**Effect of Rhizobium, Nano Nutrients (NPK), and Organic manures on the growth of pea (*Pisum sativum* L)**

**Abstract**

The present study evaluated the effects of Rhizobium inoculation, nano-nutrients (NPK 19:19:19), and organic manure on the growth, chlorophyll content, and yield of *Pisum sativum* L. during the 2024 Rabi season under subtropical conditions. Six treatments were tested using a randomized block design, including control, nano-nutrients, Rhizobium inoculation, their combinations, and organic manure. Treatments involving integrated nutrient management, particularly T5 (Rhizobium inoculation + nano-NPK + organic manure) and T6 (Rhizobium inoculation + organic manure), significantly enhanced plant height, number of leaves, and branches, with T6 exhibiting the highest early vegetative growth parameters. Chlorophyll content was highest in T5, indicating enhanced photosynthetic activity. Yield attributes, including number of pods per plant, pod length, seeds per pod, and seed yield per plant, were markedly improved in both T5 and T6, with T5 recording the highest total seed yield at 23.0 g per plant. The control treatment consistently showed the lowest growth and yield values. The results demonstrate that the combined application of Rhizobium, nano-nutrients, and organic manure synergistically promotes physiological development and productivity in pea, suggesting an effective strategy for sustainable and high-yield pea cultivation.

**1. Introduction**

Pea (Pisum sativum L.) is a cool-season leguminous crop of significant agronomic and nutritional importance. It serves as a major source of plant-based protein (20–25%), making it especially valuable in vegetarian and plant-based diets. Furthermore, peas are rich in dietary fiber (5–7%), aiding in digestive health and glycemic control, and are an excellent source of essential vitamins such as A, B-complex, and C, thereby contributing to food and nutritional security. The carbohydrate content, which ranges between 60–70%, mainly comprises starch, offering a sustained energy source. Additionally, peas contain minimal fat (1–2%), mostly composed of unsaturated fatty acids, further enhancing their nutritional value (Lalmawiberi et al., 2023).

Globally, pea cultivation plays an important role in food systems. According to the Food and Agriculture Organization (FAO), the global production of dry peas is around 10.5 million tonnes, while fresh peas account for approximately 7 million tonnes (FAO, 2024). Peas are best suited to temperate and subtropical regions, performing optimally under moderate climatic conditions with temperatures between 18°C and 25°C. However, the crop is sensitive to abiotic stresses such as excessive heat, which can significantly impair flowering, pod setting, and yield. Adequate moisture is critical during the early vegetative and flowering stages, but waterlogging during sensitive growth periods can severely affect productivity. Key developmental stages—germination, flowering, and pod formation are especially vulnerable, and stress during these phases often results in reduced biomass and lower grain yield (Kumar et al., 2024).

From an ecological and agronomic perspective, Pisum sativum offers several benefits beyond nutrition. As a leguminous crop, it forms a symbiotic association with Rhizobium bacteria, which colonize the root system and form nodules. These nodules harbor nitrogenase enzymes that convert atmospheric nitrogen into ammonia, a plant-usable form of nitrogen. This biological nitrogen fixation enhances soil fertility, reduces the need for chemical nitrogen fertilizers, and supports environmentally sustainable practices. The incorporation of Rhizobium inoculants in pea cultivation has been shown to promote root development, improve nitrogen uptake, and increase both vegetative and reproductive growth parameters (Devi et al., 2023).

Recent innovations in sustainable agriculture have introduced Nano nutrient (NPK) formulations as efficient nutrient delivery systems. Nano-sized particles of essential nutrients—nitrogen (N), phosphorus (P), and potassium (K) possess a larger surface area and enhanced reactivity, allowing for improved absorption and reduced nutrient losses. These characteristics enable a more controlled and efficient nutrient release, supporting healthy plant growth and optimizing resource use. Nano nutrients (NPK) have been observed to enhance chlorophyll content, root proliferation, and biomass accumulation, ultimately leading to higher yields (Singh et al., 2024).

Organic manures, such as farmyard manure and compost, also play a crucial role in sustainable crop production. They contribute to soil organic matter content, enhance microbial activity, and improve soil structure, water-holding capacity, and nutrient availability. When used in combination with Rhizobiuminoculation and Nano nutrient (NPK) applications, organic manures create a synergistic effect, significantly boosting plant physiological processes and agronomic performance. The integration of these inputs results in a balanced nutrient supply, improved soil health, and reduced environmental impact compared to conventional high-input systems (Singh et al., 2024).

The combined application of Rhizobium, nano-nutrients, and organic manures has emerged as a promising strategy for enhancing the growth and productivity of pea crops. This integrated nutrient management approach not only supports vigorous plant development but also contributes to long-term soil fertility and sustainability. Investigating the interactive effects of these inputs on pea growth parameters such as germination percentage, shoot and root length, leaf number, dry matter accumulation, and yield provides valuable insights into optimizing resource-efficient, eco-friendly cultivation practices for Pisum sativum L.

**2. Materials and Methods**

The present investigation was conducted during the Rabi season of 2024 at the research farm of the Amity Institute of Organic Agriculture, affiliated with Amity University, Noida. The experimental site is geographically located at 28.544°N latitude and 77.3330°E longitude, with an elevation of 200 meters above mean sea level. The region experiences a tropical to subtropical climate, with average temperatures during the cropping season ranging between 18°C and 25°C, providing favorable conditions for pea cultivation. The experimental field was characterized by loamy soil with good drainage and moderate fertility. A Randomized Block Design (RBD) was employed to ensure statistical validity, comprising six treatments with three replications each. Individual plots were sized at 7 meters by 3 meters, and the spacing maintained was 30 cm between rows and 10 cm between plants within each row. The pea variety selected for this study was IPFD-12-2, known for its adaptability and yield potential. For treatments involving Rhizobium inoculation, seeds were treated with a slurry of 100 g of Rhizobium culture per 500g of seeds. Thereafter, the seeds were thoroughly mixed and allowed to shade-dry before sowing. Manual sowing was carried out in well-prepared plots, adhering strictly to recommended agronomic practices throughout the crop cycle. Organic manure was applied at the rate of 10.5 kg per plot, incorporated into the soil 15 days before sowing to enhance the nutrient availability. Nano-nutrient formulations of Nano Nutrients (NPK)- (19:19:19) @1 % concentration were applied as foliar sprays at 25 and 45 DAS, using commercially available Nano Nutrients (NPK) at the recommended concentration of 1%. These nano-nutrients were intended to enhance nutrient uptake efficiency and stimulate plant physiological processes.

**2.1 Treatment Details**

**List 1: Treatment Details**

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| --- | --- | --- |
| **Sr. No.** | **Treatment** | **Treatment Details** |
| 1. | T1 | Control |
| 2. | T2 | [Nano nutrients (NPK)-19:19:19 @ 1% (foliar spray)] |
| 3. | T3 | [Rhizobium inoculation (100 g per 500 g seeds)] |
| 4. | T4 | [Nano Nutrients(NPK)-(19:19:19) @ 1% + Rhizobium inoculation(100 g per 500 g seeds)] |
| 5. | T5 | [Rhizobium inoculation-(100 g per 500 g seeds) + Nano Nutrients(NPK)-(19:19:19) @ 1% + Organic manure(FYM)-10.5kg per plot] |
| 6. | T6 | [Rhizobium inoculation-(100 g per 500 g seeds) + Organic manure(FYM)-10.5kg per plot] |
|  |  |  |

**2.2 Field Parameters**

Growth and yield parameters were recorded at 15, 30, and 45 days after sowing (DAS) to evaluate the developmental progress of the pea plants under different treatment conditions. Plant height (in cm) was measured using a standard measuring scale from the base to the apex of the main stem. Simultaneously, observations were made on the number of leaves and the number of branches per plant to assess vegetative vigor. To further evaluate plant growth and biomass accumulation, measurements of shoot and root lengths were taken, and the respective fresh and dry weights were recorded for biomass estimation. For yield assessment, parameters include the number of pods per plant, the number of seeds per pod, and test weight. Additionally, the total grain yield per plot was calculated and expressed in kilograms per hectare to determine the impact of treatments on crop productivity.

**2.3 Chlorophyll Content Estimation**

Chlorophyll content in pea leaves was analyzed during the vegetative stage using a standardized laboratory procedure. Fresh, healthy leaf samples were collected from each treatment plot and promptly transferred to the laboratory. To remove any adhering dust and contaminants, the leaves were thoroughly washed with distilled water. A sample of 0.5 grams of leaf tissue was weighed and homogenized using 80% acetone to extract chlorophyll pigments. The homogenate was then centrifuged to separate the supernatant, which was used for spectrophotometer analysis. Absorbance readings were taken at 645 nm and 663 nm using a UV-visible spectrophotometer.

The total chlorophyll content was calculated using Arnon’s modified formula (1949) as follows:

Total Chlorophyll (mg/g FW) = [(22.2 × A645) + (8.02 × A663)] × V / (W × 1000)

Where:

* A645and A663represent absorbance values at 645 nm and 663 nm, respectively,
* Vis the volume of the extract in milliliters,
* W is the fresh weight of the leaf tissue (g).

**3. Results and discussion:**

**3.1. Growth Parameter:**

Plant height in Pisum sativum L. showed significant variation among the six treatments from 15 to 45 DAS, reflecting the impact of different nutrient management practices during the early vegetative phase. At 15 DAS, treatment T6, which combined Rhizobium inoculation, nano nutrients, and organic manure, recorded the highest plant height (12.0 cm), followed by T5 (9.5 cm), T4 (9.0 cm), T2 (8.0 cm), and T1 (6.0 cm). The lowest height was observed in T3 (5.0 cm). The early vigor in T6 is likely due to enhanced nitrogen fixation by Rhizobium, improved micronutrient availability from nano fertilizers, and better soil conditions provided by organic manure (Shyam et al., 2024). By 30 DAS, plant height increasedacross all treatments, with T6 maintaining the lead at 15.0 cm, followed by T5 (13.0 cm), and T4 and T2 (12.0 cm each). T1 and T3 showed comparatively lower growth (8.0 cm and 7.0 cm). The enhanced growth in treatments involving nano nutrients supports essential physiological functions such as chlorophyll synthesis and cell division, leading to faster elongation and leaf expansion (Singh et al., 2024). At 45 DAS, T6 continued to exhibit the highest plant height (30.0 cm), while T1 showed the lowest (20.0 cm). The superior performance of T6 indicates sustained nutrient availability and improved soil microbial activity, facilitating continuous growth during this stage (Samantaray et al., 2024). The synergy between biological nitrogen fixation, nano nutrient supplementation, and organic amendments is crucial for maximizing early vegetative growth in pea plants, consistent with the findings of (Oyege et al., 2023).

Fig.1 (a). Effect of different treatments on plant height (cm).

The number of leaves per plant in *Pisum sativum* L. showed clear differences among treatments from 15 to 45 DAS. At 15 DAS, treatment T6 recorded the highest leaf count (19 leaves), followed by T5 (15), T4 (14), T2 (13), and T1 (11), with T3 showing the lowest (10). This early advantage in T6 is likely due to the combined effects of Rhizobium inoculation, nano nutrients, and organic manure enhancing early leaf development. By 30 DAS, the number of leaves increased significantly, with T6 maintaining the lead at 26 leaves, followed by T5 (24). T2 and T4 showed moderate growth with 21 and 22 leaves, respectively, while T1 and T3 recorded similar counts of 21 leaves. The improved leaf production under nano nutrient treatments suggests enhanced photosynthetic capacity and nutrient assimilation, supporting overall plant growth (Verma & Singh, 2024). At 45 DAS, T6 again exhibited the highest leaf number (34), demonstrating sustained vegetative vigor. T5 followed with 31 leaves, while T2 and T4 each had 28 leaves. T1 recorded the lowest leaf count (25), reflecting the limited nutrient availability in this treatment. The superior leaf production in T6 and related treatments likely results from better nutrient cycling, microbial activity, and hormonal regulation promoting leaf expansion and development (Biswas et al., 2023).

Fig.1 (b). Effect of different treatments on the number of leaves per plant.

The number of branches per plant in Pisum sativum L. varied significantly across treatments from 15 to 45 DAS. At 15 DAS, treatments T6 and T5 recorded the highest number of branches (5 each), followed by T4, T3, and T2 with 4 branches each, while T1 had the lowest count (2). By 30 DAS, T6 maintained the lead with 6 branches, followed by T5 with 4 branches. Treatments T4 and T2 recorded 3 branches each, T1 had 2, and T3 showed the lowest number (1). At 45 DAS, T6 again showed the maximum number of branches (8), followed by T5 (7). Treatments T4, T3, and T2 each had 6 branches, while T1 had the lowest count (5). The higher branching under T6 and T5 treatments could be attributed to better nutrient availability through rhizobium inoculation, nano nutrients, and organic manure, which promote enhanced cell division and shoot development (Kumar et al., 2023). Lower branch numbers in T1 and T3 indicate a limited nutrient supply restricting vegetative growth.

Fig.1 (c). Effect of different treatments on Number of branches per plant.

**2.2 Chlorophyll Content Estimation**

Chlorophyll content in Pisum sativum L. leaves varied significantly across the six treatments (T1 to T6). Chlorophyll a content was highest in T5 (0.76 mg/g), followed by T4 (0.65 mg/g), T3 (0.63 mg/g), and T6 (0.60 mg/g). Moderate levels were observed in T2 (0.56 mg/g), with the lowest content in the control treatment T1 (0.50 mg/g). Similarly, chlorophyll b content peaked in T5 (0.87 mg/g), followed by T4 (0.75 mg/g), T3 (0.71 mg/g), and T6 (0.61 mg/g). Treatments T2 and T1 showed comparatively lower values of 0.65 mg/g and 0.60 mg/g, respectively. Total chlorophyll content was also highest in T5 (1.61 mg/g), followed by T4 (1.45 mg/g), T3 (1.34 mg/g), and T6 (1.31 mg/g). Lower total chlorophyll content was recorded in T2 (1.25 mg/g) and T1 (1.15 mg/g). The superior chlorophyll content in treatments involving nano nutrients and organic manure (especially T5 and T4) suggests enhanced nutrient availability, which supports chlorophyll synthesis and photosynthetic efficiency (Kumari et al., 2023). The slightly lower chlorophyll in T6 compared to T5 may reflect treatment composition differences, but both outperformed control and single-factor treatments, highlighting the importance of integrated nutrient management in improving photosynthetic pigments and overall plant health (Kaur et al., 2024).

**Fig.2. Effect of treatments on Chl-a (mg g-1), Chl-b (mg g-1), and Total-Chl (mg g-1) content**

**3.3. Yield Parameter**

The number of pods per plant varied significantly among treatments (Fig. 3), with T5 and T6 recording the highest counts of 13 and 14 pods per plant, respectively. The control treatment T1, produced the lowest number of pods (8). Pod length was also greatest in T5 and T6, reaching up to 10.0 cm, while T1 and T2 had shorter pods, measuring less than 9.0 cm. Regarding seed number per pod, T5 again showed superiority with 9.5 seeds per pod, closely followed by T6 with 9 seeds. T2 had the lowest seed count, approximately 7.5 seeds per pod. Seed yield per plant and total seed weight were significantly higher in T5, with 11.0 g per plant and 23.0 g total seed weight, followed by T6 (24.0 g total seed weight). The lowest yields were recorded in T1, with 8.0 g per plant and 18.0 g total seed weight. These results indicate that treatments combining nano nutrients and organic manure (T5 and T6) enhance pod development and seed production, likely due to improved nutrient availability and physiological activity during reproductive stages (Sheershwal et al., 2023). The integrated nutrient management strategies positively influenced both yield components and total productivity of Pisum sativum L.

**Fig.3.Effect of different treatments on yield parameters of pea; Number of pods, Pods length (cm), seeds per pods, seed yield per plants (g) and seed weight (g).**

**4. Conclusion**

This investigation confirms that integrating Rhizobium inoculation with nano-nutrients and organic manure significantly improves vegetative growth, chlorophyll content, and yield in *Pisum sativum* L. Treatments T5 (Rhizobium + Nano nutrients + Organic manure) and T6 (Rhizobium + Organic manure) notably enhanced plant height, leaf number, branching, chlorophyll synthesis, and pod and seed development compared to the control and single-factor treatments. The increased nitrogen fixation, nutrient availability, and improved soil health under these treatments contributed to enhanced biomass accumulation and reproductive performance, ultimately leading to higher seed yield. The superior photosynthetic pigment content observed in T5 suggests better physiological functioning that supports yield formation. These findings highlight the potential of integrated nutrient management approaches combining biofertilizers, nano-fertilizers, and organic amendments for boosting pea productivity sustainably while maintaining soil fertility and environmental balance.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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