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Efficacy of electrolytically-derived disinfectant against dispersal of *Fusarium oxysporum* and *Rhizoctonia solani* in hydroponic tomatoes

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ABSTRACT

Demand for conservation and recycling of water has increased significantly. Therefore irrigation water used for horticultural or agricultural purposes needs to be treated before being reused to eradicate plant pathogens and thereby reducing the risk of pathogen dispersal and losses due to disease. The economically important fungal plant pathogens *Fusarium oxysporum* (Synder and Hans) and *Rhizoctonia solani* (Kühn) were selected to examine the efficacy of nutrient solution treatment by electrolytic disinfection to prevent the dispersal of these pathogens in the hydroponic production of tomatoes (*Solanum lycopersicum* Mill.).

First, we determined the efficacy of the disinfectant to inactivate *F. oxysporum* and *R. solani in vitro*. The electrolytically generated potassium hypochlorite (KClO) was tested at five concentrations of free chlorine (0.2, 0.5, 0.8, 1.0, 2.0 mg/L) in nutrient solutions of pH 5.5, 6.0 and 6.5 with four contact times (5, 30, 60, 120 min). Best sanitation was achieved in nutrient solution at pH 6.0. *In vitro, F. oxysporum* required 2 mg/L at 30 min for complete inactivation whereas chlorination had only a minimal effect on viability of *R. solani.* Subsequent trials under practical conditions applied the disinfectant *via* a new sensor-based disinfection procedure. Potassium hypochlorite solution produced on site and injected into a recirculating nutrient solution once a week for 60 min at a free chlorine concentration of 0.5 mg/L (ORP 780 mV) inhibited the dispersal of *F. oxysporum* and *R. solani* during the entire test period of 16 weeks. In contrast all tomato test plants irrigated with untreated nutrient solution became infected with *F. oxysporum* and a third of them additionally with *R. solani*. At the applied dose no plant damage occurred. Thus, the treatment proved to be effective and applicable to prevent dispersal of fungal pathogens by nutrient solution under simulated field conditions.

1. Introduction

Tomato (*Solanum lycopersicum* Mill.) is considered one of the most economically important vegetable crops in the world. Production is currently around 130 million tons, of which 88 million are destined for the fresh market and 42 million are processed (Anonymous, 2016). In the European Union, the tomato also holds the number one position among vegetables, with 16.6 million tons, representing 12% of global production. Tomatoes are characterized by a high water requirement. Particularly in arid and semi-arid areas irrigation is required for both the quantity and quality of tomato production. Several sources of water can be used for irrigation purposes: municipal water, groundwater, water collected from roofs and paved surfaces, run-off water and surface water from ponds, lakes, streams and rivers. Some of these sources pose a high risk of disseminating plant pathogens. Whereas groundwater and municipal water tend not to harbor plant pathogens, run-off water collected by channels and stored in ponds or tanks poses a high risk for dispersal of plant pathogens (Moorman et al., 2014). Pathogens may be introduced to run-off water directly from crops in cultivated fields or natural vegetation surrounding the fields. Numerous species of zoosporic organisms, fungi, bacteria and viruses have been found in surface water and recirculating nutrient solution (Hong and Moorman, 2005; Hong et al., 2014; Mehle and Ravnikar, 2012). The latter greatly facilitates the spread of waterborne pathogens within crops, since pathogens washed or leached from the crop can accumulate in the holding tank and be delivered back to the crop repeatedly with each irrigation cycle.

Due to this potential risk, physical and chemical disinfection methods are used in greenhouse facilities to minimise the occurrence and spread of plant pathogens (Stewart-Wade, 2011). Recently

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Raudales et al. (2014) summerised established water treatments to control plant pathogens including the mode of action of each technology. Currently the grower can choose from physical treatments such as filtration, heat, and ultraviolet (UV) radiation or chemical water treatments such as bromine, chlorine, chlorine dioxide, ozone, hydrogen peroxide, and ionized copper or silver.

Chlorine has been used successfully in the disinfection of public water, seeds and in post-harvest processes (Van Haute et al., 2015). To treat irrigation water sodium hypochlorite or calcium hypochlorite are commonly used as they are easy to apply, relatively persistent and inexpensive (Fisher et al., 2014). When hypochlorite is introduced to water it reacts to form free chlorine species of hypochlorous acid (HOCl) and hypochlorite (OCl^-) ions, which oxidise organic materials including any pathogens present in the water (Zheng et al., 2016). This chemical compound destroys pathogens through penetration of the cell wall, damaging proteins and membranes and disrupting metabolic processes (Fisher et al., 2014). HOCl predominates at a solution of pH below 7.5 and is a much stronger sanitizer than hypochlorite which predominates at a pH greater than 7.5 (De Hayr et al., 1994). The contact time and dose required for the chlorination of irrigation water to eradicate plant pathogens varies with species and life stage (Cayanan et al., 2009; Hong and Richardson, 2004; Scarlett et al., 2016). Furthermore the effectiveness of chlorine is influenced by water-soluble fertilizer and organic matter (OM) as it is readily oxidised by OM, and it reacts with the nitrogen to form chloramines (Hong et al., 2003). Chloramines are more stable but have a lower biocidal effect with only 4% the disinfection efficacy of hypochlorous acid (White, 2010).

Electrolysed oxidised water has drawn significant attention in the food industry as a non-thermal method of sanitation and microbial inactivation (Rahman et al., 2016), and is a promising technology for the treatment of irrigation water (Elmer et al., 2014). It is a solution with disinfecting properties which is generated by passing a dilute salt solution (commonly sodium or potassium chloride) through an electrolytic cell. This application constitutes a sustainable and green method, which has several advantages compared to other sanitation techniques including cost effectiveness, ease of application, on-the-spot production, human safety and protection of the environment. It should be emphasized that neither transport nor storage of hazardous substances is required and the disinfecting effect can be adjusted according to the particular on-site chlorine demand. The efficacy of a sensor-based disinfection with electrolytically-derived potassium hypochlorite to inhibit the dispersal of plant viruses in tomato crops was recently determined for *Pepino mosaic virus* (Bandte et al., 2016).

The present study was undertaken to evaluate the efficacy of the potassium hypochlorite treatment of nutrient solution in eliminating two common undesirable fungal plant pathogens in tomato production, *Fusarium oxysporum* f. sp. *lycopersici* (Synder and Hans) and *Rhizoctonia solani* (Kühn). *F. oxysporum* causes a highly destructive disease leading to extensive crop losses in both field and protected tomatoes, and remains a major limiting factor for tomato production (McGovern, 2015). The fungus is soil-borne and causes vascular wilt by infecting plants through the roots and spreading internally through the cortex to the vascular tissue. It can survive in the form of mycelium and chlamydospores in substrate and plant debris for longer periods of time. This persistence of resting spores combined with the limited range of effective fungicides complicates disease management. *R. solani* also ranks among the most important soil-borne fungal pathogens (Cao et al. 2004). It is a species complex composed of a diverse assemblage of soil fungi that vary with respect to host specificity and morphology (Bartz et al., 2010). These fungi can cause diseases in more than 500 genera of plants. Typical symptoms such as seedling damping-off, root necrosis, basal stem cankers, and fruit rot, result from the colonization of plant tissues by fungal hyphae or sclerotia present in the substate (Jones et al., 2014).

To evaluate the efficacy of controlling fungal pathogens and the suitability of the sensor-based disinfection system we conducted extensive investigations. *In vitro* studies were carried out to ascertain dose-effect relations of the disinfectant. *In vivo* studies were conducted to elucidate the efficacy and suitability under simulated field conditions

2. Material and methods

2.1. Plant pathogens and inoculum

Conidia and mycelial fragments of *F. oxysporum f.* sp. *lycopersicum* (DSM-62059, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were prepared by growing the culture on “Synthetic Low Nutrient Agar” (SNA, Nirenberg, 1976) at 22 °C, under an 8 h light: dark regime, for 10 days. Plates were flooded with high-purity water and scraped with a sterile rod to detach spores. The resulting suspension was added to the liquid medium and incubated at 30 °C for 10 days, shaking at 120 rpm. To remove large mycelial fragments the spore suspension was filtered through two layers of cheesecloth. *R. solani* (PM-5, collection of the Division Phytomedicine, Humboldt-Universität zu Berlin, Berlin, Germany) was cultured on “Potato Dextrose Agar” (PDA) at 22 °C under an 8 h light:dark regime for 12 days. The inoculum was prepared by placing colonised PDA plugs (5 × 5 mm) in 100 ml of Potato dextrose broth (PDB) and incubated at 30 °C for 12 days in the dark on an orbital shaker. Mycelium was harvested and homogenized in a blender (Clatronic, model SM2452) for 30 s with high-purity water.

Suspensions of all fungal propagules were quantified using a hemocytometer and diluted with sterile deionised water to obtain 10^6 propagules/mL prior to testing the chlorine treatment. The viability of these propagules was checked on PDA and determined in colony forming units (CFU)/ml; the average value over all tests was 91%.

2.2. Potassium hypochlorite solution

Hypochlorite was produced on-site in a single chamber brine electrolysis plant (nt-BlueBox mini; newtec Umwelttechnik GmbH; Berlin, Germany) as described by Schuch et al. (2016). A direct current of 10 A with a voltage of 13 V was applied with titanium electrodes to a brine solution containing potassium chloride (KCl) and fresh water leading to the formation of chlorine (Cl_2). In turn Cl_2 was disproportionated to hypochlorous acid (HClO) and Cl^- in the presence of hydroxyl ions (OH-) in an aqueous solution. The potassium hypochlorite (KClO) solution produced by the device contained 36.6 mg of free chlorine/L. The content of free chlorine in this electrolytically generated stock solution as well as in the working solutions was checked manually using a handheld apparatus (Pocket Colorimeter II, Hach Lange GmbH, Germany). Following the manufactors instructions the measurement was carried out at 528 nm in a volume of 10 mL with a photometric precision of ± 0.0015 Abs.

2.3. In vitro experiments

The antifungal efficacy of the potassium hypochlorite solution was tested *in vitro* on *F. oxysporum* and *R. solani.* It was tested in nutrient solution at three different pH values representinga horticulturally-relevant range: 5.5, 6.0, and 6.5.

The nutrient solution consisted of tap water and a stock solution of macronutrients (calcium nitrate 1.7 mmol/l, magnesium sulfate 2.6 mmol/l, potassium nitrate 3.3 mmol/l, monopotassium phosphate 0.4 mmol/l, ammonium nitrate 0.4 mmol/l and 10 mg/l Fe EDTA 13%) and micronutrients according to Göhler and Molitor (2002). The pH value was adjusted to 6.0, the electrical conductivity (EC) value to 1.8. Testing covered a range of different concentrations (0, 0.2, 0.5, 0.8, 1.0 and 2.0 mg free chlorine/L displayed by the electrolytic processed potassium hypochlorite) and contact times (5, 30, 60, and 120 min). These doses can be expected not to cause plant damage in practical use. Contact times were achieved by using 0.01 M sodium thiosulfate