International Journal of Medical Toxicology & Legal Medicine **Volume 27, No. 4S, 2024**
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V27I4S/061

Effect of Thalassiatestudinum flour on the growth and production of polyphenols of Phaseolus vulgaris

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Received: 15.09.2024 Revised: 28.10.2024 Accepted: 14.11.2024

ABSTRACT
Remains of Thalassiatestudinum abound and accumulate on the coasts of Campeche and represent a pollution problem when decomposing; However, the biomass of this marine plant is rich in biomolecules, both primary metabolites (proteins, carbohydrates and lipids) and secondary (polyphenols), which can be used to nourish crops as a biofertilizer. Therefore, the objective of this work was to evaluate the effect of the dust obtained from the drying and grinding of Thalassiatestudinum collected on the beaches of San Francisco de Campeche on the germination and growth of Phaseolus vulgaris. The P. vulgaris seeds were placed in individual wells of 20 mL of 0.5% agar-agar, the control only contained the seeds and the problem groups were placed in each well with 10, 30 and 100 mg of dry sargassum powder in the agar after boiling, when cooled to 40ºC; The vials were placed in a humid chamber for 7 days for germination and growth. At the end of the period, the seedlings were harvested and their morphometry was measured (size, root size and weight of the plant) and the content of pigments and polyphenols were determined. and the antioxidant activity of methanolic extracts from the leaves of harvested plants. The results obtained demonstrate an increase in the growth of P. vulgaris treated with T. testudinum, which is why it is recommended to continue the study to evaluate its potential as a biofertilizer.

Keywords: sustainable development, biofertilizer, organic agriculture

INTRODUCTION

The increase in human population demands a greater amount of food; For this reason, agrochemical products are used to improve crop production. However, the use of pesticides and fertilizers affects food safety and represents a severe risk to the health of consumers; Consequently, currently it has been proposed as an alternative to this problem, the use of biofertilizers to provide nutrients and improve crop production, increasing tolerance to stress and resistance to pathogens, they are also eco-friendly, low cost and their Continuous use improves soil fertility[1-3]. Although the term fertilizer is frequently associated with microorganisms that are added to crops, it can also be called organic fertilizers from different sources such as plant residues or a combination of both[3,4].

Plant residues are often a source of water and soil contamination; but they are a rich source of nutrients that must be adapted to modify the physical and chemical structure of the materials that favors chemical speciation to increase the availability of nutrients[5,6]. Such is the case of Thalassiatestudinum, which is a very abundant marine phanerogam due to its high production of organic matter and which provides shelter, food and spaces for fauna; This species is very abundant in the Gulf of Mexico and the coasts of the State of Campeche tend to be covered by remains of the plant[7-9], T. testudinum is a producer of secondary metabolites with biological activity of interest, among the chemical compounds synthesized by the plant are phenols, flavonoids, phytosterols, coumarins, terpenes and saponins that can be used by the pharmaceutical and agricultural industries[9-11].

Therefore, the objective of this research was to evaluate the effect of Thalassiatestudinum flour on the growth of Phaseolus vulgaris to estimate its potential sustainable use as a biofertilizer in the production of bean crops and prevent its degradation and contamination of the coasts.

MATERIALS AND METHODS

The plant material was collected on the coast of the State of Campeche (Mexico) and transported to the laboratory for mechanical cleaning and washing with plenty of running water; The washed material was dried at 40º C in an electric oven until constant weight and the dry material was ground in an electric mill to obtain flour; Finally, the flour was sifted with a number 12 sieve and kept refrigerated until use.

Certified bean seeds (Phaseolus vulgaris) were used that were selected according to their physical appearances that did not show signs of decomposition and that they were intact; The seeds were washed with running water to remove dust and then disinfected by immersion in a 5% aqueous solution of sodium hypochlorite for five minutes and finally washed with plenty of water to eliminate the rest of the hypochlorite. The seeds were placed in a sterile absorbent paper to dry them and subsequent planting.

Each group consisted of 36 seeds that were grown on 0.5% agar-agar in a humid chamber at 25±1 ºC for 7 days; To the problem groups, 10 (S10), 30 (S30) and 100 mg (S100) of Thalassiatestudinum flour were added to the agar after boiling and cooling to 40º C. At the end of period the seedlings that grew were harvested for morphometric analysis and the determination of their photosynthetic pigments, polyphenols. and DPPH inhibitory activity; To do this, the leaves were extracted by maceration with absolute methanol in a 1:5 (W/V) ratio, allowed to rest for 30 minutes and then the extract was filtered and centrifuged at 14,000 r.p.m. for 10 minutes.

Determination of chlorophylls and carotenes[12-15].

Chlorophyll quantification was performed spectrophotometrically at wavelengths of 649 and 665 nm and total chlorophyll was calculated using Sumanta equations [1] and [2] for methanol as a solvent. Likewise, the concentration of total carotenoids was quantified spectrophotometrically by measuring the absorbance of the extract at a wavelength of 470 nm and using equation [3] of Sumanta et al., each determination was performed in triplicate.

$$C_a = 12.47A_{665} - 3.62A_{649} \quad [1]$$

$$C_b = 25.06A_{649} - 6.5A_{665} \quad [2]$$

$$C_c = \frac{1000\,A_{470} - 1.63\,C_a - 104.96\,C_b}{221} \quad [3]$$

Quantification of total polyphenols[16,17].

100 □L of the P. vulgaris leaf extract was added to 1.0 mL of distilled water and 100 □L of the Folin Ciocalteu Reagent was added. The mixture was allowed to react for 30 minutes and then 500 □L of a solution was added. of 10% Na_2CO_3 in water and let it rest for 30 minutes at room temperature in the dark. Finally, the absorbance was measured at 760 nm; A five-point calibration curve was prepared using gallic acid as a standard. The polyphenol content was expressed as mg equivalents of gallic acid (GAE) per g of fresh leaf; each determination was performed in triplicate.

Determination of the inhibitory capacity of Radical DPPH[16-18].

2.0 mL of a 150 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in 80% methanol was used and 25, 50 and 100 □L of the extract were added, incubated for 30 minutes in the dark and at room temperature; Subsequently, the absorbance was measured at 520 nm in a UV Visible spectrophotometer, gallic acid was used as a standard solution. Each sample was analyzed in triplicate and the results are expressed as mg EAG/g fresh leaf.

Ferric Reductor Activity Power[19-20].

30 μL of the sample was taken and 900 μL of the FRAP solution was added (prepared by adding 25 mL of acetic acid/acetate buffer pH 3.6, 2.5 mL of TPTZ [Tris (2-pyridyl)-S-triazine] 10 mM/40 mM HCl and 2.5 mL of 20 mM $FeCl_3$) and 120 μL of distilled water, in 3 mL amber vials that were incubated in a water bath at 37º C for 30 minutes, subsequently the absorbance of the samples at 593 nm. A calibration curve was carried out with $FeSO_4$ $7H_2O$ and the results are calculated as μmol of Fe^{2+} produced, gallic acid was used as an antioxidant standard and the valuesof the extracts are expressed as mg EAG required to produce the reduction of the ferric ion.

Statistic analysis

The data obtained were processed in the Microsoft Excel ® program and the analyzes of variance and comparison of means (Tukey's test, $p<0.05$) were performed in the SPSS statistical program. For the calibration curves, a Pearson linear correlation analysis was performed and the respective straight line equations were obtained for determining the concentrations of the analytes.

RESULTS AND DISCUSSION

Beans are a very important crop, especially in developing countries; but the fertility of the soil and the attack of pests compromise its production and although chemical fertilizers improve its yield, they are unaffordable for farmers and affect the stability of the soil and contaminate it, which is why it is important to evaluate sustainable alternatives to offer viable options to farmers. peasants[21]. This is the case of Thalassiatestudinum, which grows in abundance and its accumulation contaminates the coasts of the State of Campeche; But this large amount of biomass, in addition to being a source of carbon and nitrogen, can provide secondary metabolites that enhance the crop and improve its quality.

Initially, one way to estimate the beneficial effect of the substances used as fertilizer is to measure the size and weight of the treated plants[21]. Figure 1 shows the effect that the T. testudinumpowder had on root growth and stem of Phaseolus vulgaris, in the case of the stem an increase is observed with respect to the control group but in the dose of 100 mg an affectation in growth appears; while in the root there is a dose-dependent effect of T. testudinum on the size of this organ.

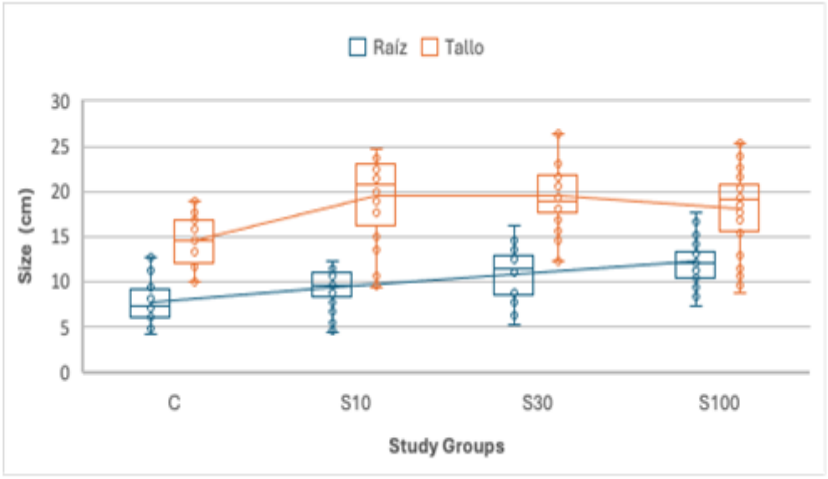

Figure 1. Size of the root and stem of Phaseolus vulgaris from the different treatments (C control group, S10 with 10 mg, S30 with 30 mg and S100 with 100 mg of Thalassiatestudinum, n=36).

Regarding the effect of T. testudinum flour on the weight of P. vulgaris, it can be seen in Figure 2 that the lowest dose (10 mg) practically did not improve this parameter but the higher doses tended to increase the biomass of the plant. This may be because T. testudinumserved as a source of nutrients (carbon, nitrogen or phosphorus) because fertilizers increase the biomass and production of crops, this is useful to improve the growth of crops in impoverished soils or with minimal access to inorganic nutrients that limit their production[22-24].

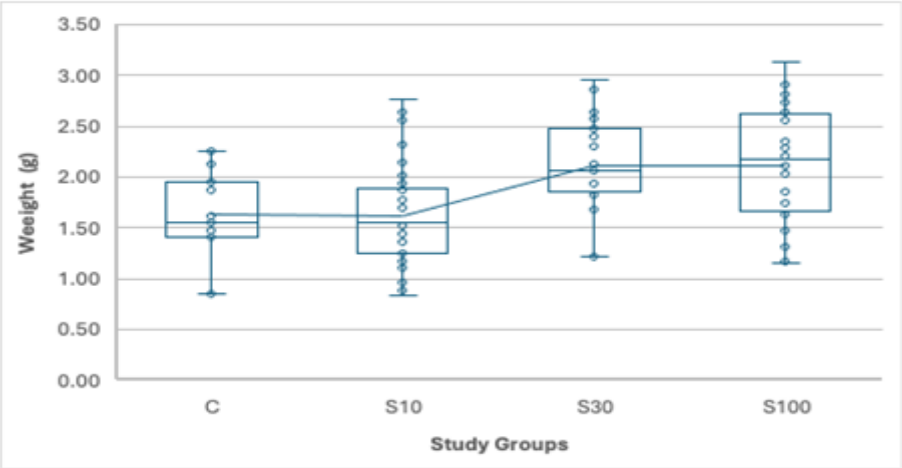

Figure 2. Weight of Phaseolus vulgaris seedlings after growth for 7 days with the different treatments (C control group, S10 with 10 mg, S30 with 30 mg and S100 with 100 mg of Thalassiatestudinum).

The total weight is a general indicator of the fertilizer effect, however some nutrient deficiencies can manifest in the growth or reduction of specific organs, thus phosphorus deficiency reduces the size of the leaves and increases the length of the roots[24-26]; Consequently, it must be verified that the increase in weight is proportional to all plant organs. Figures 3 and 4 report the percentages and weights of the plant parts of P. vulgaris treated with T. testudinum, the increase in weight mainly It is due to an increase in stem size because there was no significant variation between the biomass of leaves and roots. These results agree with reports that seaweed, beneficial microorganisms or substances such as fulvic and huic acid increase the size of the plant mainly due to the contribution of nutritional substances such as amino acids[27].

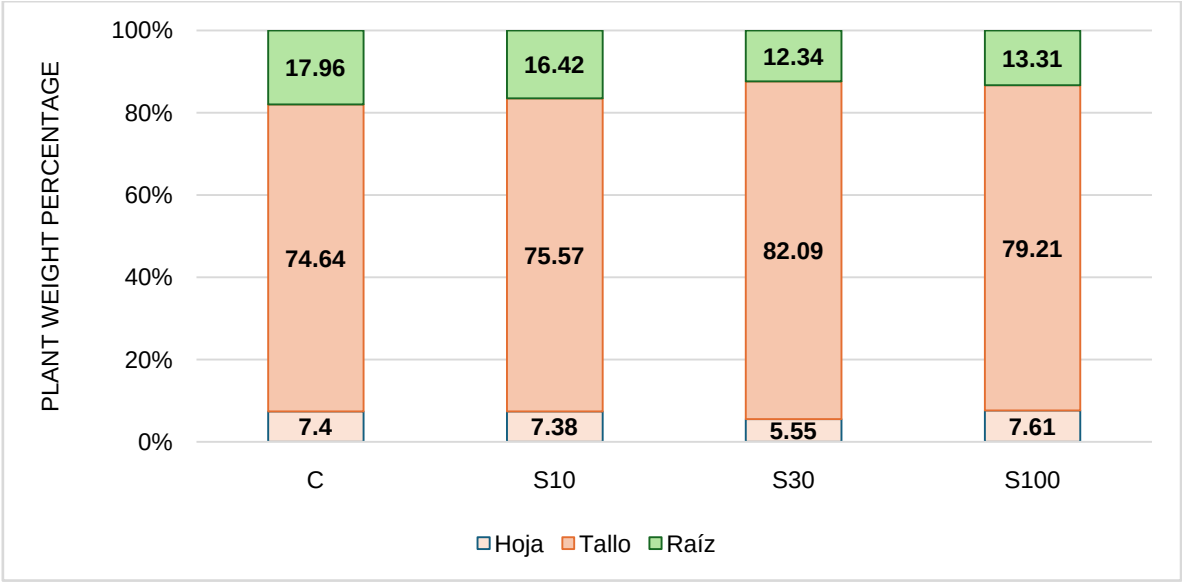

Figure 3. Percentage of weight of each part of the Phaseolus vulgaris seedling after its growth for 7 days with the different treatments (C control group, S10 with 10 mg, S30 with 30 mg and S100 with 100 mg of Thalassiatestudinum).

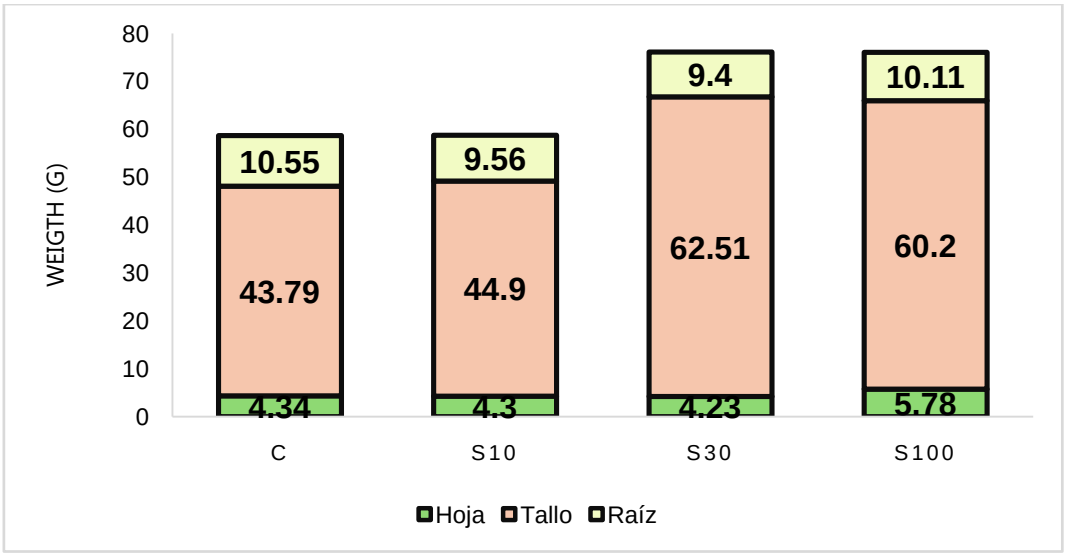

Figure 4. Weight of each part of the total Phaseolus vulgaris seedlings after growth for 7 days with the different treatments (C control group, S10 with 10 mg, S30 with 30 mg and S100 with 100 mg of Thalassiatestudinum).

Chlorophyll levels in a plant are of both ecological and physiological interest, thus the use of beneficial fertilizers tends to increase chlorophyll levels in the leaves, the main productive units of plants, and is also important for the preservation of flowers. which increases the final yield of the crop[28-30]. For their part, cartotenes are the pigments responsible for the color of some fruits and vegetables and their function is to protect the plant from ultraviolet radiation during photosynthesis because it is responsible for the neutralization of reactive oxygen species and free radicals[29].

The results of the quantification of pigments contained in the leaves of P. vulgaris are shown in figure 5. T. testudinum caused an increase in the concentration of total chlorophylls in the treated seedlings, compared to the control group; but no modification is observed in the level of carotenes. Both results coincide with the expected values according to research reports that quantified the levels of these pigments [28-30].

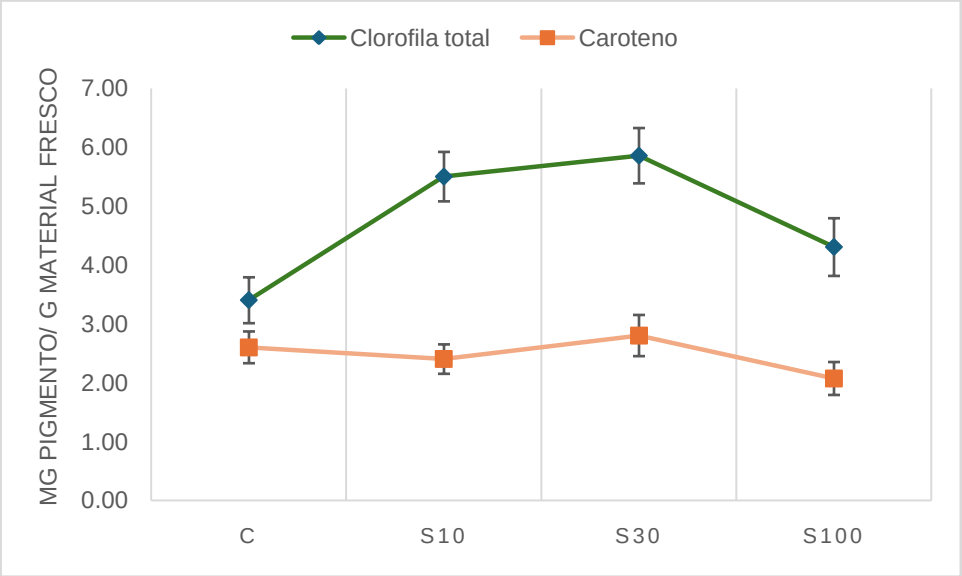

Figure 5. Total chlorophyll and carotene content in the leaves of Phaseolus vulgaris after growth for 7 days with the different treatments (C control group, S10 with 10 mg, S30 with 30 mg and S100 with 100 mg of Thalassiatestudinum).

The amount of polyphenols, like other bioactive compounds found in plants, will depend on the environmental and nutritional conditions of the crop; These secondary metabolites exert various beneficial properties for plants that modulate metabolism, homeostasis and cell proliferation. Due to their antioxidant properties, they are involved in the defense against oxidative stress and derived diseases[31-34]. The main polyphenolic compounds contained in Phaseolus vulgaris are flavonoids, anthocyanins, stilbenes, lignans and tannins; Of them, flavonoids represent around 50% of all phenolic compounds[31-34].

Figure 6 shows the phenolic valuescontained in the methanolic extracts of the leaves of P. vulgaris. In the control group, flavonoids represent 44% of the total polyphenolic compounds, while in the treated groups they were 47, 39 and 50%. , respectively S10, S30 and S100; The increase observed due to the effect of T. testudunim is mainly due to other types of polyphenols, although in the S100 group the amount of flavonoids also increased significantly.

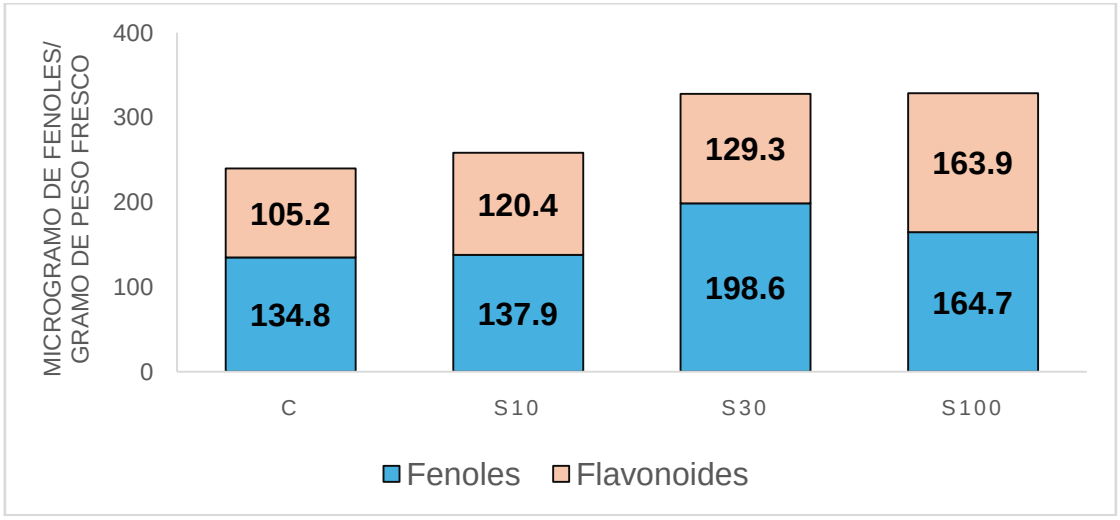

Figure 6. Content of flavonoids and other phenolic compounds in Phaseolus vulgaris leaves after growth for 7 days with the different treatments (C control group, S10 with 10 mg, S30 with 30 mg and S100 with 100 mg of Thalassiatestudinum).

The main biological activity associated with polyphenolic compounds is antioxidant; P. vulgaris is a species with a very good antioxidant activity compared to other similar grains and seeds, in addition, germination causes an accumulation of polyphenols approximately 1.5 times greater in seedlings 6 days after germination[31-34]. In Figure 7, we can observe not only an increase in the amount of total polyphenols but also an increase in their free radical neutralizing capacity (test with DPPH) and the capacity to reduce the ferric ion (FRAP); These two activities are important both for biological systems because they defend them from oxidative damage and for their nutritional value because the reduction of the ferric ion to ferrous ion increases the bioavailability of the element[31-34]. The results of the antioxidant activity are expressed in terms of mg equivalents of gallic acid (GAE) to better appreciate that although phenols are antioxidants of great importance, they are not the only compounds that exert this action and it can be seen that the improved antioxidant activity in extracts from treated seedlings may also be due to the increase in other antioxidant compounds.

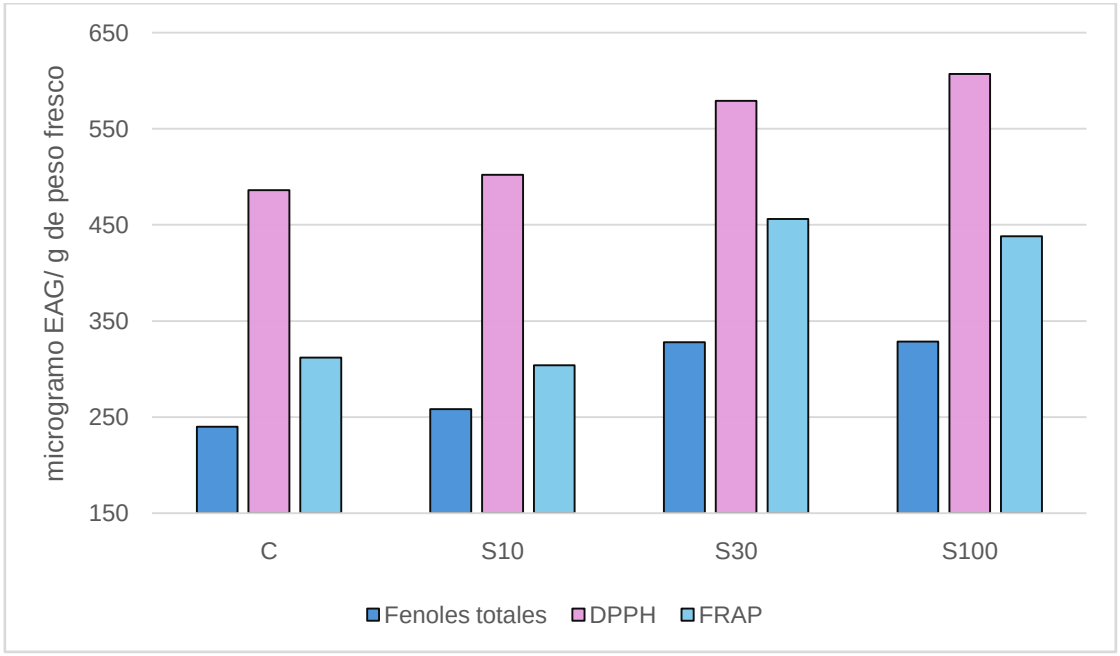

Figure 7. Total polyphenol content and antioxidant activity of the methanolic extracts of the leaves of Phaseolus vulgaris after growth for 7 days with the different treatments (C control group, S10 with 10 mg, S30 with 30 mg and S100 with 100 mg from Thalassiatestudinum).

CONCLUSION

The powder obtained from Thalassiatestudinum exerted a fertilizing effect on the growth of Phaseolus vulgaris in agar-agar by increasing the size and weight of the treated seedlings and also increased the content of chlorophylls and total polyphenols; This may be an alternative to take advantage of the seagrass biomass that accumulates on the marine coasts of the Gulf of Mexico and use it sustainably in agriculture.

REFERENCES

1. Daniel, A. I., Fadaka, A. O., Gokul, A., Bakare, O. O., Aina, O., Fisher, S., Burt, A. F., Mavumengwana, V., Keyster, M., & Klein, A. (2022). Biofertilizer: The Future of Food Security and Food Safety. Microorganisms, 10(6), 1220. https://doi.org/10.3390/microorganisms10061220.
2. López, Marisol, Martínez Viera, Rafael, Brossard Fabré, Mariluz, Bolívar, Angela, Alfonso, Nidia, Alba, Amelia, & Pereira Abreo, Heydi. (2008). Efecto de biofertilizantes bacterianos sobre el crecimiento de un cultivar de maíz en dos suelos contrastantes venezolanos. Agronomía Tropical, 58(4), 391-401.
3. Chávez-Díaz, Ismael Fernando, Zelaya Molina, Lily X., Cruz Cárdenas, Carlos Iván, Rojas Anaya, Edith, Ruíz Ramírez, Santiago, & Santos Villalobos, Sergio de los. (2020). Consideraciones sobre el uso de biofertilizantes como alternativa agro-biotecnológica sostenible para la seguridad alimentaria en México. Revista mexicana de ciencias agrícolas, 11(6), 1423-1436. https://doi.org/10.29312/remexca.v11i6.2492
4. Lara Mantilla Cecilia, García Támara Liliana Pahola, Oviedo Zumaqué Luis E. Efecto biofertilizante del preparado: residuos vegetales -bacteria nativa diazótrofa, sobre las variables biométricas en plántulas de Rhapanus sativus. Rev. colomb. biotecnol [Internet]. 2011 July [cited 2024 Apr 03] ; 13(1): 156-162.

5. Hernández-Rodríguez, Ofelia Adriana, Hernández-Tecorral, Ana, Rivera-Figueroa, César, Arras-Vota, Ana María, & Ojeda-Barrios, Dámaris. (2013). Calidad nutrimental de cuatro abonos orgánicos producidos a partir de residuos vegetales y pecuarios. Terra Latinoamericana, 31(1), 35-46.
6. Hernández Rodríguez, Ofelia Adriana, Rivera Figueroa, César H., Díaz Ávila, Elías E., Ojeda Barrios, Dámaris L., & Guerrero Prieto, Víctor M.. (2017). Plant and livestock waste compost compared with inorganic fertilizer: nutrient contribution to soil. Terra Latinoamericana, 35(4), 321-328.
7. Prieto, Antulio, Sant, Sybil, Méndez, Elizabeth, & Lodeiros, César. (2003). Diversidad y abundancia de moluscos en las praderas de Thalassia testudinum de la Bahía de Mochima, Parque Nacional Mochima, Venezuela. Revista de Biología Tropical, 51(2), 413-426.
8. Álvarez-Rocha, Mariana, & Ortegón-Aznar, Ileana. (2022). ¿Puede afectar el arribazón de sargazo en el Caribe Mexicano a la cobertura y morfología de Syringodium filiform (Cymodoceaceae)?. Hidrobiológica, 32(1), 51-58.
9. García-Granados, R.U., Cruz-Sosa, F., Alarcón-Aguilar, F.J., Nieto-Trujillo, A., & Gallegos-Martínez, M.E.. (2019). Análisis fitoquímico cualitativo de los extractos acuosos de Thalassia testudinum Banks ex Köning et Sims de la localidad de Champotón, Campeche, México, durante el ciclo anual 2016-2017. Polibotánica, (48), 151-168. Epub 15 de junio de 2020.https://doi.org/10.18387/polibotanica.48.12
10. Delgado-Roche L, Santes-Palacios R, Herrera JA, Hernández SL, Riera M, Fernández MD, Mesta F, Garrido G, Rodeiro I, Espinosa-Aguirre JJ. Interaction of Thalassiatestudinum Metabolites with Cytochrome P450 Enzymes and Its Effects on Benzo(a)pyrene-Induced Mutagenicity. Mar Drugs. 2020 Nov 19;18(11):566. doi: 10.3390/md18110566. PMID: 33227946; PMCID: PMC7699293.
11. de la Torre Núñez, Eliane, Rodeiro Guerra, Idania, Menéndez Soto del Valle, Roberto, & Pérez Carrasco, Dalmis. (2012). Thalassia testudinum, una planta marina con potencialidades de uso terapéutico. Revista Cubana de Plantas Medicinales, 17(3), 288-296.
12. GONZÁLEZ, JAVIERA, PASTENES, CLAUDIO, & HORTON, PETER. (2001). Efecto de la temperatura, el estrés hídrico y luminoso sobre la heterogeneidad del fotosistema II en cuatro variedades de poroto (Phaseolus vulgaris L.). Revista chilena de historia natural, 74(4), 779-791. https://dx.doi.org/10.4067/S0716-078X2001000400006
13. Serpa Ibáñez, Román Felipe, & Calderón Rodríguez, Abelardo. (2006). Efecto de diferentes fuentes de nitrógeno en el contenido de carotenoides y clorofila de cuatro cepas peruanas de Dunaliella salina Teod.. Ecología Aplicada, 5(1-2), 93-99.
14. More, A.D., & Borkar, A.T. (2016). Improvement in Protein and Chlorophyll Content through Physical and Chemical Mutagens in Phaseolus vulgaris L. Int. J. Curr. Microbiol. App. Sci., 5(10), 583-591. http://dx.doi.org/10.20546/ijcmas.2016.510.065
15. Sumanta, Nayek, Haque, Choudhury Imranul, Nishika, Jaishee, &Suprakash, Roy. (2014). Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. Research Journal of Chemical Sciences, 4(9), 63-69.
16. Rochín-Medina, Jesús Jaime, Mora-Rochín, Saraid, Navarro-Cortez, Ricardo Omar, Tovar-Jimenez, Xochitl, Quiñones-Reyes, Guillermo, Ayala-Luján, Jorge Luis, & Aguayo-Rojas, Jesús. (2021). Contenido de compuestos fenólicos y capacidad antioxidante de variedades de frijol sembradas en el estado de Zacatecas. Acta universitaria, 31, e3059. https://doi.org/10.15174/au.2021.3059
17. Oliveira Silva, Maira, Brigide, Priscila, Viva de Toledo, Nataly Maria, & Canniatti-Brazaca, Solange Guidolin. (2018). Phenolic compounds and antioxidant activity of two bean cultivars (Phaseolus vulgaris L.) submitted to cooking. Brazilian Journal of Food Technology, 21, e2016072. http://dx.doi.org/10.1590/1981-6723.7216
18. Mujica, María V, Granito, Marisela, & Soto, Naudy. (2012). Variación de los compuestos fenólicos de Phaseolus vulgaris L. durante el almacenamiento y su relación con el endurecimiento. Bioagro, 24(3), 163-174.
19. Benítez-Estrada, Abigail, Villanueva-Sánchez, Javier, González-Rosendo, Guillermina, Alcántar-Rodríguez, Víctor Eduardo, Puga-Díaz, Rubén, & Quintero-Gutiérrez, Adrián Guillermo. (2020). Determinación de la capacidad antioxidante total de alimentos y plasma humano por fotoquimioluminiscencia: Correlación con ensayos fluorométricos (ORAC) y espectrofotométricos (FRAP). TIP. Revista especializada en ciencias químico-biológicas, 23, e20200244. Epub 05 de marzo de 2021.https://doi.org/10.22201/fesz.23958723e.2020.0.244
20. Mercado-Mercado, Gilberto, Rosa Carrillo, Laura de la, Wall-Medrano, Abraham, López Díaz, José Alberto, & Álvarez-Parrilla, Emilio. (2013). Compuestos polifenólicos y capacidad antioxidante de especias típicas consumidas en México. NutriciónHospitalaria, 28(1), 36-46. https://dx.doi.org/10.3305/nh.2013.28.1.6298
21. Mkindi, Angela G., Yolice L. B. Tembo, Ernest R. Mbega, Amy K. Smith, Iain W. Farrell, Patrick A. Ndakidemi, Philip C. Stevenson, and Steven R. Belmain. 2020. "Extracts of Common Pesticidal Plants

Increase Plant Growth and Yield in Common Bean Plants" Plants 9, no. 2: 149. https://doi.org/10.3390/plants9020149

22. Escalante-Estrada, José Alberto Salvador, & Rodríguez-González, María Teresa. (2017). Biomass and Yield of Common Bean (Phaseolus vulgaris) as a function of the nitrogen Source. Annual report of the Bean improvement cooperative, 60, 77-78.
23. Ranjbar-Moghaddam, Faegheh, &Aminpanah, Hashem. (2015). Green bean (Phaseolus vulgaris L.) growth and yield as affected by chemical phosphorus fertilizer and phosphate bio-fertilizer. Idesia (Arica), 33(2), 77-85. https://dx.doi.org/10.4067/S0718-34292015000200010
24. Mndolwa, E. , Collins, H. and Miklas, P. (2018) Plant Growth Response of Eight Andean Dry Bean (Phaseolus vulgaris L.) Genotypes to Phosphorus Fertilizer in the Greenhouse. Agricultural Sciences, **9**, 1269-1285. doi: 10.4236/as.2018.910089.
25. Rojas Victoria, Nestor Jorge, Escalante Estrada, José Alberto Salvador, Aguilar Carpio, Cid. (2023). Analysis of growth fertilization system. Tropical and subtropical Agroecosystems, 26 (1), doi: http://dx.doi.org/10.56369/tsaes.4306.
26. Romero-Félix, Celia Selene, Paso, Damaris Fabiola Pellegaud-del, Salas-Arellanes, Juan Antonio, Sauceda-Acosta, Raúl Hipólito, Buelna-Tarín, Salomón, & López-Valenzuela, Blanca Elvira. (2023). Bioestimulantes en el crecimiento y rendimiento de Phaseolus vulgaris L. en el Norte de Sinaloa. Ecosistemas y recursos agropecuarios, 10(2), e3650. Epub 22 de septiembre de 2023.https://doi.org/10.19136/era.a10n2.3650
27. Espinoza-Galaviz, J. Y., Romero-Félix, C. S., Sánchez-Soto, B. H., Sauceda-Acosta, R. H., Almada- Ruíz, V. G., & Lugo-García, G. A. (2023). Biostimulants on yield and its components in common bean (Phaseolus vulgaris L.). Agro Productividad. https:// doi.org/10.32854/agrop.v15i4.2462
28. Carreño Siqueira, J.A., Marques, D.J., Silva, M.C.G. et al. The use of photosynthetic pigments and SPAD can help in the selection of bean genotypes under fertilization organic and mineral. Sci Rep **13**, 22610 (2023). https://doi.org/10.1038/s41598-023-49582-4
29. Guerrero-Martin, C. A., Ortega-Ramírez, A. T., Silva-Marrufo, Ó., Casallas-Martín, B. D., Cortés-Salazar, N., Salinas-Silva, R., Camacho-Galindo, S., Da Silva Fernandes, F. A., Guerrero-Martin, L. E., Paulo de Freitas, P., & D V Duarte, E. (2023). Biofortification of Kidney Bean (Phaseolus vulgaris L.) Crops Applying Zinc Sulfate and Ferric Sulfate: Pilot Crop in Colombia. Molecules (Basel, Switzerland), 28(5), 2004. https://doi.org/10.3390/molecules28052004
30. Mfilinge, A. , Mtei, K. and Ndakidemi, P. (2014) Effect of Rhizobium Inoculation and Supplementation with Phosphorus and Potassium on Growth and Total Leaf Chlorophyll (Chl) Content of Bush Bean Phaseolus vulgaris, L.. Agricultural Sciences, **5**, 1413-1426. doi: 10.4236/as.2014.514152.
31. Alcázar-Valle, M., Lugo-Cervantes, E., Mojica, L., Morales-Hernández, N., Reyes-Ramírez, H., Enríquez-Vara, J. N., & García-Morales, S. (2020). Bioactive Compounds, Antioxidant Activity, and Antinutritional Content of Legumes: A Comparison between Four Phaseolus Species. Molecules (Basel, Switzerland), 25(15), 3528. https://doi.org/10.3390/molecules25153528
32. Rodríguez Madrera, Roberto, Ana Campa Negrillo, Belén Suárez Valles, and Juan José Ferreira Fernández. 2021. "Phenolic Content and Antioxidant Activity in Seeds of Common Bean (Phaseolus vulgaris L.)" Foods10, no. 4: 864. https://doi.org/10.3390/foods10040864
33. García-Díaz, Y.D., Vera-Guzmán, A.M., Aquino-Bolaños, E.N., Muciño-Serrano, S. and Chávez-Servia, J.L. (2022). Phenolic Compounds, Antioxidant Activity and Mineral Contents in Grains of Phaseolus lunatus L. and P. coccineusL. Landraces. Legume Research. 45(9): 1155-1160. DOI: 10.18805/LRF-690.
34. Puertas Mejía, Miguel Angel, Mosquera-Mosquera, Nerly, & Rojano, Benjamín. (2016). Estudio de la capacidad antioxidante in vitro de Phaseolus vulgaris L. (frijol) mediante extracción asistida por microondas. Revista Cubana de Plantas Medicinales, 21(1), 42-50.