

EFFECT OF *ASPERGILLUS NIGER* STRAIN XF-1 ON SOIL NUTRIENTS AND GROWTH OF *AMORPHA FRUTICOSA*

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Abstract. In order to reveal the interaction effect between *Aspergillus niger* (*A. niger*) and *Amorpha fruticosa* (*A. fruticosa*), to guide the large-scale production and cultivation of *A. fruticosa* in ecological restoration, and to promote the greening of rock slope and improve the ecological environment, a study on the effect of fermentation broth with different dilutions of *A. niger* strain XF-1 on soil nutrient elements and the growth of *A. fruticosa* was conducted by pot experiment in a green house. The results showed that the contents of chlorophyll a, chlorophyll b and total chlorophyll in the leaves of *A. fruticosa* significantly increased with the 10⁻² dilution. *A. niger* strain XF-1 fermentation broth, and the plant height, fresh weight and dry weight of *A. fruticosa* significantly increased. Whether the condition of planting *A. fruticosa* or not, the contents of available phosphorus, iron, copper, and zinc in soil by watering were higher in *A. niger* strain XF-1 fermentation broth than that of control (no watering with the *A. niger* strain XF-1 fermentation broth), and the contents of the above-mentioned nutrients by watering with the 10⁻² dilution were the highest in soil in *A. niger* strain XF-1 fermentation broth, which could significantly promote the growth of *A. fruticosa*. Therefore, *A. niger* strain XF-1 has a good growth promotion effect on *A. fruticosa*.

Keywords: *Aspergillus niger*, nutrient element, growth-promoting, *Amorpha fruticosa*, weathering

Introduction

Aspergillus niger (*A. niger*) can dissolve the elements in rocks and minerals, releasing phosphorus, potassium, calcium, silicon and other elements in a form that is easy to be absorbed by plants (Wu, 2018). Meanwhile, it can also secrete oxalic acid, tartaric acid and citric acid to promote the growth of plants (Wang et al., 2018). It is a good strain used as a biological fertilizer. Zhang Lizhen et al. isolated and screened a strain of *A. niger* from caragana rhizosphere soil from a saline-alkali land, and found that this strain could convert insoluble inorganic phosphorus into available phosphorus nutrients for plants to absorb through metabolism, and improve the effective utilization rate of phosphorus (Zhang et al., 2011). Qian Linzhao found in his study that after adding *A. niger* fermentation broth, the pH value in the soil was significantly reduced, and the effective P, exchangeable Ca, Mg and effective Fe, Cu and Zn in the soil were effectively released in the acidic environment, resulting in a significant increase in the content of nutrient elements in the soil (Qian, 2014). Wang Yanqiu et al. used *A. niger* Ap-2 strain to produce biophosphorus bacterial fertilizer, and found that the content of available phosphorus in soil increased by 141.94%. The weight of single leaf, the

proportion of superior tobacco and the internal quality of tobacco leaf were all better than the control (Wang et al., 1993). Li Song et al. found that secondary metabolites of *A. niger* could promote the growth of potted tomatoes and reduce the incidence of tomato root knot nematode disease (Li et al., 2011). Gong Mingbo et al. inoculated *A. niger* in the corn field experiment, and the corn yield increased by about 15% compared with the control treatment (Gong et al., 2010). Lu Jing et al. found that the chlorophyll content, photosynthetic rate and the accumulation of carbon assimilation products in wheat leaves were enhanced when 100 times of *A. niger* fermentation broth was irrigated. It also significantly promoted the nitrate reductive activity of wheat leaves and roots ($p < 0.05$), and the nitrogen metabolism rate, protein content, total phosphorus content of plant tissues in wheat seedlings (up to 61%) and biomass accumulation in wheat parts above ground all increased significantly (up to 21%). After applying *A. niger* fermentation broth diluted 100 times, the available phosphorus content of the soil increased by 122% during the experiment. The decrease of soil total phosphorus was greater than that of the corresponding control group (Lü et al., 2015). Relevant studies have shown that microorganisms can resist diseases and insect pests, absorb heavy metals, dissolve phosphorus and release potassium, therefore, they can be successfully used in bioremediation, promotion of crop growth and improvement of crop quality.

Amorpha fruticosa (*A. fruticosa*), also called *Shrubby falseindigo*, is a perennial deciduous shrub, that has a strong vitality, resistance to drought (Yan et al., 2017), resistance to water dipping, resistance to cold, resistance to the sand, resistance to pests, resistance to stress, and resistance to pollution (Cui et al., 2016). It is currently an important plant in the flood control and highway defence in China, and it is also one of the common species of slope greening trees. *A. fruticosa* is a good green fertilizer and animal feed, leaves of which are large and rich in nutrition. At the same time, the root wart of *A. fruticosa* in the part of root plays an important role in soil improvement. When Yang Chuanxing et al. investigated the weathering soil thickness of gangue hill in semi-arid areas, they found that the weathering soil thickness of *A. fruticosa* was the thickest in the five years old young trees among *A. fruticosa*, *Robinia pseudoacacia*, *Ulmus pumila* and Seabuckthorn. Compared with other tree species, *A. fruticosa* can save 50% of the cost in the greening of gangue hill. Planting *A. fruticosa* can accelerate the surface weathering of gangue, forming of soil, thus afforestation cost is lower (Yang, 2008).

Plant beneficial rhizospheric microorganisms (PBRMs) are able to colonize the rhizosphere and to improve plant growth, development and nutrient use efficiency by means of a wide variety of mechanisms like organic matter mineralization, biological control against soil-borne pathogens, biological nitrogen fixation, potassium, phosphorous and zinc solubilization and root growth promotion (Meena et al., 2017; Eriola, 2018). The growth promotion effect of *A. niger* XF-1 on *A. fruticosa* was studied in a green house. We tested the effect of different concentrations of fermentation broth of *A. niger* XF-1 on the growth of *A. fruticosa*, the number of soil microorganisms, available phosphorus, iron, copper, zinc, manganese, exchangeable calcium and magnesium in soil. The physiological and biochemical characteristics of *A. fruticosa* seedling were analysed under different concentrations of *A. niger*, and the interaction effect between the *A. niger* and the *A. fruticosa* was revealed, so as to provide theoretical and technical support for guiding the large-scale production and cultivation in the ecological restoration of rock side slope.

Materials and methods

The tested seeds of A. fruticosa

Seeds of *A. fruticosa* were collected from Taihang mountain in Henan province. It was preserved in the Key Laboratory of Soil and Water Conservation and Ecological Restoration in Nanjing Forestry University.

Collection of soil and rock samples

Soil samples were taken from the closed mine in Jindingshan (E 120°30', N31°17') in Suzhou, Jiangsu. The content of soil organic matter (OM) was 13.67 g·kg⁻¹, total nitrogen (N) 0.92 g·kg⁻¹, pH 7.10, available P was 9.01 mg·kg⁻¹, exchangeable calcium was 9.70 cmol·kg⁻¹, exchangeable magnesium was 3.16 cmol·kg⁻¹, effectiveness of iron, manganese, copper, zinc was 2.66, 5.24, 0.50, 1.23 mg·kg⁻¹ respectively in tested soil. Samples were sterilized at 121 °C for 20 min.

The rocks used in this study are porphyritic granites. The collected samples were placed in a sterile kraft bag and stored in a refrigerator at -20 °C. Fresh rock samples used in the experiment were washed with distilled water, dried naturally, ground, screened with 100 mesh, particle size < 90 µm, and sterilized under high pressure at 121 °C for 20 min.

Testing strain

A. niger strain XF-1 was activated, inoculated and fermented in PD culture solution for 72 h for later use.

Medium and preparation

PD medium (liquid): 200 g of diced potatoes were boiled for 20 min, filtered by 8 layers of gauze to remove residue. 20 g of glucose was added in it, and distilled water was added to 1000 mL. Then, the potatoes were thoroughly stirred and sterilized at 115 °C for 20 min (Wu, 2018).

Seed treatment and seedling cultivation of A. fruticosa

Seeds of *A. fruticosa* were soaked in sodium hypochlorite solution (2.5% of active chlorine) for 10 min to eliminate the bacteria on the surface of the seed, washed several times with sterile water, put on the sterilized tray containing quartz sand (hand knead as dough, openhanded as powder), covered with a sterile gauze, then put in the darkness of the incubator at 28 °C. Right amount water was sprayed to maintain appropriate humidity every day, sprouting seed germinated after 4 d, the consistent germinated seeds were selected and transferred to the pot for culture.

The pots (12.5 cm in diameter and 14.5 cm in height) used in the experiment were filled with 1.0 kg of soil and 100 g of granite powder each, and were divided into two groups: one group was planted with *A. fruticosa*, and the other group was not planted with *A. fruticosa*. Three plants were planted in each pot. When the plants emerged for 10 days, *A. niger* fermentation broth was diluted by 50, 100 and 200 times respectively, and poured into the two groups of the test bowls. A total of 5 mL irrigation was applied at one time, and tap water irrigation was applied for control treatment. The experimental treatments and their codes were shown in *Table 1*. The treatments of planting *A.*

fruticosa were CK (T₀-A), T₅₀-A, T₁₀₀-A, T₂₀₀-A. The treatments without *A. fruticosa* were CK (T₀), T₅₀, T₁₀₀ and T₂₀₀, as shown in Table 1. Each treatment was repeated 10 times. The experiment began on May 20, 2017, with a growth cycle of three months, and 50 mL water was sprayed on every other day to maintain appropriate humidity.

Table 1. The different treatments and corresponding codes

Treatment	Without <i>A. fruticosa</i> group	Planting <i>A. fruticosa</i> group
CK	CK (T ₀)	CK (T ₀ -A)
50 times nutrient solution of <i>A. niger</i> XF-1	T ₅₀	T ₅₀ -A
100 times nutrient solution of <i>A. niger</i> XF-1	T ₁₀₀	T ₁₀₀ -A
200 times nutrient solution of <i>A. niger</i> XF-1	T ₂₀₀	T ₂₀₀ -A

Determination of microbial quantity in rhizosphere

A. fruticosa was removed from soil, 0.2 g plant roots were carefully cut and placed in 100 mL sterile water, diluted and spread on PDA solid medium, cultured at 28 °C for 72 h and counted. *A. niger* PDA plates were cultivated, 1 g of the soil of various treatments were taken and put in a 100 mL conical flask, 50 mL sterile water was added in it, it was diluted it into four gradient, namely 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ in super clean workbench after 3 h, and then last two gradient coating to its corresponding medium were selected and put through the shaking table at 28 °C. After 4 d, the number of tested fungi in the soil was observed and recorded.

Determination of soil pH value, effective P, Cu, Zn, Fe, Mn, exchangeable Ca, and Mg

PH value of soil was determined by PHS-3CT. The available phosphorus in soil was determined by sodium bicarbonate extraction–molybdenum antimony anticolorimetric method (Olsen method). The contents of exchangeable Ca and Mg in soil were determined by 1 mol·L⁻¹ ammonium acetate exchangeable atomic absorption spectrometry (Bao, 1981). The soil availability of Cu, Zn, Fe and Mn were determined by DTPA solution extraction-atomic absorption spectrometry (Zhang, 2004).

Determination of chlorophyll content

Chlorophyll content of *A. fruticosa* was extracted by ethanol and 80% acetone mixture ($V_{\text{ethanol}}: V_{80\% \text{ acetone}} = 1:1$), and determined by colorimetry at wavelengths of 646 nm and 663 nm, respectively, to calculate chlorophyll content of $w(\text{Chla})$, $w(\text{Chlb})$ and $w(\text{Chl})$ (Wang et al., 2017).

Determination of plant height and biomass of *A. fruticosa*

Put the whole pot of *A. fruticosa* under the tap pipe, rinse the soil with slender water. Take out the whole plant, clean it with deionized water several times, and then dry the water on the root surface with absorbent paper. The *A. fruticosa* roots were cut off, the height, root length and fresh weight were measured respectively. The whole plant of *A. fruticosa* were washed with deionized water, and then water was removed at 105 °C, and dried at 85 °C to a constant mass. The dry and fresh mass of each part of *A. fruticosa* were recorded, and the root-crown ratio was calculated.

Scanning electron microscope observation of root complex of *A. fruticosa*

The root part of *A. fruticosa* was cut off with scissors, put in a 1.5 mL centrifuge tube, and placed in an oven at 40 °C. After drying, the samples were fixed on the copper table of scanning electron microscope, and sprayed metal. The root of the plant was analyzed by Hitachi-S3400N scanning electron microscope and energy spectrometer.

Statistical analysis method of data

Excel 2010 was used to process the data, and SPSS 20.0 was used for variance analysis.

Results

Influence of *A. niger* strain XF-1 on the number of microorganisms tested in soil rhizosphere

The determination results from *Figure 1* showed that all the quantity of rhizosphere microorganisms of *A. fruticosa* inoculated with *A. niger* were above 10^3 cfu·g⁻¹, which was significantly higher than that of control. With the decrease of irrigation concentration, the number of rhizosphere microorganisms decreased gradually. The number of microorganisms of *A. niger* XF-1 treated with T₅₀-A was maximum, reached 3.16×10^4 , which was significantly higher than other treatments. It indicated that *A. niger* XF-1 could colonize the rhizosphere of *A. fruticosa*, and then interacted with plants to form a soil-microbe-plant system with soil.

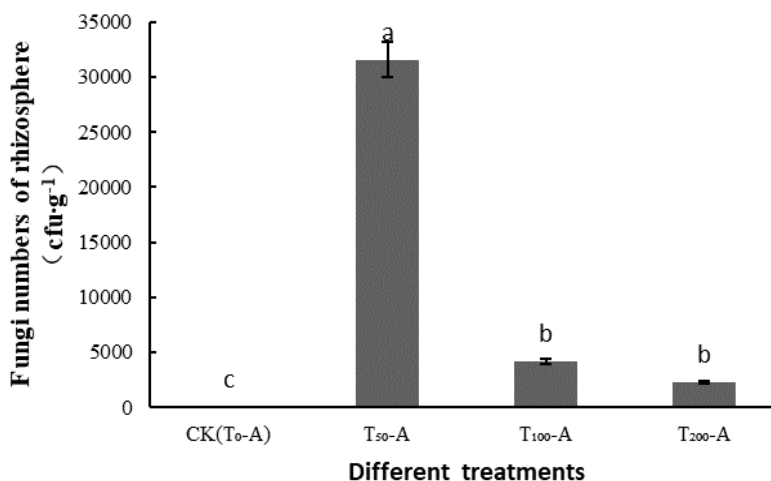


Figure 1. Fungi numbers in *A. fruticosa* rhizosphere in different treatments

Effects of *A. niger* XF-1 on soil pH value

As shown in *Table 2*, the pH values of the treated group after irrigation of *A. niger* XF-1 fermentation broth were significantly lower than that of the control group and were slightly acidic, indicating that irrigation of *A. niger* XF-1 nutrient solution had a significant impact on the soil pH value. The pH value of soil of planting *A. fruticosa* treated with the same *A. niger* concentration was lower than that without planting *A. fruticosa*, but the difference was not significant, indicating that the growth of *A. niger*

strain XF-1 secreted organic acids to reduce the soil pH value. Compared with the control without *A. fruticosa*, the soil pH values of treatments of the control group of planting *A. fruticosa*, diluted 50 times, diluted 100 times and diluted 200 times decreased to 7.0, 6.2, 6.1 and 6.3 respectively, and the pH value of the treatment diluted 100 times decreased to the lowest, indicating that the organic acid produced by watering at this concentration was the most.

Table 2. pH values of different dilutions of fermentation broth of *A. niger* strain XF-1

Treatment	pH value
CK (T ₀ -A)	7.0 ± 0.09a
CK (T ₀)	7.1 ± 0.12a
T ₅₀ -A	6.2 ± 0.11b
T ₅₀	6.3 ± 0.08b
T ₁₀₀ -A	6.1 ± 0.19b
T ₁₀₀	6.2 ± 0.07b
T ₂₀₀ -A	6.3 ± 0.13b
T ₂₀₀	6.4 ± 0.08b

Different letters after the same column of numbers indicate significant differences at the 0.05 level

Influence of A. niger strain XF-1 on effective P, Cu, Zn, Fe and Mn in soil

As can be seen from *Table 3*, after watering *A. niger* strain XF-1 nutrient solution, the content of effective P in soil was generally higher than that of CK (T₀-A and T₀). For dilution of 50 times, 100 times and 200 times, the available P content of soil planted with *A. fruticosa* increased by 16.9%, 35.91% and 26.76%, respectively compared with CK (T₀-A), and that of soil without *A. fruticosa* increased by 17.69%, 32.24% and 23.32%, respectively compared with CK (T₀). After watering *A. niger* strain XF-1 nutrient solution, the contents of effective Fe, Cu, zinc, and Mn in the soil were higher than CK (T₀-A and T₀). The content of effective Fe of treatments of planting *A. fruticosa* diluted 50 times, 100 times and 200 times in the soil after 90 days increased by 24.04%, 27.10% and 25.95% than CK (T₀-A), respectively, the content of effective Cu in soil increased by 4.91%, 11.48% and 6.56% than CK (T₀-A), respectively, the content of effective zinc in soil increased by 6.30%, 7.87% and 7.09% than CK (T₀-A), respectively, the content of available Mn in soil increased by 6.65%, 11.49% and 6.85% than CK (T₀-A), respectively. For dilution of 50 times, 100 times and 200 times, the content of effective Fe of treatments without *A. fruticosa* in soil after 90 days increased by 26.89%, 26.89% and 25.76% than CK (T₀), respectively, and the content of effective Cu in soil increased by 1.61%, 11.29% and 6.45% than CK (T₀), respectively, the content of available Zn in soil increased by 5.43%, 6.30% and 4.65% than CK (T₀), respectively, and the content of available Mn in soil increased by 6.85%, 11.49% and 6.85% than CK (T₀), respectively, but the difference was not significant.

Effects of A. niger strain XF-1 on exchangeable Ca and Mg in soil

As can be seen from *Table 4*, after watering of *A. niger* XF-1 nutrient solution, the contents of exchangeable Ca and Mg in soil were higher than CK (T₀) and CK (T₀-A), but the difference were not significant. 90 days after watering, the contents of

exchangeable Ca of treatments diluted 50 times, 100 times and 200 times with planting *A. fruticosa* in soil were 1.23%, 1.91% and 0.90% higher than CK (T₀-A), and the contents of exchangeable Mg of that in soil were 0.61%, 1.22% and 0.91% higher than CK (T₀-A), respectively. The contents of exchangeable Ca of treatments diluted 50 times, 100 times and 200 times without planting *A. fruticosa* in soil after 90 days increased by 1.57%, 2.68% and 1.12% than CK (T₀) respectively, and the content of exchangeable Mg of that in soil increased by 0.61%, 1.21% and 0.91% than CK (T₀), respectively, but there was no significant difference.

Table 3. Effect of different dilution fermentation broth of *A. niger* XF-1 on available P, K, Cu, Zn, Fe, Mn (mg·kg⁻¹) in soil

Treatment	P	Cu	Zn	Fe	Mn
CK (T ₀ -A)	7.10 ± 0.12b	0.53 ± 0.01b	1.27 ± 0.03b	2.62 ± 0.05b	4.96 ± 0.02b
CK (T ₀)	7.63 ± 0.19b	0.62 ± 0.02a	1.29 ± 0.04b	2.64 ± 0.06b	4.96 ± 0.06b
T ₅₀ -A	8.30 ± 0.09b	0.64 ± 0.02a	1.35 ± 0.02a	3.25 ± 0.04a	5.29 ± 0.04ab
T ₅₀	8.98 ± 0.13ab	0.63 ± 0.01a	1.36 ± 0.01a	3.29 ± 0.03a	5.30 ± 0.03a
T ₁₀₀ -A	9.65 ± 0.08a	0.68 ± 0.01a	1.37 ± 0.03a	3.33 ± 0.02a	5.53 ± 0.05a
T ₁₀₀	10.09 ± 0.15a	0.69 ± 0.02a	1.37 ± 0.02a	3.35 ± 0.01a	5.53 ± 0.03a
T ₂₀₀ -A	9.00 ± 0.08a	0.65 ± 0.01a	1.36 ± 0.01a	3.30 ± 0.03a	5.30 ± 0.06a
T ₂₀₀	9.41 ± 0.12a	0.66 ± 0.01a	1.35 ± 0.02a	3.32 ± 0.02a	5.30 ± 0.07a

Different letters after the same column of numbers indicate significant differences at the 0.05 level

Table 4. Effect of different dilution fermentation broth of *A. niger* on exchangeable Ca and Mg (mg·kg⁻¹) in soil

Treatment	Ca	Mg
CK (T ₀ -A)	8.92 ± 0.04a	3.29 ± 0.07a
CK (T ₀)	8.94 ± 0.05a	3.30 ± 0.09a
T ₅₀ -A	9.03 ± 0.06a	3.31 ± 0.05a
T ₅₀	9.08 ± 0.13a	3.32 ± 0.07a
T ₁₀₀ -A	9.09 ± 0.08a	3.33 ± 0.08a
T ₁₀₀	9.18 ± 0.15a	3.34 ± 0.07a
T ₂₀₀ -A	9.00 ± 0.11a	3.32 ± 0.05a
T ₂₀₀	9.04 ± 0.12a	3.33 ± 0.06a

Same letters after the same column of numbers indicate no significant differences at the 0.05 level

Effect of *A. niger* strain XF-1 on chlorophyll content of *A. fruticosa*

As can be seen from Figure 2, the photosynthetic pigment content of *A. fruticosa* seedlings could be increased to different degrees after watering dilution nutrient solution of *A. niger* at different multiples. The contents of chlorophyll a, chlorophyll b and total chlorophyll in treatments of watering diluted 100 times were the highest, higher than that in treatments of watering diluted 50 times, but the difference was not significant ($p < 0.05$). The content of chlorophyll a and total chlorophyll were significantly higher than that of watering diluted 200 times, and significantly higher than that of control ($p < 0.05$). There was no significant difference in chlorophyll b content. The contents of

chlorophyll a, chlorophyll b and total chlorophyll in treatments of watering diluted 50 times were higher than that in treatments of watering diluted 200 times, but the difference were not significant ($p < 0.05$). The contents of chlorophyll a and total chlorophyll were significantly higher than that of the control group (T_0 and T_{0-A}), while the content of chlorophyll b was not significantly different ($p < 0.05$). The contents of chlorophyll a, chlorophyll b and total chlorophyll in the watered locust with a concentration of 200 times dilution were higher than that of the control (T_{0-A}), the contents of chlorophyll a and total chlorophyll were significantly higher than that of the control (T_{0-A}), and the content difference of chlorophyll b was not significant ($p < 0.05$). To sum up, the results showed that the contents of chlorophyll a, b and total chlorophyll of *A. fruticosa* in treatments of watering diluted 100 times were higher than that in treatments of watering diluted 50 times, and the contents of chlorophyll a, b and total chlorophyll of *A. fruticosa* in treatments of watering diluted 200 times were the lowest. The chlorophyll content was $T_{100-A} > T_{50-A} > T_{200-A}$ in turn.

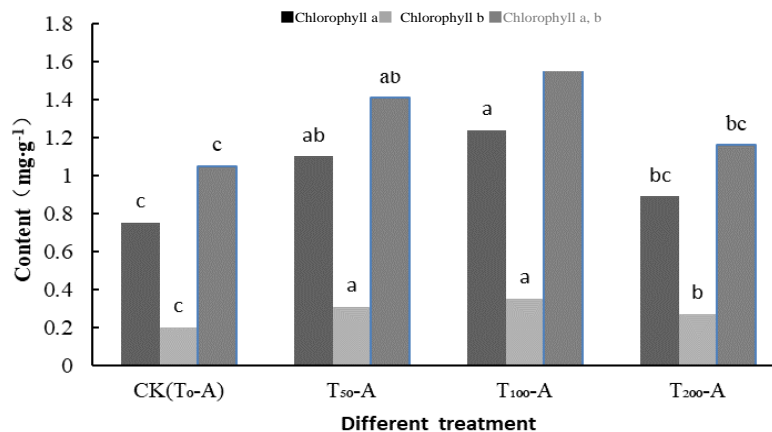


Figure 2. Effect of different dilution fermentation broth of *A. niger* XF-1 on chlorophyll content of *Amorpha fruticosa*

Effect of *A. niger* XF-1 on plant height and biomass of *A. fruticosa*

Effect of watering three kinds of different dilution concentrations of *A. niger* fermentation broth on plant height, root system and biomass were shown in *Figures 3* and *4* and *Tables 5* and *6*.

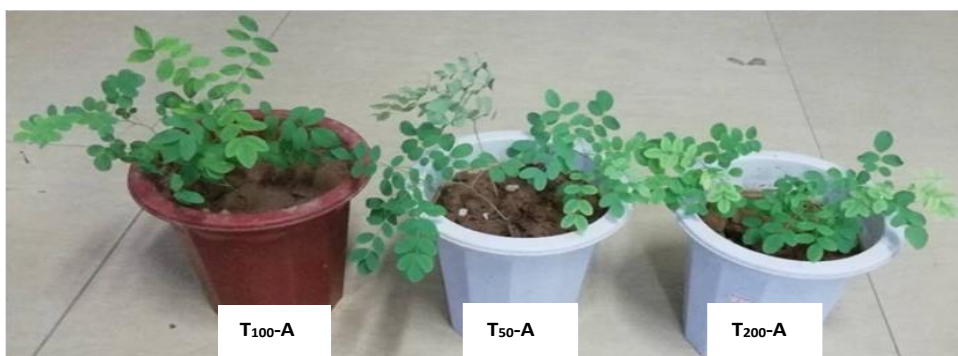


Figure 3. Effect of different dilution fermentation broth of *A. niger* XF-1 on the growth of *A. fruticosa*

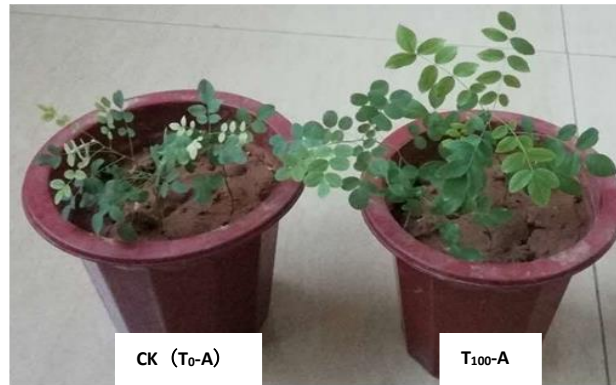


Figure 4. Comparison of plant height of *A. fruticosa* between treatment T_{100-A} and $CK (T_0-A)$

Tables 5 and 6 show that treatments of watering different dilution concentrations of fermentation broth of *A. niger* XF-1 can obviously promote the growth of *A. fruticosa* above ground. The analysis of plant height, fresh and dry weight above ground of *A. fruticosa* showed that the promoting effect of T_{100-A} was the best, of T_{200-A} was the second, of T_{50-A} was the worst. The plant height of *A. fruticosa* for T_{100-A} was 6.09 cm, 2.02 cm and 4.20 cm, which was significantly higher than that for T_0-A , T_{50-A} and T_{200-A} respectively. The fresh weight above ground for T_{100-A} was 0.11 g, 0.21 g and 0.24 g, which was significantly lower than that for T_{50-A} , T_{200-A} and T_0-A , respectively. The dry weight above ground for T_{100-A} was 0.04 g, 0.08 g and 0.12 g, which was significantly lower than that for T_{50-A} , T_{200-A} and T_0-A , respectively. The promoting ability of the three kinds of *A. niger* XF-1 nutrient solution to root length of *A. fruticosa* was $T_{100-A} > T_{50-A} > T_{200-A}$, and the root length of *A. fruticosa* for T_{100-A} was 2.34 cm, 3.92 cm, and 3.15 cm, which was significantly higher than that for T_{50-A} , T_{200-A} and T_0-A , respectively. The root diameter circumference and dry weight of *A. fruticosa* for the three kinds of *A. niger* XF-1 nutrient solution were significantly lower than that for T_0-A . The root diameter circumference of *A. fruticosa* for T_{100-A} was 0.26 mm and 0.58 mm, which was significantly higher than that for T_{50-A} and T_{200-A} respectively, but 0.21 mm was significantly lower than that for T_0-A . Root fresh weight of *A. fruticosa* for T_{100-A} was significantly higher (0.04 g and 0.05 g) than that for T_{50-A} and T_{200-A} respectively, but 0.04 g was significantly lower than that for T_0-A . The dry weight of root system of *A. fruticosa* for T_{100-A} was significantly lower than that for T_{50-A} and T_0-A by 0.02 cm and 0.04 cm respectively, and significantly higher than that for T_{200-A} by 0.02 cm. Compared with the control T_0-A , the root cap ratio of *A. fruticosa* with different concentrations of *A. niger* XF-1 fermentation broth was significantly decreased, indicating that *A. niger* XF-1 nutrient solution improved the condition of nutrition supplies of *A. fruticosa*, was advantageous to the rapid growth of above round parts. Among them, T_{100-A} treatment promoted the growth of *A. fruticosa* the fastest, but there was no significant difference with the other two concentrations of *A. niger* XF-1 nutrient solution. On the whole, T_{100-A} treatment has the best effect in promoting the growth of *A. fruticosa*, which is suggested as a reference for ecological restoration.

Scanning electron microscope (SEM) observation of root complex of *A. fruticosa*

The above studies indicated that *A. niger* XF-1 had a significant effect on promoting *A. fruticosa*. In order to better study the weathering of plants and microorganisms on

granite rocks, the effect of *A. niger* XF-1 on the granite and the roots of *A. fruticosa* was observed by SEM. As can be seen from *Figure 5*, the surface of the granite which is not planted with *A. fruticosa* and not inoculated with *A. niger* XF-1 was relatively smooth, angular and without a large number of fragments. However, *A. niger* XF-1 was distributed on the surface of the granite treated by inoculation without *A. fruticosa*, and there were a large number of granular substances, even the presence of fungal secretions. It can be seen that the granite underwent different degrees of weathering under the action of *A. niger* XF-1.

Table 5. Effect of different dilution fermentation broth of *A. niger* XF-1 on plant height, root length and diameter circumference of *A. fruticosa*

Treatment	Plant height (cm)	Root length (cm)	Diameter circumference (mm)
CK (T ₀ -A)	7.43 ± 0.19d	9.42 ± 0.22c	2.03 ± 0.45a
T ₅₀ -A	11.50 ± 0.17b	10.23 ± 0.26b	1.56 ± 0.09c
T ₁₀₀ -A	13.52 ± 0.35a	12.57 ± 0.69a	1.82 ± 0.06b
T ₂₀₀ -A	9.32 ± 0.23c	8.65 ± 0.19d	1.24 ± 0.03d

Different letters after the same column of numbers indicate significant differences at the 0.05 level

Table 6 Effect of different dilution fermentation of *A. niger* XF-1 on the above ground part and root biomass of *A. fruticosa*

Treatment	Fresh weight (g·plant ⁻¹)			Dry weight (g·plant ⁻¹)			Root cap ratio
	Overground	Root system	Total	Overground	Root system	Total	
CK (T ₀ -A)	0.40 ± 0.09d	0.45 ± 0.09a	0.85c	0.26 ± 0.09c	0.25 ± 0.09a	0.51 ± 0.09c	0.96a
T ₅₀ -A	0.55 ± 0.09b	0.37 ± 0.09c	0.92b	0.34 ± 0.09a	0.21 ± 0.09c	0.55 ± 0.09b	0.62b
T ₁₀₀ -A	0.66 ± 0.09a	0.41 ± 0.09b	1.07a	0.38 ± 0.09a	0.23 ± 0.09b	0.61 ± 0.09a	0.61b
T ₂₀₀ -A	0.45 ± 0.09c	0.36 ± 0.09c	0.80c	0.30 ± 0.09ab	0.19 ± 0.09d	0.49 ± 0.09d	0.63b

Different letters after the same column of numbers indicate significant differences at the 0.05 level

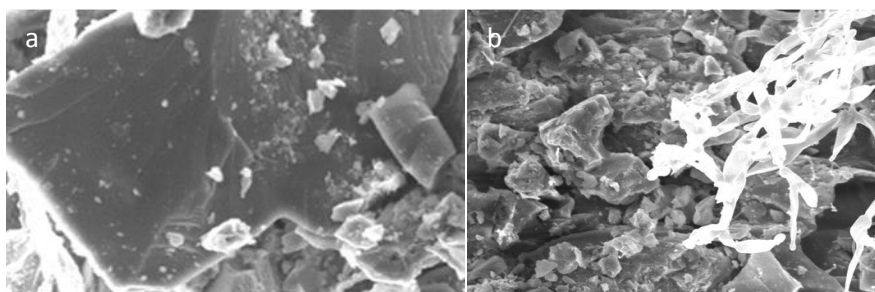


Figure 5. SEM photos of granite surfaces without planting *A. fruticosa*. (a) Granite surface without *A. niger* strain XF-1. (b) Granite surface with *A. niger* XF-1

Figure 6 showed the scanning electron microscope (SEM) images of the root system of plant and the attached granitoid particle complexes planted with *A. fruticosa* without inoculation. As can be seen from the picture, the root (or root hair) of the *A. fruticosa* can absorb fragments of granite. According to the result of energy spectrum analysis, the weathering effect of granites adsorbed on the root surface of *A. fruticosa* is not obvious.

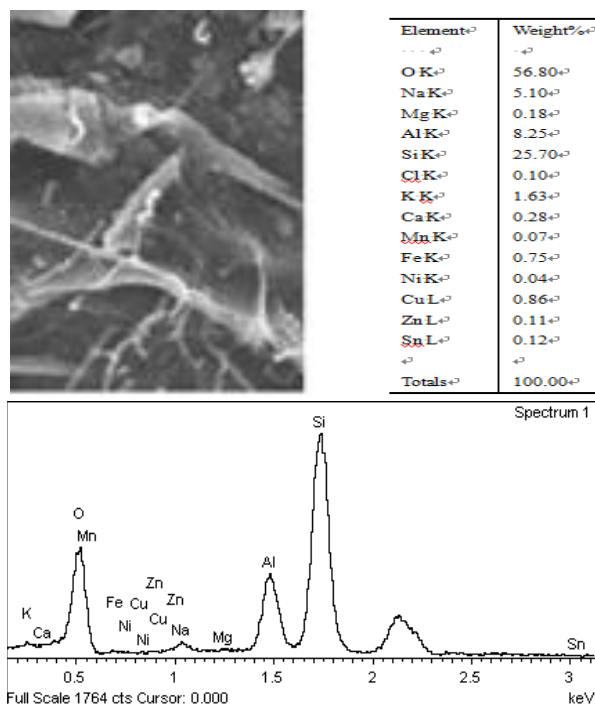


Figure 6. SEM photos of granite planted *A. fruticosa* without *A. niger* XF-1

Figure 7 is a scanning electron microscope image of the root system and the attached granitoid particle complex of *A. fruticosa* by inoculation. It can be seen from the figure that there were a large secretions of *A. niger* XF-1 and granites on the root system surface of *A. fruticosa* treated by inoculation. It can be seen that *A. niger* XF-1 could colonize the surface of granite rocks and root of *A. fruticosa*, and multiply to produce hyphae and secrete metabolites. According to the results of energy spectrum analysis, the surface of *A. niger* XF-1 and root system of *A. fruticosa* adsorbed a lot of elements. *A. niger* XF-1 had a significant effect on the granite weathering of the root surface of *A. fruticosa*.

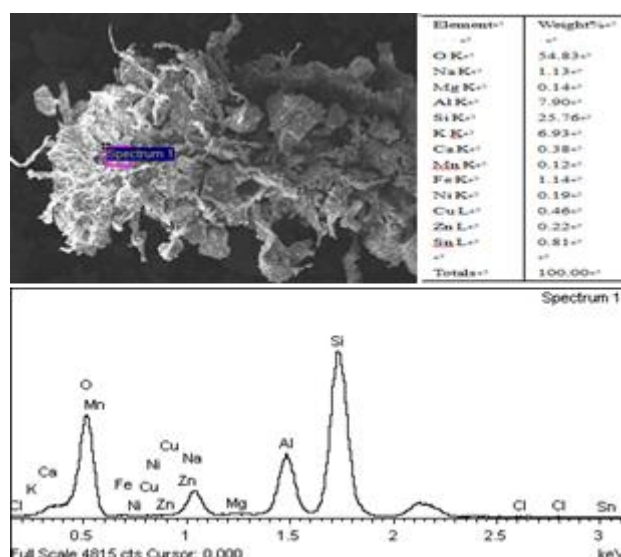


Figure 7. SEM photos of granite, that plant *A. fruticosa*, treated with *A. niger* XF-1

Discussion

A. niger XF-1 can well colonize surfaces of plant roots and rock, appropriately reduce the soil pH value, increase the content of available phosphorus, iron, copper, zinc and manganese in the soil, and promote the growth of plants and the increase of biomass. It is a strain that can effectively dissolve soil elements and promote the growth of plants. The results of this experiment showed that the pH value of soil was decreased from 7.1 to 6.1 after watering the fermentation broth of *A. niger* strain XF-1. The decrease of pH value was due to the strain producing a large amount of oxalic acid, lactic acid and succinic acid (Wu, 2018). The secretion of organic acids is the main mechanism of releasing nutrient elements from rock minerals (Wu et al., 2017a, b). During the reproduction process in soil, microorganisms secrete organic acids, cause the increase of the number of H⁺ in soil reduce the pH value in soil and can chelate with some plasmas such as iron, aluminum, calcium and so on, which makes the undissolved phosphorus render into effective phosphorus (Zhong et al., 2015), and the calcium, magnesium, iron, copper, zinc, silicon and other nutrients in soil can be activated with the increase of H⁺ to improve the availability of these nutrients in the soil (Wu, 2018; Wu, 2019). Meanwhile, microorganisms can also secrete polysaccharides, amino acids and other substances to directly improve plant stress resistance (Huang et al., 2009; Li et al., 2012).

In the process of screening microbial strains of weathered rock minerals from soil, in addition to its ability to weathering rock minerals, its role in plant-microbial systems should be considered. As the rhizosphere microorganism, the function of microbial strains may be influenced by other factors (Benoit et al., 2015). As living organisms, microbial strains also secrete metabolites, which may affect plant growth and development. For example, metabolites affect the effective absorption, and deposition of nutrients, the activity of plant pathogens, the occurrence and degree of plant diseases, and the stress resistance of plants, so as to promote or inhibit the growth of plants (Jiang et al., 2000). Saxena et al. have shown that applying bioinoculated *A. niger* K7 and biochar simultaneously is a good measure to improve soil fertility and crop yield. In the soil-plant experiment, all treatments significantly increased the growth, root nodule and yield of soybean plants ($p < 0.05$) (Saxena, 2016). Rhizosphere microorganisms stimulate plant growth because they produce a variety of plant growth hormones and vitamins in solution and in plants. These compounds can promote plant growth to some extent.

Nitrogen, phosphorus and potassium are essential elements for plant growth and development. Released nutrients by weathering rock mineral and substances secreted by metabolism for single strain cannot completely meet the requirements of plant growth. Therefore, using the mutual composite of microorganisms with different functions in soil, such as compound utilization of phosphorus-soluble microorganisms and microbial strains which can resolve nitrogen or potassium, can meet the plant's nutritional requirements. At present, the compounding and use of compound bacterial preparation has become a hot research problem, and if microbial strains are used in combination with microorganisms that can decompose the remaining chemical fertilizers and pesticides in the soil, or kill pests, or sterilize bacteria, and or improve physical and chemical properties of soil, it is bound to strengthen the ability of microorganisms to adapt to the environment, optimize the structure of soil, stimulate the accumulation of the microbial metabolites, and meet the supply of plant growth nutrients, which will benefit the rapid coverage of bare rock slope vegetation and promote the sustainable development of ecological restoration.

Conclusion

In this study, the number of rhizosphere microorganisms was significantly higher than that of control group after inoculation. Moreover, with the increase of irrigation concentration, the number of rhizosphere microorganisms of *A. fruticosa* increased gradually. It indicated that *A. niger* XF-1 can colonize the rhizosphere of *A. fruticosa*, which is conducive to the formation of soil-microbial-plant system.

After inoculation with *A. niger* XF-1 fermentation broth, the pH value of soil decreased significantly. The contents of exchangeable Ca and Mg in soil were generally higher than those in control. Different concentration of *A. niger* XF-1 fermentation broth could increase the photosynthetic pigment content and the growth of *A. fruticosa* seedlings significantly.

SEM results showed that the root surface of *A. fruticosa* had a large number of metabolites and granites after inoculation of *A. niger* XF-1. It indicated that XF-1 could colonize on the granite and the root surface of *A. fruticosa*, produce a large number of mycelia and secrete metabolites. The granite weathering effect on *A. fruticosa* was obvious. This study provides a new idea for ecological restoration of degraded habitats and a new model for rapid soil formation and wall greening of exposed rocks in abandoned mining areas. At present, the use of microorganisms for ecological restoration is still in the experimental stage, and the effect of mountain slope greening remains to be further studied.

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REFERENCES

- [1] Benoit, I., van den Esker, M., Patyshakuliyeva, A. et al. (2015): *Bacillus subtilis* attachment to *Aspergillus niger* hyphae results in mutually altered metabolism. – *Environmental Microbiology* 17(6): 2099-2113.
- [2] Bao, S. D. (1981): *Soil Agrochemical Analysis*. 3rd Ed. – China's Agricultural Publishing House Beijing.
- [3] Cui, B. X., Zhang, X. X., Han, G. et al. (2016): Antioxidant defense response and growth reaction of *Amorpha fruticosa* seedlings in petroleum. – *Contaminated Soil, Water, Air, & Soil Pollution* 227(4): 121.
- [4] Eriola, V., Glenda, S., Astrit, B. (2018): Tripartite relationships in legume crops are plant-microorganism-specific and strongly influenced by salinity. – *Agriculture* 8(8): 117.
- [5] Gong, M. B., Fan, B. Q., Jin, Z. G. et al. (2010): Screening and application of phosphate-dissolving microorganism suitable for corn production. – *Acta Microbiologica Sinica* 50(12): 1619-1625.
- [6] Huang, Y. H., Tang, R. S., Ye, X. Q., et al. (2009): Effect of ABA on the germination of white grain wheat seeds and growth of its seedlings. – *Journal of Triticeae Crops* 29(3): 503-507.
- [7] Jiang, X. J., Huang, Z. X., Xie, D. T., et al. (2000): Promoting effects of the metabolites of silicate for plant growth. – *Journal of Southwest Agricultural University* 2(2): 116-119.

- [8] Li, M., Ma, Q. Q., Zhao, H. J., et al. (2012): Effect of micro-derived hardness agent on endogenous hormones content in maize roots. – Heilongjiang Agricultural Sciences 29(8): 47-49.
- [9] Li, S., Duan, Y. X., Zhu, X. F. et al. (2011): Effects of adding secondary metabolites of *Aspergillus niger* on resistance to tomato root-knot nematode. – China Vegetables 1(4): 44-49.
- [10] Lü, J., Tian, X. H., Y, H. et al. (2015): Effect of *Aspergillus niger* fermentation liquid on the growth of wheat seedlings in calcareous soil. – Journal of Northwest Agriculture and Forestry University (Natural Sciences Edition) 43(5): 100-106.
- [11] Meena, V. S., Meena, S. K., Verma, J. P. et al. (2017): Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: a review. – Ecological Engineering 107: 8-32.
- [12] Qian, L. Z., Gong, M. B., Gu, J. G. et al. (2014): Effect of two different fungi on nutrient dynamic changes in soil. – Chinese Soil and Fertilizer 41(5): 86-89.
- [13] Saxena, J., Rawat, J., Sanwal, P. (2016): Enhancement of growth and yield of glycine max plants with inoculation of phosphate solubilizing fungus *Aspergillus Niger* K7 and biochar amendment in soil. – Communications in Soil Science and Plant Analysis 47(20): 2334-2347.
- [14] Wang, X. H., Wang, C. D., Sui, J. K. et al. (2018): Isolation and characterization of phosphofungi, and screening of their plant growth-promoting activities. – AMB Express 8(1): 63.
- [15] Wang, Y. Q., Xu, H. L., Xu, G. Y. et al. (1993): The applicable of AP-2 *Aspergillus niger* dissolve phosphorus microorganism in the planting tobacco. – Biotechnology 3(1): 4-38.
- [16] Wang, Y. X., Zhang, J. C., Wu, Y. W. et al. (2017): Effects of soil bacteria inoculation in spray seeding matrix on photosynthesis characteristics and chlorophyll fluorescence parameters of *Amorpha fruticosa*. – Research of Environmental Sciences 30(6): 902-910.
- [17] Wu, Q. F., Hu, H. B., Zhang, X. (2018): Effect of *Aspergillus niger* and its metabolites on weathering of granite. – Journal of Nanjing Forestry University (Natural Sciences Edition) 42(1): 81-88 (in Chinese).
- [18] Wu, Q. F., Hu, H. B. (2019): The influence of environmental factors on *Aspergillus niger* granite weathering. – Applied Ecology and Environmental Research 17(1): 395-408.
- [19] Wu, Y. W., Zhang, J. C., Guo, X. P., et al. (2017a): Identification of efficient strain applied to mining rehabilitation and its rock corrosion mechanism: based on boosted regression tree analysis. – Environmental Science 38(1): 283-293 (in Chinese).
- [20] Wu, Y. W., Zhang, J. C., Wang, L. J. et al. (2017b): A rock-weathering bacterium isolated from rock surface and its role in ecological restoration on exposed carbonate rocks. – Ecological Engineering 101(1): 162-169.
- [21] Yan, W. M., Zheng, S. X., Zhong, Y. q. W. et al. (2017): Contrasting dynamics of leaf potential and gas exchange during progressive drought cycles and recovery in *Amorpha fruticosa* and *Robinia pseudoacacia*. – Scientific Reports 7(1): 4470.
- [22] Yang, C. X. (2008): *Amorpha fruticosa* L. is a superior afforestation tree species in semi-arid waste dump area. – Forestry Science and Technology information 4(03): 30-31.
- [23] Zhang, J. (2004): Contrast determination of available Cu, Zn, Fe, Mn in soil by DTPA and M3 extraction. – Journal of Shanxi Agricultural Sciences 32(3): 30-33.
- [24] Zhang, L. Z., Fan, J. J., Niu, W. et al. (2011): Isolation of phosphate solubilizing fungus (*Aspergillus niger*) from *Caragana* rhizosphere and its potential for phosphate solubilization. – Acta Ecologica Sinica 31(24): 7571-7578.
- [25] Zhong, W. Y., Shi, F. C., Hong, M. J. et al. (2015): Phosphate solubilization and promotion of maize growth by *Penicillium oxalicum* P4 and *Aspergillus niger* P85 in a calcareous soil. – Canadian Journal of Microbiology 61(12): 1-11.