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Analysis of the soil physicochemical factors affecting the distribution of cordyceps in gannan hezuo regional

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ABSTRACT

Literature survey, Interview and sampling analysis were carried out in the four streets and seven villages in Hezuo City of Gannan to research the effects of soil physicochemical factors on the distribution of CORDYCEPS. Soil layers 0-5, 5-10, 10-15 and 15-20 cm in 21 sampling sites were analyzed. The plant number of CORDYCEPS was recorded, as well as the vegetation component, plant coverage, density and height, distribution status and growth status. The results show that pH, TN, TP and AP had no significant differences; while WC, OM, TK and AK showed extremely significant differences in different soil layers; in soil layer 5-10 cm, WC significantly affected the distribution of CORDYCEPS; while pH and TK had extremely significant impacts. The number of CORDYCEPS was the maximum in soil layer 5-10 cm. In the first principal component, TP was the major factor affecting the population distribution of CORDYCEPS. In the second principal component, pH was the major factor. So CORDYCEPS had strict requirements for soil layer, TP and soil acidity-alkalinity. At the same time, WC, OM and TK also affected the distribution of CORDYCEPS.

KEYWORDS

Soilenvironment; CORDYCEPS; Soillayer; Physicochemicalfactor.

INTRODUCTION

CORDYCEPS is the complexes of larva body and stroma of *Cordyceps sinensis* (BerK.) Sacc after fungus parasitism[1]. CORDYCEPS is mild in nature and sweet in taste, having extremely high medicinal value. It has the functions of anti-leukemia, antidepressants, anti-radiation, anti-cancer, tonifying lung, tonifying the kidney, relieving cough and asthma, stopping bleeding, reducing phlegm, tonifying lung and kidney, enhancing the immune function and hemopoietic ability, and regulating the immune system. Therefore, CORDYCEPS is mainly used to cure chronic cough, hemoptysis, and weakness after the illness, insomnia, emaciation, impotence, emission, waist and knee pain and so on[2-4]**.** The life cycle of CORDYCEPS is very complex, which transforms through a relatively independent conidia stage and ascospore stage. Under nature conditions, the host bat moth needs 5-6 years to complete a whole generation. Among them, more than 85% of the time is underground. Overlapping generations appear throughout the whole year; and the very special requirement for the habitat is needed[5]. As the increasing demand for CORDYCEPS, the wild environment is greatly destroyed; the natural resources have reduced gradually and have been faced with the situation of exhaustion completely[6]. Therefore, there is no time to lose in strengthening the research and protection of wild environment of CORDYCEPS.

The growth of CORDYCEPS is strict with the environmental conditions, which is mainly distributed in Qinghai-Tibet Plateau and the marginal area, including Tibet, Qinghai, Sichuan, Gansu, Yunnan and other provinces. CORDYCEPS grows in the alpine shrub meadow and alpine meadow with the altitude of 3600-4500 m. The suitable soil is the fertile and loose alpine meadow soil with thick soil layer and moderate moisture. The distribution of CORDYCEPS shows significant zonal and vertical distribution law, which is consistent with the distribution of host[7]. The host of CORDYCEPS relies heavily on soil habitat. In recent years, researches on the habitat of CORDYCEPS are mainly focused on the altitude, associated plants and eating patterns of bat moth[8,9]. However, there are no reports on the soil microenvironment of CORDYCEPS distribution area in Hezuo City of Qinghai-Tibet Plateau. Based on these, relationship between the soil nutrient and population distribution of CORDYCEPS in this area was researched, aiming at providing scientific references for the habitat protection of CORDYCEPS, and the realization of semi-artificial cultultivation and sustainable utilization of wild resources.

EXPERIMENTA CONTENT

General status of research region

The research region includes four streets and seven villages in Hezuo City of Gannan Tibetan Autonomous Prefecture, which were Dangzhou Street, Yiheang Street, Jianmukeer Street, Tongqin Street, Kajiaman Village, Kajiadao Village, Zuogaiduoma Village, Zuogaimanma Village, Jiamaogong Village, Lexiu Village and Nawu Vilalge. Hezuo City (102°47′51″-103°22′00″E, 35°18 ′50″N) is located in the northern area of Tibetan Autonomous Prefecture of Gannan, which is south to Luqu County, west to Xiahe County, north to Hezheng and Linxia Counties. This region has unique geographical position and significant regional advantages, with the average altitude being more than 3 000 m. The climate of Hezuo City belongs to the plateau climate with great temperature difference between day and night, 1.7℃ annual average temperature, and no absolute frost-free period.

Research methods

Literature survey

By consulting to the Medicinal Plants of Qinghai-Tibet Plateau, Flora of Tibetan Medicine in Qinghai-Tibet Plateau, and other relevant literatures[10-15], distribution status of CORDYCEPS in the eastern margin of Qinghai-Tibet Plateau was researched, as well as the vegetation and ecological conditions of distribution region, which provided references for the selection of investigation routs and investigation regions.

Interview and investigation

The peasants, Forestry Bureaus, Agricultural Bureaus, Tibetan medicine companies, Tibetan medicine hospitals, Food and Drug Administrations and CORDYCEPS markets were interviewed in Dangzhou Street, Yiheang Street, Jianmukeer Street, Tongqin Street, Kajiaman Village, Kajiadao Village, Zuogaiduoma Village, Zuogaimanma Village, Jiamaogong Village, Lexiu Village and Nawu Vilalge. The resources, distribution, yield and price of local CORDYCEPS were investigated.

Sampling analysis

Based on the information obtained by literature investigations and interviews, the investigation region, time and route were selected, as well as the sample survey areas and the quadrat schemes.

Sample plot selection and soil sample collection

Under the guidance of local peasants, investigation on sampling sites and quadrat combining with the questionnaire visit was carried out from early May to late June in the years 2011-2014. When selecting the sampling sites and quadrat, regions that could reflect both the wild resources distribution of CORDYCEPS and the distribution characteristics of

CORDYCEPS were selected. Quadrat selection was carried out in the investigation region by the method of random sampling. During the investigation, 10 typical major quadrates were selected with the size being 10 m × 10 m. In each major quadrat, 5 small quadrates (1 m × 1 m) were designed. There were in all 50 small quadrates. During sampling selection, 5 points method was used in the 10 major quadrates to record the sample characteristics and the surrounding environment information, including longitude, latitude, altitude, slope gradient, grassland type and major vegetation. A deep hole (30-40 cm) was dug in the quadrat. Soil samples at the layers of 0-5, 5-10, 10-15 and 15-20 cm were collected. There were in all 3 samples. The soil type was recorded. In the same sample site, soil samples in 5 quadrates at the layers of 0-5, 5-10, 10-15 and 15-20 cm were strictly mixed, which was used as the soil sample of corresponding quadrates. There were in all 21 soil samples. Soil sample was sealed by diagonal method. After coded, they were put in freshness protection bags and were detected in the laboratory.

Statistics of the number of CORDYCEPS[4]

Investigation and analysis of each quadrat were carried out, including the CORDYCEPS number, vegetation component, plant coverage, density and height, distribution status, growth status, soil type, and soil physicochemical properties. In each quadrat, one CORDYCEPS was dug; and the biomass was weighed. The stroma length and bat moth length were weighed. After baked in the oven at 80 ℃ to dryness, it was weighed again. The resource quantity was calculated according to the sample plant method. At the same time, 1 kg soil sample in corresponding quadrate was collected. Based on the objective of this investigation, the reserved quadrate was kept in each investigation region for dynamic investigation.1 quadrat was randomly selected from each major quadrat. The hole of 50 cm was dug. The number of CORDYCEPS and host was calculated in the small quadrat. Due to the serous damage of most major quadrates by peasants, the number of CORDYCEPS could not accurately calculated, and the holes in quadrat was used to represent the number of CORDYCEPS.

Analysis of soil index[5]

(1) Detection of soil organic matter content (OM). Electric sand bath and potassium dichromate titrimetric method was adopted. (2) Detection of total nitrogen (TN). Semimicro Macro Kjeldahl method was adopted. (3) Detection of total phosphorus in soil (TP). NaOH melting method —Mo-Sb Anticolorimetric method was adopted. (4) Detection of total potassium (TK). NaOH melting method- flame photometer method was adopted. (5) Detection of hydrolyzable nitrogen (HN). Alkaline hydrolysis diffusion method was adopted. (6) Detection of rapidly-available phosphorus (AP). Sodium bicarbonate method was adopted. (7) Detection of rapidly-available potassium (AT). Ammonium acetate—flame photometer method (1 mol/L neutral NH_4OAc extraction). (8) Detection of water content (WC). Oven drying method was adopted. (9) Detection of pH value. Potentiometry was adopted.

Data treatment

Principal component analysis

Data dimension reduction was carried out. Under the premise of no loss of information, less variables were selected to take the place of original variables, so as to eliminate the overlapping information.

Cluster analysis

The research object was analyzed. All the individual cases were classified into different categories, so that the individuals in the same category had relatively great similarity; and those in different categories had great differences, which was called cluster analysis. This method of mathematical statistics was suitable for the situation of unclear sample quantitative analysis method of two principal component clusters was used for the comprehensive evaluation and category of tested sample. Data were processed by software DPS7.5; and principal component analysis was carried out. Hierarchical clustering method was used for the data of principal component, so as to determine the principal component of soil nutrition for CORDYCEPS. Effects of different factors on the population distribution of CORDYCEPS was analyzed by discriminant analysis, such as free water, hygroscopic water, pH value, organic carbon, organic matter, total nitrogen, hydrolyzable nitrogen, total phosphorus and available phosphorus.

RESULT AND DISSCUSS

Environmental characteristics of the main distribution areas of CORDYCEPS

TABLE 1 reported that CORDYCEPS mainly distributed in Kajiaman Village, Kajiadao Village, Zuogaiduoma Village, Zuogaimanma Village and Nawu Vilalge, which had more than 340 mm annual precipitation, was above 3000 m altitude and at upper slope. The medicinal and nutritional value was high. However, there were few CORDYCEPS in Jiamaogong Village and Lexiu Village with relatively poor values. Vegetations in the habitat were mainly Gentianaceae, Scrophulariaceae, Compositae, Equisetaceae, Rosaceae, Polygonaceae and Gramineae. Soil types were mainly the wet and dark felty

Soils, dark felty soils, felty soils, thin felty soils, cold and brown calcic soils, cold calcic soils, cold and dark calcic soil. Plants were mainly the Kobresia myosuroides, Potentilla anserine, Equisetum arvense, Bistorta macrophylla, Polygonum viviparum, Potentilla fruticosa and so on. The vegetation coverage rate was 75%; and the soil thickness was 30-40 cm.

TABLE 1 : Environmental characteristics of the main distribution areas of CORDYCEPS

Soil factor	Kajiadao Village	Kajiaman Village	Zuogaiduoma Village	Zuogaimanma Village	Jiamaogong Village	Lexiu Village	Nawu Village
Altitude	>3200m	3210 m	>3500 m	>3220 m	2860 m	2700 m	3000 m
Annual average precipitation	516 mm	510 mm	530 mm	590 mm	250 mm	250 mm	340 mm
Annual average temperature	0.5 ℃	2.8 ℃	0.3 ℃	2 ℃	8 ℃	12 ℃	1.6 ℃
Soil altitudinal zone	Dark brown soil	Dark brown soil	Brown felty soil	Dark brown soil	Brown soil	Brown soil	Brown soil
Vegetation type	Gentianaceae Scrophulariaceae Compositae Equisetaceae Rosaceae	Compositae Papaveraceae Leguminosae Polygonaceae	Papaveraceae Gramineae Rosaceae Compositae Polygonaceae	Gramineae Rosaceae Equisetaceae Compositae	Primulaceae Gramineae Rosaceae Polygonaceae	Gramineae Compositae Rosaceae Polygonaceae	Gentianaceae Gramineae Rosaceae Compositae、
Soil type	Wet and dark felty soils	Dark felty soils	Felty soils	Thin felty soils	cold and brown calcic soils	Cold calcic soils	Cold and dark calcic soil
Slope position	Upper slope	Upper slope	Upper slope	Upper slope	Middle slope	Middle slope	Upper slope

Effects of the soil physicochemical properties of different soil layers on the distribution of CORDYCEPS

Soil physicochemical properties of different soil layers of 21 quadrates were researched by single-factor variance analysis. Results showed that the pH value, TN, TP and AP in three soil layers had no significant differences ($P > 0.05$). Therefore, the mean values of the pH value, TN, TP and AP in four soil layers were used for the analysis and statistics. Results showed that WC, OM, TK and AK had extremely significant differences ($P < 0.01$). HN showed no significant differences at layers 0-5cm, 10-55 cm and 20-25 cm.

TABLE 2 : Investigation on the soil physicochemical properties of different soil layer

Variables	Soil layer //cm			
	0-5	5-10	10-15	15-20
WC	39.52±0.05aA	37.76±0.07bB	34.38±0.06cC	29.24±0.05dD
OM	34.31±0.05bB	35.28±0.09aA	32.30±0.05cC	26.35±0.10dD
PH	7.02±0.09aA	7.04±0.13aA	7.05±0.05aA	7.04±0.06aA
TN	2.21±0.04aA	2.29±0.06aA	2.18±0.029aA	2.16±0.01aA
HN	31.82±0.06bB	32.05±0.08aA	31.71±0.05bB	31.70±0.08bB
TP	1.41±0.05aA	1.43±0.07aA	1.42±0.06aA	1.38±0.09aA
AP	11.49±0.05aA	11.57±0.05aA	11.44±0.05aA	11.44±0.06aA
TK	2.80±0.06bB	2.99±0.06aA	2.72±0.05cC	2.57±0.05dD
AK	182.41±0.06cC	183.6±0.10aA	182.89±0.05bB	176.92±0.09dD

Note: Lowercases after the data indicated significant differences at 5% level; capital letters indicated significant differences at 1% level, the same as follows.

The correlation coefficient matrix of principal component analysis of soil physicochemical properties affecting the distribution of CORDYCEPS.

In the 21 quadrates, DPS7.05 was used for the principal component analysis of nine parameters (WC, OM, pH, TN, HN, TP, AP, TK and AK). And nine correlation coefficient matrixes of different soil layers were obtained. TABLE

3 reported that the absolute values of more than 85% data were greater than 0.30. Each variable had relatively great correlation coefficient with at least one variable. TABLE 3 reported that OM had significant correlations with WC, TN, HN, AP and AK (a = 0.05, r = 0.9500). TN had significant correlation with TP and TK; TP and AK showed significant correlation; HN had extremely significant correlation with AP and AK (a = 0.01, r = 0.9900); and AP and AK showed significant correlation.

TABLE 3 : Correlation coefficient matrix

Variables	WC	OM	PH	TN	HN	TP	AP	TK	AK
WC	1								
OM	0.9532	1							
PH	0.6365	0.3791	1						
TN	0.8845	0.9786	0.2037	1					
HN	0.8981	0.9717	0.3208	0.9374	1				
TP	0.7565	0.9176	0.005	0.9507	0.9439	1			
AP	0.9022	0.9743	0.3247	0.9405	0.9999	0.9436	1		
TK	0.8111	0.9071	0.1182	0.9655	0.8148	0.8733	0.8201	1	
AK	0.853	0.9603	0.2086	0.9481	0.9931	0.9755	0.9927	0.8355	1

The eigenvalues and variance contribution rates of correlation coefficient of principal component analysis of soil physicochemical properties

Principal component analysis in TABLE 4 showed that the eigenvalues of the first two were K1=7.5256＞1.0000 and K2=1.1866＞1.0000, with relatively high variance contribution rates; and the accumulative contribution rate was 96.8023＞85%, indicating that the first two principal components included the 100% information of nine parameters (WC, OM, pH, TN, HN, TP, AP, TK and AK) in soil layers 0-5, 5-10, 10-15 and 15-20 cm.

TABLE 4 : The eigenvalues and variance contribution rates of soil physicochemical properties

Principal component	Eigenvalue	Contribution rate //%	Accumulative contribution rate //%
1	7.5256	83.6177	83.6177
2	1.1866	13.1845	96.8023
3	0.2878	3.1977	100
…	…	…	…

Factor rotation of soil physicochemical properties

Based on principal component analysis, the first two principal components in factor matrix after varimax orthogonal rotation were selected to calculate the eigenvector of variable. TABLE 5 showed that the first principal component had the highest correlation coefficient with TP, reflecting that TP was the major factor affecting the population distribution of CORDYCEPS. In the second principal component, PH had relatively high correlation coefficient, indicating that CORDYCEPS had strict requirements for soil acidity-alkalinity.

TABLE 5 : Transposed matrix of the variable eigenvector of soil habitat of CORDYCEPS

Variable	The first eigenvector	The second eigenvector
WC	0.8042	0.5870
OM	0.9463	0.3204
PH	0.0625	0.9980
TN	0.9793	0.1422
HN	0.9466	0.2630
TP	0.9935	-0.0568
AP	0.9475	0.2669
TK	0.9205	0.0591
AK	0.9727	0.1489

Effects of soil depth on the morphology and number of CORDYCEPS

Based on the comprehensive statistics of morphology and number of CORDYCEPS in investigation zone, variance analysis was carried out. Results showed that the larva number in different soil depths had extremely significant differences. The larva lengths, larva diameters, stroma lengths and stroma diameters in different soil layers were in the order of 5-10 cm layer ＞15-20 cm layer ＞0-5 cm layer ＞ 20-25 cm layer. TABLE 6 reported that as the soil depth increased, the number of CORDYCEPS firstly enhanced and then reduced. The diameter and size of CORDYCEPS also showed the same changes. The number of CORDYCEPS was the maximum in soil layer 5-10 cm, which was 2.10 on average. The number of CORDYCEPS was the minimum in soil layer 20-25 cm, which was only 0.21. The number of CORDYCEPS in soil layer 20-25 cm reduced significantly by 90.00%, compared with the soil layer 5-10 cm. CORDYCEPS number was relatively great in soil layer 15-20 cm, which was 1.38 on average, indicating that CORDYCEPS mainly lived in soil layer 5-10 cm in Hezuo City in Gannan Tibetan Autonomous Prefecture; and the soil layer more than 20 cm was not suitable for the survival of CORDYCEPS.

TABLE 6 : Effects of soil depth on the morphology and number of CORDYCEPS

Soil layer//cm	Larva length//cm	Larva diameter//mm	Stroma length //cm	Stroma diameter//mm	Larva number
0-5	2.78±0.51bB	2.89±0.31cB	3.84±0.38bB	0.45±0.07bB	0.48±0.51cC
5-10	3.00±0.53aA	4.29±0.23aA	6.26±0.37aA	0.63±0.09aA	2.10±1.04aA
15-20	2.39±0.12bAB	2.94±0.18bB	4.44±0.21bB	0.40±0.06bB	1.38±0.86bB
20-25	2.03±0.47bB	2.28±0.17cB	4.37±0.16bB	0.36±0.08bB	0.21±0.76dD

Cluster analysis of the effects of soil physicochemical properties on the distribution of CORDYCEPS

Results showed that in soil layer 0-5 cm, PH and TN significantly affected the distribution of CORDYCEPS; while WC and TK had extremely significant impacts. In soil layer 5-10 cm, WC significantly affected the distribution of CORDYCEPS; while PH and TK had extremely significant impacts. In soil layer 15-20 cm, OM and TK significantly affected the distribution of CORDYCEPS; while WC, PH and TN had extremely significant impacts. In soil layer 20-25 cm, OM, PH and TK significantly affected the distribution of CORDYCEPS; while WC and TN showed extremely significant impacts. HN, AP and AK in three soil layers showed no impacts on the distribution of CORDYCEPS (TABLE 7).

TABLE 7 : Effects of soil physicochemical properties on the distribution of CORDYCEPS

Variable	Soil layer//cm			
	0-5 Wilk's	5-10 Wilk's	15-20 Wilk's	20-25 Wilk's
WC	0.68bB	0.79bA	0.67cC	0.62cC
OM	0.87aA	0.89aA	0.81bA	0.79bA
PH	0.72bA	0.74bB	0.74bB	0.76bA
TN	0.74bA	0.89aA	0.72bB	0.71bB
HN	0.95aA	0.93aA	0.92aA	0.90aA
TP	0.92aA	0.95aA	0.91aA	0.88aA
AP	0.95aA	0.99aA	0.95aA	0.95aA
TK	0.63bB	0.72bB	0.82bA	0.82bA
AK	0.72aA	0.97aA	0.95aA	0.93aA

CONCLUSIONS

Research results showed that CORDYCEPS in Hezuo City of Gannan Tibetan Autonomous Prefecture was mainly distributed in the upper slope with the altitude above 3000 m. The vegetation types were alpine meadow and alpine brush meadow; the soil types were mainly the dark felty soils and felty soils.

The average water content in soil layer 0-5 cm was the maximum, which was related to the precipitation of this region. The water content in soil layer 15-20 cm showed relatively great changes. The larva preferred high humidity (soil humidity 40%-46%)[6]. Other soil physicochemical properties (pH, TN, TP and AP) in different layers showed no significant differences, which was basically stable. Research results showed that WC, OM, TK and AK in three soil layers showed extremely significant differences. HN content had no significant differences in soil layers 0-5, 15-20 and 20-25 cm.

Results showed that larva mainly lived in the soil layer 5-10 cm. The host of CORDYCEPS mainly lived in soil all through its life cycle. Therefore, soil directly affected the population distribution of CORDYCEPS. It could be concluded

from this research that pH, TN, TP and AP were not the major soil factors affecting the growth and distribution of CORDYCEPS. The host larva might be affected by other biological factors and vegetation types, which needed further research and verification in future.

As the soil depth increased, the number, size and diameter of CORDYCEPS firstly enhanced and then reduced. The number of CORDYCEPS was the maximum in soil layer 5-10 cm. In this region, the soil was slightly alkaline; and the pH values in three soil layers showed no significant differences, which was consistent with the research result by Wu Qinggui et al[5].

In general, soil physicochemical properties had relatively great impacts on the distribution and survival of CORDYCEPS. Results of principal component analysis showed that in the first principal component, TP was the major factor affecting the population distribution of CORDYCEPS. In the second principal component, PH had relatively high correlation coefficient, indicating that CORDYCEPS had strict requirements for soil acidity-alkalinity. The number of CORDYCEPS was the maximum in soil layer 5-10 cm; and the soil layer more than 20 cm was not suitable for the survival of CORDYCEPS. TP, PH and TK were the key factors affecting the distribution of CORDYCEPS in research region; while HN, AP and AK in three soil layers showed no impacts.

Investigation showed that peasants' consciousness of protecting grassland vegetation enhanced in recent years. They got into the habit of backfilling during excavation, which had certain relaxation effects on the damage of ecological environment. However, almost all the family members, except the old and the small child, engaged in finding the CORDYCEPS during harvest period, which led to the yield reduction and extinction of CORDYCEPS. Therefore, it was necessary to strengthen the artificial cultivation research of CORDYCEPS and to actively protect and utilize CORDYCEPS. This research provided scientific references for the semi-artificial cultivation and sustainable development of wild resources and the habitat protection of CORDYCEPS.

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