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Culture Medium Based on Tomato Leaves for Abundant Production of Conidia from *Septoria lycopersici*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: This study aimed to perform further experiments about the massive production of spores in alternative culture medium based on tomato leaves with different recipes to foment artificial inoculation for scientific purposes.
Study Design: The experiments were performed in completely randomized designed with three replicates.
Place and Duration of Study: The study was conducted on the Caçador experimental station of the Agricultural Research and Rural Extension Enterprise of Santa Catarina (EPAGRI) from May to November 2018.
Methodology: The study tests 13 amounts of tomato leaves to choose the concentration which better improve the conidia production within two weeks of incubation at 25ºC and photoperiod of 12 h. After the incubation period, the number of spores was counted in a Neubauer chamber. The

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statistical analysis were performed with Scott-Knot (P<.05). Once the more appropriate quantity of leaves was determined, the conidia production on different media was compared using PDA, King B, NDA, tomato extract, PMA and tomato leaf extract medium incubated at the same conditions as previously described. The amount of conidia produced in tomato leaf extract medium was then compared with those produced in PDA (considered a standard medium for fungus cultivation). After the incubation period, the number of spores was counted in a Neubauer chamber. The statistical analysis was performed with Tukey test (P < .05). Experiments were performed in a completely randomized design with three replicates.

Results: Results from analysis of variance indicated that quantities superior to 13 g per 100 mL rises the amount of conidia produced within two weeks from incubation at 25ºC and photoperiod of 12 h. In addition, all culture media tested supported the mycelial growth, but the production of cirrus was only evident when tomato leaves were used as the main component of the culture medium. Comparatively with the standard medium PDA, the tomato leaf extract medium is more appropriate for experiments dealing with *Septoria lycopersici* sporulation.

Conclusion: The culture medium based on fresh tomato leaves can foment a large production of cirrus of *Septoria lycopersici*, reason why it better than the standard medium PDA for research based on septoria leaf spot.

Keywords: Spores; fungus; septoria leaf spot; inoculum production; cirrus.

1. INTRODUCTION

Septoria leaf spot is one of the most common foliar disease of tomato plants caused by the fungus *Septoria lycopersici*. Infection can occur at any stage of plant development, but appears more frequently at the beginning of the warm and rainy season. It begin as small, water-soaked and rounded spots that gradually develop a grayish white center with dark borders [1]. Fruiting bodies appears as tiny black specks under favorable conditions. When in favorable conditions and without control measures this disease can cause extreme defoliation and necrosis over the stem [2].

When in an appropriate conditions of humidity and substrate the pycnidia may ooze spores out forming a cirrus (mass of spores). The cirrus seems like a gel composed of proteinaceous and saccharide compounds [3,4], which the primary roles of cirrus components are protection of pycnidiospores from dessication and prevention of premature germination [4,5]. The presence of cirrus can indicated a large production of spores *in vitro* and in tomato crops.

The mass production of conidia can be a challenge in scientific works that intend to use artificial inoculation of pathogens. The fungus *Septoria lycopersici* is easily isolated in a standard culture medium known as PDA (Potato – Dextrose – Agar) [6,7,8]. However, the production of conidia is low in this culture medium after two weeks of incubation, despite the abundant and vigorous mycelial growth. A different recipe using the boil for 30 minutes of 20 g of tomato leaves per 100 mL H_2O (the unique amount tested) was employed for *S. lycopersici* sporulation. After 13 days of incubation at 24 ± 5ºC, the sporulation was classified as good with 15-24 conidia per lower microscopic field, but the observation of cirrus was not mentioned [9]. Thus, the objective of this work was perform further experiments about the methodology for the abundant production of conidia of *Septoria lycopersici* in order to provide a source of artificial inoculum for experiments with tomato crops. This study aimed to improve the production of spores in tomato leaves based culture medium.

2. MATERIALS AND METHODS

2.1 Mycelial Growth and Sporulation in Culture Medium with Different Amounts of Tomato Leaves

The culture medium was composed of 1 g, 3 g, 5 g, 7 g, 9 g, 11 g, 13 g, 14 g, 29 g, 57 g, 71 g, 86 g and 100 g of fresh tomato leaves and agar 2 g for 100 ml distilled H_2O. The leaves were processed in a blender with 80 mL H_2O, and subsequent sieved to remove the leaves, and then the volume was filled to 100 mL. After pouring into the Petri dishes, a disc of culture medium containing mycelium (9 mm) was inserted into the center of each plate. The Petri dishes were incubated for 14 days at 25°C and photoperiod of 12 h. The experiment was performed in a completely randomized design

with three replicates. After the incubation period, the number of spores was counted in a Neubauer chamber. The statistical analysis was performed with Scott-knot test ($P < .05$).

2.2 Mycelial Growth and Sporulation Comparison Using Different Culture Media

For the production of cirrus by *Septoria lycopersici* the following culture media were used: commercial PDA produced by *Himedia laboratories* (39 g per 1 L); King B (Glycerin 15 mL, peptone 20 g, $MgSO_4(H_2O)$ 3 g, K_2HPO_4 2 g, agar 16 g per 1 L); NDA (Peptone 5 g, meat extract 3 g, dextrose 10 g, agar 20g per L); tomato extract medium (tomatoes 2 kg, agar 20 g and dextrose 20 g per L), PMA (Potato – Malte – Agar, 43 g per L) and tomato leaves extract medium (fresh tomato leaves 13 g and agar 2 g per 100 mL distilled H_2O). The experiment was performed in a completely randomized design with three replicates. The analysis of the mycelial growth and sporulation were done qualitatively by the observation of the mycelial growth and the production of cirrus, respectively.

2.3 Sporulation Comparison in PDA and Tomato Leaf Extract Media

For PDA were used 39 g per 1 L of the commercial product and for tomato leaf extract medium were used 13 g of fresh tomato leaves and 2 g agar per 100 mL distilled H_2O. After pouring into the Petri dishes, a disc of culture medium containing mycelium (9 mm) was inserted into the center of each plate. The Petri dishes were incubated for 27 days at 25 °C and photoperiod of 12 h. The experiment was performed in a completely randomized design with three replicates. After the incubation period, the number of spores was counted in a Neubauer chamber. The statistical analysis was performed with Tukey test ($P < .05$).

3. RESULTS AND DISCUSSION

3.1 Mycelial Growth and Sporulation in Culture Medium with Different Amounts of Tomato Leaves

All concentration supported the mycelial growth, but cirrus was only observed when the amount of fresh leaves was above to 7 g / 100 mL. Concentration of fresh leaves higher than 13 g per 100 mL H_2O was more efficient as a substrate to foment the production of spores (Fig. 1).

Tomato leaves as the unique source of nutrition are rich in carbohydrates such as sucrose [10], and macronutrients (N, P, K, Ca, Mg, S) and micronutrients (Fe, Zn, Cu, Mn, B) [11], the leaves also has various trace elements [12], that might be involved in the sporulation process of *Septoria lycopersici*.

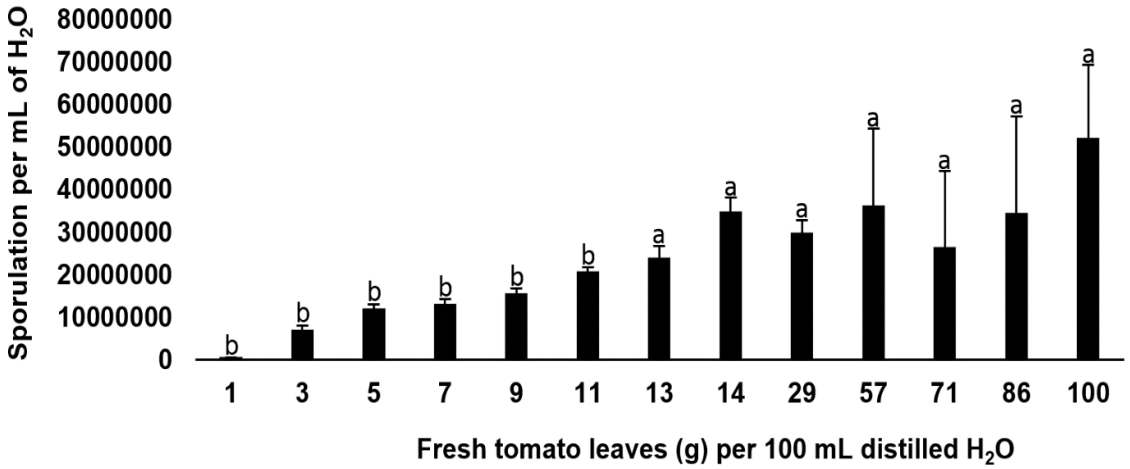

Fig. 1. Sporulation of *Septoria lycopersici* in culture medium containing different concentrations of tomato leaves

Statistical analysis: Scott-knot test, P < .05

Black bars = Mean values with standard deviation of means of three replicates

3.2 Sporulation Comparison Using Different Culture Media

All culture media supported the mycelial growth of *Septoria lycopersici*, but cirrus of spores was only observed in the tomato leaves extract medium (Fig. 2 and Fig. 3). Besides the appropriated humidity, the nutrient source seems to be crucial to the abundant production of cirrus.

In the course of time, many attempts were performed to find the appropriate culture medium for sporulation of *Septoria*. Many pathogenic species compose this gender, which one with specific requirements in nutrition for sporulation. For *S. tritici* the best solid media for conidia production was YMDA, a medium based on yeast extract, malt extract, dextrose and agar [13]. For *S. glycines* the Fries medium composed by $[(NH_4)_2\ C_4H_4O_6$ 5 g, NH_4NO_3 1g, KH_2PO_4 1g, $MgSO_4.7H_2O$ 0.5 g, $CaCl_2$ 0.13 g, NaCl 0.1 g, yeast extract 1 g, sucrose 30 g, agar 15 g and H_2O 1L was considered the best [14]. For *S. carvi* a medium based on malt with decoction of the leaves or schizocarps of caraway was considered one of the best media to raise the number of pycnidia and conidia formed [15]. The use of tomato leaves as source of nutrient takes advantage of a waste that is usually discarded in tomato crop. In fact, a medium based on tomato leaves are already commercialized by U$ 1,623.59 each 50 g by Sigma-Aldrich, but without instruction of use [16].

The mycelial growth on PDA is not flatted and seems to deform the culture medium. In comparision with the standard medium PDA, the culture medium based on fresh tomato leaves foment the production of cirrus, which was not observed on PDA (Fig. 4). The production of spores was statistically different in the media tested. The amount of spores produced in fresh tomato leaves medium was $1.06x10^5 \pm 2,32x10^4$, while in PDA was $1,36x10^3 \pm 1.11x10^2$. The longer the incubation time, the greater will be the production of conidia while there is space in the Petri dish (Fig. 4).

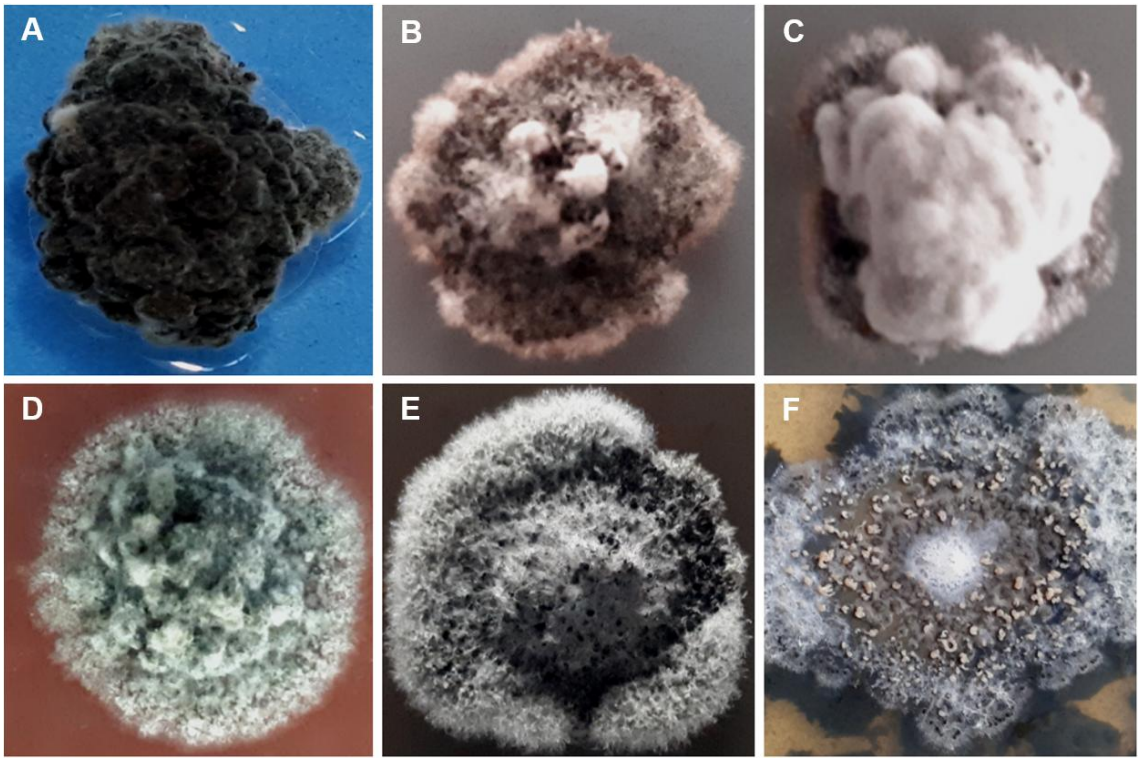

Fig. 2. Cirrus formation by *Septoria lycopersici* in different culture media. Mycelial growth and production of cirrus in PDA (A), King B (B), NDA (C), tomato extract medium (D), PMA (E) and tomato leaf extract medium (F)

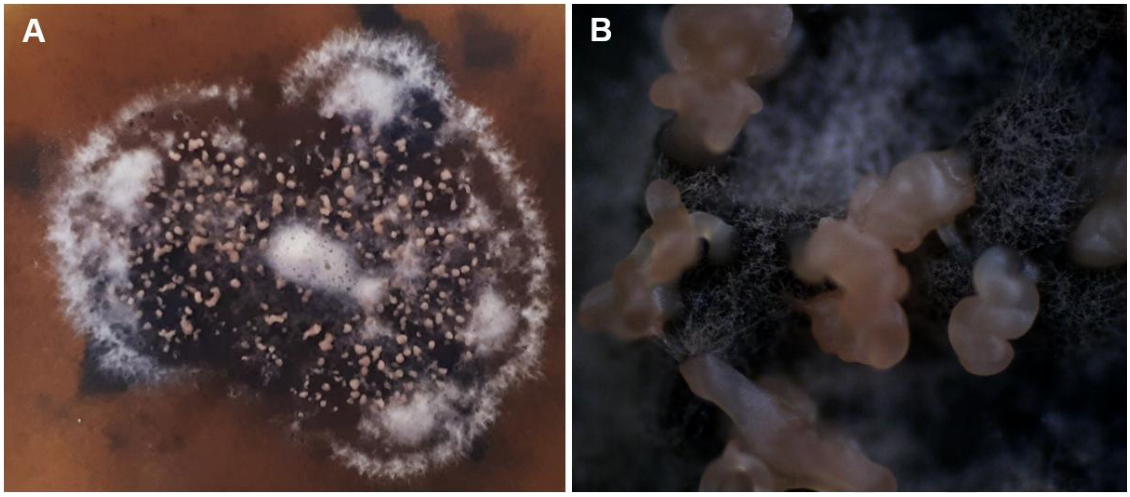

Fig. 3. Mass production of cirrus in culture medium based on fresh tomato leaves (13 g/100 mL)

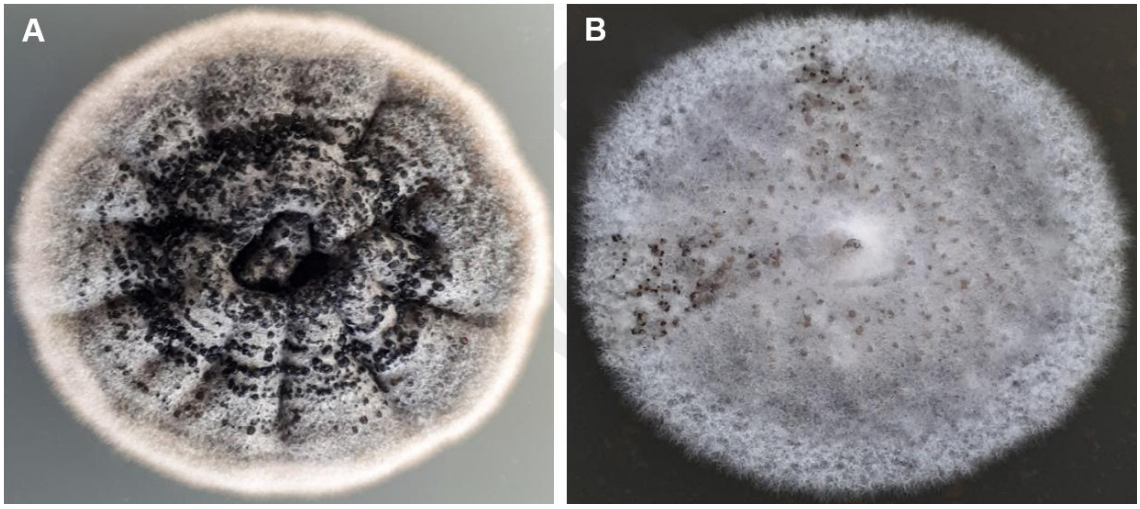

Fig. 4. Comparision of spore production in PDA (A) and fresh tomato leaves (B)

4. CONCLUSION

The culture medium based on fresh tomato leaves can foment a large production of cirrus of *Septoria lycopersici*, reason why it is better than the standard medium PDA for research based on septoria leaf spot of tomato crops.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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