater protein content (22.2%), which was statistically identical to that of all other treatments except the control (Fig. 2).

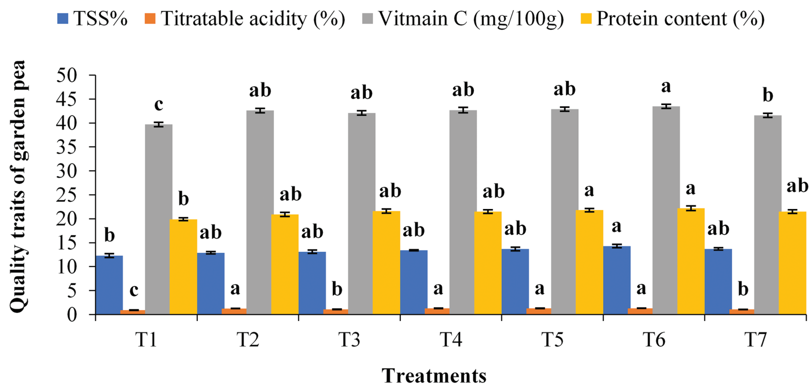

Figure 2: Total soluble solids (TSSs), titratable acidity, vitamin C content and protein content of garden pea affected by the application of Zn, B and Mo with a *rhizobium* inoculant. Error bars represent the mean ± standard error of the mean (n = 3). The means indicated by the different letters in the bars are significantly different at the 5% level according to Tukey's honestly significant difference (HSD) test. T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1 + T_2, T_4 = B_2Mo_1 + T_2, T_5 = Zn_3B_2 +T_2, T_6 = $Zn_3B_2Mo_1$ + T_2, T_7 = $Zn_3B_2Mo_1$

3.5 Rhizobium and Micronutrients Influence the Nutrient Content of Garden Pea Seeds

The year of cultivation affected the nitrogen (N), potassium (K), sulphur (S), zinc (Zn) and boron (B) contents in the seeds of garden peas but not the phosphorus (P) content (Table 4). In the second year (2020-21), relatively high N (34.7 g kg^{-1}), K (11.2 g kg^{-1}), S (3.90 g kg^{-1}), Zn (0.038 g kg^{-1}) and B (0.041 g kg^{-1}) levels were detected in the seeds, while all of these values decreased in the first year (2019–20) (Table 4). The application of Zn, B and Mo combined with *Rhizobium* inoculation affected the nitrogen, phosphorus, potassium, sulphur, zinc and boron contents in the seeds of the garden peas (Table 4). A significant increase in the nitrogen content (35.5 g kg^{-1}) was detected in T_6, similar to most of the treatments, although the minimum nitrogen content was detected in the T_1 (control) treatment. The maximum phosphorus content (8.86 g kg^{-1}) was comparable between the T6 treatment and the T_5 and T_7 treatments. The highest potassium content (13.3 g kg^{-1}) was in the T_6 treatment, while the lowest potassium content (6.91 g kg^{-1}) was in the control treatment. The sulphur content measured at the highest level (6.63 g kg^{-1}) in the same T6 treatment was statistically similar to that in the T_7 and T_5 treatments, whereas it was lower (2.61 g kg^{-1}) in the T_1 treatment (control). The maximum zinc content in the seeds (0.042 g kg^{-1}) was noted in $T_{6,}$ which was statistically similar to that in the T_7 and T_5 treatments. The same T_6 treatment exhibited an increase in the content of boron (0.045 g kg^{-1}), which was statistically similar to that in the T_7 treatment. Both the Zn and B contents were minimal in the T_1 (control) treatment (Table 4).

3.6 Nutrient Contents in Straw Influenced by Rhizobium Inoculant and Micronutrients

The year of cultivation affected the nitrogen, potassium, sulphur and boron contents but not the phosphorus and zinc contents in the straw of garden peas (Table 5). Increased contents of nitrogen (14.3 g kg^{-1}), potassium (15.3 g kg^{-1}) and sulphur (3.04 g kg^{-1}) were detected in 2020–21, while the contents of all nutrients decreased in 2019–20 (Table 5).

Table 4: Effect of the year of cultivation, the application of Zn, B and Mo with *Rhizobium* inoculant and their interaction (Y * T) on the N, P, K, S, Zn and B contents in the seeds of garden pea

Cultivation years	N	P	K	S	Zn	B
				$g\ kg^{-1}$		
2019–20	33.6 ± 0.35^{b}	7.68 ± 0.21^{a}	10.3 ± 0.49^{b}	3.67 ± 0.15^{b}	0.035 ± 0.001^{b}	0.037 ± 0.001^{b}
2020–21	34.7 ± 0.40^{a}	7.71 ± 0.22^{a}	11.2 ± 0.53^{a}	3.90 ± 0.16^{a}	0.038 ± 0.001^{a}	0.041 ± 0.001^{a}
Level of significance	*	ns	**	**	**	**
MSD value	0.93	0.21	0.19	0.14	0.001	0.0007
CV (%)	4.29	4.39	2.86	5.67	5.31	2.98
Treatment						
T_1	31.9 ± 0.49^{b}	6.16 ± 0.08^{d}	6.91 ± 0.13^{f}	2.61 ± 0.09^{d}	0.029 ± 0.0009^{d}	0.031 ± 0.001^{e}
T_2	33.4 ± 0.65^{ab}	6.86 ± 0.11^{c}	7.82 ± 0.15^{e}	3.17 ± 0.12^{c}	0.032 ± 0.001^{cd}	0.034 ± 0.0003^{d}
T_3	34.6 ± 0.65^{a}	7.21 ± 0.11^{bc}	10.8 ± 0.36^{d}	3.76 ± 0.10^{b}	0.038 ± 0.0008^{b}	0.035 ± 0.0005^{d}
T_4	34.5 ± 0.59^{ab}	7.77 ± 0.18^{b}	11.8 ± 0.27^{c}	3.72 ± 0.09^{b}	0.035 ± 0.0005^{c}	0.041 ± 0.001^{c}
T_5	34.8 ± 0.58^{a}	8.56 ± 0.15^{a}	11.9 ± 0.30^{c}	4.26 ± 0.11^{a}	0.039 ± 0.0009^{ab}	0.043 ± 0.001^{bc}
T_6	35.5 ± 0.73^{a}	8.86 ± 0.20^{a}	13.3 ± 0.25^{a}	4.63 ± 0.11^{a}	0.042 ± 0.001^{a}	0.045 ± 0.001^{a}
T_7	34.4 ± 0.63^{ab}	8.47 ± 0.11^{a}	12.7 ± 0.21^{b}	4.36 ± 0.10^{a}	0.041 ± 0.0006^{ab}	0.044 ± 0.001^{ab}
Level of significance	**	**	**	**	**	**
MSD value	2.70	0.62	0.57	0.40	0.004	0.002
CV (%)	4.29	4.39	2.86	5.67	5.31	2.98
Interaction (Y × T)	ns	ns	ns	ns	ns	ns

Note: T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1 + *Rhizobium*, T_3 = Zn_3Mo_1 + *Rhizobium*, T_4 = B_2Mo_1 + *Rhizobium*, T_5 = Zn_3B_2 + *Rhizobium*, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium* and T_7 = $Zn_3B_2Mo_1$. This means that a column that includes the same letters is not significantly different at the 5% level according to Tukey's HSD test. MSD = minimum significant difference, CV = coefficient of variation, Y year, T treatment. The values are the means ± standard errors (n = 3). ns indicates not significant at $p > 0.05$, * indicates significant at $p \leq 0.05$ and ** indicates significant at $p \leq 0.01$ according to ANOVA.

Table 5: Effect of the year of cultivation, the application of Zn, B and Mo with *Rhizobium* inoculant and their interaction (Y * T) on the N, P, K, S, Zn and B contents in the straw of garden pea

Cultivation year	N	P	K	S	Zn	B
				$g\ kg^{-1}$		
2019–20	13.4 ± 0.22^{b}	4.66 ± 0.13^{a}	14.5 ± 0.36^{b}	2.81 ± 0.12^{b}	0.0380 ± 0.001^{a}	0.0328 ± 0.001^{b}
2020–21	14.3 ± 0.21^{a}	4.86 ± 0.13^{a}	15.3 ± 0.38^{a}	3.04 ± 0.13^{a}	0.0378 ± 0.001^{a}	0.0335 ± 0.001^{a}
Level of significance	**	ns	**	*	ns	**
MSD value	0.53	0.25	0.43	0.18	0.0003	0.0002
CV (%)	6.03	8.15	4.57	9.79	1.26	0.98
Treatment						
T_1	12.7 ± 0.43^{b}	3.81 ± 0.14^{d}	11.6 ± 0.38^{d}	1.81 ± 0.10^{c}	0.0297 ± 0.0002^{f}	0.0278 ± 0.0002^{f}
T_2	13.3 ± 0.38^{ab}	4.42 ± 0.18^{cd}	14.2 ± 0.34^{c}	2.72 ± 0.13^{b}	0.0336 ± 0.0001^{e}	0.0292 ± 0.0002^{e}
T_3	14.2 ± 0.40^{ab}	4.61 ± 0.21^{bc}	14.9 ± 0.26^{bc}	2.93 ± 0.10^{b}	0.0403 ± 0.0001^{c}	0.0288 ± 0.0003^{e}
T_4	13.9 ± 0.27^{ab}	5.02 ± 0.12$^{a–c}$	15.5 ± 0.39^{ab}	3.15 ± 0.08^{ab}	0.0358 ± 0.0002^{d}	0.0342 ± 0.0002^{d}

(Continued)

Table 5 (continued)

Cultivation year	N	P	K	S	Zn	B
				$g\ kg^{-1}$		
T_5	14.3 ± 0.42^{a}	$4.88 \pm 0.11^{a–c}$	15.7 ± 0.26^{ab}	3.20 ± 0.18^{ab}	0.0414 ± 0.0004^{b}	0.0362 ± 0.0002^{c}
T_6	14.8 ± 0.44^{a}	5.42 ± 0.16^{a}	16.6 ± 0.35^{a}	3.52 ± 0.14^{a}	0.0427 ± 0.0001^{a}	0.0387 ± 0.0002^{a}
T_7	14.2 ± 0.30^{ab}	5.17 ± 0.07^{ab}	16.1 ± 0.29^{ab}	3.17 ± 0.08^{ab}	0.0417 ± 0.0002^{b}	0.0376 ± 0.0002^{b}
Level of significance	**	**	**	**	**	**
MSD value	1.54	0.71	1.25	0.53	0.0009	0.0006
CV (%)	6.03	8.15	4.57	9.79	1.26	0.98
Interaction (Y * T) significance	ns	ns	ns	ns	ns	ns

Note: T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1+ *Rhizobium*, T_3 = Zn_3Mo_1 + *Rhizobium*, T_4 = B_2Mo_1 + *Rhizobium*, T_5 = Zn_3B_2 + *Rhizobium*, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium* and T_7 = $Zn_3B_2Mo_1$. This means that a column that includes the same letters is not significantly different at the 5% level according to Tukey's HSD test. MSD = minimum significant difference, CV = coefficient of variation, Y year, T treatment. The values are the means ± standard errors (n = 3). ns indicates not significant at $p > 0.05$, * indicates significant at $p \leq 0.05$ and ** indicates significant at $p \leq 0.01$ according to ANOVA.

Table 5 shows that the increased nitrogen content in straw (14.8 g kg^{-1}) in T6 was comparable to that in most of the treatments, although a lower nitrogen content (12.7 g kg^{-1}) was detected in the control treatment. The T_6 treatment resulted in a higher phosphorus content (5.42 g kg^{-1}) that was statistically identical to that of the T_7, T_5 and T_4 treatments, while the control treatment was responsible for the minimum phosphorus content (3.81 g kg^{-1}). The maximum potassium content (16.6 g kg^{-1}) was recorded in the T6 treatment, which was comparable to that in the T_7, T_5 and T_4 treatments, although the minimum potassium content (11.6 g kg^{-1}) was recorded in the T_1 treatment. An increase in the sulphur content (3.52 g kg^{-1}) was also detected in the T6 treatment group, followed by the T_7, T_5 and T_4 treatments, while a decrease in the sulphur content (1.81 g kg^{-1}) was detected in the control group (T_1). Significantly, the maximum zinc (0.0427 g kg^{-1}) and boron (0.0387 g kg^{-1}) contents were detected in T_6, while both were minimal in the control treatment (Table 5).

3.7 *Effect of Zn, B and Mo Combined with Rhizobium Inoculant on Total Nutrient Uptake by Garden Pea*

Figs. 3a and 3b show the effect of micronutrients (Zn, B & Mo) combined with rhizobium inoculation on the total uptake of different nutrient elements by garden peas. The cultivation year affected the total uptake of N, P, K, S, Zn and B by garden pea (seed + straw). The second year (2020–21) favored the maximum total uptake of N (86.6.0 kg ha^{-1}), P (22.2 kg ha^{-1}), K (45.9 kg ha^{-1}), S (12.2 kg ha^{-1}), Zn (0.132 kg ha^{-1}) and B (0.131 kg ha^{-1}), although the minimum uptake of all nutrients occurred from 2019–20 (data not shown).

The highest total uptake of nitrogen (92.5 kg ha^{-1}) was detected in the treatments involving the application of 3 kg Zn, 2 kg B and 1 kg Mo with Rhizobium inoculation (50 g kg^{-1} seed) (T_6), which was statistically similar to the T_7 treatment, while the lowest total uptake value (62.7 kg ha^{-1}) was detected in the control treatment. Compared with the T7 treatment, the T_6 treatment was more effective at attaining the greatest phosphorus uptake (26.2 kg ha^{-1}) in garden peas, although the control treatment (T_1) had the lowest P uptake (Fig. 3a). The highest total potassium uptake (54.3 kg ha^{-1}) was obtained from the T_6 treatment, while the lowest was from the T_1 treatment (control). The maximum sulphur uptake by garden pea (14.9 kg ha^{-1}) in the T_6 treatment was comparable to that in the T_7 treatment, while the minimum was in the T_1 (control) treatment (Fig. 3a). The greatest zinc (0.154 kg ha^{-1}) and boron uptake (0.153 kg ha^{-1}) were recorded in T_6, which were significantly greater than those in the other treatments but were statistically similar to those in T_7. Both Zn and B uptake were the lowest in the T_1 (control) treatment (Fig. 3b).

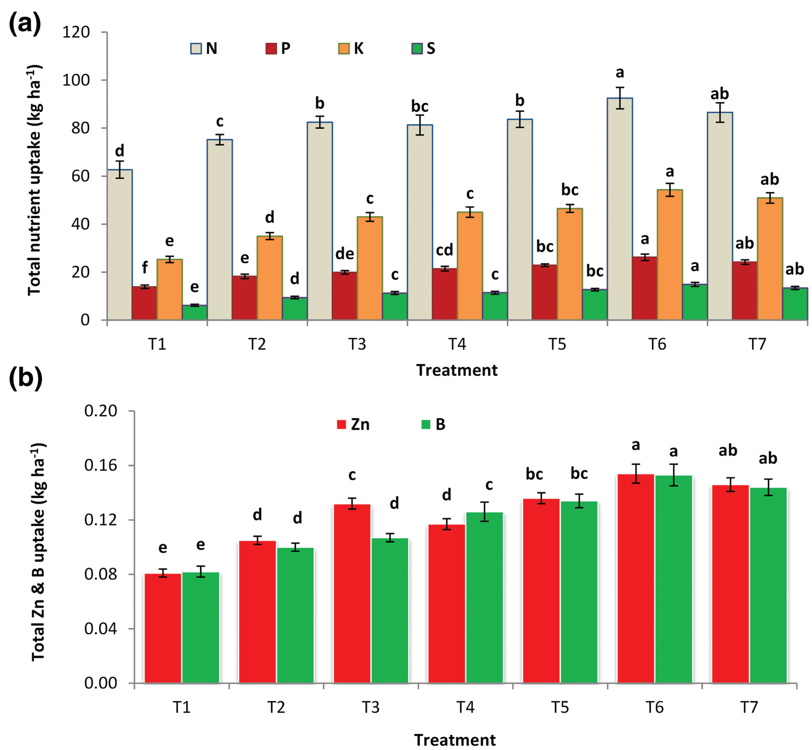

Figure 3: (a) Total uptake of N, P, K and S and (b) total uptake of Zn and B by garden pea (seed + straw) as affected by the application of Zn, B and Mo with *Rhizobium* inoculant. Error bars represent the mean ± standard error of the mean (n = 3). The means indicated by the different letters in the bars are significantly different at the 5% level according to Tukey's honestly significant difference (HSD) test. T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1 + *Rhizobium*, T_4 = B_2Mo_1 + *Rhizobium*, T_5 = Zn_3B_2 + *Rhizobium*, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium*, T_7 = $Zn_3B_2Mo_1$

3.8 Effect of Zn, B and Mo Combined with Rhizobium Inoculation on the Microbial Population in Soil

Zn, B and Mo combined with *Rhizobium* inoculation affected *Rhizobia*, total bacteria, fungi, actinomycetes, PSB and free-living bacteria in the postharvest soil of garden peas (Table 6). In the case of Rhizobia, a significantly increased population (600 × 10^5 cfu/g soil) was found in $T_{6,}$ while a decreased population was noted in the T_1 (control) treatment.

The greatest population of total bacteria (3300 × 10^5 cfu/g soil) was detected in the T_4 treatment, and the lowest population (2.0 × 10^5 cfu/g soil) was detected in the T_1 treatment (control). Significantly, the maximum fungal population (5400 × 10^5 cfu/g soil) was detected in the T_6 treatment, while the minimum fungal population was detected in the T_7 and T_2 treatments. The population of actinomycetes reached a maximum (1000 × 10^5 cfu/g soil) in $T_{6,}$ which was significantly greater than that in the other treatments. However, the actinomycete population was least abundant in the T_2 treatment (Table 6). The largest population of phosphate-solubilizing bacteria (400 × 10^5 cfu/g soil) was observed in the T_2 and T_6 treatments, which was comparable to that in the T_3 and T_7 treatments, and a smaller population was observed in the T_5 treatment. The highest population of free-living bacteria (4200×10^5 cfu/g soil) was recorded in the T_6 treatment, followed by the T_3 treatment, and the lowest population (11×10^5 cfu/g soil) was registered in the T_1 (control) treatment (Table 6).

Table 6: Effect of Rhizobia inoculation with Zn, B and Mo on the microbial population of Rhizobia, total bacteria, fungi, actinomycetes, PSB and free-living bacteria in postharvest soil of garden pea after the second year of 2020–21

Treatments	Rhizobia	Total bacteria	Fungus	Actinomycetes	PSB	Free-living bacteria
	Population ($\times 10^5$ cfu/g soil)					
T_1	100 ± 0.17^{bc}	2.00 ± 0.17^{d}	1100 ± 57.7^{b}	300 ± 57.7^{b}	100 ± 5.77^{bc}	11 ± 0.58^{e}
T_2	200 ± 6.93^{b}	500 ± 57.7^{d}	100 ± 28.8^{d}	2.00 ± 0.29^{c}	400 ± 57.7^{a}	200 ± 28.8^{e}
T_3	200 ± 17.3^{b}	100 ± 11.5^{d}	1400 ± 57.7^{b}	200 ± 57.7^{bc}	300 ± 57.7^{ab}	4100 ± 57.7^{a}
T_4	2.00 ± 0.13^{c}	3300 ± 173.2^{a}	600 ± 57.7^{c}	100 ± 5.78^{bc}	100 ± 5.77^{bc}	2700 ± 57.7^{c}
T_5	200 ± 28.8^{b}	1600 ± 173.2^{c}	1400 ± 115^{b}	200 ± 28.8^{bc}	12 ± 1.15^{c}	700 ± 57.7^{d}
T_6	600 ± 57.7^{a}	2400 ± 115.4^{b}	5400 ± 115^{a}	1000 ± 57.7^{a}	400 ± 57.7^{a}	4200 ± 57.7^{a}
T_7	200 ± 34.6^{b}	1700 ± 57.7^{c}	100 ± 11.5^{d}	100 ± 8.66^{bc}	$200 \pm 28.8^{a-c}$	3300 ± 57.7^{b}
MSD value	152.06	563.68	373.47	211.06	210.61	266.36
Level of significance	**	**	**	**	**	**
CV (%)	24.80	14.38	9.06	27.18	34.12	4.29

Note: T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1+ *Rhizobium*, T_3 = Zn_3Mo_1 + *Rhizobium*, T_4 = B_2Mo_1 + *Rhizobium*, T_5 = Zn_3B_2 + *Rhizobium*, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium* and T_7 = $Zn_3B_2Mo_1$. This means that a column that includes the same letters is not significantly different at the 5% level according to Tukey's HSD test. MSD = minimum significant difference, CV = coefficient of variation. The values are the means ± standard errors (n = 3). ns indicates not significant at $p > 0.05$, * indicates significant at $p \leq 0.05$ and ** indicates significant at $p \leq 0.01$ according to ANOVA.

3.9 Cost and Return Analysis Due to the Application of Rhizobium Inoculant and Micronutrients in Garden Pea

The use of Zn, B, and Mo with *Rhizobium* inoculation had an encouraging effect on the cost and return analysis of garden pea production (Table 7). The maximum gross returns of USD 4240 ha^{-1} for green pods and USD 1596 ha^{-1} for dry seeds of garden peas were recorded for the T_6 ($Zn_3B_2Mo_1$ + *Rhizobium*) treatment, followed by the T_7 treatment. The lowest gross return was recorded in the control treatment group. However, the benefit-cost ratio (BCR) was 5.70 for the green pod treatment and 2.29 for the dry seed from the T_2 (only rhizobium inoculation) treatment. The lowest BCR of 3.61 for the green pods of garden peas occurred in the T_3 treatment, and the minimum BCR of 1.48 for dry seeds occurred in the T_4 treatment (Table 7). The decreasing trend of the benefit-cost ratio might be related to the higher market price of Mo-containing fertilizer.

Table 7: Cost and return analysis for garden pea cultivation as influenced by the application of Zn, B and Mo combined with *Rhizobium* inoculation

Treatments	Total variable cost (USD ha^{-1})	Gross return (USD ha^{-1})		Gross margin (USD ha^{-1})		The benefit-cost ratio	
		Green pod	Seed	Green pod	Seed	Green pod	Seed
T_1	577	2927	1231	2350	654	5.07	2.13
T_2	607	3460	1391	2853	784	5.70	2.29
T_3	985	3556	1466	2571	481	3.61	1.49
T_4	985	3655	1453	2670	468	3.71	1.48
T_5	735	3602	1474	2867	739	4.90	2.01
T_6	1040	4240	1596	3200	556	4.08	1.53
T_7	1016	3888	1531	2872	515	3.83	1.51

Note: T_1 = Control, T_2 = *Rhizobium* inoculation, T_3 = Zn_3Mo_1 + *Rhizobium*, T_3 = Zn_3Mo_1 + *Rhizobium*, T_4 = B_2Mo_1 + *Rhizobium*, T_5 = Zn_3B_2 + *Rhizobium*, T_6 = $Zn_3B_2Mo_1$ + *Rhizobium* and T_7 = $Zn_3B_2Mo_1$. Input price: Urea = USD 0.20 kg^{-1}, Triple super phosphate = USD 0.29 kg^{-1}, Muriate potash = USD 0.21 kg^{-1}, Gypsum = USD 0.18 kg^{-1}, Zinc sulphate = USD 1.71 kg^{-1}, Boric acid = USD 1.95 kg^{-1}, Ammonium molybdate = USD 171 kg^{-1}, Provex = USD 4.88/100 g, Ribcord = USD 1.46/100 ml, Bio-fertilizer (Rhizobium) = USD 1.22/100 g, Garden pea seed = USD 0.98 kg^{-1}, Ploughing = USD 17.1 ha^{-1} (single pass), Wage rate = USD 6.10 day^{-1}. Output price: Garden pea green pod at USD 0.49 kg^{-1} and Garden pea dry seed at USD 0.85 kg^{-1}. Gross returns are calculated on the farm gate price of Gazipur district in Bangladesh. 1 USD = 82 BDT. BDT is Bangladesh currency.

4 Discussion

Zinc, boron, and molybdenum are crucial microelements for plant growth, flowering, fruiting, and quality improvement [46]. These micronutrients are also involved in plant physiological, biochemical, and metabolic activities, and their insufficiency promotes growth abnormalities in plants [22,46]. Improvements in garden pea growth, yield, and quality were amplified by the application of Zn, B, and Mo with Rhizobium inoculant. Rhizobia are good sources of biofertilizers that are widely used to enrich nitrogen in soils and plant roots through biological nitrogen fixation [47,48]. The experimental soil was deficient in micronutrients, viz., Zn, B, and Mo. The present study revealed significantly increased garden pea yields (green pod, seed, and straw yield) in response to the combined application of 3 kg Zn, 2 kg B, and 1 kg Mo per hectare combined with *Rhizobium* inoculation (50 g kg^{-1} seed). Micronutrients (Zn, B, and Mo) assisted in the translocation of photosynthates, increasing pod formation and quality, which eventually increased the number of green pods and the seed yield of garden peas. A similar result was corroborated by Hossain et al. [49], who reported that the combined application of 3 kg Zn, 2 kg B, and 1 kg Mo per hectare contributed to achieving greater seed and straw yields of lentils. Quddus et al. [50] also reported that the combined application of 3 kg Zn and 2 kg B resulted in the highest seed yield of field peas. Rhizobium can directly influence plant metabolism by solubilizing phosphates and producing hormones, which consequently promotes plant growth and ultimately improves crop yields [51,52]. In the present study, we compared the performance of Rhizobia singly or in combination with micronutrients (Zn, B, and Mo). However, the combined use of Zn, B, and Mo with Rhizobium inoculant significantly increased the seed yield by approximately 29.7% compared with that of the control group and by 14.7% compared with that of the *Rhizobium* inoculation group. Yadav et al. [51] reported a similar result in chickpeas in which seed yield significantly increased due to the application of phosphorous, zinc, and *Rhizobium* inoculation compared with the control. In our study, a single application of *Rhizobium* inoculant contributed to a 13.0% greater increase in seed yield compared to that of the control. Bhuiyan et al. [20] and Tena et al. [13] noted that the use of effective *Rhizobia* strains can enhance the yield of legume crops. Kumar et al. [10] also reported that plant-associated microbes play a key role in increasing plant biomass and crop yield.

Growth and yield attributes are very important factors for achieving a higher yield of garden peas. However, the combined use of Zn, B, and Mo along with *Rhizobium* inoculation was more effective than single *Rhizobium* inoculation or the application of paired micronutrients (ZnB, ZnMo, or BMo) combined with *Rhizobium* or three micronutrients, excluding *Rhizobium* inoculation, in terms of the growth and yield of garden pea. The greatest plant height, the greatest number of branches, the maximum number of pods per plant, and 100 seed weight were achieved by the joint application of 3 kg Zn, 2 kg B, and 1 kg Mo per hectare along with *rhizobium* inoculation at 50 g kg^{-1} seed. Similarly, Islam et al. [53] reported that the combined application of micronutrients (Zn, B, and Mo) effectively contributed to increasing plant height, the number of branches, and pods per plant, and the number of lentil seeds. Mohanty et al. [3] reported a similar effect in garden peas: the combination of micronutrients with *Rhizobium* inoculation, and N, P, and K significantly improved the yield attributes and yield. We observed that the combination of *Rhizobium* inoculant with micronutrients was more effective than a single application of *Rhizobium* inoculant. However, the results of the experiment demonstrated that the joint application of *Rhizobium* inoculant with micronutrients (Zn, B, and Mo) contributed 9.0% more to the number of pods per plant than the single use of *Rhizobium* inoculant. The treatment combination also contributed 3.0% more to the number of pods per plant than did the combined application of Zn, B, and molybdenum fertilizers. The combined application of *Rhizobium* inoculant with Zn, B, and molybdenum fertilizers might be more effective in influencing the availability of soil nutrients for plant uptake, leading to the generation of more pods and increasing yields of garden peas. The results of the study also revealed that *Rhizobium* inoculation alone resulted in better growth and yield of garden peas than no inoculation. This result agreed with the observations of Kumar et al. [10] and Tilman et al. [54]. Sayed et al. [5] reported that the inoculation of legume seeds with associative N_2-fixing bacteria resulted in improved growth, yield attributes, and yield.

Micronutrients, especially Mo, are crucial constituents of the nitrogen-fixing enzyme nitrogenase, which assists in atmospheric nitrogen fixation and is converted to NH_3, which is assimilated by plants [55]. Zinc is also involved in the process of nodule formation [24]. Boron is also beneficial for increasing root length, nodulation, and the size of root nodules [15]. The inoculation of *Rhizobium* enhances the content of chlorophyll, protein, and nitrogen fixation, and subsequently the rate of photosynthesis, and thus carbohydrate accumulation in the plant. In our study, the combination of 3 kg Zn, 2 kg B, and 1 kg Mo per hectare with *Rhizobium* inoculation (50 g kg^{-1} seed) increased the number of nodules per plant, increased root length, and weight, and improved nodule size. The results of the experiment showed that the increase in nodulation was 21.1% greater than that in the control due to the joint application of 3 kg Zn, 2 kg B, and 1 kg Mo per hectare with *Rhizobium* inoculant. This result is in agreement with the findings of Das et al. [56], who reported that micronutrients combined with *Rhizobium* sp. improved the nodule number in chickpea plants by 14.3% compared with that in the uninoculated control group. Gupta et al. [57] also reported in chickpeas that the increase in nodule number per plant was related to sufficient Mo application with *Rhizobium*, phosphate-solubilizing bacteria, and the recommended dose of other fertilizers. Similarly, Allito et al. [58] reported that inoculation with *Rhizobium* significantly increased nodulation in faba beans. Bejandi et al. [59] observed that the application of micronutrients and chickpea seed inoculation with rhizobium contributed to an increase in the chlorophyll, and protein content in plants. Wang et al. [60] reported that micronutrients combined with *Rhizobium* inoculation are beneficial for nitrogen fixation, although they are a basic compound of both chlorophyll and protein.

Micronutrient application combined with *Rhizobium* inoculation might help improve the nutritional quality of garden peas. In our study, greater TSS in the green seeds of garden peas was found with the application of 3 kg Zn, 2 kg B, and 1 kg Mo per hectare with *Rhizobium* inoculation (50 g kg^{-1} seed), but the cause of such an effect is unclear. We found that micronutrient application combined with

Rhizobium inoculation improved the vitamin C and protein contents in garden pea seeds. However, inoculation of *Rhizobium* with micronutrients might be favourable for improving vitamin C and protein contents. Similar findings were verified by the reports of Khiangte et al. [15], and Raj et al. [23].

The experimental soil was deficient in Zn, B, and Mo; thus, the test crop was responsive to the application of those micronutrients, which caused increased accumulation and uptake of several nutrients (N, P, K, S, Zn, and B) by the combined application of 3 kg Zn, 2 kg B, and 1 kg Mo per hectare with *Rhizobium* inoculation (50 g kg^{-1} seed). However, the joint application of *Zn3B2Mo1* + *Rhizobium* enhanced the nutrient (N, P, K, S, Zn, and B) content in the plants, causing greater uptake of 23.0% N, 43.9% P, 55.1% K, 58.1% S, 46.6% Zn, and 53.0% B in the aboveground part of the garden pea than did the single application of Rhizobium (data not shown). Hossain et al. [49] reported a similar result in lentils in which the uptake of nutrients increased due to the combined application of Zn, B, and Mo fertilizers. Similar results were also reported by Das et al. [56] in chickpeas, who reported that nutrient uptake was greatest after micronutrient application and rhizobium inoculation. In our study, a single application of *Rhizobium* resulted in better uptake of all nutrients compared to that in the uninoculated plot. Consequently, the combination of Zn, B, and Mo with *Rhizobium* inoculation was more efficient than single *Rhizobium* inoculation or noninoculated treatment. These phenomena might be related to the greater seed and straw yields of garden peas with higher nutrient concentrations. Rihana et al. [46] reported that nutrient acquisition and uptake were greater when appropriate essential micronutrients were supplied to appropriate *Rhizobium* strains, which also ensured proper microbial activity and a favourable environment.

Microbial populations such as *Rhizobium*, total bacteria, fungi, actinomycetes, phosphate-solubilizing bacteria (PSB), and free-living bacteria in postharvest soils are influenced by the application of micronutrients combined with *Rhizobium* inoculation. Most of the treatments in our study exhibited inconsistent variations in the populations of different microbes. Population variations in microbes depend on the soil environment (pH, moisture, etc.) conditions, which influence the survival of microbes in soil [61]. More rhizobium, fungus, and actinomycete populations were isolated in the T_6 ($Zn_3B_2Mo_1$ + *Rhizobium*) treatment. The highest population of *Rhizobium* might be favored for increasing yield and nutrient uptake because *Rhizobium* facilitates better nutrient mobilization and nutrient availability to plants [62]. The bacteria are capable of living in soil as well as inside legume root nodules, which involve atmospheric nitrogen fixation for supplying N to plants [10]. This observation is in agreement with the findings of Purwaningsih et al. [63] in *Arachis hypogaea* L., who reported that *Rhizobium* spp. bacteria can increase the host plant's survival capacity; influence many physiological processes, including cell enlargement and division; and produce more lateral roots, root hairs, and root nodules, which help the plant take up available nutrients and fix biological nitrogen.

In our study, the combined application of Zn, B, and Mo with *Rhizobium* inoculation improved the postharvest soil organic matter and total N, P, Zn, and B contents (data not shown). Micronutrients and *Rhizobium* might influence plant biomass production, and increase microbial activities. However, the incorporation of legume biomass into the soil and seed inoculation with *Rhizobium* might support the availability of nutrients and increase the organic matter content in the soil. Kebede [64] and Yadav et al. [51] also reported that the incorporation of legume crops had a marked effect on rebuilding organic matter and conserving soil quality. In our study, we found a greater gross return with the application of 3 kg Zn, 2 kg B, and 1 kg ha^{-1} *Rhizobium* inoculation (50 g kg^{-1} seed). However, the benefit-cost ratio decreased due to the higher market price of Mo fertilizer in Bangladesh. Hence, the combined contribution of Zn, B, and Mo with *Rhizobium* inoculation is highlighted by a positive change in soil fertility through biological nitrogen fixation and nutrient availability to plants for maximizing crop productivity and quality.

5 Conclusions

The results indicated that the application of Zn, B, or Mo at 3, 2 or 1 kg ha^{-1}, respectively, combined with *Rhizobium* inoculation (50 g kg^{-1} seed), contributed to enhancing the growth, yield, nodulation, and quality traits of garden pea. The same treatment resulted in the formation of more pods per plant and increased the yield of green pods and dry seeds. The treatment also increased the protein and vitamin C contents and improved the economic benefits. The application of 3 kg Zn, 2 kg B, and 1 kg Mo ha^{-1} along with *Rhizobium* inoculation improved postharvest soil fertility. The fertility can be used for the succeeding crop. According to the results and discussion, the recommendation can be made that the application of 3 kg Zn, 2 kg B, and 1 kg Mo ha^{-1} with *Rhizobium* inoculation (50 g kg^{-1} seed) support the maximum yield and quality improvement of garden pea in Zn-, B- and Mo-deficient soil. This result has potential for the region where there is no use of *Rhizobium* inoculant or imbalanced or no use of Zn, B, or Mo fertilizer in garden pea cultivation. Hence, more comprehensive research is needed in the future to determine the specific involvement of micronutrients and *Rhizobium* inoculants and to measure the ability of specific microbes to change soil health.

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