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Inhibition of the spread of *Sclerotinia sclerotiorum* in aquaponics

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SUMMARY

Sclerotinia sclerotiorum, which causes white mold, is a widespread pathogen. In 2020, a new host plant of this fungus, the watercress (Nasturtium officinale) was identified in Hungary in an aquaponic system. During the cultivation of watercress S. sclerotiorum was detected on the plant, the fungus caused a 30% yield loss. Fungicides should not be used against fungi in aquaponic systems. Non-chemical methods of integrated pest management should be used. These include biological control (resistant species, predators, pathogens, antagonist microorganisms), manipulation of physical barriers, traps, and the physical environment. In the aquaponic system, the removal of the growing medium (expanded clay aggregate pellets) solved the damage of Sclerotinia sclerotiorum 100%. By removing the expanded clay aggregate pellets, the environmental conditions became unfavorable for the development and further spread of the S. sclerotium fungus.

Keywords: *watercress; Sclerotinia sclerotiorum; aquaponic system; expanded clay aggregate pellets*

INTRODUCTION

Watercress (*Nasturtium officinale* R. Br.) belongs to *Brassicaceae* (also called *Cruciferae*) family (Giallourou et al., 2016) which is a perennial aquatic plant growing in fresh floating water. Watercress appertains to genus *Nasturtium,* and it is apparently native to much of European and Asian countries, stretching from the British Isles probably as far as western China (Gonçalves et al., 2009). The species is native in the Northern hemisphere, but due to cultivation, it spread worldwide. Generally, it is sold in the fresh form and consumed as a vegetable in salads, soups, and other recipes (Blüthner, 2020). The leaves of this plant are also widely used as an aromatic food and medicinal plant as a depurative, diuretic, expectorant, hypoglycaemic, odontalgic, stimulant, and stomachic, and also for treating hypertension and cardiovascular diseases (Bown, 1995; Hamzeh, 2012; Zargari, 1987). In addition, it can be used for water phytoremediation (Kara et al., 1999; Kara, 2002).

Watercress production is endangered by several diseases and pests (*Spongospora, Cercospora, Xanthomonas, Fasciola, Lemna, Plutella*) (Blüthner, 2020). There is a fungus on the watercress characterized by whitish mycelium with spheroid or elongated, dark sclerotia, identical to *Sclerotinia sclerotiorum* (Lib.) de Bary (Mordue et al., 1976). *S. sclerotiorum* is a necrotrophic fungal pathogen causing disease in a wide range of plants. This fungus is capable of colonizing over 600 plant species found worldwide (Ibrahim et al., 2021). Most of these species are dicotyledonous, although several agriculturally significant monocotyledonous plants are also hosts (Bolton et al., 2006; Downey and Rimmer, 1993). An index of plants reported to be susceptible to this fungus was compiled from the scientific literature. The index contains 42 subspecies or varieties, 408 species, 278 genera, and 75 families (Boland and Hall, 1994). This pathogen has already been reported on watercress in Italy (Garibaldi et al., 2019). The white mold fungus was described by Csüllög et al. (2022) on watercress in an aquaponic system in Hungary.

Watercress is an excellent choice for aquaponic systems because it is relatively easy to grow (Smith, 2007). The growing food insecurity, uncontrollable rise in food prices, water scarcity, and poverty, especially in developing countries, coupled with concerns for climatic patterns, have resulted in a significant global challenge (Bulya et al., 2020; Gosh and Chowdhury, 2019; Lennard and Goddek, 2019). Aquaponics, the combined culture of fish and plants in recirculating systems, has become increasingly popular (Rakocy et al., 2006). Hence, aquaponic system will expose agriculturists to venture into new technology to enhance the production and growth of plants (Palm et al., 2014). Fungicides should not be used to control fungus on aquaponic plant production. Nonchemical methods of integrated pest management must be used. These include biological control (resistant cultivars, predators, pathogens, antagonistic organisms), physical barriers, traps, and manipulation of the physical environment. There are more opportunities to use biological control methods in enclosed greenhouse environments than in exterior installations (Mori and Smith, 2019; Rakocy et al., 2006; Rivas-Garcia, 2020).

MATERIALS AND METHODS

Fungicides should not be used to control fungus on aquaponic systems. The aim of our experiment was to determine the role of the medium (expanded clay aggregate pellets) in infection.

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We set up our experiment started on the 24th of November in 2020 in the Fish Biology Laboratory of the University of Debrecen, in a double sheet plastic tunnel greenhouse under shade cloth. We used eight classical recirculation aquaponic systems. Four aquaponics was designed with expanded clay aggregate pellets as a growing medium. The other four systems were made without a media bed. Instead of the clay pebbles, in the units without a media bed, we used a mole net stretched to a frame and submerged in water to support the roots of the plants. The area of the plant part of the units was 0.41 m^2. At the beginning of the experiment, we planted four carp (*Cyprinus carpio* L.) with an average weight of 150 grams, whose waste products and fodder residues ensured a continuous supply of nutrients to the plants in the aquaponic systems. The optimal amount of feed was determined at 2 % day^{-1} of total biomass. We used commercially available feed with 45% protein and 20% fat content, in 2 mm particle size. We planted 30 watercress plants into every unit. In each case, 15 plants were infected and placed on one side of all eight units. 15 control plants (without any infections) were placed on the other side of all eight units.

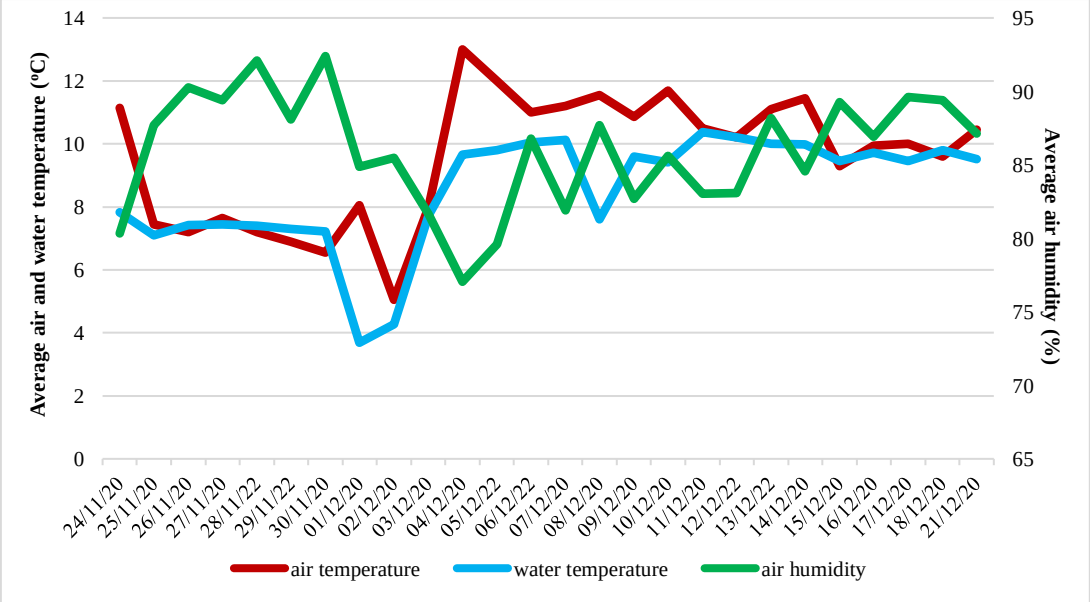
We used for artificial inoculation a *Sclerotinia sclerotiorum* strain (Genbank number MW959042.1). This fungus was detected first in the aquaponic system on the watercress in 2020. During the primary surveys, we found the white mycelium of a *Sclerotinia* species, and a few days later we also observed its sclerotia. Subsequently, we used molecular biology methods for identification of this species. PCR amplification was performed with primers ITS1/ITS4 for the internal transcribed spacer region. Specific polymerase chain reaction was performed with specific primers SSasprF/SSasprR. Based on the molecular biology identification we detected *Sclerotinia sclerotiorum* fungus on watercress. After that we made pure culture of this fungus as follows: mycelia from infected plants were placed on potato dextrose agar (PDA) and incubated under dark conditions at 25 °C for seven days. 5 mm diameter PDA discs containing 7-day-old *Sclerotinia sclerotiorum* fungus were placed on the leaf axils of 20 cm watercress plants with 2 healthy nodules.

During the experiment we measured different environmental parameters, such as the air temperature (°C), air humidity (%) (PCE-THB 40 Humidity/Baro/Temp. DATA RECORDER), water temperature (°C), dissolved oxigen (DO) (mg l^{-1}) (HACH HQ30d), pH values (HANNA Combo pH&ORP Waterproof) and Electrical Conductivity (EC) (µS) (Adwa AD 332 EC/TDS Meter).

RESULTS AND DISCUSSION

In this study, we examined for four weeks the following parameters: the air temperature (°C), the air humidity (%), the water temperature (°C), the dissolved oxigen (DO) (mg l^{-1}), the pH values and electrical conductivity (EC) (µS). *Figure 1* showed the measured average air temperature (°C), the air humidity (%) and the water temperature (°C) values in the tent. The temperature of the air and water changed to a similar extent, the rise and fall of water temperature followed the change of the air data.

Figure 1: **Average air temperature (°C), air humidity (%) and water temperature (°C) values in the tent**



During the first week, the air temperature decreased from 11 °C to 6.9 °C. It increased to 11 °C at the end of the second week. The air temperature values were around 10–11 °C in the third week. During the fourth week, we measured slightly lower values around 9–10 °C. In the first week, the water temperature showed

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around 7 ºC values. At the beginning of the second week, a dynamic decrease was observed then the values increased to 7–8 ºC again. In the third week, higher values were detected than in the first and second weeks. The water temperature showed similar values between 9 ºC and 10 ºC in the last two week.

The average air humidity fluctuated more at the beginning of the experiment than at the end. These values ranged from 77.05% to 92.40% over the entire four weeks. We detected between 80% and 90% air humidity values since the third week.

The dissolved oxygen (DO) (mg l^{-1}) values increased from 10.43 to 12.60. The pH values ranged from the acidic (6.7) to the alkaline range (9.7). The electrical conductivity (EC) (µS) values changed between 614.00 and 737.75. During the experiment, these parameters did not show significant differences.

We investigated the spread of *Sclerotinia sclerotiorum* in the different growing medium. We planted 30 watercress plants (20 cm height) with 2 healthy nodules into every unit. In every case, 15 plants were infected and placed on one side of all eight units. 15 control plants (without any infections) were placed on the other side of all eight units. *Table 1* showed the *Sclerotinia sclerotiorum* spread in the aquaponic system with expanded clay aggregate pellets or mole net.

Table 1: ***Sclerotinia sclerotiorum*** **spread in the aquaponic system with expanded clay aggregate pellets or mole net**

Expanded clay aggregate pellets				**Mole net**			
the name of the repetition number	number of plants number with visual symptoms from 15 infested plants	number of plants with visual symptoms from 15 control plants	number of all plants with visual symptoms from 30 plants	the name of the repetition number	plants number with visual symptoms from 15 infested plants	plants number with visual symptoms from 15 control plants	number of all plants with visual symptoms from 30 plants
I_{ecap}	11	6	17	I_{mn}	11	0	11
II_{ecap}	14	8	22	II_{mn}	10	0	10
III_{ecap}	13	5	18	III_{mn}	13	0	13
IV_{ecap}	12	8	21	IV_{mn}	12	0	12

I_{ecap}=first repetition number of expanded clay aggregate pellets

I_{mn}= first repetition number of mole net

In I_{ecap} we detected 11 plants with white mold symptoms from 15 infested plants. In II_{ecap} 14 plants, in III_{ecap} 13 plants, and in IV_{ecap} 12 plants showed visual symptoms from 15 infested plants. After four weeks in I_{ecap} six control plants, in II_{ecap} eight control plants, in III_{ecap} five control plants, and in IV_{ecap} eight control plants showed white mold symptoms.

In I_{mn} we detected 11 plants with *Sclerotinia sclerotiorum* fungus from 15 infested plants. In II_{mn} ten plants, in III_{mn} 13 plants, and in IV_{mn} 12 plants showed visual symptoms from 15 infested plants. Due to the lack of the expanded clay aggregate pellets the control plants did not show visual symptoms.

During the data analysis, we used Pearson correlation test to prove the growing medium's role in the spread of the infection. We found extremely closed relationship between all infected plants number and the growing medium (r=0.933) on 1% significant level. There is another significant (P=0.01) relationship with the growing medium, the control infections number (r= 0.971), which proved that the expanded clay aggregate pellets, are helps the infection of the *Sclerotinia sclerotiorum* (*Table 2*).

There is a 1% level significant correlation between the control infected and all infected groups (r=0.951), which means the spread of the infection (*Table 3).*

Table 2: **Correlational relationships between the growing medium and the spread of infection**

	growing medium	**infected**	**control infected**	**all infected**
growing medium	1	0.408	0.971**	0.933**

**. Correlation is significant at the 0.01 level.

Table 3: **Correlational relationships with the number of all infected plants**

	growing medium	**infected**	**control infected**	**all infected**
all infected	0.933**	0.615	0.951*	1

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CONCLUSIONS

In this study, we examined the effect of the growing medium on the spread of the *Sclerotinia sclerotiorum* pathogen fungus. We hypothesized that the pathogen propagation strategy was highly dependent on the presence of the growing medium, so we removed it. We set up the experiment for the cooler winter months because this fungus prefers cool, rainy, wet, and humid conditions. We used classical recirculation aquaponic system in four repetitions, which were designed with expanded clay aggregate pellets and without a media bed. We planted 30 watercress plants into every unit. In each case, 15 plants were infested, and 15 control plants were not infested. The spread of the pathogen was monitored for 4 weeks. We measured the air temperature, the water temperature, and the air humidity. The environmental conditions were excellent for the adhesion, infection, and rapid spread of the pathogen. In the high humidity environment, the pathogen was able to spread and infect extremely quickly the control plants through the expanded clay aggregate pellets. However, in the medium-free aquaponics (only used mole net) the pathogen had not possible growing medium of transmission, so the control plants were not infected in this case. The presence of the growing medium greatly contributes to the rapid spread of the *S. sclerotiorum*. By removing the expanded clay aggregate pellets, the environmental conditions became unfavorable for the development and further spread of the fungus.

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