Impact of salinity and fertilization on soil properties, and root development in fenugreek (Trigonella foenum-graecum) cultivation

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Abstract

Salinity is a paramount factor that poses challenges to agricultural productivity and sustainability. At the same time, fenugreek is valued as a forage crop for its medicinal properties in addition to its extensive edible use. The objective of this study is to explore how fertilization and salinity impact soil physical properties and root system development in fenugreek cultivation. A field experiment was established at the Agricultural University of Athens during growing seasons 2019-2020 (1st GS) and 2020-2021 (2nd GS) in a split-splot design with the 2 main salinity treatments (High salinity; HS & Conventional salinity; CS) and 5 fertilization treatments (biocyclic-vegan (BHS), manure (FYM), compost (COMP), inorganic fertilization (11-15-15) and the control (C). Soil porosity was statistically significantly affected by both salinity (p<=0.05) and fertilization (p<=0.001). Also, organic matter was significantly affected by fertilization (p<=0.001). HS (59.78±2.65) resulted in 20.02% fewer nodules on plant roots compared to CS treatments (71.75±2.65). The maximum number of nodules was recorded in the FYM treatment (68.93±0.77). In addition, mean root diameter was affected by fertilization (p<=0.01) COMP (2.92±0.31 mm) and NPK treatments (2.83±0.31 mm) resulted in 19.52% and 23.32% smaller root diameter respectively compared to BHS, while FYM (2.68±0.31 mm) resulted in a 30.22% smaller diameter. A significant increase of seed yield was noticed under organic fertilization where the highest yield of 2.1 t ha$^{-1}$ was recorded in BHS (2nd GS). Although fenugreek was affected by high salinity, it demonstrated considerable resistance and maintained its yields, rendering it a crop suitable for challenging soils.

Keywords: biocyclic - vegan agriculture; fenugreek; salinity; soil porosity; root density

Introduction

Fenugreek originated in the Mediterranean region of Europe (Hilles and Mahmood, 2021). Due to its adaptability, fenugreek has the ability to be grown in different regions of the world, in different climates and growing environments. In this respect, fenugreek allows farmers to grow this plant in different soil types and...
climatic conditions, allowing its production in a variety of geographical areas, including central Europe, the
United Kingdom, the United States, and other regions. Fenugreek is suitable for areas with moderate to low
rainfall (Sharma and Maloo, 2022). A temperate and cool growing season without temperature extremes is
favourable for its best growth. Current uses of fenugreek are mostly culinary and medical. As a spice in parts of
South Asia, the Middle East, North Africa, and Mediterranean Europe, as an ingredient of Indian subcontinent
curries. Fenugreek is therefore a well-known traditional spice and is an important ingredient in widespread
South Asian cuisine throughout the world (Shahrajabian et al., 2021). It has been renowned for its medicinal
applications, owing to its rich nutritional and therapeutic properties, for treating various diseases and disorders
in Ayurvedic, Greek and Tibetan medicine since ancient times. Both fenugreek seeds and leaves are highly
valued for their content of pharmaceutically significant phytochemicals, including alkaloids, carbohydrates,
steroidal saponins, amino acids, and other vital organic and inorganic compounds and minerals (Idris et al.,
2021; Ghosh and Thakurdesai, 2022; Ahmad et al., 2023).

The use of organic fertilizers is regarded and documented as crucial for the improvement of soil
characteristics (Bilalis et al., 2017; Ansari et al., 2017; Meddich et al., 2020). Soil structure, texture, density and
organic matter content control the water holding capacity of the soil; therefore, any management practice that
improves these soil properties, in turn, improves the water holding capacity of the soil (Huntington, 2006; Lal,
2020). The addition of organic matter through the application of organic fertilizers improves soil aggregation
and increases soil density, presenting the soil with more space for soil particles surrounded by water films
(Shepherd et al., 2002).

Several research papers have observed additional benefits to diverse crops with the application of organic
fertilizers compared to the application of equivalent nutrients through inorganic fertilizers (Giller, 2002; Palm
et al., 1997; Vanlauwe et al., 2006; Tabaxi et al., 2021). Following that result, mechanisms have been proposed
to explain these additional benefits to crops from the application of organic fertilizers. Some of them are based
on nutrient timing, the priming effect and general improvement in fertility. Regarding the mechanism of
improved nutrient timing proposed by Vanlauwe et al. (2001), when organic fertilizers are applied, the carbon
they contain is provided to microbes and they bust the decomposition processes. This leads to interstitial
immobilization of soil N (Myers and McGarity, 1968; Palm et al., 2001) to create their body tissues. Immobilized
nitrogen becomes accessible at a later stage of plant growth, when microbes decompose organic
material to release nutrients, or when some microbes lyse, thereby releasing their nutrients to the plant during
its increased nutrient demand (Badalucco and Kuikman, 2000).

Although soil organic matter binds soil particles together, it also promotes the activity of soil
microfauna. Their movement creates micro- and macropores in the soil, providing additional space for water
infiltration (Voroney, 2024). Therefore, enhancing the soil’s ability to retain water can be achieved through the
addition of organic fertilizers. Given the unpredictability of droughts due to climate change, utilizing
organic fertilizers to bolster soil moisture retention is a prudent approach (FAO, 2005).

While conventional agriculture and livestock farming are included in the main causes of greenhouse gas
emissions, and environmental degradation (Chirinda et al., 2010; Tal, 2018; Seymour and Utter, 2021; Edberg,
2023), a new organic farming standard is being created, unburdened by animal inputs. In 2017, a new standard,
Biocyclic Vegan Agriculture, was introduced to the family of organic standards. The use of the Biocyclic Vegan
label is based on an accredited certification system, ensuring complete transparency for consumers at every level
of the supply chain, “from farm to fork.” This guarantees that a Biocyclic Vegan product is not only organic and
plant-based but also produced according to vegan criteria (Oudshoorn et al., 2019). The fundamental principle
of biocyclic-vegan agriculture, globally, is the maintenance or restoration of healthy life cycles (from Greek:
bios=life and kyklos=cycle). This requires a responsible interaction with the environment, which humans use
and significantly impact. Therefore, every personal and economic activity considered within a holistic
framework aims for conscious and sustainable contribution. It represents a suitably adapted development for
the future of agriculture and food industry, where healthy soil leads, through healthy plants, to healthy people.
In this way, the biocyclic or "cycle of living matter" (as referred to by Dr. Hans-Peter Rusch) can be sustainably influenced and enhanced, in harmony with the laws of nature. In this sense, biocyclic-vegan agriculture aims to activate the self-healing potential of an agricultural ecosystem by providing growth conditions as close to nature as possible, thus increasing ecosystem services overall (Seymour, 2023). This activation essentially starts at the macromolecular level and the life of the soil, from where it can then positively impact the entire food chain (Mann, 2020).

Salinity is one of the most significant factors limiting the geographical distribution of plants and negatively impacting the productivity and quality of crops worldwide. Salinity affects approximately 30% of the world’s irrigated land, with this area increasing by about 1-2% annually due to lands affected by salt (FAO, 2018). Excessive concentrations of Na+ in the soil cause irreversible nutrient imbalances, inhibit growth, and can even lead to plant death, with the extent of damage depending on the salt concentration and the specific tolerance of the plants (Shrivastava and Kumar, 2015). Fenugreek can also be cultivated on lands with slight to moderate salinity. Specific traits can be used as criteria to identify fenugreek cultivars that are tolerant to salt stress Banakar et al. (2022).

Roots are vital for absorbing soil nutrients. Root exudates play a crucial role in the conversion and efficient use of nutrients by plants. Plant roots can modify the chemical, physical, and biological properties of the rhizosphere through the release of organic acid anions, protons, signaling molecules, and other compounds (Marschner, 2012). Additionally, plants can regulate the morphological characteristics of their roots to adapt to changing soil environmental conditions. All these processes influence the nutrient use efficiency of plants (Calleja-Cabrera, 2020).

There are various ways to enhance nutrient use efficiency, including root system traits (e.g., genetics, root architecture). Research by Zhang et al. (2010) and Shen et al. (2013) demonstrated how maximizing the root zone can provide a unique opportunity to increase crop productivity, improve nutrient use efficiency, and minimize environmental impacts.

Roots determine the ability of crops to explore and exploit soil resources (water and nutrients). Thus, they are key targets for breeding programs aimed at improving yield stability, resource use efficiency, and resilience to environmental stresses (Hammer et al., 2009, Siddique et al., 2015; Duncan et al., 2018). Enhancing root systems can be a promising strategy, particularly in the context of sustainable intensification (Collette et al., 2011), low-input agriculture (including organic farming), and climate change resilience.

Additionally, a substantial body of literature confirms the essential role of the abundant and diverse bacteria in the rhizosphere in plant growth and adaptation to extreme conditions (Kalam et al., 2022). This presents opportunities to improve stress resilience and crop productivity by developing and applying appropriate biofertilizers. However, their formulation must consider the positive interactions between rhizobacteria and mycorrhizal fungi (Jabborova et al., 2021). Biofertilizers are recognized as a sustainable tool for agriculture, particularly for enhancing plants’ ability to cope with adverse environmental conditions (Sahoo et al., 2012).

The natural fertility of soil and soil structure affects its ability to resist degradation processes such as compaction and erosion in the context of land management. This study aims to investigate the effects of fertilization and salinity on soil physical properties and root system development in a fenugreek crop. In particular, it seeks to understand how different fertilizers affect soil porosity and soil organic matter. It will also examine the effects of salinity on soil characteristics. The study will evaluate how the type of fertilizer applied affects the growth and morphology of fenugreek plant root systems, and how soil salinity affects the distribution and density of fenugreek roots. Finally, the relationships between physical soil properties and fenugreek root development will be investigated.
Materials and Methods

Location and Experimental Design

Fenugreek cultivation experiments were conducted in the experimental field of the Agricultural University of Athens, Agronomy Laboratory (experimental field of arable crops) (37°59'02.1 "N 23°42'08.4 "E and altitude 28.04 m) for three consecutive growing seasons. The first growing season of the experiment was 2019-2020 (1st GS) followed by 2020-2021 (2nd GS). One variety of fenugreek (Trigonella foenum graecum) was cultivated throughout the experiment. The previous crop was organic tobacco with green manuring of vetch. The total precipitation was 217.89 mm in 1st GS, and 309.00 mm in 2nd GS.

![Figure 1. Meteorological data at experimental area for the growing seasons 2019-2020 and 2020-2021](image)

The soil pH is characterized as clay loam (CL), slightly alkaline and with a satisfactory organic matter content (2.37 %). The CaCO$_3$ was 29.9%, and the N, P, K were 101.3 ppm, 20.3 ppm and 235 ppm respectively.

The experimental design was split plot design. In total, there were 15 large experimental units where the 2 main salinity treatments (High salinity; HS & Conventional salinity; CS) and 30 small plots with the 5 fertilization treatments (biocyclic-vegan (BHS), manure (FYM), compost (COMP), inorganic fertilizer (11-15-15) and the control (C) in three blocks. Sowing for the experiment was done manually with a row spacing of 30 cm. The seeding rate was 30 kg ha$^{-1}$. The treatment of salinity was carried out 1 week after sowing. The quantity of 200 kg ha$^{-1}$ NaCl was applied on the surface of the large experimental units. The CS plots received zero amount of NaCl.

For the treatment of fertilization, biocyclic-vegan (BHS), manure (FYM), compost (COMP), inorganic fertilizer (11-15-15) and the control. Fertilizer rates were applied to be the same units of nitrogen per treatment and was it 110 kg N ha$^{-1}$.

Biocyclic Humus Soil is recommended as a substitute for manure or other animal-based fertilizers for producers following the Biocyclic Vegan Standard. The applied amount of BHS was 3.928 tons per hectare. It contained 46.3 g of organic matter per 100 g, 2.8 g of nitrogen per 100 g, and had a pH of 7.6. The compost was a commercial preparation, with an application rate of 9.166-ton ha$^{-1}$. The composition of the compost was 70% compost, 15% black peat, organic materials, 10% perlite, and 5% soil, with a pH of 5.5 - 6.8 and 1.2% nitrogen.
The applied FYM came from the stables of the Agricultural University of Athens and the applied amount was 6.875-ton ha⁻¹. The physico-chemical composition of the manure was pH 7.39, total N 1.60%, P (Olsen) 8.9 ppm, and organic C 4.4%. The applied amount of NPK was 1 ton ha⁻¹.

During the two growing seasons (GS), the soil treatment remained consistent. Immediately after the previous crop, primary tillage was performed using a cultivator, with the main goal of restructuring the soil’s porosity. For soil preparation prior to sowing, light tillage was carried out with a rotary tiller. Weeds were managed as needed through manual hoeing. There was no irrigation applied.

**Sampling and methods**

**Soil samples**

Soil samples were taken from the 0-25 cm soil layer. The total nitrogen in the soil was measured according to ISO 11261:1995 protocol (ISO, 1995). Cation exchange capacity was determined following International Organization for Standardization (ISO) 11260 (ISO, 1994). Electrical conductivity was determined in a soil water extract according to ISO 11265:1994 standard. Organic matter was calculated using the Walkley and Black method (1934). The total porosity (St) of soil was estimated from the following equation (Flint and Flint, 2002): St (%) = (1-Db/Dp) where: St – total pore spaces, Dp – particle density (2.5 g cm⁻³), Db – soil bulk density

For each plot, soil bulk density was determined by taking undisturbed soil cores with 100 cm³ cylinders from a depth of 0-10 cm. Three samples of 100 cm³ per plot were taken at 100 DAS. The undisturbed samples were finally oven-dried at 100 °C for 24 h to obtain soil dry mass and the soil bulk density was calculated as follows: Db = dry mass (g)/100 cm³.

**Root samples**

Root samples were taken from the 0-30 cm soil layer using a cylindrical auger (25 cm in length and 10 cm in diameter) at the midpoint between consecutive plants within a row. Three samples were analyzed per plot at 90, 110, and 130 DAS. Firstly, every sample, the roots were separated from the soil after being soaked in a solution of water + (NaPO₃)₆ + Na₂CO₃ for 36 h and then decanted into a 0.1% trypan blue FAA staining solution (a mixture of 10% formalin, 50% ethanol and 5% acetic acid solutions). The number of nodules were recorded (No 100 cm⁻³). For the determination of Root density (mm² of root 100 cm⁻³ soil), Mean root diameter (mm of root 100 cm⁻³ soil), as well as root volume (ml of root 100 cm⁻³ soil) and Root length (mm 100 cm⁻³ soil) the root samples were placed on a high-resolution scanner. Then, the digital images of the root were investigated by DT software.

**Vegetation and yield measurements**

The number of plants that had sprouted from the soil was measured in 40 plots of land. The measurement was conducted using quadrats of 0.25 m². The Leaf Area Index (LAI) was measured using an automatic leaf area meter (Delta-T Devices Ltd., Cambridge, UK) at 110 DAS. The seed yield (kg ha⁻¹) and the weight of 1000 seeds (g) were measured on the day of the harvest. Each plot was harvested at full seed maturity (seed moisture was at 14%) and the harvest was conducted by hand.

**Statistical analysis**

The experimental data were evaluated using analysis of variance (ANOVA) across all years. Additionally, homogeneous groups of means were formed using Tukey’s method, as the experiment is factorial. For the homogeneous groups, the null hypothesis of equality of means within them was not rejected. The ANOVA analysis was conducted using Sigma Plot (ver. 10; Systat Software Inc., CA, USA). Finally, the Pearson correlation coefficient (PCC) was calculated using the R software. All analyses were performed at a significance level of α = 0.05 (5%).
Results

The factor that significantly affected soil total nitrogen was fertilization ($p \leq 0.001$), and the interaction between fertilization and salinity ($p \leq 0.01$) for the 1st and 2nd GS. The maximum soil nitrogen value was recorded in the BHS intervention ($2.45 \pm 0.06$ mg g$^{-1}$), and the minimum in the C intervention ($1.13 \pm 0.06$ mg g$^{-1}$) (Table 1). For the 2nd GS, the factor that significantly affected soil nitrogen was fertilization ($p \leq 0.001$), and the interaction between fertilization and salinity ($p \leq 0.05$). FYM ($2.34 \pm 0.07$ mg g$^{-1}$) gave 8.51% less soil nitrogen compared to BHS, while NPK ($2.17 \pm 0.07$ mg g$^{-1}$) and COMP ($2.10 \pm 0.07$ mg g$^{-1}$) gave 17.51% and 21.43% less, respectively.

### Table 1. Two-way ANOVA analysis of the fertilization (BHS: Biocyclic Humus Soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11-15-15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on soil total nitrogen (STN), Cation exchange capacity (CEC), electrical conductivity (EC), porosity and organic matter (OM)

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<th>EC</th>
<th>Porosity</th>
<th>OM</th>
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</table>

The F-test indicators are from the ANOVA. Different letters (a, b, c, and d) within a column indicate significant differences according to the Tukey test. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant ($p > 0.05$).

For the 1st GS, COMP ($18.66 \pm 0.31$) and FYM ($18.33 \pm 0.31$) had 7.18% and 9.11% less CEC compared to BHS, while NPK ($17.33 \pm 0.31$) had 15.41% less. For the 2nd GS, the maximum CEC value was recorded in the BHS intervention ($19.83 \pm 0.30$) and the minimum in the C intervention ($15.66 \pm 0.30$). COMP ($18.33 \pm 0.30$) and FYM ($18.33 \pm 0.30$) gave 8.18% less CEC compared to BHS, while NPK ($17.33 \pm 0.30$) gave 14.43% less.
Moreover, in 1st GS, soil porosity was statistically significantly affected by both salinity (p<=0.05) and fertilization (p<=0.001). With HS, soil porosity was 43.02±0.29%, while in the CS, it was 44.95±0.29%. The maximum value for soil porosity was recorded in the BHS and FYM treatments (45.87±0.36%), and the minimum was in the NPK (41.08±0.36%). For the 2nd GS, soil porosity was also significantly influenced by salinity (p<=0.05) and fertilization (p<=0.001). With HS, soil porosity was 49.62±0.35%, while in CS it was 51.84±0.35% (Table 1). The maximum value for soil porosity was noted in the FYM treatment (52.92±0.45%), and the minimum in the COMP treatment (47.38±0.45%).

Additionally, for both the 1st and 2nd GS, organic matter was significantly affected by fertilization (p<=0.001), with no significant differences observed between C and NPK treatments. For the 1st GS, the maximum value for organic matter was documented in the BHS treatment (3.76±0.15%), and the minimum in the NPK treatment (2.43±0.15%). For the 2nd GS, the maximum value for organic matter was observed in the FYM treatment (52.92±0.45%), and the minimum in the COMP treatment (47.38±0.45%).

In Table 2 is presented that throughout the growth stages (90, 110, and 130 DAS) for both crop cycles (1st and 2nd GS), root density was statistically significantly affected by the fertilization factor (p<=0.001). For the 1st GS at 90 DAS, the FYM (4294.80±214.05 mm$^2$) and NPK (3941.70±214.05 mm$^2$) resulted in 3.33% and 12.59% smaller root density compared to BHS, while COMP (3482.70±214.05 mm$^2$) resulted in a 27.42% smaller area. In the same year, at 130 DAS, the maximum root density was documented in the BHS treatment (4247.7±218.6 mm$^2$) and the minimum in the C treatment (2144.9±218.6 mm$^2$). FYM (4171.3±218.6 mm$^2$) and NPK (3826.7±218.6 mm$^2$) resulted in reduced root density by 1.83% and 11% respectively compared to BHS.

### Table 2. Two-way ANOVA analysis of the fertilization (BHS: Biocyclic humus soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11-15-15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on root density and mean root diameter at 90, 110 and 130 DAS

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<th>Fertilization</th>
<th>Root density 90 DAS</th>
<th>Root density 110 DAS</th>
<th>Root density 130 DAS</th>
<th>Mean root diameter 90 DAS</th>
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The F-test indicators are from the ANOVA. Different letters (a, b, c, and d) within a column indicate significant differences according to the Tukey test. Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001, ns: not significant (p > 0.05).
For the 2nd GS, at 90 DAS, FYM \((4659.00±254.52 \text{ mm}^2)\) and NPK \((4290.1±254.52 \text{ mm}^2)\) led to 3.31% and 12.19% smaller root density compared to BHS, while COMP \((3758.1±254.52 \text{ mm}^2)\) resulted in a 28.07% smaller area. At 110 DAS, FYM \((4596±243.39 \text{ mm}^2)\) and NPK \((4198.6±243.39 \text{ mm}^2)\) resulted in reduced root density by 2.59% and 12.30% respectively compared to BHS. At 130 DAS, the maximum root density was noted in the BHS \((4612.4±232.5 \text{ mm}^2)\) and the minimum in the C \((2332.7±232.5 \text{ mm}^2)\). FYM \((4538.7±232.5 \text{ mm}^2)\) and NPK \((4177.6±232.5 \text{ mm}^2)\) resulted in reduced root density by 1.62% and 10.41% respectively compared to BHS, while COMP \((3727.7±232.5 \text{ mm}^2)\) resulted in a 13.73% smaller area (Table 2).

Mean root diameter was statistically significantly affected by fertilization \((p<=0.01)\) throughout the growth stages (90, 110, and 130 DAS) for both growing seasons. For the 1st GS at 90 DAS, the maximum mean root diameter was registered in the BHS treatment \((3.14±0.28 \text{ mm})\) and the minimum in the C \((1.66±0.28 \text{ mm})\). COMP \((2.62±0.28 \text{ mm})\) and NPK \((2.54±0.28 \text{ mm})\) resulted in 19.85% and 23.62% smaller mean root diameter respectively compared to BHS. At 110 DAS, BHS and COMP, as well as COMP with FYM, did not show statistically significant differences between them. The maximum mean root diameter was observed in the BHS treatment \((3.08±0.28 \text{ mm})\) and the minimum in the C treatment \((1.63±0.28 \text{ mm})\). COMP \((2.57±0.28 \text{ mm})\) and NPK \((2.49±0.28 \text{ mm})\) resulted in 19.84% and 23.69% smaller mean root diameter respectively compared to BHS. For the 2nd GS, at 90 DAS, the maximum mean root diameter was recorded in the BHS treatment \((3.64±0.33 \text{ mm})\) and the minimum in the C treatment \((1.93±0.33 \text{ mm})\). At 130 DAS, the maximum mean diameter was noted in the BHS treatment \((3.49±0.31 \text{ mm})\) and the minimum in the C treatment \((1.86±0.31 \text{ mm})\). COMP \((2.92±0.31 \text{ mm})\) and NPK \((2.83±0.31 \text{ mm})\) resulted in 19.52% and 23.32% smaller root diameter respectively compared to BHS, while FYM \((2.68±0.31 \text{ mm})\) resulted in a 30.22% smaller diameter (Table 2).

The root volume was significantly influenced both by fertilization \((p<=0.001)\) and salinity \((p<=0.05)\) for both cropping periods. The root volume was statistically significantly affected by both salinity \((p<=0.05)\) and fertilization \((p<=0.001)\) (Table 3). For the 1st GS, at 90 DAS, HS \((14.39±0.20 \text{ ml})\) resulted in 7.51% less root volume compared to CS treatments \((15.47±0.20 \text{ ml})\). At 110 DAS, BHS and FYM did not show a statistically significant difference between them, while the differences among the rest were evaluated as statistically significant. FYM \((17.68±0.31 \text{ ml})\) had only 0.23% less root volume compared to BHS, while COMP \((15.53±0.31 \text{ ml})\) and NPK \((13.94±0.31 \text{ ml})\) had 14.10% and 27.12% less, respectively. The same year, at 130 DAS, HS \((13.96±0.21 \text{ ml})\) resulted in a reduced root volume by 7.59% compared to CS treatments \((15.02±0.21 \text{ ml})\).

For the 2nd GS, at 90 DAS, HS \((16.66±0.23 \text{ ml})\) decreased the root volume by 7.44% compared to CS treatments \((17.90±0.23 \text{ ml})\). BHS and FYM did not show a statistically significant difference between them. The same year, at 130 DAS, HS \((16.09±0.26 \text{ ml})\) led to a reduced root volume by 2.90% compared to CS treatments \((17.32±0.26 \text{ ml})\). FYM \((20.10±0.35 \text{ ml})\) resulted in only 0.20% less root volume compared to BHS (Table 3).
Table 3. Two-way ANOVA analysis of the fertilization (BHS: Biocyclic Humus Soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11-15-15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on root volume at 90, 110 and 130 DAS

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Table 4 presents the data for the 1st GS at 90 DAS, showing that root length was statistically significantly affected by both salinity (p<=0.005) and fertilization (p<=0.001). HS (1884.00±20 mm) resulted in a 28.66% decrease in root length compared to CS treatments (2423.9±20 mm). The maximum root length was observed in FYM treatment (2465.50±43.46 mm), and the minimum in the control (C) (1818.60±43.46 mm). At 110 DAS, root length was also significantly affected by both salinity (p<=0.001) and fertilization (p<=0.001). HS (1855.50±15.85 mm) led to a 38.78% shorter root length compared to CS treatments (2575±15.85 mm). BHS (2188±12.06 mm) and NPK (2140.1±12.06 mm) resulted in 31.67% and 34.62% shorter root length, respectively, compared to FYM. At 130 DAS, root length was affected by both salinity (p<=0.01) and fertilization (p<=0.001). HS (2140.1±39.00 mm) led to a 38.23% decrease in root length compared to CS treatments (2970.7±39.00 mm). BHS (2524±29.66 mm) and NPK (2468.7±29.66 mm) resulted in 31.71% and 34.66% shorter root length, respectively, compared to FYM, while COMP (2423.4±29.66 mm) led to a 37.18% decrease.

For the 2nd GS, root length at 90 DAS was statistically significantly affected by both salinity (p<=0.01) and fertilization (p<=0.001). Similarly, at 110 DAS, HS (2165.10±35.35 mm) resulted in plants with roots 38.90% shorter compared to CS treatments (3007.40±35.35 mm). BHS (2554.4±26.88 mm) and NPK (2498.3±26.88 mm) resulted in 31.77% and 34.73% shorter root length, respectively, compared to FYM. At 130 DAS, root length was affected by both salinity (p<=0.01) and fertilization (p<=0.001). HS (2140.1±39.00 mm) led to a 38.23% decrease in root length compared to CS treatments (2970.7±39.00 mm). BHS (2524±29.66 mm) and NPK (2468.7±29.66 mm) resulted in 31.71% and 34.66% shorter root length, respectively, compared to FYM, while COMP (2423.4±29.66 mm) led to a 37.18% decrease.

In Table 4, for both the 1st and 2nd GS, the number of nodules was statistically significantly influenced by both salinity (p<=0.05) and fertilization (p<=0.001). HS (63.46±1.34) resulted in plants with 13.73%
fewer nodules on their roots compared to CS treatments (72.17±1.34). BHS and COMP did not show any difference between them. At 130 DAS, it was statistically significantly affected by both salinity (p<0.05) and fertilization (p<0.001). HS (59.78±2.65) resulted in 20.02% fewer nodules on plant roots compared to CS treatments (71.75±2.65). The maximum number of nodules was recorded in the FYM treatment (68.93±0.77).

At 110 DAS of 2nd GS, COMP (79.57±0.96) and BHS (79.38±0.96) resulted in plants with 1.41% and 1.65% fewer nodules on their roots compared to FYM, while NPK (76.40±0.96) resulted in 5.60% fewer nodules. At 130 DAS, the factor that statistically significantly affected the number of nodules was fertilization (p<0.001). COMP (78.38±0.83) and BHS (78.19±0.83) resulted in 1.17% and 1.42% fewer nodules on plant roots compared to FYM, while NPK (75.30±0.83) resulted in 5.31% fewer.

Table 4. Two-way ANOVA analysis of the fertilization (BHS: Biocyclic Humus Soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11-15-15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on root length and nodules number at 90, 110 and 130 DAS

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<td>630.05***</td>
<td>503.41***</td>
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<tr>
<td>Salinity *</td>
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<td>505.16***</td>
<td>403.63***</td>
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</table>

The F-test indicators are from the ANOVA. Different letters (a, b, c, and d) within a column indicate significant differences according to the Tukey test. Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001, ns: not significant (p > 0.05).
For both the 1st and 2nd GS, plant emergence in 40 DAS plots was statistically significantly affected by fertilization (p<=0.001). Fertilization treatments showed statistically significant differences compared to the C, however, the differences between them were not evaluated as statistically significant (Table 5).

The maximum seed yield was recorded in FYM (2013.5 ± 100.13 kg ha\(^{-1}\)) and the minimum in C (1168.4 ± 100.13 kg ha\(^{-1}\)) for the 1st GS. NPK (1866.8 ± 100.13 kg ha\(^{-1}\)) yielded 7.86% less seed compared to FYM, while COMP (1750.5 ± 100.13 kg ha\(^{-1}\)) and BHS (1721.8 ± 100.13 kg ha\(^{-1}\)) yielded 15.02% and 16.94% less, respectively. For the 2nd GS, the maximum seed yield was observed in the BHS intervention (2101.6 ± 68.06 kg ha\(^{-1}\)) and the minimum in the C intervention (1300.9 ± 68.06 kg ha\(^{-1}\)). BHS and FYM (1994.4 ± 68.06 kg ha\(^{-1}\)) did not differ significantly in seed yield.

The TSW was statistically significantly affected by the fertilization (p<=0.001). For the 1st GS, the TSW reached its maximum value in the BHS treatment (17.00±0.33g), and its minimum in the C (9.87±0.33g). FYM (16.00±0.33g) resulted in 6.25% lower weight compared to BHS, while NPK (15.09±0.33g) and COMP (13.40±0.33g) yielded 12.66% and 26.87% lower, respectively. For the 2nd GS, the highest TWS was recorded in the BHS intervention (17.71±0.47g), and the lowest in the C intervention (11.85±0.47g). FYM (16.91±0.47g) resulted in 4.73% lower weight compared to BHS, while NPK (15.71±0.47g) and COMP (15.18±0.47g) yielded 12.73% and 16.67% lower, respectively (Table 5).

### Table 5. Two-way ANOVA analysis of the fertilization (BHS: Biocyclic humus soil, COMP: compost, C: control, FYM: farm yard manure, NPK: 11-15-15) and salinity levels (CS: conventional salinity, HS: high salinity) effect on plant emergence, seed yield and thousand seed weight (TSW)

<table>
<thead>
<tr>
<th>Plant emergence</th>
<th>Seed yield</th>
<th>TSW</th>
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<tr>
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<td>2013.50a</td>
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<tr>
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<td>HS</td>
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<td></td>
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<td>Df</td>
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<td>ns</td>
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<td>Error</td>
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<tr>
<td><strong>Fertilization</strong></td>
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<td></td>
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<tr>
<td>BHS</td>
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<tr>
<td>COMP</td>
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<tr>
<td>C</td>
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<td>FYM</td>
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<td>1994.40ab</td>
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<td>NPK</td>
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<td><strong>Salinity</strong></td>
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<td>CS</td>
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<tr>
<td>Df</td>
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<td><strong>Total</strong></td>
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<td>ns</td>
</tr>
</tbody>
</table>

The F-test indicators are from the ANOVA. Different letters (a, b, c, and d) within a column indicate significant differences according to the Tukey test. Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001, ns: not significant (p > 0.05).
Discussion

The application of BHS has been shown to offer a wide range of advantages for the development of fenugreek root systems and soil improvement. The same beneficial properties of BHS have been observed in sweet potato cultivation (Eisenbach et al., 2018) and industrial tomato production (Eisenbach et al., 2019; Kakabouki et al., 2021).

The porosity of the soil was statistically significantly affected by both salinity and fertilization. Salinity led to reduced porosity through the formation of aggregates, connecting soil particles with each other, thus reducing the total pore space, and limiting water movement (Głąb, 2014; Pessarakli et al., 2019). Organic fertilization may contribute to increasing soil organic matter content (Brady and Weil, 2016). This result is consistent with the findings of this study. Organic matter plays a crucial role in maintaining soil structure and porosity by promoting the stability of aggregates (Evanylo et al., 2008). This is also inferred from our experiment as a significant positive correlation was noted between organic matter and porosity ($r=0.72$, $p<=0.001$) (Figure 2).

Figure 2. Pearson’s correlation matrix with $r$ and $p$-values between soil and root characteristics

Conversely, inorganic fertilizers when used excessively may contribute to soil compaction, which can reduce soil porosity. Additionally, organic fertilizers BHS, FYM, and COMP did not differ statistically among themselves.

However, this effect is often associated with inappropriate management practices (Brady and Weil, 2016). Changes in soil porosity can affect plant root growth and water uptake (Głąb, 2014; Singh et al., 2020). Understanding the combined effects is vital for predicting plant responses to soil conditions (Efthimiadou et al., 2010; Marschner, 2012).

The STN was statistically significantly affected by the type of fertilization. The highest STN was recorded with BHS. Due to the stability of BHS and its resistance to nutrient leaching, the risk of overfertilization is virtually eliminated, even with the application of high rates. Consequently, BHS could play an important role in addressing the current global nitrogen challenge (Anders and Eisenbach, 2017). Furthermore, Singh (2005) and Jani et al. (2015) demonstrated that the presence and decomposition of legume roots have little positive effect on soil nitrogen increase. Therefore, the presence of fenugreek roots in the soil
after seed harvesting may have a positive effect on soil nitrogen increase. STN shows a positive correlation with LAI ($r=0.58$, $p<0.001$) and soil organic matter ($r=0.62$, $p<0.001$).

Organic matter was statistically significantly affected by fertilization in fenugreek culture. This was confirmed in a multitude of crops (Galantini and Rosell, 2006; Plaza et al., 2016; Šimanský et al., 2019; Balík et al., 2022). Biological fertilization and in particular BHS fertilization gave the highest organic matter values. This is confirmed by several studies, which claimed that biological amendments promote better plant growth, which can be linked to improved root development and more efficient use of water and nutrients (Chen and Aviad, 1990; Oleńska et al., 2020; Elnahal et al., 2022).

Root density was statistically significantly affected by the type of fertilization in fenugreek cultivation, with greater root density observed with organic fertilizers. This conclusion is confirmed in alfalfa cultivation (Vasileva and Kostov, 2015). This can be explained by the fact that organic fertilizers can improve soil structure and nutrient content, creating a more favorable environment for root growth (Kopke et al., 2015; Olmo et al., 2016). Additionally, organic fertilization contributes to the development of beneficial microbial communities in the soil, which can improve nutrient availability to plants and positively influence root density (Eghball and Power, 1999; Zhang et al., 2019). This is confirmed in our experiment as root density showed a high positive correlation with soil nitrogen ($r=0.84$, $p<0.001$) (Figure 2). On the other hand, when applied judiciously, inorganic fertilizers provide specific nutrients necessary for root growth and overall plant development (Sinha and Tandon, 2020). The root density in fenugreek cultivation showed a moderate to low positive correlation with soil porosity and soil organic matter ($r=0.33$ and $r=0.42$ respectively, $p<0.001$).

Environmental stressors, such as nutrient deficiencies or salinity, can influence root morphology (Vadez et al., 2007). Measuring the mean root diameter allows researchers to assess how plants respond to these stressors and adjust their cultivation practices accordingly (Fageria and Moreira, 2011; Parkash, 2020). In our experiment, the mean diameter of fenugreek roots was larger with organic fertilizers compared to inorganic ones, specifically with BHS. It is noteworthy that the mean root diameter of fenugreek roots was not affected by high salinity. Therefore, fenugreek cultivation can develop a healthy root system even in soils with high salinity. The root diameter of fenugreek roots showed a moderate positive correlation with soil nitrogen and soil organic matter ($r=0.60$ and $r=0.37$ respectively, $p<0.001$).

Root volume is a key indicator of a plant’s root system spatial extent, affecting its ability to access nutrients and water in the soil (Lynch, 2013). In our experiment, root volume in fenugreek cultivation was significantly influenced by fertilization and salinity. High salinity led to a reduction in root volume. This result is consistent with Munns (2002) and Flowers and Colmer (2008), who demonstrated that high soil salinity can decrease root volume due to osmotic stress and hindered water uptake, affecting root growth and development. Changes induced by salinity in root morphology, such as reduced root length and branching, contribute to an overall decrease in root volume (Zhu, 2002; Munns and Tester, 2008). In our experiment, root volume in fenugreek cultivation was larger under organic fertilization. This conclusion arises because organic fertilization enhances root volume by shaping the spatial distribution of the rhizosphere and promoting microbial interactions and nutrient recycling in the soil, providing a rich substrate for microbial growth and activity (Windisch et al., 2021; Biswas and Kole, 2017). Additionally, root volume showed a positive correlation with BHS ($r=0.73$, $p<0.001$).

Root length was significantly affected by both salinity and fertilization in fenugreek cultivation. High soil salinity negatively impacted the root length of fenugreek plants. This finding aligns with Benmoussa et al. (2022) in bean cultivation. High salinity induces ionic toxicity, limiting water uptake and causing cellular damage, ultimately resulting in reduced root elongation (Munns, 2002; Flowers and Colmer, 2008). In contrast, organic fertilizers significantly promoted root length in fenugreek cultivation by improving nutrient availability, enhancing nutrient uptake efficiency, and overall plant health, contributing to increased root length (Dadresan et al., 2015; Saadatian et al., 2017). Additionally, while root length appeared to be influenced by salinity, it showed a relative correlation with LAI ($r=0.38$, $p<0.01$). This, combined with the observation
by some researchers that the impairment of root water uptake under high salinity conditions correlates with plant salt tolerance (Rewald et al., 2011; Ramana et al., 2012), leads us to conclude that fenugreek plants are moderately tolerant to salinity. Furthermore, Tuncturk (2011) demonstrated that fenugreek plants exhibited the highest concentration of macro and micronutrients in the roots compared to shoots under saline conditions.

The number of nodules was significantly affected by salinity in fenugreek cultivation. High soil salinity led to a decrease in the number of fenugreek nodules (a 20% reduction), as salinity stress inhibits the symbiotic interaction between rhizobia and nitrogen-fixing bacteria. This conclusion aligns with Raje et al. (2002). In our experiment, the number of nodules was also significantly influenced by fertilization. Fertilization practices can affect nitrogen fixation, with imbalanced nutrient levels, especially excessive nitrogen, potentially inhibiting ozone formation in legume crops, including fenugreek plants (Abd-Alla, 2023). The number of fenugreek root nodules showed a strong positive correlation with soil nitrogen and TSW (r=0.52 and r=0.41 respectively, p<=0.01). This finding is consistent with Otieno et al. (2009).

In our experiment, it was demonstrated that fertilization, and not salinity, influenced plant emergence. The tolerance to salinity is confirmed by Saberali and Moradi (2019). Additionally, adequate moisture is essential for seed imbibition and initiation of germination (Mehrafarin et al., 2011). Our results align with the above as the highest number of plants per square meter was noted in the 40 HAS during the 3rd growing period, following the highest rainfall compared to the other three years. Although the number of plants emerged per square meter in the 40 HAS was statistically significantly affected by fertilization, no significant differences were observed between organic and inorganic fertilizers. Furthermore, plant emergence showed a positive correlation with soil porosity (r=0.77, p<0.001) and soil organic matter (r=0.46, p<0.001).

Seed yield in fenugreek was statistically significantly affected only by fertilization. This result is in agreement with Zandi et al. (2011) who recorded 1300-1468 kg ha⁻¹ with the highest recorded with most nitrogen units; in the 2nd GS BHS gave the highest yields. Godara et al. (2012) demonstrated that the recommended nutrient dose through inorganic fertilizers produced significantly higher yield than organic manures (vermicompost and poultry FYM). Specifically, application of the recommended dose of nutrients provided through chemical fertilizers resulted in the highest grain yield (27.75 q/ha).

Conclusions

In conclusion, the application of BHS provides significant benefits for fenugreek root system development and soil improvement, reflecting its advantages observed in other crops. Fertilization practices, particularly the use of organic fertilizers such as BHS, have a notable impact on soil properties, enhancing soil porosity, organic matter content, and overall soil structure. These improvements are crucial for maintaining soil health and promoting robust root growth. Salinity, on the other hand, tends to reduce soil porosity and root volume, underscoring the importance of managing salinity levels to ensure optimal plant development. Organic fertilization contributes significantly to soil organic matter and root density, fostering a more favorable environment for root proliferation and nutrient uptake. Additionally, BHS’s stability and resistance to nutrient leaching make it an effective solution for enhancing soil nitrogen levels and improving overall plant health. This is particularly important in addressing global nitrogen challenges. The positive correlations between soil characteristics such as nitrogen content, organic matter, and root traits like density, volume, and diameter further emphasize the interconnectedness of soil health and plant development. Although fenugreek was affected by high salinity, we judge that it is quite salinity tolerant and did not affect its yields. Ultimately, the use of BHS and other organic fertilizers can lead to improved plant emergence and seed yield, demonstrating their critical role in sustainable agricultural practices and effective soil management.
Authors’ Contributions

Conceptualization: AF and DB; Data curation: AF and DB; Formal analysis AF and AM; Funding acquisition; Investigation: AF and KT; Methodology: DB; Project administration: AF; Resources: Software: AF and IK; Supervision: DB; Validation: IK; Visualization: AF and DB; Writing - original draft: AF, KT, PS, AM; Writing - review and editing: AF, KT, PS, AM, DB AND IK. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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