Isolation Of The Pathogens Causing Hemp Stem Disease

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***Abstract* — Hemp, with very strong vitality, is a kind of annual tall herbaceous plant. Hemp is naturally hardy and drought tolerant which grows well in a cold climate. Hemp plants can resist various plant diseases. Besides, hemp can fight against insect pests without toxic pesticides and chemical fertilizers and thus effectively improve the soil salinization. In this work, two strains of pathogenic fungi (DN1 and DN2) were isolated from lesion site of the hemp plant, by an observation of hypha and spore morphology, the DN1 and the DN2 are respectively determined as deuteromycotina Alternaria (Alternaria alternata) and Verticillium (M.Verticillium). At pH values between 4 and 14, the two fungi can grow normally and show a great ability to adapt to the environment. Effects of hemp on soil pH were determined by pot experiment. The results showed that hemp could repair the weak alkaline soil.**

Keywords - hemp stem disease; pathogenic isolation; pH value of soil

I. INTRODUCTION

Crop rotation of such grains as hemp, soybean, and corn can not only maintain soil fertility, but also repair soil pollution caused by long-term fertilization. Hemp fiber, which is long and tough, is strong in corrosion resistance. The woven hemp fiber has many advantages, such as good air permeability, strong hygroscopicity, strong wear resistantance, antibacterial and deodorizing ability [1-2]. Therefore, the rotation of hemp and other crop can improve soil and as a result, bring great economic income in agriculture.

A few stem diseases of hemp cause the bast fiber swelling and decaying, which seriously affects the quality and harvest of hemp fiber. In addition, the high disease incidence can cause large losses to local farmer [3-11]. Therefore, enough attention should be paid to the occurrence and a new method should be applied to control the hemp stem disease. In this study, pathogenic bacteria were isolated from the hemp stem at the onset of a disease. The colony morphology and structure are observed, and the growth conditions will be figured out then. A preliminary exploration shows the effects from the hemp plant to soil pH value, the study of which will lay the foundation for the further study of the hemp plant impact on the soil environment. That is why we need to determine the preliminary exploration, aiming at the difference which hemp will make on the soil.

II. MATERIALS AND METHODS

A. Separation And Purification Of Bacteria

A freshly taken hemp lesions stem is washed repeatedly, after a surface sterilization of diseased tissue, it is soaked respectively with 75% ethanol and sterile water for 3 minutes. The operation above should be repeated 3 times for a thoroughly sterilize . After surface disinfection, the tissue is placed in the prepared PDA (potato dextrose agar) medium plate. Then the tissue was cultured for 3 to 7 days. A single colony will be picked out from the grown colonies. The chosen colony is purified to insure a precise experiment [12].

B. Morphological Structure Of Bacteria

The morphology and structure of bacteria is observed with a microscope after the mycelium pellet is scraped. The colony was rinsed with sterile water to make spore suspension. A drop of spore suspension was dropped on the central of glass slide. Then we use a microscope of more precision to study the morphology and structure of the spore.[13].

C. Effects Of Different Temperature On Bacteria Growth

The two pathogen fungi cultured in PDA culture medium are made into cakes of diameter 5mm and transferred
to the PDA panel and cultured respectively at 15℃, 20℃, 25℃, 28℃, 30℃, 35℃, 40℃ in the same period. Then the colony growth diameter is measured so that the growth of bacteria could be determined.

D. Effects Of Different pH On Bacteria Growth

Six fungus cakes of 5 mm diameter were made of the pathogenic bacteria in the PDA culture medium, they are transferred to the PDA plates with pH values of 2, 4, 6, 8, 10, 12, respectively. Under the condition of 28℃, these colonies are trained for the same period, and the growth diameters of colonies are measured to determine the growth of bacteria.

E. Contrast Test Of Soil Ph

By a pot experiment, a preliminary study on the hemp plant effect to soil pH value is made: the soil is fixed in light saline land and its pH is 10.4(the test is repeated 10 times), each pot planted three strain of

hemps, where a pot remained empty as control. After hemp harvesting, changes in soil pH value are measured.

III. RESULTS

A. Separation And Purification

Three strains of fungi are isolated from the lesions of hemp stem, after several generations of purification, again transferred back to the hemp plants, where, two strains of bacteria, respectively named DN1 and DN2, which have a high virulence to hemp plants. By use of needle punching, the DN1 and the DN2 are respectively transferred back to the hemp plants, susceptible symptoms of the plants manifest themselves 5-7 days later, see Fig.1. With collecting the lesions, the pathogens are isolated once more and they are the same as the DN1 and the DN2, the pathogens under the microscope. Bacteria in the humid and high temperature conditions can easily cause disease. In the initial period of disease infection, the hemp stalk appear irregular tan plaques, and the contour is not obvious, the center has black mold layer. In the later, the disease could cause stem bending, dry, and serious disease causing the stalk broken.

Fig.1 Hemp Stalk Rot Symptoms Of Local Inoculation

B. Morphological Observation

(1) Colony Morphology

The two strains of isolated pathogenic fungus colonies are shown in Fig.2. Under the condition of 28℃, the fungal pathogens in PDA medium, named DN1 colony, was cultured for 72 hours, the mycelia grow vigorously with a radial growth trend and formed into an regular circular colony. Another fungal colony in PDA medium, named DN2, grow relatively slow and need to be cultivated for 144 hours. In the beginning, the DN2 colony is white, after a period of incubation, it becomes yellow, most individuals of the colony are aerial mycelium, taking on villous projections.

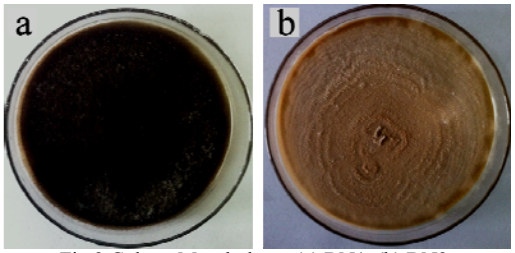

Fig.2 Colony Morphology. (a) DN1. (b) DN2.

(2) Mycelium And Spore Morphology Observation

The DN1 hypha together with its spore morphology is shown in Fig. 3, its mycelium, with less branches, is long and grew vigorously. The DN1 has very strong ability of sporulation. In the field of vision, a lot of brick-like septate conidia with the color from light brown to dark brown and an oval shape could be seen, and there are beak-like cells in their tops. According to the morphological characteristics of hyphae and spores, it could be determined that the DN1 hypha belongs to deuteromycotina fungus, Alternaria (Alternaria alternata) [10].

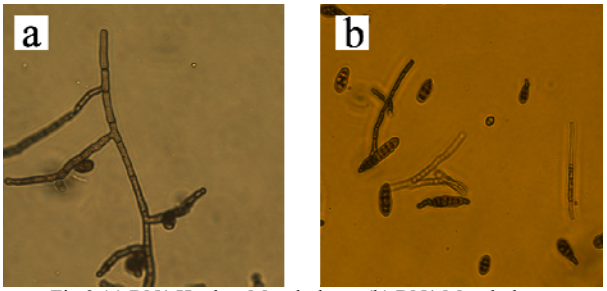

Fig.3 (a) DN1 Hyphae Morphology. (b) DN1 Morphology.

The DN2 hypha together with its spore morphology is shown in Fig. 4, the mycelium, without any branch, grows very vigorously, the conidiophore is very short and takes on a wheel-like distribution in the hyphae. The conidia in the DN2 are small, solitary, round and in large number. Due to the unobvious morphological characteristics, it could be speculated that the DN2 hypha might be deuteromycotina, Verticillium (M.Verticillium) [10].

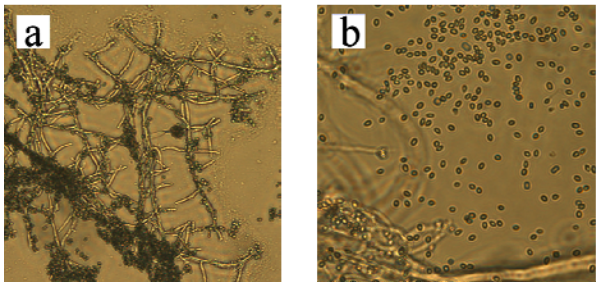

Fig.4 (a) DN2 Hyphae Morphology. (b) DN2 Morphology

C. Temperature And Bacteria Growth

In the PDA medium, the growth of two pathogen fungi are measured at 10℃, 15℃, 20℃, 25℃, 30℃, 35℃, 40℃ and the results are listed in Table I. It could be seen that: (1) the two strains of fungi could grow at 15℃ to 35℃, where, the optimum growth temperature

range of the DN1 was 25℃ to 30℃ and whose colony growth diameter respectively reaches 64.00 mm to 62.67mm; (2) the colony growth diameter of DN2 at 30℃, is 55mm; (3) when the temperature is below 15℃ or above 35℃, the two colonies only show a slight growth phenomena due to their very low activity. The above shows that the environment temperature from 25℃ to 30 ℃ is benefit to the disease and disease could not occur at a too high or too low temperature.

TABLE I. THE COLONY GROWTH DIAMETERS OF THE DN1 AND DN2 AT DIFFERENT TEMPERATURES.

Temperature	10℃	15℃	20℃	25℃	30℃	35℃	40℃
DN1 diameter	7.33±0.6	28.67±2.5	40.67±2.9	64.00±3.3	62.67±2.5	44.33±3.0	6.33±1.5
DN2 diameter	8.00±1.0	20.67±1.2	26.33±2.1	52.00±2.6	55.00±1.7	44.33±3.1	5.67±2.1

D. pH And Bacterial Growth

In the PDA medium, the growth of two kinds of pathogenic fungi are tested under the conditions of pH values respectively of 2, 4, 6, 8, 10, 12 and 14, and the results were listed in Table I. It could be seen from the results in Table II that the DN1 gets stronger adaptable ability to the environment and could grow normally in a wider range of pH values. From Table II, it also could be seen that the DN1 and DN2 did not grow when the pH value is less than 4. However, They grow very well in the range of pH values of 6 to 12. The control results showed: the alkali resistance of the DN1 is stronger than that of the DN2, when pH value is larger than 12, the DN2 began to stop growing, while the DN1 still remained strong growth momentum.

The data in Table II also shows the adaptability of the two strains of pathogenic fungi (the DN1 and the DN2) to the pH value, the occurrence of diseases has a wide range of pH value. That is to say, they get a very strong adaptable ability to the environment.

TABLE II. THE COLONY GROWTH DIAMETERS OF THE DN1 AND DN2 UNDER DIFFERENT PH VALUES.

pH value	2	4	6	8	10	12	14
DN1 diameter	0	22.5±3.3	69.8±1.8	70.5±3.1	69.2±3.8	57.8±3.6	36.8±4.1
DN2 diameter	0	16.8±2.8	56.1±4.2	56.9±2.2	59.7±3.1	58.2±1.9	10.1±1.7

E. Soil pH And Cultivation Of hemp Carrying The Pathogenic Fungi Of DN1 And DN2

The results in Table III showed: when the hemp plants carrying the two pathogenic fungi are harvested, the soil pH value had significant changes, the pH values decrease more obviously.

TABLE III. THE PH VALUE CHANGES OF SOIL AFTER PLANTING HEMP, CK IN THE 1ST ROW, THE 2ED COLUMN THE TABLE IS ABBREVIATION OF CONTROL CHECK.

	CK	1	2	3	4	5	6	7	8	9	10
pH value after harvest	10.8	8.2	8.4	7.9	10.7	8.8	8.0	9.4	8.8	9.0	8.3

The other test results for the adaptability of the two strains of pathogenic fungi to pH value also show that they had a stronger adaptability to pH value and were able to grow well under the environment of the pH values of 4 to 12. The DN1 had a wider adaptability to the pH value than the DN2. When the pH value reached 14, the DN1 still remained the certain growth momentum, showing that the disease occurrence has a strong ability to the soil pH value environment.

IV. DISCUSSION

As we can see from the experiment, There is tremendous room for alkaline improvement of hemp which is planted on large area. The rotation of hemp and other crop can both improve the quality of soil and increase farmers' income. The mechanism of the other soil improvement should be made a further research.

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