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Effect of site conditions and fertilization treatments on morphological traits and mineral content of *Aloe vera plants*

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Summary

The aim of this study was to investigate the effects of various fertilizer treatments on the morphological traits and mineral content of Aloe vera plants. The experiment was conducted in a greenhouse in Rabat, as well as two other fields in Skhirat and Sidi Bettach. The treatments including the 'no application', control F0 and three treatments which is compost (Organova) F1, compost combined with humus (Humivital) F2, and nitrogen (ammonitrate) 33% F3. The productivity of biomass was estimated based on the variables of morphological traits and mineral content. The effect of organic and inorganic fertilizers on the different variables of Aloe vera depends on the site. Significant effects were found for the most of the variables such as length, width, dry matter; ash content, manganese Mn, copper Cu, zinc Zn, calcium Ca, potassium K, carbon C, nitrogen N, sulfur S contents. Site effect was significant, with the higher values obtained for length, Width, Dry matter, Ash contents, Ca and N in Skhirat. Site effect was also significant for Mn and Cu in Sidi Bettach, and K in the greenhouse. The effect of the treatment F1 was significantly higher for the length and width of the leaves than F3 and the control F0, in addition F2 was significantly higher for the width of the leaves, nitrogen N and sulfur S contents.

Keywords: *Aloe vera*, Compost, Economic Sustainability, Humus, Nitrogen, Organic crops, Organic Fertilization

Introduction

Aloe vera (L.) Burm. F. (= Aloe barbadensis Mill.) is widely recognized as a medicinal plant and is cultivated in many countries for its medicinal, cosmetic, and food uses. Aloe vera belongs to the monocotyledonous order Asparagales and the Asphodelaceae family. It is well adapted to xerophytic conditions and can tolerate water deficits (SILVA et al., 2010; DELATORRE-HERRERA et al., 2010). This plant is characterized by succulent leaves, which can store water due to the presence of polysaccharides. It employs the crassulacean acid metabolism (CAM), which is an adaptation in hot conditions that require the synthesis of malic acid (SALINAS et al., 2016). These characteristics allows for greater water use efficiency and reduce evapotranspiration rates during the day (NOBEL, 1997; SUSHRUTA et al., 2013).

The economic value of *Aloe vera* is attributable to its chemical composition with high contents of polysaccharides, followed by essential amino acids, sugars, minerals calcium (Ca), sodium (Na), potassium (K), iron Fe, manganese (Mn), magnesium (Mg), copper

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(Cu), zinc (Zn), chromium (Cr), and antioxidant selenium Se. It further contains proteins, and fatty acids, vitamins, and several types of enzymes (PANDEY and SINGH, 2016). *Aloe vera* gel contains vitamins including A, C, and E, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), choline and folic acid. It also contains secondary metabolites, such as alkaloids, aloins, lectins, lignin, saponins, tannins, and phenolic compounds (VEGA-GÁLVEZ et al., 2009; PANDEY and SINGH, 2016). These compounds work synergistically and contribute to various pharmacological activities, including anti-inflammatory, antiviral, and immunostimulatory effects (SAHU et al., 2013; KUMAR et al., 2016; KUMAR et al., 2017a; KUMAR et al., 2017b).

Economic yields are obtained for 5 years, after which the *Aloe* needs replanting. Harvesting is a labor-intensive process. Only mature well developed leaves are collected, which are 60 to 80 cm in length and 8 to 10 cm width at the base. Employing good agronomic practices and with 20,000 plants ha⁻¹, it can provide two harvests per year between May and October (BASSETTI and SALA, 2003; CRISTIANO et al., 2016). The leaf of *Aloe vera* plant is primarily composed of a thick green exterior rind with white teeth at the edges, a viscous jelly-like mucilage layer on the inner side of the rind, and fillet fluid, which serves as the plant's water storage region (CHOWDHURY et al., 2021).

The chemical composition of *Aloe vera* is influenced by various factors, including geographical location and soil type. These environmental interactions directly affect the quality and quantity of the plant's primary active constituents. Studies have shown that the phytochemical composition of *Aloe vera* gel is influenced by irrigation regime, light intensity, temperature, and fertilization practices (LI et al., 2020; ANJUM et al., 2022).

Aloe vera is known for its relatively easy cultivation requirements. The succulence property of the plant makes it more responsive to nutrient. Fertility management in *Aloe vera* field may be one of the strategies for increasing of the yield of *Aloe vera* (SAHA et al., 2005). Currently, agronomic techniques continue to be explored to improve the quantity and quality of *Aloe* leaves to meet domestic and international market demand (CARDARELLI et al., 2013; MURILLO-AMADOR et al., 2015; PEDROZA-SANDOVAL et al., 2015).

Quality growth and crop productivity in *Aloe vera* differ with soil characteristics, availability of the nutrient elements and overall soil fertility (CHOWDHURY et al., 2021). In addition, chemical fertilizers enhance a good leaf quality. So, it may be necessary to find out a suitable recommendation for fertilization in *Aloe vera* farming.

Inorganic fertilizers, particularly ammonium nitrate, have been found to enhance the vegetative characteristics, gel, and aloin contents of *Aloe vera*. Nitrogen plays a physiological role in the molecular structure of chlorophyll pigments and the cytochromes, which are essential in photosynthesis and respiration, and plays an important role in synthesis of the plant constituents through the action of different enzymes activities and protein synthesis. This is reflected in the increase in growth of several medicinal plants such as anise, coriander and sweet fennel plants (MOHAMED et al., 2016). Ammonium nitrate is a commonly used nitrogen (N) source, and it is an odorless salt containing approximately 33 to 34% N. It can be applied to the soil surface or incorporated into the soil. Additionally, the nitrate component provides an immediately available source of nitrogen for the plant. Ammonium nitrate also has an acidifying effect on the soil due to the presence of ammonium, which can increase the availability of other soil nutrients (WATSON, 1987; KHANDELWAL et al., 2009; MOHAMED et al., 2016).

Organic fertilizer such as compost offers several benefits when used alone or in combination with synthetic chemical fertilizers or other organic fertilizers (AL-KAHTANI and AHMED, 2012). ROTHÉ et al. (2019) reported that organic fertilizer used alone or in mixture have a great potential, applied once at planting, compared to mineral fertilizers applied at regular intervals. Humic acid has a significant potential in soil improvement and remediation efforts. Additionally, it can serve as a nutrient source for crops, as humic acid-based fertilizers, humates, fulvic acid, and activated fulvic acid derived from humic acid are used to provide nutrients. Humic acid fertilizers are considered green, organic, and environmentally friendly options for sustainable agriculture (LI, 2020).

The leaf nutrient analysis can be the best method for diagnosing the economical nutritional status of plants and represents an important tool for determining the reduction potential of future fertilization requirements (AL-KAHTANI and AHMED, 2012). Subsequently, it is

important to analyse different parameters of Aloe leaves to provide information on plant development and to understand the physiological effects induced during plant growth. In our study, we evaluated the effects induced by conventional and optimised, organic and inorganic fertilizers on different variables including morphological characteristics and mineral content.

Materials and methods

Study area

The experiment was carried out in three different locations (Fig. 1). The first location was a greenhouse, in the National Agricultural Institute of Research Rabat INRA located in a littoral zone of Rabat, and the second experimental field was located in a coastal zone of Skhirat, south of Rabat Morocco (33°53'01.8"N 7°00'56.6"W), with mean annual rainfall around 600 mm per year, however, it is highly variable and can range between 250 and 800 mm. Morocco is characterized by arid and semi-arid climates. The average temperature is 17 °C due to the Atlantic Ocean influence, resulting in monthly minimal temperature of 10 to 12 °C in January and monthly maximal temperature of 20 to 24 °C in July/August. Mean annual rainfall is around 600 mm; however, it is highly variable and can range between 250 and 800 mm (ZOUAHRI et al., 2015). The third experimental field was located in Sidi Bettach, Benslimane province, Morocco (33°30'35.0"N 6°51'08.0"W. Characterized also by a semi-arid climate, Sidi Bettach has a mediterranean climate with dry summer. According to the Köppen-Geiger classification, during the year, the average temperature in Sidi Bettach is 17.3 °C and the average rainfall is 527.9 mm (ZOUAHRI et al., 2015).



Fig. 1: Aloe vera samples in the different locations; a: Greenhouse, b: Skhirat, c: Sidi Bettach.

Plant material

The *Aloe* plants were kindly supplied by the company Best *Aloe* SARL (*Sidi Abbad* – Marrakech, Morocco). The plants were identical in size, health, and appearance, and reaching 30 cm in height. A total of 120 *Aloe vera* plants around 18-month-old, 40 plant were transplanted in each location (Fig. 1). Each plant transplanted and cultivated in 25 cm high circular plastic pots supplied with a mixture of sand and peat-moss with a 20 cm diameter at the upper surface and drainage holes at the bottom.

Soil analysis

The soil samples were collected from the surveyed sites and taken to the laboratory of the Research Unit on the Environment and the Conservation of Natural Resources (URECRN), of the National Institute of Agricultural Research in Rabat. The soil samples were collected from the surveyed locations, were air-dried, crushed and then sieved. Tab. 1 presents the physical and chemical properties of the soils as well as the corresponding analytical protocols used (KILMER and ALEXANDER, 1949; RHOADES et al., 1989).

Tab. 1: Physicochemical properties, and textures of soils.

| Parameter | GH | SK | SB | Method |
|-------------------------------------|------------|------------|-------|-----------------------------|
| рН | 7.6 | 7.4 | 7.2 | Saturated soil-paste |
| EC (mS/cm) | 1.8 | 1.4 | 1.1 | extract soil: water $(1:5)$ |
| OM (%) | 1.1 | 2.9 | 2.1 | Chromic acid wet oxidation |
| Dry matter (%) | - | 99.5 | 99.7 | |
| N (%) | 0.1 | 0.2 | 0.2 | Kjeldahl |
| K ₂ O (ppm) | 530.2 | 54.2 | 180.8 | Flame Photometry |
| P ₂ O ₅ (ppm) | 103.1 | 86.7 | 2.6 | |
| Clay (%) | 27.0 | 15.0 | 25.0 | |
| Loam (%) | 32.8 | 30.1 | 37.9 | Pipette |
| Sand (%) | 40.2 | 54.9 | 37.1 | |
| Texture | Sandy loam | Sandy loam | Loam | |

GH: greenhouse, SK: *Skhirat*, SB: *Sidi Bettach*, EC: Electrical conductivity (measured at 25 °C), OM: Organic matter.

Tab. 2: Analysis of water irrigation of the sites.

Water analysis

The physico-chemical analysis of the irrigation water was carried out in the laboratory of the Research Unit on the Environment and the Conservation of Natural Resources of the National Institute of Agricultural Research in Rabat. The frequency of irrigation was the same: once a week. Tab. 2 shows irrigation water tests report. pH and electrical conductivity were measured using the electrometric method (CARTER, 1947) using a pH meter and a direct-reading conductivity meter using a pH-meter micropH 2000 and a conductometer microCM 2200 (CRISON Instruments, S.A., Barcelona, Spain), respectively. The elements Na and K were quantified after preparing appropriate dilutions (1:50) by flame emission photometry using a Jenway[™] PFP7 industrial flame photometer (Thermo Fisher Scientific Inc., Göteborg, Sweden) and as calibrators standard solutions of Na and K in the range 0-15 ppm.

The elements Ca and Mg were determined by complexometric titration with EDTA using the Patton and Reeder's (Ca) and Eriochrome black T (Ca, Mg) indicators (PRENTØ and PRENTØ, 1981). Mohr's method (BELCHER and MACDONALD, 1957) was used to determine Cl- concentration by titration with silver nitrate. A soluble chromate salt (K_2CrO_4) was added as indicator.

Fertilization treatments

In this study, fertilization trial was conducted in the greenhouse at the INRA in Rabat and in two distinct locations (*Sidi Bettach* and *Skhirat*) as mentioned previously. The treatments were applied in each location at random, including the no fertilized samples F0. Two commercial organic fertilizers (ORGANOVA[®] and *Humivital*) and ammonium nitrate as inorganic fertilizer were used. ORGANOVA[®] (EléphantVert, Meknès, Morocco), compost F1 is an organic amendment 100%, resulting from the recovery by composting of agricultural waste, agro-industry, and livestock. It contains a minimum of 30% organic matter and a humidity of 35% (\pm 5%). This fertilizer was applied manually in solid form in a single application at the beginning of the study, mix it into the soil of the rhizosphere around each plant. The dose used was 10 t/ha equal to 1 kg/m² on the field, and 0.01 kg per pot on the greenhouse. The characteristics of each fertilizer are in the following descritption in (Tab. 3).

The experimental setup was carried out and evaluated with two organic fertilizers from two industrial companies *Organova* as a solid form of compost and liquid fertilizer from Fertinagro company

| Location | pН | EC (mS/cm) | Na+ | K+ | Ca ²⁺ | Mg ²⁺ | Cl- | HCO ₃ - | |
|----------|-----|------------|-------|------|------------------|------------------|------|--------------------|--|
| GH | 7.3 | 2.0 | 10.61 | 0.1 | 7.2 | 3.2 | 20.3 | 5.0 | |
| SK | 8.4 | 1.4 | 11.5 | 0.16 | 6.2 | 4.2 | - | - | |
| SB | 7.3 | 2.8 | 10.9 | 0.05 | 6.8 | 3.9 | - | - | |

GH: greenhouse, SK: Skhirat, SB: Sidi Bettach, EC.: Electrical conductivity (measured at 25 °C); K+: Potassium; Na+: Sodium.

Tab. 3: Characteristics and dosis of the fertilizer treatments, compost : (Organova) F1, humus : (Humivital) combined with compost F2, and Ammonitrate F3.

| Fertilizer | pН | OM (%) | N (%) | C/N ratio | OC (%) | Dosis (kg/ha) | | |
|------------------|----|--------|-------|-----------|--------|---------------|-----|------|
| | | | | | | F1 | F2 | F3 |
| ORGANOVA® | - | 30 | 1.5 | 10 | 17.44 | 15 | 15 | - |
| HIMIVITAL | 8 | 27 | 3 | 43.5 | 15.69 | - | 50 | - |
| Ammonium nitrate | - | - | 33 | - | - | - | - | 50 |
| Dosis N (kg/ha) | | | | | | 0.2 | 1.7 | 16.5 |
| Dosis OM (kg/ha) | | | | | | 4.5 | 18 | - |

O.C: Organic carbon; % N: Nitrogen percentage; C/N: Carbon nitrogen ratio; % OM: Organic matter.

named *Humivital*, the third fertilizer is nitrogen as inorganic fertilizer. The other organic fertilizer used in this work was *Humivital* (humus), a liquid fertilizer based on humic substances (10% humic acids and 15% fulvic acids). *Humivital* was applied in combination with ORGANOVA[®] (2 ml of humus in liquid form is added after applying compost in solid form, compost humus F2) according to the dose of 10 t/ha or 1 kg/m² for compost which was the liquid added 2 mL of humus, at a rate of 50 L/ha in a single application already in a liquid form. As inorganic fertilizer, ammonium nitrate with a nitrogen content of 33% was used. The ammonium nitrate F3 was applied in a single dose of 50 kg/ha on field and 0.15 g per pot in greenhouse. The detailed characteristics of each fertilizer (compost F1, compost with humus F2, and nitrogen F3) are given in (Tab. 3).

The experiment was carried out following a randomized complete block design (RCBD) with five replications for each treatment applied.

Morphological traits

During the experiment, mature leaves (about 20-30 cm in length) were cut at the base using a sharp knife. The following qualities were evaluated for all leaves; A standard scale was used to determine the fresh weight (g) (Ohaus, CT600-S model, series 18939). To acquire the dry weight, *Aloe vera* leaves collected were put in a pre-heated oven (Shel-Lab, model Fx-5, serie-1000203) at 70 °C until constant weight was achieved (g). Morphological traits were measured in each plant by leaf length and width, fresh weight and dry matter. The observation of leaf length and width has been done during six months between February and October 2019.

Mineral content

The mineral content was determined using spectro-chemical procedures as follows. *Aloe vera* leaves were dried in oven at 70 °C for 48 hours. Dry leaves were crushed and milled in a grinder. Then, 1 gram of milled leaves was calcinated at 550 °C for 4.5 hours. The ashes obtained after calcination were weighted to perform a percentage calculation, recovered, and dissolved using 2 mL of distilled water and 1 mL of HCl 37%. This solution was filtered and made up to 50 mL with distilled water in a volumetric flask.

The final solution contains the micronutrients Fe, Mn, Cu, and Zn which were quantified using atomic absorption spectrophotometry. A Perkin Elmer Analyst 100 equipped with an IntensitronTM hollow cathode lamp (Perkin Elmer, Norwalk, CT, USA) operating in a continuous mode at 25 mA was used. Acetylene-air flame, at a flow of 2 and 4 L/min, respectively, was used for atomization of micronutrients where absorbance was measured at 248.3 nm (slit 0.2 nm) for Fe, 279.5 nm (slit 0.2 nm) for Mn, 324.8 nm (slit 0.7 nm) for Cu, and 213.9 nm (slit 0.7 nm) for Zn,, respectively. Calibrations were obtained by serial dilution of 1 g/L stock solutions in the range 0-5 ppm. All correlation coefficients were > 0.98. The results were expressed as μ g of element/g of dry matter.

To determine the foliar phosphorus (P), the solution is obtained after mineralization of the leaves with acid digestion. In this solution, P is in the form of H_3PO_4 , which, in the presence of orthophosphoric acid and molybdic acid and a reducing agent, forms a blue color complex with Murphy's method (MURPHY and RILEY, 1962; OLSEN and SOMMERS, 1982).

For the determination of macronutrients Ca and Mg, the extracts were analyzed using atomic absorption (AA) spectroscopy. N, C and S were determined by calcination in a CNS Macrosample CNS-2000 Leco Elemental Analyzer at the CITIUS agricultural analysis service (University of Seville, Spain), for potassium K and sodium Na, the analysis is performed with emission spectrometry.

Statistical analysis

The data were analyzed using SPSS®Statistics software (version 25). Analysis of variance (ANOVA) was used to evaluate the effects of the factors site (S) and fertilizer type (F) on the means of the morphological traits and mineral content of *Aloe* plants as dependent variables. In addition, interactions between factors were investigated. Tukey's HSD test was used to find means that are significantly different from each other.

Results and discussion

According the statistical analysis, site effect was significant for the most of the variables such as length, width, dry matter; ash content, Mn, Cu, Zn, Ca, K, C, N, S. The effect of the site depends on the edaphoclimatic properties. Site effect was significant, with the higher values obtained for length, width, dry matter, ash contents, Ca and N in Skhirat. Site effect was also significant for Mn and Cu in Sidi Bettach, and K in the greenhouse in Tab. 5.

In our study, the treatment effect was significant for the length and width of the leaves, F1 induced the highest average of the leaves for both variables compared to F3 and F0 in Tab. 4. Treatment effect was significant also for some minerals, F2 induced significantly the highest average of N and S in Tab. 7.

LIU et al. (2007) reported that the types and texture of soil were closely related to the growth and development of medicinal plants and loam soil was the ideal type for the cultivation of root/stem-type medicinal plants. The performance variation of different soils for Aloe vera cultivation might be due the physical and chemical properties of the soils. Among the properties, pH, organic matter content, salinity, nutrient contents and their availability could be the prime factors controlling the growth and yield of any crop (CHOWDHURY et al., 2018). In addition, crop productivity varies with the contents and different combinations of nutrient elements, pH, EC and organic matter present in soil (BROADLEY et al., 2012; HAWKESFORD et al., 2011). If the soil pH is too low or too high, it can affect nutrient availability and uptake, leading to stunted growth and smaller leaves. However, different types of soil have quite different physicochemical properties, which have substantial effects on the growth, development and the active constituents of medicinal plants (LI and XIAO, 2012). Soil fertility is also important for Aloe vera growth. Soil organic carbon is also a crucial parameter for soil fertility as it enhances soil physical, chemical and biological properties (LÜTZOW et al., 2006; BIRKHOFER et al., 2008).

Also, several studies investigated the impact of various inorganic fertilizers and organic manures on the yield and quality of Aloe vera gel (KUMAR et al., 2016). Adequate levels of nitrogen, phosphorus, and potassium are necessary for the plant to produce healthy leaves. Nitrogen fertilizer may enhance both growth and yields because the nitrogen supply helps in the full expansion of the leaf, chlorophyll content, and photosynthetic rates subsequently increases the supply of carbohydrates to the plants (KHANDELWAL et al., 2009). If the soil is deficient in these nutrients, the leaves may be smaller and lighter in weight. Organic fertilizers release N over time as much as several years from the time of application. Fertilizer additions to any particular crop should consider the cumulative effects of previous applications of organic nutrient sources, which will continue releasing N (HUE and SILVA, 2000). Furthermore, the high N treatment showed high levels of leaf N, chlorophyll and soil available N, while the dry matter weight of the optimized fertilization treatment was higher than that of the high N treatments and the N-deficient treatments (YAN-LI et al., 2023).

Length and width

Leaf length and leaf width were measured for each plant during the study period to assess the main effects of fertilizations (F) and sites

(S) in Tab. 4. Based on the Tukey posthoc test, significant effect of the site and fertilization treatment (p < 0.001) was recorded on morphological traits. It was significant interaction between the two factors, meaning that the ranking of the treatments depends on the site. The average mean of the leaf length with 47.0 ± 8.1 cm, at the Skhirat was higher compared to Sidi Bettach and the greenhouse. The effect of the treatment was also significant (p<0.001) on the length of the leaves; Plants treated with F1 treatment had higher mean value of $44.2 \pm$ 7.8 cm compared to F0 with an average of 37.2 ± 10.9 cm and higher than the other treatments F2 and F3. The mean value of the width of the leaves, at the Skhirat was higher with 6.3 ± 0.7^{a} cm compared to the greenhouse $5.5 \pm 1.5^{\text{b}}$ cm and *Sidi Bettach* $5.3 \pm 1.0^{\text{b}}$ cm. The Tukey posthoc test showed that F0 was clearly different from others treatments. However, the effect of the treatment on average width of the leaves for F2 and F1 treatments, were also significantly higher with 6.2 ± 1.0^{a} cm and 6.1 ± 1.1^{a} cm, respectively compared to F3 with 5.5 ± 1.3^{b} cm and F0 with 5.1 ± 1.1^{c} cm. The mean value of the with for the plants treated with F2 treatment had the highest mean value which means that compost combined with humus increased the width of the leaves and plants treated with F1 tends to increase the length.

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Aloe vera grows well in all kinds of soils but well drained soil rich in organic matter is preferable (KUMAR and YADAV, 2014). Aloe vera prefers well-draining, sandy soil that is rich in organic matter. If the soil is too heavy or compacted, it can affect root growth and nutrient uptake, leading to smaller and lighter leaves. Soil can have a significant effect on the growth and weight of *Aloe vera* leaves. Plants reduce their chlorophyll content in response to N deficiency (COLE-MAN et al., 1988), which is considered as a biochemical biomarker for detection of plant N status in plants in agricultural fields (YUE et al. 2020; CARDIM FERREIRA LIMA et al. 2020). In soils, N must be present as either NH_4^+ or NO_3^- before plants can absorb and use it. The application of 150 kg N·ha⁻¹ resulted in an increased both number and size of leaves, and total yield (BHARADWAJ, 2011).

Tab. 4: The main effect of type of treatment and sites on the mean values of length and width (cm) leaves of *Aloe vera* measures duplicated in each plant (N=5) in different fertilizer treatments; Control F0, Compost F1, Compost plus Humus F2, Nitrogen F3; in greenhouse, *Sidi Bettach*, and *Skhirat*.

| Experimental factor | Level | Length (cm) | Width (cm) |
|---|---------------------------------------|--|---|
| Fertilizer treatment (F) N = 15 | F0 F1 F2 F3 | $\begin{array}{c} 37.2 \pm 10.9^{\mathrm{c}} \\ 44.2 \pm 7.8^{\mathrm{a}} \\ 42.3 \pm 7.9^{\mathrm{ab}} \\ 41.2 \pm 10.7^{\mathrm{b}} \end{array}$ | $5.1 \pm 1.1^{c} \\ 6.1 \pm 1.1^{a} \\ 6.2 \pm 1.0^{a} \\ 5.5 \pm 1.3^{b}$ |
| Site (S) N = 20 | Greenhouse Sidi Bettach Skhirat | $\begin{array}{c} 35.2 \pm 7.6^{c} \\ 43.2 \pm 9.4^{b} \\ 47.0 \pm 8.1^{a} \end{array}$ | $\begin{array}{l} 5.5 \pm 1.5^{\mathrm{b}} \\ 5.3 \pm 1.0^{\mathrm{b}} \\ 6.3 \pm 0.7^{\mathrm{a}} \end{array}$ |
| Two-way ANOVA | | p-value | p-value |
| Main effect of F Main effect of S Interaction (F×S) | | <0.001 <0.001 <0.001 | <0.001 <0.001 <0.001 |

Within columns, mean (\pm standard deviation) values followed by the different letters*, same lower-case letter were not significantly different by the minimum significant difference of Tukey's test at P \leq 0.05, according to the Two ways ANOVA test.

Fresh weight, dry matter and ash percentage

The fresh weight, dry weight and the ash content of the leaves of *Aloe vera* were measured for each plant at the end of the experiment to evaluate the main effects of the fertilizing treatments (F) and of the sites (S) in Tab. 5. According to the Tukey posthoc test, site

effect (S) was significant for the dry matter and ash content. Site effect was pronounced in *Skhirat*, the highest value of the dry matter was 4.7 ± 0.8^{a} g and ash percentage was 20.2 ± 1.7^{a} %, the mean value of each variable was higher than the greenhouse and *Sidi Bettach*. In our study, there is no significant effect for fertilizers. This finding is similar to those reported by NúÑEZ-COLIMA et al. (2018), not significant differences in the yield of forage oats when applying compost. The absence of effect could be related for this study to the dose used.

These results partially coincide with those of PEDROZA-SANDOVAL et al. (2015), who point out that the application of compost in combination with humic acids had no effect on *Aloe* growth, but higher plant height does occur when applying 14 l·ha⁻¹·year⁻¹ algae extract. Accumulation of dry matter is essential for crop yield formation, and dry weight is an extensively used parameter for assessing the growth conditions of plants (TANG et al., 2019). The effect of biofertilizers was significant in a study by CARDARELLI et al. (2013), when guano doses were doubled, from 4 to 8 g·L⁻¹, it resulted in greater fresh and dry weight of *Aloe* leaf, suggesting that the effect of fertilizers is linked to the doses. SAHA et al. (2005) obtained highest value with 17.8% of the ash content of the *Aloe* gel, when organic treatment was applied, with the vermicompost plus worm-wash treatment.

Micronutrients Fe, Mn, Cu and Zn

According to the tukey posthoc test, site effect was significant for the mean values of Mn, Cu, and Zn in Tab. 5. The fertilization treatment was not significant for micronutrients. Site effect was significant (p <0.001) regarding Mn and Cu in Sidi Bettach with (118.3 ± 57.2^a and 15.9 ± 16.1^a µg·g⁻¹) respectively, higher than Skhirat and the greenhouse. Site effect was significant for Zn in the greenhouse with 5.1 ± 0.7^{a} µg·g⁻¹ higher than Skhirat and Sidi Bettach.

Minerals are usually required in small amount for the development of plants and organisms. Excessive uptake of these trace elements affects the metabolic activities of plant as well as animals. Fe has several vital functions inside the body of animal and plants as well which involves in oxidation-reduction reactions (ETC), hemoglobinoxygen transport and transport and also a co-factor for numerous other enzymes. Cu is also serve as constituents of many enzymes which contain copper in the active sites and catalyze the oxidation reactions. It plays a key role in the hemoglobin synthesis. Trace elements present in Aloe vera have a significant antidiabetic activity. Zn is a versatile element which has been well known to be an important trace element in diabetes as a cofactor for insulin. It's important for plants to have Fe and other metals such as Mn, Cu, and Zn because they are involved in many metabolic processes that are part of the essential enzyme system for plants to do many things that need to happen for them to grow (RAVET and PILON, 2013). It was reported that the addition of poultry manure alone or in combination with organic fertilizers increased the uptake of Zn and Fe by wheat and rice (AL-KAHTANI and AHMED, 2012).

Numerous field and laboratory experiments have shown the dependence between the Mn content of the nutrient medium and the synthesis of ascorbic acid. Mn deficiency reduces vitamin C concentrations. Higher concentration of reduced form of ascorbate in the Mn deficient plants might be a plant mechanism of keeping the redox status of plant cells under control (MUZOLF-PANEK et al., 2017).

Macronutrients Ca, Mg, Na, K, P, C, N and S

Site effect was significant for (Ca, K, C, N, S) and significant effect of fertilizers on N and S. According the Tukey posthoc test in Tab. 6; the mean value of Ca was higher in *Skhirat* with 29.2 ± 7.5^{a} mg·g⁻¹ than *Sidi Bettach* and the greenhouse, the mean value of K was higher in the greenhouse with 28.9 ± 7.1^{a} mg·g⁻¹ than *Sidi Bettach* and *Skhirat*,

Tab. 5: Effects of treatments and sites on fresh weight (g), % dry matter, % ash content, and minerals Fe, Mn, Cu, and Zn, (µg·g⁻¹dw) in dry weight of leaves of Aloe vera L. Plants subjected to four treatments, Control F0, Compost F1, Compost plus Humus F2, and Nitrogen F3, in three sites: Greenhouse, Sidi Bettach, and Skhirat. Mean ± standard deviation values).

| Experimental facto | or Level | Fresh weight | Dry matter | Ash | Fe | Mn | Cu | Zn |
|------------------------|---------------|------------------|----------------------------|-----------------------------|-----------------|------------------------------|----------------------------|----------------------------|
| Fertilization | F0 | 107.5 ± 53.0 | 4.4 ± 0.6 | 17.8 ± 2.2 | 69.3 ± 17.5 | 67.6 ± 56.6 | 7.2 ± 3.9 | 4.6 ± 0.4 |
| treatment (F) | F1 | 116.3 ± 28.8 | 4.2 ± 1.1 | 19.5 ± 1.6 | 88.4 ± 77.8 | 56.0 ± 55.7 | 12.3 ± 18.8 | 4.7 ± 0.2 |
| N = 15 | F2 | 107.9 ± 60.3 | 4.1 ± 1.0 | 18.2 ± 2.2 | 53.5 ± 12.9 | 46.6 ± 32.8 | 4.9 ± 2.5 | 4.7 ± 0.4 |
| | F3 | 83.7 ± 43.9 | 4.4 ± 1.0 | 18.5 ± 3.6 | 69.1 ± 49.6 | 67.8 ± 74.6 | 8.7 ± 9.7 | 5.0 ± 0.9 |
| | Greenhouse | 95.3 ± 47.4 | 3.6 ± 0.7 ^b | 17.6 ± 2.9 ^b | 51.4 ± 17.8 | $15.8 \pm 6.7 ^{b}$ | 4.3 ± 0.9^{b} | 5.1 ± 0.7 ^a |
| Site (S) N = 20 | Sidi Bettach | 94.1 ± 49.1 | 4.4 ± 0.9 ab | 17.7 ± 1.8 ^b | 77.5 ± 72.3 | 118.3 ± 57.2 ^a | 15.9 ± 16.1 ^a | 4.7 ± 0.3 ab |
| | Skhirat | 122.1 ± 44.4 | $4.7\pm0.8~^{a}$ | 20.2 ± 1.7 $^{\rm a}$ | 81.3 ± 30.4 | 44.4 ± 15.7 ^b | 4.5 ± 2.8 ^b | 4.5 ± 0.3 ^b |
| Two-way ANOVA | | p-value | p-value | p-value | p-value | p-value | p-value | p-value |
| Effect of Fertilizer t | reatments (F) | 0.475 | 0.830 | 0.425 | 0.531 | 0.519 | 0.386 | 0.289 |
| Effect of Site (S) | | 0.255 | 0.012 | 0.013 | 0.291 | < 0.001 | 0.006 | 0.009 |
| Interaction (F×S) | | 0.232 | 0.744 | 0.483 | 0.813 | 0.435 | 0.260 | 0.159 |

Within columns, mean (\pm standard deviation) values followed by the same letter were not significantly different by the minimum significant difference (MSD) of Tukey's test at P \leq 0.05.

and significant interaction was observed for Mg. According the Tukey posthoc test (Tab. 7); Site effect was significant for C, the mean value was higher in Sidi Bettach with 37 ± 0.1^{a} % and the greenhouse with 36.8 ± 0.8^{a} % than Skhirat. Site effect was significant for N with a mean value of 0.87 ± 0.11^{a} % in Skhirat, higher than Sidi Bettach and the greenhouse. The mean value of S content was higher in Sidi Bettach. Fertilization effect was significant for N and S, the mean values were higher for F2 treament compared to F0 and the other treatments (F1 and F3). For CARDARELLI et al. (2013), the N, K, Ca, Mg, Na, Mn, and Zn concentrations in leaf tissue were significantly affected by the fertilization level with the higher values recorded with standard fertilization than with the reduced fertilization. The importance of N has been evaluated on theoretical grounds because it may be pointed out that CAM plants might need less N than C3 plants, and thus having higher nitrogen use efficiency (RAVEN and SPICER, 1996; GARCÍA-HERNÁNDEZ et al., 2006). Nutrient levels and element responses have also been studied in CAM desert succulents. In the chlorenchyma of cultivated cacti and agaves, levels of Ca, Mg and Mn tended to be higher than in most other agronomic plants, and thus these CAM plants are calcitrophic species (LÜTTGE, 2004). Ca can bind to negatively charged groups of proteins and lipids, and hence decrease membrane fluidity/permeability (LÜTTGE, 2004; GARCÍA-HERNÁNDEZ et al., 2006). MARSCHNER (1986) has reported antagonism between Ca and K, and WALWORTH and SUMNER (1987) as a contrast related to K dilution and Ca accumulation over time. This result agrees with the soil calcareous nature and the low K solubility in the substrate. By this way, there is the possibility that K dilution does not occur when plants accumulate enough K under dry matter basis in leaves of Aloe vera and present lower Ca leaf concentration without ability to promote K dilution. For most nutrients, there is significant difference due to site effect in Skhirat area such as Ca, K as well as manganese Mn and Cu in Tab. 5 and 6. These results are similar to those found by CHOWDHURY et al. (2021), which the highest Ca concentration was recorded in plants under 100% of poultry manure fertilization treatment and the lowest value was recorded in the case of no fertilization treatment (CHOWDHURY et al., 2021).

Tab. 6: Effects of treatments and sites on the mean dry weight (mg·g⁻¹) of mineral content Ca, Mg, Na, K, and P in leaves of Aloe vera plants subjected to treatments: Control F0, Compost F1, Compots plus Humus F2, and Nitrogen F3, the three sites: Greenhouse, Sidi Bettach, and Skhirat. Mean ± standard deviation.

| Experimental factor | Level | Ca | Mg | Na | K | Р |
|---------------------------|-------------|-------------------------|---------------|----------------|-----------------------------|-----------------|
| Fertilization | F0 | 23.6 ± 4.7 | 4.0 ± 3.2 | 8.7 ± 1.9 | 22.9 ± 3.7 | 68.1 ± 2.24 |
| treatment (F) | F1 | 26.1 ± 9.7 | 4.2 ± 0.8 | 10.8 ± 0.2 | 26.6 ± 1.7 | 61.0 ± 1.16 |
| N = 15 | F2 | 21.7 ± 0.2 | 3.5 ± 0.5 | 10.1 ± 1.4 | 37.7 ± 1.2 | 74.7 ± 1.15 |
| | F3 | 24.4 ± 4.5 | 2.5 ± 0.9 | 9.2 ± 1.2 | 28.5 ± 8.9 | 82.0 ± 0.29 |
| Gi | reenhouse | 18.6 ± 9.0 ^b | 2.7 ± 2.1 | 9.7 ± 1.4 | 28.9 ± 7.1 ^a | 62.5 ± 1.2 |
| Site (S) $N = 20$ Side | di Bettach | 24.1 ± 6.2 ab | 3.7 ± 1.9 | 9.0 ± 1.9 | 18.1 ± 7.1 ^b | 76.2 ± 0.8 |
| | Skhirat | $29.2\pm7.5~^{a}$ | 4.3 ± 2.2 | 9.3 ±1.1 | 16.6 ± 5.4 ^b | 75.7 ± 1.2 |
| Two-way ANOVA | | p-value | p-value | p-value | p-value | p-value |
| Effect of Fertilizer trea | atments (F) | 0.568 | 0.148 | 0.832 | 0.141 | 0.884 |
| Effect of Site (S) | | 0.003 | 0.095 | 0.558 | < 0.001 | 0.809 |
| Interaction (FxS) | | 0.028 | 0.009 | 0.456 | 0.379 | 0.708 |

Within columns, mean (\pm standard deviation) values followed by the same letter were not significantly different by the minimum significant difference (MSD) of Tukey's test at P ≤ 0.05 .

Tab. 7: Effects of treatments and sites on dry weight (%) in the mineral content of C, N, and S in leaves of *Aloe vera* plants subjected to treatments: Control F0, Compost F1,Compots plus Humus F2, and Nitrogen F3, the three sites: Greenhouse, *Sidi Bettach*, and *Skhirat*. Mean ± standard deviation

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| Experimental factor | Level | С | N | S |
|------------------------------------|--------------|-----------------------------|------------------------------|--------------------------------|
| | F0 | 35.6 ± 0.1 | 0.69 ± 0.15 ^b | 0.059 ± 0.014 ^b |
| Fertilization | F1 | 36.7 ± 0.8 | $0.78\pm0.19~^{ab}$ | 0.068 ± 0.022 ab |
| treatment (F) $N = 15$ | F2 | 38.1 ± 0.5 | 0.90 ± 0.10 a | 0.082 ± 0.012 ^a |
| 11 - 15 | F3 | 36.9 ± 1.8 | $0.82\pm0.29~^{ab}$ | 0.064 ± 0.063 ab |
| | Greenhouse | 36.8 ± 0.8 ^a | 0.67 ± 0.21 ^b | 0.067 ± 0.025 ^{ab} |
| Site (S) $N = 20$ | Sidi Bettach | 37.0 ± 0.1 $^{\rm a}$ | $0.84\pm0.21~^a$ | 0.078 ± 0.021 ^a |
| N = 20 | Skhirat | $35.6\pm0.3~^{\rm b}$ | 0.87 ± 0.11 $^{\rm a}$ | 0.060 ± 0.019 b |
| Two-way ANC | OVA | p-value | p-value | p-value |
| Effect of Fertil treatments (F) | izer | 0.146 | 0.024 | 0.019 |
| Effect of Site (| S) . | < 0.001 | 0.002 | 0.027 |
| Interaction (F× | :S) | 0.073 | 0.005 | 0.004 |

Within columns, mean (\pm standard deviation) values followed by the same letter were not significantly different by the minimum significant difference (MSD) of Tukey's test at P ≤ 0.05 .

Conclusions

The aim of this study was to determine the influence of organic and inorganic fertilization on the yield, the morphological characteristics and the mineral content of the Aloe vera plants in two regions of Morocco. The effect of organic and inorganic fertilizers on different variables of Aloe vera depends on the site. Significant effects were found for the most of the variables such as length, width, dry matter; ash content, Mn, Cu, Zn, Ca, K, C, N, S. Site effect was significant, with the higher values obtained for length, width, dry matter, ash content, Ca and N in Skhirat. Site effect was also significant for Mn and Cu in Sidi Bettach, and K in the greenhouse. The effect of the treatment F1 was significantly higher for the length and width of the leaves than F3 and F0, in addition F2 was significantly higher for the width of the leaves, N and S. Organic fertilization remains the preferred approach due to the advantages it offers to the environment, soil fertility, and crop yield. Nevertheless, these must be tuned to optimal results in the Aloe vera plant. It was demonstrated that the effect of fertilizer treatment depends on the site and tends to affect the level of nutrients in Aloe vera which leads to a good quality of the plant.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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