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# The role of mycorrhiza and humic acid on quantitative and qualitative traits of faba bean plant under different fertilizer regimes

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## Abstract

In sustainable agricultural ecosystem management, a key focus is on minimizing chemical fertilizer use and maximizing the use of non-chemical alternatives. Our study was designed to explore the impact of mycorrhizal fungi and humic acid (HA) on the quantitative and qualitative characteristics of faba beans under varying chemical fertilizer levels. The experiment involved different treatments, which included HA and mycorrhiza in four variations (control, HA, HA+ mycorrhiza, and mycorrhiza alone), and nitrogen, phosphorus, and potassium (NPK) fertilizers at three different dosages (50%, 75%, and 100% of the recommended amount). The findings revealed that combining HA with mycorrhiza significantly influenced root colonization, with the highest chlorophyll a concentration (1.58 mg $g^{-1}$) observed in the HA+ mycorrhiza treatment at 75% NPK. This treatment also led to the highest counts in seeds per pod, number of pods, and weight of 100 seeds. Between the 100% and 75% NPK levels in the HA+ mycorrhiza treatment, no significant differences were noted in terms of grain and biological yield. The greatest grain yields were measured at 4356 kg $ha^{-1}$ and 4322 kg $ha^{-1}$ for the HA+ mycorrhiza treatment at 100% and 75% NPK, respectively. Additionally, the highest concentrations of Fe, N, P, K, and Zn were observed with the HA+ mycorrhiza application at the 100% NPK level.

## INTRODUCTION

Legume plants are crucial in sustainable agriculture, providing both economic and environmental benefits. They have a unique ability to fix atmospheric nitrogen in their root nodules due to their symbiotic relationship with rhizobia. This important capacity is key to their role in sustainable farming practices. (Fouda, 2017). Faba beans, a crucial legume crop in the winter season, serve as a primary protein source for both human and animal diets. (Dawood et al., 2019). The global cultivation of the Faba bean (*Vicia faba* L.) covered an area of 2.67 million hectares in 2020. Worldwide, the production of this bean amounted to 5.67 million tons. In Iran, the area dedicated to Faba bean cultivation was 7,918 hectares (FAO, 2022).

Nitrogen (N) is an essential nutrient that is often limited in both natural environments and managed ecosystems. Arbuscular Mycorrhizal (AM) fungi, which form symbiotic relationships with the majority of terrestrial plants, are key in enhancing plant N uptake from soil. This can help overcome N limitations, significantly influencing plant growth and soil nutrient dynamics. Although AM fungi do not possess the genetic machinery for decomposition, they are capable of deriving substantial amounts of mineral N from organic matter. Recent studies have shed light on the role of other soil organisms with decomposing abilities in supporting AM fungi's N acquisition and its transfer to plants. However, the nature of these

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interactions, whether competitive or synergistic in terms of nutrient uptake, remains a subject of debate. The impact of global environmental changes, like N enrichment, on these complex interactions is also a critical area of ongoing research.

To enhance the production and quality of faba beans, the utilization of organic and biological fertilizers is a viable approach. These fertilizers can serve as alternatives to traditional phosphorus and nitrogen sources. One such organic fertilizer is humic acid, which is formed through the decomposition of organic materials under certain conditions, facilitated by microorganisms (El-Kholy et al., 2019). Humic acid has both indirect and direct effects on soil and plants. Indirectly, it enhances soil structure, aids in plant rooting, increases water retention, promotes growth-supporting bacteria, and reduces soil toxicity from fertilizers and nutrients (Samavat, 2007). Direct effects of humic acid occur after plants absorb it, leading to various biochemical changes like improved protein and nucleic acid synthesis, hormone-like activities, heightened photosynthesis, and enzyme activity (Daur and Bakhashwain, 2013). It also boosts nutrient availability by converting mineral elements into forms plants can use (Büyükkeskin et al., 2015). Studies by Bayoumi and Selim (2012) revealed that faba bean plants treated with humic acid, nitrogen, and biological fertilizers showed the highest grain weight. Humic acid application also increased N, P, K levels and the K/Na ratio in cowpea shoots, while decreasing Na content (El-Hefny, 2010).

Phosphorus is a crucial nutrient for legumes and often the most limiting factor for their production. It has been demonstrated that the application of arbuscular mycorrhiza can significantly enhance the growth and yield of agricultural crops. This biological approach not only improves crop performance but also reduces the need for inputs like phosphorus fertilizer, thereby offering a more sustainable and efficient way of meeting the phosphorus requirements of legumes. (Saia et al., 2014; Sawers et al., 2010). Mycorrhiza has the ability to convert essential nutrients from an inaccessible form into a usable form of the plant (Khaitov and Teshaev, 2015) and increases the plant's ability to absorb phosphorus and micronutrients, while providing the plant with the carbohydrates necessary for the fungus (Tawaraya, 2003). Also, plant coexistence with fungi protects the plant from diseases and improves the ability to absorb water from the soil (Zhu et al., 2012). The results showed that at low concentrations of phosphorus and nitrogen fertilizers, mycorrhizal fungi increase the rate of stabilization in legume plants (Leij, 1998). The results of Pereira et al. (2019) study showed that inoculation of faba bean plant with mycorrhiza increased yield-related trait.

Our understanding of how cropping management influence temporal and spatial changes of soil health indicators could be used to develop effective and cost-effective agricultural management activities (Mirbakhsh et al., 2022). Addressing air pollution, soil degradation, and water resource management necessitates comprehensive consideration of scientific principles, including those related to traffic, climate, and crop management (Mirbakhsh et al., 2023). These practices are environmentally friendly and enhance the sustainability of cropping systems by decreasing the reliance on chemical fertilizers. This approach is echoed in studies by Mirbakhsh et al. (2023) and Jamshidi et al. (2019). Consequently, this research aimed to explore the potential for reducing chemical fertilizer usage in faba bean cultivation, focusing on the impact of mycorrhiza and humic acid on the nitrogen, phosphorus, and potassium (NPK) levels, and how these factors influence the quantitative and qualitative characteristics of the faba bean plant.

## MATERIALS AND METHODS

### Analysis of soil physical and chemical properties at the research location

In 2019–2020, an experiment was carried out at the Islamic Azad University's Research Farm in Chalus Branch, situated at coordinates 40° 55′ N, 53° 72′ E, and an elevation of 4 meters above sea level. The site's average temperature, humidity, and precipitation data are detailed in Table 1. Soil samples from the top 30 cm layer were collected for analysis before planting, as shown in Table 2. Using Robinson's method (Baize, 1988), the soil texture was analyzed. The pH and electrical conductivity (EC) of the soil were evaluated using a water-based solution. The measurements for both total organic carbon (TOC) and total organic matter (TOM) were conducted in accordance with the procedures outlined in Aubert's method (1978). The Olsen and Sommers (1982) methods were used to gauge available phosphorus (P), and total nitrogen (N) content was determined using the Kjeldhal method (Rodier et al., 1984).

**Table 1.** Meteorological parameters for the field sites during experiment (Mazandaran Province Meteorological Office)

| Months | Mean temperature (°C) | | Relative humidity (%) | | Precipitation (mm) | |
|---|---|---|---|---|---|---|
| | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 |
| November | 12.98 | 14.06 | 79.87 | 84.32 | 148.36 | 193.12 |
| December | 10.52 | 12.13 | 85.10 | 79.80 | 185.30 | 122.90 |
| January | 8.90 | 10.60 | 80.30 | 82.40 | 95.20 | 94.30 |
| February | 10.30 | 12.20 | 84.70 | 88.20 | 87.90 | 94.30 |
| March | 8.10 | 11.20 | 83.40 | 86.50 | 110.10 | 118.40 |
| April | 11.40 | 14.15 | 83.10 | 79.90 | 70.30 | 125.10 |
| May | 16.40 | 18.90 | 80.70 | 82.10 | 18.10 | 41.20 |
| June | 24.20 | 21.50 | 73.20 | 79.30 | 10.30 | 26.30 |

**Table 2.** Physical and chemical properties of soil

| Texture | Ec (ds $m^{-1}$) | pH | K (ppm) | N (%) | P (ppm) | OM (%) | TOC (%) |
|---|---|---|---|---|---|---|---|
| Sandy clay loam | 0.57 | 0.99 | 17.03 | 0.12 | 74 | 7.2 | 0.79 |

Potassium (K) levels were measured using a flame spectrophotometer (AFP100, BEM, and UK).

## Plant materials and experimental conditions

This study was structured as a factorial experiment within a randomized complete block design, encompassing three replications. The experiment involved two main factors: the application of humic acid and mycorrhiza, and the use of NPK fertilizer. The humic acid and mycorrhiza factor had four treatments: a control using distilled water, application of humic acid alone (400 mg $L^{-1}$ $ha^{-1}$), a combination of humic acid and mycorrhiza, and mycorrhiza alone. The NPK fertilizer was applied at three different levels, which were 50%, 75%, and 100% of the recommended dosage.

A commercial humic acid (HA) product, HUMIFIRST, was utilized. This product, originating from Zarin Gostar Bastan CO., Industrial Town, Shiraz, Iran, is 100% organic and comprises 17% total fertilizer extract, 12% HA, and 5% fulvic acid.

The experimental field consisted of 36 plots, each measuring 1.50 meters in width and 3 meters in length, arranged with six planting rows. Plant spacing was maintained at 10 cm within rows and 25 cm between rows, resulting in a plant density of 40 plants per meter. For mycorrhiza inoculation, approximately 50 g of arbuscular mycorrhizal fungi were mixed with 1 kg of faba bean seeds before planting. Seeds of the "Barakat" variety, sourced from the Agricultural Research, Education, and Extension Organization in Karaj, Iran, were planted in mid-December. This planting schedule was chosen to align with the local climate and weather patterns of the region.

Basal fertilization included 75 kg per hectare of calcium phosphate ($Ca(PO_4H_2)_2$), 50 kg per hectare of potassium sulfate ($K_2SO_4$), and 50 kg per hectare of urea, as determined by soil analysis conducted prior to the experiment. Additionally, 100 kg per hectare of urea fertilizer was applied before seed planting.

## Measured characteristics

Plants were harvested on 24 June until September in 2020. During the maturation phase, five plants from each plot were randomly chosen and brought to the laboratory. There, analyses were conducted to examine seed yield per square meter (1.0 $m^2$) and the nutritional content of the seeds. In September, mycorrhizal symbiosis was investigated, and root colonization percentage of bean plant was determined, roots were stained according to Phillips and Hayman's (1970) method. Root colonization percentage was determined according to the method of Biermann and Linderman (1981).

The assessment of nitrogen content in the seeds was conducted using the Kjeldahl method, utilizing a Semi-automatic Kjeldahl Distillation Unit (Model UDK 139) from VELP Company, Italy. Total chlorophyll a and b in the shoots were quantified using a modified

Arnon's method from 1949. Prior to nutrient analysis, samples were washed with distilled water and dried in an oven (Model OVO3-D/K.J 55, PARS AZMA Co., Iran) at 65 °C until constant dry weight was achieved. These dried samples were then weighed and pulverized into a fine powder using an electric mill.

For ash analysis, 1.0 gram of the dried plant material was incinerated in porcelain crucibles in an electric furnace (Model FTMF-701, Fine Tech, South Korea) at 550 °C. Afterwards, 5 ml of 2 N HCl was added to the ash, and the crucibles were put in a water bath for 30 minutes. The mixture was then filtered, and the resulting solution was diluted to a volume of 50 ml.

The concentrations of zinc and iron in this solution were measured using an atomic absorption spectrophotometer (Varian-(spectrAA -240), Italy). The potassium content was determined using flame emission photometry, employing a flame photometer (Model XP-5, BWB, England).

### Statistical analysis

Data variance was analyzed using SAS software, version 1. To compare the means of the desired traits, Duncan's multiple range tests were employed, setting the level of significance at a 5% probability threshold.

## RESULTS AND DISCUSSION

### Colonization

The results of analysis of variance of the effect of non-chemical fertilizer and NPK levels on root colonization are shown in Table 3. Root colonization at NPK levels and in mycorrhizal inoculated treatments is shown in Figure 1. The results showed that with the application of 100% NPK, there was no significant difference between mycorrhiza and mycorrhiza + humic acid treatments in terms of root colonization percentage, and the highest root colonization percentage was observed in these treatments at 67

**Table 3.** Analysis of variance of chlorophyll of faba bean under different levels of humic acid, mycorrhiza and NPK in two years

| S.O.V | df | AMF colonization | Chlorophyll a | Chlorophyll b | Chlorophyll total |
|---|---|---|---|---|---|
| Year (Y) | 1 | 15.32 | 0.0003 | 0.002 | 0.004 |
| Block (Year) | 4 | 136.03 | 0.0108 | 0.001 | 0.016 |
| Fertilizer (F) | 3 | 72.43 * | 0.1189 * | 0.115 ** | 0.421 ** |
| Y × F | 3 | 2.38 | 0.0046 | 0.003 | 0.002 |
| NPK | 2 | 1782.44 | 0.2534 | 0.141 * | 0.773 * |
| Y × NPK | 2 | 172.10 ** | 0.0163 | 0.004 | 0.037 |
| F × NPK | 6 | 136.53 * | 0.0483 ** | 0.017 * | 0.051 * |
| Y × F × NPK | 6 | 17.43 | 0.0055 | 0.004 | 0.008 |
| Error | 44 | 19.38 | 0.0075 | 0.003 | 0.012 |

Remarks: * and ** at 5% and 1%, significant, respectively.

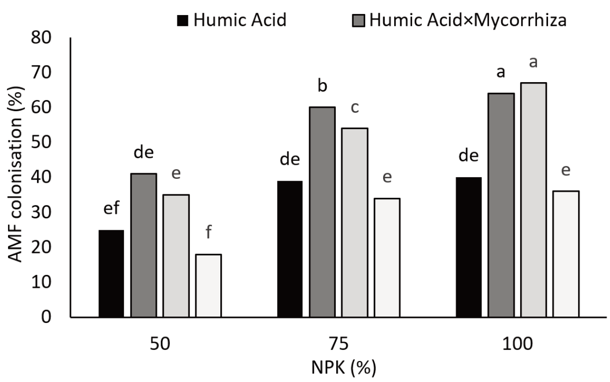


**Figure 1.** Effects of NPK fertilizer application in treatments inoculated with mycorrhiza on root colonization

and 64%, respectively. The lowest percentage of root colonization was obtained under the conditions of application of 50% NPK and control (without inoculation). The data showed that a high dose of NPK resulted in high AMF colonization (Figure 1).

It seems that stimulation of root and shoot growth and increase of branching and development of hairy roots by applying HA+ Myc+ 75 % NPK significantly increased root colonization. Studies have shown that humic acid increases the percentage of plant symbiosis with mycorrhizal fungi due to root morphological changes (Wright and Lenssen, 2013). The application of humic acid alone had a higher percentage of root colonization than the control. By increasing the permeability of root cells, humic acid helps in better absorption of nutrients and further plant growth. Also, it has been proven that this compound increases the rate of cell proliferation in the whole plant, especially in the roots, by producing more nucleic acids and amino acids (Liu et al, 1998). The use of mycorrhiza alone with the application of 10, 75 and 50% NPK increased the root colonization percentage by 86.11, 58.82 and 94.44% respectively. It can be concluded that with mycorrhizal inoculation, the population of this fungus has increased significantly compared to the control, and for this reason, it has improved the percentage of root symbiosis in beans.

## Chlorophyll

The records of analysis of variance of the effect of non-chemical fertilizer and NPK levels on chlorophyll indices are shown in Table 3. The results showed that the interaction effect of non-chemical and NPK fertilizer on chlorophyll a, chlorophyll b and total chlorophyll was significant. The highest chlorophyll a was observed with the application of humic acid + mycorrhiza at the level of 75% NPK in the amount of 1.58 mg $g^{-1}$, and the lowest was obtained in the control treatment with the use of 50% of the NPK. Under 50% NPK application conditions, the use of humic acid and mycorrhiza together or separately increased the plant chlorophyll content compared to the control (Table 3). Bitterlich et al. (2018) stated that due to the spread of mycorrhizal mycelium in soil pores, the rate of water and nutrient uptake increased, and sufficient water was provided for physiological activities in the plant, thus increasing the chlorophyll content in the plant. On the other hand, the study of Ding et al. (2021) showed that plants treated with humic acid and growth-promoting bacteria increased leaf chlorophyll content by 36% compared to the control, which is consistent with the results of this study.

Chlorophyll content mostly affected by environmental alternation and stress (Mirbakhsh and Sedeh, 2022). In this study, the results of chlorophyll b and total chlorophyll also showed that the highest chlorophyll b and total chlorophyll with application of humic acid + mycorrhiza were observed in 75% and 100% of NPK, and the lowest in humic acid treatment with 50% NPK (0.54 mg $gr^{-1}$) was obtained (Table 3). Humic acid has increased growth and chlorophyll in the plant by increasing the chelation of nutrients and reducing evapotranspiration (Rady et al., 2016) and mycorrhiza by increasing the level of water uptake and improving water relations and uptake of nutrients by the plant.

In general, the results showed that the application of humic acid + mycorrhiza and separate application of mycorrhiza had a significant effect on increasing the chlorophyll of the plant. Also, with the combined use of humic acid and mycorrhiza, the amount of chlorophyll has increased at the level of 75% NPK compared to 100% NPK, which indicates that the use of these fertilizers can reduce fertilizer consumption by 25%.

## Yield and yield components

The analysis of variance revealed that the use of non-chemical fertilizers and NPK significantly affected both the number of seeds per pod and the number of pods per plant, as detailed in Table 4. The application of humic acid combined with mycorrhiza resulted in the highest count of pods per plant (6) and seeds per pod (5), whereas the lowest counts were observed in the control group, which did not receive any fertilizer, as shown in Figure 2. The effectiveness of humic acid can be attributed to its hormone-like properties (Zhang et al., 2018), and the benefits of mycorrhizal fungi are linked to improved nutritional and water uptake due to changes in root morphology (Dodd and Pérez-Alfocea, 2012). These enhancements lead to increased growth and flowering. This study's findings align with those of El-Kholy et al. (2019), where a reduction in NPK fertilizer use correlated with a decrease in the number of pods per plant and seeds per pod. The highest and lowest counts for both pods per plant and seeds per pod were observed with 100% and 50% NPK application, respectively (Figure 3).

The interaction effect of non-chemical fertilizer and NPK on the weight of 100 faba bean was significant

**Table 4.** Analysis of variance of yield and yield components of faba bean under different levels of humic acid, mycorrhiza and NPK in two years

| S.O.V | df | Number of pods per plant | Number of seed per pods | 100 seeds weight | Seed yield | Biologic yield |
|---|---|---|---|---|---|---|
| Year (Y) | 1 | 0.03 | 2.05 | 35.23 | 204904.90 | 192286.81 |
| Block (Year) | 4 | 0.78 | 7.93 | 340.08 | 2792020.86 | 1938930.42 |
| Fertilizer (F) | 3 | 7.47 * | 4.74 * | 166.58 * | 8008554.62 * | 931210.64 |
| Y × F | 3 | 0.29 | 0.21 | 5.96 | 497745.83 | 167516.87 |
| NPK | 2 | 3.91 * | 2.82 * | 4099.62 | 13268904.99 * | 1405084.48 |
| Y × NPK | 2 | 0.22 | 0.16 | 430.25 ** | 6384.99 | 349677.15 |
| F × NPK | 6 | 0.38 | 0.29 | 314.01 * | 2154273.57 * | 759904.70 * |
| Y × F × NPK | 6 | 0.16 | 0.10 | 43.58 | 354146.38 | 121896.02 |
| Error | 44 | 0.34 | 0.25 | 44.58 | 469134.30 | 381065.42 |

Remarks: * and ** at 5% and 1%, significant, respectively.

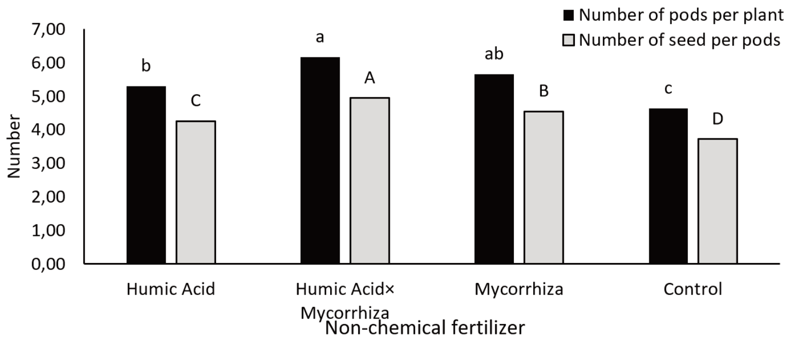


**Figure 2.** Effects of humic acid and mycorrhiza on the number of seeds and pods of faba bean plant

(Table 3). The highest weight of 100 seeds was observed with the application of humic acid + mycorrhiza and the application of humic acid at 100% in NPK level at the rate of 142.15 and 142.12 g, respectively, and the lowest was in the control treatment using 50% NPK. Under 50% NPK application conditions, the use of humic acid and mycorrhiza together or separately increased the weight of 100 seeds faba bean compared to the control (Table 5). The results of El-Kholy et al. (2019) also showed that the use of humic acid increased the grain weight per faba bean plant, which is consistent with the results of this study.

The results of analysis of variance showed that the interaction effect of humic acid and mycorrhiza and NPK on grain yield and biological yield was significant (Table 4). The highest grain yield with humic acid + mycorrhiza application was observed in NPK 100 and 75% at 4356 and 4322 kg ha$^{-1}$, respectively. The lowest grain yield was obtained with 50% NPK application and no fertilizer application (Figure 3). El-Kholy et al. (2019) stated that the use of humic acid reduced the use of phosphorus fertilizer in the faba bean plant, and the highest yield of faba bean seeds was achieved in the application of humic acid and a reduction of 50% of the recommended phosphorus fertilizer. At different levels of NPK, the application of humic acid + mycorrhiza had higher grain yield than other treatments. In general, fertilizer application increased grain yield compared to the control treatment. It seems that humic acid by affecting the physical and chemical properties of soil and increasing water holding capacity in soil and mycorrhizal fungi by forming a colony in the roots and increasing the level of water and nutrient uptake have led to increased growth period and improved yield.

The results showed that the highest biological yield with humic acid + mycorrhiza application was observed in 75 and 100% NPK at the rate of 6155 and 6021 kg ha$^{-1}$, respectively. At 75 and 100% NPK levels, the application of humic acid + mycorrhiza had higher biological yield than other treatments (Table 5).

**Table 5.** Chlorophyll, 100 seeds weight and biologic yield of faba bean under different levels of humic acid, mycorrhiza and NPK

| Treatments | NPK (%) | Chlorophyll a (mg gr⁻¹) | Chlorophyll b (mg gr⁻¹) | Chlorophyll total (mg gr⁻¹) | 100 seeds weight (gr) | Biologic yield (kg ha⁻¹) |
|---|---|---|---|---|---|---|
| Humic acid | 50 | 1.12 ef | 0.54 f | 1.66 e | 115.41 defg | 5353.71 bcd |
| | 75 | 1.25 cde | 0.71 de | 1.95 bcd | 117.66 def | 5279.49 cd |
| | 100 | 1.32 bcd | 0.64 e | 1.96 bcd | 142.12 a | 5454.36 abcd |
| Humic acid × Mycorrhiza | 50 | 1.22 def | 0.68 de | 1.90 cd | 112.84 efg | 5056.79 d |
| | 75 | 1.58 a | 0.88 a | 2.46 a | 137.19 ab | 6154.89 a |
| | 100 | 1.39 b | 0.91 a | 2.30 a | 142.15 a | 6020.87 ab |
| Mycorrhiza | 50 | 1.20 def | 0.68 de | 1.87 cd | 108.60 fg | 5236.89 d |
| | 75 | 1.30 bcd | 0.74 cd | 2.04 bc | 123.37 cde | 5957.83 abc |
| | 100 | 1.28 bcd | 0.84 ab | 2.12 b | 131.29 abc | 5553.44 abcd |
| Control | 50 | 1.10 f | 0.69 de | 1.79 de | 104.92 g | 5035.91 d |
| | 75 | 1.20 def | 0.80 bc | 2.00 bc | 125.13 cd | 5020.67 d |
| | 100 | 1.38 bc | 0.73 cd | 2.11 b | 130.19 bc | 5576.00 abcd |

Remarks: Values within a column followed by same letters are not significantly different at LSD (P≤0.05).

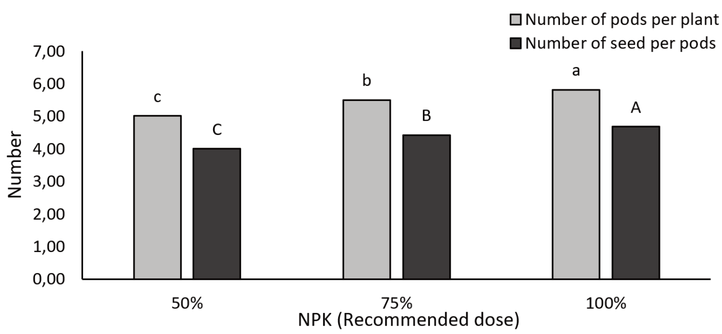


**Figure 3.** Effects of NPK fertilizer on the number of seeds and pods of faba bean plant

In general, fertilizer application had a positive effect on increasing the biological yield of faba bean. The study of Ding et al. (2021) showed that plants treated with humic acid and growth-promoting bacteria along with 50% P of the recommended amount had the highest growth and yield in the faba bean plant, which is consistent with the results of this study. Ayas and Gulser (2005) note that humic acid (HA) enhances plant growth and height, thereby boosting biological yield, primarily by increasing plant nitrogen content. Ulukan (2008) and Roudgarnejad et al. (2021) suggest that the yield improvement due to HA is linked to its stimulation of plant growth. This stimulation occurs through various mechanisms, such as the metabolism of micro and macro elements, activation of enzymes, changes in membrane permeability, and protein synthesis, all contributing to increased plant biomass.

The study's findings revealed that treatments involving humic acid and mycorrhiza led to an increase in chlorophyll content. This, in turn, elevated the rate of photosynthesis and the plant's materialization capacity, culminating in enhanced vegetative growth and a higher biological yield.

## Qualitative traits

The analysis of variance regarding the effects of non-chemical fertilizers and NPK levels on seed quality traits, as presented in Table 6, indicates significant interaction effects on the contents of nitrogen, phosphorus, zinc, potassium, and iron in whole grains. The highest nitrogen content in seeds, at 2.60%, was found in the humic acid + mycorrhiza treatment with 100% NPK application, comparable to the humic acid + 100% NPK treatment (2.47%).

**Table 6.** Analysis of variance results for qualitative traits of faba bean under different levels of humic acid, mycorrhiza and NPK in two years

| S.O.V | df | Seed N | Seed P | Seed Zn | Seed K | Seed Fe |
|---|---|---|---|---|---|---|
| Year (Y) | 1 | 0.308 ** | 8.49 ** | 1.02 ** | 0.567 ** | 0.226 ** |
| Block (Year) | 4 | 0.002 | 0.13 | 0.01 | 0.001 | 0.0001 |
| Fertilizer (F) | 3 | 0.570 * | 0.86 | 0.04 | 0.155 | 0.055 |
| Y × F | 3 | 0.027 * | 0.18 * | 0.02 * | 0.043 ** | 0.016 ** |
| NPK | 2 | 0.902 * | 1.89 | 0.26 * | 1.055 * | 0.415 * |
| Y × NPK | 2 | 0.013 | 0.19 | 0.01 | 0.029 ** | 0.013 ** |
| F × NPK | 6 | 0.241 * | 1.12 * | 0.13 * | 0.343 * | 0.126 * |
| Y × F × NPK | 6 | 0.008 | 0.06 | 0.009 | 0.002 | 0.0005 |
| Error | 44 | 0.008 | 0.07 | 0.01 | 0.003 | 0.001 |

Remarks: * and ** at 5% and 1%, significant, respectively.

**Table 7.** Qualitative traits of faba bean under different levels of humic acid, mycorrhiza and NPK

| Treatments | NPK (%) | Seed N | Seed P | Seed Zn | Seed K | Seed Fe |
|---|---|---|---|---|---|---|
| Humic acid | 50 | 1.75 h | 2.81 g | 1.46 de | 1.08 ef | 0.90 d |
| | 75 | 1.88 gh | 3.71 bc | 1.61 c | 1.18 cde | 0.99 c |
| | 100 | 2.47 ab | 3.54 bcde | 1.86 b | 1.52 a | 1.40 a |
| Humic acid × mycorrhiza | 50 | 2.34 bc | 3.29 cdef | 1.49 de | 1.10 ef | 0.92 d |
| | 75 | 2.38 bc | 4.40 ab | 1.55 cd | 1.15 cde | 0.96 cd |
| | 100 | 2.60 a | 4.55 a | 2.26 a | 1.24 bcd | 1.20 b |
| Mycorrhiza | 50 | 2.04 ef | 3.38 cdef | 1.53 cde | 1.13 de | 0.95 cd |
| | 75 | 2.31 bc | 3.66 bcd | 1.81 b | 1.26 bc | 1.12 b |
| | 100 | 2.29 cd | 4.38 ab | 1.90 b | 1.33 b | 1.18 b |
| Control | 50 | 1.84 gh | 3.16 efg | 1.32 f | 0.97 f | 0.82 e |
| | 75 | 2.00 fg | 3.25 def | 1.47 de | 1.08 eff | 0.91 d |
| | 100 | 2.18 de | 3.06 fg | 1.44 e | 1.07 eff | 0.91 d |

Remarks: Values within a column followed by same letters are not significantly different at LSD (P≤0.05).

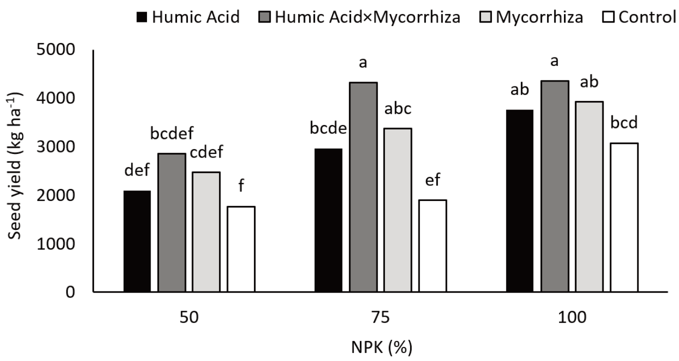


**Figure 4.** Seed yield of faba bean under different levels of humic acid, mycorrhiza and NPK

The highest phosphorus content, at 4.55%, was observed in the humic acid + mycorrhiza treatment with 100% NPK, similar to the results of humic acid + mycorrhiza with 75% NPK and mycorrhiza + 100% NPK treatments. For potassium, the highest percentage (2.26%) was also in the humic acid + mycorrhiza treatment with 100% NPK. Regarding seed zinc and iron contents, the peak levels were in treatments involving humic acid with 100% NPK, at 1.52% and 1.40%, respectively. The lowest percentages of seed nitrogen and phosphorus were seen in the humic acid + 50% NPK treatment, while the minimum levels of potassium, zinc, and iron were in the control treatment with 50% NPK at 1.32%, 0.97%, and 0.82%, respectively.

Büyükkeskin et al. (2015) mention that humic acid enhances the content of sodium, potassium, manganese, and zinc in faba bean plants compared to controls. Various studies, including those by Ding et al. (2021) and Eyheraguibel et al. (2008), have highlighted a significant link between humic acid use and nutrient uptake in plants. Verlinden et al. (2009) emphasized the substantial benefits of humic acid, particularly in chelating nutrients such as Na, K, Mg, Zn, Ca, Fe, Cu, and others, which helps address nutritional deficiencies and improves plant fertility and production.

Haghighi and Kafi (2010) reported that humic acid boosted water and essential nutrient absorption, such as potassium, nitrogen, and iron. This is achieved by promoting root growth in length and volume, increasing the number of root branches, and expanding the surface area of root hairs, thus enhancing the plant's nutrient uptake efficiency.

Mycorrhiza application, both alone and in combination with humic acid, increases nutrient absorption in plants. Franken (2012) observed that mycorrhiza mitigated the adverse effects of nutrient deficiency and stimulates plant growth through nutrient uptake and transport, including nitrogen, phosphorus, potassium, magnesium, zinc, iron, and manganese. Mycorrhizae also enhance root growth and development by producing plant hormones like cytokines and increasing enzyme activity, as noted by Casson & Lindsey (2003), leading to improved nutrient transfer from roots to aerial parts.

## CONCLUSIONS

In conclusion, the study found that both chemical and non-chemical fertilizers affected faba bean root colonization and various plant traits. The combined application of mycorrhiza and humic acid had the most significant positive effects on chlorophyll content, seed production, and pod formation. This approach reduced the reliance on chemical fertilizers, with no significant difference observed between 100% and 75% NPK fertilizer when combined with humic acid and mycorrhiza. The use of humic acid and mycorrhiza improved nutrient uptake, leading to increased nutrient content in seeds and overall plant growth and yield. This combined approach effectively reduced the need for chemical fertilizers, offering a sustainable alternative to boost faba bean growth and mitigate environmental concerns associated with chemical fertilizers.

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