

# Mulberry popcorn disease occurrence in Korea region and development of integrative control method

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## Abstract

Mulberry fruits also have tremendous potential for providing various valuable industrial products of very high economic value for human beings. Nevertheless, through global warming, the popcorn disease caused by sclerotia forming fungi reduces the productivity of mulberry fruits in worldwide. So, in this study, we investigated damage ratio of mulberry popcorn disease in mulberry fruit production farm (Buan, Jeongueb, Sangju, Gochang in Korea). In Jeonbuk Buan, popcorn disease rate was the highest about 30%, on the other hand, in case of Gyungbuk Sangju and Jeonbuk Gochang, not damage. Also, we investigated about popcorn disease prevention by various of chemical treatment methods.

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## Introduction

Globally, mulberry (*Morus* sp.) is exploited for feeding leaf to silkworms in order to obtain silk fiber or for animal feedstock production. Especially, mulberry fruit is known to a by-product that was produced from mulberry tree after harvesting leaves for silkworm rearing, as a yield and consumption of mulberry fruit was increased, it has been fixing to a new income crop. Mulberry fruits are used for the health benefits of human beings (Singhal *et al.*, 2003). Mulberry fruit has been effectively in natural medicine for the treatment of sore throat, fever, hypertension and anemia (Alakbarov and Aliyev, 2000; Shivakumar *et al.*, 1995). Mulberry fruit contains not only high amounts of anthocyanins, but non-anthocyanin phenolics including rutin and quercetin known to have multi-bioactive functions including neroprotective effects (Kim *et al.*, 1996).

Recently, through global warming, the popcorn disease caused

by sclerotia forming fungi reduces the productivity of mulberry fruits in worldwide. The disease in other countries has been known as popcorn disease, swollen fruit disease. (Sultana *et al.*, 2013; Hong *et al.*, 2007a, Kishi, 1998; Kohn and Nagasawa, 1984; Whetzel and Wolf, 1945). Mulberry farms have been collectivized over recent years, mulberry popcorn disease, which damages mulberry production most severely, has increasingly occurred every year to cause considerable damages to farmers. In Korea, *Ciboria shiraiana* and *Scleromitrla shiraiana*, have been reported to be pathogens for mulberry popcorn disease (Sultana *et al.*, 2013; Cho and Shin, 2004; Hong *et al.*, 2007a). Swollen fruit disease of mulberry tree in Asia including China and Japan was reported as *C. shiraiana* (Ling, 1948; Kishi, 1998) and *S. shiraiana* was first identified from sclerotia of mulberry fruits in Korea (Hong *et al.*, 2007a). In the spring, when mulberries are flowering, the sclerotia produce cup-like apothecia from which spores are released into the air to infect developing fruit.

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It is a serious disease if the tree is being cropped for commercial purposes leading to substantial losses in product yield and quality. But, there have still been no effective control method for mulberry popcorn disease, registered agricultural pesticides, and eco-friendly agricultural materials. Therefore, in the current study, occurrence and scale of damage of mulberry fruits popcorn disease was surveyed in several locations in Korea. Also, our findings provide a basis for development of chemical and non-chemical controls against popcorn disease on mulberry fruits.

## Materials and Methods

### Survey about damage rate of mulberry fruits by popcorn disease

It was investigated damage ratio for mulberry popcorn disease in mulberry fruit production farm of four Korea regions (Jeonbuk Buan, Jeonbuk Jeongueb, Gyeongbuk Sangju, Jeonbuk Gochang). The investigation period was Buan (15.5.26), Jeongueb (15.6.5) Gochang (15.6.5), Sangju(15.6.3). The surveyed mulberry tree variety was Gwasang 2 ho having a higher incidence of popcorn disease. The research was focused on the status of the disease such as the weekly morbidity rate. Survey methods was firstly counted total mulberry fruit of the longest branch in mulberry tree and disease incidence mulberry fruit in mulberry tree. Sample size was over the five and in the following method has been calculated.

※ Disease incidence ratio (%) = (Total mulberry fruits - Disease incidence mulberry fruits)/Total mulberry fruits × 100

### Investigation of popcorn disease prevention by a various of chemical treatment

For investigation of chemical and non-chemical controls methods against popcorn disease on mulberry fruits, as the treatments, chemical agent, fixing agent, biopesticide, liquid fertilizer were applied in Buan mulberry tree farm. Gwasang 2-ho having a higher incidence of popcorn disease was used in these treatments. The chemical treatment methods was as follows.

- I. Chemical treatment
- II. Chemical + Fixing agent
- III. Biopesticide treatment
- IV. Liquid fertilizer treatment

On these treatment methods, it was treated three times for each 5 d from bloom season in farm (2015. 4. 10, 4.15, 4.20). Survey methods was firstly counted total mulberry fruit of the longest branch in mulberry tree and disease incidence mulberry fruit in mulberry tree. Sample size was over the five and in the following method has been calculated. Also, the mulberry fruits treated in chemical agent was got for investigation of pesticides residue amount.

※ Disease incidence ratio (%) = (Total mulberry fruits - Disease incidence mulberry fruits)/Total mulberry fruits × 100

### Statistical analysis

Each experiment was carried out in triplicate, all data were the average of three independent experiments and analyzed by SPSS (version 18.0), and expressed as mean ± standard deviation (SD). Results were considered significant at  $p < 0.05$ .

## Results and discussion

### Survey about damage rate of mulberry fruits by popcorn disease

Mulberry fruits popcorn disease's occurrence rate was surveyed for Gwasang 2 Ho in four regions of Korea (Table 1). In Jeonbuk Buan, popcorn disease rate was the highest about 30%, on the other hand, in case of Gyeongbuk Sangju and Jeonbuk Gochang, not damage. Gwasang 2 Ho was the most susceptible to popcorn disease. The occurrence and severity of popcorn disease varied depending on temperature, moisture, geographical location, and mulberry plant variety (Yang, 1959; Hong *et al.*, 2007b; Sultana and Kim, 2016). The life cycle of popcorn disease on mulberry fruits was infected fruit drop from the tree and serve as the source of spores next season and symptoms develop in May and June as the fruit matures (Hong *et al.*, 2007a; Hu *et al.*, 2011; Hua *et al.*, 2011). Also, ascospores that are sprayed from apothecia infect the female flowers of the mulberry plant (family Moraceae, genus *Morus* L.) from February to April in China (Huang *et al.*, 1992). It was reported that diseased mulberry fruits have ovaries filled with fungal mycelium rather than having normal plant tissues. The pathogen releases vast number of fungal spores, resembling a waxy substance on outside ovary wall (Gray and Gray 1987; Siegler

**Table 1.** Survey about damage rate of mulberry fruits by popcorn disease

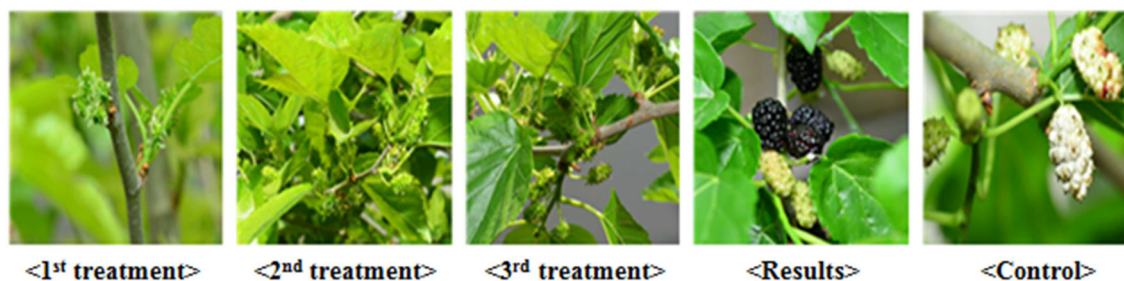
Regions	Disease incidence (%)					Average
	Farm I	Farm II	Farm III	Farm IV	Farm V	
Jeon-buk Buan	32.07±2.83	40.05±4.04	35.13±2.92	15.28±1.74	28.35±2.72	30.17±2.85
Jeon-buk Jeongueb	15.48±3.02	21.45±3.04	17.42±1.63	18.53±2.61	19.21±3.82	18.41±2.82
Gyeong-buk Sangju	1.2±0.85	2.1±0.4	1.48±0.57	2.58±0.23	0.92±0.41	1.65±0.49
Jeon-buk Gochang	0	0	0	0	0	0

Values are mean ± SD of triplicates

**Table 2.** Survey of popcorn disease prevention by a various of agent treatment

Control agent	Disease incidence ratio (%)			Prevention (%)
	1 <sup>st</sup> test port	2 <sup>nd</sup> test port	Average	
Chemical agent	25.14±3.5	26.42±2.4	25.75±2.95	44.3
Chemical+Fixing agent	28.45±4.2	32.14±5.3	30.3±4.75	34.4
Biopesticide	43.21±3.4	43.74±3.1	43.47±3.25	5.9
Liquid fertilizer	44.21±1.45	45.97±2.17	45.09±2.17	2.4
control	45.28±4.27	47.21±3.45	46.24±3.86	0

Values are mean ± SD of triplicates



**Fig. 1.** Photograph on mulberry fruits treated chemical agent.

and Jenkins, 1922). And Hong *et al* (2007b) was reported that *Ciboria shiraiana* and *Sclerotinia shiraiana* was occurred at the ratio of about 6 to 4 in the fields.

### Investigation of popcorn disease prevention by a various of chemical treatment

On the prevent effect of mulberry popcorn disease by using chemical agent, fixing agent, biopesticide, and liquid fertilizer, chemical and non-chemical agent spray treatment was carried out in Buan mulberry farm.

As that result, when chemical A as thiophanate-methyl pesticide was applied, the prevention rate was 44.3%, however, in treatment of the selected biopesticide and liquid fertilizer, it did not effective (Table 2). It is respectively showed agent spraying photograph (Fig. 1 and Fig. 2). Chai *et al* (2005) reported that 25% Amistar SC was more effective on popcorn disease when compared with conventional pesticides carbendazim and under field conditions. The fungicides as carbendazim, thiophanate-methyl, Glyphosate were used to study further on the basis of their economics and active mechanisms on controlling pathogen in crop field. Also, the other chemical control's method was



**Fig. 2.** Photograph on mulberry fruits treated biopesticide and liquid fertilizer.

used 95% glyphosate, Bordeaux mixture, lime sulfur mixture against mulberry fruit popcorn disease (Hua *et al.*, 2011; Ahn *et al.*, 2013; Ye *et al.*, 2014; Sultana and Kim, 2016). But, the application of such chemical treatments is being decreased due to their impacts on environmental pollution and food safety.

The treatment of chemical agent mixed fixing agent was showed 34.4% prevention. The fixing agent was prevent damages due to acid rain and protects crops from hot and cold weather. It was useful on reduce the usage of chemicals and spray frequency and keeps effects lasting longer as it can prevent crops from being washed away by wind or rain by keeping pesticides and nutrients adhered to crops. Fig. 3 was shown by morphological observation of mulberry fruit scanning electron microscope (SEM) that whether mulberry fruit surface

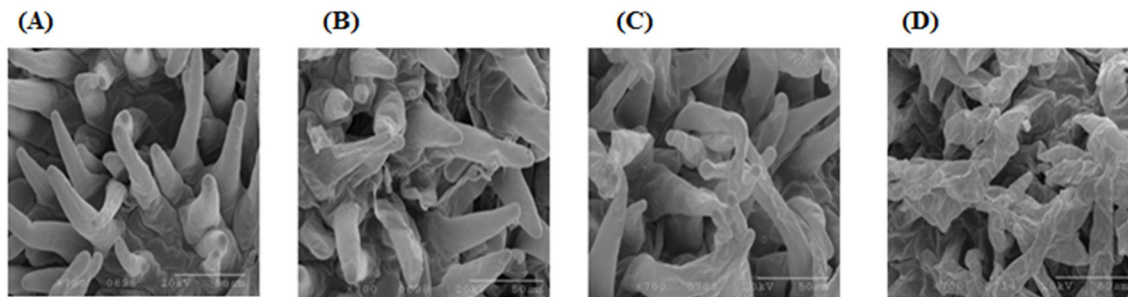
treated fixing agent was effected or non-effected. In fig. 3b, when 100 times diluted fixing agent was applied, surface could be maintained. Finally, the residue amount of pesticide about chemical agent as thiophanate-methyl was surveyed. As that result, it was appropriated for permissible level (Table 3). But, there have still been no effective control method for mulberry popcorn disease, registered agricultural pesticides, and eco-friendly agricultural materials. Therefore, to prevent against popcorn disease, it is necessary clean management of mulberry tree fields and appropriate pesticides.

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**Fig. 3.** Morphological observation of mulberry fruit treated fixing agent scanning electron microscope (SEM). (A) Control, (B) 100 times dilution, (C) 500 times dilution, (D) 1000 times dilution.

**Table 3.** Survey of the residue amount of pesticide about chemical agent

Component name	Chemicals	Carbendazim residue amount (mg/Kg)	Remarks
Thiophanate-methyl	Chemical	0.11	-Application MRL: 2.0 (strawberry)
	Chemical + Fixing agent	0.16	
	Chemical	1.10	-Recommendation MRL: 5.0 (mulberry fruits)
	Chemical + Fixing agent	1.72	

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