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Technical note

INFLUENCE OF *Ascophyllum nodosum* EXTRACT ON ROOTING OF *Mentha spicata* L.

Pablo Fernando Luna-Rodríguez[1/], Hans Marcelino Munguia-Falcon[2], Isabel Elena Mogollón-Ortega[3], Excela Elizabeth Saavedra-Flores[4], Claudia Paz Romero-Ayala[5], Maria Fernanda Siancas-Carrasco[6], Martha Andrea Márquez-Bautista[7], Cecilia Emperatriz Figueroa-Serrudo[8], Isabel Maximiliana Montes-Yarasca[9], Héctor Baroni Cántaro-Segura[10]*

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ABSTRACT

Introduction. The production of medicinal plants is a market that has been growing rapidly in recent years and requires sustainable agronomic techniques to meet this demand. The herb spearmint (*Mentha spicata* L.) is one of these plants in demand worldwide. However, there are not many techniques for its commercial cultivation, whether in terms of harvest, post-harvest, or propagation. As for propagation, this is usually done vegetatively, occasionally with the use rooting agents. Among the wide variety of rooting agents available in the market, the use of algae extracts such as *Ascophyllum nodosum* is

* Corresponding author.
Email: 20210579@lamolina.edu.pe
1 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Círculo de Investigación en Plantas Nativas Medicinales, Lima, Perú.
0000-0001-6332-5652.
2 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Círculo de Investigación en Plantas Nativas Medicinales, Lima, Perú.
0000-0003-0540-973X.
3 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Círculo de Investigación en Plantas Nativas Medicinales, Lima, Perú.
0009-0003-0495-4362.
4 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Círculo de Investigación en Plantas Nativas Medicinales, Lima, Perú.
0009-0000-2178-1270.
5 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Círculo de Investigación en Plantas Nativas Medicinales, Lima, Perú.
0000-0001-5311-4341.
6 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Círculo de Investigación en Plantas Nativas Medicinales, Lima, Perú.
0009-0004-6540-2809.
7 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Círculo de Investigación en Plantas Nativas Medicinales, Lima, Perú.
0009-0007-0100-8388.
8 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Lima, Perú.
0000-0002-3800-132X.
9 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Lima, Perú.
0000-0002-1285-8413.
10 Universidad Nacional Agraria La Molina, Faculty of Agronomy, Lima, Perú.
0000-0002-6194-0675.

a sustainable alternative. **Objective.** To evaluate the influence of *Ascophyllum nodosum* extract on the rooting of spearmint cuttings. **Materials and methods.** The trial was carried out under greenhouse conditions at National Agricultural University La Molina, Peru with different doses of the extract and different evaluation times. Doses of 0, 1, 5 and 10 mL L^{-1} of the extract and 3 evaluation moments were used, which were 21, 28 and 35 days after cutting. In this way, an appropriate dose and transplanting time would be obtained. The variables evaluated were root number, root length, rooting percentage, fresh weight of stem, root and leaf, and dry weight of stem, root and leaf. **Results.** Spearmint cuttings showed significant differences (a = 0.05) for root length, rooting percentage, and root fresh weight with the application of the extract, with better results in the 10 mL L^{-1} dose for root length and root fresh weight. **Conclusion.** *Ascophyllum nodosum* extract proved to be efficient in rooting *Mentha spicata* cuttings.

RESUMEN

Influencia del extracto de *Ascophyllum nodosum* en el enraizamiento de *Mentha spicata* L. Introducción. La producción comercial de plantas medicinales es un mercado que está creciendo rápidamente en los últimos años y que requiere de técnicas agronómicas sostenibles para hacerle frente a esta demanda. La hierbabuena (*Mentha spicata* L.) es una de estas plantas demandadas a nivel mundial. No obstante, no existen muchas técnicas para su cultivo comercial, ya sea en términos de cosecha, poscosecha o propagación. En cuanto a la propagación, esta suele ser de forma vegetativa y ocasionalmente con algún enraizante. De la gran variedad de enraizantes que se pueden encontrar en el mercado, el uso de extractos de algas como *A. nodosum* resulta una alternativa sostenible como bioestimulante. **Objetivo.** Evaluar la influencia del extracto de *A. nodosum* en el enraizamiento de esquejes de hierbabuena. **Materiales y métodos.** El ensayo se llevó a cabo en condiciones de invernadero en la Universidad Nacional Agraria La Molina, Perú con diferentes dosis del extracto y diferentes momentos de evaluación. Se usaron dosis de 0, 1, 5 y 10 mL L^{-1} del extracto y 3 momentos de evaluación que fueron a los 21, 28 y 35 días después de realizado el esqueje. Las variables evaluadas fueron número de raíces, longitud de raíces, porcentaje de enraizamiento, peso fresco de tallo, raíz y hoja, y peso seco de los mismos. **Resultados.** Los esquejes de hierbabuena demostraron diferencias significativas (α = 0,05) para longitud de raíz, porcentaje de enraizamiento, y peso fresco de raíces ante la aplicación del extracto, con mejores resultados en la dosis de 10 mL L^{-1} para longitud de raíz y peso fresco de raíces. **Conclusión.** El extracto de *A. nodosum* probó ser eficiente en el enraizamiento de esquejes de *Mentha spicata*.

INTRODUCTION

Medicinal plants are of great importance in all regions both for their value in traditional medicine and for the economic livelihood they provide to small producers when marketed (FAO 1997, as cited in Membreño 2019). Particularly *Mentha spicata* L. is one of the most important species of the *Mentha genus* worldwide (Mokhtarikhah *et al.* 2022), with a wide use in gastronomy, pharmaceuticals, beverages and confectionery, being recognized as a safe plant by the FDA (Food and Drug Administration) as it does not cause negative effects during prolonged uses (Guzmán *et al.* 2017). The plants of this genus have also been reported as insecticidal, antibacterial, antifungal and allelopathic, with great potential for use in the agricultural sector (El Hassani 2020). On the other hand, within the framework of food security, one of the great challenges is to propitiate production systems that are oriented to meet future food needs and thus generate a contingency plan that allows families, with scarce resources, to live adequately (García Flores *et al.* 2016). Therefore it is necessary to give greater importance to these plants and generate systems that help to maintain them.

Mentha spicata is native to the European continent, and is one of the most popular and common species of the *Mentha genus*. It is perennial plant, with a plant height between 30 to 50 cm; its stem has a reddish-purple coloration, a conical terminal inflorescence of lilac or violet colors and subsessile leaves (Resquín *et al.* 2011). *Mentha spicata* contains volatile oils, resins, tannins, coumarins, flavonoids, steroids and alkaloids (Salvá 2016). It is used to treat ailments such as vomiting, nausea, pain and gastrointestinal disorders (Kumar *et al.* 2011). Two of the main chemical compounds are limonene (Telci *et al.* 2010) and rosmarinic acid, a phenolic compound associated to anti-inflammatory, anticancer, antioxidant, antifungal and antibacterial properties (Mahendran *et al.* 2021).

Cultivation of medicinal plants in Peru is minimal, making the implementation and promotion of sustainable practices challenging due to limited information availability (Nolasco Cruz 2016). Furthermore, this production primarily occurs in rural areas where technical agronomic expertise is scarce, reflecting broader issues within Peruvian agriculture (MIDAGRI 2015). Various propagation methods are available, each better suited to specific crops. For plants like perennial herbs, which root easily, propagation is typically achieved through cuttings or ratoons (Quintero 1985). Given its importance and ease of implementation, this method warrants consideration and further exploration. Therefore, it is imperative to adapt and generate knowledge for effective agronomic management in the propagation of these plants.

Biostimulants are gaining importance in the agricultural field due to their ability to be used in sustainable systems (Rouphael & Colla 2020). In general, they have the capacity, at low concentrations, to improve flowering, growth, the qualitative properties of the commercial product and its shelf life (Basile *et al.* 2020). Among them, some of the most studied are those made from macroalgae (Kapoore *et al.* 2021). One of these macroalgae is *Ascophyllum nodosum*, which is important for its content of phenolic compounds, polysaccharides, amino acids and phytohormones (Battacharyya *et al.* 2015). The use of this biostimulant has shown in crops efficient nitrogen use, increased water use efficiency, and biomass gain (Łangowski *et al.* 2022, Frioni *et al.* 2021).

Agrostemin is a commercial product obtained from extracts of *Ascophyllum nodosum*. It has shown to stimulate growth in vegetables, increasing their quality and yield. It contains natural hormones that affect different physiological processes of the plant (Serfi 2022). It is also used as a rooting agent (Telenchana 2017, Jacome 2011).

The present works aims to evaluate the effects of 3 concentrations of *Ascophyllum nodosum* extract on the rooting of *Mentha spicata*.

MATERIALS AND METHODS

The plant material was obtained from the Vegetable Research and Social Projection Program of the Universidad Nacional Agraria La Molina, Peru. It was collected from *Mentha spicata* L. mother plants on November 12, 2022, using sterile pruning shears. Propagation was conducted following the protocol outlined by Amaro *et al.* (2013), with certain modifications. Apical cuttings 6.8 to 8.8 cm long, 2 to 3 mm in diameter and 5 nodes, were collected. The leaves were removed, (except for those of the upper node) and they were placed in containers with water to avoid dehydration before treatment.

The rooting agent used for the treatments was an extract of *Ascophyllum nodosum* with the commercial name Agrostemin. From this, three solutions of one liter of water were prepared with different concentrations of the extract. The cuttings were submerged for 5 minutes.

A 4x3 completely randomized factorial design was used with 10 cuttings per treatment, with a total of 120 cuttings. The first factor (D) corresponds to the doses of Agrostemin used for the rooting solutions and the second factor (T) corresponds to the evaluation times of the cuttings since they were cut. The Agrostemin doses used were 0, 1, 1, 5 and 10 mL per liter of water. The evaluation times were 21, 28 and 35 days after cutting. The variables evaluated were root length of the largest root, root number (the number of primary roots), rooting percentage, fresh weight of leaves, fresh weight of stem, fresh weight of roots, dry weight of leaves, dry weight of stem, and dry weight of roots.

Propagation was carried out inside a protective structure of the Vegetable Research and Social Projection Program at the Universidad Nacional Agraria La Molina, Peru with recorded temperature and relative humidity. During the experiment, the minimum temperature reported was 14.4°C, the maximum temperature was 29.1°C and the average temperature was 19.6°C. The maximum relative humidity was 95%, the minimum was 58% and the average relative humidity was 83.74%. For propagation, 12 trays of 25 spaces, each 8.8 cm high, 5.4 cm in diameter and with one cutting were used. In these trays, fine river sand was used as substrate and the cuttings were watered 6 days a week.

The evaluation of the variables root number, root length and rooting percentage was carried out on the 10 cuttings of each treatment, while the other variables were evaluated on only 3 cuttings per treatment.

Statistical analyses were performed with R Studio software and graphs with Graph Pad Prism software. The ANOVA table was performed for each variable to determine the statistical significance of the factors. Following this, Tukey's test of comparisons (p-value < 0.05) was conducted to identify significant differences between treatments.

RESULTS AND DISCUSSION

According to the ANOVA table, for the variable root number, only the time factor was highly significant. In the Tukey test, no significant differences between treatments were observed (Table 1). However, a significant increase in root number was reported as time increased (Figure 1).

Table 1. Average values of the variables root number (RN), root length (RL) in centimeters and rooting percentage (RP) of the 12 treatments.

Treatments	DAA	DA (mL L^{-1})	RN	RL (cm)	RP (%)
1	21	0	2.00 a	1.16 c	20 f
2	21	1	3.00 a	1.20 c	50 e
3	21	5	3.66 a	0.93 c	70 cd
4	21	10	2.33 a	1.00 c	50 e
5	28	0	4.17 a	2.00 c	70 cd
6	28	1	7.17 a	4.20 bc	80 bc
7	28	5	4.33 a	3.48 bc	60 de
8	28	10	5.00 a	5.17 abc	80 bc
9	35	0	3.00 a	4.80 bc	80 bc
10	35	1	7.33 a	7.63 ab	100 a
11	35	5	7.33 a	5.03 abc	80 bc
12	35	10	9.16 a	9.48 a	90 ab

Note: DAA: days after application of Agrostemin, DA: dose of Agrostemin, Different letters indicate significant differences between treatments (p-value<0.05).

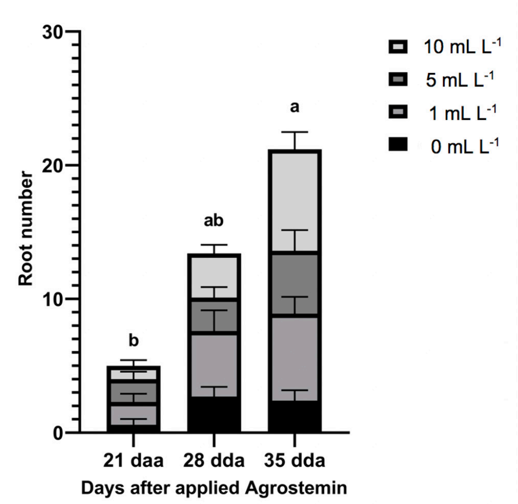

Figure 1. Root number at 21, 28, 35 DAA and according to the doses of Agrostemin.

As for the variable root length (Figure 2), the factors time and dose were highly significant, but not their interaction. At each dose there is an increase in root length as time increases.

In the study carried by Gomes *et al.* (2018), they observed that as *Ascophyllum nodosum* extract concentration increases, root length of *Passiflora actinia* Hook cuttings increases. However, in the present experiment, just at 35 days after the application of Agrostemin, there was a significant difference in root length between the dose of 10 mL L^{-1} and the control (Figure 3).

The rooting percentage variable presented high significance for the time and dose factors, as well as for their interaction. Significant differences were found between each time, with 35 dda showing the highest percentage of rooting. On the other hand, for the dose factor, 1 and 10 mL L^{-1} showed the highest percentage of rooting. The data reported in Table 1 indicate that the highest percentage of rooting is obtained at 35dda and with a dose of 1 mL L^{-1} of Agrostemin.

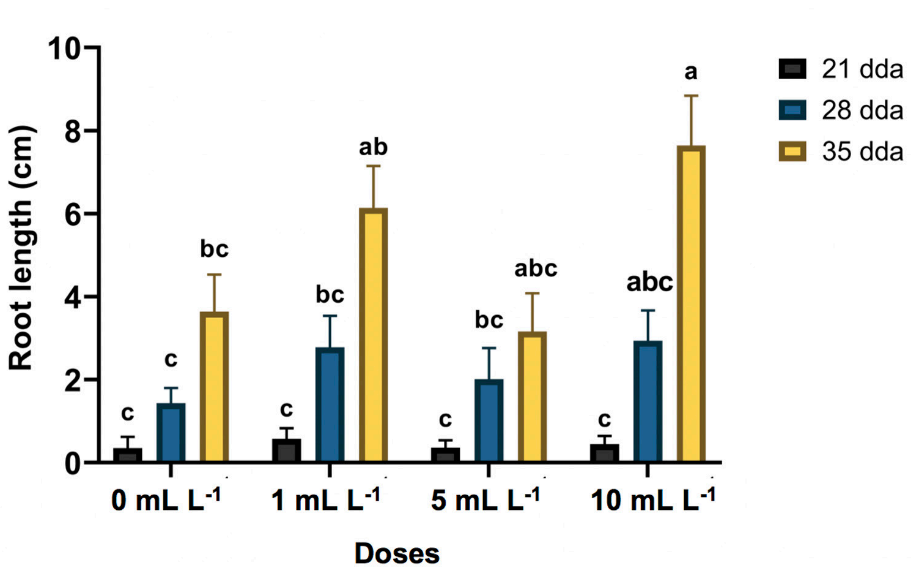

Figure 2. Root length in different doses of Agrostemin and according to three evaluation moments.

Figure 3. Spearmint cuttings at 35 days with 0 (A), 1 (B), 5 (C) and 10 (D) mL L^{-1} of Agrostemin.

The evaluations of fresh weight of leaves and roots, and dry weight of leaves, stem and roots did not show significant differences between any of the treatments. The root fresh weight variable shows significant differences in the time and dose factors, but there is no significant interaction between them. In the treatments of time 3, the fresh weight of roots has significant differences compared to the other times (Figure 4). Even if we consider the time factor, it achieved a significantly higher root dry weight at 35 days after cutting. On the other hand, the treatments to which Agrostemin was applied had a significantly higher root weight than the control. These results are similar to those reported by Elansary (2017), where no significant differences were reported between the dry weights of *Mentha spicata* in the control group and those that received *Ascophyllum nodosum*.

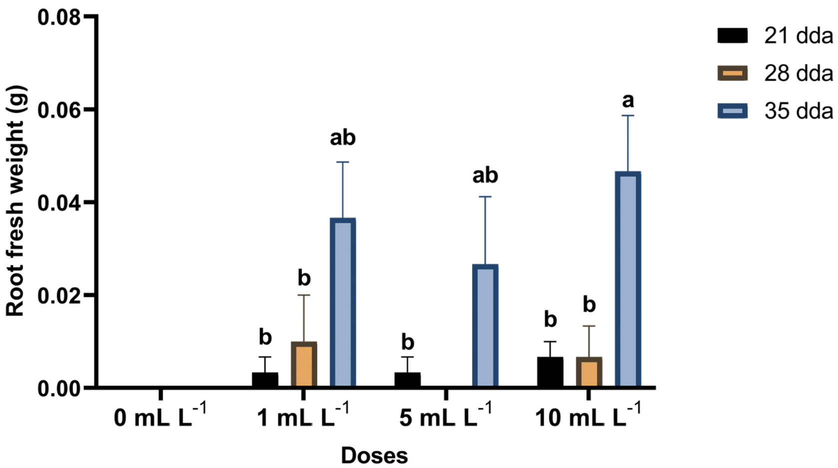

Figure 4. Fresh weight of roots in different doses of Agrostemin and according to three times from its application.

CONCLUSIONS

Ascophyllum nodosum as a biological rooting agent for *Mentha spicata* L. cuttings proved to enhance root length, rooting percentage and root fresh weight in contrast to the control. Particularly, the application of a 10 mL L^{-1} dosage yielded the most pronounced improvements in these variables.

Most of the observed variables exhibited an increase with time after cutting. Nevertheless, the fresh and dry weights of leaves and stems did not demonstrate significant differences despite the passage of time since cutting.

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