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Effect of Organic Fertilization and Mycorrhization on the Growth and Yield of *Artemisia annua* L. Plants in Soil Fortified with Exogenous Nitrogen

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Abstract

The production of *Artemisia annua*, as well as that of other cultivated plants, is influenced by environmental factors which disrupt all vital functions. Therefore, finding a healthy production method remains a major challenge in meeting the demand for these plants. The aim of this study is therefore to evaluate the influence of organic fertilization on the growth of *A. annua* plants, with the aim of meeting demand in a sustainable manner. To achieve this, 1.5-month-old *Artemisia annua* plants are transplanted into fields in an experimental design consisting of two blocks separated by 1 m. The first block is fertilized with AMF and the second with ENTO, with three repetitions in each block; Both are irrigated with purified water rich in minerals, mainly the two forms of nitrogen that can be absorbed by plants: NO_3^- (79.65 ± 07.6) mg/l, NH^{4+} (1250 ± 121.3) (mg/l) with a pH of 7.4 ± 0.3. The mineral elements in the leaves and the agronomic and biochemical parameters are evaluated. The results show that fertilization with AMF and ENTO, followed by irrigation with purified water, increases the mineral absorption of *A. annua*, except for sodium in acidic soil. A significant negative correlation ($p < 0.05$, $r = -0.57$) was observed between proteins and chlorophylls, between proteins and flavonoids ($r = -0.45$), as well as between flavonoids and phenols. This would be an appropriate biological fertilization method for improving Artemisia production, as it is a valuable crop.

Keywords

Artemisia annua L., Biofertilization, Secondary Metabolites, Growth

1. Introduction

In the tropical zone in general and in Cameroon in particular, malaria is an endemic disease caused primarily by *Plasmodium falciparum* [1], which is sensitive to quinine and its derivatives, but especially to artemisinin [2]. The latter is extracted from the Artemisia plant, scientifically known as *Artemisia annua* L., making its cultivation the only way to obtain this compound [3]. In addition to artemisinin, this plant contains several other secondary metabolites, namely proteins, flavonoids, and phenolic compounds, which also contribute to its effective curative properties. Artemisia is also used as a spice and dietary supplement due to its high iron, calcium, phosphorus, iodine, selenium, and zinc content. This plant is also rich in essential oils and polysaccharides, saponins, coumarins [4]. It has been used effectively to combat Covid-19 in sub-Saharan Africa [5]. Thus, in Cameroon, IRAD, through the National Herbarium, has set up the production of *Artemisia* and has been working for some time on the production of herbal teas, capsules and tablets based on *Artemisia* [6] [7]. However, production remains insufficient to meet national demand as part of endogenous measures to respond to malaria but also of the national strategy to combat the Covid-19 coronavirus. The prospects for the production of medicinal plants in Cameroon, as recorded by the Ministry of Scientific Research and Innovation (MINRESI) following its interministerial meeting on traditional pharmacopoeia held on June 15, 2020, can only be achieved through several levers, including increasing the production of *Artemisia* plants, knowing that conventional mineral fertilization is incompatible with the economic context of the Cameroonian farmer due to the high cost of approved fertilizers [8] and their impact on soil health. On the other hand, organic and biological fertilizers could compensate for this low level of nutrients and restore these tropical soils. Thus, the aim of this work is to evaluate the influence of fertilization on the production of *A. annua* plants in a context of proven sustainability. Soil productivity in the tropics is declining even with the continued use of chemical fertilizers [8] [9]. Organic and biological fertilizers such as purified water, would increase crop yields and maintain soil fertility in the long term, and compensate for the low level of nutrients in these tropical soils [10]. These fertilizers, through their beneficial effects on the physicochemical and biological properties of the soil, and therefore on plant growth, would allow the latter to be not only of good quality but also to retain their curative and organoleptic properties. Thus, improving the production of *A. annua* plants by providing beneficial fertilizers would contribute to having information on the influence of the types of fertilizers applied and their impact on the quality aspect. Hence, the general objective of this work was to evaluate the effect of fertilization on increasing the production of good quality *A. annua* plants, while highlighting their implication on the agro-morphological parameters of growth; variation in the content of some metabolites and mineral elements in plant leaves.

2. Materials and Methods

The study was conducted from February to June 2023 on the experimental plot at

the University of Yaoundé I. The geographical coordinate of the cultivation site was 13˚51'30" N and 11˚29'57" E. The plant material consisted of healthy, seven-week-old *Artemisia annua*. seedlings obtained from the nursery department of the Agricultural Research Institute for Development (IRAD) in Nkolbisson. The organic purified water solution used in the irrigation solutions was obtained from the water treatment plant of the University of Dschang campus; because it is rich in minerals compounds mainly the two forms of assimilable nitrogen and contains microorganisms that can promote plant growth and development.

3. Analysis of the Physicochemical Parameters of the Soil at the Growth Site

The physicochemical parameters of the soil at the study site were previously determined using the current methods of [11], and in compliance with ISO standards. AFNOR NF and EN, on an average sample of 1kg of this soil, taken randomly. The physical analyses consisted of determining the texture by evaluating the rates of clay, fine silt, coarse silt, fine sand and coarse sand using the Robinson-Kôhn pipette method based on [12] law after destruction of organic matter by hydrogen peroxide; Iron oxides as well as carbonates with hydrochloric acid (NFX 31 - 107 standard) followed by sedimentation of fine to very fine particles according to [12] law. The mineral texture of the soil is determined according to its relative proportion of clay, silt, and sand according to the texture triangle reference. Total nitrogen was determined by sulfuric acid mineralization of 2 g of sample using the Kjeldahl method, NFISO 11261 [13], and the ratio of carbon to total nitrogen was calculated from the previously determined carbon and total nitrogen. The soil cation exchange capacity (CEC) and the saturation rate of exchangeable bases were determined using the modified method of [14].

4. Effect of Applied Biofertilizers on Production in *A. annua*

The experimental design was a complete block consisting of two sub-blocks corresponding to biofertilization and biofertilizers plus purified water, with two replicates, each separated by 1 m. Each sub-block consisted of 18 experimental units, each 1.5 m wide and 2.5 m long, with a distance of 0.5 m between them. The plants used were transplanted into holes of 10 cm wide and 10 cm deep and made from the standard sowing density of *A. annua* 20,000 plants/ha, namely two lines of three plants per EU for a total of 216 plants [15]. After transplanting, the plants are fed every two days in the morning with 36 L of tap water/EU until the rains return. The maintenance consisted of pulling out weeds and loosening the soil around the plants. The treatments applied were Control (Cont), Arbuscular Mycorrhizal Fungi (AMF), Entomocompost (ENTO), Control + purified water (Cont + PW), Arbuscular Mycorrhizal Fungi + purified water (AMF + PW), and Entomocompost + purified water (ENTO + PW). For the AMF and ENTO treatments used, 30g was applied. Fertilization consisted of applying purified water every two weeks by a 4V/V mixture of tap water and purified water, composed of NO_3^-

(mg. l^{-1}): 79.65 ± 77.6, $NH4^{+}$ (mg. l^{-1}): 1250 ± 121.3, PO4 (ml.l^{-1}): 61.1 ± 9) at a pH of 7.4 ± 0.3 stored at a temperature of (28.28 ± 1.8) ˚C).

5. Effect of Biofertilizers Applied on the Agronomic Parameters of *Artemisia annua* Plants

Agronomic parameters were measured monthly on five plants/EU. Plant height and stem diameter at the root collar were measured using a tape measure and a caliper, respectively. The fresh and dry weights of plant samples were determined using an electronic scale, while the number of flowers, buds, secondary branches of the five basal branches, and the number of roots were manually counted. During the growth of *Artemisia* plants in the field, samples of non-senescent adult leaves were collected for the physiological and biochemical analyses described below.

6. Evaluation of the Effect of Biofertilizers on Plant Physiological Parameters in *A. annua*

6.1. Total Chlorophyll Content

Total chlorophyll concentration was determined in *A. annua* leaves using the [16] method: approximately 0.5 g of fresh leaves were ground and homogenized with 4 ml of 80% acetone. The resulting homogeneous mixture was filtered using filter paper (Wattman No. 4), and the filtrate, constituting the crude chlorophyll extract, was collected in a test tube for quantitative analysis. The optical densities of these extracts were read using a spectrophotometer at 652 nm against 80% acetone, and the concentrations were determined using the [16] equation before being adjusted to the levels in mg. g^{-1} of fresh matter.

6.2. Relative Water Content of *A. annua* Leaves

Each month during the cultivation period, leaf samples were collected, weighed to determine fresh mass (FM), then immediately soaked in demineralized water and kept in the dark before being reweighed to determine turgor mass (TM). They were then placed in an incubator at 85˚C for 48 h and reweighed upon removal to determine dry mass (DMS). The values obtained for each sample were used to determine the relative water contents (RWC) (%) using the formula [(FM − DM)/(TM − FM)] × 100 [17].

6.3. Evaluation of the Effect of Biofertilizers on the Mineral Content of *A. Annua* Plants

Mineral elements were extracted from the leaves using the mineralization method proposed by [10]. This method consists of drying the sample in an oven at 105˚C for 24 hours, calcining it at a high temperature (550˚C) in a muffle furnace for 4 hours, and adding a mineral acid, followed by gentle heating after the ash has cooled. The resulting solution is used to determine the phosphorus, sodium, calcium, magnesium, zinc, and iron contents.

6.4. Phosphorus Content

Phosphate ions are determined based on the principle of the formation of the yellow phospho-vanado-molybdate complex, measurable by molecular absorption spectrophotometry at 430 nm in the presence of the nitro-vanado-molybdate reagent [10]. Thus, 6 ml of distilled water and 2 ml of nitro-vanado-molybdate reagent are added to each test tube containing 2 ml of the previously prepared extract. The mixture is homogenized, incubated for 1 hour, and the optical density is read using a UV-VI spectrophotometer at 430 nm against the blank.

6.5. Potassium, Sodium, and Calcium Contents

The principle for extracting K^+, Na^+, and Ca^{2+} ions is based on capturing light emissions from their excitation on heated leaf samples [10]. 5 g of the previously obtained leaf powder sample is diluted in 20 mL of 2N nitric acid; the resulting solution is transferred into a 50 ml volumetric flask, and the volume is adjusted to the mark with distilled water. The solution is filtered, and the optical densities are read using a flame photometer.

6.6. Magnesium, Zinc, and Iron Contents

Mg^{2+}, Zn^{2+}, and Fe^{2+} ions are determined using the atomic absorption spectrometry method [10], based on the theory of atomic energy quantification. Thus, 5 g of the sample and 20 mL of 2 N nitric acid were added to a 50 mL volumetric flask, and the solution was adjusted with distilled water to the fill line. The optical densities were then read using an atomic absorption spectrophotometer. The contents of these mineral elements were automatically obtained using the Spectra Manager software, which calculated the concentration in mg/L of the extract run through the spectrophotometer from the calibration curve.

7. Effect of Biofertilizers Applied on the Biochemical Parameters of *A. annua* Plants

7.1. Phenolic Compound Contents

The extraction was performed according to the modified protocol of [18]; Approximately 0.5 g of fresh adult leaves of *A. annua* were cold-ground in 2 ml of hydrogen chloride. The resulting mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant is collected for measurement, which is based on the reduction of the Folin-Ciocalteu reagent in an alkaline medium due to the oxidation of phenolic compounds, producing a blue color whose absorption at 760 nm is proportional to the phenolic compound content in the extract. Thus, to a volume of 100 µl of the extract obtained previously, add 2.5 ml of distilled water, 10 µl of Folin-Ciocalteu and 0.5 ml of 20% Na_2CO_3. Incubate the mixture obtained in a water bath at 40˚C for 20 minutes and read the DO values using a spectrophotometer at 720 nm relative to distilled water. The phenolic compound content is expressed in mg. g^{-1} of fresh matter by reference to the calibration curve established with hydrochloric acid (0.1 mg. ml^{-1}).

7.2. Flavonoid Content

The extraction was performed according to the modified protocol of [19]. Approximately 0.5 g of freeze-dried leaves were ground and transferred to test tubes with 4 ml of a 70% acetone and 2% acetic acid (V/V) mixture. After homogenization, the mixture was incubated for one hour in the dark and centrifuged at 4500 rpm for five minutes. The first supernatant, which constitutes the crude flavonoid extract, was collected. Quantification of total flavonoids was determined using the modified colorimetric method [20]. Thus, 2.5 ml of distilled water and 500 µl of 5% sodium nitrite were added to each test tube containing 100 µl of extract. The mixture was incubated in the dark for 6 minutes. Then, 15 µl of 10 % aluminum trichloride and 500 µl of 1 M sodium hydroxide were added. The DO were read using a spectrophotometer at 510 nm against the blank. The flavonoid content was expressed in milligrams per liter of quercetin equivalent extract (mg EQ.ml^{-1}) of fresh material and converted to the levels in mg. g^{-1} of fresh material.

7.3. Soluble Protein Content

Total protein extraction was performed according to the modified protocol of [21]. Approximately 0.5 g of fresh leaves was cold-ground in 2 ml of phosphate buffer. The resulting mixture was vortexed for 10 minutes, incubated, and centrifuged at 3000 rpm for 10 minutes at 7˚C. The collected supernatant constituted the crude protein extract. Successively introduce 2 µl of Molarite pH phosphate buffer, 10 µl of extract, and 2000 µl of Bradford reagent into a test tube, and, after 30 minutes of incubation at room temperature, the ODs are read at 595 nm using a spectrophotometer against distilled water. Protein quantities are expressed in mg. g^{-1} of fresh matter by reference to the calibration curve established with BSA (Bovine Serum Albumin) 0.1 mg.ml^{-1}.

7.4. Statistical Analysis

Data presented as (mean ± standard deviation) with (n = 5) were analyzed descriptively using GraphPad Prism 8 software; comparison of means was performed by analysis of variance (ANOVA) using the Student-Newman-Keuls test at the 5% threshold. The results obtained are represented in the form of graphs and tables using Microsoft Excel 2013 software and the Simultaneous multiple correlation comparisons using the Bonferroni method.

8. Results and Discussion

8.1. Soil Physicochemical Characteristics

The data from the soil substrate analysis of the cultivation site are shown in **Table 1**. The analysis reveals that this soil has a clayey texture and an acidic pH. These characteristics are unsuitable for growing *A. annua* [22]. Indeed, the ideal pH range for optimal plant production of this plant species is between 5.0 and 6.4 [23]. Nevertheless, its average fertility (CEC 11.35 meq/100g of soil) and very low available phosphorus content (6.48 mg.kg^{-1}) and high C/N ratio demonstrate a

slow organic matter mineralization and a low total nitrogen level [24]. The exchangeable cations and average cation exchange capacity in this field show that the clay-humic soil complex is dominated by calcium and potassium ions, with high levels, in contrast to calcium, magnesium, and sodium.

Table 1. Physicochemical characteristics of the soil.

	ELEMENTS	VALEURS
Texture (%)	Clay (%)	45.5
	Loam (%)	25.5
	Sand (%)	34
	Textural Class	**C**
	Apparent Density (g/cm^3)	0.89
Soil Reaction	pH-H_2O	5.2
	pH-KCL	4.7
	Electrical Conductivity (µS/cm)	0.28
Organic Matter	CO (%)	5.48
	MO (%)	5.48
	Total N (g/kg)	0.08
	C/N	68.5
Exchangeable Cations (meq/100 g)	Calcium	1.76
	Magnesium	0.04
	Potassium	1.25
	Sodium	0.06
	Sum of Bases	3.12
Cation Exchange Capacity (meq/100 g)	CEC pH7	11.35
	Saturation (%)	27.47
Phosphorus Assimilable Bray II	Bray II (mg/kg)	6.48
C (clay), defined all the other classes		

8.2. Effect of Biofertilizers on Growth Parameters and Biomass Production of *A. annua* Plants

Growth parameters, fresh biomass, and water content assessed in the different treatments varied significantly ($p \leq 0.05$) over time (Table 2). Analysis showed that plants fertilized with ENTO + PW exhibited a significant increase ($p \leq 0.05$) in the number of secondary branches of the five basal branches, the average number of axillary buds, average plant height, and stem collar diameter at month 3 compared to the positive control (Cont + PW). Similar observations were made with AMF compared to the control, and in the average number of roots from month 1 compared to the negative control. Similarly, the application of AMF resulted in a significant increase ($p < 0.05$) in the root collar diameter, the average number of second-

ary branches of the last five branches of the plants at month 3, and the average number of primary roots at the same period, compared to the negative control (cont.). While the highest plant height compared to the negative control was observed in the plot treated with Entomocompost (ENTO) compared to the negative control.

Similarly, the average fresh weight of the leaves as well as the water contents increased significantly ($p \leq 0.05$) in all treatments compared to the negative and positive controls.

Table 2. Growth parameters and biomass assessed in *A. annua* plants in the applied treatments.

Treatment	Month	Average of plant sizes (Cm)	Average of collar diameters (mm)	NRSB	Average number of roots	Average fresh weight (g)
Cont	**1**	48.5 ± 1.82ad	5.63 ± 01.58e	21 ± 2.03e	20.33 ± 1.58e	296.35 ± 1.42ab
	2	96.83 ± 3.26a	11.93 ± 2.39e	95.05 ± 4.61ac	20.66 ± 1.52cde	253.2 ± 5.55a
	3	120.33 ± 2.37a	12.52 ± 2.41e	122.8 ± 3.77ac	32.33 ± 5.13ad	335.22 ± 8.50a
AMF	**1**	37.83 ± 2.25e	08.54 ± 4.64e	19 ± 2.59e	45 ± 1ab	238.13 ± 5.87a
	2	85.66 ± 3.38a	13.22 ± 3.56e	105.05 ± 5ab	38.33 ± 1.40acd	423.13 ± 1.72a
	3	105 ± 1.33a	17.34 ± 3.36d	130.6 ± 4.96a	46 ± 1.16ab	513.57 ± 1.20a
ENTO	**1**	39.66 ± 1.21e	7.723 ± 1.84e	10.00 ± 1.35e	54.33 ± 2.36a	296.35 ± 12.42a
	2	99.83 ± 2.46a	14.39 ± 2.85e	94.83 ± 5.77ab	39 ± 8.54ab	669.91 ± 1.54a
	3	123.83 ± 2.48a	14.08 ± 3.64e	129.33 ± 4.88a	34 ± 5.56ab	741.58 ± 3.74a
Cont. + PW	**1**	38.83 ± 1.94e	07.40 ± 2.70e	14.5 ± 1.47e	32 ± 1.69abcd	166.77 ± 2.57d
	2	102.66 ± 3.62ac	12.03 ± 3.86e	100 ± 2.30ad	32.33 ± 2.51acd	368.8 ± 1.91a
	3	122.33 ± 2.83ae	12.96 ± 4.13e	121.05 ± 5.21ab	36 ± 1.53ac	931.07 ± 1.93a
AMF + PW	**1**	44.16 ± 1.22cde	08.24 ± 1.08e	16.33 ± 1.46e	39 ± 1.12ab	237.55 ± 1.83a
	2	91.33 ± 1.05a	13.66 ± 2.14e	102.8 ± 3.54ab	32.33 ± 2.03acd	545.94 ± 1.13a
	3	123.83 ± 3.20a	12.98 ± 3.76e	129.33 ± 4.88a	36.33 ± 1.69bce	616.67 ± 2.18a
ENTO + PW	**1**	42.66 ± 2.42de	08.45 ± 2.85e	10.83 ± 1.52e	36 ± 4.58ac	189.01 ± 1.58a
	2	107.16 ± 2.36ac	13.74 ± 5.12e	110.6 ± 1.60ad	25 ± 7.93e	236.11 ± 2.05a
	3	132.5 ± 2.24a	16.28 ± 6.94e	138.05 ± 2.08a	34.66 ± 0.59b	475.46 ± 1.88a

Control (Cont.); Arbuscular mycorrhizal Fungal (AMF); Entomocompost (ENTO); Control + Purified water (Cont. + PW); Arbuscular Mycorrhizal Fungi + Purified water (AMF + PW) and Entomocompost + Purified water (ENTO + PW). Values are the averages of five repetitions.

with ENTO + PW, the fresh biomass increased significantly ($p \leq 0.05$) at month 2 compared to the negative control and the positive control. In the sub-block of plants watered only with purified water (positive control), there was no significant difference in fresh weight between the plants of the treated batches and those of the negative control. However, the contribution of AMF and ENTO in the culture medium led to a significant increase ($p \leq 0.05$) of this parameter after month 1 compared to the control. The increase in the size of *A. annua* plants over time would be the result of the physiological response due to the supply of necessary nutrients through AMF

on the one hand and watering with purified water (PW) on the other. This corroborates the results of [25] who showed that other biofertilizers, namely chicken droppings and cow purse, lead to an increase in the growth parameters of *A. annua*. Thus, fertilization of plants with AMF would contribute to the increase in the volume of hydromineral absorption following the development of a network of fungal hyphae that colonize the soil and offer a larger absorption surface to the plant [26]. These beneficial soil microorganisms also provide favorable conditions, namely a favorable pH, for plant development [27]-[29]. The results presented by plants treated with ENTO + PW followed by those treated with ENTO on these different parameters could be explained by the richness of this fertilizer in mineral elements, namely N, P, and K, essential for plant growth [30] [31], which contributes to water balance and chlorophyll synthesis, which is explained by an increase in the total chlorophyll content in the leaves of *A. annua* as well as fresh biomass.

The slowdown in growth observed at month 1 in *A. annua* plants is thought to be due to the time taken to recognize the symbiotic partners (plants-AMF). The plant wastes energy feeding these non-productive microorganisms. From month 2 onwards, the microorganisms then become beneficial to the plant because the AMF would have already sufficiently branched the plant roots for better hydromineral nutrition, complementing the texture, nature, and pH of the soil, which play an important role in plant growth [27] [29]. However, the decrease in the number of plant roots over time in the different treatments could be explained by root renewal, as was the case with ferns [32].

9. Effect of Biofertilizers on the Physiological Parameters of *A. annua* Plants

9.1. Total Chlorophyll Content in *A. annua* Plant Leaves

The results of plant biofertilization on total chlorophyll content assessed in *A. annua* leaves after harvest are presented in **Figure 1**. Analysis of this figure shows that: in plants watered with purified water, total chlorophyll content increased significantly ($p \leq 0.05$) at month 1 in plants fertilized with AMF compared to the positive control and at month 2 in plants fertilized with AMF and ENTO compared to the two controls. Similarly, total chlorophyll content in *A. annua* leaves decreased over time.

The peak in chlorophyll levels in month 3 could be explained by the fact that young *Artemisia* plants are full of numerous non-senescent adult leaves that perform optimal photosynthesis during growth. However, over time, flowering and fruiting lead to a decline in photosynthetic activity due to leaf aging and the inhibition of new leaf growth [33]. The higher chlorophyll content during the first month and its decline from month 2, unlike the variation in phenolic compounds, could be explained by the fact that during the first month, the sugars produced primarily supported the fungi (AMF) in establishing the symbiosis [34]. Then, during the second and third months, the protein biosynthesis pathway was favored to the detriment of the phenylalanine ammonia lyase (PAL) pathway.

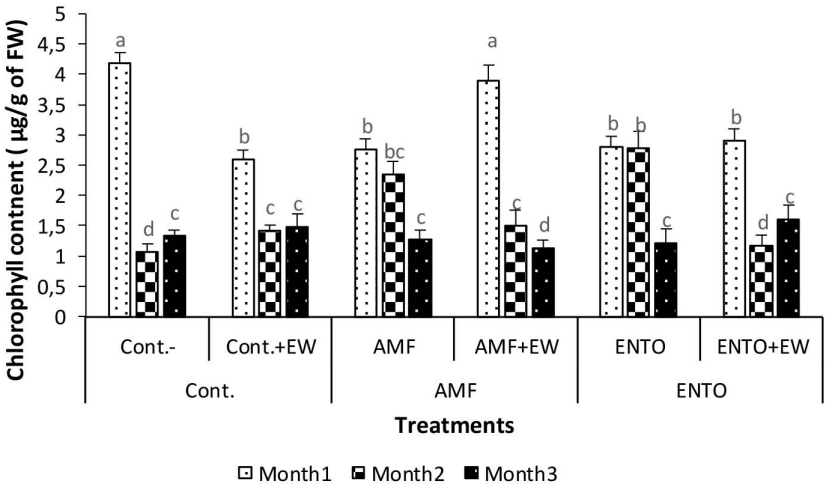

Figure 1. Leaf chlorophyll content of *A. annua* (µg. g-1 of FW) after applying the purified water (PW) irrigation. Control (Cont.); Arbuscular mycorrhizal Fungal (AMF); Entomocompost (ENTO); Control + Purified water (Cont + PW); Arbuscular Mycorrhizal Fungi + Purified water (AMF + PW) and Entomocompost + Purified water (ENTO + PW). Values are the averages of five repetitions.

9.2. Relative Water Content of *A. annua* Plants

The different treatments applied showed significant effects ($p < 0.05$) on the water content of *A. annua* plants in the field compared to the controls (**Figure 2**). Indeed, the addition of purified water resulted in a significant decrease ($p < 0.05$) in relative water content in both AMF fertilized and ENTO-fertilized plots. This could be explained by a high dry biomass following an accumulation of reserves in the leaves and an enrichment of the cytoskeleton [35] because, the location of the experimental site in an urban area result in a high CO_2 content available inside the cells which consequently leads to the accumulation of synthesized organic compounds.

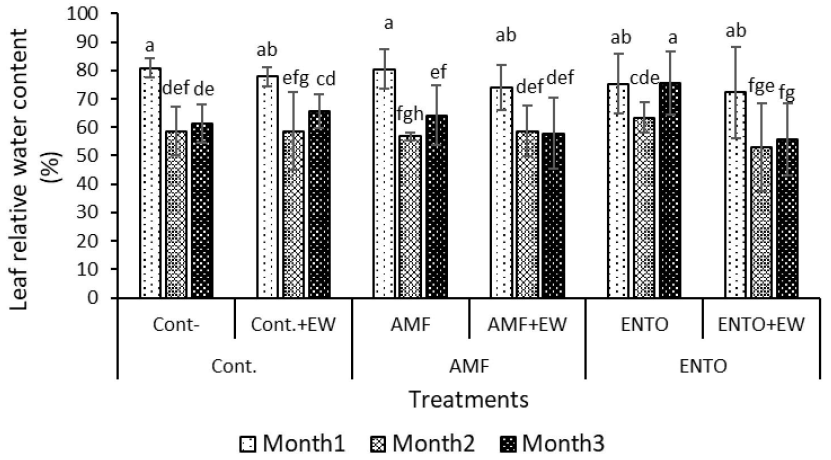

Figure 2. Leaf relative water content (%) of *A. annua* applying the purified water (PW) irrigation. Control (Cont.); Arbuscular mycorrhizal Fungal (AMF); Entomocompost (ENTO); Control + Purified water (Cont + PW); Arbuscular Mycorrhizal Fungi + Purified water (AMF + PW) and Entomocompost + Purified water (ENTO + PW). Values are the averages of five repetitions.

9.3. Mineral Content of *A. annua* Leaves

The mineral composition of *A. annua* leaves after three months of field cultivation is shown in Table 3. Analysis of this table shows that ENTO fertilization promotes calcium absorption, while CMA fertilization also promotes iron and zinc absorption. Furthermore, the addition of purified water to ENTO-enriched media promotes the absorption of Ca, Mg, Fe, P, and K, and limits that of Na. Fertilization with CMA and ENTO promotes the absorption of mineral elements in *A. annua*.

Mineral content is higher in plants irrigated with purified water. AMF promotes the absorption of mineral elements by these plants. These results corroborate those of [26], who showed that mycorrhizae allow plants to benefit from better phosphorus absorption thanks to the network of fungal hyphae that colonize the soil and provide a larger absorption surface with the substrate.

The high calcium content in plants fertilized with ENTO + PW could be explained primarily by the composition of the purified water, which is rich in mineral elements, and by the fact that ENTO provides nutrients that are easily assimilated by these plants to the soil [36]. Thus, these variations in peak levels of essential mineral elements are explained by the nature of the fertilizer applied and the soil.

Table 3. Mineral content of *A. annua* leaves according to the treatments applied in the presence of purified water.

Treatment		PO_4^{3-} (mg/100 g)	Na^+ (%)	K^+ (%)	Zn^{2+} (mg/100g)	Ca^{2+} (%)	Mg^{2+} (%)	Fe^{2+} (mg/100g)
Artemisia annua + Epure water	**Cont**	400.5	0.036	1.144	4.309	0.187	0.301	72.100
	AMF	399.5	0.035	1.096	5.745	0.1855	0.268	125.300
	ENTO	383.5	0.047	1.125	4.191	0.2005	0.266	46.890
	Cont + PW	271	0.124	1.105	5.372	0.164	0.216	40.575
	AMF + PW	416.5	0.066	1.100	4.193	0.151	0.284	75.500
	ENTO + PW	339	0.037	1.125	4.721	0.2055	0.293	84.450

Cont (Control), Cont (-) (Negative control), AMF (Arbuscular Mycorrhize Fungal), ENTO (Entomocompost), EW (Epure Water). PO_4^{3-} (Phosphore). Na^+ (Sodium). K^+ (Potassium). Zn^{2+} (Zinc). Ca^{2+} (Calcium). Mg^{2+} (Magnesium) & Fe^{2+} (Fer).

10. Effect of Biofertilizers on the Biochemical Parameters of *A. annua* Plants

10.1. Phenolic Compound Contents in *A. annua* Leaves

The results of total phenolic compound contents evaluated in adult *A. annua* leaves are presented in Figure 3. Analysis of this figure shows that in plants watered with purified water (PW), the phenolic compound content increased significantly ($p \leq 0.05$) at months 1 and 2 in plants enriched with AMF compared to the controls, as well as in plants fertilized with ENTO at month 1 under the same conditions compared to the positive control and at month 3 of the same treatment compared to both controls. The high phenolic compound contents would protect

plants against abiotic stresses generated by soil acidity, which has an acidic pH [33], although corresponding to the crop requirements; These results corroborate those of [34] who showed that by fertilizing *X. sagittifolium* with poultry manure and NPK fertilizer, the polyphenol content increased in the leaves which were resistant against biotic and abiotic attacks.

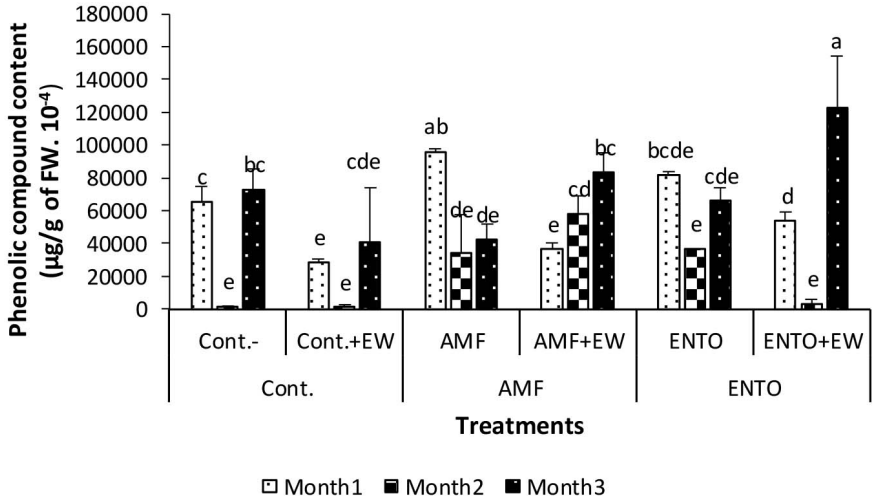

Figure 3. Total phenolic compound content of the *A. annua* plant (mg/g of FW) applying the purified water (PW) irrigation. Control (Cont.); Arbuscular mycorrhizal Fungal (AMF); Entomocompost (ENTO); Control + Purified water (Cont + PW); Arbuscular Mycorrhizal Fungi + Purified water (AMF + PW) and Entomocompost + Purified water (ENTO + PW). Values are the averages of five repetitions.

10.2. Flavonoid Contents in *A. annua* Leaves

The results of flavonoid content assessed in *A. annua* leaves are presented in **Figure 4** and its analysis shows that: In the sub-block of plants watered with purified water (PW), flavonoid content increased significantly ($p < 0.05$) at month 2 in media fertilized with AMF compared to the two controls. This was also the case for months 2 and 3 respectively, with the maximum obtained at month 3 (19.49 ± 5.68 of FWM). In media fertilized with ENTO, a significant increase ($p < 0.05$) in leaf flavonoid content was observed at month 3 compared to the positive control. Similarly, the addition of purified water (PW) leads to a significant increase ($p < 0.05$) in the flavonoid content at months 2 and 3 in the media fertilized with ENTO compared to the two controls with the maximum content 19.58 ± 2.80 µg. g^{-1} of FWM at month 3. Overall, the results show that the total flavonoid contents of the leaves in *A. annua* are very low during the first two months of growth for all treatments applied. Flavonoids are constituents of phenolic compounds that are involved in the regulation of plant cell growth [37]. The results show that these contents are lower than those of polyphenols and proteins. However, they increase over time. This could be explained by the slow and progressive mineralization in the media fertilized with ENTO and the complete establishment of the root-AMF exchange network.

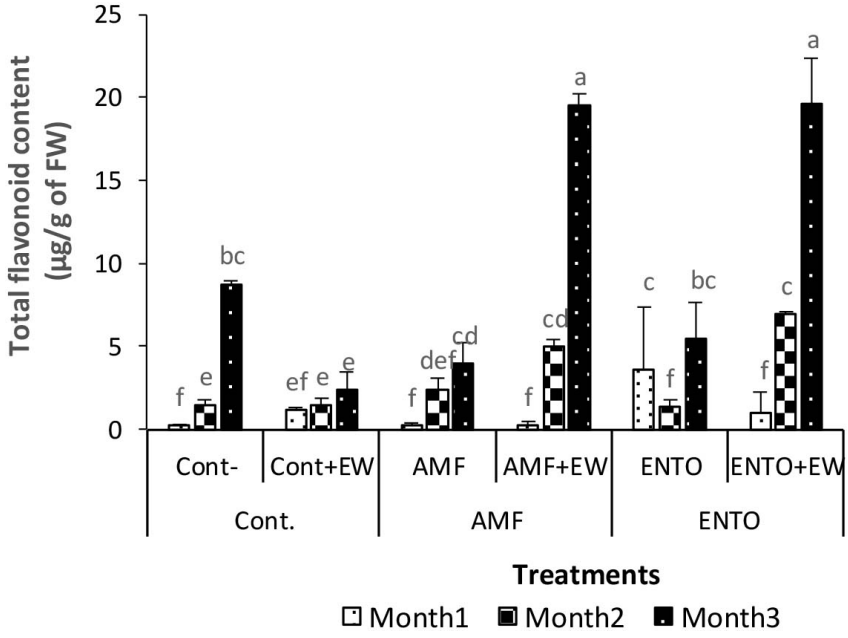

Figure 4. Total flavonoid content of the *A. annua* plant (µg/g of FW) applying the purified water (PW) irrigation. Control (Cont.); Arbuscular mycorrhizal Fungal (AMF); Entomocompost (ENTO); Control + Purified water (Cont + PW); Arbuscular Mycorrhizal Fungi + Purified water (AMF + PW) and Entomocompost + Purified water (ENTO + PW). Values are the averages of five repetitions.

10.3. Protein Content in *A. annua* Leaves

The results of the soluble protein content of *A. annua* L. leaves evaluated are presented in **Figure 5**. Analysis shows that the addition of purified water (PW) leads to a significant increase ($p < 0.05$) in this content at month 3 in media enriched with CMA compared to the two controls. In media fertilized with ENTO, a significant increase ($p < 0.05$) in protein content is observed at month 3 compared to the controls. In contrast, the addition of purified water (ENTO + PW) leads to a significant decrease ($p < 0.05$) in soluble protein content at month 3. Generally, this content increases over time in the leaves of plants watered with purified water. Metabolically, the higher soluble protein contents at month 3 obtained in ENTO+ CMA-enriched media would be due to the increase in the plant's nutrient absorption surface (CMA). Similar results are obtained by the work of [38] who showed protein accumulation in the leaves of corn fertilized with nitrogen fertilizer.

The lower levels of proteins, flavonoids and phenolic compounds during the first month and higher levels from month 2 onwards could be explained by the fact that during the first month the sugars produced by photosynthetic activity rather maintained the fertilizers (AMF) which would be entirely dependent on them in order to allow the establishment of the symbiosis [34] then during the second and third months it was the protein biosynthesis pathway which was favored to the detriment of the phenylalanine ammonia lyase (PAL) pathway, demonstrating the effect of correlations between parameters assessed on plants.

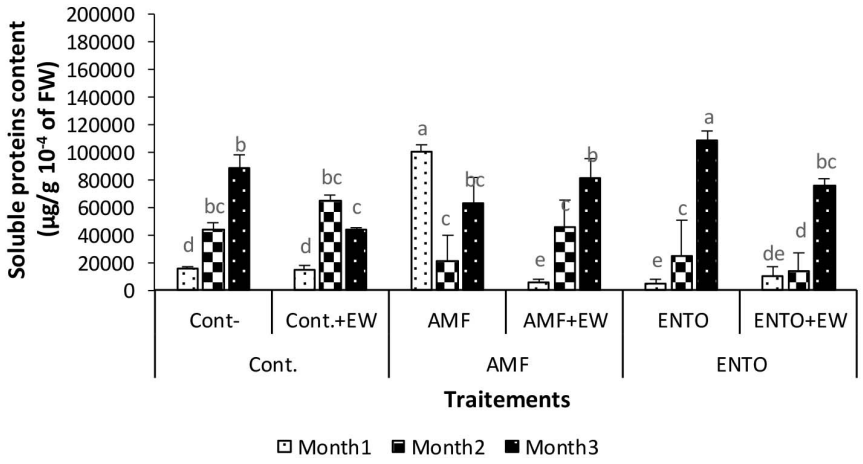

Figure 5. Soluble proteins content of the *A. annua* plant (µg/g of FW) applying the purified water (PW) irrigation. Control (Cont.); Arbuscular mycorrhizal Fungal (AMF); Entomocompost (ENTO); Control + Purified water (Cont + PW); Arbuscular Mycorrhizal Fungi + Purified water (AMF + PW) and Entomocompost + Purified water (ENTO + PW). Values are the averages of five repetitions.

The expression of the correlation of parameters assessed on *A. annua* L. plants after purified water (PW) irrigation in each treatment is presented in Figure 6. Its analysis reveals a significantly ($p < 0.05$) negative correlation ($r = 0.57$) between proteins and chlorophylls on the one hand and between proteins and flavonoids ($r = 0.45$) on the other hand (Figure 6).

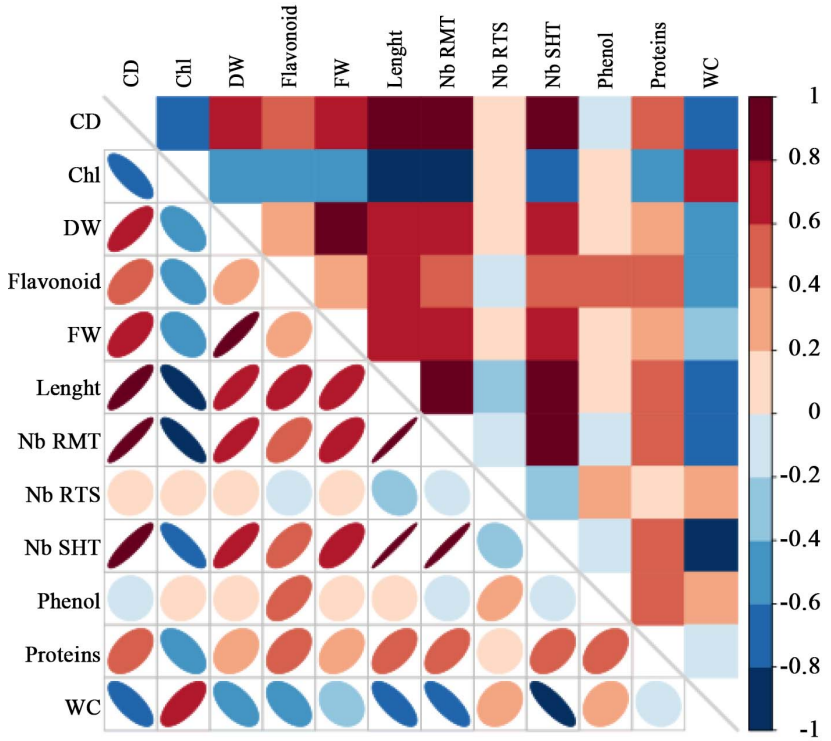

Figure 6. Correlation diagram of the parameters assessed on *A. annua* plants after purified water (PW) irrigation in each treatment.

Otherwise, in the PAL pathway, a significantly ($p < 0.05$) positive correlation ($r = 0.54$) is observed between flavonoids and phenols. Similarly, a strongly positive correlation is observed between the fresh and dry masses of the samples, confirming active chlorophyll synthesis and accumulation of reserves in the leaves with an enrichment of the cytoskeleton. Although this study shows the good performance of *Artemisia annua* L. under organic fertilization in the field, it was conducted over only one cycle and its level of soil depletion remains to be evaluated.

11. Conclusion

At the end of this study, which aimed to evaluate the influence of biofertilization on the production of *Artemisia annua* of good organoleptic quality, it emerged that the site's soil is clayey with an acidic pH. Fertilization with AMF and ENTO followed by irrigation with purified water promotes an increase in plant size, stem collar diameter, average root number, and basal branching. The same is true for an increase in the average number of axillary buds. Both fertilization and irrigation types promote biomass production. Biochemically, fertilization with AMF and ENTO followed by irrigation with purified water promotes total chlorophyll synthesis. Fertilization with ENTO followed by irrigation with purified water favored the synthesis of phenolic compounds. On the other hand, both types of fertilization favored the accumulation of soluble proteins and flavonoids and the addition of purified water in the media enriched with ENTO much more. Similarly, they favor the absorption of mineral elements in *A. annua* However, the addition of purified water under these conditions inhibits the absorption of Na. Thus, plants fertilized with Entomocompost (ENTO) and those watered with purified water (ENTO + PW) present better results and interesting organoleptic qualities.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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