

Efficacy of Chemical Preservatives to Control *Perilla* Rust

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Abstract: The rust fungi are biotrophic fungal pathogens that cause serious disease on *Perilla* leaves in Korea. The effect of some commonly used chemical preservatives (sorbic acid, D-sorbitol, propionic acid and benzoic acid) against *Perilla* rust was investigated in this study. Results of this investigation showed that all the preservatives were effective against *Perilla* rust except benzoic acid. There was no growth of rust spores on the *Perilla* leaves treated with 0.1% of preservative even after 21 days of preservation. However, 0.01% of preservative also showed remarkable reduction of rust spores on the *Perilla* leaves as compared to the control groups. Thus, the results of this study indicated that the chemical preservatives used might be useful to control the growth of rust fungi on *Perilla* leaves.

Key Words: Chemical preservatives, Sorbic acid, Anti-rust activity, *Perilla* leaves

Introduction

Food and feed spoilage fungi cause great economic losses worldwide. It is estimated that between 5 and 10% of the world's food production is wasted due to

fungal deterioration (Pitt and Hocking, 1997). These fungi cause losses in dry matter or quality and some species can produce health damaging mycotoxins. The rust fungi are a monophyletic group of approximately 7000 species in the basidiomycota and are highly specialized obligate parasites of plants. Rust fungi cause diseases in economically important plant species such as cereals, legumes, composites and many trees (Kolmer *et al.*, 2009). *Perilla* rust is a damaging disease in *Perilla* cultivation in Korea. For the *Perilla* production, leaf diseases are cumbersome and damaging the crop. The *Perilla* leaf diseases caused by fungi include leaf spot, gray mold, anthracnose, rust, etc (The Korean Society of Plant Pathology, 2004; Kim *et al.*, 2001; Moon *et al.*, 1998), among which rust is frequently encountered and widely spread in Korea.

The life cycle of rust can vary from two to five distinct spore stages. Some rusts (heteroecious) require two taxonomically unrelated hosts (alternate) to complete their life cycle, while some rusts (autoecious) require only one host. In fields and green houses, the *Perilla* rust occurs anytime during the growing season, (late June to mid August). Initially, the symptoms appear as tiny yellowish projections on the lower surface of the leaves usually spreading inwardly from terminal leaf edge, accompanied by yellow to brown flecks formed on their opposite upper side and covering the whole leaves with spore masses within 2-3 weeks.

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The enlarged pustules can be seen as yellow spore masses in the beginning but later turn whitish. The leaf tissues become dry and dead (Yun *et al.*, 2007).

Most food is only available fresh for a relatively short period but we want to enjoy them throughout the year. This therefore is a good enough reason why we need to preserve food. Food must be preserved not only to prevent losses but also to protect them from toxin which is produced by microorganisms and then transmitted to the food. Prescott *et al.* (2002) defined preservatives as a group of chemical compounds deliberately added to food or that appears in food as a result of pre-processing treatment, processing or storage. Chemical preservation has become an increasingly important practice in modern food technology with the increase in production of processed and convenience foods. These preservatives are deliberately added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes and thus increasing its shelf life (Saad *et al.*, 2005). This work was therefore aimed at determining the effect of some chemical preservatives (sorbic acid, D-sorbitol, propionic acid and benzoic acid) to control the rust disease of *Perilla* leaves.

Materials and Methods

Collection of *Perilla* leaves

Fresh *Perilla* leaves with average size of 9cm × 5 cm were collected from the open field near Daegu University, Kyoungsan, Republic of Korea, in August 2010.

Chemical preservatives

Some commonly used chemical preservatives sorbic acid, D-sorbitol, propionic acid and benzoic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). These kinds of preservatives can generally permit in KFDA (Korean Food and Drug Administration) for food preservation.

Morphological characteristics of *Perilla* rust spores

Perilla rust spores were observed with a light microscope. Morphology and surface structures of spores were also investigated by scanning electron microscopy (SEM) to support the light microscopic features of the spores. For SEM, spores were dusted on double-sided adhesive tape on specimen holders, coated with gold using a sputter coater (Hitachi E-1010 Ion Sputter, Japan) and then observed under a

SEM (Hitachi S-3000N, Japan) at 15 kV.

In vivo anti-rust activity of chemical preservatives

The efficacy of the chemical preservatives used was evaluated to control rust fungus on *Perilla* leaves during the storage period. For each experiment, four leaves were taken into one group and all leaves were dipped into the preservatives at the tested concentrations (0.01 and 0.1%) except control. Then the leaves were artificially inoculated with a spore suspension of *Perilla* rust during which thorough mixing was carried out to ensure equal distribution of the fungal uredospores (1×10^5 spores/mL). All groups of treated and untreated leaves were kept into plastic basket (18×11×6 cm). Two baskets as replicates were used for each particular treatment. All baskets were stored at room conditions (temperatures of $24 \pm 1^\circ\text{C}$ and relative humidity of 55 – 75%). The storage period extended for three weeks.

Results and Discussion

We confirmed the rust fungal spore before artificial inoculation of the spores on experimental *Perilla* leaves by microscopic evaluations. The light microscopic features of urediospores were shown in Fig 1A. They were single celled, bi-nucleate, outer wall spiny and

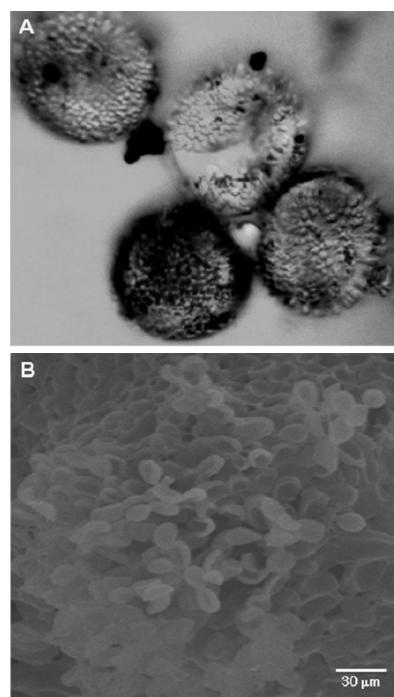


Fig. 1. (A) uredospore under light microscope; (B) uredinium under scanning electron microscope.

Table 1. Control of *Perilla* rust by using different concentration of chemical preservatives

Chemical preservatives	Control (RS)	Efficacy of chemical preservatives			
		RS	0.01% Preservative PR (%)	RS	0.1% Preservative PR (%)
Propionic acid	>1×10 ⁴	21 ± 2.5	97.90	0	100
Sorbic acid	>1×10 ⁴	17.7 ± 2.1	98.23	0	100
D-Sorbital	>1×10 ⁴	27 ± 2.8	97.30	0	100
Benzoic acid		<i>Perilla</i> leaves were damaged			

Control means without preservative;

RS, No. of rust spores on *Perilla* leaf; PR, Prevention rate

Values in the same column with different significance ($P<0.05$).

yellow or orange in color when young. Fig 1B shows the scanning electron micrographs of uredinium. The mycological data are well matched as comparison with the previously described related rust fungi (Hiratsuka and Kaneko, 1975; Hiratsuka *et al.*, 1992; Kaneko, 1981).

From the results shown in Table 1, it can be deduced that the chemical preservatives used were effective against the rust fungi. This is shown by the growth or no growth of the rust spores on *Perilla* leaves, which contrasted in the case of the leaves without preservatives. Preservatives have been used to store food substances and they act by inhibiting, retarding or arresting the growth of microorganisms or of any such deterioration resulting from their presence or of masking the evidence of any such deterioration (Ihekoronye and Ngoddy, 1995). To be in accord with good manufacturing practices, the use of preservatives should not adversely affect the nutritive value of food or should not permit the growth of food poisoning organisms while suppressing the growth of others that would make spoilage evident (Ihekoronye and Ngoddy, 1995).

Investigation of the anti-rust activity of the preservatives revealed that all the preservatives were effective against *Perilla* rust at the concentration of 0.1% except benzoic acid, which had lost colour (turned dark brown) and even odorous of the *Perilla* leaves. However, the leaves treated with 0.01% of preservatives showed remarkable reduction of rust fungi as compared to the control group. As the results shown in Table 1, 100% rust fungal spore was inhibited at 0.1% preservatives, whereas at 0.01% of preservatives, sorbic acid inhibited highest amount of rust spore (98.23%). On the other hand, propionic acid and D-sorbital inhibited 97.9 and 97.3% rust spore, respectively. In this study, even after 21 days

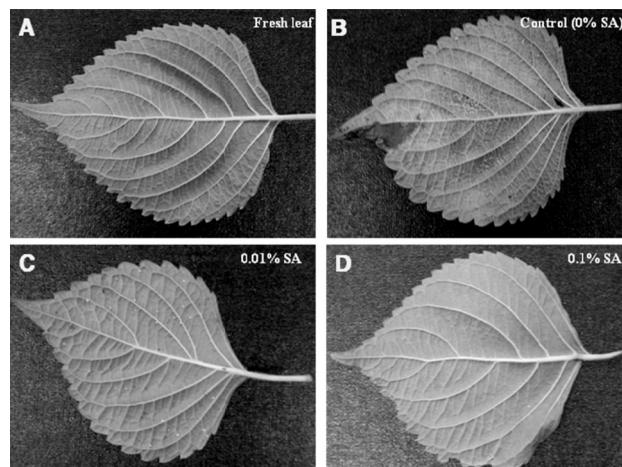


Fig. 2. Control of *Perilla* rust by using sorbic acid. (A) Fresh leaf at 0 day; (B, C, D) leaves after 21 days of incubation at different concentration of sorbic acid. SA denotes sorbic acid.

of preservation, samples containing chemical preservatives had more keeping quality than leaves without preservatives (Figure 2). Thus from the above findings, it can be concluded that some commonly used chemical preservatives could be considered as the fast and reliable anti-rust agents to control *Perilla* rust diseases.

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