Effects of fungicides and antifungal agents on *Olpidium* root rot of spinach in a hydroponic culture

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**Abstract**

*Olpidium* root rot is a disease of spinach grown in hydroponic culture. It causes browning of the roots of spinach, growth delay, and a serious reduction in yield. However, there is no information about control measures. Thus, we obtained preliminary data to explore the efficacy of fungicides and antifungal materials, which have potential to control *Olpidium* root rot. Azoxystrobin, oxathiapiprolin, phosphorous acid, and silver were found to be effective against *Olpidium* root rot.

**Key words:** spinach, *Olpidium virulentus*, olpidium root rot, hydroponic culture

*Olpidium virulentus* (Sahtiy.) Karling is a soil-born fungus. *Olpidium* is an obligate parasite belonging to the family, Olpidiaceae. It produces zoosporangium, resting spore, and zoospore. The resting spore persists in soil or water, as it waits for new hosts. *O. virulentus* has been regarded as a nonpathogenic parasite of spinach. However, in May 2018, significant browning and carling of the roots and a serious reduction of yield were observed in a commercial hydroponic culture of spinach in Osaka prefecture, Japan. Similar symptoms were also reported as the Olpidium syndrome in 2013 (Muramoto et al.). The causative agent was identified as *O. virulentus* and named Olpidium root rot (Nishimura et al., 2019). *O. virulentus* infects and propagates in the root cells of spinach and makes zoosporangia and resting spores, then transmits from spinach to spinach by zoospores through the nutrient water. In this study, the effectiveness of five substances (azoxystrobin, hymexazol, oxathiapiprolin, silver and phosphorous acid) for this disease were examined. However, the use of fungicides on hydroculture spinach, except the silver and phosphorous acid nutrient, is prohibited by Agricultural Chemicals Regulation Law, in Japan. The data on azoxystrobin, hymexazole and oxathiapiprolin obtained in this study were to serve as reference data.

**Materials and Methods**

**Pathogen and host**

All experiments were conducted using spinach (*Spinacia oleracea* L. cv. Mirage; SAKATA SEED CORPORATION) as a host. Spinach seeds were planted in vermiculite in a growth chamber at 18°C. The seedlings were transplanted into hydroponics and grown for 14 days in a greenhouse. A single sporangium isolate of *O. virulentus* isolated from spinach in a commercial hydroponic culture in Osaka prefecture was maintained on spinach seedlings growing by hydroculture. The spinach seedlings grown for 3 weeks in Olpidium culturing hydroponic was used as an inoculum.

**Chemical influence studies**

Five plants were placed in each hydroponic bath (9 liters of aerated Enshi standard nutrient solution, EC 2.4 dS/m, pH 6.5). Chemicals used were azoxystrobin-containing fungicide (10 ppm, Amister 10 flowable, 10% a.i.; Syngenta), hymexazol-containing fungicide (10 ppm, Tachigaren solution, 30% a.i.; Mitsui chemicals agro), oxathiapiprolin-containing fungicide (10 ppm, Zorvec enicade, 10.2% a.i.; Dupont), silver-coated cloth (5 × 6.5 cm, Ooctcloth, 0.23 mg a.i./cm²; Mishima OA system) as a source of silver and phosphorous acid nutrient (1000 ppm, Phosplus, 31% a.i.; OAT agrio). One day after the placement of plants in the hydroponic bath, the substances were respectively added to the hydroponic water before inoculation. All roots of two spinach colonized by *O. virulentus* were shredded and mixed, then inoculated into each bath with 5 g (fresh weight) on the next day of the substance application. After 14 days from inoculation, the roots of all spinach in each treatment were harvested.

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Five 1-cm-long root segments (0.4–0.5 mm in diameter) from each plant were randomly selected and examined microscopically for the counting of sporangia and resting spores of *O. virulentus*. As the total sporangia and resting spores numbers of inoculated-only plots varied between experiments, the result was indexed using the total numbers of inoculated plot as 100 and showed an average of indexes of two experiments. Percentage infection was calculated from the following formula.

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\text{Percentage infection (\%)} = \left( \frac{\text{total number of Olpidium zoosporangia and resting spores in treated roots}}{\text{total number of Olpidium zoosporangia and resting spores in inoculated-only roots}} \right) \times 100.
\]

Ten plants per the treatment were used for the experiment. The experiment was conducted two times in a greenhouse.

**Results and Discussion**

The data were showed as relative values, with inoculated plot as 100% (Fig. 1). No sporangia or resting spores of *O. virulentus* were observed in roots of the non-inoculated plants. Azoxystrobin, oxathiapiprolin, phosphorous acid and silver ion decreased the number of sporangia and resting spores of *O. virulentus* in less than 10% of inoculated plants. The sporangia and resting spores were barely observed in the roots treated with azoxystrobin. Azoxystrobin has efficacy for lettuce big-vein disease (Tanaka et al., 2012). Hymexazol reduced the number of sporangia and resting spores in the roots to 22% of inoculated plants. Oxathiapiprolin and hymexazol inhibit zoospores of *Phytophthora* and *Pythium*, respectively. In contrast, the roots of the inoculated spinach plants were browning, and almost all cells contained either sporangia or resting spores of *O. virulentus*.

Phosphorous acid is registered as a fertilizer permitted for use in hydroculture. Many studies have reported that phosphorous acid inhibited *Pythium* root rot (Förster et al., 1998; Jee et al., 2002; Kusakari, 2011; Watanabe et al., 2016). Phosphorous acid is known to suppress the growth of hypha and the release of zoospore of fungi, while also inducing disease resistance in host plants (Afek and Sztejnberg, 1989; Coffey and Joseph, 1985). Present result suggests that phosphorous acid can suppress release of zoospores of *O. virulentus* from the sporangia. Nishiguchi et al. (2014) found that Phosphorous acid controlled lettuce big-vein disease by reducing the pH of soil. They also clarified that acidic soil conditions suppressed zoospore release from zoosporangia in *O. virulentus*, the vector of *Mirafiori lettuce big-vein virus* (Iwamoto et al., 2017). The silver is one of the few fungicides available for use in hydroculture. It is used in commercial hydroculture to avoid *Pythium* disease. Silver is known to inhibit the zoospore motility and germination of *Pythium* (Kusakari et al., 1998). The inhibitory effect of silver ion for *O. virulentus* could be attributed to a similar mechanism. Several plants treated with silver of one bath of the second experiment caused minor growth inhibition. It is important to monitor the dose of this material because a young root of spinach might be damaged from a high silver concentration. The mechanisms of effectiveness of azoxystrobin, oxathiapiprolin and hymexazol remain unknown. Further study is necessary to clarify them.

In conclusion, phosphorous acid nutrient and silver ion are effective for the control of Olpidium root rot of spinach. However either material has preventive efficacy and probably does not work after infection of spinach by *O. virulentus*. For effective disease control in hydroponics culture, it is important to have some fungicides that are effective after the disease has developed.

**References**

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