Preliminary Results on Fungicidal Efficacy of Microwave Treatment on *Fusarium oxysporum*

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Abstract

Fusarium oxysporum is a saprophyte parasite that can infect many crop species inducing severe yield loss in agricultural production. For this reason, its control in the soils is a very significant topic and great efforts from the scientific community are focused on this goal. The aim of this work was to study the die-off of *Fusarium oxysporum* after microwave heating. A set of experiments have been carried out on a saline solution mimicking soils conductivity properties and considering different fungus concentrations. The results obtained show a great efficacy of the treatment, with a die-off greater than 90% already at 70°C temperature.

1. Introduction

World food production should increase by 50 % in the next decades to meet the growing demand for food [1]. To achieve this objective main world crops require protection from pests. It has been estimated that in the years 2001-2003 plant pathogens were responsible for a global crop yield loss varying between 14% and 35% depending on the different geographical areas of the world [2]. *Fusarium oxysporum* is the most common fungal specie among communities in various soil types worldwide [3]. *Fusarium oxysporum* additionally results an autochthonous constituent of fungal biota in the rhizosphere of plants [4]. All strains of *Fusarium oxysporum* are saprophytic and capable of living for long periods on soil organic matter [5]. However, some strains are considered pathogenic to various plant species. Their mechanism of action consists in the penetration in the roots and the invasion of the vascular system, causing loss of turgor in plants. The consequence of such pathogenicity is reflected in damage of varying magnitude and economic importance for many crop species [6]. Starting from the early 1960s protection of crop species from pathogens has been realized by an increasing use of pesticides and chemical fertilization. The massive use of chemical products in agriculture is threatening Earth's ecosystems with serious environmental damages. It is urgent to implement a reversal of the management of agricultural practices towards forms of sustainable agriculture that do not irreversibly damage the earth's natural resources and safeguard agroecosystems.
Microwave exposure is one of the most promising eco-compatible techniques for soil and plants disinfection. Different works in literature have already demonstrated the potential of microwave heating treatments [7, 8, 9, 10]. The aim of this work was to evaluate the fungicidal efficacy of microwave treatment on *Fusarium oxysporum isolate HSGF01* in saline solution matrix (NaCl 0.1 M) mimicking a soil [11]. This matrix was artificially spiked with known concentrations of the target microorganism. The samples have been exposed in a rectangular waveguide applicator to reach different temperature levels (60, 70, 80, 90° C). After exposure the heating effect has been evaluated considering the fungus die-off. Moreover, the results have been validated with a biosensor. This paper is divided into four sections. In the second section materials and methods will be introduced. In the third section the results will be presented and finally in the fifth section conclusions of are reported.

2. Materials and Methods

2.1 Biological samples

The fungus was grown in laboratory on *Potato Dextrose Agar* (PDA) at 22°C for 5 days. Colonies were picked up from the PDA plates and diluted in the saline solution. A spectrophotometer was used to measure the absorbance at 590 nm to determine accurate concentration of cells/mL. A value of 1.125 OD was observed corresponding to a concentration of 10^7 cells/mL. Then appropriate serial dilutions were disposed to obtain 10^6 cells/mL and 10^5 cells/mL concentrations. Aliquots of 15.0 mL inocula were prepared and subsequently exposed to microwave-treatments. Aliquots not exposed were considered as controls. All analyses were performed in triplicate.

2.2 Exposure system

The exposure system is a well consolidated setup at 2.45 GHz allowing the treatment of samples in controlled dosimetric and environmental conditions [12, 13, 14]. It is shown in Fig.1. The samples, positioned in a cylindrical plastic container (38 mm diameter, 20 mm height) without a cover, were introduced in a customized microwave applicator fed by a magnetron (Alter TMA20) and were exposed to the minimum incident power density of 8.1 W/cm^2 available by the magnetron source (300 W). The incident and reflected powers from the sample were remotely monitored during the exposure. The applicator is a rectangular waveguide (WG, WR340, 8.6 cm × 4.3 cm) connected to a dummy load. A cylindrical waveguide (diameter of 35 mm, 100-mm long, centered with respect to the wider side of the WG and located 7 cm from the flange), allows temperature measurements through an infrared thermal camera (FLIR camera E60) with negligeable electromagnetic field perturbation [12, 15]. The temperature distribution on the surface of the samples were recorded during the exposure. Different time exposures were employed depending on the target temperature. Samples not exposed and kept at ambient temperature (21°C) were used as controls.

Figure 1: Exposure system setup.

2.3 Experimental protocol

Tests were conducted on two different concentrations of *Fusarium oxysporum isolate HSGF01* in saline solution 0.1 M: 10^6 CFU/mL and 10^5 CFU/mL. The two fungal inocula were exposed in the microwave applicator to reach 4 different temperature levels T1-60°C, T2-70°C, T3-80°C, T4-90°C. The output power was set for all the experiments at 300 W and the generator was turned off when the sample reached the target temperature.
Treatments were followed by microbiological analyses to assess the die-off of *Fusarium oxysporum isolate HSGF01*. Serial ten-fold dilutions were prepared for saline solution spiked with the fungus and controls. Then 100 µl of each sample was spread plated on Rose Bengal Agar (Thermo Fisher Scientific, USA). Petri dishes were incubated at 22 ± 1 °C for 96 hours. Typical colonies were counted and statistical analysis allowed residual concentration of *Fusarium oxysporum* after treatments.
An additional analysis has been carried out using a biosensor employing electrochemical impedance. The sensor has been optimized and developed to measure rapidly the soil concentration of *Fusarium oxysporium* directly in the field. Moreover, it is integrable into an automated system capable of conveying the soil extract directly onto the sensor chip, enabling periodic measurements even in the absence of an operator.

3. Results

Lab-scale liquid tests showed the high die-off efficacy of the treatments on *Fusarium oxysporum.* In figures 2-3 results, respectively for the 10^6 and 10^5 fungus concentration, are reported in terms of percentage of die-off rate.

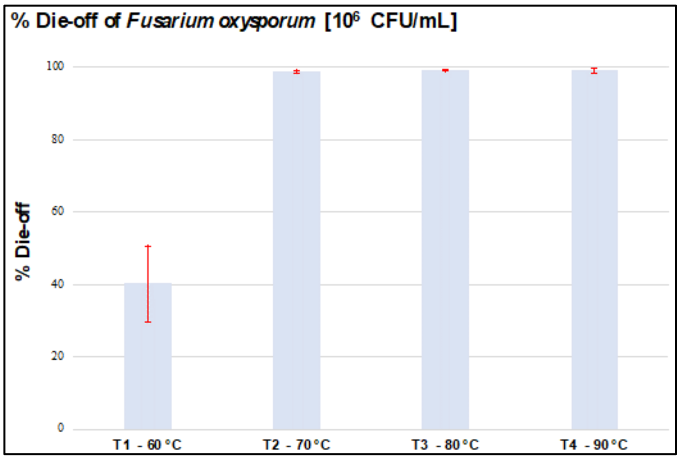

Figure 2. *Fusarium oxysporum* [10^6 CFU/mL] concentration die-off means (in blue) and standard error (in red) after microwave exposure. Analyses were performed in triplicate.

Data indicate that temperatures at 70° C allowed over 90% die-off rates. At both conditions of 80°C and 90°C die-off rates about 99% were obtained. Moreover, apart from the temperature T1, the experiments have a good repeatability: especially at 90° C the standard error is less than 0.65 for both concentrations. In the Fig. 2 and Fig. 3 the average values and standard errors of triplicate experiments are shown.

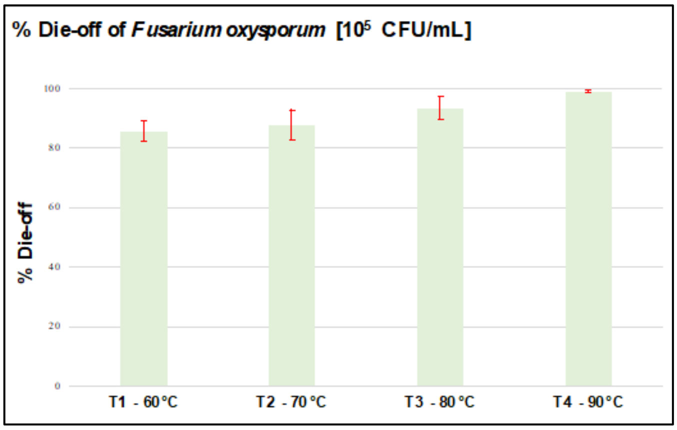

Figure 3. *Fusarium oxysporum* [10^5 CFU/mL] concentration die-off means (in green) and standard error (in red) after microwave exposure. Analyses were performed in triplicate.

Moreover, preliminary results using biosensor confirm the reliability and robustness of the experiments, as comparable outcomes were achieved to plate growth (data non shown).

4. Conclusion

In this work an experimental study on *Fusarium oxysporum isolate HSGF01* suppression through microwave heating has been presented. The fungus has been inoculated with two different concentrations in a saline solution that mimics soils properties. Samples have been exposed in a rectangular waveguide applicator to reach four different temperature levels. Results have been validated both with microbiological analysis and a biosensor. The die-off percentages obtained are very promising and a good agreement was found with previous works [8, 9]. Future assays will be addressed to inoculate *Fusarium oxysporum* in agricultural soils samples and the same experiment procedure here presented will be applied. This work will be particularly useful for on-site evaluation of microwave treatments in the real environmental conditions in which also the biosensor can be tested.

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