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# Effect of Chemical Fertilization of Food Legumes on the Physical, Chemical and Microbiological Properties of Soil: Case of Chlef and Mostaganem, Algeria

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## ABSTRACT

**Background:** The use of chemical fertilizers is gaining importance for increasing crop productivity in Algeria. Application of these fertilizers in food legumes are also increasing due to poor native soil nutrients. Use of chemical fertilizers in food legumes may have varying impact on soil properties, which needs to be evaluated.

**Methods:** In this study, various techniques were employed to evaluate the effect of chemical fertilizers on soil properties. Physicochemical and microbiological properties of soils of Chlef and Mostaganem were studied and correlations between the different parameters were assessed and compared with natural soil properties.

**Result:** The comparison of the results clearly demonstrated the effect of chemical fertilizers on soil microbial life, particularly evident in root colonization and the mycorrhizal potential index (MPI). Chemical fertilizers reduced arbuscular mycorrhizal fungi by threefold or more compared to natural soil. Among the microbiological parameters studied, a significant increase was recorded in natural soil samples compared to those treated with chemical fertilizers.

**Key words:** AMF, Chemical fertilizers, *Cicer arietinum* L., MPI, Soil physicochemical analyses, *Vicia faba* L.

## INTRODUCTION

Significant amounts of chemical fertilizers are applied annually to improve soil fertility and crop productivity (Garba *et al.,* 2022; Tyagi *et al.,* 2022; Yang *et al.,* 2024). Application of these chemical fertilizers pose risks, including soil degradation, pollution and indirect environmental effects (Hicks *et al.,* 2020). Chemical fertilizers, also referred to as inorganic fertilizers, are synthetic amendments employed to supply nutrients to crops. The natural nutrients present in soil, which are vital for plant growth such as nitrogen, phosphorus and potassium are synthetically produced from inorganic materials and applied to the soil in the form of chemical fertilizers (Dikir, 2023). While chemical fertilizers enhance plant growth and increase fruit and vegetable yields within a relatively short timeframe, their application is not without drawbacks. They adversely affect the physical, chemical and biological properties of the soil in comparison to naturally derived organic fertilizers (Zaman *et al.,* 2019; Dikir, 2023; Bangre *et al*., 2023). These soil properties are critical for maintaining soil health and promoting crop growth (Iqbal *et al.,* 2021). Chickpea (*Cicer arietinum* L.) ranks as Algeria's second most important legume crop after broad beans, predominantly cultivated in Tlemcen and Ain Temouchent's humid climates (Madr, 2014). *Vicia faba* L., another key legume, provides protein and supports nitrogen fixation, crucial for Mediterranean agriculture (Crépon *et al.,* 2010; Kumar *et al.,* 2017; Wang *et al.,* 2017; Choudhary and Bhushan, 2024). This study compares the effects of *Cicer arietinum* L. and *Vicia faba* L. in Chlef and Mostaganem, examining mycorrhizal fungi interactions with and without NPK fertilizers on soil and plant development.

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## MATERIALS AND METHODS

### Study area

The Wilayas of Chlef and Mostaganem, located in northwest Algeria, cover 4,074 km$^2$ and 2,269 km$^2$, respectively. Chlef, 200 km west of Algiers, borders the Mediterranean Sea to the north and features a subtropical climate with mild, rainy winters and hot, sunny summers. Mostaganem, with a semi-arid climate, has a population of 737,118. The study sites, Ténès (53 km north of Chlef) and Stidia (14 km from Mostaganem), are coastal towns along the Mediterranean.

**Plant material**

Plant samples were collected in Algeria, November 2023, from Ténès (Chlef) and Stidia (Mostaganem). Three root and leaf samples of *Cicer arietinum* L. and *Vicia faba* L. were taken from natural and fertilized plots. Aerial parts were sampled after 65 days, underground parts after three months. Root samples, collected at ~20 cm depth with a hatchet, targeted fine mycorrhizal roots for assessment. Fertilized plots used NUTAGRA (NPK: 12/12/18),″ providing 12% nitrogen, 12% phosphorus, 18% potassium.

**Soil**

Three soil samples were collected around each legume in both Ténès (Chlef) and Stidia (Mostaganem) for physicochemical analysis and the Mycorrhization Potential Index (MPI). Soil sampling was conducted at a depth of 20 to 30 cm.

**Physicochemical soil analysis**

Soil samples were air-dried, sieved (2-mm mesh), homogenized and stored. Analyses, including phosphorus, nitrogen and carbon, were conducted in Oran's Pedology Laboratory using automated instruments and laboratory methods.

**Evaluation of soil mycorrhizal infectious potential (MIP)**

The Mycorrhizal Infectious Potential (MIP) refers to soil's capacity to form mycorrhizal associations using spores, mycelium, or root debris with vesicles naturally present (Plenchette *et al.,* 1989).

To assess MIP, ten *Sorghum bicolor* L. seeds, a highly mycotrophic plant, were planted in 150 ml pots with 100 g of non-sterilized soil at different dilutions (Table 1). A portion of the soil was heated (120°C, 20 minutes) and mixed with sterilized soil in ratios from 3% to 100%. Seeds were watered daily with sterilized distilled water.

After two weeks, roots were harvested to determine MIP, following the method suggested by Philippe and Hayman (1970). Roots showing any infection point indicating hyphal penetration were classified as mycorrhizal. Results were expressed as the percentage of mycorrhizal plants per pot. Linear regression models (Y = aX + b) were created for each soil type, correlating mycorrhizal plant percentages with the logarithmic value of unsterilized soil quantity (Duponnois *et al.,* 2001).

**Estimation of natural root colonization degree**

Root samples were taken from three randomly selected plants for each of the three target species. The roots were characterized using a staining technique outlined by Phillips and Hayman (1970). The frequency and intensity of colonization were measured according to established protocols (Trouvelot *et al.,* 1986).

**Statistical analysis**

The results were analyzed using MANOVA (5% significance level) and Tukey's post-hoc test with Statistica 7.1. Correlation coefficients and PCA were used to explore parameter interrelationships.

## RESULTS AND DISCUSSION

**Physicochemical soil analysis**

**Physicochemical analysis of soil**

The results of the physicochemical characteristics of soil samples from different collections of the species *Cicer arietinum* L. and *Vicia faba* L., both natural and treated with chemical fertilizers, from the two regions of Chlef and Mostaganem, are presented in Table 2. According to the Soil Texture Triangle, granulometric analyses conducted on soil samples from the Chlef region indicate that the texture of the studied soils is clayey, with over 40% clay content and sand percentages ranging between 28% and 32%. Silt content, however, varies between 23% and 28% (Table 2). In contrast, granulometric analysis of the soils from the Mostaganem region (Table 2) reveals a sandy texture, with more than 68% sand and significantly lower clay (4-24%) and silt (4-16%) contents.

Soil or sediment conductivity is a measure of the quantity of ions present that could dissolve in the presence of water. According to the results shown in Table 2, conductivity values are lower in the rhizosphere of species from the Chlef region (0.16 to 0.593 mS/cm) compared to those from the Mostaganem region (0.123 to 0.433 mS/cm). Soil pH, an indicator of environmental conditions, directly influences microbial life and the chemical forms of nutrients. The soils sampled from both the Chlef and Mostaganem regions exhibit alkaline pH values, as per the pedological reference of Baize and Jabiol (1995), ranging from 7.91 to 8.84 (Table 2).

Soil organic matter consists of living organisms, plant and animal residues and decomposing products. Typically, it constitutes only a small percentage (0.5% to 10%) of the soil mass. Results from the rhizospheric soils of the studied plant species *Cicer arietinum* L. and *Vicia faba* L., both natural and treated with chemical fertilizers, indicate that organic matter content does not exceed 1% in either the Chlef or Mostaganem regions (Table 2). A similar trend is observed for carbon content, which remains below 0.6% across all samples. Nitrogen and phosphorus levels in soils from the Chlef and Mostaganem regions treated with

**Table 1:** Soil dilutions with varied proportions of sterilized and non-sterilized soils.

| Dilution | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Non-sterilized soil (g) | 3 | 6 | 12 | 24 | 48 | 100 |
| Sterilized soil (g) | 97 | 94 | 88 | 76 | 52 | 0 |

chemical fertilizers for both species (*Cicer arietinum* L. and *Vicia faba* L.) are higher compared to natural soils (Table 2).

In this study, changes in the physicochemical characteristics of the soils of the studied species (*Cicer arietinum* L. and *Vicia faba* L.) were observed under the influence of symbiotic associations and chemical fertilizers. Alterations in edaphic parameters have been noted in several studies following plant-microorganism symbiotic associations (Hatimi and Tahrouche, 2007). Soil pH is one of the most critical indicators of soil quality (Barrow and Hartemink, 2023). It can be influenced by climate, vegetation and the decomposition of parent rock (Smith *et al*., 2000). Its elevation is attributed to limited leaching due to low precipitation, a characteristic of these regions (Wezel *et al.,* 2000; Sanon *et al.,* 2013), as observed in the ecosystems of Chlef and Mostaganem. These findings are corroborated by Iqbal *et al*. (2021), who note that the application of mineral fertilizers significantly influences soil pH, potentially increasing acidity and thus reducing pH. Tao *et al*. (2025) report that soil pH decreases rapidly under the influence of ammonium-based nitrogen fertilizers.

Nitrogen levels in the soils of Chlef and Mostaganem were moderately rich, influenced by nitrogen-fixing legumes and chemical fertilizers (Kjeldahl, 1883; Tamiru *et al*., 2023). An increase in nitrogen content in soils treated with mineral nitrogen compared to natural soils has also been observed by Jiang *et al.* (2021) and Tadesse *et al.* (2024). Assimilable phosphorus levels in soils treated with chemical fertilizers were higher (phosphorus-rich) compared to natural soils of the two studied plant species. While soils may contain phosphorus reserves several thousand times greater than what is required for plant growth, only a small soluble fraction is available for plant uptake (Sohrt *et al.,* 2017). According to the reference framework (Olsen, 1954), Hatimi and Tahrouche (2007) and Benelhadj Djelloul *et al*. (2024), sandy soils are generally poor in phosphorus and nitrogen and legumes, along with their fungal symbionts, are considered key contributors to soil fertility. Additionally, Yan *et al*. (2008) identified a positive correlation between total soil nitrogen and root colonization by arbuscular mycorrhizal fungi (AMF).

**Mycorrhizal inoculum potential of the soil (MIP)**

The mycorrhizal inoculum potential (MIP) of soil reflects the mycorrhizal fungi population and its ability to form mycorrhizae under poor conditions. After two weeks of sorghum cultivation, microscopy revealed fungal structures like hyphae and vesicles (Fig 1). The MIP50 values for natural soils in Chlef and Mostaganem ranged from 2.93 to 4.25, while chemically treated soils of *Cicer arietinum* L. and *Vicia faba* L (Table 3). had MIP50 values from 12.8 to 14.88, requiring three times more inoculum for colonization. This negative impact of chemical fertilizers on microbial populations aligns with Ashwin and Bagyaraj (2023). Chemical fertilizers alter the composition and functional structure of soil microbial communities, thereby impairing

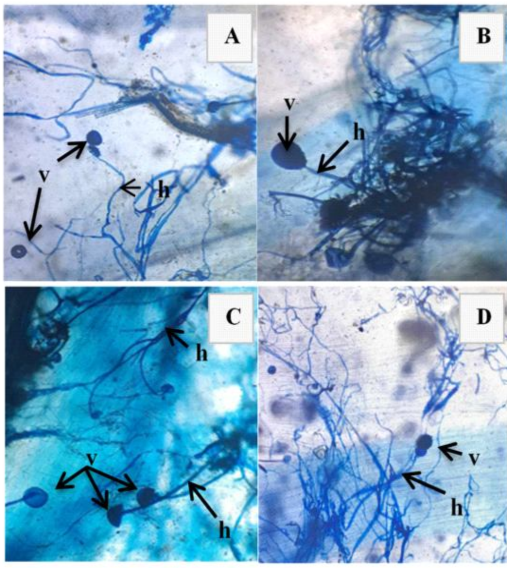
**Table 2:** Physicochemical analysis of various soil samples from the plant species *Cicer arietinum* L. and *Vicia faba* L., both natural and treated with chemical fertilizers, from the chlef and Mostaganem regions.

| Soil | Particle size % | | | Organic matter % | Conductivity (mS/cm) | Total carbon % | PH | Total nitrogen N2*% | Available phosphorus ppm |
|---|---|---|---|---|---|---|---|---|---|
| | Sands | Clay | Silts | | | | | | |
| VFN (C) | 28±00$^{a}$ | 48.33±0.51$^{a}$ | 23.66±0.51$^{a}$ | 0.716±0.02$^{a}$ | 0.16±0.005$^{a}$ | 0.413±0.01$^{a}$ | 8.51 ±0.07$^{a}$ | 0.171±0.009$^{ag}$ | 4.883±0.065$^{a}$ |
| CAN (C) | 28±00$^{a}$ | 44±00$^{b}$ | 28±00$^{b}$ | 0.777±0.017$^{b}$ | 0.16±00$^{a}$ | 0.448±0.009$^{a}$ | 8.55±0.03$^{a}$ | 0.175±0.001$^{a}$ | 4.420±0.017$^{b}$ |
| VFE (C) | 32±00$^{a}$ | 40±00$^{c}$ | 28±00$^{b}$ | 0.397±0.020$^{c}$ | 0.593±0.011$^{b}$ | 0.227±0.007$^{a}$ | 8.03±0.05$^{b}$ | 0.229±0.004$^{b}$ | 20.936±0.158$^{c}$ |
| CAE (C) | 34±00$^{a}$ | 40±00$^{c}$ | 26±00$^{c}$ | 0.956±0.065$^{d}$ | 0.586±0.015$^{c}$ | 0.523±0.003$^{c}$ | 7.91±0.09$^{c}$ | 0.297±0.003$^{c}$ | 25.770±0.293$^{d}$ |
| VFN (M) | 68±00$^{a}$ | 24±00$^{d}$ | 8±00$^{d}$ | 0.952±0.010$^{d}$ | 0.129±0.010$^{d}$ | 0.536±0.015$^{a}$ | 8.76±0.04$^{c}$ | 0.085±0.005$^{d}$ | 10.800±0.100$^{e}$ |
| CAN (M) | 80±00$^{a}$ | 16±00$^{e}$ | 4±00$^{e}$ | 0.931±0.022$^{d}$ | 0.123±0.005$^{d}$ | 0.553±0.005$^{b}$ | 8.84±0.03$^{d}$ | 0.093±0.005$^{e}$ | 9.966±0.030$^{f}$ |
| CAE (M) | 80±00$^{a}$ | 4±00$^{f}$ | 16±00$^{f}$ | 0.466±0.005$^{e}$ | 0.433±0.020$^{e}$ | 0.270±0.010$^{a}$ | 8.24±0.04$^{f}$ | 0.106±0.012$^{f}$ | 14.966±0.057$^{g}$ |
| VFE (M) | 68±00$^{a}$ | 24±00$^{d}$ | 8±00$^{d}$ | 0.44±0.009$^{f}$ | 0.256±0.005$^{f}$ | 0.23±0.092$^{a}$ | 8.38±0.05$^{g}$ | 0.171±0.016$^{g}$ | 18.400±0.100$^{h}$ |
| CD= | 0 | 0.35378 | 0.35378 | 0.050393 | 0.019417 | 0.013722 | 0.09472 | 0.012051 | 0.235841 |
| SEm± | 0 | 0.116642 | 0.116642 | 0.016615 | 0.006402 | 0.004524 | 0.03123 | 0.005305 | 0.077758 |

VFN (C): *Vicia faba* L. Natural (Chlef), CAN (C): *Cicer arietinum* L. Natural (Chlef), VFE (C): *Vicia faba* L. treated with chemical fertilizers (Chlef), CAE (C): *Cicer arietinum* L. treated with chemical fertilizers (Chlef), VFN (M): *Vicia faba* L. Natural (Mostaganem), CAN (M): *Cicer arietinum* L. Natural (Mostaganem), CAE (M): *Cicer arietinum* L. treated with chemical fertilizers (Mostaganem), VFE (M): *Vicia faba* L. treated with chemical fertilizers (Mostaganem). Data sharing the same letter within the same column are not significantly different according to the Tukey test (p<0.05).

soil fertility and subsequently decreasing biological fertility, including the efficacy of arbuscular mycorrhizal fungi (AMF) (Hicks *et al*., 2020; Liu *et al*., 2023).

Increased fertilizer use reduces microbial populations (Choudhary *et al.,* 2023) and disrupts microbial activity (Enebe and Babalola, 2020; Tosi *et al.,* 2021; Pavlov *et al.,* 2023). Ashwin and Bagyaraj (2023) observed reduced microbial populations and dehydrogenase activity with nitrogen fertilizers (Kaur and Kaur, 2021).




Grx40. h: hypha, v: vesicle.

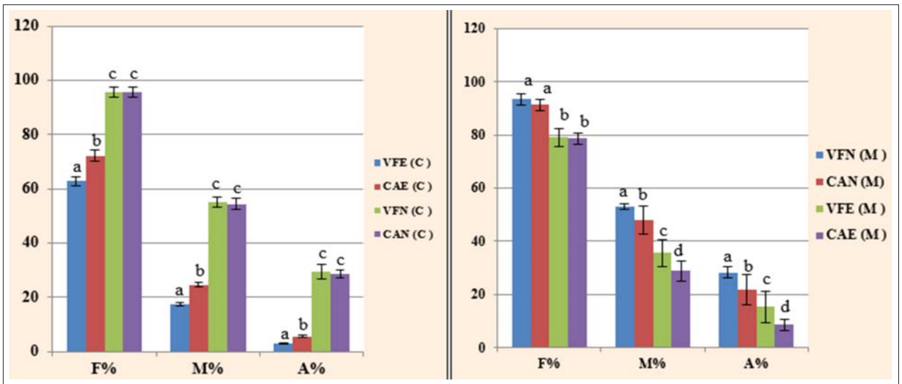
**Fig 1:** Endomycorrhizal structures in the roots of sorghum grown in the Chlef region (A, D) and the Mostaganem region (B, C).

**Root colonization by AMF**

Microscopic analysis of roots from *Cicer arietinum* L. and *Vicia faba* L., stained with trypan blue revealed mycorrhizal fungal structures (hyphae, vesicles and arbuscules). According to Fig 1, natural plant roots from both the regions of study area showed over 91% mycorrhization frequency, whereas the roots of plants in chemically fertilized soils reach only 78% mycorrhization frequency. Root cortex colonization intensity ranges between 47.94% and 55.16% in natural species but decreases to 17.44%-35.66% in treated plants. Arbuscular richness is higher in natural species (21.83%-29.26%) compared to treated ones (2.90%-14.37%) (Fig 2).

Arbuscules, key nutrient exchange structures, signify functional symbiosis (Brundrett and Tedersoo, 2018; Martin *et al.,* 2024). Bohrer *et al*. (2004) reported that multiple factors influence arbuscular mycorrhizal fungi (AMF), including plant species, fungal species and external factors such as chemical fertilizers, which exert a detrimental effect on the plant-mycorrhiza association. This negative impact arises because the availability of chemical fertilizers to plants restricts the establishment of this cooperative relationship, a finding that is evident in their study.

The application of chemical fertilizers adversely affects mycorrhizal symbiosis, reducing the diversity of mycorrhizal species and limiting the benefits that these symbioses confer to their host plants (Sreenivasa and Bagyaraj, 1989). Indeed, several studies, including those by Ekblad *et al*. (1995), Bâ *et al*. (2001), Hijri *et al*. (2006), Breuillin *et al.* (2010) and Iqbal *et al*. (2021), have demonstrated that soil fertility levels particularly elevated phosphorus concentrations inhibit the plant AMF symbiosis and, in certain instances, completely suppress AMF activity.




F%: Frequency of mycorrhization. M%: Intensity of colonization in the root system. A%: Arbuscular richness of the root system. VFN (C): *Vicia faba* L. Natural (Chlef), CAN (C): *Cicer arietinum* L. Natural (Chlef), VFE (C): *Vicia faba* L. treated with chemical fertilizers (Chlef), CAE (C): *Cicer arietinum* L. treated with chemical fertilizers (Chlef). VFN (M): *Vicia faba* L. Natural (Mostaganem), CAN (M): *Cicer arietinum* L. Natural (Mostaganem), CAE (M): *Cicer arietinum* L. treated with chemical fertilizers (Mostaganem), VFE (M): *Vicia faba* L. treated with chemical fertilizers (Mostaganem). Data sharing the same letter within the same column are not significantly different according to the Tukey test ($p<0.05$).

**Fig 2:** Percentage of root infection in the studied leguminous species from the Chlef and Mostaganem regions.

**Table 3:** Determination of MIP50 for soils sampled under the plant species *Cicer arietinum* L. and *Vicia faba* L., both natural and treated with chemical fertilizers, from the Chlef and Mostaganem regions.

| Sol | Linear regression y=Ax+B | $R^2$ | MIP50 |
|---|---|---|---|
| VFN (C) | y=-11.71x+62.66 | 0.894 | 2.93[a] |
| CAN(C) | y=-12.85x+70 | 0.863 | 4.25[b] |
| VFE (C) | y=-15.94x+129.2 | 0.874 | 13.50[c] |
| CAE (C) | y=-17.04x+131.3 | 0.861 | 12.8[c] |
| VFN (M) | y=-11.42x+63.33 | 0.863 | 3.15[a] |
| CAN (M) | y=-10.28x+56 | 0.956 | 2.33[a] |
| CAE (M) | y=-15.56x+132.3 | 0.710 | 14.48[d] |
| VFE (M) | y=-14.85x+128.6 | 0.552 | 14.88[d] |
| SEm±0,01801 | | | |
| CD=0.01342 | | | |
| α=0.05 | | | |

VFN (C): *Vicia faba* L. Natural (Chlef), CAN (C): *Cicer arietinum* L. Natural (Chlef), VFE (C): *Vicia faba* L. treated with chemical fertilizers (Chlef), CAE (C): *Cicer arietinum* L. treated with chemical fertilizers (Chlef), VFN (M): *Vicia faba* L. Natural (Mostaganem), CAN (M): *Cicer arietinum* L. Natural (Mostaganem), CAE (M): *Cicer arietinum* L. treated with chemical fertilizers (Mostaganem), VFE (M): *Vicia faba* L. treated with chemical fertilizers (Mostaganem). Y: Linear regression of the percentage of mycorrhizal plants as a function of the logarithm of the quantity of non-sterilized soil. MIP50: Quantity of non-sterilized soil corresponding to 50% mycorrhizal infection. $R^2$: Correlation coefficient. Data sharing the same letter within the same column are not significantly different according to the Tukey test (p<0.05).

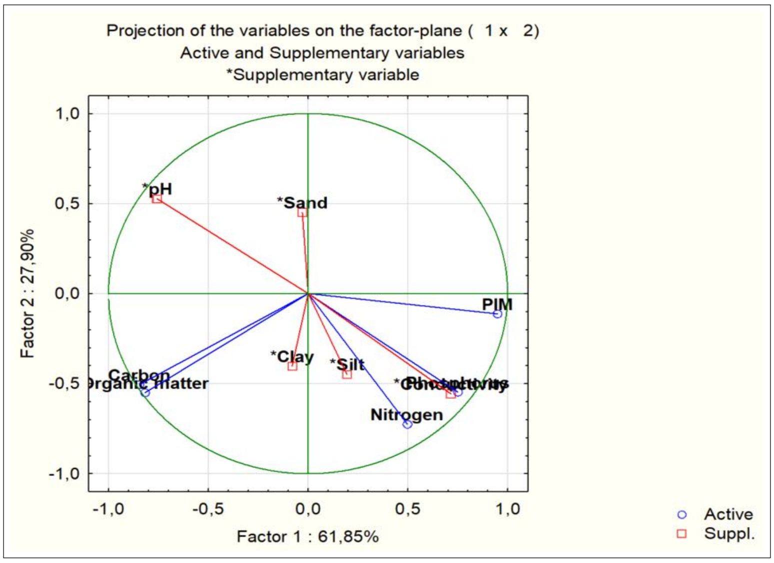



**Fig 3:** Principal component analysis of the physicochemical and microbiological parameters of soils from the different plant species *Cicer arietinum* L. and *Vicia faba* L.

### Principal component analysis (PCA)

Fig 3 shows the factorial plane projection (F1×F2) of data on the physicochemical and microbiological characteristics of soils from *Cicer arietinum* L. and *Vicia faba* L. PCA effectively visualized the relationships between physicochemical parameters and mycorrhizal infectious potential (MIP). The first axis accounted for 61.85% of variation, grouping phosphorus, nitrogen, conductivity and MIP with significant positive correlations (0.78 to 0.86). A negative correlation between MIP and carbon (-0.71) was observed. The second axis explained 27.90% of the variation, with a significant positive correlation between organic matter and carbon (0.89).

## CONCLUSION

This study investigates the effects of chemical fertilizers and mycorrhizal symbiosis (MSA) with leguminous species on soil characteristics and the development of *Cicer arietinum* L. and *Vicia faba* L. in Chlef and Mostaganem. Chemical fertilizers influence physicochemical properties and microbial populations, facilitating species adaptation to nutrient inputs. Our results offer insights into the Mycorrhizogenic Infectious Potential of different soils, suggesting that introducing herbaceous legumes can improve soil quality and reduce reliance on fertilizers.

**Conflict of interest**

The authors declare no competing of interests.

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