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EFFECT OF MANY DOSES OF ROSEMARY MANURE ON EARLY SEEDLINGS GROWTH OF HOLM OAK IN NURSERY STAGE

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Abstract

Description of the subject: Since the morphological attributes of the seedling in the nursery stage determine widely the success of the plant after afforestation, the producers of forest seedlings must find available, cheap and nature-friends products to replace chemical fertilizers but with maintaining their ability to improve the morphological traits of seedlings.
Objectives : The main objective of this study was to investigate the effect of 04 doses of rosemary (*Rosmarinus officinalis* L.) manure used as bio fertilizer on the early growth of holm oak (*Quercus ilex* L.)
Methods: 5 treatments in total, 4 are resulting from the dilution of the stock solution of rosemary manure (5%, 10%, 15% and 20%) compared to a control treatment (tap water), are used in root application weekly in the aim to assess the growth parameters after harvesting the seedlings in the end of the 9th week.
Results: The tested bio fertilizer had a large positive effect on all measured morphological parameters of *Q. ilex*, the dose of 5% gave the greatest values for plant height, root collar diameter, number of leaves and of internodes, and also of fresh and dry biomass.
Conclusion: Thus, rosemary manure with a dose of 5% is recommended as suitable bio fertilizer to nurture seedlings of *Q. ilex* and increase their morphological qualities in nursery stage.
Keywords: *Rosmarinus officinalis*, *Quercus ilex*, seedlings, bio fertilizer, morphological qualities, nursery stage, sustainable agriculture.

EFFET DE NOMBREUSES DOSES DE PURIN DE ROMARIN SUR LE DEBUT DE CROISSANCE DES PLANTS DE CHENE VERT AU STADE DE PEPINE

Résumé

Description du sujet: Vu que les qualités morphologiques du plant en pépinière déterminent largement le succès de la plante après boisement, les producteurs de plants forestiers doivent trouver des produits disponibles, pas chers et amis de la nature pour remplacer les engrais chimiques mais en conservant leur capacité à améliorer les qualités morphologiques des plants.
Objectifs: L'objectif principal de cette étude était d'étudier l'effet de 04 doses de purin de romarin (*Rosmarinus officinalis* L.) utilisé comme biofertilisant sur lu chêne vert (*Quercus ilex* L.) en début de croissance.
Méthodes: 5 traitements au total, dont 4 sont issus de la dilution de la solution mère de purin de romarin (5%, 10%, 15% et 20%) comparés au traitement témoin (eau du robinet), sont utilisés hebdomadairement dans le but d'évaluer les paramètres de croissance après la récolte des plants à la fin de la 9ème semaine.
Résultats: Le biofertilisant testé a eu un effet positif important sur tous les paramètres morphologiques mesurés de Q.ilex, la dose de 5 % a donné les plus grandes valeurs pour la hauteur de plants, le diamètre au collet, le nombre de feuilles et d'entre-nœuds, ainsi que la biomasse fraîche et sèche.
Conclusion: Ainsi, le purin de romarin avec une dose de 5% est recommandé comme biofertilisant approprié pour nourrir les plants de Q.ilex et augmenter leurs qualités morphologiques en phase pépinière.
Mots clés: *Rosmarinus officinalis*, *Quercus ilex*, plants, biofertilisant, qualités morphologiques, stade pépinière, agriculture durable.

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INTRODUCTION

The nursery stage is a critical phase in the production of forest seedlings. Furthermore, the race is accelerating towards improving the morphological properties of seedlings for economic ends that interest producers. In arid and semi-arid regions, a successful afforestation is based on using vigorous seedlings [1]. Controlling seedling morphological quality is a factor based on nutrient management in the medium in which the seedling lives [2]. The humanity and especially the agricultural network is facing a double impact challenge, firstly is to face the global warming with compensating the lost forest areas by afforestation, and secondly to reduce using the synthetic fertilizers. Consisted of various known nutrients (N, P, K, Mg, Ca…), these chemical fertilizers became harmful not only for human but unfortunately causing many ecological and environmental disorders, and this may be due to their exaggerated use in many agricultural practices [3, 4], moreover, they are not cheap nor eco-friendly [5]. As there is a direct dependence of growth and development of roots on soil and soil conditions [6], bio-fertilizers, which are substances added to plants to increase their nutrient efficiency [7], increase soil fertility and therefore help plants to absorb nutrients and increase productivity and yield through its direct action on root systems in the medium [3]. Many researchers worldwide have proven the significance of bio-fertilizers in terms of quality and quantity of agricultural products. Among these bio-fertilizers, plant-based fertilizers and their uses which are widespread nowadays [8, 9, 10], even their excessive use doesn't cause soil [11], water or environment pollution [12]. Thus, the use of these bio-products seems to be an urgent need considering their many advantages, less toxicity to living beings and whole ecosystem, yields and crops high productivity, chemical fertilizers being replaced or reduced [13]. Rosemary (*Rosmarinus officinalis* L.) is an abundant spontaneous perennial herb belonging to Lamiaceae, with upright stems and dark green leaves. It is known since long time for its medicinal, culinary and cosmetic use, this is due to its flavor and antioxidant properties [14], and yet its pharmaceutical and therapeutic potential persists [15]. In Algeria, despite its abundance, and its presence in all regions of the country, especially the north, but it remains undesirable and not valued except for some decorative purposes on the sidewalks and on the edges of green spaces. Holm oak, (*Quercus ilex* L.), which is one of the very important trees in the Mediterranean basin, this sclerophilous tree which constitutes with the Aleppo pine together the main constituent of the afforestation operations has witnessed a significant decline in its areas in recent decades [16]. The alarming situation facing the world caused by climate change and consequently the imbalance in ecosystem requires us to consider the evergreen oak species with great importance [17]. Wild spontaneous plants manure is largely used and studied, especially stinging nettle (*Urtica dioica* L.) but we didn't find researches about rosemary aqueous manure, in this experience we aimed to highlight if there is an effect using rosemary aqueous manure as a bio fertilizer on *Q. ilex* seedlings quality as a bio fertilizer used in root application hypothesizing that, with its richness of multitude of benefic bio components, it enhances the early seedling growth of holm oak.

MATERIALS AND METHODS

This work was conducted in the greenhouse of the faculty of Nature and Life Sciences in Blida 1 University, during a period of 64 days (9 weeks) between January and March 2020. In complete random block, the experiment was assessed under greenhouse in semi controlled conditions, the temperature ranged between 18 – 32°C.

1. Materials

1.1. Study species

We have chosen the holm oak (*Q. ilex*) in our experiment for its large use in the Algerian programs of afforestation for many years because of its plasticity, and mainly for the aim of seeking a low-cost ways for nurseries to produce vigorous seedlings. The rosemary used in the experiment was gathered from the yard of the faculty.

1.2. The aqueous manure

The aqueous manure is obtained by maceration, a dose of 100g of fresh material (green parts: leaves and stem of the rosemary cut into small pieces) per 1L of spring water, and with occasional stirring, the aqueous rosemary manure had been ready after 20 days, the fermentation process had finished when bubbles disappear from the surface of the manure. Then

it is filtered, stored in labeled opaque containers and diluted with distilled water: 5%, 10%, 15% and 20% as needed.

2. Methods

2.1. Seedlings production and treatments application

Acorns of *Q. ilex* collected from mature healthy holm oak trees of the dominical forest in Lazharia, the town of Tissemsilt, are subjected to the flotation method, we used this technique to eliminate bad ones, and then they were put to germinate half buried in dishes filled of sieved sand, autoclaved at 121°C for 20 minutes [18], then sown directly after germination in pots of 1L full of 1/3 sand and 2/3 soil. The soil used in our experiment was brought from the experimental station of the faculty, this soil has a clay-silty texture [19]. The experimental design had 6 replicates for each of the 5 treatments which were as follows: 05%, 10%, 15%, 20% of rosemary manure and the fifth is the control treatment (irrigation only with tap water). We watered the pre-germinated acorns directly after sowing, the application of the bio fertilizer was applied by an irrigating method (root application), and that was regularly continued once a week, 50 ml for each seedling for 9 weeks. In the meantime, we were watching over the need of seedlings to water especially in high temperature times.

2.2. Growth measurements

Growth measurements of seedlings aboveground and belowground parts were performed in the end of the 9th week starting from the beginning of the bio fertilizer treatments. Right before the seedlings being harvested, the shoot's height and the root collar diameter were measured, the number of leaves [20] and internodes [21, 22] were then counted. And quickly after harvesting seedlings, the aboveground (stem, branches and leaves) and the belowground (roots) were weighed separately, before weighing roots, they were washed with tap water softly many times to clean them from soil and any other particles. Both aboveground and belowground parts were put to dry in 65°C for one week, and then we measured the new dry weight for each part. Moreover, Dickson quality index of the seedlings was calculated according to the formula: *DQI = TDW/(H / RCD) + (SDW / RDW)* [23]. With: *DQI:* Dickson quality index; *TDW*: Total dry weight (g) = Shoot dry weight (g) + Root dry weight (g); *H:* Shoot's height (cm); *RCD*: Root collar diameter (mm); *SDW*: Shoot dry weight (g); *RDW*: Root dry weight (g).

2.3. Data analysis

The possible difference between treatments was verified by analysis of variance (ANOVA). Analyses were conducted using STATGRAPHICS Centurion 18. The data obtained were subjected to analysis of variance with a single classification criterion. Normality and homogeneity of variance were checked before any analyses. The comparison of the means was made with Student-Newman-Keuls test method of the smallest significant difference at risk of error of 5%.

RESULTS

1. Effect of rosemary manure application on height of Q. ilex seedlings

The effect of rosemary manure on the shoot's height of the seedlings is shown in figure 1. The highest effect was recorded by the dose of 5% of rosemary manure (T_1) with a height of 11.53cm. The lowest value of the plant's height (8.8cm) was recorded on seedlings treated with 20% of rosemary manure (T_4). The 2 other treatments of the rosemary manure (T_2 and T_3) were varied and irregular (2.7cm and 2.65cm respectively) and both of them were less than the height's value of the seedlings subjected to control treatment T_0 (10.83cm).

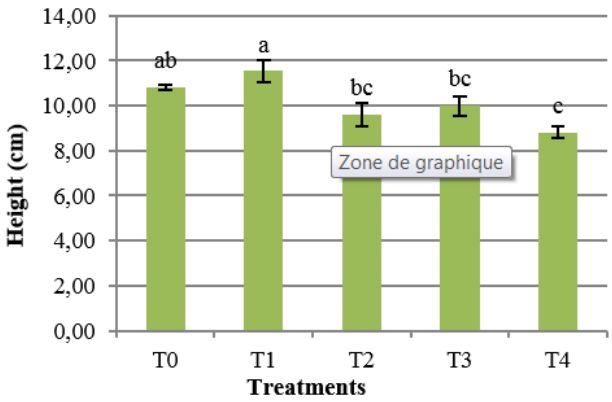

Figure 1: Effect of rosemary manure on the shoot's height of *Q. ilex*. The bars are means ± sd. Means marked with different letters indicate significant difference ($p < 0.05$)

2. Effect of rosemary manure application on the root collar diameter of Q. ilex seedlings

The rosemary manure affected positively the root collar diameter of the *Q. ilex* seedlings, as shown in figure 2,

the highest value (3.17mm) was recorded on seedlings treated with T_1, the effect of the 3 other treatments (T_2, T_3 and T_4) was negative, their values were less than T_0 value (2.83mm), while the lowest value of the root collar diameter (2.63mm) was recorded on seedlings treated with T_4.

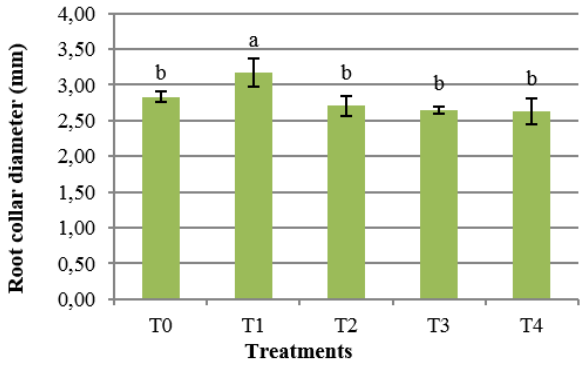

Figure 2: Effect of rosemary manure on the root collar diameter of *Q. ilex*, The bars are means ± sd. Means marked with different letters indicate significant difference ($p < 0.05$)

3. Effect of rosemary manure application on the leaves number of Q. ilex seedlings

The results presented on figure 3 show that *Q. ilex* seedlings recorded the maximum leaves number when treated with T_1 (14.00) compared to control treatment T_0 (11.00), All other treatments (T_2, T_3 and T_4) had led to leaves number less than those of control treatment but T_3 treatment recorded the lowest number of leaves (10.00).

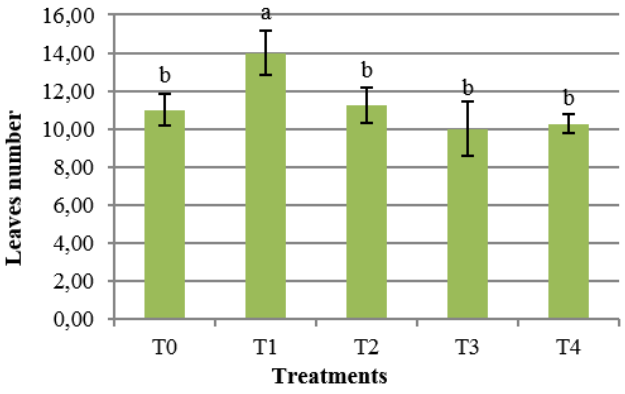

Figure 3: Effect of rosemary manure on the number of leaves of *Q. ilex*. The bars are means ± sd. Means marked with different letters indicate significant difference ($p < 0.05$)

4. Effect of rosemary manure on the number of internodes of Q. ilex seedlings:

Results of figure 4 show clearly that treatment T_1 of rosemary manure had the greatest positive effect on internodes number of *Q. ilex* seedlings (10.5), followed respectively by T_4 (9.25), T_3 (9.00) and T_2 (8.25). While all used rosemary doses had greater effect on seedlings internodes number than those of control treatment (8.00).

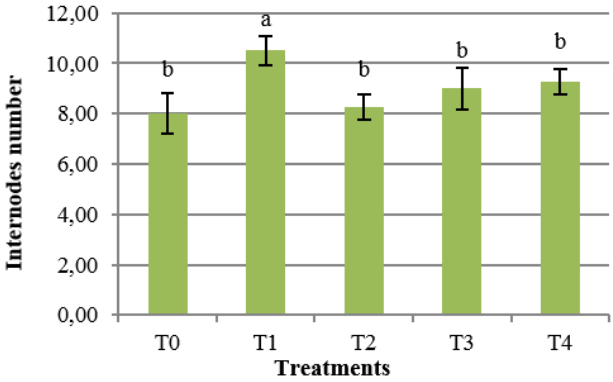

Figure 4: Effect of rosemary manure on the number of internodes of *Q. ilex*. The bars are means ± sd. Means marked with different letters indicate significant difference ($p < 0.05$)

5. Effect of rosemary manure on the biomass accumulation of Q. ilex seedlings

The results on the figure 5 show that *Q. ilex* seedlings shoot's biomass was affected by the rosemary manure, the highest value of shoot's fresh biomass being observed in T_1 treatment (1.94g) and that was the only positive effect recorded. The other treatments (T_2, T_3 and T_4) gave values less than control's treatment (1.75, 1.48 and 1.43g) respectively (5A). In contrary, the application of rosemary manure with all treatment (T_1, T_2, T_3 and T_4) gave better values of root fresh weigh than control treatment, the values have been graded from T_1 (3.64g) which was the highest measured value, followed by T_2 (3.07g), then T4 (2.82g) and the lowest was T_3 with (2.81g). (5B). On the other side, the application of rosemary manure decreased the biomass of the shoot dry weight except the T_1 which had a positive effect and increased it (0.87g) compared to control treatment (0.73g) (5C). In contrast of that, the dry biomass of the roots had increased with all the treatments except control (0.76g), T_1 on the top (1.10g), followed by T_2, T_3 and T_4, (0.97, 0.88 and 0.84g) successively. (5D)

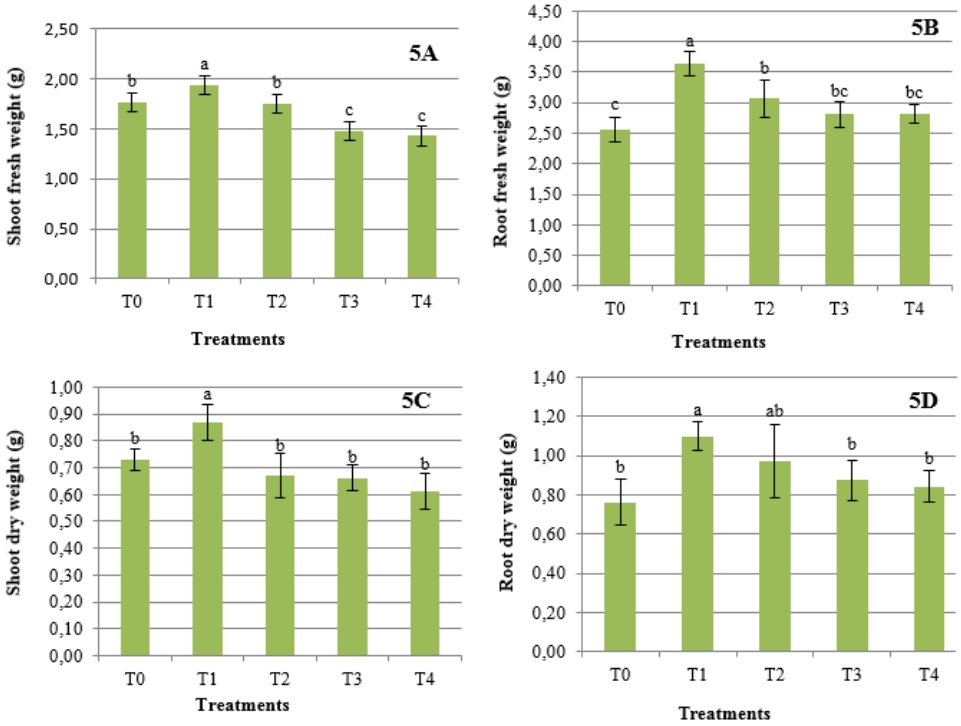

Figure 5: Effect of rosemary manure on the fresh and dry biomass accumulation of *Q. ilex* seedlings. The bars are means ± sd. Means marked with different letters indicate significant difference (p<0.05).

6. Dickson quality index of Q. ilex seedlings treated with rosemary manure:

DQI is a quality indicator used to qualify seedlings, it is a measure that integrates several measured important parameters, it predicts the quality of the seedling according to the medium in which it lived [23], the higher the value of DQI, the better the seedling regarding its vigor and robustness and also distribution of the biomass. In our experiment (Table 1), the seedlings subjected to T_1 recorded higher value of DQI (0.45), the seedlings subjected to T_2, T_3 and T_4 recorded less than those of T_1 (0.39, 0.34 and 0.36 respectively), however their values were higher than those of control treatment (0.31).

Table 1: Dickson quality index of *Q. ilex* seedlings according to applied treatments

Treatments	T_0	T_1	T_2	T_3	T_4
TDW (g)	1.49	1.97	1.64	1.54	1.45
H (cm)	10.8	11.53	9.60	10.00	8.80
RCD (mm)	2.83	3.17	2.7	2.65	2.63
SDW (g)	0.73	0.87	0.67	0.66	0.61
RDW (g)	0.76	1.10	0.97	0.88	0.84
DQI	**0.31**	**0.45**	**0.39**	**0.34**	**0.36**

DISCUSSION

The rosemary manure used in this experiment has promoted the growth of the seedlings of *Q. ilex*, and this was clearly shown on the seedlings treated with the dose of 5%, they had higher values of shoot height, root collar diameter, leaves and internodes number, and also the fresh and dry biomass. This is probably due to the richness of rosemary manure with fertilizing elements and therefore, to its virtue of improving the mineral nutrition of seedlings and created better nutrient absorption which favored faster vegetative growth. Thus, a better morphology of *Q. ilex* seedlings is caused by the presence of many nutrients in the manure which go into the construction of proteins and plant tissues. This richness and containment of multitude of bioactive compounds in rosemary made of it a source of fertility of the medium. According to findings of Mena et *al.* [14], rosemary extract contains 57 polyphenols, 24 flavonoids, 24 diterpenoids, 5 phenolic acids, 3 lignans and 1 triterpenoid.

The potassium present in rosemary, in addition of his role in cellular integrity and enzymatic activities [24], it affects the production of proteins and ATP, which was, in our case, necessary for *Q. ilex* seedlings to grow more. Nikitina et al. reported in their study that rosemary shoots contain many macro and micro elements, especially raw material of this species which is rich of 25 elements, 11 essentials and 4 conditionally essential, among them: K, Ca, Si , Mg, Na, P, Al, Fe, Mn, Sr, Zn, B, Ti, Ba and Cu [25], no doubt this huge amount of minerals made of our manure a store of nutrients for the benefit of seedlings. We believe that the richness of rosemary manure with calcium and strontium promoted *Q. ilex* seedlings growth, these suggestions are consistent with Nianwei et *al*. [26] who mentioned in their recent study that the growth of Chinese cabbage seedlings was enhanced and that was due to the presence of strontium in the medium conditionally with calcium. Similarly in this context, Benrebha et *al*. [27] reported that nettle manure enhanced morphological and nutritional traits of lettuce when used in combined root-foliar application. In contrary, Garmenida et *al*. [28] stated that nettle manure used in foliar application did not give significant results on potato yield, whereas Argyropoulou et *al*. [29] found that the use of rosemary as amendment of soil had increased tomato growth. he tested bio fertilizer containing boron, which has also a role in morphological configuration of plants [30], gave it a strong boost to enhance growth. The magnesium present in the manure, which is important in chlorophyll synthesis, production, transportation, and utilization of photoassimilates, enzymes activation, and protein synthesis [31], had enhanced *Q. ilex* seedlings physiological processes. We suggest also that the availability and assimilability of chlorophyll and nitrogen from rosemary leaves released in the manure were easily used by the seedlings, accordingly, their vegetative growth increased. On the other hand, the measured pH of the rosemary manure dose of 5% (T_1) was 6.70, it is slightly acidic to neutral, this value is quite favorable for roots of Q.ilex for nutrients uptake and for the seedlings growth [32], thus, the irrigating by the bio fertilizer (rosemary manure) when seedlings need water helped roots to use the nutritive elements present therein.

CONCLUSION

The obtained results in our experiment have confirmed our hypothesis that rosemary manure used in root application could enhance *Q. ilex* seedlings growth. The use of the rosemary manure as a bio fertilizer gave significantly positive results by improving the growth of holm oak in the nursery stage considerably. All measured morphological traits were enhanced, we recorded an increase of 6.57% in height, 12.01% in root collar diameter, 27.27% in leaves number, 9.6% in shoot fresh weight, 19.17% in shoot dry weight, 42.74% in root fresh weight and 44.73% in root dry weight comparing to control treatment. Contributing in the sustainability of agricultural systems and environmentally non-damaging, we can advise forest seedlings producers to use this bio fertilizer with the dose of 05% in root application.

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