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Physico-Chemical Parameters of Humic Substances of Brown Coal and their Effect on the Growth of *Phoenix dactylifera*

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Abstract: The motivation for the study was the need to obtain data on the effect of humic acids isolated from brown coal on soil fertility and plant development. In our study, we conducted experiments with plants to isolate humic substances from brown coal and study its physicochemical properties and the effect of the resulting fraction on the growth of *Phoenix dactylifera* date palm plants in laboratory conditions. The isolated humic acids had a density of 1.0416 g/cm^3 and the dynamic viscosity was 1.84*10^{-3} Pa*s. The mass fraction of humic substances was 56%. As a result, when watering date palm plants with a 1% solution of humic substances, plant height increased by 41.5 in 1 month, leaf width by 80, and leaf length by 15%, compared with the control variant. The results of physicochemical experiments prove that humic substances from brown coal promote the level of plant nutrition.

Keywords: Humic Substances, Brown Coal, Soil, *Phoenix dactylifera*

Introduction

According to the World Meteorological Organization (WMO, 2023) every year about 12 million ha of productive land becomes infertile due to desertification and drought. Kazakhstan is no exception. Currently, out of 182 million ha of pasturelands, 14 million ha have been completely withdrawn from rotation and the total area of degradation has exceeded 50 million ha. Thus, studies in the search for environmentally friendly methods of farming and landscaping to reduce the level of land degradation using affordable materials are relevant in all countries of the world.

According to the Bureau of National Statistics, more than 3.9 million t of brown coal were mined in Kazakhstan in 2020 (Financial Market, 2020). Due to their higher oxidation state and lower calorific value, they are not suitable for use as fuel for electricity or coking coal production. HA is abundant and naturally occurring in coal. However, HA obtained directly from coal often has problems with low yield, high ash content, and a limited amount of active functional group (De Melo *et al*., 2016). As two determining factors, the choice of raw materials and the isolation procedure will affect the chemical nature and efficiency of extraction of the obtained HA from a potential organic source (Janeba-Bartoszewicz *et al*., 2019).

Many studies have shown the effect of HA on the growth of date palms both in natural and artificial conditions (Al-Mayahi, 2021). This crop has the potential to be grown in conditions of salinity, drought, and high temperatures (Tama and Hameed, 2021). The leading components of landscaping cities and towns are perennial and annual crops that carry a certain functional load, affect the microclimate, increase ionization and air purification, create an anti-noise effect, and perform health functions. Increasing the plantings of perennial ornamental crops is economically beneficial for urban greening. Recently, there has been a strong trend in demand for various types of palms that do not need special care and are resistant to the prevailing number of diseases. The introduction and expansion of the range of palm trees in large Kazakh cities depend on scientific research to strengthen their resistance to harsh ecotopic conditions, biotic factors, and insect pests in Northern Kazakhstan. In the conditions of a changing climate and the sharply increased need to prevent desertification, the date palm has great prospects.

The properties of HS from various sources with a certain composition, isolated by different technologies, in the form of soil dressing or foliar dressing, still require research for many crops, both agricultural and ornamental ones.

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Therefore, the purpose of this study is to evaluate the effect of the physicochemical characteristics of HS isolated from brown coal from the Maikobe deposit (Republic of Kazakhstan) on the growth and development of the *P. dactylifera* date palm to increase the level of plant adaptation. The study consists of five sections, namely the introduction, materials and methods, results, discussion, and conclusion.

Materials and Methods

The study was conducted in the period from March 2021-March 2022 at the Institute of Coal Chemistry and Technology LLP, Astana, Kazakhstan, and Kazakh Agrotechnical University named after S. Seifullin NJSC, Astana, Kazakhstan. Date palm plants were grown under controlled conditions of a climate chamber for 1 year, which was sufficient to obtain reliable data on the stimulation of plant growth and development when using HS.

Coal Samples

Coal samples were taken from five different points of the Maikube brown coal basin, including the Shoptykol, Sarykol, and Taldykol deposits. The Maikube section has a design capacity of 20 million t/year (Ermagambet *et al*., 2020). The coals of the basin are humus, brown, with a high degree of carbonification (B_3) and medium ash (25-28%). Refractory ash with a high content of $A1_2O_3$ (up to 30), low-sulfur coals (0.5-1.0), and multi-phosphorous coals (0.1%). The heat of combustion per combustible mass is 29.3-31.4 kJ/kg. The total coal reserves of the basin are estimated at 5.3 billion t, including 1.8 billion t suitable for open-pit mining at stripping up to 10 m^3/t (Yermagambet *et al*., 2021).

Oxidized brown coal of the Maikube deposit, pre-crushed to a size of less than 0.5 mm and having the following characteristics (wt.%) was used as a feedstock for humate production: A^d 66.09; W^r 5.73; V^d 17.78; S_t^d 0.71; C_t^d 21.01; H_t^d 1.68; N_t^d 2.09; Na 0.61; Al 0.89; K 0.58; Ca 0.31; Ti 0.22; Fe 1.11; Zr 0.08.

The size of coal particles was 2.95 microns (10), 63.8 microns (50), and 452 microns (90%). The X-ray phase composition of the sample contains halloysite ($Al_2Si_2O_5(OH)_4$), Silicon Oxide (SiO_2), and albite Na ($AlSi_3O_8$). The yield of HS from brown coal was 56%. Further, the physicochemical properties of the obtained fraction were studied.

Isolation of HS

The study of the fraction of isolated HS according to the described technology and its effect on plants is necessary to confirm the bio *dactylifera* chemical properties of the drug, its non-toxicity for plants, and the possibility of using it as a growth stimulant. Each experiment was carried out in three replicates.

Coal samples for HA extraction were first oxidized using 2% HNO_3. A 40-gram sample of each coal was mixed with 100 mL of 2% HNO_3 solution in beakers. The contents of the glass were gently stirred for 1 h. The contents were then filtered and washed to remove unreacted acid from the oxidized coals. Samples of oxidized coal were dried in a furnace at 40°C and stored in sealed plastic bags for use in the extraction of HA.

The oxidized samples of each coal were treated with 3% KOH solutions and stirred for 3 h and then the contents were filtered out. The filtrate of each sample containing soluble acids was stored in hermetically sealed bottles for further testing (Zhang *et al*., 2017).

The following instruments were used to determine the quality of raw materials and the content of HS: Scanning electron microscope *SEM (Quanta 3D 200i) (Netherlands)* with a prefix for energy dispersion analysis from *EDAX (USA)*, Infrared (IR) Fourier spectrometer *(NicoletiS 10) (USA)*, CHNS/O element analyzer *(Perkin Elmer) (Germany)*, particle size analyzer *(Mastersizer 3000) (UK)*.

Experiments with Plants

Decorative cultures of the *P. dactylifera* genus were used as the object of the study. To obtain reliable statistical data, we used a total of 200 date palm seeds, which were distributed into the treated and control variants, 100 seeds in each group. A solution of 0.5, 1, and 1.5% humate was used as an experimental material for the work. Before planting, the seeds were soaked in water (control variant) and 1% humate solution (treated variant) for 2 days. After that, the seeds were placed in a soil mixture for germination in a light room (humidity: 60%, temperature: 24-26°C, illumination: 32,000 lux, light period: 16/8 h). The first shoots were observed after 25 days. The control variants were watered as the soil dried, depending on the time of year. The treated plants were watered with a solution of humate of a given concentration every 12 days as a soil dressing. In the case of drying the soil, irrigation was carried out with tap water. The plants were grown in a soil mixture (peat + perlite) at a temperature of 18-26°C Fig. 1.

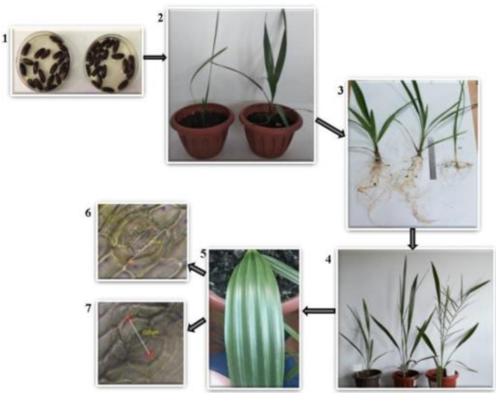

Fig. 1: Abstraction of the experiment: 1-seeds soaking; 2-palm plants after 1 month; 3-phenological measurements; 4-palm plants 2 years after planting in the soil mixture; 5-palm leaf treated with 1% hamate; 6, 7-the size of the stomata of the leaf

Despite the positive results obtained on the effect of the obtained fraction of HS from brown coal on the growth of date palms in the phase of germination and primary growth (1 month), the monitoring of plants was continued for the next 12 months. Since the plants were grown under artificial conditions in vegetative vessels, HS could show toxicity, since they have properties to accumulate in the soil and enhance the effect on plant growth. During the growth process, visual observations were carried out, plant growth characteristics (plant height, leaf width, and length) were measured and leaf sections were microscoped using the method of casts (prints), thin sections were made and microscopic objects were fixed and measured.

The determination of the Percentage of (P) was based on the ability of phosphoric acid to give a blue coloration with ammonium molybdenum acid in the presence of stannous chloride in a dried leaf sample using hydrochloric acid. The content of N and K was determined by ashing a sample of leaves with 0.2 g of concentrated sulfuric acid and hydrogen peroxide. In the resulting solution, we determined nitrogen colorimetric ally and potassium flame photometrically (Ibrahim and Al-Wasfy, 2014). The method for determining Ca was based on the ability of calcium to form a pink complex compound with the indicator murexide (ammonium salt of purple acid $C_8H_4O_6N_{15}NH_4$) (Moran, 1982). The determination of Mg (Chapman and Pratt, 1961) was based on the creation of stable complexes with EDTA. With the formation of colorless complex compounds, the equivalent point is determined by the change in color of the black chromogen.

Statistical Analysis

Standard statistical analysis was conducted using Student's T-test (t) and the p-values (p) in Microsoft Excel 2010.

Results

The main indicators according to international standards are the content of substances such as Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S), and Oxygen (O) in humate. The saturation of the fraction with these components determines the quality and value of HS. The main elements are always present, regardless of their origin, country, or continent. After the isolation of HA from brown coal using a 3% KOH solution, the elemental composition was studied. The data are presented in Table 1.

The resulting HS was a dark brown liquid with a density of 1.0416 g/cm3 and the dynamic viscosity of the resulting sample was 1.84*10-3 Pa*s. The isolated fraction contained 22.205 C and 20.4435% O, which characterizes it as saturated with these elements. The H and N content was at the level of 1.9 and 3.5%, respectively. The S content was the lowest (0.6775%), which is typical for HS. According to the obtained atomic and percentage ratio, the chemical formula of the resulting extract was C95N95O65N15S1.

Besides, one of the main indicators of the quality of HA is the particle size, which determines the availability of HS for plants, their solubility in soil, and the absence of large agglomerates. Figure 2 shows the distribution of the particle size of HS and the volume of fractions.

After the rotary cavitation machine and the ultrasound machine, the particle size averaged from 19.2 nm-3.57 microns. According to the results, two fractions with a particle size from 0.01-0.1 microns were isolated, with a maximum content of more than 40% of the volume, as well as the second fraction containing particles from 0.15-1.0 microns in size.

When studying the isolated fraction of HS using IR spectrophotometry (Fig. 3), wide peaks were noted in the region of 3,262 cm-1, which were attributed to the valency fluctuations of such bonds as OH and COOH.

Bending vibrations of the methyl and methylene groups at 1,363 cm-1 and stretching vibrations of the C-O bond in alcohols, phenols, and others also confirm the presence of these functional groups.

Further, the influence of HS on the germination energy, growth, and development of date palm plants was studied. The results of the experiment are presented in Table 2.

The results showed that the treatment with HS promoted the growth of seedlings. On the 20th day after soaking, germination in the treated variant was 76%, which was 34% more than in the control variant. On the 25th day, germination increased significantly, but in the control variant, it was 29% lower than in the treated one. The total germination of seeds treated with HS was 92%, while in the control variant, it equaled 63%. These results indicate that HS, even with short-term soaking, contributes to an increase in the energy of seed germination.

According to the conducted studies, the date palm was responsive to the introduction of HS during the growth period.

Figures 4-5 show the observation data for 1 month and 1 year of cultivation.

As a result of the conducted experiments, a visual difference between the control and the treated variants was noted. The height of palm plants a month after germination in the control variant was 18.8 cm and in the treated variant, it was 26.6, which is 41% higher. Significant differences were also noted in the length of the stem. Thus, in the control variant, this indicator was 4.8 cm, while in the treated variant, it was 7.4 cm. The difference between the control and treated variants was 56%. The length of the leaf is the most important indicator for the date palm, as it performs the main decorative functions and acts as the main photosynthetic organ. In the treated variant, as in all indicators, the value was higher than in the control variant and amounted to 17.3 cm. In the control variant, an average value of 15.4 was noted, which was 15% lower than the treated variant. The leaf width in the control variant was 0.56 cm, which is 52% less than the treated variant (0.85 cm). According to the results obtained, it can be concluded that with the introduction of a 1% humate solution, the growth rates are significantly higher than in the control variant. The remaining three indicators (the length of the stem, the length of the leaf, and the width of the leaf) in these variants were almost identical and significantly higher than the control variant.

Table 1: Average values of C, H, N, S and O

	Content, %					Atomic relations				
Name	C	H	N	S	O	C/H	C/O	C/N	C/S	Chemical formula
Humate	22.205±0.1	1.9065±0.3	3.5±0.3	0.6775±0.1	20.4435±0.1	0.979	0.447	7.411	87.49	$C_{95}H_{95}O_{65}N_{15}S_1$
Atomic Fraction	01.85	1.89	0.25	0.02	01.28					
Percentage						C/H	C/O	C/N	C/S	

Table 2: Effect of HS on seed germination (% of germinated seeds)

	Duration of the germination period, days	
A variant	on day 20	on day 25
HS	76±1.3	92±1.7
Control (%)	42±2.0	63±1.5

Note: The p-value was calculated between the control and treated variant ($p<0.001$)

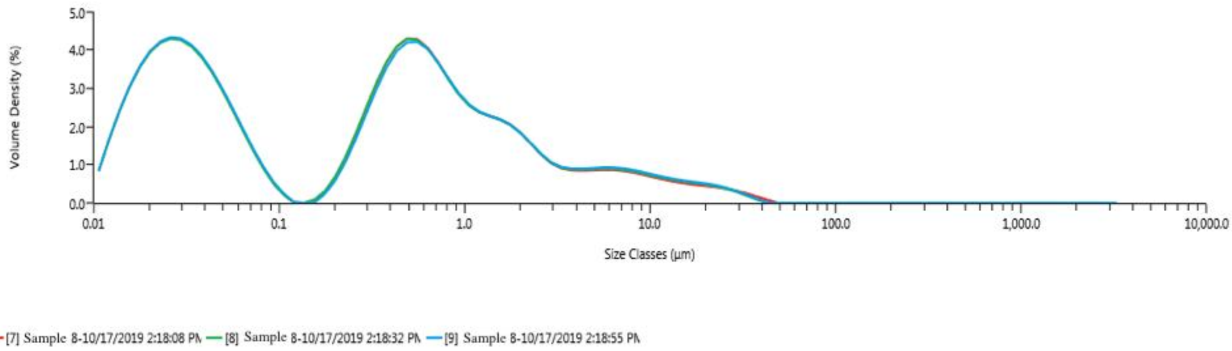

Fig. 2: Distribution of particle sizes of HS

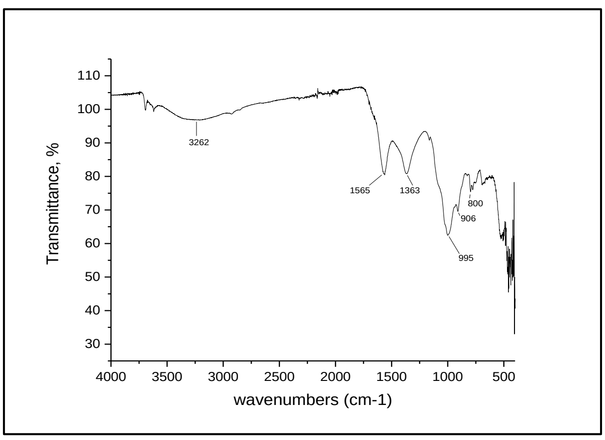

Fig. 3: IR spectrum of HS

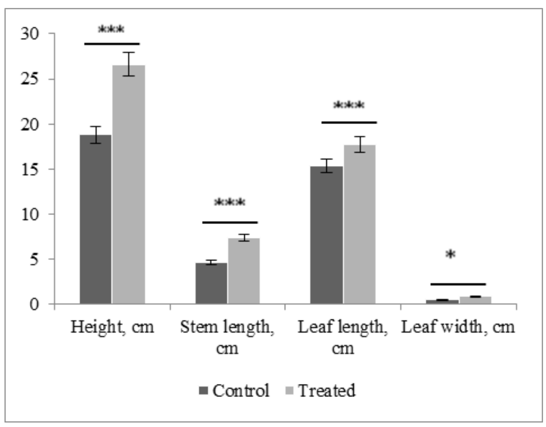

Fig. 4: Results of monitoring of date palm growth during the intervals of 1 month (***$p<0.001$, **$p<0.01$, *$p<0.05$)

Table 3: Effect of soil dressing of 1% HS on the total content of chlorophylls and the content of N, P, K, Ca, and Mg in date palm leaves

A variant of the experiment	Total chlorophylls (mg/g of raw mass)	N, %	P, %	K, %	Ca, %	Mg, %
Control	48.5±0.03[b]	1.41±0.01[b]	0.16±0.03[b]	1.32±0.03[a]	1.86±0.01[a]	0.40±0.01[a]
Treated	52.4±0.03[a]	1.73±0.02[a]	0.28±0.02[a]	1.51±0.03[a]	2.03±0.02[a]	0.49±0.01[a]

*The letters a and b indicate the reliability of the data; the same letters mean the absence of significant differences between the data

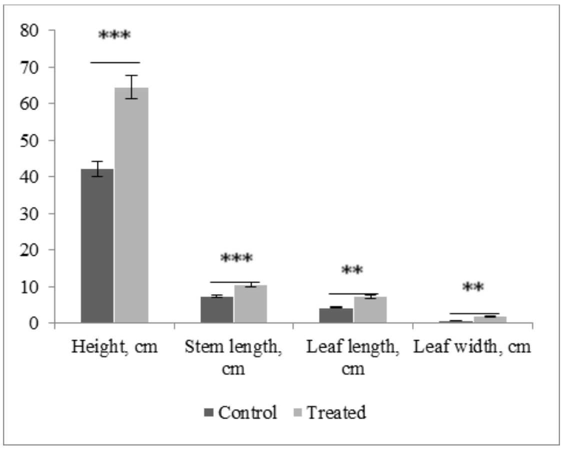

Fig. 5: Results of monitoring of date palm growth during the intervals of 1 year (***p<0.001, **p<0.01, *p<0.05)

After 1 year of observations, no toxic effect of HS was observed on date palm plants. Thus, the height of plants in the treated variant was 53% higher compared to the variant (42 cm). The length of the stem in the treated variant was 10.5 cm, while in the control variant, it was 7.4 cm, which is 43% lower. The length of the leaf with the use of soil dressing of 1% HS every 12 days was 2.5 times bigger in the treated variant. The width of the leaf was 190% more in the treated variant, compared with the control variant.

The conducted studies indicate that the introduction of 1% HS into the soil will significantly increase the growth rates of the date palm while increasing all growth indicators.

Table 3 presents the data from biochemical studies of the state of plants, the accumulation of macronutrients, and the amount of chlorophyll.

HS had a significant effect on the chemical properties of the leaves of the date palm plants and caused an increase in all the studied indicators. A 1% solution of HS increased the percentage of total chlorophyll in the leaves by 8% compared to the control variant. Concerning the five main components of the tissue, similar results were obtained: An increase in N content by 22, Ph by 75, P by 14, Ca by 9, and Mg by 22.5%.

Discussion

The analysis of the literature data demonstrated that according to the elemental composition of HS, the percentage composition of C, O, H, and N varies in the range as follows: C (45-60), O (25-45), H (4-7), N (2-5) and inorganic elements (ash) 0.5-5. HA has different contents of these components depending on the source, the limits varying within the following range: C: 37.2-75.8, O: 7.9-56.6, H: 1.6-11.7, N: 0.5-10.5, and S: 0.1-8.3%.

Zara *et al*. (2017) obtained a fraction of HS from 13.5 to 29.6% when isolating with 3.5% KOH. For example, the yield of HA from rice compost was 13.4% (Ch'ng *et al*., 2018). In F. Noureen's studies (Fatima *et al*., 2021), the isolation of HS from low-quality brown coal yielded up to 57.8%, while the yield from high-quality bituminous coal equaled 49.6%. The extraction of HS from the soil in one of the regions of India for the cultivation of tea with the use of sodium hydroxide as an extractant was 61%.

The data obtained by IR spectrometry are consistent with similar studies, where HS were isolated from the soil, which showed a wide absorption centered in the areas of 3,360, 1,406, 1,233, and 1,060 cm^{-1} (De Hita *et al*., 2020). Subbituminous coal as a source shows the presence of aliphatic bands (2,900-2,860 cm^{-1}, 1,460 cm^{-1}) and aromatic components (1,600 cm^{-1}). Oxygen-containing groups are represented by bands ∼3,600, ^{2,600}, 1,715, and 1,200 cm^{-1} (Zhou *et al*., 2019).

The isolated HS fraction from coal from one of the deposits in China shows relative absorption at 3,427 cm^{-1} C = C in aromatic rings and stretching of O-H into alcohol and phenolic groups. Weak absorption peaks of COO are at 1,580 cm^{-1} with COO-symmetric stretching, N-H deformation, and C ≡ N stretching. Absorption peaks at 1,382 cm^{-1} are associated with OH deformation and C-O stretching in phenolic OH, deformation of C-H groups CH_2 and CH_3, and CO-antisymmetric stretching. HA has the weakest absorption peak at 1,108 cm^{-1}, which is associated with the stretching of C-O polysaccharides or polysaccharide-like substances and Si-O from silicate impurities. The absorption peak at 619 cm is caused by deformation and vibration of the surface of the C-N (Fekry *et al*., 2020).

Thus, HS obtained from brown coal by particle size and volume of fractions, by elemental composition and saturation with O and H bonds, may have the potential of being used as a biofertilizer/growth stimulator for plants.

Regarding experiments to observe the growth and development of the date palm, no similar studies have been found in the literature. However, Ahmed *et al*. (2021) noted that when applied to the soil with a frequency of 1 every 2 weeks at a concentration of 15 mL/l, it stimulated the growth of the date palm.

Conclusion

The results obtained from the study of the effect of HS on the growth and development of date palms prove that HS obtained from brown coal stimulates the energy of germination, growth indicators, and biochemical parameters in plant leaves. The fact that the level of total chlorophyll increased by 8% after 1 year of cultivation indicates the ability of HS to increase photosynthetic activity, thereby increasing the level of O and Carbon Dioxide (CO) consumption from the air. An increase in nutrients in the leaves indicates the availability of the obtained HS fraction for plants. The obtained results of this study confirm that HS can become an effective component of biofertilizers and plant growth stimulants. The introduction of both soil and foliar dressing, albeit in a minimal concentration, affects the growth of crops. The results obtained in the course of the study prove that the HS obtained from brown coal according to the described method complies with international requirements and quality standards and can be recommended for use in crop production to increase the growth of ornamental plants. In the field of application of humates, future research is needed to obtain data on the effect on the growth and development of other types of ornamental and agricultural plants.

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Author's Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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