

Effects of flavonoids from potato-onion on *Fusarium* wilt fungus of tomato

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ABSTRACT

We studied, the effects of total flavonoids extracted from potato-onion on the *Fusarium* wilt fungus (*Fusarium oxysporum* (Sch1) f. sp. *Lycopersici* (Sacc) Snyder et Hansen) of tomato. Total flavonoids extracted from the potato-onion decreased the spore germination and their growth. The degree of inhibition was concentration dependent.

Key words: *Allium cepa* var. *agrogatum*, flavonoids, fungus, fusarium wilt, *Fusarium oxysporum*, inhibitory effect, potato-onion, tomato.

INTRODUCTION

Fusarium wilt of tomato caused by *F. oxysporum*, reduces the quality and yield of tomatoes. It is generally controlled by using fungicides, but this has environmental concerns (20). Therefore, it is important to find pollution-free alternative methods to prevent and control the tomato *Fusarium* wilt. Potato-onion, a variant of onion, (*Allium cepa* var. *agrogatum* Don) is widely cultivated in Northeast, China because of its nutritional and medicinal properties (32). Previous studies have reported that the high content of antioxidants in potato-onion, mainly flavonoids, has the potential to control *Fusarium* wilt (2,10,20) and are also effective against the pathogens such as *Fusarium graminearum* (3,34,35,36). Furthermore, flavonoids from banana peels, papaya fruits (24), leaves and stems extracts of *Lycium barbarum* (25) and leaves of dandelion (5) are inhibitory to various fungi (*Verticillium dahlia*, *Sclerotinia sclerotiorum*). The potato-onion is inhibitory to *Fusarium* wilt of tomato (21), thus, flavonoids can act as biocides against soil-borne pathogens (1). However, whether the flavonoids in this plant play key role to control the *Fusarium* wilt of tomato is unknown. This study aimed to determine the *in vitro* effects of flavonoids from potato-onion on the tomato pathogen *F. oxysporum*.

MATERIALS AND METHODS

Potato-onion variety 'wuchang' was obtained from the Laboratory of Vegetables Physiological Ecology, Northeast Agricultural University, Harbin, China (45°41'N, 126°37'E). *Fusarium* wilt fungus (*F. oxysporum*) was isolated from the infected plants of tomato grown in greenhouse and was maintained on potato dextrose agar medium (extract from 200 g potato, 20 g glucose, 0.25 g magnesium sulfate, 0.4 g potassium nitrate and 0.4 g potassium dihydrogen phosphate).

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Figure 1. The photograph of potato-onion plant.

Extraction of total flavonoids from potato-onion bulbs

Disease free bulbs of potato-onion without mildew, were washed with water and dried to constant weight at 60 °C in an oven. The dried potato-onion bulbs were ground to powder and 4 g powder was soaked in 40 ml of 1% acidified methanol for 30 min. It was then centrifuged at 10,000 rpm for 15 min. The supernatant was dried at 45 °C and the dry residue was suspended in 40 ml methanol.

Total flavonoids in methanol extract were determined using the aluminium chloride assay (7). Briefly, 2 ml extract was taken in test tube, diluted with 2 ml distilled water and then added 150 µL sodium nitrite (5% NaNO₂, w/v). After 6 min, the mixture was treated with 150 µL aluminium chloride (10% AlCl₃, w/v) and incubated for 6 min. Two ml sodium hydroxide (NaOH 1N) was then added and made up to 6 mL with distilled water. After 15 min, the absorbance was measured at 510 nm using spectrophotometer (7).

Preparation of medium containing total flavonoids

The final concentration of the total flavonoids solution tested was 1.25, 2.5, 5.0, 10.0, 20.0 mg/ml by dilution method with distilled water under sterile conditions. Test medium containing total flavonoids was prepared as per the method of Nefzi *et al.* (18) with slight modification. PDA medium was autoclaved at 120 °C for 30 min. When the medium was about 53 °C, 2 ml total flavonoids solution (filtered through 0.22 µm filter membranes) from potato-onion was added to 18 ml of PDA medium, thoroughly mixed and poured into Petri dishes. Control (CK) consisted of medium with distilled water in place of extract.

Spore germination

The spore suspension was prepared according to method of Yong-Hong *et al.* (29). The pathogen was cultured in potato dextrose medium (potato, 200 g; glucose, 20 g) on a shaker (110 r/min, 28 °C) for 7 days, and then the solution was filtered under sterile conditions with sterilized gauze to get suspension. The spore number in the suspension was determined microscopically using a hemocytometer and a spore suspension having a concentration of 1.0×10^3 spores/mL was prepared.

To test the effects of flavonoids on spore germination, spore suspension (20 µL) was mixed with 20 µL of total flavonoids solutions of different concentrations. The mixture was then incubated for 4, 6 and 8 h at 25 °C and the number of spores germinated was determined. A spore was considered germinated, when the length of spore tube was more than half the diameter of spore and the percent spore germination was calculated by examining 100 spores (29).

Fungal growth

To determine the effects of the extracts on fungal growth, a mycelial disk (5 mm dia) taken from a 7-days-old fungal culture on PDA, was transferred to the centre of freshly prepared PDA medium on plates supplemented with different concentrations (0,1.25,2.5,5.0,10.0,20.0 mg/mL) of total flavonoids. The plates were incubated at 28 °C in dark. The colony diameter was measured after 72 h and 120 h. There were six treatments in total and 15 plates for each treatment (total of 90 plates) (15). Inhibition of Fungal growth Rate (IR %) was calculated using the formula:

$$IR (\%) = \left(\frac{dt-dx}{dt} \right) * 100$$

Where *dt* : Colony diameter in control plates; *dx* : Colony diameter in treated plates (12).

Fungal biomass was also measured quantitatively. The pathogen was inoculated on PDA medium containing total flavonoids and cultured at 28 °C for 72 h. Five ml phosphate buffer (pH 7, 0.2 M) was put into the plate, fungal mat was scraped off the surface with a sterile coating rod. The fungal suspension was transferred to pre-weighed centrifuge tube and centrifuged at 3500 rpm for 10 min. The supernatant was discarded and the tube was dried to constant weight at 105 °C for 30 min in an oven. The mycelium was then transferred to a clean test tube containing 1 ml distilled water. Two ml of potassium dichromate-sulfuric acid mixture was added and kept in boiling water bath for 30 min, followed by dilution with 2 ml distilled water. The OD₆₂₀ of the solution was then measured using a spectrophotometer (14,16) and the biomass calculated with reference to glucose standard.

Relative conductivity of *F. oxysporum*

One ml fungal spore suspension was inoculated into 100 mL potato dextrose medium broth and incubated on shaker (110 r/min, 28°C) for 48 h. Then, the broth was filtered through 0.22 µm membrane (Linghang, China). Two ml filtrate was mixed with 2 ml of different concentrations of flavonoid extract (1.25, 2.5, 5.0, 10.0, 20.0 mg/mL). Distilled water was used as control.

To determine the effects of flavonoids on the cell membrane permeability of fungus, the conductivity of suspension was measured at intervals. A fraction of this mixture (0.5 ml) mixture was withdrawn every 12 h, centrifuged at 5000 rpm for 5 min, and the conductivity of supernatant was determined using conductivity meter (28).

Statistical analysis

The raw data was processed by Excel software. Significant differences in means among the groups were analyzed using SPSS 17.0 software with the Tukey test at the 0.05 level. Data were expressed as mean values and pooled standard error of the mean (S.E.M.).

RESULTS AND DISCUSSION

Spore germination and fungal growth

With the increase in total flavonoids concentration of potato-onion, spore germination and fungal biomass were decreased ($P < 0.05$) (Fig. 2 a,b). Flavonoids from potato-onion affected the growth of fungus in a dose dependent manner. Inhibition was noticed even at 2.5 mg/ml level of total flavonoids and was $> 50\%$ when the concentration of total flavonoids exceeded 10.0 mg/ml (Table 1).

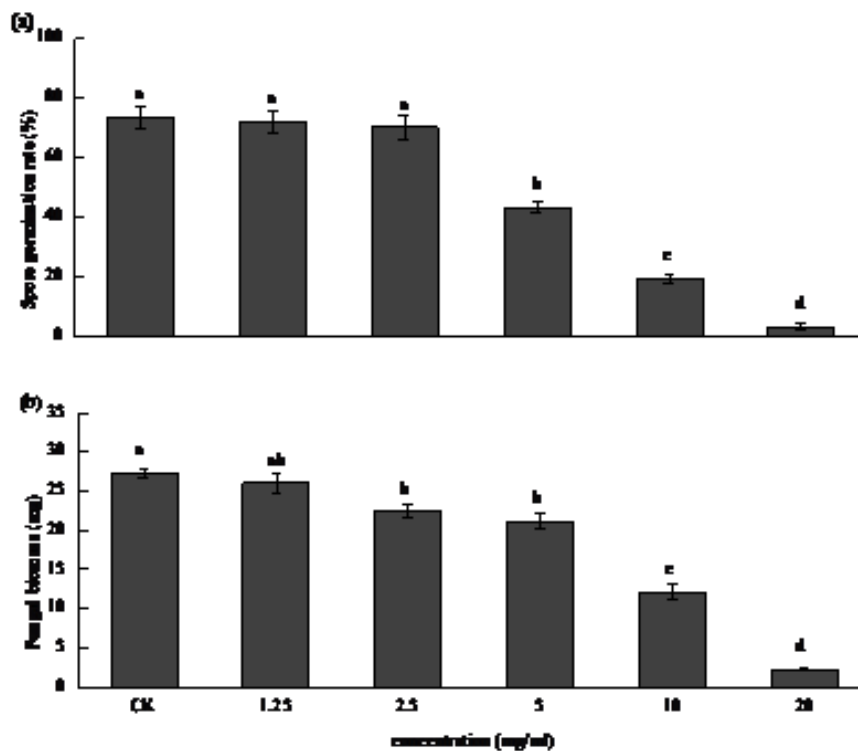
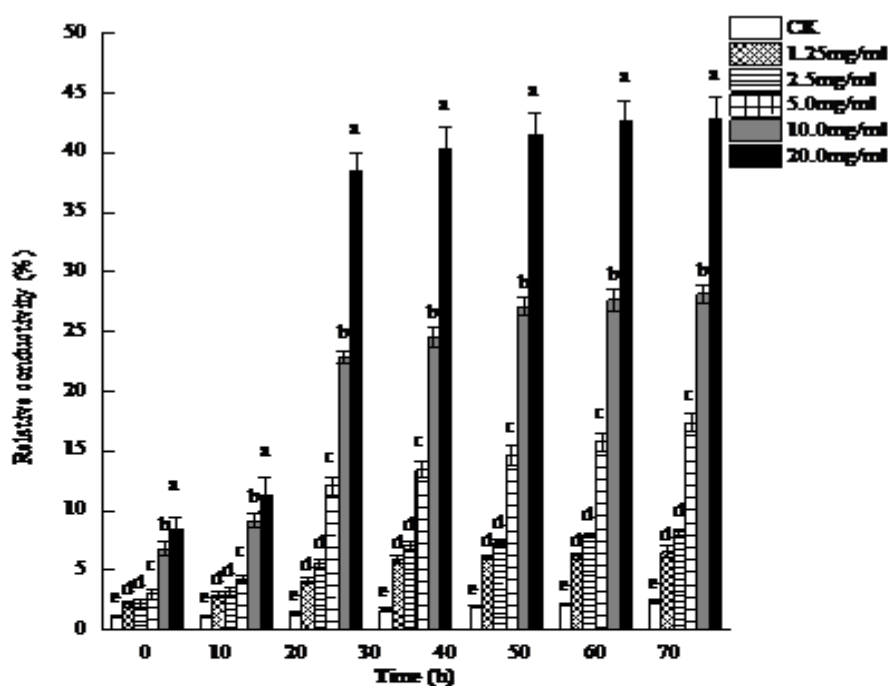


Figure 2. Effects of different concentrations of total flavonoids from potato-onion on spore germination rate and fungal biomass

Many pathogenic microorganisms are strongly inhibited by flavonoids (11,19,23). Hence flavonoids can be used to control infection by pathogenic microorganisms (31). A concentration dependent inhibition by flavonoids has been also reported earlier (6,15).

Table 1. Effects of total flavonoids from potato-onion on growth of *F. oxysporum*

Total Flavonoids Concentration (mg/mL)	Mycelial growth diameter (cm)		Inhibition rate (%)	
	72 h	120 h	72 h	120 h
CK	4.29±0.16a	6.96±0.45a	0	0
1.25	4.21±0.78a	6.87±1.01a	1.86	1.29
2.5	3.98±0.65b	6.63±0.13b	7.23	4.74
5.0	2.86±0.10c	5.28±0.09c	33.33	24.13
10.0	2.09±0.13d	3.84±0.22d	51.28	44.83
20.0	0.53±0.01e	0.57±0.03e	87.65	91.81

Figure 3. Effects of concentrations of total flavonoids from potato-onion on relative conductivity of *F. oxysporum* culture medium

Note: CK: Sterile water; 1.25, 2.5, 5.0, 10.0 and 20.0 mg/ml represented different concentrations of total flavonoids, respectively.

Low concentrations are stimulatory, while higher concentrations are inhibitory (17,33). However, in our study we found that even the lowest concentration that we used inhibited the spore germination. Perhaps this may be due to the concentration of flavonoids being higher than reported earlier. These results showed that total flavonoids from potato-onion have a strong inhibitory effect on spore germination and growth of *Fusarium wilt* of tomato.

The relative conductivity of *Fusarium mycelium* increased by increasing flavonoids concentration ($P < 0.05$) (Fig. 3). Flavonoids changes the membrane permeability and this is related to the relative conductivity of the medium (12,22). The results of this study showed that the relative conductivity of *Fusarium* medium increased significantly, indicating that the flavonoids cause a change in the cytoplasmic membrane structure of the fungus and thus has an antifungal effect (9,22,27). Thus, it weakens the pathogenic ability of *Fusarium* and this is consistent with the conclusions of previous studies (4).

Flavonoids may play an inhibitory role in the growth of *Fusarium* and this study has comprehensively has shown that spore germination and growth of *F. oxysporum* were inhibited by the flavonoids. Thus, it is speculated that flavonoid-containing plants, such as potato-onion, when grown as companion crop with tomatoes and releasing flavonoids through the roots or bulbs may prevent spore germination and growth of tomato *Fusarium* wilt, and thus control the development of tomato *Fusarium* wilt. This study therefore provides a basis for further studies for control of *Fusarium* wilt by flavonoids.

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