# Study on the Control Effect of Chinese Herbal Medicine Regulator on Leaf Spot Nematode Disease of *Chloranthus spicatus*

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**Abstract** [**Objectives**] This study was conducted to investigate the control of Chinese herbal medicine regulators on leaf spot nematode disease, the main pest of *Chloranthus spicatus* (Thunb.) Makino. [**Methods**] *C. spicatus* plants infected with nematodes were irrigated with a Chinese herbal medicine regulator at two different concentrations, and the control effect on leaf spot nematode disease of the plant was studied by measuring the number of nematodes, the contents of chlorophyll, malondialdehyde, soluble sugar and free proline in the leaves of the plants in the soil. [**Results**] Compared with the control, the two concentrations of Chinese herbal medicine regulator significantly reduced the number of nematodes in the soil, increased the contents of chlorophyll (mainly chlorophyll a), soluble sugar and free proline in the leaves of *C. spicatus*, and decreased the content of malondialdehyde. It indicated that the Chinese herbal medicine regulator could effectively control the spread of nematodes and had certain effect on the recovery of *C. spicatus* plants. [**Conclusions**] The use of Chinese herbal medicine regulators is helpful to curb the occurrence of leaf spot nematode disease in *C. spicatus* by biological prevention and control means, and provides theoretical guidance for the development of the green industry of *C. spicatus*.

**Key words** *Chloranthus spicatus*; Leaf spot nematode disease; Chinese herbal medicine regulator; Physical signs
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*Chloranthus spicatus* (Thunb.) Makino, also known as Jinsulan, Zhenzhulan and Jizhualan, is a perennial herb evergreen flower of *Chloranthus* in Chloranthaceae[1]. The inflorescence of *C. spicatus* is conical and looks like a panicle, and the flowers are as small as pearls, yellow in color as ivory, and have elegant and rich aroma. Because of its unique floral fragrance, since the Qing Dynasty, *C. spicatus* has been used to smoke tea, so that famous tea and floral fragrance are organically combined, and chloranthus tea with excellent color, aroma and taste is prepared. *C. spicatus* is known as "the four major fragrant flowers for tea in China" together with jasmine, *Michelia alba* and *Citrus aurantium* L. var. *amara* Engl. With the increasing concern for health and environmental protection in modern society, chloranthus tea as a drink can make people experience a more fresh and natural feeling of life. Chloranthus tea also has the effects of beautifying the feature, fixing the mind, nourishing the body, relieving pain and reducing weight, so it is especially favored by female consumers. In addition, the stems, leaves and roots of *C. spicatus* all can be used as medicines, which have the effects of expelling wind and dampness, connecting bones and muscles, removing food stagnation, expelling parasite, relieving pain and stopping bleeding[2].

In the history of China, *C. spicatus* was mainly produced in some tea-growing areas in Fujian, Anhui, Jiangsu, Zhejiang, Jiangxi and Sichuan Provinces[3]. At present, the main planting area of *C. spicatus* in Anhui Province is Shexian County, Anhui Province. The history of chloranthus tea in Shexian County is very long. According to *Shexian Annals*, the cultivation of *C. spicatus* began during the Daoguang period of the Qing Dynasty. After the liberation of New China, Shexian Tea Factory began to produce chloranthus tea in batches in 1950. In the national flower tea appraisal conducted by the Ministry of Commerce in 1979, chloranthus tea selected by Shexian tea factory won the first place. As a result, Shexian chloranthus tea enjoys a great reputation at home and abroad, and its market sales are steadily leaving others behind in their dust. Shexian chloranthus tea almost occupies 90% of the chloranthus tea market in China, especially in North China and Northeast China such as Shandong, Hebei and Tianjin. After the reform and opening up, tea enters the market and is freely traded, which leads to a sharp increase in the income for farmers who plant *C. spicatus*.

However, with the increase of planting density, diseases and pests of *C. spicatus* occur from time to time. Leaf spot nematode disease caused by *Aphelenchoides fragarae* first occurred in Fujian in 1965, and then spread to Jiangxi, Nanjing and other places. In 1984, it broke out on a large scale in Shexian County, Anhui

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Province, destroying more than 3 000 pots of seriously-diseased plants and causing direct economic losses of 200 000 yuan[4]. Stomata are the only way for *A. fragarae* to invade the leaves of *C. spicatus* without damage[5]. After *A. fragarae* enters the leaves of *C. spicatus*, at the initial stage of the disease, the diseased leaves turn pale yellow, then turn reddish brown, and the disease spots gradually expand and finally turn dark brown. The diseased leaves are easy to fall off early, and the flowers of the infected plants are small and few, and most of the leaves of seriously-infected plants fall off, which has a great impact on the yield[6-7]. Meanwhile, *A. fragarae* spreads very fast in the leaves of *C. spicatus*, and has the ability of active infection. In March – May, when there is more rainfall, the nematodes can move quickly with the help of water flow or spread with the help of rain splash, and the rate of diseased leaves decreases in October – December, which is related to the fact that the pathogenic nematodes gradually enter the overwintering state[7]. *A. fragarae* can overwinter in axillary buds and dead diseased leaves of *C. spicatus*. If the diseased leaves on the plants are not removed in time, the nematodes overwintering in the axillary buds or dead leaves will infect healthy plants in the next year, resulting in a serious reduction in the yield of *C. spicatus*[6-7]. Leaf spot nematodes of *C. spicatus* are harmful and spread quickly. Once a plant is infected, it can only be removed by pulling out the whole plant, resulting in serious economic losses. So far, the research on the susceptible mechanism of leaf spot nematodes of *C. spicatus* at home and abroad is still blank.

Chemical control has always been an important means to control nematode diseases, but agricultural control, physical control and biological control have not achieved good results in this kind of diseases caused by nematodes[8]. It has been reported that abamectin, fenamiphos, aldicarb and other pesticides have certain effects on the prevention and control of the development of leaf spot nematode disease of *C. spicatus*[9-10]. However, with the extensive use of chemical pesticides, the agricultural environment is polluted and the quality of agricultural products is deteriorated, which greatly harms people's health. In 2017, the No. 1 Central Document proposed to promote the zero growth of chemical pesticides, requiring that highly toxic chemical pesticides should not be used in the production process of flowers, vegetables and fruits. Five agricultural books with a long history (*Qimin Yaoshu*, *Wangzhen Nongshu*, *Nongsang Jiyao*, *Nongzheng Quanshu* and *Shoushi Tongzhe*) all record the application of Chinese herbal medicine in agriculture. Euphorbiae Ebracteolatae Radix, Radix Astragali, Herba Euphorbiae Helioscopiae, Radix Sophorae Flavescentis contain many kinds of bioactive substances (such as propylene, lignin, coumarins, flavonoids and terpenoids), which have good effects on the prevention and control of crop diseases and pests, and strong killing activities on cabbage worms, diamondback moths, aphids and nematodes[11-12]. Using Chinese herbal medicine to control plant diseases and pests is the most promising "ancient" and "new" field, and "Chinese medicine agriculture" will be a highlight of crop disease and pest control in China in the future. At present, the research on the prevention and control of leaf spot nematode disease of *C. spicatus* with Chinese herbal medicine is still in a blank stage. In this study, the control effect of Chinese herbal medicine regulator on leaf spot nematode disease of *C. spicatus* was investigated, hoping to expand green and efficient control methods of *C. spicatus* and inject new power into further reduction of production cost, improvement of product quality and ecological sustainable development.

## Materials and Methods

### Plant materials

*C. spicatus* used in this experiment was planted in Shexian County, Anhui Province. At the beginning of April, 2024, Chinese herbal medicine regulator (Shandong Yuanjiang Biotechnology Co., Ltd., mainly composed of *Stellera chamaejasme*, *Astragalus membranaceus* and *Crocus sativus*) was irrigated to two-year-old *C. spicatus* plants with similar disease severity according to two gradients of 1 ∶ 150 ($T_1$) and 1 ∶ 75 ($T_2$), and clear water was used as the control (CK). Healthy *C. spicatus* plants were treated with clean water only (JK). The plants were watered once a week and treated three times in successive. Samples were taken before treatment and on days 7, 14, 21 and 28 after treatment. Leaves of five *C. spicatus* plants were taken from each treatment to determine physiological and biochemical indexes, and three biological replicates were made.

### Determination of nematode content

Nematodes in soil were separated using Baermann funnel separation method and counted[13]. Specifically, a funnel with a diameter of 10 cm was placed on a wooden rack, and its lower end was connected with a rubber tube 10 cm in length with a flatjaw pinchcock. Next, 5 g of fresh soil was wrapped in double layer of gauze and added in the funnel filled with clear water. After 24 h, due to its water repellency and its own weight, the nematodes left the plant tissue, swam in the water, and finally settled in the rubber tube at the end of the funnel. Finally, the flatjaw pinchcock was opened, and the number of nematodes in the water was checked with a counting dish.

### Determination of chlorophyll content

Several representative leaves were selected from the plants, cut into pieces after removing coarse veins, and mixed evenly. Next, 0.2 g was quickly weighed, and added in a 25 ml graduated test tube, which was then added with 10 ml of 80% ethanol, plugged and heated in a water bath at 80 ℃ for extraction of chlorophyll until all the leaves turned green. After cooling, 80% ethanol was used to dilute the extract to constant volume, and the liquid was chlorophyll extract.

A cuvette with an optical diameter of 1 cm was added with chlorophyll extract 1 cm away from the mouth of the cuvette, and measured for the absorbance ($A$) at 663 and 645 nm with 80% ethanol as the control.

The calculation method is as follows:

$$C_a = 12.70A_{663} - 2.69A_{645} \quad (1)$$

$$C_b = 22.9A_{645} - 4.68A_{663} \quad (2)$$

$$C_T = C_a + C_b = 20.21\ A_{645} + 8.02A_{663} \quad (3)$$

$C_a$ and $C_b$ are the concentrations of chlorophyll a and b, and $C_T$ is the total chlorophyll concentration in (mg/L). The above formulas (1), (2) and (3) were used to calculate chlorophyll a, b and total chlorophyll concentration, respectively.

The measured absorbance values of $A_{663}$ and $A_{645}$ were substituted into the above formulas (1), (2) and (3) to calculate $C_a$, $C_b$ and $C_T$ (*i.e.* chlorophyll a, chlorophyll b and total chlorophyll concentration). Then, chlorophyll a, chlorophyll b and total chlorophyll contents were calculated according to following formulas.

$$\text{Chlorophyll a content (mg/g Fw)} = \frac{C_a \times \text{Total amount of extract (ml)}}{\text{Fresh weight of sample (g)} \times 1\,000}$$

$$\text{Chlorophyll b content (mg/g Fw)} = \frac{C_b \times \text{Total amount of extract (ml)}}{\text{Fresh weight of sample (g)} \times 1\,000}$$

$$\text{Total chlorophyll content (mg/g Fw)} = \frac{C_T \times \text{Total amount of extract (ml)}}{\text{Fresh weight of sample (g)} \times 1\,000}$$

Finally, the ratio of chlorophyll a/chlorophyll b was calculated and analyzed.

**Determination of malondialdehyde content**

First, 0.1 g of leaf was added with 10% trichloroacetic acid (TCA) to 10 ml and ground. The obtained paste was centrifuged at 4 000 r/min for 10 min to obtain the supernatant as the sample extract.

Next, 2 ml of supernatant was added with 2 ml of 0.6% thiobarbituric acid (TBA) and shaken to mix well. The liquid was boiled in a water bath at 100 ℃ for 10 min (timing was started from the appearance of bubbles in the test tube), and should be centrifuged again after rapid cooling if there was any precipitation. The absorbance values of the supernatant at 450, 532 and 600 nm were determined respectively, with distilled water as the control.

The calculation method is as follows:

$$C_{MDA}\,(\text{mol/L}) = 6.45\ (A_{532} - A_{600}) - 0.56A_{450}$$

The MDA concentration in the extract was calculated according to following formula:

$$\text{MDA concentration in extract (μmol/ml)} = C_{MDA} \times \frac{\text{Volume of reaction liquid (ml)}}{1\,000} \Big/ \text{Amount of extract used in determination (ml)}$$

The MDA content in the sample was calculated according to following formula:

$$\text{MDA content (μmol/g Fw)} = \frac{\text{MDA concentration in extract (μmol/m)} \times \text{Total amount of extract (ml)}}{\text{Fresh weight of plant tissue (g)}}$$

**Determination of free proline content**

(1) Reagents and preparation methods are given below.

Preparation of 2.5% acidic ninhydrin solution: First, 1.25 g of ninhydrin was dissolved in 30 ml of glacial acetic acid and 20 ml of 6 mol/L phosphoric acid. Next, the liquid was stirred and heated (70 ℃) to dissolve ninhydrin. The obtained solution was stored in a refrigerator.

Preparation of 3% sulfosalicylic acid: A 3 g of sulfosalicylic acid sample was dissolved in distilled water and diluted to 100 ml.

Preparation of 10 μg/ml proline standard moth liquor: Accurately, 20 mg of proline was weighed, poured into a small beaker, and dissolved with a small amount of 80% ethanol. Next, the solution was poured into a 200 ml volumetric flask, and diluted with 80% alcohol to constant volume (100 μg/ml proline mother liquor). Next, 10 ml of the solution was pipetted and diluted with distilled water to 100 ml to give a 10 μg/ml proline standard solution.

(2) Drawing of proline standard curve: Six test tubes were numbered, and proline standard solutions with contents within 0 – 12 μg per tube were prepared according to Table 1.

After adding the reagents in the table, each tube was heated in a boiling water bath for 30 min. After taking out and cooling, 4 ml of toluene was added to each test tube, which was shaken for 30 s. After standing for a while, all the pigments were transferred to the toluene solution.

**Table 1 Preparation of proline standard solutions**

| Reagent | Tube No. | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 10 μg/ml proline standard solution//ml | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
| Distilled water//ml | 2 | 1.8 | 1.6 | 1.4 | 1.2 | 1.0 |
| Glacial acetic acid//ml | 2 | 2 | 2 | 2 | 2 | 2 |
| 2.5% acidic ninhydrin//ml | 2 | 2 | 2 | 2 | 2 | 2 |
| Proline content per tube//μg | 0 | 2 | 4 | 6 | 8 | 10 |

The toluene solution of proline was gently transferred from the upper layer of each tube into a cuvette with a syringe, and measured for the absorbance (A) value at the wavelength of 520 nm with toluene solution as a blank control.

A standard curve was drawn with proline contents of No. 1 – No. 5 tubes as the x-axis and the absorbance values as the y-axis.

(3) First, 0.5 g of plant leaves was weighed from different treatments and placed in large test tubes, respectively. Next, 5 ml of 3% sulfosalicylic acid solution was added to each tube to perform extraction for 10 min in a boiling water bath (with frequent

shaking during the extraction process). After cooling, the solution was filtered into a clean test tube to obtain the filtrate as the proline extract.

(4) First, 2 ml of extract was transferred into a test tube with a glass stopper, added with 2 ml of glacial acetic acid and 2 ml of 2.5% acidic ninhydrin reagent, and heated in a boiling water bath for 30 min, and the solution turned red. Next, after cooling, 4 ml of toluene was added, and each solution was shaken for 30 s. After standing for a while, the supernatant was added into a 10 ml centrifuge tube and centrifuged 3 000 r/min for 5 min.

(5) The upper red toluene solution of proline was transferred into a cuvette with a straw, and measured for the absorbance (A) value at the wavelength of 520 nm with toluene solution as a blank control.

(6) The calculation method is as follows:

The proline content in each sample determination solution was found out from the standard curve, and the proline content in the sample was calculated according to following formula:

$$\text{Proline content}(\mu g/g\ \text{Fw}) = \frac{X \times \text{Total amount of extract}(ml)}{\text{Fresh weight of sample}(g) \times \text{Amount of extract used in determination}(\mu g)}$$

In the formula: $X$ is the proline content found from the standard curve (μg).

(7) Precautions: The prepared acidic ninhydrin solution was only stable within 24 h, so it should be prepared freshly for use. The results would be more significant if the samples were subjected to osmotic stress treatment. The addition order of reagents should not be wrong.

**Determination of soluble sugar content**

(1) Reagents and preparation methods are given below.

Anthrone reagent: A 200 mg of anthrone sample was weighed and dissolved in 100 ml of concentrated sulfuric acid. The reagent should not be stored for a long time, that is to say, it should be prepared freshly before use.

Preparation of 100 μg/ml sucrose standard mother liquor: A 100 mg of sucrose sample was accurately weighed in a beaker and added with a little water to dissolve it. Next, it was added into a 100 ml volumetric flask and diluted to constant volume to obtain a 100 μg/ml sucrose standard mother liquor.

(2) Drawing of sucrose standard curve: Six test tubes were numbered, and sucrose standard solutions with contents within 0 – 100 μg per tube were prepared according to Table 2.

After adding the reagents listed in the table, 6.5 ml of anthrone reagent was quickly added to each tube along the tube wall, and each tube was immediately shaken to mix evenly, and placed on a test tube rack for color development at room temperature. After cooling, the liquid was poured into a cuvette and measured at the wavelength of 620 nm, taking No. 0 tube as a blank control, to make a standard curve by the multi-point calibration quantitative method.

**Table 2 Preparation of sucrose standard solutions**

| Reagent | Tube No. | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 100 μg/ml Sucrose standard mother liquor // ml | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
| Distilled water // ml | 2.5 | 2.3 | 2.1 | 1.9 | 1.7 | 1.5 |
| Sucrose content per tube // μg | 0 | 20 | 40 | 60 | 80 | 100 |

(3) First, 0.1 g of fresh sample was weighed and added into a test tube, which was then added with 10 ml of distilled water to perform extraction in a boiling water bath for 20 min. Afer cooling, the liquid was filtered into a 100 ml volumetric flask, and the residue was rinsed with hot water for 2 – 3 times, and the liquid obtained from rinsing was filtered into the volumetric flask together. After cooling to room temperature, the volume was adjusted to the graduation to get the sample solution to be measured.

(4) First, 0.2 ml of the liquid to be tested (sugar content 30 – 80 μg) was pipetted into a test tube, added with 2.3 ml of distilled water, and shaken to mix well. Next, 6.5 ml of anthrone reagent was quickly added along the test tube wall, and the test tube was immediately shaken to mix evenly, and placed on a test tube rack for color development. Afer cooling to room temperature, the sugar content of the extract in the tube to be tested was determined by the multi-point calibration quantitative method at the wavelength of 620 nm, with a blank tube as the control.

(5) The calculation method of the result is as follows:

$$\text{Soluble sugar content}(\mu g) = \frac{\dfrac{\text{Sugar content of extract}(\mu g)}{1\,000 \times \text{Amount of to-be-tested liquid}(ml)} \times \text{Total amount of extract}(ml)}{\text{Dry weight (mg) or fresh weight (g) of tissue} \times 1\,000}$$

## Results and Analysis

**Changes of nematode number in soil after applying Chinese herbal medicine regulator**

The Berman funnel separation method was applyed to count the number of nematodes in the soil before and after the use of Chinese herbal medicine regulator. The results showed that the number of nematodes in the soil gradually decreased with the time, from 66 nematodes and 103 nematodes in each gram of soil before treatment to only 3 and 1 in each gram of soil after 28 d of treatment (Table 3, Fig. 1). Although the number of nematodes in the CK decreased from 96 nematodes in each gram of soil to 41 nematodes in each gram of soil, there were still a large number of nematodes in the soil at day 28 of treatment. However, no nematodes were observed in the soil free of nematode infection. The above results showed that the use of Chinese herbal medicine regulator could effectively reduce the number of nematodes in the soil.

**Table 3 Changes in the number of nematodes in soil**

| Sample name | Time | Nematode number// nematodes/g | Sample name | Time | Nematode number// nematodes/g |
|---|---|---|---|---|---|
| JK | 0 d | 0.00 ±0.00 | CK | 0 d | 96.53 ±12.52 |
| | 7 d | 0.00 ±0.00 | | 7 d | 65.93 ±5.51 |
| | 14 d | 0.00 ±0.00 | | 14 d | 62.67 ±8.73 |
| | 21 d | 0.00 ±0.00 | | 21 d | 42.13 ±3.94 |
| | 28 d | 0.00 ±0.00 | | 28 d | 41.07 ±5.80 |
| $T_1$ | 0 d | 66.13 ±4.68 | $T_2$ | 0 d | 103.20 ±19.05 |
| | 7 d | 60.93 ±9.87 | | 7 d | 89.60 ±14.26 |
| | 14 d | 14.27 ±2.84 | | 14 d | 34.87 ±3.23 |
| | 21 d | 19.53 ±4.52 | | 21 d | 25.60 ±1.90 |
| | 28 d | 3.60 ±0.80 | | 28 d | 1.20 ±0.20 |

## Changes of chlorophyll content in leaves after applying Chinese herbal medicine regulator

Before and afterapplication of the Chinese herbal medicine regulator, we analyzed chlorophyll content in the leaves of *C. spicatus*. As can be seen from Fig. 1, except the high chlorophyll content in the leaves of *C. spicatus* on day 0 of treatment $T_1$, the chlorophyll content in the leaves of *C. spicatus* increased with the treatment time on days 7, 14, 21 and 28, and reached the highest values on day 28 of treatment, at 1.57 and 1.06 mg/g respectively. Although the change of chlorophyll content in leaves of the CK (without the Chinese herbal medicine regulator) was similar to that of treatment $T_1$, its chlorophyll content was lower than that of $T_1$ in almost every treatment period. It can also be seen from Fig. 1 that the change of chlorophyll content in the leaves of *C. spicatus* was mainly caused by the change of chlorophyll a, and the change of chlorophyll b was less affected by the Chinese herbal medicine regulator.

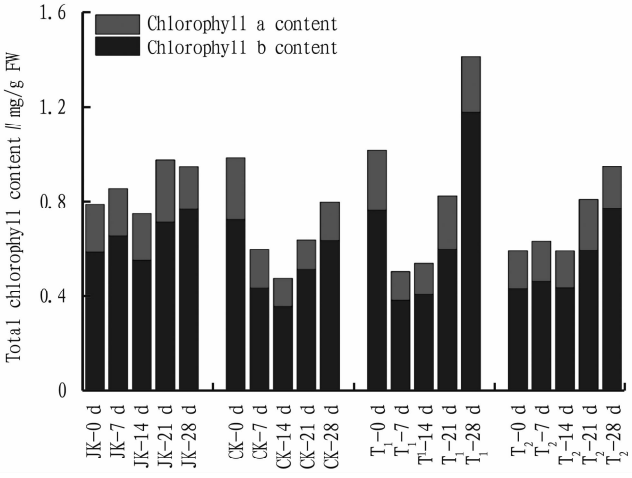


**Fig. 1 Effects of Chinese herbal medicine regulator on chlorophyll content in leaves of *C. spicatus***

## Changes of MDA content in leaves after applying Chinese herbal medicine regulator

The content of malondialdehyde can directly reflect the peroxide level of plant cell membrane. It can be seen from Fig. 2 that under treatment $T_2$, the malondialdehyde content in the leaves of *C. spicatus* decreased first and then increased, reaching the lowest value of 0.031 μmol/g on day 14 of treatment, and reaching the highest value of 0.053 μmol/g on day 28 of treatment, which was the same as that in the leaves of *C. spicatus* before treatment with the Chinese herbal medicine regulator. The changes of malondialdehyde content in leaves of *C. spicatus* in treatment $T_1$ were similar to the CK, and the malondialdehyde content in leaves decreased on days 7 and 21. However, the content of MDA in leaves of healthy *C. spicatus* was lower on days 7 (0.031 μmol/g) and 28 (0.042 μmol/g). The above results showed that the application of higher concentration of Chinese herbal medicine regulator could effectively reduce the content of MDA in the leaves of *C. spicatus*.

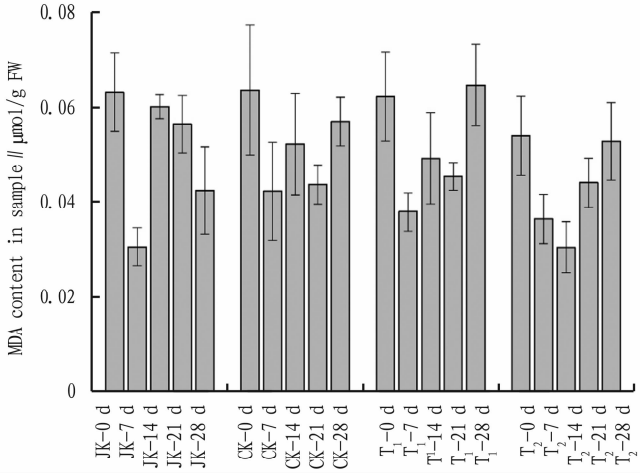


**Fig. 2 Effects of Chinese herbal medicine regulator on MDA content in leaves of *C. spicatus***

## Changes of soluble sugar content in leaves after applying Chinese herbal medicine regulator

Soluble sugar content is usually related to the stress resistance of plants. As can be seen from Fig. 3, the soluble sugar content in leaves of *C. spicatus* showed an upward trend after application of the Chinese herbal medicine regulator, and reached the highest values on day 28 after treatment, which were 0.06% and 0.66% respectively. Although the change trend of soluble sugar content in leaves of the CK and healthy plants was similar to that after treatment with Chinese herbal medicine regulator, the soluble sugar content in leaves of *C. spicatus* without Chinese herbal medicine regulator was only 0.34% on day 28 of treatment, while the soluble sugar content in leaves of healthy plants was 0.55% on day 28. It showed that the use of Chinese herbal medicine regulator

could increase the soluble sugar content in the leaves of *C. spicatus* and enhance the ability of *C. spicatus* leaves to maintain water and osmotic pressure.

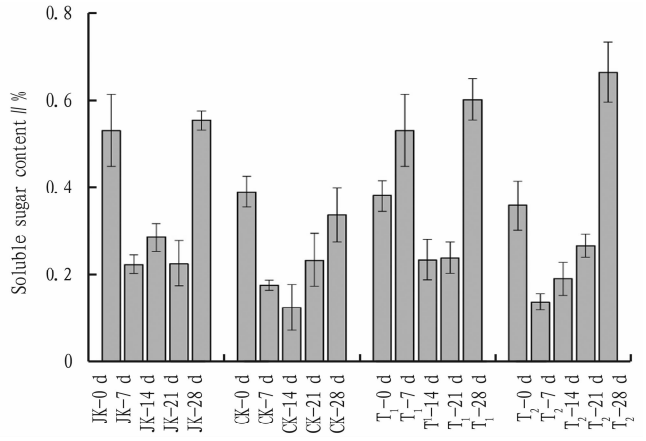


**Fig. 3 Effects of Chinese herbal medicine regulator on soluble sugar content in leaves of *C. spicatus***

### Changes of free proline content in leaves after applying Chinese herbal medicine regulator

Free proline has many biological functions in plants, and the increase of its content is the metabolic mechanism of plants adapting to biotic and abiotic stresses. Compared with the CK, the two concentrations of Chinese herbal medicine regulator significantly increased the content of free proline in the leaves of *C. spicatus* on day 14 after treatment, reaching 9.14 and 9.10 μg/g respectively. Except for day 28 of treatment $T_2$ when the content of free proline in the leaves of *C. spicatus* was slightly lower than that of the CK, the content of free proline in the leaves of *C. spicatus* treated with two concentrations of Chinese herbal medicine regulators was higher than that of the CK. The content of free proline in the leaves of healthy *C. spicatus* was also higher than that in the CK. The above results showed that the use of Chinese herbal medicine regulator could improve the content of free proline in the leaves of *C. spicatus*, and its many important physiological functions played an important role in enhancing the resistance of *C. spicatus* to nematodes.

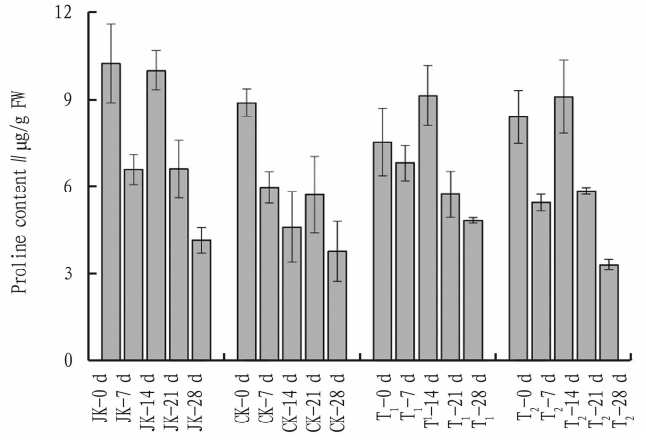


**Fig. 4 Effects of Chinese herbal medicine regulator on free proline content in leaves of *C. spicatus***

## Conclusions and Discussion

*C. spicatus* is a pure natural herb. Because of its unique fragrance and medicinal efficacy, it has been widely favored by female consumers in recent years. At present, reports on *C. spicatus* mainly focus on cultivation, planting techniques, deep processing techniques or research on effective components, and few studies have been conducted on pests and diseases. The disease caused by *A. fragarae* spreads quickly, and it is difficult to eradicate it, thus causing a great impact on the yield of *C. spicatus*. So far, no effective treatment has been found. Agriculture and forestry are deeply harmed by nematodes. Besides using disease-resistant varieties, rationally intercropping and strengthening field management, chemical control is the most convenient, rapid and effective control measure[14-15]. It has been reported that the long-term use of chemical agents leads to the emergence of drug resistance in nematodes[16]. Therefore, reducing the use of chemical pesticides plays an important role in food safety, human health and the maintenance of the ecological environment. Using plants, microorganisms or their metabolites to control nematodes is a green, environmentally friendly and efficient biological control method for the sustainable development of agricultural production. China is rich in plant germplasm resources, and traditional Chinese medicine based on Chinese herbal medicines has a long history. In recent years, many studies at home and abroad show that Chinese herbal medicines have a good effect on preventing and controlling crop diseases and pests, and can reduce the use of chemicals in agriculture[17-18]. At present, the effect of plant-derived Chinese herbal medicines on biological control of nematodes has not been reported.

In this study, the control effect of Chinese herbal medicine regulator chelated with various Chinese herbal medicines on leaf spot nematode disease of *C. spicatus* was investigated by comparing the changes of nematode content in the soil for cultivating *C. spicatus* and the changes of chlorophyll, malondialdehyde, soluble sugar and free proline content in *C. spicatus* leaves before and after the application of Chinese herbal medicine regulator. The application of the two concentrations of Chinese herbal medicine regulator (1∶150 and 1∶75) both significantly reduced the number of nematodes in the soil (less than 4 nematodes/g) on day 28 after treatment, which indicated that the use of Chinese herbal medicine regulator effectively reduced the number of nematodes in the soil. The main functions of Chinese herbal medicines in controlling pests are contact killing, anesthesia and repelling[19-20]. Although the mechanism of killing nematodes in soil by Chinese herbal medicine regulators is still unclear, alkaloids, flavonoids, terpenoids and other substances in Chinese herbal medicine regulators can effectively reduce the number of nematodes in the soil for cultivating *C. spicatus*.

In the early stage of the disease, the leaves of *C. spicatus* infected with *A. fragarae* show pale yellow lesions, then turn reddish brown, and finally the lesions expand and become dark brown, which eventually leads to leaf shedding[7]. Chlorophyll is an important substance in plant photosynthesis, and its content is closely related to the photosynthetic rate of leaves[21]. When plants are stressed by pests and diseases, the chlorophyll content in leaves

will decrease. For example, the photosynthetic rate of wheat infected with powdery mildew is obviously weakened, and the chlorophyll content in lily leaves infected with gray mold also decreases with the extension of the onset time[22-23]. After *A. fragarae* infects the leaves of *C. spicatus*, the yellowing and browning of the diseased spots will inevitably lead to the decrease of chlorophyll content in the leaves. After applying Chinese herbal medicine regulator, compared with the control, the chlorophyll content in the leaves of *C. spicatus* under the two treatment concentrations began to rise on day 21, and reached a higher level on day 28, while the chlorophyll content in healthy plants remained at a higher level. Specifically, the content of chlorophyll a changed greatly, and the content of chlorophyll b was almost unaffected by the Chinese herbal medicine regulator. It showed that after applying the Chinese herbal medicine regulator, with the extension of treatment time, the number of nematodes in soil decreased significantly, which made the growth of leaves of *C. spicatus* recover and the chlorophyll content increase gradually. Malondialdehyde (MDA) is the main product of lipid peroxidation, and its content reflects the degree of plant damage under adversity, and has a certain relationship with plant disease resistance. After plants are subjected to biological stress, the content of malondialdehyde in the body will increase[24-25]. The content of malondialdehyde (MDA) in the leaves of *C. spicatus* without Chinese herbal medicine regulator had been at a high level, but the use of higher concentration of Chinese herbal medicine regulator could effectively reduce the content of MDA in the leaves of *C. spicatus*. Although the two concentrations of Chinese herbal medicine regulators significantly reduced the number of nematodes in the soil, there was difference in malondialdehyde content in the leaves of *C. spicatus* after treatment with the two concentrations of Chinese herbal medicine regulator. It showed that the treatment of higher concentration of Chinese herbal medicine regulator could keep malondialdehyde content in the leaves of *C. spicatus* at a low level.

After plants are infected with diseases and pests, it will cause a series of metabolic changes in the body, and also affect the changes of osmotic adjustment substances such as soluble sugar and free proline. So far, these osmotic adjustment substances have shown positive effects on the resistance of pathogens. After wheat is inoculated with Bipolaris sorokinian, the soluble sugar content in susceptible leaves increases significantly, especially in the early stage of susceptibility, and the soluble sugar content is positively correlated with disease resistance[26]. The research results of cucumber downy mildew showed that the higher the sugar content in cucumber leaves, the stronger its disease resistance, and vice versa[27]. After applying the Chinese herbal medicine regulator, the soluble sugar content in leaves of *C. spicatus* was higher than that of the CK at different stages, which indicated that the increase of sugar content might play a positive role in resisting the stress of pests, and it could make cells produce more secondary metabolites through pentose phosphate pathway to improve the defense ability of plants. Free proline is also an important osmotic adjustment substance in plants[28]. In this study, it was found that the content of free proline in the leaves of *C. spicatus* was higher than that of the CK after applying the Chinese herbal medicine regulator. The accumulation of free proline in cells can improve the metabolic capacity of plants, and improve the stability of membrane structure by increasing the water binding force between protein molecules in biofilm, thus protecting plant cell membrane[29].

In this study, a Chinese herbal medicine regulator was applied to *C. spicatus* infected with leaf spot nematodes, and the contents of chlorophyll, malondialdehyde, soluble sugar and free proline in leaves of *C. spicatus* were determined. It was found that application of the Chinese herbal medicine regulator could effectively reduce the number of nematodes in soil and improve the ability of *C. spicatus* to resist biological stress, which indicated that the Chinese herbal medicine regulator could be used as a biological agent to prevent and treat leaf spot nematode disease of *C. spicatus*. Although the control mechanism of Chinese herbal medicine regulators on leaf spot nematode disease of *C. spicatus* is not clear at present, Chinese herbal medicine regulators may reduce nematodes in soil by contact killing, anesthesia and repelling, and simultaneously restore the growth of *C. spicatus*, increase the contents of chlorophyll, soluble sugar and free proline in its leaves, and reduce the content of malondialdehyde, finally restoring the growth ability of *C. spicatus* plants. The specific mechanism of Chinese herbal medicine regulators in controlling leaf spot nematode disease of *C. spicatus* needs further study.

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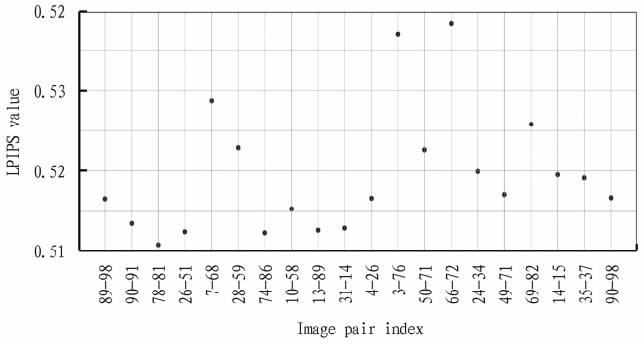


**Fig. 1 LPIPS value distribution map**

## Conclusions

In this study, a method of generating and processing two-dimensional slices of sandstone samples based on CT scanning was proposed, and diversity verification and generation were performed by combining with the StyleGAN2-ADA model. The redundancy of data was significantly reduced and the differences between slices were improved, by selecting training data at an interval of 30 slices, and the problem of pattern collapse in the training process of generative adversarial network was avoided. The calculation results of LPIPS value showed that the selection of spaced slices could maintain enough differences in visual perception, thus improving the quality and diversity of images generated by the model. This study provides a new method for the training of models for generating images on small samples, and also provides an effective solution for the accurate segmentation and analysis of porous medium structures.

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