EXPLORING THE POTENTIALITIES OF *CHAMAEROPS HUMILIS* L. SOIL TO IMPROVE GROWTH AND QUALITY OF SAFFRON

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Abstract. Native soils are characterized by an indigenous symbiotic microflora, with several beneficial effects. This work aims to explore the potential beneficial effect of the microflora of *Chamaerops humilis* L. soil used as a live organic substrate on the growth and flowering of saffron (*Crocus sativus* L.), with a special focus on the potential role of arbuscular mycorrhizal fungi (AMF). An experiment was conducted, for 2 years, using two treatments: sterile (ST) and live (LT). Several parameters on soil fertility, growth and quality of saffron were assessed. The obtained results demonstrate that the main physico-chemical properties of the soil significantly improved in LT, compared to ST at harvesting. Moreover, LT significantly improved growth traits, photosynthetic pigments, and mycorrhization colonization of roots compared to ST. Specifically, the quality assessment based on picrocrocin, safranal, and crocetin concentrations qualified the saffron stigmas under LT in category I, and ST in category II. Overall, the application of LT demonstrates its beneficial effects on soil fertility, yield, and quality of saffron. Therefore, *Ch. humilis L.* soil may be recommended as a potential bio-fertilizer and/or a nursing plant, with the potential positive effect of AMFs, able to enhance productivity and quality of saffron culture.
Keywords: *indigenous soil, Chamaerops humilis L., arbuscular mycorrhizal fungi, bio-fertilizer, yield, saffron, agriculture*

Introduction

Native soils are recognized by an indigenous symbiotic microflora with several beneficial effects (Luecke et al., 2021). This microflora includes diverse communities of bacteria and fungi, known by their potential to promote plant growth (PGPM). PGPM improve plant growth by enhancing the availability of nutrients, regulating phytohormones, and increasing plant tolerance against biotic and abiotic stresses (Lopes et al., 2021). Among the fungal community, arbuscular mycorrhizal fungi (AMF) form a symbiotic association with the roots, which facilitates the assimilation of nutrients (Magurno et al., 2015), and participates in the improvement of productivity (Harman et al., 2020).

In southern Mediterranean and sub-Saharan Africa dryland farming areas, a native flora proliferates in adaptation to the thermo-mediterranean biotopes (Vargas, 2020). This vegetation could act as a nurse plant, with very important beneficial interests in agriculture productivity and soil rehabilitation (Manaut et al., 2015).

In Khouribga area (Morocco), a special Mediterranean hilly landscape dominates the surrounding rural lands under direct rainfall regime. These dryland farms are generally

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cultivated by cereals. In this area, the landscape is often dominated by two spontaneous plants (*Chamaerops humilis* L., *Ziziphus lotus* (L.*)* Desf.) that well individualized; or coexist in clear association over the hills.

The attempt to introduce certain adapted and value-added crops could constitute a promising alternative in the valorization of these types of lands, especially if this approach is based on the exploitation of their indigenous soil microbes as bioinoculants for supplying nutrients and/or boosting plant growth as a sustainable strategy to enhance agricultural productivity.

Saffron is one of the most important crops in the world. 90% of world production of saffron comes from Iran, followed by India, Spain, Morocco, Greece, and Italy (Cardone et al., 2020). Despite the importance of saffron cultivation in Morocco, as a high value- added culture, the extension of cultivation outside local region (Taliouine), traditionally recognized, as the saffron land, remains very limited; although the landscape and climate of Morocco offer various similar regions (Mzabri et al., 2019).

This work aims to explore the potential beneficial effect of the rhizosphere microflora of *Chamaerops humilis* L. as a live organic substrate on growth and flowering of saffron, with special focus on the potential role of AMFs in improving the yield and quality of saffron cultivation. Through this experimentation, we anticipate the possibility of the first introduction of saffron culture in these special Mediterranean lands (Khouribga aerea).

Materials and methods

Soil sampling

The rhizospheric soil of *Ch. humilis* L. was used as a substrate, comprising a potential native microbial inoculums. Rhizospheric soil and root samples (25-35 cm depth) were collected using sterilized material according to Manaut et al. (2015). Briefly, from a homogeneous and undisturbed *C. humilis* steppe (32°85’39” N; 6°9’02” W), 10 shrubs, spaced 20 m apart from each other, were randomly selected for sampling.

Experimental design and culture conditions

Plastic pots were used as a growing support. In each pot (d = 10 cm), one corm of saffron was inoculated, with an equivalent planting density of 100 corms/m^2. The selected saffron corms are 2 years old, with an average diameter between 2.8-3.2 cm, able to give flowering in the future season. Two treatments were considered, with 20 repetitions each:

- Control treatment = Sterile treatment (ST): 100% of rhizosphere soil of *C. humilis* sterilized at 180°C for a 1-h cycle, with 3 repeated cycles, after every 4 h.
- Native soil treatment = Live treatment (LT): 100% of non-sterilized rhizosphere of *C. humilis* soil (TM).

The plastic pots were placed in a private garden, located on top of a hill (762 m) in the rural region of Khouribga (32°86’28” N; 6°86’86” W). The plants are grown under ambient conditions throughout two flowering seasons (October 2021 – April 2023). During the cultivation period, the minimum and maximum temperatures are recorded in

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January 2022 (8°C), and in July 2022 (40°C), respectively. The minimum and maximum precipitations are recorded in October 2022 (1.8 mm), and in July 2022 (122.08 mm). Meteorological data were taken from the online weather forecasting website (www.worldweatheronline.com).

After 15 to 25 days from the first inoculation (October 2021), 100% of the corms in both treatments germinated and produced leaves and flowers, with pestles and stamens. Afterwards, the plants were monitored until the second reproductive (October 2022), and vegetative periods (April 2023). Flowers have been harvested and discarded. Before degeneration, the leaves were cut. Materials were used for growth measurements, physiological, biochemical and phytochemical and quality analyzes.

Spore extraction and identification

The spores are concentrated on a sucrose solution (Gerdemann and Nicholson, 1963), and counted under a binocular magnifying glass (Magnification: X40). The different morphotypes are identified according the description available at the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) website (http://invam.wu.edu, last accessed: July 2023).

The parameters of assessment

Physicochemical analysis

The physico-chemical parameters of the soil such as pH, electrical conductivity (EC), total organic carbon (TOC), organic matter (OM), available phosphorus (AP) and total nitrogen (N) were determined. Soil pH and electrical conductivity were measured according to ISO 3632 (2010). TOC and OM were determined by the method of Anne (Nelson and Sommers, 1982). AP was determined by the Olsen method (Olsen et al., 1954). N was determined according to the method described by Kjeldahl (1883).

AMF colonization

Fragments of lateral roots were carefully washed and cleared with 10% of KOH at 90°C for 30 min. Then, they acidified (1% HCl) and stained with Trypan blue at 90°C for 20 min (Phillips and Hayman, 1970). AMF infection frequency and intensity were evaluated in root fragments according to Trouvelot (1986).

AMF infection frequency (F) was determined using *Equation 1:*

$$F(\%) = \frac{\text{number of colonized root fragments}}{\text{number of total root fragments analyzed}} \times 100 \quad \text{(Eq.1)}$$

AMF infection intensity (M) was determined using *Equation 2:*

$$M(\%) = (95xn5 + 70xn4 + 30xn3 + 5xn2 + n1) / \text{total roots segments} \quad \text{(Eq.2)}$$

Growth parameters

Morphological traits

Various growth parameters were measured at harvested of plants: number of leaves, leaf length, and dried stigmas.

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Photosynthetic pigments

Photosynthetic pigments from leaves were extracted according to Arnon (1949). Briefly, 200 mg of the fresh leaves from three plants was crushed with mortar and pestle in 10 ml 80% of acetone and centrifuged at 5000 rpm for 10 min. Then, the Optical density of the supernatant was measured using a UV/visible spectrophotometer (Metash-UV-Vis-5100) at optical densities (OD) 663, 645 and 453 nm. Total chlorophyll (Chl-t), chlorophyll a (Chl-a), chlorophyll b (Chl-b), were estimated according to the formulas provided by Arnon (1949). Carotenoid content (T. cartot.) (mg/g F.W) was estimated according to Maclachalan and Zalik (1963).

Phytochemical characterization

Samples of harvested stigmas were analyzed according to the ISO 3632 (2010). The aqueous saffron extract was analyzed by using an UV-Vis spectrophotometer (Metash-UV-Vis-5100). Picrocrocin, safranal, and crocin were assessed by direct readings of the specific absorbance of 1% at 257, 330, and 440 nm, respectively. Total phenolic (TPs), Total flavonoids (TFs) were quantified in saffron stigmas, using Folin–Ciocalteu method (Folin and Ciocalteu, 1927), and the method described by Kim et al. (2003), respectively.

Statistical analysis

Tests of means comparison of two groups were used to Data analysis. Mean values were considered using T-student and Wilcoxon tests carried out after normality pre-test ($P = 0.05$). Results were presented as mean values ± standard error (SE). Principal component analysis (PCA) was performed to assess correlation between soil properties and quantitative and qualitative traits of saffron. Statistical analyses were performed using the software SPSS Statistics, IBM Ver. 26.0.

Results

Soil fertility assessment

Physicochemical properties

Results of soil analyses of saffron cultivation under both sterile (ST) and live (LT) treatments are shown in *Table 1*. The main soil's physicochemical properties were improved compared to the initial state and significant difference were recorded between ST and LT at harvesting ($P < 0.05$). Indeed, the obtained results have shown an effect on the saffron culture under LT on pH, which increased from 6.33 to 8.13. EC values were slightly decreased from 0.27 to 0.21 mS/cm). Otherwise, a significant increase ($P < 0.05$) was recorded for N (%), TOC (%), OM (%), and AP (mg/kg) content (*Table 1*).

Spore identification and AMF colonization

The taxonomic identification showed the presence of 30 taxa belonging to 9 genera: *Acaulospora, Claroideoglomus, Dentiscutata, Entrophorospora, Funneliformis, Gigaspora, Scutellospora, Rhizophagus, Septoglomus* (*Fig. 1*). To assess root colonization, microscopic observations revealed the presence of fungal infection criteria (hyphae, vesicles, spores) (*Fig. 1*). The overall colonization of saffron roots under LT showed more infectivity with frequencies ($F = 71.5\%$), compared to that of ST

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(F = 13%). Likewise, the intensity of colonization (M%) of the saffron roots under LT reaches 100%, compared to ST (45.7%).

Table 1. *Physicochemical parameters under ST and LT at the first (T0) and 2nd y (Tf) of cultivation*

	pH		EC (µs/cm)		TOC (%)		OM (%)		N (%)		AP (mg/g sol)	
	T0	Tf	T0	Tf	T0	Tf	T0	Tf	T0	Tf	T0	Tf
S.T	7.74 ±0.15 a	8.45 ±0.21 a	287 ±1 a	220 ±1 a	0.62 ±0.14 a	0.42 ±0.04 a	1.08 ±0.23 a	0.72 ±0.06 a	0.24 ±0.01 a	0.16 ±0.005 a	1.01 ±0.29 a	1.50 ±0.66 a
L.T	6.33 ±0.15 b	8.13 ±0.21 a	267 ±1 b	212 ±1 b	1.66 ±0.30 b	3.04 ±0.09 b	2.86 ±0.51 b	5.24 ±0.15 b	0.19 ±0.01 a	0.22 ±0.01 b	0.61 ±0.48 a	2.21 ±0.15 b

Values with different letters are significantly different as determined by T-student test, carried out after normality pre-test (P = 0.05). Numbers after ± represent the standard deviation of the mean (n = 3).

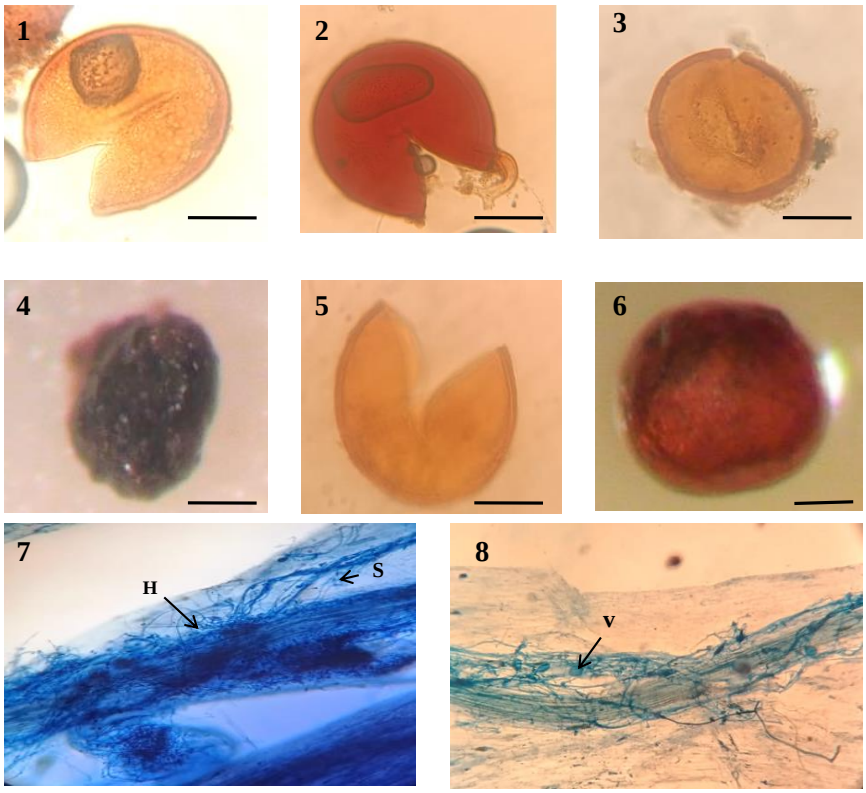

Figure 1. *Identification of certain AMF-spore taxa recorded in Ch. Humilis soil (1-6) and different structures of AMF colonization of saffron roots (7, 8) (inter and intraradical hyphae (H), vesicles (V), and spores (S)). 1. Acaulospora morrowiae, 2. Septoglomus constrictum 3. Rhizophagus fasciculatis, 4. Scutellospora nigra, 5. Entrophorospora sp., 6. Gigaspora sp. Scale bar 100 µm (40× magnification)*

Growth assessment

Morphologic parameters

As shown in *Figure 2*, LT significantly ($P < 0.05$) improved the number of leaves, leaf length, and dried stigmas, with high yield values compared to that of ST, (5.87%),

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(6.81%), (16.72%), respectively (*Fig. 2*). However, no significance was observed for the number of flowers ($P^{¨} > 0.05$).

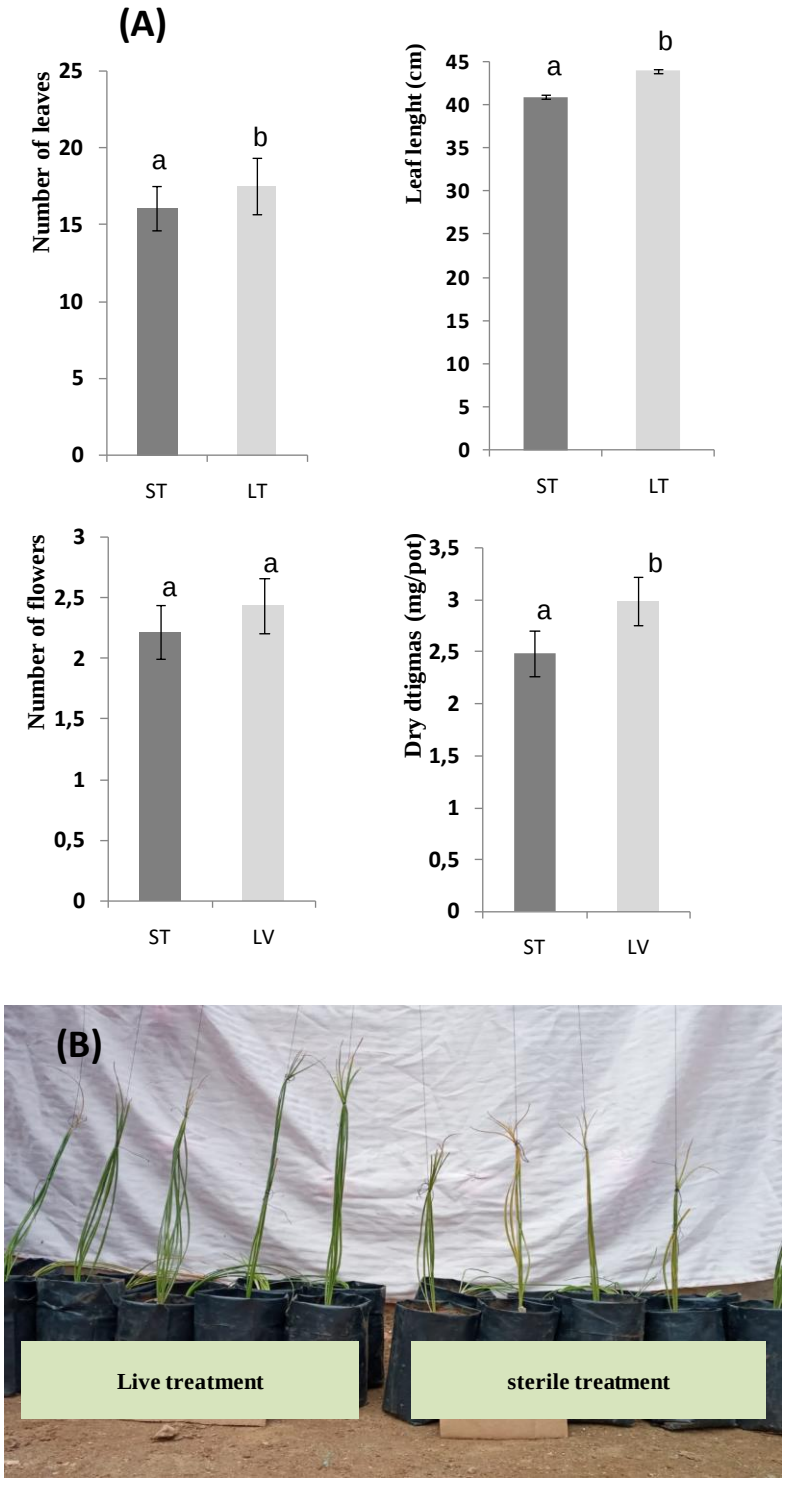

Figure 2. *Growth parameters under ST and LT at 2nd y of cultivation (A), with a photo (B) of the experiment showing the height of the aerial part (leaves) at the end of the vegetative period. Values with different letters are significantly different as determined by Wilcoxon test carried out after normality pre-test (P = 0.05). The lines above the bars represent the standard deviation of the mean (n = 20)*

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Photosynthetic pigments

LT showed the highest amounts (mg/FWg) for Chl-t (0.51), Chl-a (0.35), Chl-b (0.17), and carotenoids (0.15) (*Fig. 3*). It appears that LT showed a significantly positive effect ($P < 0.05$) and improved the photosynthetic pigments compared to ST, with very high yields for Chl-a, Chl-b, Chl-t, and total carotenoids, with (82.97%), (64.62%), (76.83%), (8.3%), respectively.

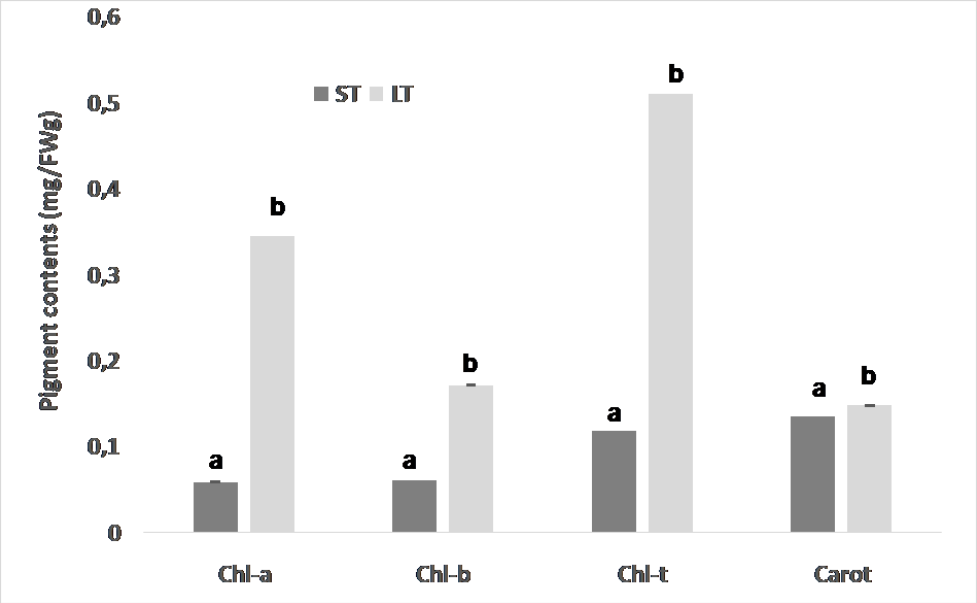

Figure 3. *Pigment contents under ST and LT at 2nd y of cultivation. Values with different letters are significantly different as determined by T-student test carried out after normality pre-test (P = 0.05). The lines above the bars represent the standard deviation of the mean (n = 3)*

Saffron quality indicators

Total phenolics and total flavonoids characterization

Total phenolics (TPs), total flavonoids (TFs) were quantified in saffron stigmas at harvesting in the 2nd y. The results of the phytochemical characterization are shown in *Figure 4*. The content of TPs is significantly higher ($P < 0.05$) in LT extract (3.37 ± 0.09 mg eq GA/g extract) than ST extract (2.36 ± 0.05 mg eq GA/g extract). Similarly, TFs content showed significantly higher amounts in LT extract (2.27 ± 0.045 mg Cat/g extract) than ST extract (1.47 ± 0.064 mg Cat/g extract) (*Fig. 4*).

Picrocrocin, safranal, and crocin contents

As presented in *Table 2*, the mean percentages of crocin, picrocrocin and safranal in the saffron stigmas obtained from LT and ST extracts were compared according to ISO 3632 (2010). The mean comparison of the picrocrocin (77.98), safranal (38.29), and crocetin (226.82) concentrations in the LT extract showed very interested values as compared to ST extract (69.87), (34.02), (173.17), respectively. This measures qualified the saffron stigmas under LT in the category I, and ST in the category II, with good significance for both picrocrocin and crocetin ($P < 0.05$).

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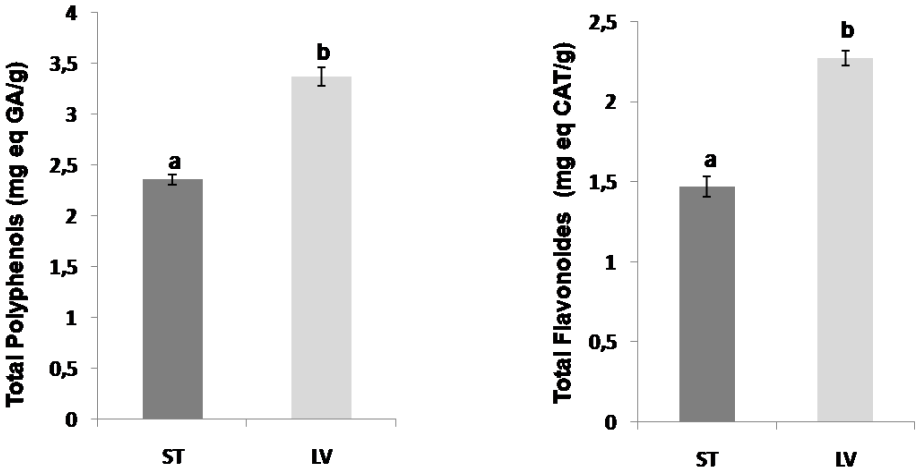

Figure. 4. *Phenolic contents in flowers under ST and LT at 2nd y of cultivation. Values with different letters are significantly different as determined by T-student test carried out after normality pre-test (P = 0.05). The lines above the bars represent the standard deviation of the mean (n = 3)*

Table 2. *Quality indicators evaluated under ST and LT at 2nd y of cultivation*

Treatments	Qualitatives traits					
	A1%1 cm (257 nm)	**ISO category (Picrocrocin)**	**A1%1 cm (330 nm)**	**ISO category (Safranal)**	**A1%1 cm (440 nm)**	**ISO category (Crocetin)**
LT	77.98 ±0.1a	I (>70)	38.29 ±0.12a	>20<50	226.82 ±0.0001a	I>200
ST	69.87 ±0.14 b	II (>50)	34.02 ±0.12a	>20<50	173.17 ±2.11b	II>170
Signifiance	*		n.s		*	

Values with different letters are significantly different at (*P* = 0.05) (*) as determined by T-student test carried out after a normality pre-test. N.S: No significance

Potential interdependencies between all quantified parameters

A PCA was carried out. From the analysis, it is shown that almost all of the variables have been reduced to two main factors, constituting two axes, with a total accumulation of 83.67% (*Fig. 5*). The first one (F1) retains (52.47%) information and the second (F2) (31.19%). F1 axis consists mainly of the association of variables: Mycorrhization intensity (INMYCLT), Chlorophyll (LVChlb, LVChlt), Picrocrocin (LVpirco), safranal (LVSafranal), pH, TOC, and OM, with correlation coefficients greater than 0.9. From this correspondence, it seems a dependence of two quality indicators of saffron, namely safranal and picrocrocin with the Mycorrhization intensity, and pH, TOC and OM in LT. F2 axis consists mainly of the Mycorrhization frequency (Freq ST), conductivity (CE), TPs (LVPT), TFs (LVFT), which seem to depend with the growth parameters of saffron.

Discussion

The direct application of the native soil of *Ch. humilis* L. as a live organic substrate has been significantly promoted the yield and quality of saffron. In this sense, the results of

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the physicochemical analysis show an improvement in the soil fertility approved by the increase of the contents of N, AP, OM, TOC, and pH. However, EC value was slightly decreased (*Table 1*). These results are consistent with those of previous studies demonstrated that slightly alkaline soil with pH (6.3 - 8.3) and electrical conductivity ranging (90 -300 ds/m) are most suitable for increasing saffron productivity (Ganaie and Singh, 2019). In our investigation, results related to the improvement of soil fertility may be due the involvement of soil microflora, in particular AMFs. In fact, under the LT treatment, the AMFs showed an increase of Mycorrhization frequency of roots (F = 13.33 to 71.5%), which may demonstrate their potentialities in improving Saffron yield (Ghanbari et al., 2019). AMFs are one of the most important groups of plant symbionts that positively affect several aspects of plant life, i.e., improved nutrition, better growth, stress tolerance, and disease resistance (Campo et al., 2020). Furthermore, this enhancement can be explained by the beneficial effect of the plant on the fertility of its rhizosphere via the excretion of root exudates that they can alter the rhizospheric soil's and activate it by dissolving nutrients (Shi, 1993).

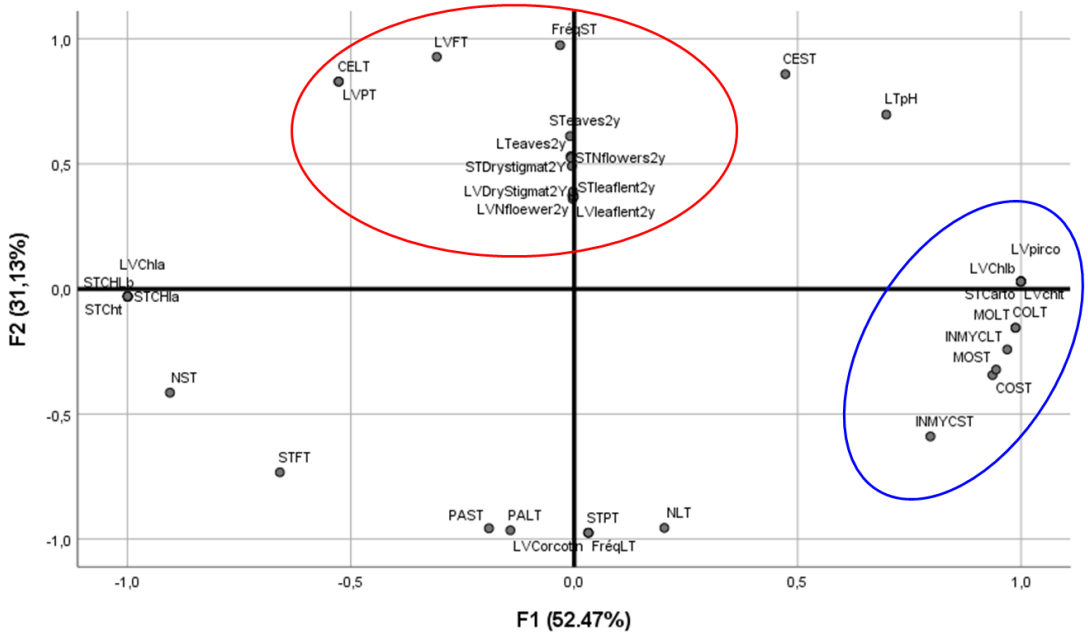

Figure 5. *Principal component analysis of the studied parameters in both ST and LV treatments of Saffron experimentation. ST - Sterile treatment; LV - Live treatment; Leaves - number of leaves; Nflowers - number of flowers; leaflent - leave lent; Drystigmat - dried stigmas; Chl - Chlorophyll a, b, total, Carto - Carotenoids; Fréq - frequencie of Mycorhization; INMYC - Intensity of infection; PA - Available phosphore; N - Azote; CO - Total organic carbon; M.O - Organic matter; CE - Electrical conductivity; pH - potential hydrohen; FT - Total flavonoids; PT - Total phenolics; pirco - Picrocrocin; Corcotin - crocin; Saf - safranal; 2y - at harvesting in 2ndy*

The obtained results of the dry stigma yield (kg/ha) show that productivity under LT treatment (6.9 kg/ha) is greater than that of ST (5.6 kg/ha) in the 2ndy, with an increase in yield (18.8%). Indeed, these experimental results can be considered as an important finding since it shows a comparative yield within the recognized range in the Mediterranean producing countries: Morocco (5–6 kg/ha) (Aziz and Sadok, 2015), Italy

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(3.4 to 10.0 kg/ha), Greece (4.0–7.0 kg/ha), Spain (2.5–6.0 kg/ha) (Gresta et al., 2008; Kothari et al., 2021).

Moreover, LT application improved growth parameters of both vegetative (leaf numbers, leaf length) and reproductive (numbers of flowers and dried stigmas) organs of the saffron cultivation (*Fig. 2*). Thus, the increase in vegetative yield was translated by a remarkable improvement in the parameters of photosynthetic activity. Indeed, an increase in pigment contents (Chl-a, Chl-b, Chl-t, and t. carot.) in saffron leaves has been demonstrated under LT application (*Fig. 3*). The improvement of photosynthetic traits in plants may be explained by the increased availability of mineral elements such as nitrogen and phosphorus, and their uptake by plant roots, particularly under the facilitating effect of an intense endomycorrhizal activity (Campo et al., 2020).

Indeed, according to PCA analysis (*Fig. 5*), it was demonstrated the dependence of the frequency of mycorrhization with all the growth parameters, conductivity, and TPs and TFs productions. LT seems to induce the enhancement of secondary metabolites with high antioxidant power, namely TPs (337.45 mg/eqGA100 g) and TFs (227.02 mg/eqCat100 g) (*Fig. 4*). Hence, in various studies, strong evidence linking between TPs and TFs contents and the antioxidant activity of saffron stigmas has been well demonstrated (Baba et al., 2015; Ghanbari et al., 2019). Furthermore, this high antioxidant power could have a beneficial effect on the health of the plant by showing a significant improvement in yield compared to the control.

More specifically, we found that the LT application was able to increase the saffron quality indicators (picrocrocin, safranal, and crocetin) allowing to qualify the saffron stigmas under LT treatment in category I, while that of ST is qualified in category 2 (*Table 2*). Crocin, picrocrocin, and safranal, the metabolites that give saffron its red color, bitter flavor, and aroma, are completely responsible for its quality (Parizad et al., 2019; Zhang et al., 2019). From the PCA analysis, it is demonstrate the association of mycorrhization intensity, Chlorophyll, Picrocrocin, safranal, pH, TOC, and OM. This dependence demonstrates the important role of AMFs as a biofertilizer to enhance nutrition and organic traits (Rabani-Foroutagheh et al., 2014)**.** Our results corroborate others having shown that the mode of biofertilization have a significant impact in improving saffron quality (Siracusa et al., 2011; Ghanbari et al., 2019).

Conclusion

The application of LT demonstrates its beneficial effects on soil fertility, yield, and quality of saffron. Therefore, it can be concluded that the application of *Ch. humilis L.* soil as a live organic substrate having shown an effect on the productivity and quality of saffron, and could be considered as a potential bio-fertilizer and/or a nursing plant, with potential positive effect of AMFs, able to enhance productivity and quality of saffron culture in this special Mediterranean area. These promising results encourage us to design future optimization research, with a view; to reduce the quantity of soil to be used as amendment, to research and select strains of certain beneficial microbes, in particular AMFs, and to produce effective inoculums based on this soil.

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REFERENCES

[1] Arnon, D. I. (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. – Plant Physiology 24(1): 1-15.

[2] Aziz, L., Sadok, W. (2015): Strategies used by the saffron producers of Taliouine (Morocco) to adapt to climate change. – Journal of Alpine Research (Revue de géographie alpine) 103(2). http://journals.openedition.org/rga/2902. DOI: https://doi.org/10.4000/rga.2902.

[3] Baba, S. A., Malik, A. H., Wani, Z. A., Mohiuddin, T., Shah, Z., Abbas, N., Ashraf, N. (2015): Phytochemical analysis and antioxidant activity of different tissue types of Crocus sativus and oxidative stress alleviating potential of saffron extract in plants, bacteria, and yeast. – South African Journal of Botany 99: 80-87.https://doi.org/10.1016/j.sajb.2015.03.194.

[4] Campo, S., Martín-Cardoso, H., Olivé, M., Pla, E., Catala-Forner, M., Martínez-Eixarch, M., San Segundo, B. (2020): Effect of Root Colonization by Arbuscular Mycorrhizal Fungi on Growth, Productivity and Blast Resistance in Rice. – Rice (N Y) 13(1): 1-14. DOI: 10.1186/s12284-020-00402-7. PMID: 32572623; PMCID: PMC7310045.

[5] Cardone, L., Castronuovo, D., Perniola, M., Cicco, N., Candido, V. (2020): Saffron (Crocus sativus L.), the king of spices: an overview. – Scientia Horticulturae 272: 109560. DOI: 10.1016/j.scienta.2020.109560.

[6] Folin, O., Ciocalteu, V. (1927): On tyrosine and tryptophane determinations in proteins. – Journal of Biological Chemistry 73: 627-650. https://doi.org/10.1016/S0021-9258(18)84277-6.

[7] Ganaie, D. B., Singh, Y. (2019): Saffron in Jammu & Kashmir. – International Journal of Research in Geography 5(2): 1-12.

[8] Gerdemann, J. W., Nicolson, T. H. (1963): Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. – Transactions of the British Mycological Society 46(2): 235-244.

[9] Ghanbari, J., Khajoei-Nejad, G., van Ruth, S. M., Aghighi, S. (2019): The possibility for improvement of flowering, corm properties, bioactive compounds, and antioxidant activity in saffron (Crocus sativus L.) by different nutritional regimes. – Industrial Crops and Products 135: 301-310.

[10] Gresta, F., Lombardo, G. M., Siracusa, L., Ruberto, G. (2008): Saffron, an alternative crop for sustainable agricultural systems. A review. – Agronomy for Sustainable Development 28: 95-112.

[11] Harman, G., Khadka, R., Doni, F., Uphoff, N. (2020): Benefits to plant health and productivity from enhancing plant microbial symbionts. – Frontiers in Plant Science 11: 610065.

[12] ISO 3632: Saffron (Crocus sativus L.). ISO-3632 Part 1: Specifications, ISO 3632:(2010) Part 2: Test Methods. – International Organization for Standardization, Geneva, Switzerland (2010).

[13] Kim, U. K., Jorgenson, E., Coon, H., Leppert, M., Risch, N., Drayna, D. (2003): Positional cloning of the human quantitative trait locus under lying taste sensitivity to phenylthiocarbamide. – Science 299(5610): 1221-1225.

[14] Kjeldahl, J. (1883): A new method for the determination of nitrogen in organic matter. – Zeitschrift für Analytische Chemie 22: 366-382. http://dx.doi.org/10.1007/BF01338151.

[15] Kothari, D., Thakur, M., Joshi, R., Kumar, A., Kumar, R. (2021): Agro-climatic suitability evaluation for saffron production in areas of western Himalaya. – Frontiers in Plant Science 12: 657819.

[16] Lopes, M. J. S., Dias-Filho, M. B., Gurgel, E. S. C. (2021a): Successful plant growth-promoting microbes: inoculation methods and abiotic factors. – Frontiers in Sustainable Food Systems 5(606454): 1-13. DOI=10.3389/fsufs.2021.606454.

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DOI: http://dx.doi.org/10.15666/aeer/2106_59775988

[17] Luecke, N. C., Mejia, A. J., Crawford, K. M. (2021): Native soil amendments combined with commercial arbuscular mycorrhizal fungi increase biomass of Panicum amarum. Scientific Reports 11(1): 17865. https://doi.org/10.1038/s41598-021-97307-2.
[18] Maclachalan, S., Zalik, S. (1963): Plastid structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant of barley. – Canadian Journal of Botany 43: 1053-1062.
[19] Magurno, F., Sasvári, Z., Posta, K. (2015): Assessment of native arbuscular mycorrhizal fungi assemblages under different regimes of crop rotation. – Applied Ecology and Environmental Research 13: 1215-1229. https://doi.org/10.15666/aeer/1304_12151229.
[20] Manaut, N., Sanguin, H., Ouahmane, L., Bressan, M., Thioulouse, J., Baudoin, E., Galiana, A., Hafidi, M., Prin, Y., Duponnois, R. (2015): Potentialities of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. – Ecological Engineering 79: 113-119.
[21] Mzabri, I., Addi, M., Berrichi, A. (2019): Traditional and modern uses of saffron (Crocus sativus). – Cosmetics 6(4): 63.
[22] Nelson, D. W., Sommer, L. E. (1982): Total Carbon, Organic Carbon and Organic Matter. Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. 2nd Ed. – The American Society of Agronomy (ASA-SSSA), Madison, pp. 595-579.
[23] Olsen, S. R. (1954): Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate (No. 939). – US Department of Agriculture, Washington.
[24] Parizad, S., Dizadji, A., Habibi, M. K., Winter, S., Kalantari, S., Movi, S., Lorenzo Tendero, C., Alonso, G. L., Moratalla-Lopez, N. (2019): The effects of geographical origin and virus infection on the saffron (Crocus sativus L.) quality. – Food Chemistry 295: 387-394. https://doi.org/10.1016/j.foodchem.2019.05.116.
[25] Phillips, J. M., Hayman, D. S. (1970): Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. – Transactions of the British Mycological Society 55: 158-IN18.
[26] Rabani-Foroutagheh, M., Hamidoghli, Y., Mohajeri, S. A. (2014): Effect of split foliar fertilisation on the quality and quantity of active constituents in saffron (Crocus sativus L.). – Journal of the Science of Food and Agriculture 94(9): 1872-1878.
[27] Shi, W. M. (1993): Root exudates and soil nutrient availability. – Soil 25: 252-256.
[28] Siracusa, L., Gresta, F., Ruberto, G. (2011): Saffron (Crocus sativus L.) Apocarotenoids: A Review of Their Biomolecular Features and Biological Activity Perspectives. – In: Yamaguchi, M. (ed.) Carotenoids: Properties, Effects and Diseases. Nova Science Publishers Inc., UK, pp. 1-33.
[29] Trouvelot, A., Kough, J. L., Gianinazzi-Pearson, V. (1986): Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de methods d'estimation ayant une signification fonctionnelle. – In: Gianinazi-Pearson V., Gianinazzi S. (eds.) Physiology and Genetics Aspects of Mycorrhizae. INRA Publisher, Paris, pp. 217-221.
[30] Vargas, P. (2020): The Mediterranean Floristic Region: High Diversity of Plants and Vegetation Types. – In: Di Paolo, D. (ed.) Encyclopedia of the World′S Biomes. Grasslands and Shrublands. – Elsevier, San Diego, pp. 602-616.
[31] Zhang, A., Shen, Y., Cen, M., Hong, X., Shao, Q., Chen, Y., Zheng, B. (2019): Polysaccharide and crocin contents, and antioxidant activity of saffron from different origins. – Industrial Crops and Products 133: 111-117.

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DOI: http://dx.doi.org/10.15666/aeer/2106_59775988