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INFLUENCE OF PLANTING REGION AND SOIL CHEMICAL PROPERTIES ON MEDICINAL COMPOUND CONTENTS IN *Cistanche tubulosa*

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Abstract

Cistanche tubulosa is a valuable herbal remedy in China. In this study, we aimed to identify and analyze how bioactive compounds are affected by planting region and soil properties. Plant and soil samples in desert and alkaline-saline environments were collected, and measurements were obtained for (i) dry weight and medicinal compounds of plant samples and (ii) soil salinity and pH of soil samples. The effect of soil salinity on the medicinal compounds revealed that the dry weight per plant of *C. tubulosa* was relatively high in low salinity soil. However, under high salinity conditions, echinacoside and verbascoside contents increased. No visible effects of individual soil pH were found on dry weight and echinacoside, verbascoside, and mannitol contents. Regarding the interaction between soil salinity and pH, only mannitol content showed a significant difference. The mannitol content was unaffected by pH in the presence of slight salinity conditions; meanwhile, in severe salinity conditions, high pH dramatically lowered the mannitol content. In loam with saline soil, a lower dry weight per plant was observed but the planting density was higher. The good water and fertilizer retention abilities of loam can lead to a higher yield per unit area. To improve yield and quality simultaneously in the introduction regions, the crucial points for cultivation techniques of *C. tubulosa* in seashore saline land include a high seed dosage of *C. tubulosa* and moderate soil salinity.

Keywords: Cultivation. Echinacoside. Environmental factors. Pondus hydrogenii. Soil salinity. Verbascoside.

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1. Introduction

Cistanches Herba, commonly known as “desert ginseng” or Rou Cong Rong, is a well-known traditional Chinese herbal medicine. The official plants listed in the Chinese pharmacopoeia are Cistanche deserticola Y.C. Ma and Cistanche tubulosa (Schenk) Wight (ChPC 2020). The dried fleshy stem of Cistanches Herba has been used as a tonic in China for millennia (Gu et al. 2016; Li et al. 2018). It is also used as a health supplement in Iran, India, Japan, and Mongolia (Wang et al. 2017a). Cistanche has anti-senescence, anti-oxidation, neuroprotection, anti-inflammation, hepatoprotection, immunomodulation, anti-neoplastic, and anti-osteoporosis effects (Gu et al. 2016; Zhang et al. 2017; Xu et al. 2019).

More than 150 compounds have been identified in Cistanche, including phenylethanol glycosides (PhGs), oligosaccharides, polysaccharide benzyl glycosides, iridoids, and lignans. PhGs are the major bioactive compounds in Cistanche species and play a major role in the treatment of kidney deficiencies. Many PhGs, such as echinacoside and verbascoside, exhibit a variety of curative effects, including anti-aging and anti-Alzheimer’s disease (Jimenez and Riguera 1994; Tian et al. 2021). In the Chinese Pharmacopoeia, echinacoside and verbascoside contents are important indices for evaluating the quality of Cistanches Herba (ChPC 2020). Aqueous extracts of Cistanches Herba have been shown to be effective against constipation (Xu et al. 2019). Carbohydrates such as mannitol and galactoglucan have laxative effects (Jimenez and Riguera 1994; Tian et al. 2021).

Presently, the Cistanches Herba industry is booming in China, with a cultivation area of 84,000 ha and an annual output of 6,000 tons, after continuously struggling in the past decades (Song et al. 2021). As the scale of planting increases in Inner Mongolia, Xinjiang, and Gansu provinces in China, the problem of Cistanche Herb sources has been solved, and the output value of related industries is expected to reach 20 billion yuan by 2021. C. tubulosa parasitizes the roots of Tamarix. The host plant C. tubulosa, which is primarily distributed in saline soils and margins of dunes, riversides, and lakesides, is highly tolerant to salinity and drought (Shrivastava and Kumar 2015). Soil salinization has become a significant factor restricting the stable development of ecological environments and crop production.

Almost 20 % of cultivated land is adversely affected by salinity and alkalinity (Hazman et al. 2015; Shrivastava and Kumar 2015; Machado and Serralheiro 2017), and an increasing trend in soil salinization has been observed (Cuevas et al. 2019; Singh 2021). It is estimated that there are more than 99.13 million ha of saline-alkali soil in China, accounting for 10 % of the saline-alkali land worldwide. Because of oxidative and salinity stress, only few plants can survive in saline-alkaline soil, but the output of these plants is significantly diminished (Qiao et al. 2015). Fortunately, Tamarix has excellent stress resistance to salinity and drought and grows well in soil with 1 % salt content (Liu et al. 2020). Wild Tamarix plants are abundant in the saline-alkaline soil of the North China Plain. These conditions are favorable for C. tubulosa industry in coastal saline lands. Therefore, C. tubulosa was introduced from Xinjiang to North China during the first decade of the 21st century by Guo (Machado and Serralheiro 2017).

The environment significantly influences the growth and development of Cistanche (Sun et al. 2020a). The physical and chemical characteristics of the soil, including salinity, texture, and pH, can affect plant metabolism, particularly secondary metabolism. Under soil salinity stress, high levels of secondary metabolites are found in plants such as soybean (Radhakrishnan et al. 2012), maize (Wei and Pan 2014), and tomatoes (Borghesi et al. 2011). A higher acetylacteoside content has been reported in Cistanche grown in saline-alkali land than in grassland and sandy land (Zheng et al. 2014; Sun et al. 2020b). Under field conditions, the effects of soil texture, salinity, and pH on the medicinal compounds of C. tubulosa have not been well documented. Therefore, further detailed experiments are required to fully elucidate these aspects. In this study, we aim to determine how plant growth, development, and bioactive compounds are affected by planting region and chemical properties of the soil.

2. Material and Methods

Sample Collection

One hundred and twenty-three C. tubulosa samples were collected from Hotan Prefecture (35°14′ N–39°29′ N latitude, 81°9′ E–82°51′ E longitude) in Xinjiang autonomous region. The soil type was Haplic

Solonchaks (Calcaric, Yermic) according to the World Reference Base for Soil Resources (4th edition) (Xinping et al. 2007; Shiheng et al. 2020; WRB 2022). It features a moderate continental desert climate, which includes drought, lack of rainfall, intense evaporation, and a large temperature differential between days and years (Liu et al. 2020).

Sixty-three samples were collected from Wuqiao (37°29′ N–37°47′ N latitude, 116°19′ E–116°24′ E longitude), in Hebei Province. The study area has a temperate continental monsoon climate with warm humid summers and dry cold winters. The soil type was Chloridic Solonchaks (Aric, Loamic) (WRB 2022). The sampling locations are shown in Figure 1.

Figure 1. Map of sampling locations. The box in red represents Hotan, and the dot in red represents Wuqiao.

Thirty soil samples from 0 to 20 cm layers in ten areas in Wuqiao, Hebei Province, were collected for the analysis of soil salinity and pH. At the same time, 30 Cistanche tubulosa samples were collected for the determination of medicinal compound content and dry weight. All C. tubulosa samples were oven dried at 60 °C to record the dry weight. The dried samples were crushed and sieved through a 60-mesh sieve.

HPLC Analysis

The echinacoside and verbascoside contents were determined following the method of the Chinese Pharmacopoeia (ChPC, 2020). Mannitol content was determined using a well-defined HPLC-ELSD method (Xiong et al. 2016). The HPLC method was validated before sample testing, achieving a favorable linear relationship (r > 0.9990) and recovery (98.5 %–103.8 %) of the determination method. Therefore, the contents of the three medicinal compounds can be accurately determined (KuiJun et al. 2012).

Soil Analysis

Soil salinity was determined using the residue-drying quality method (KuiJun et al. 2012). Air-dried soil (20 g) was blended with 100 mL distilled water. The mixture was stirred for 3 min and filtered through a filter paper. Then, 50 mL filtered mixture was transferred to a weighed beaker and evaporated to dryness. The dried substance was treated with 15 % hydrogen peroxide solution and dried for 4 h in a drying oven at 100–105 °C. The weight of the beaker was measured again after drying to a constant weight.

Soil salinity=(W2-W1)/Ws×100/50×100 %, where Ws denotes the weight of the air-dried soil, W1 is the weight of the beaker, and W2 denotes the weight of the beaker measured again.

Soil sample (10 g) was mixed with 25 mL deionized water. The mixture was shaken for 30 min and then kept static for 5 min. The pH of each soil solution was measured using a pH meter (PB-10; Sartorius) (Wang et al. 2017b).

Data Processing

All results were visualized using the ggplot2 package in R Core Team (2022) version 4.1.3. Descriptive statistics and multiple comparisons were performed using R version 4.1.3..

3. Results

Performance of Dry Weight and Medicinal Compound Contents in Two Planting Areas

To better analyze the effects of planting area on medicinal compound content in *C. tubulosa*, the dry weight and content of the three medicinal compounds in all 186 samples were determined. The results are shown in the violin plots in Figure 2. The dry weight of *C. tubulosa* collected from Hotan was in the range of 5.31 g to 349.68 g per plant, and the dry weight of those collected from Wuqiao were from 1.07 g to 79.69 g. The mean values for the dry weight of plants collected from Hotan and Wuqiao were 81.34 g and 19.32 g, and the medians were 58.89 g and 13.48 g, respectively. The contents of echinacoside in *C. tubulosa* were 1.92–535.45 mg g^{-1} and 72.05–442.22 mg g^{-1} in Hotan and Wuqiao, respectively. The mean values of echinacoside contents in Hotan and Wuqiao were 166.80 mg g^{-1} and 259.02 mg g^{-1}, and the medians were 95.99 mg g^{-1} and 270.27 mg g^{-1}, respectively. The contents of verbascoside were 0.78–212.88 mg g^{-1} and 20.82–104.70 mg g^{-1} in Hotan and Wuqiao, respectively. The mean values of verbascoside contents were 41.50 mg g^{-1} and 57.41 mg g^{-1}, and the medians were 32.80 mg g^{-1} and 55.2 mg g^{-1}, respectively. The minimum, maximum, mean, and median values of mannitol contents were 12.52 mg g^{-1}, 88.70 mg g^{-1}, 28.81 mg g^{-1}, and 24.78 mg g^{-1} in *C. tubulosa* from Hotan and 33.47 mg g^{-1}, 77.43 mg g^{-1}, 45.93 mg g^{-1}, and 44.68 mg g^{-1} in *C. tubulosa* from Wuqiao.

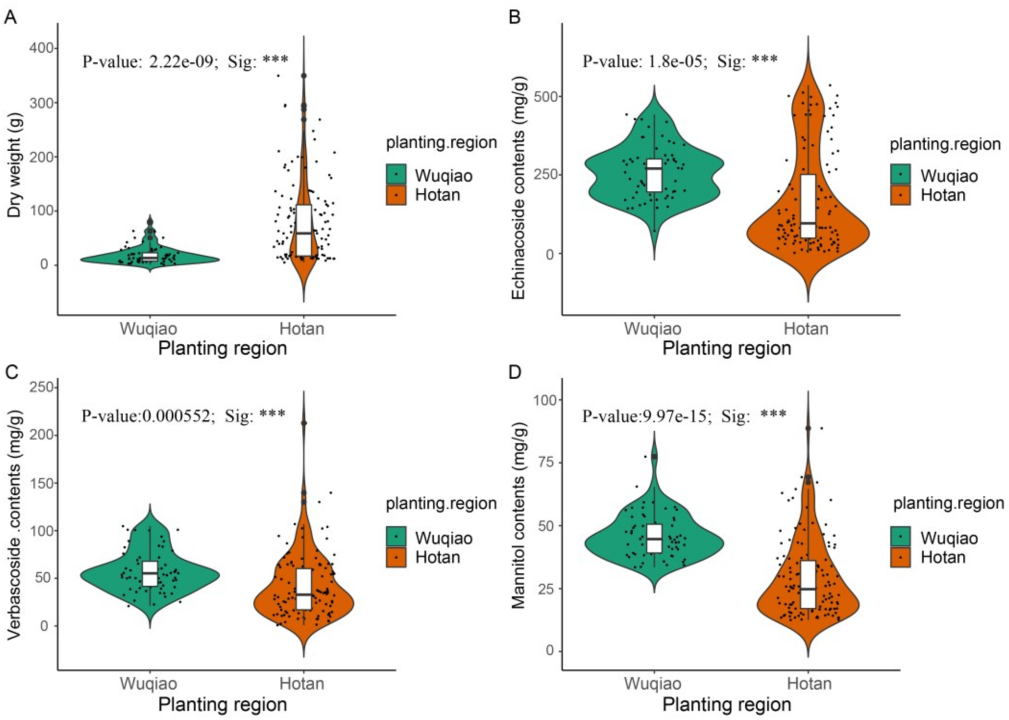

Figure 2. A Dry weight, B echinacoside content, C verbascoside content, and D mannitol content in *Cistanche tubulosa*. The two sampling regions were Wuqiao (n = 63) and Hotan (n = 122).

As shown in Figure 2 and the descriptive statistics above, the dry weight and echinacoside, verbascoside, and mannitol contents of C. tubulosa samples from Hotan had much wider size distributions than those of samples from Wuqiao. Figure 1A shows that the descriptive statistical values of the dry weight of *C. tubulosa* from Wuqiao were lower than those of *C. tubulosa* from Hotan. The dry weights of 51 samples from Hotan were higher than the maximum values in samples from Wuqiao (79.69 g). The average contents of echinacoside, verbascoside, and mannitol in *C. tubulosa* collected from Wuqiao were 55.29 %, 38.34 %, and 59.43 % higher than those of samples from Hotan, respectively.

Performance of Dry Weight and Medicinal Compound Content under Saline Conditions

Soils were sampled, and salinity in ten areas of Wuqiao region was determined. All soils were divided into three categories by salinity according to the reported literature: slight (salinity < 0.2 %), moderate (0.2 % ≤ salinity < 0.4 %), and severe (salinity > 0.4 %) (Bao 2000; Hong et al. 2018). Thirty samples from ten areas were collected for the determination of dry weight and content of medicinal compounds, and the results are shown in Table 1. The mannitol content showed no significant differences among the three categories of saline conditions. The remaining three parameters (dry weight and echinacoside and verbascoside contents) were significantly affected by the saline conditions. The highest echinacoside and verbascoside contents were observed under severe salinity conditions. The mean value of echinacoside contents under severe (291.09 mg g^{-1}) was 73.6 % higher than that under the slight salinity conditions. By contrast, the dry weight of *C. tubulosa* was significantly reduced under high salinity conditions (moderate and severe).

Scatter plots of echinacoside, verbascoside, mannitol, and dry weight contents under saline conditions for all samples are shown in Figure 3. Data analysis and curve fitting were performed using the R software. The visual results were very similar to those listed in Table 1. An increasing trend was observed in the echinacoside and verbascoside contents with increasing salinity below the moderate saline condition. Stable trends in echinacoside and verbascoside contents were noted in the moderately and severely saline soils. While the change in dry weight showed the opposite trend, i.e., high dry weight values under slightly saline conditions and vice versa.

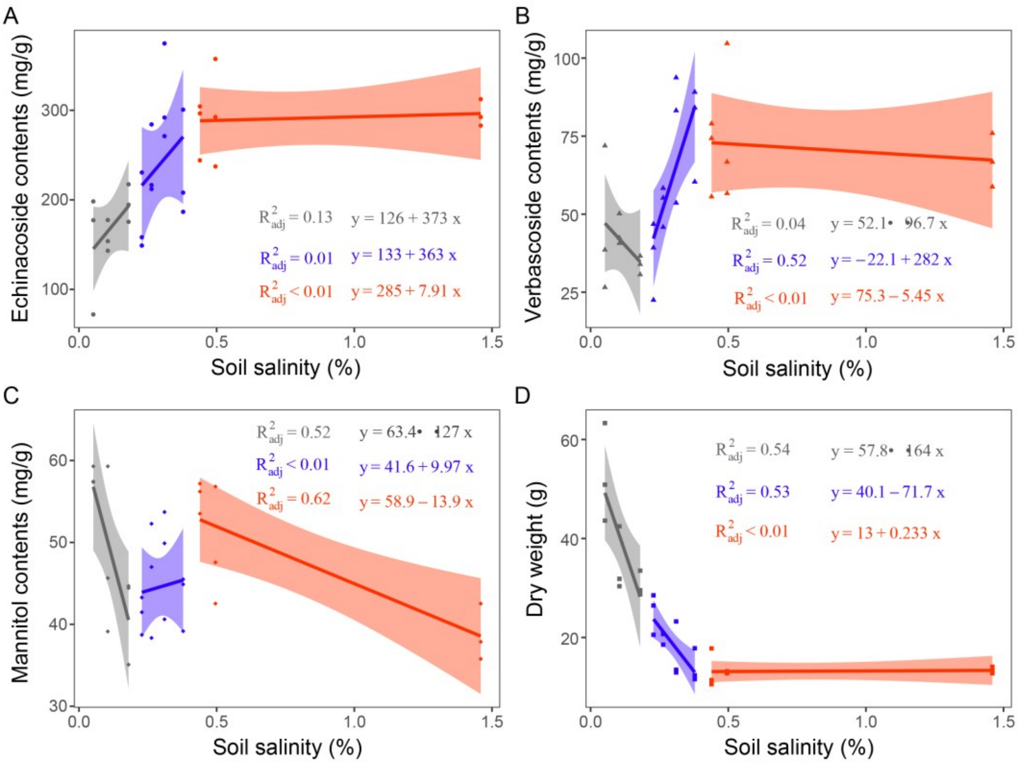

Figure 3. Contents of A echinacoside, B verbascoside, and C mannitol and D dry weight of 30 samples of *Cistanche tubulosa* under saline conditions. Saline conditions were divided into three major categories based on soil salinity. Curve fitting and statistical analysis were performed in R with ggplot2 package. Color scheme: gray = slightly saline soil, blue = moderately saline soil, red = severely saline soil.

Table 1. Dry weight and echinacoside, verbascoside, and mannitol contents in *Cistanche tubulosa* under three saline conditions.

	Echinacoside content (mg g^{-1})	Verbascoside content (mg g^{-1})	Mannitol content (mg g^{-1})	Dry weight (g)
Slight	167.72±42.294^{c}	41.28±13.38^{b}	51.37±13.14^{a}	39.36±11.81^{a}
Moderate	240.30±65.84^{b}	61.00±22.10^{a}	44.57±5.28^{a}	18.92±5.57^{b}
Severe	291.09±35.74^{a}	70.93±15.263^{a}	47.78±8.45^{a}	13.19±2.029^{b}

One-way analysis of variance Tukey's multiple comparison test were used for multiple group comparisons. Lower-case letters represent significance at P < 0.05.

Performance of Dry weight and Medicinal Compound Content under the Interaction Effect of Saline Conditions and pH

Soil pH is generally considered an important factor affecting plant growth and development. Different plant species have different optimal soil pH for growth. Unfavorable soil pH may decrease the quantity and quality of crop yield (Yue et al. 2010; Dong et al. 2012). The pH values of 30 Wuqiao soil samples were in the range of 8.24–8.84, indicating that all samples were alkaline. According to soil pH grading standards (Lager et al. 2010; Geng et al. 2021), all 30 soils were classified as low (alkaline soil, pH values from 7 to 8.5) or high (strongly alkaline soil, pH values above 8.5). Statistical comparisons (two-way analysis of variance) between the saline and pH conditions, as well as their interactions, in 30 samples of the three medicinal compounds and dry weight of *C. tubulosa* are shown in Table 2. No significant differences (P > 0.05) were noted among the four parameters under different pH conditions. Furthermore, the contents and dry weight of the three medicinal compounds were not affected by soil pH.

Table 2. Dry weight and medicinal compound content under the interaction effect of saline conditions and pH in *Cistanche tubulosa.*

	Echinacoside content	Verbascoside content (mg g^{-1})	Mannitol content (mg g^{-1})	Dry weight (g)
	F-value	F-value	F-value	F-value
salinity	12.0900***	6.0300**	1.2250^{ns}	31.0090***
pH	0.1620^{ns}	0.3160^{ns}	2.9470^{ns}	0.8630^{ns}
interaction	0.0170^{ns}	0.0030^{ns}	4.8610*	0.7460^{ns}

*** Significant at less 1%; **Significant at 1%; *Significant at 5%; ^{ns} not significant.

Only mannitol content showed a significant difference under the interaction between saline and pH conditions. Figure 4 shows the interaction effect of salinity and pH on mannitol content. Under low salinity conditions, mannitol content was not affected by pH. However, a high pH significantly reduced the mannitol content under severe salinity conditions.

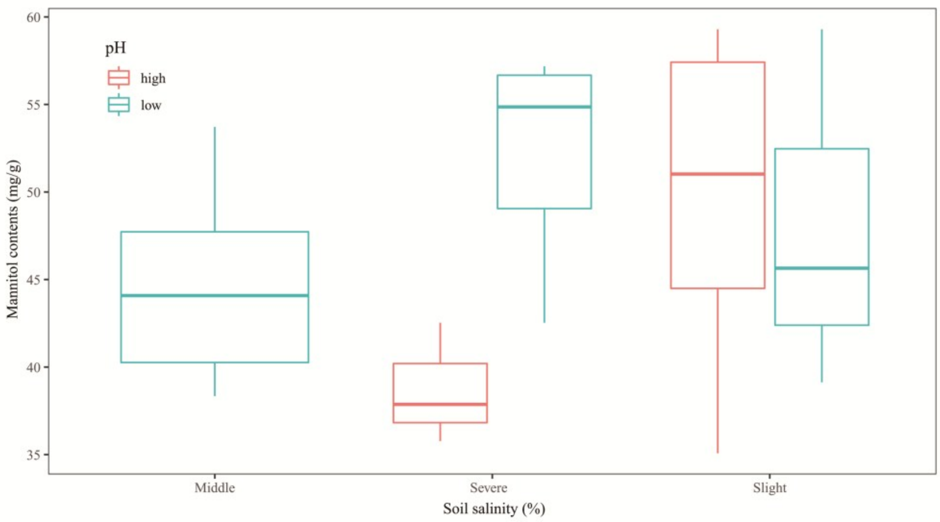

Figure 4. Mannitol content in *Cistanche tubulosa* under the interaction effect of salinity conditions and pH. The abscissa indicates three salinity conditions, and different colors represent two pH conditions.

Linear Regression Analysis between Medicinal Compounds Contents and Dry Weight

In a previous study, the three medicinal compounds were found to be synthesized at different levels at different growth stages (Ben Abdallah et al. 2018). Mounting evidence suggests that dry weight has a significant effect on the content of the three medicinal compounds, especially echinacoside and verbascoside contents. Considering the above, scatter plots were used to show the linear relationships of the three medicinal compounds and dry weight, and the line of best fit (±95 % confidence interval) was added to the scatter plot.

As shown in Figure 5, the echinacoside and verbascoside contents were maintained at high levels in the low dry weight samples. The echinacoside and verbascoside contents in the 13.48 g sample were 374.66 mg g^{-1} and 93.75 mg g^{-1}, respectively. Meanwhile, the mannitol content was slightly low with an increase in dry weight, when the sample dry weight was below 30 g; nevertheless, this difference was not significant. When the dry weight of *C. tubulosa* exceeded 30 g, mannitol content increased rapidly.

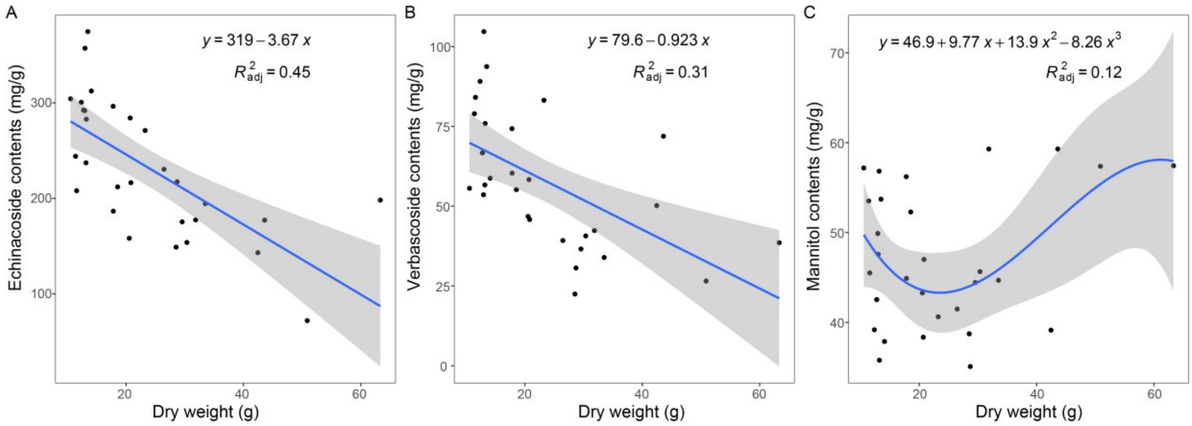

Figure 5. Scatter plots showing the relationship between dry weight and contents of A echinacoside, B verbascoside, and C mannitol in *Cistanche tubulosa*. Continuous blue lines and gray areas represent the best fit and corresponding 95 % confidence limits estimated by linear regression.

4. Discussion

C. tubulosa was introduced from Xinjiang to North China for the better use of seashore saline land in the first decade of the 21st century (Liu et al. 2020b). Large-scale planting of *C. tubulosa* and its hosts on saline land has resulted in great economic and ecological benefits. The yield of *C. tubulosa* reached 500 kg per mu (667 m^2) in Shandong Province, which is significantly higher than that in native places (Xinjiang and Inner Mongolia), and the yield can be further increased by improvements in cultivation techniques. The development of *C. tubulosa* industry is considered promising with respect to social, health, environmental, and economic benefits (Shahrajabian et al. 2019).

C. tubulosa is a parasitic plant that can grow in dry sandy lands and under stressful conditions, such as in saline-alkali lands. Wild *C. tubulosa* are primarily found in the southern Xinjiang Autonomous Region of China, the region bordering the Taklamakan Desert (sandy land) (Wang et al. 2017c). *C. tubulosa* was introduced to northern coastal China (loamic land) to identify whether the medicinal compound contents in *C. tubulosa* vary across two distinct soil habitats and determine the link between these variations and soil properties. Therefore, in this study, we verified the influential impact of environmental factors (planting region and soil properties) on the medicinal compound content in *C. tubulosa*.

Impact of Soil Physical Properties on Dry Weight and Medicinal Compound Content

For medicinal plants, the contents of medicinal compounds are more associated with economic value than crop yield. The main compounds in medicinal plants are combinations of secondary metabolites (V et al. 2010; Yamani et al. 2016), which are the active compounds and have important implications on the interaction of plants with their environments for adaptation and defense. Environmental factors, such as soil and temperature, affect plant growth, development, and reproduction (Wang and Guo 2017; Hesami et al. 2019; Yamamoto et al. 2019). Therefore, environmental factors that influence plant growth and development also influence secondary metabolite production (Davies et al. 2009). Important plant secondary metabolites, such as phenolics, flavonoids, terpenoids, and alkaloids, produced during various biochemical processes, are affected by varying degrees of crucial environmental stresses, including light irradiation, temperature, soil water, soil fertility, and salinity (Yang et al. 2018).

The effects of soil factors on the main medicinal compound contents of *C. deserticola* have been reported in Alxa, China (Wang et al. 2020). The effects of soil physicochemical properties on plant growth, development, and crop yield are a popular research topic. Larger air-filled pore spaces and lower soil nutrients occur in sandy soil. These are beneficial for the growth of underground plant parts (such as roots), especially those of barren-tolerant plants (Liu et al. 2020a). These findings prompted us to examine the changes in dry weight and medicinal compound contents of *C. tubulosa* in different soil ecotypes.

As a root parasitic plant, the entire vegetative growth stage of *C. tubulosa* occurs underground. Therefore, it is easier for *C. tubulosa* tubers to expand in sandy soil. In this study, the mean value for dry weight per plant of *C. tubulosa* in sandy soil (Hotan) was 4.2-fold more than that in loamy soil (Wuqiao, 81.34–19.32 g). The median dry weight, which is another measure of tuber expansion, showed a similar trend (58.89 g in Hotan and 13.48 g in Wuqiao). Our results predicted that that sandy soil was more favorable for *C. tubulosa* tuber expansion, as confirmed by the dry weight values per plant in Hotan and Wuqiao, representing two different soil ecotypes.

Impact of Soil Salinity Conditions and pH on Dry Weight and Medicinal Compound Contents

Soil salinity is a major stress factor that can adversely affect plant cell turgor pressure (Dexter 2004). Furthermore, plant growth, development, and crop yield are negatively affected by soil salinization (Singh et al. 2015). High salt concentrations can halt plant growth or even cause death (Cao et al. 2016). Fortunately, *C. tubulosa being* a root-parasitic plant, fulfils its water and nutrient requirements from its host. The host plant, *Tamarix* can grow well in soil with 1 % salt content. Secondary metabolites in *Cistanche*, such as glycosides and terpenoids, are products of the plant's long-term adaptation to drought and salinity stress. Drought stress has a significant stimulatory effect on the accumulation of astragalin and verbascoside in suspended cells of *C. tubulosa* (Liu et al. 2008).

Thirty soil samples and 30 *C. tubulosa* samples grown in the soil from ten areas in Wuqiao were collected for analysis. The results showed that the dry weight of *C. tubulosa* was relatively higher in low salinity soil (Figure 3 and Table 2). With increasing salt content, the dry weight decreased continuously (Figure 3). Conversely, echinacoside and verbascoside contents increased under high salinity conditions. Soil salinity had no significant effect on mannitol content in *C. tubulosa*. No visible effects were observed for individual soil pH values on dry weight or echinacoside, verbascoside, and mannitol contents. Regarding the interaction between soil salinity and pH, only the mannitol content showed a significant difference. Under low salinity conditions, the mannitol content was not affected by pH. Meanwhile, a high pH significantly reduced the mannitol content.

For crops, the yield was negatively correlated with quality. Therefore, it is difficult to simultaneously improve yield and quality (Soma et al. 2020). Correlation analysis of the three medicinal compound contents and dry weight showed (Figure 5) that the echinacoside and verbascoside contents were maintained at a high level in low dry weight samples. The best-fitting straight-line equation of the echinacoside and verbascoside contents with dry weight were $y = 319-3.67x$ and $y=79.6 -0.923x$, respectively. Nonetheless, the fitting curve of mannitol content to dry weight was not linear but a monistic cubic equation. From the equation, the mannitol content was found to decrease somewhat by the increase in dry weight when the sample dry weight was below 30 g; the mannitol content increased rapidly with an increase in the dry weight above 30 g.

Photosynthesis in the leaves of *Tamarix chinensis*, the host of *C. tubulosa*, may decrease owing to stomatal limitation under salt stress treatment (Xia et al. 2017). The biomass of *T. chinensis* significantly decreased under salt stress (Dawalibi et al. 2015). The yield and medicinal compound contents were affected by soil salinity through changes in the source–sink relationship between *C. tubulosa* and *T. chinensis*. In the present study, the dry weight of *C. tubulosa* was significantly reduced under saline conditions. For alkaline soil, there was a negative correlation between the N, P, and K contents and pH value of the rhizospheric soil of *T. chinensis* (Lihua et al. 2015). However, it is unclear whether soil pH can affect plant growth and metabolic activity of *T. chinensis*. In this study, the results showed that dry weight and echinacoside and verbascoside contents were not affected by soil pH.

Improvements in *C. tubulosa* Cultivation Techniques in Seashore Saline Land

Crop yield depends on the dry weight of individual plants and planting density (van Zelm et al. 2020). Owing to the detrimental effects of soil type and soil salinity, the dry weight of individual plants decreased in the seashore saline land of North China compared with that in Xinjiang. Simultaneously, a high yield was observed in Shandong Province. A reasonable initial estimate was obtained for a higher planting density. Structurally, loam has higher porosity and smaller pores than sandy loam (Dathe et al. 2016). As the physical properties differ dramatically, mineral nutrition in sand is easily lost and sand also has a low water retention capacity. On the contrary, loam has the opposite effect and has good water and fertilizer retention capacity. Good water and fertilizer retention capacities in loams can maintain a high density of hosts of *C. tubulosa*. A lower dry weight also ensures higher medicinal compound content in *C. tubulosa*. To improve yield and quality simultaneously, the key points for cultivation techniques of *C. tubulosa* in seashore saline land include high host density, high seed dosage of *C. tubulosa*, and moderate soil salinity (0.2 %–0.4 %).

5. Conclusions

Our analysis of the effect of soil salinity on medicinal compounds revealed that the dry weight per plant of *C. tubulosa* was relatively high in slightly saline soil. However, under high salinity conditions, echinacoside and verbascoside contents increased. No significant effects of individual pH of the soils were noted on the dry weight and echinacoside, verbascoside, and mannitol contents. Regarding the interaction between soil salinity and pH, only mannitol content showed a significant difference. The concentration of mannitol is unaffected by pH in the presence of slight salinity conditions; meanwhile, in severe salinity conditions, high pH dramatically lowers the mannitol content. A lower dry weight per plant was found in loam with saline soil, but the planting density was higher. The good water and fertilizer retention capacities of loam can lead to a higher yield per unit area. To improve the yield and quality simultaneously in the introduction regions, the crucial points for cultivation techniques of *C. tubulosa* in seashore saline land include high host density, high seed dosage of *C. tubulosa*, and moderate soil salinity.

Authors' Contributions: WANG, X.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; MA, X.: acquisition of data, analysis and interpretation of data, drafting the article; LI, L.: acquisition of data, analysis and interpretation of data; Liu, Y.: drafting the article; LI, J.: acquisition of data, analysis and interpretation of data; Xuan, J.: acquisition of data, analysis and interpretation of data; MA, D.: conception and design, drafting the article; ZHENG, L.: conception and design; SHU, J.: drafting the article. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Ethics Approval: Not applicable.

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