Common Strategies to Control Pythium Disease

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ABSTRACT

Pythium species are soil-borne pathogens which can cause serious economic loss worldwide and threatening agricultural production. Traditional management methods like chemical fungicides are effective against Pythium spp. But as people pay more attention to human health and environmental issues, alternative methods that are ecofriendly and harmless to health are urgently needed. Currently, various approaches had been made including using natural extract, regulating planting conditions, using plant growth-promoting rhizobacteria and screening disease tolerance plants. Hereby, we review the recent achievements, particularly chemical and physical methods, biocontrol and host plant defense which can be used to control Pythium disease.

Keywords

biocontrol, physical and chemical control, plant defense, Pythium species

1. Introduction

Soil-borne pathogens such as Pythium spp. is basically difficult to control due to its highly contagious, wide range of host, and longevity, that will increase the cost of fungicide application. For instance, Pythium spp. can infect not only food crops (e.g. rice, wheat, and maize), but also vegetables and ornamental plants (e.g. cucumbers, sweet peppers, potatoes, tomatoes, peanut, and roses) (Johnstone et al., 2005; Li et al., 2007; Al-Mawaali et al., 2012; Okubara et al., 2014; Wheeler et al., 2016; Tabli et al., 2018). After the host is infected by Pythium, symptoms such as plant stunting, water-soak root rot, crown rot, and leaf blight could occur, resulting in host death (Kageyama et al., 2002; Higginbotham et al., 2004; Deng et al., 2005; Lin et al., 2018).

Pythium root rot can cause serious loss of agricultural production worldwide. In Kenya and Rwanda, Pythium root rot caused up to 70% yield losses in traditional local bean cultivars, while 35-54% and 12-17% loss at 18°C and 28°C in hydroponically grown lettuce (Stanghellini and Kronland, 1986; Nzungize et al., 2012). Besides, Pythium root rot also reduced ginger (Zingiber officinale Rosc.) production by 50% to 90% in severely affected areas (Rai et al., 2018). Interestingly, Johnstone et al. (2005) reported that the net carbon exchange rate had no difference between inoculated and noninoculated bell pepper on leaf area basis, while inoculated treatment showed significantly lower on whole-plant basis. Moreover, the daily carbon gain showed a similar result with the net carbon exchange rate. These results indicated that Pythium species do not influence photosynthesis directly, but to reduce the biomass of the plant. This finding suggested that the symptoms caused by Pythium spp. at the very beginning will be difficult to notice.
Pythium spp. have a wide range of hosts. Crop rotation cannot control Pythium efficiently due to its broad hosts. For example, four *Pythium* species isolated from symptomatic seedlings of corn and soybean showed equally pathogenic on both hosts (Matthiesen et al., 2015). In another experiment, Feng et al. (2019) detected four plant-pathogenic *Pythium* from different tissue of lettuce by loop-mediated isothermal amplification (LAMP). From the Poaceae weeds grown next to the rice field in Akita Prefecture in Japan, *P. arrhenomanes* and *P. graminicola* had been isolated (Toda et al., 2015). Furthermore, 11 *Pythium* spp. isolated from bell pepper in Florida also caused root rot in tomato (Chellemi et al., 2000). In addition, a single host can be attacked by multiple species of *Pythium*. Chamswarng and Cook (1985) recovered ten species and varieties of *Pythium* from both wheat root and the soil in eastern Washington and northern Idaho. On the other hand, 11 different distinct of *Pythium* from both ginger and the soil caused ginger soft rot in Queensland, Australia (Le et al., 2016).

The longevity of *Pythium* also makes some traditional management methods ineffective. The *Pythium* can survive in the soil as oospores which has thick wall without host. Hoppe (1966) reported that *Pythium* species survived after 12 years in air-dried muck soil. The sporangia of *P. ultimum* gminated as usual after 11 months under air-dried conditions compared with the field soil maintain moistly, and the population afterward remained stable (Stanghellini and Hancock, 1971). Garren (1971) also found that *P. myriotylum* still aggressive after 10 months at room temperature in a sealed plastic bag with low inoculum. Moreover, Ichitani and Goto (1982) isolated *P. zingiberum* successfully from the field after crop rotation for more than 6 years in Japan. On the other hand, Samejima and Ichitani (1988) reported that oospores of *P. zingiberum* showed no gemination after 70 days buried in autoclaved soil with autoclaved cucumber stem segments, but the gemination ability of *P. butleri* had no change. Even in polar regions, mycelia of all tested *Pythium* in host plants survived after 3 freeze-thaw cycles (Murakami et al., 2015).

Given those above, *Pythium* can cause serious loss to many kinds of crops. Therefore, effective disease management is critically required. Using commercial fungicide could be a choice, however, people are anxious about the potential human health risk and the fungicide resistant ability of *Pythium* enhanced by chemical compound (Margni et al., 2002; Lookabaugh et al., 2015). Considering that *Pythium* root rot is more common during summer and high moisture conditions in some area, some researchers are focusing on controlling *Pythium* spp. by temperature and moisture (Matthiesen et al., 2015; Wheeler et al., 2017; Huzar-Novakowski and Dorrance, 2018). Besides, biocontrol is also a hot topic because of less human health and environmental risk (Van Os and Van Ginkel, 2001; Mavrodi et al., 2012). Furthermore, with the development of molecular biology, it had been proved that many signal pathways are involved in plant defense against *Pythium*, but the current knowledge is still not enough to help us to breed disease resistant plants (Adie et al., 2007; De Vleesschauwer et al., 2012; Sánchez-Vallet et al., 2012). The management of *Pythium* is complicated, since the aggressiveness of *Pythium*, the effectiveness of fungicide, the applicability of biocontrol agents and the application of disease resistant plants can be influenced by many factors such as air temperature, water pH, even soil type. Hereby, we had focused on common management strategies that can be used to minimize the economic loss caused by *Pythium*.

## 2. Physical and chemical control

*Pythium* species are widespread in soil and water (Senda et al., 2009; Toda et al., 2015; Uzuhashi et al., 2015). They can grow in a wide range of temperature, and the optimum temperature range from 13°C to 35°C depend on the species (Kageyama et al., 2002; Senda et al., 2009; Matthiesen et al., 2015; Huzar-Novakowski and Dorrance, 2018). Plant root necrosis caused by *P. myriotylum* was highly correlated with nutrient solution temperature in hydroponic culture, while the lowest necrosis level at 15°C (Fortnum et al., 2000). Furthermore, other researchers
also showed that low temperature could decrease the colonized and browned proportion in the root (Sutton et al., 2006). It is noteworthy that temperature not only influences the aggressiveness of *Pythium* spp., but also change the fungicide sensitivity of *Pythium*. Matthiesen et al. (2015) reported *P. oopapillum* and *P. torulosum* showed more aggressive under 18°C and 23°C, while *P. sylvaticum* showed more aggressive under 13°C. Interestingly, *P. oopapillum* and *P. torulosum* were more sensitive to fungicide at the same temperature range which they were more aggressive.

Apart from temperature, water content is also a critical factor that affects symptom severity. Gent and McAvoy (2011) showed that partial saturation reduced biomass but did not affect the rate of flower development or plant nutrient composition. For instance, in three of five experiments, plants showed less root rot symptoms, and even the recovery rate of *Pythium* on agar was lower than standard saturation in some individual experimental groups (Elmer et al., 2012). Furthermore, by irrigating and draining rapidly to simulated partial saturation, the biomass and stem height reduced 10-20%, while no symptoms occurred under partial saturation (Gent et al., 2011). On the other hand, by controlling the water content, relatively low precise substrate volumetric water content (0.2 m³/m³) showed lower root infection by *P. aphanidermatum in Petunia × hybrida ‘Dreams Red’ compared with 0.4 m³/m³ and cyclic (0.18 to 0.43 m³/m³) treatment. However, the mortality proportion was lowest in cyclic treatment (Wheeler et al., 2017). Not only controlling water content, but also changing water property such as oxidation-reduction potential (ORP) inhibit *Pythium* root rot. For example, using chlorinated water eliminated *Pythium* zoospore in a few minutes (Lang et al., 2008). On the other hand, inoculated tomato grown under hydroponic conditions remained healthy with high oxygen concentration in solution (Chérif et al., 1997). Moreover, using filter and UV light are also considered as feasible way to control *Pythium*. However, it had been showed that filter only delayed root rot transmission in one or two week depending on the pore diameter, while UV light failed to relieve symptoms and reduced yield even if it could control zoospore population (Goldberg et al., 1992; Zhang and Tu, 2000).

There are many commercial chemical fungicides that are effective against *Pythium* spp. such as metalaxyl, azoxystrobin, fosetyl-Al, pyraclostrobin, and trifloxystrobin (Taylor et al., 2002; Stiles et al., 2005; Lookabaugh et al., 2015; Matthiesen et al., 2015). However, fungicides showed different abilities among different plants, even among different *Pythium* spp. (Múnera and Hausbeck, 2015). Based upon environmental temperature, the EC50 value could increase more than 100 times in some fungicides (Matthiesen et al., 2015). Instead of chemical fungicides, some people set their sight on chemical agents which can enhance plant defense. For example, pre-treatment by Acibenzolar-S-methyl, a functional analogue of salicylic acid to turmeric (*Curcuma longa* L.) induce the activities of peroxidases and protease inhibitors, resulting in decreasing cell death after inoculation with *P. aphanidermatum* (Radhakrishnan et al., 2011). Apart from fungicide, Zhao et al. (2000) reported that the silver ion dissolved from silver-coated cloth reduced the root rot symptoms significantly. Surprisingly, nonionic surfactants completely controlled *Pythium* zoospores spread under hydroponic conditions (Stanghellini et al., 1996).

In addition to chemical products, some natural products such as *Brassica juncea* seed meal suppressed *P. abappressorum* constantly at least 12 weeks on apple seedlings (Weerakoon et al., 2012). Moreover, Gent and Elmer (2017) reported that combining with silicon, the disease symptoms of poinsettias (*Euphorbia pulcherrima*) significantly reduced under partial saturation ebb and flow system after inoculation with *P. aphanidermatum*. Meanwhile, polymer sodium silicate aqueous solution suppressed *Pythium in planta*, but not in vitro (Mohsen et al., 2015). Natural extract such as *Vitex agnus-castus* methanolic extract not only showed total antifungal ability against *P. ultimum in vitro*, but also induced certain pathogenesis-related proteins once inoculated with *Pythium* to enhance the plant defense ability, which showed unharmful to tomato seedlings (Švecová et al., 2013).
3. Biocontrol

In addition to chemical fungicides, biocontrol is also considered as a very promising way to control disease. By using the antagonism between different microorganisms, many biocontrol agents had been reported. For instance, Enterobacter cloacae and Erwinia herbicola were known to control preemergence damping-off effectively, and significantly suppressed Pythium colonization on cotton seed at 15°C as effective as fungicide (Nelson, 1988). Misk and Franco (2011) reported that all 11 isolated endophytic actinobacteria from different plants showed antimicrobial ability against P. irregulare. Furthermore, strains isolated from irrigation well also showed Pythium damping-off controlling ability in pea (Tabli et al., 2018). Biocontrol agents used to manage Pythium spp. have been provided in Table 1.

In addition, some biocontrol agents can even promote plant growth while relieving symptoms (Suwannarach et al., 2015). For example, six strains of Pseudomonas spp. were ideal biocontrol bacteria that reduced disease symptoms of both Rhizoctonia solani AG-8 and P. ultimum (Mavrodi et al., 2012). Besides, two strains of them even increased wheat seedling shoot length and root weight while suppressing pathogens (Mavrodi et al., 2012). Kipngenjo et al. (2015) also reported that the dry mass of tomato seedling was significantly increased by coating Bacillus subtilis and Trichoderma asperellum to the seeds in the presence of fertilizer. Meanwhile, the post-emergence damping-off proportions were 10.87% and 15.3%, respectively, when compared that to the control (63.9%). On the other hand, some biocontrol agents can reduce symptoms through inducing plant defense systems. For example, Pseudomonas corrugata strain 13 and Pseudomonas aureofaciens strain 63-28 produced salicylic acid, and induced endogenous salicylic acid accumulation after inoculation with biocontrol agent for 24 hours in cucumber root (Chen et al., 1999). Furthermore, applying these two plant growth-promoting rhizobacteria suppressed cucumber root rot caused by P. aphanidermatum through stimulating the phenylalanine ammonia-lyase (PAL) activity, while Pseudomonas corrugata strain 13 also stimulated peroxidase (PO) and polyphenol oxidase (PPO) activities (Chen et al., 2000). In addition, by increasing benzyl isothiocyanate and its precursor glucotropaeolin in the root of Brassicaceae plant Lepidium sativum after inoculation with two non-pathogenic Fusarium strains, the resistance ability of host plant was enhanced against P. ultimum (Ishimoto et al., 2004). By microscopy, Benhamou and Brodeur (2001) found that a mycoparasite, Verticillium lecanii (Zimm.) not only inhibited the colonization of P. ultimum, but also induced host plant defense, to restrict the pathogen penetration in the epidermis and the outer cortex.

Keeping the population of the biocontrol agent is the key to ensure the effectiveness of biocontrol. It was found that the biocontrol agent Pseudomonas chlororaphis Tx-1 will keep relative stable density after the sweet pepper was inoculated with P. aphanidermatum and P. dissotocum, but rapidly declined in the non-inoculated root (Chatterton et al., 2004). In another study, Postma et al. (2009) reported that combining with chitosan, the suppress ability of Lysobacter enzymogenes 3.1T8 against P. aphanidermatum in cucumber was enhanced and the bacterial population increased.

Except by using microorganisms directly, using compost is also an effective way to control Pythium. It was found that commercial compost strongly suppressed Pythium wilt disease on cucumber plants by the presence (0.056-0.36%) of fungal Cystobasidiomycetes and the presence (0.011-0.018%) of Acidobacteria Gp14 (Yu et al., 2015). On the other hand, the soil with higher organic matter content and lower sand and clay showed less disease index by comparing andosols and ferralsols in Cameroon (Adiobo et al., 2007). Moreover, the suppressiveness of andosols was significantly reduced by pasteurization, applying fungicide and bactericide. Similarly, using autoclaved rockwool increased disease incidence significantly compared with used or new rockwool due to more fungal population was present in used and recolonized rockwool (Postma et al., 2000). In addition, Van Os and Van...
Ginkel (2001) reported that Pythium growth rate was highest in sterilized soil, and lowest in sterilized soil with compost, suggested soil microflora can suppress Pythium.

4. Host plant defense

There is evidence that by expressing defense signaling pathways such as jasmonic acid (JA) signaling, mitogen-activated protein kinase (MAPK) signaling and wall-associated kinases (WAKs) inhibit root necrosis effectively after inoculation (Zhu et al., 2019). Furthermore, it had been reported that salicylic acid and gibberellic acid signal

| Pathogens   | Biocontrol agents                          | References                                                                 |
|-------------|--------------------------------------------|----------------------------------------------------------------------------|
| *P. aphanidermatum* | *Pseudomonas* spp.                         | Chen et al. (1999); Chen et al. (2000); Gravel et al. (2005)            |
|             | *Pseudomonas chlororaphis*                 | Chatterton et al. (2004)                                                  |
|             | *Actinoplanes campanulatus*                | El-Tarabily et al. (2009)                                                 |
|             | *Micromonospora chalcea*                   |                                                                           |
|             | *Streptomyces spiralis*                    |                                                                           |
|             | *Lysobacter enzymogenes*                   |                                                                           |
|             | *Bacillus subtilis*                        |                                                                           |
|             | *Pseudomonas* sp.                          |                                                                           |
|             | *Serratia* sp.                             |                                                                           |
| *P. coloratum* | *Actinomadura rubra*                       |                                                                           |
|             | *Actinoplanes philippinensis*              |                                                                           |
|             | *Bacillus subtilis*                        |                                                                           |
|             | *Micromonospora carbonaceae*               |                                                                           |
|             | *Pseudomonas fluorescens*                  |                                                                           |
|             | *Streptomyces* spp.                        |                                                                           |
|             | *Streptosporangium albidum*                |                                                                           |
|             | *Spartoverticillium netropsis*             |                                                                           |
| *P. dissotocum* | *Pseudomonas chlororaphis*                 | Chatterton et al. (2004)                                                  |
| *P. irregulare* | *Microbispora* sp.                        |                                                                           |
|             | *Streptomyces* sp.                         | Misk and Franco (2011)                                                   |
| *P. ultimum* | *Enterobacter cloacae*                     |                                                                           |
|             | *Erwinia herbicola*                        |                                                                           |
|             | *Stenotrophomonas maltophilia*             |                                                                           |
|             | *Pseudomonas* spp.                         |                                                                           |
|             | *Paenibacillus alvei*                      |                                                                           |

| Fungi       | *Trichoderma asperellum*                   | Kipngen et al. (2015)                                                     |
| *P. aphanidermatum* | *Gliocladium virens*                      | Lumsden and Locke (1989)                                                  |
| *P. ultimum* | *Trichoderma* spp.                        |                                                                           |
|             | *Trichoderma*                             | Cluquet and Scheffer (1996)                                               |
|             | *Verticillium lecanii*                     |                                                                           |
|             | *Fusarium*                                |                                                                           |
|             | *Aspergillus* sp.                         |                                                                           |
|             | *Trichoderma viride*                      |                                                                           |
pathways are critical for rice (Oryza sativa) to defense against P. graminicola, while JA, abscisic acid, and ethylene signal pathway are involved in Arabidopsis defense against P. irregulare (Adie et al., 2007; De Vleesschauwer et al., 2012; Sánchez-Vallet et al., 2012). Except for the critical signal pathway, Castro et al. (2016) found that constitutive expression of pathogenesis-related-10 gene in moss tissue increased resistance against P. irregulare. In another study, reducing availability of sugar in the rhizosphere due to a putative sugar transporter SWEET2 activity contributed to resistance to P. irregulare (Chen et al., 2015). Recently, Nair and Thomas (2013) found one potential resistant gene ZzR1 against Pythium in wild ginger relative viz., Zingiber zerumbet L. Smith.

Besides investigating the molecular aspect of plants, some researchers focus on breeding and screening. By examining the level of tolerance to Pythium root rot in main wheat product varieties, ‘KS93U161’, ‘OH708’, and ‘Sunco’ genotypes showed most tolerant to the disease determined by the number of root tips and total root length (Higginbotham et al., 2004). Besides, ornamental plants such as Caladium cultivars had also been screened and 4 of 19 cultivars showed partial resistance to Pythium root rot (Deng et al., 2005). Furthermore, by conducting somatic hybridization with potato ‘Aminca-Cardinal’ and ‘Cardinal-Nicola’, Nouri-Ellouz et al. (2006) obtained some hybrid lines which showed improved resistance ability against P. aphanidermatum, one line even noted as complete resistance. Among all the plants which showed tolerance against Pythium, phenolic compounds were identified as active ingredients. Temgo and Boyomo (2002) observed that the content of antifungal phenolic compounds

| Cultivars                        | Relative root rot severity | Infection rate (%) |
|----------------------------------|----------------------------|--------------------|
| *R. multiflora* ‘Matsushima No. 3’ | 0.26                       | 10.3               |
| *R. multiflora* var. *carnea*    | 0.27                       | 17.8               |
| *R. Veilchenblau*                | 0.31                       | 15.6               |
| *R. multiflora* var. *cathayensis* | 0.34                      | 17.0               |
| *R. Dance de Feu*                | 0.38                       | 46.4               |
| *R. The Garland*                 | 0.44                       | 22.5               |
| *R. Tapis Volant*                | 0.46                       | 33.6               |
| *R. multiflora*                  | 0.47                       | 31.8               |
| *R. Cecile Brunner*              | 0.49                       | 19.6               |
| *R. Apple Blossom*               | 0.52                       | 24.9               |
| *R. Rush*                        | 0.53                       | 34.7               |
| *R. Margo Koster*                | 0.57                       | 27.8               |
| *R. Ghislaine de Feligonde*      | 0.71                       | 55.6               |
| *R. Seagull*                     | 0.72                       | 38.1               |
| *R. Miss Edith Cavell*           | 0.75                       | 31.9               |
| *R. Kew Rambler*                 | 0.85                       | 38.9               |
| *R. Rambling Rector*             | 0.86                       | 40.4               |
| *R. Nathalie Nypels*             | 0.90                       | 35.1               |
| *R. Cameo*                       | 0.94                       | 45.9               |
| *R. Nakashima 91*                | 1.00                       | 47.8               |
| *R. Leonie Lamesch*              | 1.01                       | 62.4               |
| *R. Russelliana*                 | 1.08                       | 68.9               |
| *R. Coral Clustar*               | 1.19                       | 69.7               |
| *R. Blush Rambler*               | 1.41                       | 52.5               |
| *R. Claire Jacqueir*             | 1.45                       | 44.6               |
| *R. Goldfinch*                   | 1.52                       | 76.4               |

a The calculation methods were described by Li et al. (2007).
b Infection rate (%) = (Total number of plants with disease symptoms over 1)/(Total number of plants) × 100.
determined the resistance ability of cocoyam clone. Li (2006) reported that *Rosa multiflora* ‘Matsushima No. 3’ showed great resistance ability against *P. helicoides* (Table 2). Zhuang et al. (2012) further observed that the phenolic substances extracted from *R. ‘Matsushima No. 3’* inhibited the germ tube elongation of *P. helicoides in vitro* (Figure 1). Disease symptom of resistant and susceptible cultivars are showed in Figure 2.

![Figure 1: Tolerant effects of bound phenolic substances from *R. multiflora* ‘Matsushima No. 3’ and *R. ‘Nakashima 91’* on germ tube elongation. (modified from Zhuang et al., 2012)](image)

- x) Values with different superscripts are significant at p=0.05 by Fisher’s LSD test (n=3).
- y) Bound phenolic substances (original liquid) were detected in root extracts of 5 g (fresh weight) root mL−1.
- z) 5 μL extracted phenolic substances (or 99% ethyl alcohol) was added at 4 corners in a petri dish which a 5 mm *P. helicoides* B-5 inoculum was placed in the middle, and the germ tube elongation area was measured every 12 h.

![Figure 2: Roots of *R. multiflora* ‘Matsushima No. 3’ and *R. ‘Nakashima 91’* one week after inoculation with *P. helicoides* and noninoculation.](image)
Inducing the plant defense with exogenous stimulation is a hot topic during these decades. Many materials can be used to induce plant defense response. For instance, silicon can induce resistance in cucumber against \textit{P. ultimum} by forming electron-dense layers along primary and secondary cell walls, and pit membranes of xylem vessels, as well as significantly increased the percentage of cells filled with phenolic-like material (Chérif et al., 1992). On the other hand, pre-treated with salicylic acid increased the activity of peroxidases and protease inhibitors, resulting in cell death reduction after inoculation with \textit{P. aphanidermatum} (Radhakrishnan and Balasubramanian, 2009).

A classic example of using host plant defense is grafting, which is widely used in the world to manage soil-borne disease. By grafting onto cucurbit hybrid rootstock ‘Titan’ and ‘Hercules’, no symptoms occurred on cucumber, and they also increased the vegetative growth and fruit product and quality comparing with self-grafted cucumber (Al-Mawaali et al., 2012).

5. Conclusion

\textit{Pythium} spp. cause serious economic loss around the world. As understanding of \textit{Pythium} become wider and deeper, more feasible disease management strategies have been provided. Until now, the most widely used strategy is chemical fungicides, because it is easy to use and efficient. Except for fungicides, controlling water content, adjusting temperature and using compost can bring benefits to \textit{Pythium} management, however, these methods can only delay the development of the disease instead of eliminating the pathogen due to destroy optimal growth conditions of \textit{Pythium}. As biocontrol is emerging issues in these decades, many biocontrol agents have been discovered and researched. Recently, the plant growth-promoting rhizobacteria attract more attention compared to antagonistic microorganisms. So far, it seems that the biocontrol in the lab is a great success. Unfortunately, compared to the efficiency in the lab, the field test is another story. There are still some problems remaining like keeping the population of the biocontrol agents, and the potential risks to other plants and microorganisms. Plant response to \textit{Pythium} has been intensively studied these years. Many important signal pathways in the plant had been proved to involve disease resistance. However, even though tolerant individuals were found among many plants, the complete resistant has not been reported. Despite disease management, Wheeler et al. (2016) reported that linear relationship was found between average pod rot in the field and the sampling units with pod rot at low disease incidences in peanut plantation, and they believed this research will help farmers to manage the field more scientifically. There is no doubt that controlling \textit{Pythium} effectively will require more than one method. As the research further develops, more tolerant plant will be found, and more effective biocontrol agents and less harmful fungicide will be discovered.

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REFERENCES

Adie BAT, Perez-Perez J, Perez-Perez MM, Godoy M, Sanchez-Serrano JJ, Schmelz EA and Solano R (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in \textit{Arabidopsis}. Plant Cell, 19(5): 1665–1681.

Adiobo A, Oumar O, Perneel M, Zok S and Hôtele M (2007) Variation of \textit{Pythium}-induced cocoyam root rot severity in response to soil type. Soil Biol. Biochem., 39(11): 2915–2925.

Al-Mawaali QS, Al-Sadi AM, Khan AJ, Al-Hasani HD and Deadman ML (2012) Response of cucurbit rootstocks to \textit{Pythium aphanidermatum}. Crop Prot., 42: 64–68.
Benhamou N and Brodeur J (2001) Pre-inoculation of Ri T-DNA transformed cucumber roots with the mycoparasite, *Verticillium lecanii*, induces host defense reactions against *Pythium ultimum* infection. Physiol. Mol. Plant Pathol., 58(3): 133–146.

Castro A, Vidal S and Ponce de León I (2016) Moss pathogenesis-related-10 protein enhances resistance to *Pythium irregulare* in *Physcomitrella patens* and *Arabidopsis thaliana*. Front. Plant Sci., 7: 1–17.

Chamsrang C and Cook RJ (1985) Identification and comparative pathogenicity of *Pythium* species from wheat roots and wheat-field soils in the Pacific Northwest. Phytopathology, 75: 821–827.

Chatterton S, Sutton JC and Boland GI (2004) Timing *Pseudomonas chlororaphis* applications to control *Pythium aphanidermatum*, *Pythium dissotocum*, and root rot in hydroponic peppers. Biol. Control, 30(2): 360–373.

Chellemi DO, Mitchell DJ, Kannwischer-Mitchell ME, Rayside PA and Roskoskopf EN (2000) *Pythium* spp. associated with bell pepper production in Florida. Plant Dis., 84(12): 1271–1274.

Chen C, Bélanger RR, Benhamou N and Paulitz TC (1999) Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots. Eur. J. Plant Pathol., 105(5): 477–486.

Chen C, Bélanger RR, Benhamou N and Paulitz TC (2000) Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiol. Mol. Plant Pathol., 56(1): 13–23.

Chen HY, Huh JH, Yu YC, Ho LH, Chen LQ, Tholl D, Frommer WB and Guo WJ (2015) The Arabidopsis vacular sugar transporter SWEET2 limits carbon sequestration from roots and restricts *Pythium* infection. Plant J., 83(6): 1046–1058.

Chérif M, Benhamou N, Menzies JG and Bélanger RR (1992) Silicon induced resistance in cucumber plants against *Pythium ultimum*. Physiol. Mol. Plant Pathol., 41(6): 411–425.

Chérif M, Tirilly Y and Bélanger RR (1997) Effect of oxygen concentration on plant growth, lipidperoxidation, and receptivity of tomato roots to *Pythium F* under hydroponic conditions. Eur. J. Plant Pathol., 103(3): 255–264.

Cliquet S and Scheffer RJ (1996) Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* using *Trichoderma* spp. applied as industrial film coatings on seeds. Eur. J. Plant Pathol., 102(3): 247–255.

De Vleesschauwer D, Van Buyten E, Satoh K, Mauleon R, Choi IR, Vera-Cruz C, Kikuchi S and Höfte M (2012) Brassinosteroids antagonize gibberellin- and salicylate-mediated root immunity in rice. Plant Physiol., 158(4): 1833–1846.

Deng Z, Harbaugh BK, Kelly RO, Seijo T and McGovern RJ (2005) *Pythium* root rot resistance in commercial caladium cultivars. HortScience, 40(3): 549–552.

F (1997) Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. Microbiol., 143(12): 3921–3931.

Elmer W, Gent M and McAvoy R (2012) Partial saturation under ebb and flow irrigation suppresses *Pythium* root rot of ornamentals. Crop Prot., 33: 29–33.

El-Tarabily KA, Hardy GESJ, Sivasithamparam K, Hussein AM and Kurtböke DI (1997) The potential for the biological control of cavity-spot disease of carrots, caused by *Pythium coloratum*, by streptomycete and non-streptomycete actinomycetes. New Phytol., 137(3): 495–507.

El-Tarabily KA, Nasser A, Hardy GESJ and Sivasithamparam K (2009) Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. J. Appl. Microbiol., 106: 13–26.

Fatouros G, Gkizi D, Fragkogeorgi GA, Paplomatas EJ and Tjamos SE (2018) Biological control of *Pythium, Rhizoctonia* and *Sclerotinia* in lettuce: association of the plant protective activity of the bacterium *Paenibacillus alvei* K165 with the induction of systemic resistance. Plant Pathol., 67: 418–425.

Feng W, Hieno A, Kusunoki M, Suga H and Kageyama K (2019) LAMP detection of four plant-pathogenic oomycetes and its application in lettuce fields. Plant Dis., 103(2): 298–307.

Fortnum BA, Rideout J, Martin SB and Gooden D (2000) Nutrient solution temperature affects *Pythium* root rot of tobacco in greenhouse float systems. Plant Dis., 84(3): 289–294.

Garren KH (1971) Persistence of *Pythium myriotylum* in soils. Phytopathology, 61: 596–597.

Gent MNP and Elmer WH (2017) Partial saturation ebb and flow irrigation and silicon to suppress *Pythium* root rot of poinsettia. Crop Prot., 101: 95–102.

Gent MNP and McAvoy RJ (2011) Water and nutrient uptake and use efficiency with partial saturation Ebb and flow watering. HortScience, 46(5): 791–798.

Gent MNP, Elmer W and McAvoy R (2011) Rapid watering to achieve partial saturation of root medium on flooded floors. Acta Hortic., 893: 1065–1072.
Gravel V, Martinez C, Antoun H and Tweddel RJ (2005) Antagonist microorganisms with the ability to control Pythium damping-off of tomato seeds in rockwool. BioControl, 50(5): 771–786.

Goldberg NP, Stanghellini ME and Rasmussen SL (1992) Filtration as a method for controlling Pythium root rot of hydroponically grown cucumbers. Plant Dis., 76(8): 777–779.

Higginbotham RW, Paulitz TC, Campbell KG and Kidwell KK (2004) Evaluation of adapted wheat cultivars for tolerance to Pythium root rot. Plant Dis., 88(9): 1027–1032.

Hoppe PE (1966) Pythium species still viable after 12 years in air-dry muck soil. Phytopathology, 56: 1411.

Huzar-Novakowski J and Dorrance AE (2018) Genetic diversity and population structure of Pythium irregulare from soybean and corn production fields in Ohio. Plant Dis., 102(10): 1989–2000.

Ichitani T and Goto H (1982) Distribution of Pythium zingiberum causing rhizome rot in ginger-growing and its surrounding uncultivated soils. Jpn. J. Phytopathol., 48(5): 674–676.

Ishimoto H, Fukushi Y and Tahara S (2004) Non-pathogenic Fusarium strains protect the seedlings of Lepidium sativum from Pythium ultimum. Soil Biol. Biochem., 36(3): 409–414.

Johnstone M, Chatterton S, Sutton JC and Grodzinski B (2005) Net carbon gain and growth of bell peppers, Capsicum annuum “Cubico”, following root infection by Pythium aphanidermatum. Phytopathology, 95(4): 354–361.

Kageyama K, Aoyagi T, Sunouchi R and Fukui H. (2002) Root rot of miniature roses caused by Pythium helicoides. J. Gen. Plant Pathol., 68(1): 15–20.

Kerkeni A, Daami-Remadi M, Tarchoun N and Khedher MB (2007) In vitro and in vivo suppression of Pythium ultimum the causal agent of the cucumber damping-off by some compost fungi. Asian J. Agric. Res., 1: 50–58.

Kipngeno P, Losenge T, Maina N, Kahangi E and Juma P (2015) Efficacy of Bacillus subtilis and Trichoderma asperellum against Pythium aphanidermatum in tomatoes. Biol. Control, 90: 92–95.

Lang JM, Rebits B, Newman SE and Tisserat N (2008) Monitoring mortality of Pythium zoospores in chlorinated water using oxidation reduction potential. Plant Heal. Prog., 9(1): 9.

Le DP, Smith MK and Aiiken EAB (2016) An assessment of Pythium spp. associated with soft rot disease of ginger (Zingiber officinale) in Queensland, Australia. Australas. Plant Pathol., 45(4): 377–387.

Li L, Kageyama K, Kinoshita N, Yu W and Fukui H (2007) Development of bioassay for screening of resistant roses against root rot disease caused by Pythium helicoides drechsler. J. Jpn. Soc. Hortic. Sci., 76(1): 79–84.

Li L. (2006) Mechanism of resistance to Pythium helicoides in roses. Gifu University, Ph.D. Thesis.

Lin F, Wani SH, Collins PJ, Wen Z, Gu C, Chilvers MI and Wang D (2018) Mapping quantitative trait loci for tolerance to Pythium irregulare in soybean (Glycine max L.). G3, 8(10): 3155–3161.

Lumsden RD and Locke JC (1989) Biological control of damping-off caused by Pythium ultimum and Rhizoctonia solani with Gliocladium virens in soilless mix. Phytopathology, 79(3): 361–366.

Lookabaugh EC, Ivors KL and Shew BB (2015) Mefenoxam sensitivity, aggressiveness, and identification of Pythium species causing root rot on floriculture crops in North Carolina. Plant Dis., 99(11): 1550–1558.

Margni M, Rossier D, Crettaz P and Jolliet O (2002) Life cycle impact assessment of pesticides on human health and ecosystems. Agric. Ecosystems. Environ., 93(1-3): 379–392.

Matthiesen RL, Ahmad AA and Robertson AE (2015) Temperature affects aggressiveness and fungicide sensitivity of four Pythium spp. that cause soybean and corn damping off in Iowa. Plant Dis., 100(3): 583–591.

Mavrodi OV, Walter N, Elateek S, Taylor CG and Okubara PA (2012) Suppression of Rhizoctonia and Pythium root rot of wheat by new strains of Pseudomonas. Biol. Control, 62(2): 93–102.

Misk A and Franco C (2011) Biocontrol of chickpea root rot using endophytic actinobacteria. BioControl, 56(5): 811–822.

Mohsen ME, Tatsuya H, Masafumi S and Mitsuro H (2015) Suppressive effects of a polymer sodium silicate solution on powdery mildew and root rot diseases of miniature rose. Afr. J. Biotechnol., 8(10): 959–966.

Múnera JDC and Hausbeck MK (2015) Integrating host resistance and plant protectants to manage Pythium root rot on geranium and snapdragon. HortScience, 50(9): 1319–1326.

Murakami R, Yajima Y, Kida K-ichi, Tokura K, Tojo M and Hoshino T (2015) Surviving freezing in plant tissues by oomycetous snow molds. Cryobiology, 70(2): 208–210.

Nair RA and Thomas G (2013) Molecular characterization of ZzR1 resistance gene from Zingiber zerumbet with potential for imparting Pythium aphanidermatum resistance in ginger. Gene, 516(1): 58–65.
Naseby DC, Pascual JA and Lynch JM (2000) Effect of biocontrol strains of Trichoderma on plant growth, Pythium ultimum populations, soil microbial communities and soil enzyme activities. J. Appl. Microbiol., 88: 161–169.

Nelson EB (1988) Biological control of Pythium seed rot and preemergence damping-off of cotton with Enterobacter cloacae and Erwinia herbicola applied as seed treatments. Plant Dis., 72(2): 140–142.

Nouri-Ellouz O, Gargouri-Bouzid R, Sihachak D, Triki MA, Ducreux G, Drira N and Lakhous L (2006) Production of potato interspecific somatic hybrids with improved tolerance to PVY and Pythium aphanidermatum. J. Plant Physiol., 163(12): 1321–1332.

Nzungize JR, Lyumugabe F, Busogoro JP and Baudoin JP (2012) Pythium root rot of common bean: biology and control methods. A review. Biotechnol. Agron. Soc. Environ., 16(3): 405–413.

Okubara PA, Dickman MB and Blechl AE (2014) Molecular and genetic aspects of controlling the soilborne necrotrophic pathogens Rhizoctonia and Pythium. Plant Sci., 228: 61–70.

Postma J, Stevens LH, Wiegers GL, Davelaar E and Nijhuis EH (2009) Biological control of Pythium aphanidermatum in cucumber with a combined application of Lysobacter enzymogenes strain 3.1T8 and chitosan. Biol. Control, 48(3): 301–309.

Postma J, Willemsen-De Klein MJ and Van Elsaas JD (2000) Effect of the indigenous microflora on the development of root and crown rot caused by Pythium aphanidermatum in cucumber grown on rockwool. Phytopathology, 90(2): 125–133.

Radhakrishnan N, Alphonsje AJ and Balasubramanian R (2011) Effect of Acibenzolar-S-methyl (ASM) pre-treatment in inducing resistance against Pythium aphanidermatum infection in Curcuma longa. Crop Prot., 30(1): 24–32.

Radhakrishnan N and Balasubramanian R (2009) Salicylic acid induced defence responses in Curcuma longa (L.) against Pythium aphanidermatum infection. Crop Prot., 28(11): 974–979.

Rai M, Ingle AP, Paralikar P, Anasane N, Gade R and Ingle P (2018) Effective management of soft rot of ginger caused by Pythium spp. and Fusarium spp.: emerging role of nanotechnology. Appl. Microbiol. Biotechnol., 102(16): 6827–6839.

Samejima N and Ichitani T (1988) Behaviors of Pythium in a recirculating hydroponic system. Jpn. J. Phytopathol., 54(1): 15–29.

Sánchez-Vallet A, López G, Ramos B, Delgado-Cerezo M, Riviere MP, Llorente F, Fernández PV, Miedes E, Estevez JM, Grant M and Molina A (2012) Disruption of abscisic acid signaling constitutively activates Arabidopsis resistance to the necrotrophic fungus Plectosphaerella cucumerina. Plant Physiol., 160(4): 2109–2124.

Senda M, Kageyama K, Suga H and Lévesque CA (2009) Two new species of Pythium, P. senticosum and P. takayamanum, isolated from cool-temperate forest soil in Japan. Mycologia, 101(4): 439–448.

Stanghellini ME and Hancock JG (1971) The sporangium of Pythium as a survival structure in soil. Phytopathology, 61: 157–164.

Stanghellini ME and Kronland WC (1986) Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder rootlets by Pythium dissotocum. Plant Dis., 70(11): 1053–1056.

Stanghellini ME, Rasmussen SL, Kim DH and Rorabaugh PA (1996) Efficacy of nonionic surfactants in the control of Pythium blight in overseeded turfgrasses using a simple field inoculation technique. Plant Heal. Prog., 6(1): 13.

Stiles CM, Datnoff LE and Cisar JL (2005) Evaluation of fungicides for control of Pythium blight in overseeded turfgrasses using a simple field inoculation technique. Plant Heal. Prog., 6(1): 13.

Sutton JC, Sophier CR, Owen-Going TN, Liu W, Grodzinski B, Hall JC and Benchimol RL (2006) Etiology and epidemiology of Pythium root rot in hydroponic crops: current knowledge and perspectives. Summa Phytopathol., 32(4): 307–321.

Suwanarach N, Kumla J, Matsui K and Lumyong S (2015) Characterization and efficacy of Muscodor cinnamomi in promoting plant growth and controlling Rhizoctonia root rot in tomatoes. Biol. Control, 90: 25–33.

Švecová E, Proietti S, Caruso C, Colla G, Crinò P, Gogliettino M, Cocca E, Consiglio C, Bubici G and Nabti E (2018) Plant growth promoting and inducible antifungal activities of irrigation well water-bacteria. Biol. Control, 117: 78–86.

Taylor RJ, Salas B, Secor GA, Rivera V and Gudmestad NC (2002) Sensitivity of North American isolates of Phytophthora erythroseptica and Pythium ultimum to mefenoxam (Metalaxyl). Plant Dis., 86(7): 797–802.

Temgo TJC and Boyomo O (2002) Variations in the phenolic contents of cocoyam clones in correlation to resistance to Pythium myriotylum. Biol. Plant., 45(3): 433–436.
Toda T, Iwasa A, Fuji S and Furuya H (2015) Widespread occurrence of *Pythium arrhenomanes* pathogenic to rice seedlings around Japanese rice fields. Plant Dis., 99(12): 1823–1831.

Uzuhashi S, Okada G and Okhuma M (2015) Four new *Pythium* species from aquatic environments in Japan. Antonie van Leeuwenhoek, 107(2): 375–391.

Van Os GI and Van Ginkel JH (2001) Suppression of *Pythium* root rot in bulbous iris in relation to biomass and activity of the soil microflora. Soil Biol. Biochem., 33(11): 1447–1454.

Weerakoon DMN, Reardon CL, Paulitz TC, Izzo AD and Mazzola M (2012) Long-term suppression of *Pythium abappressorium* induced by *Brassica juncea* seed meal amendment is biologically mediated. Soil Biol. Biochem., 51: 44–52.

Wheeler TA, Russell SA, Anderson MG, Serrato-Diaz LM, French-Monar RD and Woodward JE (2016) Management of peanut pod rot I: Disease dynamics and sampling. Crop Prot., 79: 135–142.

Wheeler WD, Williams-Woodward J, Thomas PA, van Iersel M and Chappell MR (2017) Impact of substrate volumetric water on *Pythium aphanidermatum* infection in *Petunia ×hybrida*: A case study on the use of automated irrigation in phytopathology studies. Plant Heal. Prog., 18(2): 120–125.

Yu D, Sinkkonen A, Hui N, Kurola JM, Kukkonen S, Parikka P, Vestberg M and Romantschuk M (2015) Molecular profile of microbiota of Finnish commercial compost suppressive against *Pythium* disease on cucumber plants. Appl. Soil Ecol., 92: 47–53.

Zhang W and Tu JC (2000) Effect of ultraviolet disinfection of hydroponic solutions on *Pythium* root rot and non-target bacteria. Eur. J. Plant Pathol., 106(5): 415–421.

Zhao Y, Qian G, Chen Y, Du L and Liu F (2017) Transcriptional and antagonistic responses of biocontrol strain *Lysobacter enzymogenes* OH11 to the plant pathogenic oomycete *Pythium aphanidermatum*. Front. Microbiol., 8: 1025.

Zhao ZH, Kusakari SI, Okada K, Miyaizaki A and Osaka T (2000) Control of *Pythium* root rot on hydroponically grown cucumbers with silver-coated cloth. Biosci. Biotechnol. Biochem., 64(7): 1515–1518.

Zhu Y, Shao J, Zhou Z and Davis RE (2019) Genotype-specific suppression of multiple defense pathways in apple root during infection by *Pythium ultimum*. Hortic. Res., 6(1): 1–17.

Zhuang D, Li L, Tatematsu T, Nagaoka F, Nakano K, Kageyama K and Fukui H (2012) Relationship between tolerance against root rot disease and phenolic substances in root of *Rosa multiflora*. Hort. Res., 11(2): 153–158. (In Japanese with English abstract)