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Comparative effects of humic acid and charcoal on soil, growth, and biomass properties of lupine (*Lupinus albus* L.)

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Abstract

Recently, charcoal and humic acid application in soils is a burgeoning area of research due to its profound impact on soil properties and crop yields. Charcoal contributes to improved soil structure, enhanced water-holding capacity, and increased carbon sequestration while humic acid, a component of organic matter, enhances nutrient retention and availability, fostering healthier plant growth and long-term soil health. The experiments were conducted on November 20, 2022, at Grdarasha Research Station, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, Iraq. Two organic sources (humic acid and charcoal) were used to improve soil chemical properties, growth, and biomass characteristics of white lupines. Humic acid at the levels of (0, 10, and 15 g m^{-2}), respectively along with 1 kg m^{-2} of charcoal. Results showed that essential elements and heavy metals in soil were increased and improved with adding humic acid and charcoal, and then well-affected root ability to uptake nutrients. The great values of germination rate, leaf number (LN), and fresh and dry shoot weights were found when charcoal was stirred to the soil (83.33%, 15.67 LN $plant^{-1}$, 10.91, and 2.24 t ha^{-1}), respectively. While the longest root length was recorded when humic acid applied at rate of 10 g m^{-2} (24.22 cm), it was true about the enhancement of cluster roots. Despite that, humic acid at the rate of 15 g m^{-2} caused to improve fresh and dry root and shoot weights, which were compared to control treatment. The final results indicated that by adding humic acid (H), and charcoal (CH) could improve soil chemical properties, and then may affect positively microorganisms, which can promote plant growth by transforming, solubilizing, and mobilizing soil nutrients. As well, lupinus seemed as phytoremediation (uptake) in case of some heavy metals.

Keywords: lupin, heave metals, soil, root system, phytoremediation.

Introduction

Lupinus (*Lupinus albus* L.) have numerous coluors and forms. It is commonly known as lupin or lupine. Some of these species may annual and others under perennial herbaceous species. Generally, lupin includes 280 species, while not all of them have been appeared into the Integrated Taxonomic Information System (ITIS). Additionally, Ainouche and Bayer [1] reported that lupin is treated as a polymorphic species, while taxonomically its species name positions has been debated for a long time. White lupin belongs to Fabaceae family have the ability to nitrogen fixation, and indicated that it is tolerant to heavy metals and drought [2,3,4], furthermore lupines are also tolerant of high salinity, extra nitrate and sometimes it tolerances to extra calcium and soil acidity [3,5]. Previous researches were also indicated that lupin plant has high ability of developing ‘proteoid roots’ on which clusters of rootlets exude chelating agents: enzymes and organic anions such as probably phytase, hydrogen ions and phosphatase that this is occurred under nutrient deficiency circumstances, which is to enhance P, Fe, Mn and Zn acquisitions [6,7]. Based on the findings above about of lupinus could concluded that it's easy for planting, and also could be an excellent candidate for the initial phytoremediation of soils.

Any significant was not found between treated and untreated plants, so this was evidence of lupinus tolerances to Cd(II), Pb(II), Cr(III) and Hg(II) metals. Metals were also accumulated in roots, while Hg(II) translocated too fast for shoots compared to other metals [8].

Additionally, *Lupines albus* L. was used to P-uptake from contamination soils by phosphorus [9].

Results from the study were done previously [10] showed the impact of the application of humic acid (HA), whose stated that optimum yield in enhancing the physicochemical properties of the soil embankment recorded when 100 to 200 ml HA/0.12 m2 were added. Additionally, from the newest research, the importance of humic acid to the plants was again confirmed, which recommends that use of different types and levels of humic acid may increase the nutritive value of keanf leaves [11].

Four soils were contaminated by the addition of charcoal, so application of charcoal causes to improves microbial biomass and its activity, and also nutrient availability [12]. Amount of phosphorus and nitrate concentrations in the leachate also significantly decreased by adding each of the charcoal, sawdust and wood ash [13]. Other previous study reported that soil carbon as fulvic (10 to 20 cm) and humic acids (10 to 30 cm), and especially as humin (0 to 5 cm), increased due to increase charcoal which may be occurred by the role of the charcoal [14]. Moreover, charcoal and potassium were used to improve the growth and yield of flax plants, so from the results, the impact of both factors was clearly shown, which significantly improved the growth and productivity of flax, based on this finding do more researching on the charcoal and inorganic fertilizers on other crops are interested [15]. On the other hand, Johansson and Ekström [16] studied on Lupin mexicanus to remediate soil from arsenic contamination, this also by using biochar, which is how affect the phytoremediation of contaminants. Bahar *et al.* [17] also conclude that growth and yield parameters of faba bean (*Vcia faba* L.) were improved with adding charcoals and Nano-NPK fertilizers.

There has been little information on the potential effects of humic acid (H) and charcoal (CH) on essential elements and heavy metals in the soil and also affect growth, yield and yield components of white lupin. The purposes of doing this present study were to evaluate the probable effects of both organic substances on improving physicochemical characteristics of the soil and growth and productivity of the lupin plant. Also, to investigate the ability of lupin plant to tolerance, uptake and accumulation of several heavy metals.

Materials and Methods

Study site

The experimental area was located in Grdarasha Research Station, Department of Field Crops and Medicinal Plants, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, which is located at the governorate of Erbil, Kurdistan/Iraq (36° 00‘ 16“ N and 44° 01‘ 24“ E with 398 m above mean sea level).

Experimental design

The experiment was established on November 20, 2022 when the area was mechanically plowed, which was carried out in Grdarasha Research Station. Farm land was divided into several plots, which were replicated three times based on Randomized Complete Block Design (RCBD), white lupin seeds were received in Jordan country, which was cultivated in the same date above. A seed of lupin was put in each hole. Two factors in two separate experiments were investigated on lupin plant. The first one, used local charcoal (CH), which was applied on the soil surface, and then they were stirred together before planting (1 kg m^{-2}). In the second experiment different levels of humic acid were used, which were symbolled as (H0, H1 and H2), (0, 10 and 15 g m^{-2}), respectively added to the plants on December 27, 2022. Germination rate was calculated in the first week after planting date, while plant height, leaf number, other growth and yield parameters were collected on April 3, 2023.

Soil analysis

Table 1 shows the essential elements, heavy metals and physical properties of the soil were taken from the location of the study. Which was in the soil depth (0-30 cm), the samples were taken randomly in the several places in the farm before it was divided into plot treatments, this was only about the control treatments, while other soil samples were collected from the plot treatments (charcoal and humic acid), which were after plant harvesting. Afterward, the soil was air dried and sieved through a (2 mm) pore size sieve. Then, physicochemical parameters were determined in the laboratory.

Statistical analysis

All data were collected from this present study statistically analyzed based on the technique of analysis of variance (ANOVA), using IBM SPSS Statistics Program

(20), the mean comparison was fulfilled according to Duncan multiple range test at the level of significant ($P \leq 0.05$).

Table (1): Essential elements, heave metals and physical properties of the soil from the study site

Treatment	Essential elements			Heavy metals									Physical properties	
	N	P	K	Fe	Mn	Zn	Cu	Ca	Cr	Co	Pb	Ti	V	pH	Ec µS/cm
	(ppm)			(%)	(ppm)										
Con.	150.8	7.4	780.0	3.3	331.4	63.2	35.4	24.3	73.7	13.0	5.1	3611.9	91.6	8.5	428.0
Cha.	60.0	1.0	570.0	3.4	381.1	61.9	24.9	25.4	118.8	13.9	5.0	3977.6	63.5	8.4	276.0
H0	115.2	2.4	312.0	3.1	274.2	59.3	35.0	23.0	100.8	11.4	4.8	3933.0	20.0	8.4	232.0
H1	104.0	14.0	446.0	3.3	329.3	59.1	30.3	26.4	79.2	13.2	4.8	3959.2	81.8	8.3	323.0
H2	52.3	2.4	330.0	3.5	403.9	77.3	26.7	21.3	164.3	14.2	6.2	4354.5	99.6	8.4	249.0

Con.= control treatment, Cha.= charcoal (1 kg/m^2), H0= humic acid (0 g/m^2), H1= humic acid (10 g/m^2), H2= humic acid (15 g/m^2)N= nitrogen, P= phosphorus, K= potassium, Fe= iron, Mn= manganese, Zn= zinc, Cu= copper, Ca= calcium, Cr= chromium, Co= cobalt, Pb= lead, Ti= titanium, V= vanadium, pH= potential of hydrogen, Ec= electrical conductivity.

Results and Discussion

Germination percentages (%)

Fig. 1 illustrates the comparison impacts of humic acid and charcoal on the germination rates of *Lupinus albus* L. The x-axis denotes the treatments applied to the soil, including the control (no treated), humic acid, and charcoal. Meanwhile, the y-axis presents the percentage of successfully germinated seeds under each treatment.

The data obtained from the germination experiment showed that the highest germination rate was observed in the charcoal treatment (83.33 %), followed by the humic acid treatment (76.67%), and lastly the control treatment with the lowest germination rate (75%). These findings indicate that charcoal may have a greater capacity to promote the germination of *Lupinus albus* L. seeds compared to humic acid.

The outcomes of this study can provide valuable insights to farmers who seek to optimize the growth and yield of *Lupinus albus* L. through soil amendment strategies. Biochar application improved seed germination rate [18]. Additionally, as mentioned by previous researches seed germination and crop yields were increased, and also crop quality improved, when charcoal was added [19, 20, 21, 22, 23].

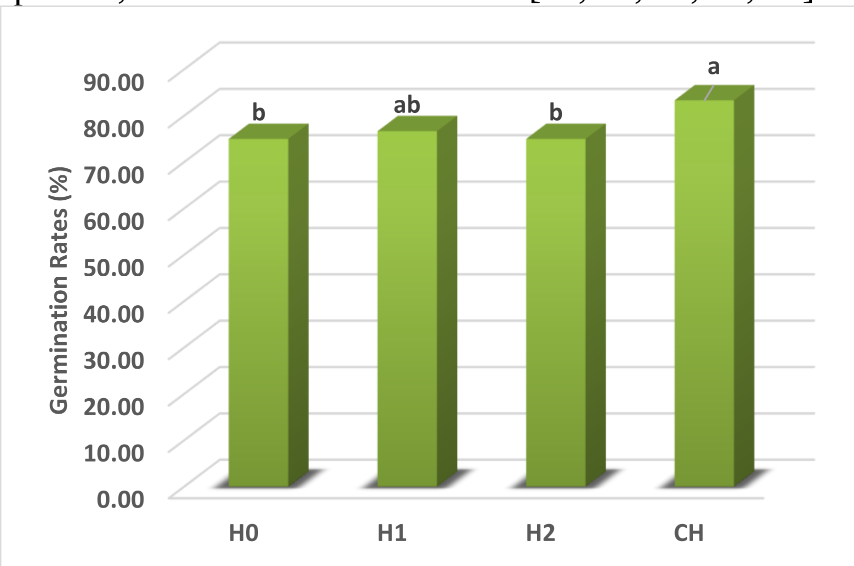

Figure (1): Effect of humic acid and charcoal on germination raties (%) of lupine plant. Bars with different letters reveal that the means are significantly different at level ≤ 0.05.

Root length (cm)

Fig. 2 presents the findings of the study on the role of humic acid (H) and charcoal to the *Lupinus* root lengths. The humic acid, charcoal, and control soil treatments are depicted on the x-axis, while the y-axis shows the root length measured in centimeters.

The data obtained from this figure revealed that the humic acid treatment (H1) resulted in a significantly higher root length (24.22 cm) compared to the charcoal and control treatments, which exhibited intermediate (21.89 cm) and lower (19.45) cm root lengths, respectively. Interestingly, the charcoal treatment significantly affect the root length of the plant compared to the control treatment. Outcomes were also suggesting that applying of humic acid and charcoal to the soil can greatly enhance the root length of L. *albus*. On the other hand, cluster roots and rootlets were enhanced especially with adding humic acid (Fig. 3). These findings are supported by the findings from previous studies, which indicated that adding humic substances and charcoal may changing soil structure; decrease bulk density, and increase porosity, additionally lead to increase infiltration capacity and finally soil erosion effects reduced [19, 24], all of these help roots take up mineral nutrients more efficiently, and for that purpose roots go to deeper soil as noted from this current study.

Martı´nez-Alcala´ *et al.* [25] stated that Zn, Cu, Cd, Pb, Al and As in their roots of white lupin plants were accumulated. It is in the same line with this present study, heavy metals were accumulated by the lupin plant roots as can be seen in table 1, which may have affected soil properties, microbial activity and crop production and quality.

Additionally, lupinus seemed efficient phytoremediator in case of Fe, Mn, Zn, Ca, Co, Pb and V heavy metals these were found when H0 treatment was compared to control treatment, soils were analyzed before and after planting lupin as shown in the (Table 1). On the other hand, by adding charcoal and humic, each of the elements (essential and heavy metals) became more active and easier to absorb by the roots of the lupinus.

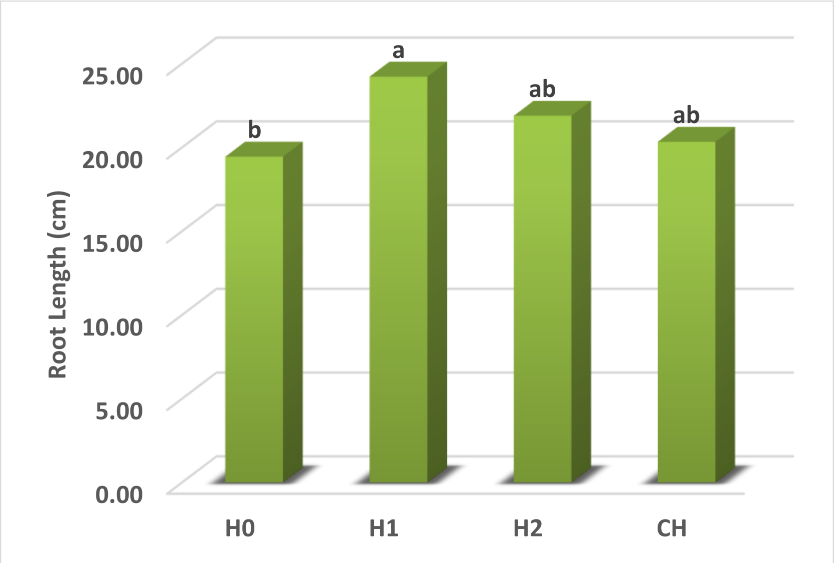

Figure (2): Effect of humic acid and charcoal on root length (cm) of lupine plant . Bars with different letters reveal that the means are significantly different at level ≤ 0.05.

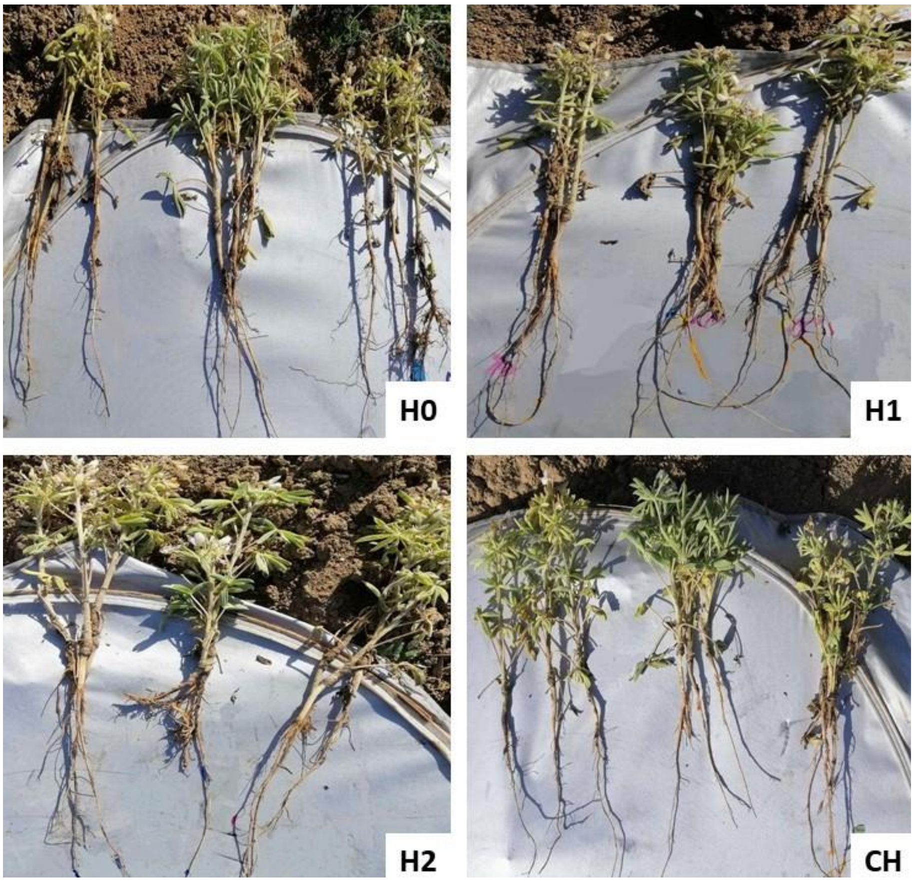

Figure (3): Lupinus root systam, cluster root, rootlets and root length; (H0) roots in the control treatment, (H1) roots of lupin when 10 g m^2 of humic acid was added to the plants, (H2) roots when 15 g m^2 of humic acid was applied, (CH) roots of lupin when charcoal was stirred to the plots.

In recently, humic acid and gibberellin were used of kenaf (*Hibiscus cannabinus* L.) and flax (*Linum usitatissimum* L.) fields, which were to enhance plant ability for absorbing heavy metals in the soil. Results showed that, each of plant have successful to absorb different heavy metals. Also, humic and gibberellin had the impact role to that purpose [26]. This once again confirms the findings of this new study, that plant types affect the absorption of heavy metals in the soil.

Plant height (cm)

Fig. 4 depicts the impact of humic acid and charcoal on the plant height of *Lupinus albus* L. The data suggests that the humic acid treatment and the charcoal treatment had a significant effect on plant height, while the control treatment resulted in the longest plant height, with an average height (17. 22 cm), followed by (16.30 cm) when humic added at the level of 15 g m^{-2} (H2). These were significantly higher than the plant heights observed in the charcoal treatment (14.89 cm) and the humic acid treatment at the level of 10 g m^{-2} (14.94 cm). These outcomes in same line with Sultan (2022) stated that plant height of kenaf plant was increased with increasing humic acid to 1.0 g 4 L^{-1} water m^{-2} (H2).

These results suggest that the application of humic acid and charcoal may have little impact on plant growth in terms of height. Despite that, it is essential to note that plant height is a crucial parameter in determining plant growth and development, which directly influences crop yield and productivity.

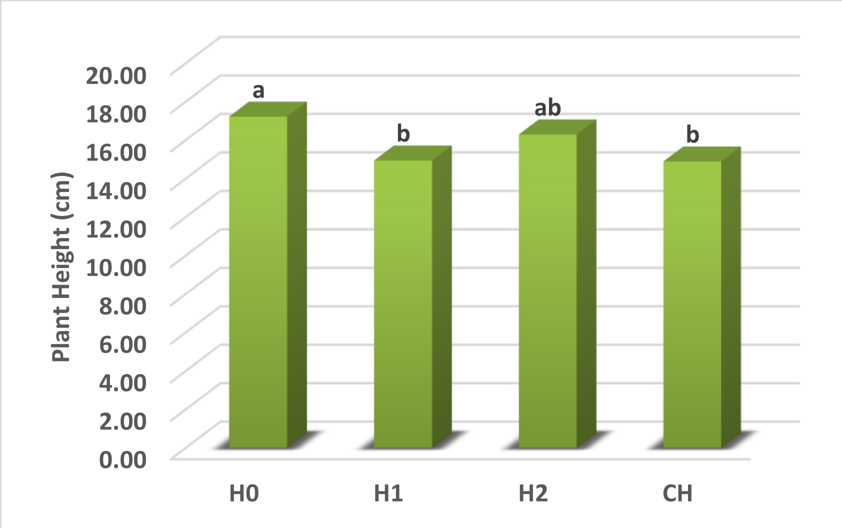

Figure (4): Effect of humic acid and charcoal on plant height (cm) of lupine. Bars with different letters reveal that the means are significantly different at level ≤ 0.05.

Leaf number plant^{-1}

Fig. 5 shows the effect of two different treatments, humic acid (H0, H1, and H2) and charcoal (CH), on the number of leaves produced by lupine plants. The statistical analysis showed that there is a significant difference in leaf numbers between treatments, as indicated by the bars with different letters. Specifically, the mean leaf number for the charcoal treatment (15.67) was significantly higher than those for the humic acid treatments (13.33, 13.00, and 12.67), which were not significantly different from each other. Same results were found about humic acid affected on leaf number of kenaf plant, which only little affected was noted [27].

Therefore, these findings suggest that the application of charcoal to lupine plants could be a more effective treatment than the application of humic acid in terms of increasing the number of leaves produced. These results may have practical implications for the cultivation of lupine plants, as increasing leaf number could improve overall plant productivity.

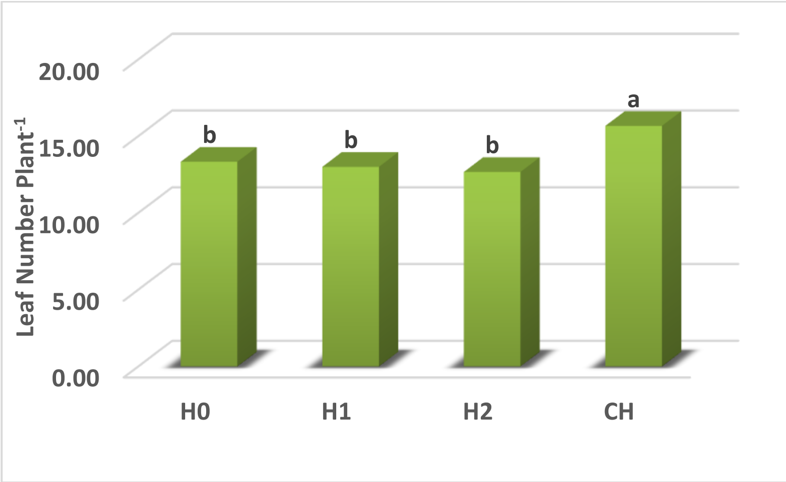

Figure (5): Effect of humic acid and charcoal on leaf number plant^{-1} of lupine plant. Bars with different letters reveal that the means are significantly different at level ≤ 0.05.

Fresh and dry root weight (t ha^{-1})

The effect of humic acid (H0, H1, and H2) and charcoal (CH), on the fresh and dry weight of roots in lupine plants were shown in (Fig. 6). The results indicate that there are some significant differences in the mean fresh and dry weight of roots among treatments, as evidenced by the bars with different letters in the figure, which denote significant differences at a level of 0.05.

The data show that the highest mean fresh and dry weight of roots were observed in the H0 treatment (4.93 and 1.42 t ha^{-1}) respectively, which were significantly higher than the other treatments. While, the lowest mean fresh and dry weight of roots were observed in the CH treatment (3.42 and 0.71 t ha^{-1}) respectively, which were significantly lower than all other treatments.

The results suggest that the application of humic acid, may have slight effective in increasing the fresh and dry weight of roots in lupine plants compared to the application of charcoal.

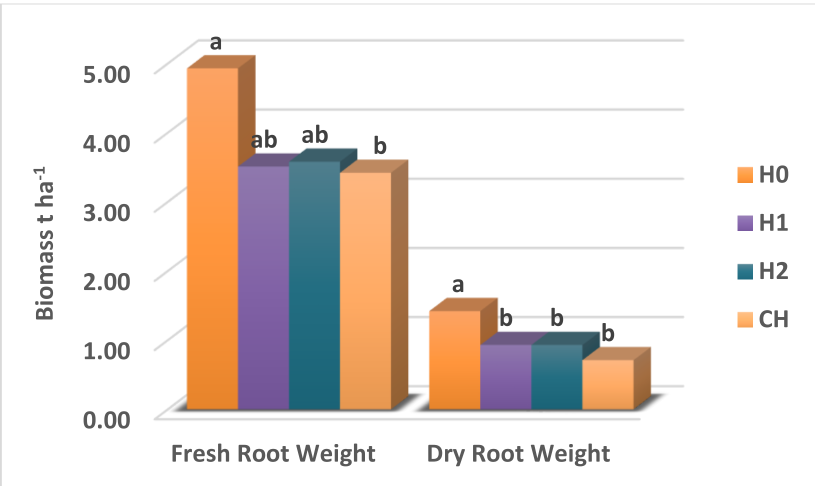

Figure (6): Effect of humic acid and charcoal on fresh and dry root weight t/ha of lupine. Bars with different letters reveal that the means are significantly different at level ≤ 0.05.

Fresh and dry shoot weight (t ha^{-1})

Figure (7) presents the results of the effect of humic acid (H0, H1, and H2) and charcoal (CH), on the fresh and dry weight of shoots in lupine plants. Statistical analysis was performed, and the results indicate that there is a significant difference in the mean fresh and dry weight of shoots among treatments, as demonstrated by the bars with different letters in the figure.

The results indicate that the mean fresh and dry weight of shoots for the CH treatment (10.91 and 2.24 t ha^{-1}), respectively were significantly higher than that of the humic acid treatments (H0: 6.22, and 1.38; H1: 6.22 and 1.33; H2: 6.8 and 1.47 t ha^{-1}), which were not significantly different from each other.

Therefore, the results suggest that the application of charcoal to lupine plants could be a more effective treatment than humic acid in terms of increasing the fresh and dry weight of shoots. These findings have practical implications for the cultivation of lupine plants, as increasing shoot weight could improve overall plant productivity.

As mentioned previously in the introduction section charcoal increased soil carbon as fulvic. That case may have significant affected in enhancing growth and biomass productivity as found in this current study (Figure 7).

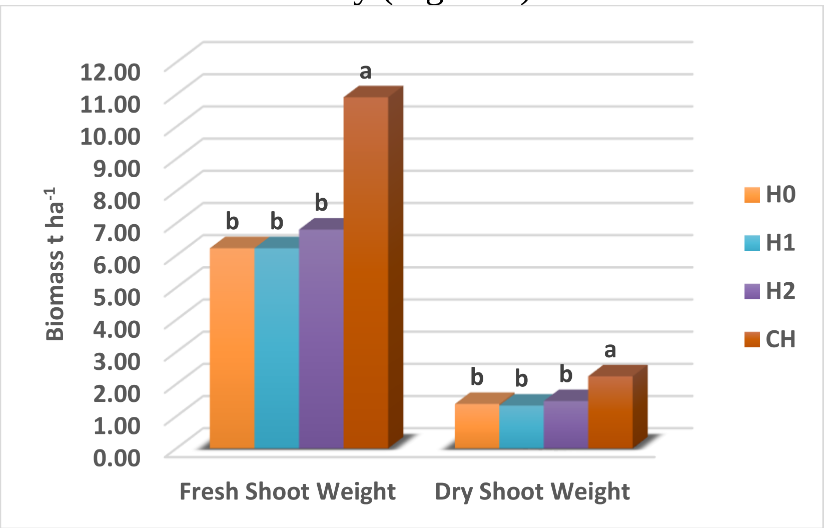

Figure (7): Effect of humic acid and charcoal on fresh and dry shoot weight t/ha of lupine plant. Bars with different letters reveal that the means are significantly different at level ≤ 0.05.

The addition of humic acid and charcoal in two separate experiments to white lupines caused to show the results of growth and biomass parameters were statistically different. The highest values of seed germination rate, leaf number, fresh and dry shoot weights were found of charcoal treatments. While, the longest root length was noted when humic acid added to the plants at the rate of 10 g m-2, followed by (H1 and CH), respectively. Cluster root was also enhancing by adding humic acid and charcoal. Despite that, the highest plant height and the great values of fresh and dry root weights were found in the control treatment. On the other hand, soil chemical properties are too different between both experiments. The addition of humic acid and charcoal improved root's ability to uptake essential elements (N, P, and K), phosphorus, and potassium were increased when humic acid was added especially at a rate of 10 g m-2. Titanium (Ti), and vanadium (V) are both heavy metals, which increased with increasing humic acid and also adding of charcoal. Both metals are importance to the human body as antioxidants and for diabetes, low blood sugar, high cholesterol, and heart disease, additionally for normal bone growth as mentioned by previous researchers. Finally, could be concluded that the addition of humic and charcoal is requested by agronomists, which are not only to improve soil chemical-physical properties but also to improve crop production in a manner that is healthy for human use. Heavy metals were also absorbed by the lupin plants which was as phytoremediation as a strategic way that has numerous benefits to the soil.

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Author Contributions

This submitted manuscript is original and it has not been published or is not under consideration for publication elsewhere.

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