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Research article

Synergistic effects of coal waste derived humic substances and inorganic fertilizer as soil amendments for barley in sandy soil

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ABSTRACT

Increasing pressures on land resources requires increased land use efficiency. Over 900 million ha of sandy soils throughout the world are extensively used for agricultural crop production, most requiring nutrient inputs. Although use of humic substances together with inorganic fertilizer as soil amendments has been introduced, their synergistic effects on plant growth in sandy soils are not well addressed. We assessed the efficacy of a lignite waste derived humic substance on barley (*Hordeum vulgare* L.) growth, with and without inorganic fertilizer. Ten treatments were applied to sandy soils, comprising sole application of the humic product at four rates (NH1, NH2, NH3, NH4), sole application of fertilizer (F), and their combinations (F + NH1, F + NH2, F + NH3, F + NH4). Synergistic effects of nano humus and fertilizer were more notable than the corresponding sole application, particularly on plant biomass and seed production. Combined application with inorganic fertilizer increased root biomass by 92 % (0.1 g per plant), shoot biomass by 80 % (0.5 g per plant), root length by 24 % (3.6 cm), and seed production by 38 % (5 seeds per head) averagely relative to the untreated control, suggesting a strong synergistic effect. The increased seed production was particularly important from an agricultural perspective. Four application rates of nano humus all showed beneficial effects on barley growth with no significant differences. The most distinct positive effect of the humic product as a sole application was on root growth. Our study confirmed that a lignite waste derived humic product, nano humus, together with fertilizer may be an effective soil amendment to enhance agricultural plant growth in sandy soil regions.

1. Introduction

Sandy soils are among the most widespread soils throughout the world, covering 900 million ha (7 %) of the land surface [1]. With increasing population and food security pressures on global land resources, sandy soils are being cultivated more intensely. Since sandy soils often have low nutrient contents, chemical fertilizers have been widely used to obtain maximum crop yield. However, sandy soils are particularly prone to nutrient losses due to their low nutrient holding capacity, resulting in limited benefits of inorganic fertilizer to plant growth [2,3]. Excessive chemical fertilizers will be wasted, adversely influencing soil health and ground water quality [4,5]. Thus, there is a growing demand for efficient and environmentally friendly materials to amend sandy soils and improve nutrient retention.

Humic substances as natural biostimulators have been widely discussed for environmental and agricultural uses [6,7]. They are

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complex, heterogeneous mixtures of polydispersed materials [8] that are naturally formed in soils, waters, sediments, and organic geological deposits [9]. Most humic substance commercial products are derived from leonardite or lignite waste from mining [10,11]. The value and potential of humic materials in environmental and agricultural applications are uncertain, with some studies showing beneficial effects on plant growth [12–14] and others without positive impacts [15,16]. Coal waste derived humic material could promote plant growth in sandy soils and its efficacy may be linked to application rate [17]. In general, within a specific range of application rates of humic materials, higher rates resulted in better plant growth relative to lower rates [18,19].

Recent research has suggested the potential for exploring humic substances - fertilizer synergisms to improve nutrient retention and slow down or control the release of nutrients into soils [20]. This has been further supported by studies recognizing the improved availability of soil nutrients [21,22]. Khan et al. [23] reported enhanced nutrient uptake in plants when fertilizer was amended with humic substances, resulting in a notable reduction (25 %) in the needed amount of fertilizer. Martins et al. [24] emphasized the significance of soil type in determining the synergistic effects, particularly in nutrient poor soils where substantial benefits were observed. Despite the possible benefits, little is known about the synergistic effects on crops in sandy soils. More research is needed to develop effective formulations and understand the implications of these synergistic effects. Hence, the main objective of our study was to assess effects of a lignite waste derived humic substance product, nano humus, with and without inorganic fertilizer, on barley growth parameters in sandy soil.

2. Materials and methods

2.1. Materials

We used barley (*Hordeum vulgare* L.) in our study as it is a common agricultural crop around the world. Barley seeds were procured from a local commercial farm (Tribend Ranch Limited). Before the experiment, a seedling emergence test was conducted in the experimental sandy soil to determine seeding density for the experiment. Seedling emergence was low in sandy soil at 36 % within 7 days.

The experimental sandy soil was collected near the town of Devon in Alberta, Canada (53°24′27.27″N, 113°45′34.94″W). The collected soil was thoroughly mixed so all pots would have the same soil. Three soil samples were analysed at a local commercial laboratory. Particle size (sand, silt, clay) was determined by hydrometer after treatment with calgon [25]. The experimental soil was a loamy sand texture (84.3 % sand, 8.8 % silt, 6.8 % clay) and neutral pH (7.3). Soil pH and electrical conductivity (1:10 soil water suspension ratio) were determined using a pH and conductivity meter (Oakton 300 Series, Oakton Instruments, IL, USA). Soil electrical conductivity was 0.7 dS/m. Total soil carbon was determined by combustion [25], available nitrate and sulphate by calcium chloride extraction [26], and available phosphorus and potassium by modified Kelowna extraction [27].Total carbon (4.5 %), available nitrogen (18.4 mg/kg), available phosphorus (18.9 mg/kg), available potassium (90.3 mg/kg), and available sulphur (206.7 mg/kg) were low.

Nano humus® is a lignite waste derived humic substance commercial product used as an organic soil amendment in the experiment. The product is black fine grained and solid, with recommendations to spray directly on the soil surface as aqueous suspensions (1:100 solid to water ratio). It contains 83.2 % organic matter, 50 % humic acid, and 1 % micronutrients (copper, iron, zinc, aluminum, manganese, boron); macronutrients include 0.86 % nitrogen, 1.11 % phosphorus (P_2O_5), 5 % potassium (K_2O), 0.29 % sulphur, and 0.34 % magnesium; with 4 % silica sand and ash by weight. Nano humus pH was 8.98 and electrical conductivity 0.08 dS/m (Oakton 300 Series, IL, USA). It has a surface area of 2.05 m^2/g, a mean particle diameters of 7 μm, and a mean pore diameter of 37.43 nm. Surface chemical characteristics were determined by Fourier transform infrared (FTIR) spectroscopy (Thermo Scientific Nicolet, model iS50, Madison, WI, USA).

A water soluble inorganic fertilizer (Plant Prod®) was used in the experiment. It was general purpose, containing 20 % total nitrogen, 20 % available phosphoric acid, and 20 % soluble potash.

2.2. Experimental design and procedures

The experiment was conducted under controlled greenhouse conditions (22 °C, 16 h photoperiod) for 110 days. A completely randomized experimental design was implemented with ten treatments, replicated six times, for a total of 60 experimental units (10 treatments x 6 replicates); there were 5 subsamples (measurements) per replicate. The 10 treatments comprised sole applications nano humus at four rates (NH1, NH2, NH3, NH4), sole application of fertilizer (F) at a fixed rate, and combined applications of nano humus and fertilizer at four rates (F + NH1, F + NH2, F + NH3, F + NH4), and an untreated control (CON). NH1 = 2.5 g nano humus per pot (750 kg/ha), NH2 = 5 g nano humus per pot (1500 kg/ha), NH3 = 7.5 g nano humus per pot (2250 kg/ha)L, NH4 = 10 g nano humus per pot (3000 kg/ha), F + NH1 = 20-20-20 fertilizer with 2.5 g nano humus (100 mg/L), F + NH2 = 20-20-20 fertilizer with 5 g nano humus (100 mg/L), F + NH3 = 20-20-20 fertilizer with 7.5 g nano humus (100 mg/L), F + NH4 = 250 ml fertilizer with 10 g nano humus per pot, CON = untreated. Four nano humus application rates were between 0.5 and 2 times the manufacturer's recommended rate for agriculture usage in sandy soil, which is equivalent to 5 g per plant pot at 1 % suspension concentration by weight. Liquid fertilizer was applied at a concentration of 100 mg/L and 250 ml per pot as recommended by the manufacturer.

Pots were 20.32 cm diameter and 13.97 cm height, with a tray under each to collect leachates which were poured back into the pot after each watering. Pots were each filled with approximately 1 gallon of sandy soil, to approximately 5 cm from the top, then randomly placed in trays on a greenhouse bench. Seeds (15 per pot) were placed at 1–2 cm depth in each pot, then covered lightly with soil. One week after germination completion in week 2, barley was thinned to 5 plants per pot. In week 3, nano humus and fertilizer were sprayed separately onto the soil surface of each treatment pot using injectors. Barley seedlings were watered twice a week to approximate a predetermined field capacity.

2.3. Plant measurements

Plant height was measured before applying fertilizer and nano humus treatments (week 3), then every week thereafter. At the end of the experiment in week 15, plant height, root length, seed numbers per head, and shoot and root biomass were determined. Plant height was measured with a ruler from the soil surface to the tallest living leaf of each plant in each pot. Roots and shoots were collected separately for each sampling plant. Shoots were cut at the soil surface and placed in paper bags. Roots were collected from the soil and dry soil was carefully removed by gentle rubbing and shaking. Roots were then washed gently with tap water and non-root material was removed using tweezers. Longest and shortest roots for each individual plant were measured with a ruler and mean length determined. Roots and shoots were oven dried at 80 °C for 48 h then weighed to determine dry biomass. Seeds were counted in each individual seed head and averaged for each plant.

2.4. Statistical analyses

Statistical analyses were conducted with R software (version 3.6.1), and significance was accepted at $p < 0.05$ for all tests. One-way multivariate analysis of variance (MANOVA) was performed to investigate treatment effects on growth variables over time (weeks 3–15, $n = 30$). Assumptions of univariate and multivariate normality (QQ plot), linearity (scatter plot matrix), low multicollinearity (Pearson's correlation test), homogeneity of covariances (Box's M-test), and variances (Levene's test) were determined before MANOVA computation. Pillai's Trace was the multivariate statistics method. When statistical significance occurred, a univariate one-way ANOVA (type II sum of squares) was conducted for each dependent variable ($n = 30$). For significant factors, Tukey's honest significant difference test was performed. Least square means were used for pairwise comparisons. Multiplicity adjustments were conducted with Tukey's honest significant difference adjustments.

3. Results

3.1. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) analysis of nano humus was performed to determine surface functional groups. Infrared spectroscopy frequency ranges, the appearance of vibration for functional groups, were characterized according to Socrates [28] and Hesse et al. [29]. Nano humus was enriched in aromatic carbon with bands at 1371 and 1559 cm-1 (C═C stretching) and phenolic groups with a broad and rounded band in the range of 3200 to 3500 cm-1 (-OH stretching); results were supported by the spectra (Fig. 1). Nano humus was characteristic of hydrophilic and hydrophobic components. Alkyne (-CΞCH) appeared as a few weak bands from 2260 to 2100 cm^{-1} and aliphatic hydrocarbon compound (C–H bending) at 1981 cm^{-1} suggesting hydrophobic components. Strong bands of symmetric C–O stretching were observed at 1006 and 1030 cm^{-1}. Background FTIR spectra indicated the presence of ambient water (~3600 cm^{-1}) and carbon dioxide (2324 cm^{-1}).

3.2. Shoot development

The stimulation effect on plant height with treatment addition was significant from weeks 4–8 throughout the barley jointing stage ($p < 0.05$), but not significant over the boot stage from weeks 9–11 ($p > 0.05$). Statistical significance increased noticeably (p values decreased) during the heading stage (weeks 12–15); being greatest in week 15 ($p = 1.3 \times 10^{-8}$). Combined application of nano humus and fertilizer yielded taller seedlings than corresponding sole applications and no treatment (Fig. 2). Although mean plant heights did not vary significantly among nano humus application rates, treatment of F + NH2, F + NH3, and F + NH4 were numerically higher

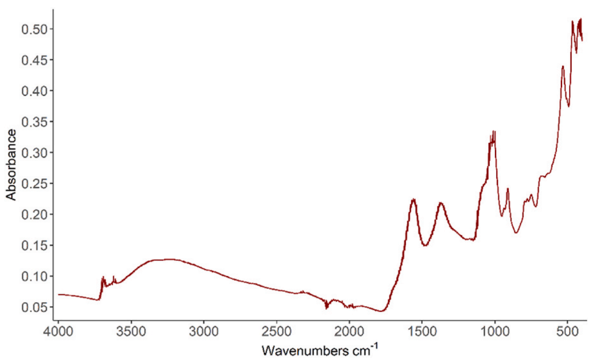

Fig. 1. Fourier transform infrared spectroscopy (FTIR) spectrum of nano humus.

values over the study period. The slight plant height stimulation effect of F + NH2 treatment was specifically noticed at the early growth stage (early stem elongation), F + NH4 treatment at the mid growth stage (later stem elongation), and F + NH3 at the late growth stage (heading stage).

In week 15, effects of combined application of nano humus and fertilizer on height were more obvious (Table 1), increasing 7 % relative to the control averagely. Greatest height enhancement was with fertilizer only (9 %), closely followed by F + NH3 (8 %). Barley height and shoot biomass were less affected by sole application of nano humus (NH1, NH2, NH3, and NH4) relative to the control. However, combined application provided a marked stimulation effect on shoot biomass, producing significantly higher biomass than the control and sole application of nano humus. A mean 80 % enhancement was evidenced by combined treatments F + NH1, F + NH2, F + NH3, and F + NH4. Shoot biomass was similar with all four nano humus rates, either with or without fertilizer.

3.3. Root development

All treatments of nano humus, with or without fertilizer, increased barley primary root length and root dry biomass in sandy soil after 15 weeks (Table 1). The beneficial effects on barley root growth varied slightly among nano humus rates. Longer roots were found in all nano humus treatments. Sole application of nano humus generally increased primary root length by 16–25 % relative to the control, and 15–24 % relative to fertilizer alone. Mean root length of barley with fertilizer alone (15.29 $\pm$ 0.52 cm) was similar to that of the control (15.17 $\pm$ 0.72 cm), indicating fertilizer may not influence barley primary root elongation. This was further confirmed by comparing root lengths between groups of nano humus with and without fertilizer, showing a lack of significant differences at each of the four rates. A clear enhancement on lateral root formation was observed in barley seedlings with nano humus alone, fertilizer alone, and their combinations, which led to greater root biomass. Among all studied growth parameters, the strongest beneficial effect was found on root biomass production by combined application and nano humus alone. Root biomass increased 92 % relative to the control with combined treatments, 49 % with nano humus alone, and 68 % with fertilizer alone. The greatest promoting effect was with the combined treatment of F + NH2, resulting in a 124 % increase.

3.4. Seed production

Barley reached maturity in week 15, with greater seed production with combined treatments of nano humus and fertilizer relative to corresponding sole applications (Table 1). Although sole application was generally not statistically different from the control, application of nano humus increased mean seed numbers (per head) by 2–11 %; fertilizer increased it by 11 %. The enhancement effect of combined application was greatest, by 38 % on four rate means. This was particularly notable for F + NH1, F + NH2, and F + NH3 which was significantly greater than the control, increasing by 55 %, 37 %, and 37 %, respectively.

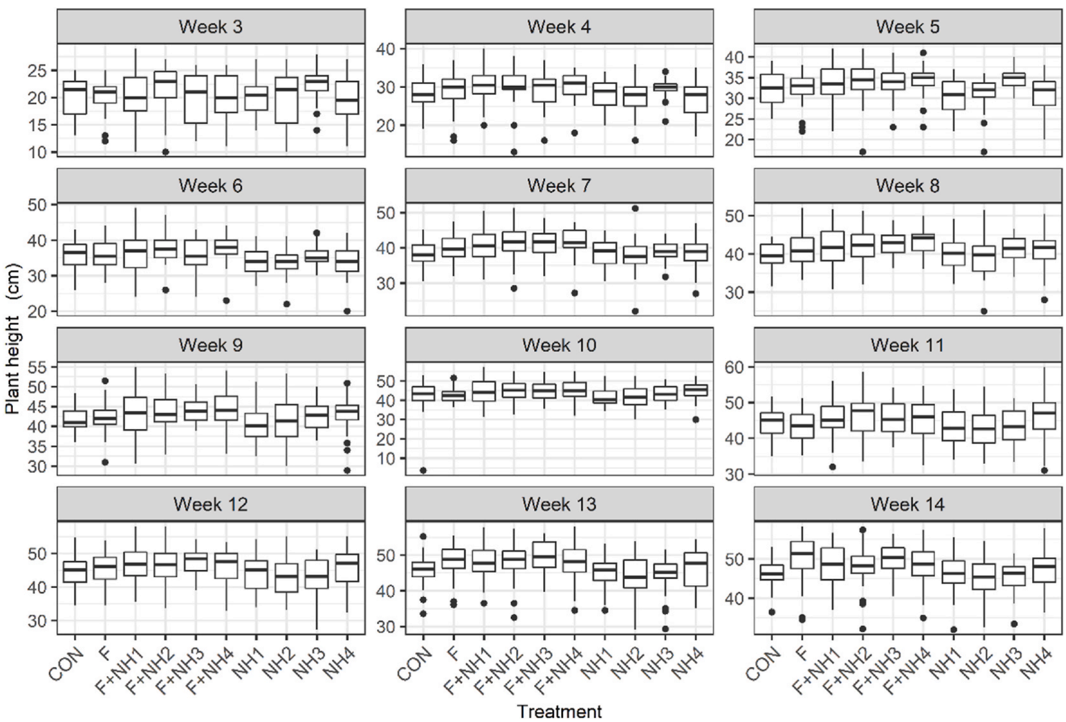

Fig. 2. Grouped boxplots for mean plant height by treatments from weeks 3–14.

Table 1
Mean and standard errors (SE) of height, shoot biomass, root length, and root biomass by treatments in week 15 (n = 6). Means in columns with different letters are significantly different.

Rate	Height	Shoot biomass	Root length	Root biomass	Seed production
	cm	g/plant	cm	g/plant	number/head
	Mean ± SE				
CON	46.57 ± 0.77bce	0.65 ± 0.04b	15.17 ± 0.72b	0.14 ± 0.01c	12.24 ± 0.70c
F	50.99 ± 0.93a	1.04 ± 0.07a	15.29 ± 0.52b	0.23 ± 0.02 ab	13.53 ± 0.83bce
F + NH1	49.82 ± 0.93 ab	1.12 ± 0.07a	18.79 ± 0.62a	0.23 ± 0.02 ab	18.86 ± 0.86a
F + NH2	49.87 ± 1.00 ab	1.17 ± 0.07a	17.77 ± 0.58 ab	0.31 ± 0.02a	16.74 ± 0.74 ab
F + NH3	50.24 ± 0.78 ab	1.22 ± 0.05a	20.07 ± 0.65a	0.27 ± 0.02 ab	16.67 ± 0.83 ab
F + NH4	49.24 ± 0.89 ab	1.17 ± 0.07a	18.63 ± 0.59a	0.24 ± 0.02 ab	15.43 ± 0.86 abc
NH1	46.32 ± 1.02bce	0.74 ± 0.04b	18.47 ± 0.57a	0.22 ± 0.02 abc	13.47 ± 0.78bce
NH2	44.85 ± 0.94c	0.59 ± 0.04b	17.58 ± 0.83 ab	0.19 ± 0.02bce	12.38 ± 0.89c
NH3	44.88 ± 0.84c	0.62 ± 0.04b	17.76 ± 0.55 ab	0.20 ± 0.01bce	12.62 ± 0.89c
NH4	47.04 ± 0.81 abc	0.75 ± 0.05b	18.95 ± 0.61a	0.21 ± 0.02bce	13.25 ± 0.80bce

4. Discussion

Without external sources of nutrients, a nutrient deficiency might occur and become one of the most limiting factors affecting barley growth in sandy soils. Nano humus, a lignite waste derived humic material, contains essential nutrients in complex nitrogen-phosphorus-potassium matrices which ensure nutrient ion assimilation and nutrition. However, similar to other humic products, nano humus does not contain sufficient nitrogen-phosphorus-potassium for barley growth. A significantly better response of barley was observed in sandy soils with both nano humus and fertilizer relative to sole applications. The combined application of inorganic fertilizer and nano humus serves as nutrient carriers in both rapid release water soluble forms and slow released complexed forms, which may help alleviate nutrient deficiencies in sandy soils.

The hydrophilic and hydrophobic domains and zwitterionic features of nano humus enable interactions with inorganic fertilizers. These characteristics function to buffer biological susceptibility to nutritional extremes, resulting in a reduction of high concentrations of salts, metals, and protons in the soil solution, and facilitating mobilization of nutrients into bioavailable forms [17]. The increased nutrient availability and plant uptake efficiency can be attributed to the formation of stable nutrient-humic complexes [30–32]. Therefore, their combinations could be utilized to develop slow or controlled release biofertilizers that align with the nutrient requirements throughout the lifecycle of plants [20].

Although there were no statistically significant differences among the four rates of nano humus, they all showed beneficial effects on barley growth, especially on root growth and seed production. Increasing the application rate did not bring more biological benefits, which implies that plant response to the application rate of lignite waste derived humic substances might be nonlinear. The recommended nano humus application rate by the manufacturer is 1500 kg/ha (5 g per plant pot) and it is now known to be suitable for soils with loamy sand texture and similar nutrient contents. The typical application rate of commercial humic products was 2–3 kg/ha was ineffective, which was further supported by our results. We found more than 500 times the typical rate was needed in sandy soils to show a notable plant growth stimulation effect, determining an appropriate soil application rate is critical for future research before field applications.

In our study, the most distinct stimulation effect of the humic product as a sole application was on root systems. This may be because the first contact organ for humic substances in soil is roots. Larger roots are associated with a greater capacity for nutrients and water uptake, which is particularly important for sandy soils with limited nutrient conditions and water holding capacity [33,34]. Our results agree with those of other studies showing beneficial effects of humic materials on root development in various nutrient poor growth media, including sandy soils. For example, Ciarkowska et al. [6] found humic materials (approximately 6–12 g/pot) tripled root dry biomass in coarse textured soils (77 % sand, 20 % silt, 3 % clay) and increased root biomass 2.5 times in medium textured soils (35 % sand, 51 % silt, 14 % clay) relative to plants in the control. Eyheraguibel et al. [35] reported a significant increase in root dry biomass (36 %) and root length (23 %) in hydroponic conditions after humic material application (50 mg C/L).

Seed production is a key factor determining agricultural production. The enhanced seed production in our study after humic substances application was also evidenced by Machiani et al. [36] who reported a 10 % increase in common bean seed production and a 16 % increase in fennel with application of humic substances (rate not mentioned) in silty clay soils. An 84 % peanut seed production increase in loamy clay soils was found by Moraditochaee [37] after spraying humic materials (40 mg/L). More work using humic materials in sandy soil applications is needed for future research to provide a direct comparison for other types of soil and facilitate understanding of the full potential of humic materials.

When combining with inorganic fertilizer, the stimulation effect on plant biomass and seed production was more obvious than with sole application. Our findings were consistent with Suman et al. [38] who found combined application significantly enhanced plant height (6 %), total biomass (7 %), leaf area index (3 %), chlorophyll content (5 %), and fruit yield (20 %) over sole application of fertilizer in sandy loam soils. Similarly, Sharif et al. [39] reported a 20–23 % increase in shoot biomass and a 32–39 % increase in root biomass in silty clay loam soils with combined addition of humic materials and inorganic fertilizer. However, there was little information on the combined effect of humic substances and fertilizer on seed production in the literature, making this a significant contribution from our study.

Humic substances, as a group of chemical compounds with supramolecular structure, can chemically interact with plant cell membranes after penetration [40,41] and thereby modify plant function. Functional groups are considered the most important property of humic materials. The beneficial effects of nano humus are likely attributed to the presence of various organic functional groups, from polar (hydroxyl, phenolic) to nonpolar (aliphatic, aromatic), which are responsible for hydrophilic and hydrophobic properties. Ojwang and Cook [42] investigating chemical interactions between humic fragments and biomembranes, found surface adsorption occurs via H bonds and then humic fragments enter the cell and modify membrane structural homogeneity. Hydrophilic structures of nano humus (phenolic –OH groups) were detected by FTIR. They are likely to constitute the interaction between nano humus and barley cell membrane surfaces. Other polar functional groups in nano humus structure may also contribute to the interaction and penetration. Hydrophilic components in the structure of humic materials could trigger nitrogen metabolism related enzymatic activities after penetration and therefore positively affect root growth and biomass production [43–45]. Further investigations on the biological effects of functional groups in humic fragments will be needed.

5. Conclusions

Our study contributes to understanding the biological effect of a coal waste derived humic material with or without inorganic fertilizer on barley growth in sandy soils, enlightening the uncertainty of synergistic effects in sandy soils. Our findings indicate that the synergism may play a role in sustainable agriculture. Synergistic effects of humic substances and inorganic fertilizer were more notable than the corresponding sole application, particularly on plant biomass and seed production. The concomitant use of humic substances with inorganic fertilizer is suggested as an effective soil amendment for enhancing agricultural plant growth in sandy soil regions. Sole application of the humic product markedly promoted barley primary root length, root biomass, and seed production; the latter a significant contribution from our study. All four rates showed beneficial effects on barley growth and plant response to application rate of humic materials might be nonlinear. Applying nano humus at equivalent to 1500 kg/ha (5 g per pot) was suitable for sandy soils, showing that more than 500 times the typical rate of commercial humic products is needed to exhibit biological efficacy in sandy soils. Our work has global implications as it can be applied in other sandy soil regions facing similar challenges.

Data availability statement

Data associated with our study has not been deposited into a publicly available repository. Data will be made available on request.

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CRediT authorship contribution statement

Yihan Zhao: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Anne Naeth:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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