

Research on Inhibition of *Cladosporium cucumerinum* and Disease Resistance of Plant by Water Extracts from Kernel Apricot and Phellodendron Leaves

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The aim of this study was to investigate allopathic effects of botanical extracts from *Armeniaca sibirica*×*vulgaris* and *Platycladus orientalis* leaves on *Cladosporium cucumerinum* and cucumber. Extracts from *A. sibirica*×*vulgaris* and *P. orientalis* were adopted and the major cucumber cultivar named Jinlv 3 was taken as the material to study the effect of prevention on *C. cucumerinum* through pot experiment. There are inhibitory effects of extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves on *C. cucumerinum* to some extent. There is a significant effect of extract from 0.5 kg/L *A. sibirica*×*vulgaris* with inhibitory effect of 22.83%. After disoperation of *C. cucumerinum* pathogen on cucumber plant, extracts from both *A. sibirica*×*vulgaris* and *P. orientalis* leaves can effectively decrease MDA content in cucumber leaves. Extract from *A. sibirica*×*vulgaris* leaves can significantly increase protein content in cucumber plant. Moreover, there is an increase of antioxidant enzyme POD and SOD in cucumber plant and disease resistance of *C. cucumerinum* after using extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves.

Key words: *Armeniaca sibirica*×*vulgaris*; *Platycladus orientalis*; cucumber; Induced resistance; *Cladosporium cucumerinum*.

Cucumber is one of the vegetable crops with a large cultivation area in China. *Cladosporium cucumerinum* is the main disease for cucumber and also the worldwide disease, affecting cucumber production in Europe, North America and Southeast Asia. With the development of protected field production in recent years, *C. cucumerinum* has caused serious damage in some regions in China. As *C. cucumerinum* has produced different levels of drug resistance to traditional chemicals according to many researches

^{1,2}, it is of vital importance to develop plant source to induce disease resistance to pathogen. Extract of some leaves can be used to improve plant's resistance to disoperation of pathogen ³⁻⁵. There is antibacterial activity and induced resistance on muskmelon powdery mildew, *C. cucumerinum*, tomato gray mold, fusarium wilt, leaf mold, and strawberry mold by extract from plant source, such as *P. orientalis* leaves ⁶⁻⁹. As one common species in Agro-forestry Integrated Ecosystem in Kerqin Sandy Land, *Armeniaca sibirica*×*vulgaris* belongs to *Armeniaca* and is the natural hybrid between *A. vulgaris* Lam. and *A. sibirica*. There are researches from various aspects on industrialization of *A. sibirica*×*vulgaris* ¹⁰⁻¹² but few researches on induced disease resistance with

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extract from *A. sibirica*×*vulgaris*. For the purpose of laying theoretical foundation for regulation and effective use of *A. sibirica*×*vulgaris* on resistance of plant, inhibitory effects of extracts from *A. sibirica*×*vulgaris* and *Platyclusus orientalis* leaves on *C. cucumerinum* and control efficiency as well as resistance mechanism of the enzyme system to *C. cucumerinum* induced by *A. sibirica*×*vulgaris* and *P. orientalis* leaves were studied in this paper.

MATERIALS AND METHODS

Pathogen for test

C. cucumerinum provided by the institute of Plant Protection, Liaoning Academy of Agricultural Sciences. Cucumber cultivar: Jinlv 3 provided by Liaoning Academy of Agricultural Sciences. *A. sibirica*×*vulgaris* was taken from “ZhangGuTai High-Efficiency Modern Ecological Agriculture Demonstration Zone of Liaoning Modern Agricultural Park. *P. orientalis* was taken from Sun Yat-Sen University. Leaf extraction method was adopted in preparation of extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves with reference to the method of Li Yonggang et al. ⁹. The test was carried out in Suntun county in

Donggang of Liaoning. Investigation methods of inoculation and disease index of *C. cucumerinum* were adopted with Lou Chunrong et al. ¹³ as the reference. Spraying method of 0.25 and 0.5 kg/L of extract from *A. sibirica*×*vulgaris* leaves and 0.25 and 0.5 kg/L of extract from *P. orientalis* leaves are the same with inoculation method of pathogen. There are 8 processes and 3 repetitions in the control group with and without *C. cucumerinum*. After spraying 7d of extract from *A. sibirica*×*vulgaris* and *P. orientalis* leaves, cucumber leaves at seedling stage were tested for activity of soluble protein, malonaldehyde, POD and SOD.

RESULTS AND DISCUSSION

Inhibitory Effects of Extracts from *Armeniaca sibirica*×*vulgaris* and *Platyclusus Orientalis* on *Cladosporium Cucumerinum*

Disease indexes and inhibitory effects of extract with different concentrations on *C. cucumerinum* were shown in Table 1. After inoculation of 3d *C. cucumerinum*, plants started to show morbidity and became worse with the growth of days. After inoculation of 7d, disease

Table 1. Disease indexes and inhibitory effects of extract with different concentrations on *C. cucumerinum*

Treatment	Concentration	Disease index				Inhibition in 7d /%
		3 d	4 d	5 d	7 d	
Sterile water-CK	44.6	63.7	76.8	84.9		
Extract of <i>Armeniaca sibirica</i> × <i>vulgaris</i>	0.25 kg/L	43.9	59.8	68.2	77.7	13.47
	0.50 kg/L	42.3	55.6	64.5	69.3	22.83
Extract of <i>Platyclusus orientalis</i>	0.25 kg/L	44.1	58.2	70.4	77.1	14.14
	0.50 kg/L	42.4	56.8	65.9	73.2	18.49

index rose to 84.9. According the test results, it can be seen that there is a significant inhibitory effect of extracts from both *A. sibirica*×*vulgaris* and *P. orientalis* leaves on *C. cucumerinum*. However, there is no significant inhibitory effect of extracts of *P. orientalis* leaves with concentration of 0.25 and 0.5kg/L on *C. cucumerinum*. With respect to inhibition of diffusion effect of *C. cucumerinum*, the effect became more significant with the increasing concentration of extracts from both *A.*

sibirica×*vulgaris* and *P. orientalis* leaves, especially 0.5 kg/L extract from *A. sibirica*×*vulgaris* leaves and 0.5kg/L extract from *Platyclusus orientalis* leaves with the inhibitory effect up to 22.83% and 18.49% respectively. Therefore, extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves can be used to improve the cucumber plant’s ability of resistance to infection from *C. cucumerinum* pathogen. The 0.5kg/L extract from *A. sibirica*×*vulgaris* is significant for the ability of resistance to infection.

Influence of Extracts from *Armeniaca sibirica*×*vulgaris* and *Platycladus Orientalis* on SOD Content of Cucumber with *Cladosporium Cucumerinum*

The SOD content after spraying extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves on cucumber leaves were showed in Figure 1. After tests with extract with several different concentrations, SOD concentration in cucumber leaves with *C. cucumerinum* rose with different levels. There was a certain effect of 2 doses of extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves on induction of SOD content in cucumber leaves with *C. cucumerinum*, which was higher for 1.28 to 1.81 times than that of the untreated cucumber leaves with *C. cucumerinum*. Moreover, 0.5 kg/L extract from *A. sibirica*×*vulgaris* showed the most significant effect. There was no significant difference for SOD and POD contents of cucumber leaves with *C. cucumerinum* after 2 doses of extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves, which showed that further exploration

should be made for appropriate dose of extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves.

Influence of Extracts from *Armeniaca sibirica*×*vulgaris* and *Platycladus orientalis* on POD Content of Cucumber with *Cladosporium Cucumerinum*

The POD content after spraying extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves on cucumber leaves with inoculation of *C. cucumerinum* was shown in Figure 2. There was a slight rise of POD content in cucumber plant with *C. cucumerinum* than that without *C. cucumerinum*, and this might be due to the response of enzyme of antioxidant system in cucumber plant after infection with the pathogen. Several kinds of dose of extract can improve the activity of POD and improve POD content in plant with and without *C. cucumerinum* by from 39.3% to 99.4% respectively. The most significant effect of stimulation on the increase of POD content was shown with 0.5 kg/L extract from *A. sibirica*×*vulgaris*. The higher concentration of

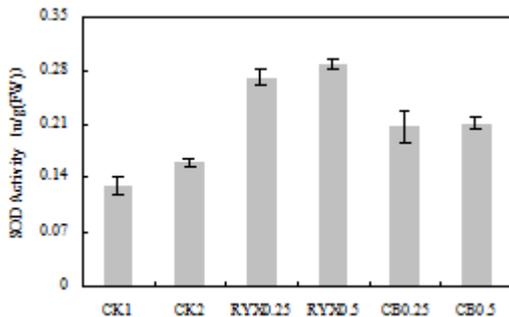


Fig. 1. SOD activity in cucumber leaves

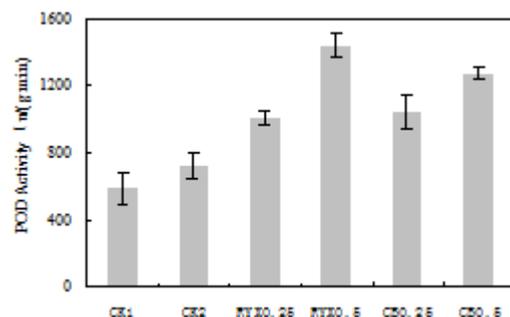
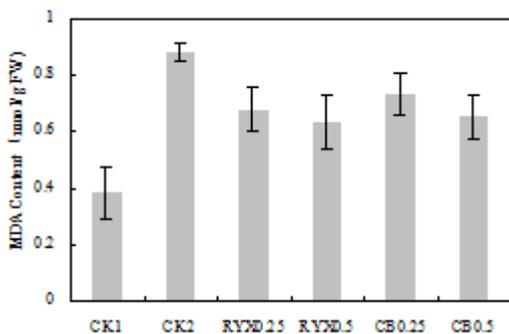


Fig. 2. POD activity in cucumber leaves



CK1: Cucumber without infecting *Cladosporium Cucumerinum*
 CK2: Cucumber infected *Cladosporium Cucumerinum*
 RYX: Extract of *Armeniaca sibirica*×*vulgaris*
 CB: Extract of *Platycladus orientalis*

Fig. 3. MDA content in cucumber leaves

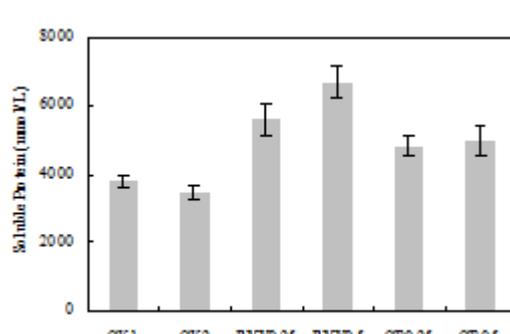


Fig. 4. Soluble protein in cucumber leaves

extracts from *A. sibirica*×*vulgaris* and *P. orientalis* were, the more significant effect of stimulation on POD would be.

Influence of Extracts from *Armeniaca sibirica*×*vulgaris* and *Platycladus orientalis* on Malonaldehyde (MDA) Content of Cucumber with *Cladosporium Cucumerinum*

The MDA content after spraying extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves on cucumber leaves with inoculation of *C. cucumerinum* was shown in Figure 3. Compared with cucumber plant without *C. cucumerinum*, MDA content in cucumber leaves with *C. cucumerinum* rose by 2.29 times. MDA content in leaves after treatment with extracts from *A. sibirica*×*vulgaris* and *P. orientalis* was significantly lower than that in leaves with *C. cucumerinum*. Moreover, MDA content decreased by 28.3% after treatment with 0.5 kg/L extract from *A. sibirica*×*vulgaris* and, showing to be the most effective. The degrees of reduced damage on membrane by *C. cucumerinum* with different decreases of MDA content was observed as 0.5 kg/L extract from *A. sibirica*×*vulgaris* > 0.5 kg/L extract from *P. orientalis* > 0.25 kg/L extract from *A. sibirica*×*vulgaris* > 0.25 kg/L extract from *P. orientalis*.

Measurement of Soluble Protein in Cucumber with *Cladosporium Cucumerinum* by Extract from *Armeniaca sibirica*×*vulgaris* and *Platycladus orientalis*

Soluble protein content after spraying extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves on cucumber leaves was shown in Figure 4. Compared with the untreated cucumber leaves with *C. cucumerinum*, protein content in cucumber leaves with *C. cucumerinum* can be raised by from 38.6% to 92% with spraying extracts from *A. sibirica*×*vulgaris* and *P. orientalis* leaves. Protein content in cucumber leaves with extract from *A. sibirica*×*vulgaris* leaves is higher than that from *P. orientalis* leaves.

CONCLUSION

Some researchers believe that the plant itself can produce disease resistance to outside intimidation by using stimulation from external factor, which is called induced disease resistance. Mechanism of induced disease resistance includes

four mature theories, which are physical mechanism, molecular mechanism, physiological, biochemical, and signaling mechanism. While being damaged under the condition of adversity, there is usually MDA generated from preoxidation of membrane in plant organs. The MDA content reflects the degree of preoxidation of membrane in plant cells^{15,17}. There is damage on bio-membrane done by free radicals generated from biological metabolism process in an adverse environment¹⁷. However, protective enzyme system in organism could clear free radicals by itself so as to reduce damage. Defensive enzyme system is generated from a series of complicated metabolism by enzyme catalysis¹⁵. As the major protective film in antioxidant enzyme system, POD coordinate with SOD and CAT so as to ensure the equilibrium state of production and clearance of free radical inside cells. The activity of enzymes, such as POD and SOD, is related with the disease resistance of plant. It is generally recognized that when plant cell are intimidated, the balance of enzymes will be broken, and preoxidation of membrane will happen, and the free radicals generated from this process are harmful from bio-membrane¹⁶. Therefore, it is time to activate the protective enzyme system of the plant itself so as to decrease or clear the damage ability of reactive oxygen species to membrane and reduce damages. As the two important protective enzymes in antioxidant enzyme system in plant, POD and SOD could react quickly for the damages of cells¹⁷⁻¹⁹, decrease or clear the damage ability of reactive oxygen species, and reduce the accumulation of MDA so as to protect the structure of membrane. Through reducing the extent of damage of the membrane to improve resistance to pathogen, POD and SOD could effectively decrease the damage ability of reactive oxygen on membrane resulted from bacteria damage, protect the structure of membrane, and repair the damages done by free radicals. Most of soluble proteins in leaves are active enzymes which participate in many important physiological metabolism processes, such as photosynthesis and respiration¹⁹. According to some researches²⁰, plant can protect the surface of membrane by accumulation of soluble protein so as to coordinate the osmotic pressure between the cell and the surrounding and reduce damages of disease.

There is inhibitory effect of extracts from

A. sibirica×*vulgaris* leaves and *P. orientalis* leaves on disease index of cucumber with *C. cucumerinum*. Moreover, there is most significant control effect of *C. cucumerinum* by extract of *A. sibirica*×*vulgaris* leaves with the inhibition rate of 22.83%. Many medicinal herbs are regarded as inducers for plant source. It has been testified that these herbs can induce and strengthen the disease resistance under the condition of adversity^{3-5, 19}. However, for the complicated pathogenesis of pathogen and epidemiology of infection of plant, there are significant differences of disease resistance of extracts from *A. sibirica*×*vulgaris* leaves and *P. orientalis* leaves for difference crops and diseases. Under the condition in this experiment, using extracts from *A. sibirica*×*vulgaris* leaves and *P. orientalis* leaves could inhibit the damage of *C. cucumerinum* to some extent, which may be related with the rise of activity of POD and SOD in cucumber plant. The disease resistance mechanism of extracts from *A. sibirica*×*vulgaris* leaves and *P. orientalis* leaves is complicated, which need to be further studied and investigated.

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