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SURVIVAL MECHANISMS OF *Fusarium oxysporum* f. sp. *passiflorae* ARE AFFECTED BY APPLICATION OF CABBAGE AND CASSAVA DEBRIS[1]

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ABSTRACT - Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *passiflorae* (Fop) is the main fungal disease in passion fruit crops. Chlamydospores, which are structures of resistance produced by Fop, allow the fungus survival in the soil for several years and have saprophytic activity. Biofumigation with incorporation of cabbage and bitter cassava has been a viable alternative, among management methods, for the control of soil pathogens. The objective of this work was to evaluate the effect of different plant debris (plant residues) on survival of Fop under laboratory conditions. In vitro tests were carried out with incorporation of leaves of yellow passion fruit, cabbage, bitter cassava, and sweet cassava plants into substrates infested with different Fop isolates. Mycelial growth and chlamydospore production and germination were evaluated. The incorporation of cabbage and bitter cassava debris had a fungistatic effect on Fop, with decreases in mycelial growth and chlamydospore production. The incorporation of cabbage into the substrate totally inhibited the chlamydospore germination in 78% of the evaluated isolates and decreased the germination percentage in the others.

Keywords: Passion fruit plant. Chlamydospores. Plant materials. Saprophytism.

MECANISMOS DE SOBREVIVÊNCIA DE *Fusarium oxysporum* f. sp. *passiflorae* SÃO AFETADOS POR APLICAÇÃO DE *DEBRIS* DE REPOLHO E MANDIOCA

RESUMO - A murcha de Fusarium causada pelo fungo *Fusarium oxysporum* f. sp. *passiflorae* (Fop) é a principal doença de origem fúngica da cultura do maracujazeiro. Os clamidósporos, estruturas de resistência produzidas por Fop, permitem a sua sobrevivência no solo por vários anos, além de possuir atividade saprofítica. Dentre os métodos de manejo, a biofumigação por incorporação de repolho e mandioca brava tem-se apresentado como uma alternativa viável no controle de patógenos veiculados pelo solo. O objetivo desse trabalho foi avaliar a influência de diferentes debris (restos vegetais) na fase de sobrevivência de Fop em condições de laboratório. Foram realizados testes *in vitro* com incorporação de folhas dos materiais vegetais maracujá amarelo, repolho, mandioca brava e mandioca mansa em substratos infestados com diferentes isolados de Fop. Foram avaliados o crescimento micelial e a produção e germinação de clamidósporos. A incorporação dos debris de repolho e mandioca brava teve efeito fungistático sobre Fop, com redução no crescimento micelial e produção de clamidósporos. A incorporação de repolho ao substrato inibiu totalmente a germinação de clamidósporos em 78% dos isolados avaliados e reduziu o percentual de germinação dos demais.

Palavras-chave: Maracujazeiro. Clamidósporos. Materiais vegetais. Saprofitismo.

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INTRODUCTION

Brazil is the main producing country of yellow passion fruit (*Passiflora edulis* Sims), with a production of 690.364 Mg in an area of 46.530 ha (14.86 Mg ha^{-1}) (IBGE, 2020). The Northeast region of the country is responsible for 71.16% of the national production, mainly the state of Bahia, with 197.160 Mg grown in an area of 17.414 ha (IBGE, 2020). Despite yellow passion fruit production in Brazil stands out in the world, several phytosanitary restrictions compromise the passion fruit production in Brazil.

Fusarium wilt is among the main diseases that affect yellow passion fruit crops; it is caused by infection by the fungus *Fusarium oxysporum* f. sp. *passiflorae* Gordon apud Purss (Fop), which is saprophyte and produces structures of resistance that ensure its survival in the soil, under unfavorable conditions, for several years (REIS; CASA, 2004). This disease causes large economic losses, decreasing the useful life of orchards (CARVALHO et al., 2015; PREISIGKE et al., 2015; FREITAS et al., 2016).

The initial infection of passion fruit plants by Fop occurs through the root system and, then, by colonization of the xylem. It is a systemic disease, which obstructs the xylem vessels, preventing the transport of water and nutrients; the main symptom of the disease is wilting, followed by the death of the plant (SILVA et al., 2013; ORTIZ et al., 2014). The conventional management adopted for other diseases, post-infection of the plant host, is inefficient for Fop because it is a soil pathogen that affects the plant vascular system and, thus, must be controlled preventively (RONCATTO et al., 2004; PREISIGKE et al., 2015; LIMA et al., 2017).

Considering the inefficiency of chemical control and absence of commercial materials resistant to Fop, the use of alternative measures is essential to decrease losses caused by Fusarium wilt. Biofumigation with plant residues (debris), which, during the plant material decomposition, releases volatile compounds that are toxic to soil phytopathogens (BLOK et al., 2000; LAZZERI; LEONI; MANICI, 2004) can be a viable and efficient process to decrease the Fop viability.

The plant biofumigation potential varies according to the species used (AMBRÓSIO et al., 2009) and is probably associated to the concentration and toxicity of substances in the plant aerial part (MOTISI et al., 2009). Determining the effects of plant debris on the Fop survival mechanisms is essential to develop an efficient biofumigation for management of Fusarium wilt. Therefore, the objective of the present study was to quantify the effect of plant materials on the Fop saprophytic growth and chlamydospore production and viability.

MATERIAL AND METHODS

Isolates of *F. oxysporum* f. sp. *passiflorae* and plant materials (debris)

The 14 isolates of *F. oxysporum* f. sp. *passiflorae* (Fop) used in the experiment, selected from the biological work collection of the Laboratory of Phytopathology of the Brazilian Agricultural Research Corporation (Embrapa Mandioca e Fruticultura), are shown in Table 1. The selection was carried out from 100% incidence of Fusarium wilt, evaluated by inoculation of Fop isolates in yellow passion fruit seedlings, and from diversification of geographical origin (Table 1).

Table 1. Origin and identification of isolates of *Fusarium oxysporum* f. sp. *passiflorae* evaluated in substrates with different plant materials.

Isolates	Municipality of origin	Host
CMF-03114	Cruz das Almas, BA	*Passiflora edulis* f. *flavicarpa*
CMF-03115	Cruz das Almas, BA	*Passiflora edulis* f. *flavicarpa*
CMF-03116	Cruz das Almas, BA	*Passiflora edulis* f. *flavicarpa*
CMF-03118	Ubaíra, BA	*Passiflora edulis* f. *flavicarpa*
CMF-03124	Porto Seguro, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0318	Paramerim, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0381	Dom Basílio, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0393	Dom Basílio, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0382	Dom Basílio, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0312	Dom Basílio, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0369	Livramento, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0399	Livramento, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0308	Livramento, BA	*Passiflora edulis* f. *flavicarpa*
CMF-0310	Livramento, BA	*Passiflora edulis* f. *flavicarpa*

The suppression of Fop was evaluated using leaves of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener), cabbage (*Brassica oleracea* var. *capitata* L.), bitter cassava, and sweet cassava (*Manihot esculenta* Crantz) as plant source materials.

Saprophytic growth in plant substrates

The plant materials were cut in fragments of approximately 1 cm² and disinfested with 70% alcohol and 1% sodium hypochlorite for one minute in each solution, washed three times in sterilized distilled water, and then dried using sterilized filter paper.

The substrate was prepared using sand (15%), maize flour (80%), plant material (5%), and sterilized distilled water (24 mL) to a final volume of 90 g. After homogenization, the substrate was transferred to glass tubes with capacity for 90 g. Then, a 1.0-cm mycelium disc containing reproduction structures of the pathogen was transferred to one of the ends of the tubes, which were sealed with cotton and laminated paper sheet. The tubes were maintained in a BOD chamber at 25±1 ºC and photoperiod of 12 hours. The mycelial growth was measured with a ruler (mm) that was placed over the tube length, with a stereoscopic microscope, in consecutive days, from the day after inoculation until the mycelial growth covers all the substrate in the tube, following the methodology established by Souza (2016). The control treatment was prepared with sand (15%), maize flour (80%), sterilized distilled water (24 mL), and a 1.0-cm mycelium disc.

Chlamydospore production

The plant material fragments were added to the substrate containing the mixture of sand and sterilized maize flour, for two consecutive times at 120 °C for 20 minutes, with 24-hour intervals. Fifteen 10-mm Fop mycelium discs, 9 mL of sterilized distilled water, and 3 g of disinfested plant material were added to 90 g of the substrate. After sealing, it was incubated in a BOD chamber for 30 days at 25±1 ºC and photoperiod of 12 hours. Then, 9 mL of sterilized distilled water was added to 1 g of the colonized substrate. The suspension was filtered in sieve, and an aliquot was taken for counting chlamydospores in a Neubauer chamber, using the A field and the correction factor 1.0×10^4 chlamydospores mL^{-1}, following the methodology established by Bueno et al. (2007).

Chlamydospore germination

The suspension used to quantify the chlamydospores was standardized for 10^6 chlamydospores mL^{-1} after filtration, and aliquots were transferred to 2-mL microtubes for each time: 0, 1, 3, 6, 12, and 24 hours. The germination was stopped by adding 0.5 µL of blue lactophenol. All germinated and non-germinated chlamydospores were counted in each time, obtaining the germination percentage. Chlamydospores that presented germination tube with length equal to or longer than their diameters were considered germinated.

Statistical analysis

The data of mycelial growth and chlamydospore production were subjected to analysis of variance and the means were grouped by the Scott-Knott test at 1% probability. The data of chlamydospore production were transformed into log (x+1) to meet the assumptions of the analysis of variance. The areas below the mycelial growth curve (ABMGC) and chlamydospore germination curve (ABCGC) were calculated. The statistical analyses were carried out using the Sisvar program (FERREIRA, 2014). A clustering analysis based on dendrograms with heatmaps was used to evaluate differences between the treatments and isolates evaluated for the variables ABMGC, ABCGC, and chlamydospore production. The gplots statistical package of the R program was used.

RESULTS AND DISCUSSION

Effects of plant materials on Fop saprophytic growth

The mycelial growth of all Fop isolates was delayed or inhibited by the treatments with cabbage and bitter cassava. The treatment with cabbage was the most efficient for fully inhibiting of pathogen growth, and its effect can be considered fungistatic (Figure 1). The yellow passion fruit and control treatments showed more pronounced mycelial growth over time (Figure 1). All plant materials tested were different from each other regarding their area below the mycelial growth curve (ABMGC), except for the yellow passion fruit and the control treatments (Figure 1b). The sweet cassava debris resulted in higher ABMGC than the cabbage and bitter cassava debris, showing efficiency for the fungus control (Figure 1b).

Studies showed that the efficiency of using residues of Brassicaceae species for the biofumigation process is connected to the production of glucosinolate compounds (LAZZERI; LEONI; MANICI, 2004). These compounds are hydrolyzed by the action of the myrosinase enzyme and produce isothiocyanate, nitrile, and thiocyanate gases which can decrease or inhibit the action of soil phytopathogens due to their biocidal effect (LAZZERI; LEONI; MANICI, 2004; MORRA; BOREK, 2010, KLEIN; KATAN; GAMLIEL, 2011;

MENG et al., 2018).

However, the quality and quantity of compounds differ between Brassicaceae species (AMBRÓSIO et al., 2009, MENG et al., 2018). The efficiency of using cassava is connected to the release of cyanogenic glycosides, such as linamarin and lotaustralin, which are hydrolyzed by the linamarase enzyme. According to Wong, Ambrosio, and Souza (2011), these substances have fungicide activity. Ambrósio et al. (2008) tested bitter cassava incorporated into the soil and found inactivation of the fungus *F. oxysporum* f. sp. *lycopersici* Race 2 after the eighth day of evaluations, considering bitter cassava as efficient as the Brassicaceae species (*Brassica oleracea* var. *capitata*).

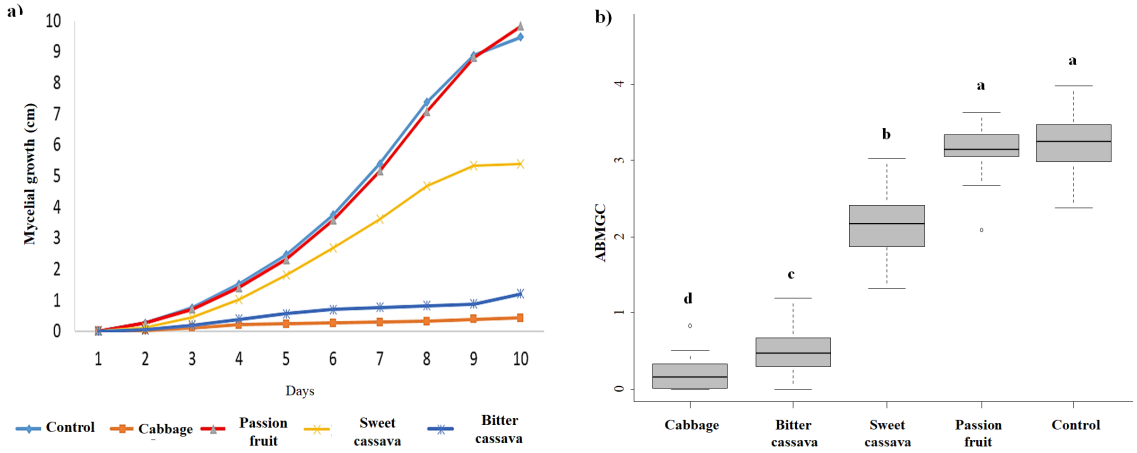

Figure 1. Mean mycelial growth of isolates of *Fusarium oxysporum* f. sp. *passiflorae* as a function of different plant materials (a), and mean area below the mycelial growth curve (ABMGC) of isolates in relation to the plant materials (b).

A clear separation of the effects of treatments on the different Fop isolates is shown in Figure 2, with the formation of 3 different groups. The first group was formed by the cabbage and bitter cassava treatments, which inhibited the mycelial growth of all isolates. The second group was formed with sweet cassava, which presented dependent effect of isolates, and caused an intermediate or late decrease of ABMGC. The third group was formed by the highest ABMGC values of the Fop isolates, which were subjected to the yellow passion fruit and control treatments (Figure 2).

Figure 2. Heatmap for area below the mycelial growth curve (ABMGC) of isolates of *Fusarium oxysporum* f. sp. *passiflorae* subjected to different treatments with plant materials, under laboratory conditions.

Isolates from a same geographical region may present differences in genetic diversity and aggressiveness (SILVA et al., 2013). However, the treatments with cabbage and bitter cassava were efficient for all isolates tested, which denotes that their use in a later biofumigation process can be

efficient, regardless of the crop region or the pathogen populational structure.

Effects of plant materials on resistance spores of *F. oxysporum* f. sp. *passiflora*

The change in the organic composition of the substrate by incorporation of cabbage and bitter cassava resulted in significant decreases in number of viable survival structures of *F. oxysporum* f. sp. *passiflorae* when compared to the control treatment (Figure 3). Regarding the chlamydospore production, the treatment with sweet cassava was grouped with the control and yellow passion fruit treatments, denoting no decreases in Fop chlamydospore production, regardless of the isolate used (Figures 3, 4; Table 2).

The bitter cassava and cabbage treatments were grouped individually, with effect of decreases in the mean of Fop chlamydospores production when compared to the other treatments (Figures 3 and 4). The effect of the cabbage treatment in decreasing the number of chlamydospores was higher than that of the bitter cassava for 28.6% of isolates (CMF0399; CMF03124; CMF0393; CMF0382) (Figure 4, Table 2). Considering the effect of treatments on each isolate, bitter cassava and cabbage decreased the Fop chlamydospore production in 64% and 50% of the isolates, respectively, when compared to the other treatments (Table 2). The decrease in chlamydospore production presented by some isolates when subjected to treatments with cabbage and bitter cassava can be associated with the release of toxic compounds that act on the initial inoculum.

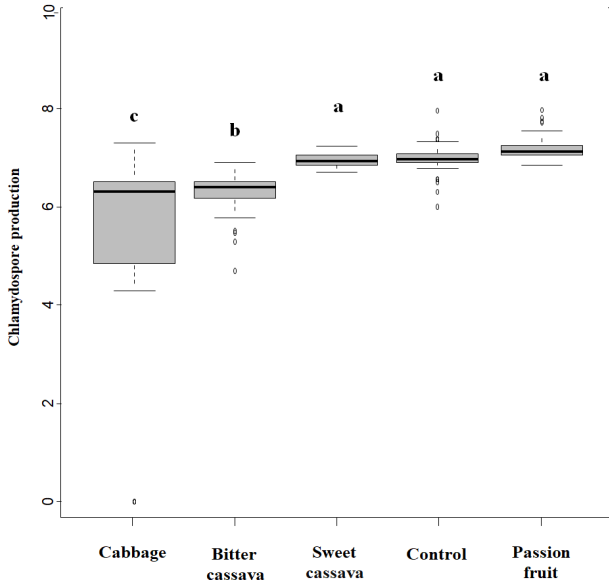

Figure 3. Mean number of chlamydospores produced by isolates of *Fusarium oxysporum* f. sp. *passiflorae* subjected to treatments with different plant materials, under laboratory conditions.

Figure 4. Heatmap for number of chlamydospores produced by isolates of *Fusarium oxysporum* f. sp. *passiflorae* subjected to treatments with different plant materials, under laboratory conditions.

Table 2. Mean number of chlamydospores of isolates of *Fusarium oxysporum* f. sp. *passiflorae* subjected to different plant materials under laboratory conditions.

Isolate	Control	Passion fruit	Bitter cassava	Sweet cassava	Cabbage
CMF-03114	6.6 aA	7.1 aA	6.3 aA	6.9 aA	6.6 cA
CMF-03115	6.5 aA	7.1 aA	6.0 aA	6.8 aA	6.3 cA
CMF-03116	7.3 aB	7.8 aB	6.3 aA	7.2 aB	6.6 cA
CMF-03118	7.1 aB	7.2 aB	6.4 aA	6.8 aB	5.7 cA
CMF-03124	7.0 aB	6.9 aB	6.5 aB	6.9 aB	3.2 aA
CMF-0399	7.0 aC	7.3 aC	6.1 aB	6.9 aC	2.4 aA
CMF-0318	6.9 aA	7.0 aA	6.6 aA	6.9 aA	6.4 cA
CMF-0381	7.3 aA	7.5 aA	6.8 aA	7.1 aA	6.9 cA
CMF-0393	6.9 aB	7.0 aB	6.4 aB	6.9 aB	4.5 bA
CMF-0369	7.0 aB	7.2 aB	6.1 aA	7.0 aB	6.5 cA
CMF-0382	7.4 aC	7.4 aC	6.1 aB	7.1 aC	4.6 bA
CMF-0308	6.7 aA	6.6 aA	6.5 aA	7.1 aA	6.1 cA
CMF-0310	7.3 aB	7.1 aB	6.3 aA	6.9 aB	6.4 cA
CMF-0312	7.0 aB	7.1 aB	5.6 aA	6.8 aB	5.9 cA

Means transformed into log (x +1). Means followed by the same lowercase letters in the columns, or uppercase letters in the rows, belong to the same group by the Scott-Knott test at 1% probability.

Different from the positive effect on decrease in mycelial growth presented by all Fop isolates, the effect of cabbage on chlamydospore production was not general, but dependent, not presenting the same efficiency for all isolates (Figure 4). The Fop geographical distribution (Table 1) was not a determinant factor for the grouping of isolates when considering the chlamydospore production in the different treatments. The isolates CMF0399 and CMF03124, from Livramento-BA and Porto Seguro-BA, were grouped in the same group, whereas the isolates CMF0382 and CMF0312, from Dom Basílio -BA, were grouped into different groups (Figure 4). Fop isolates from a same geographical region may present different results of genetic diversity and aggressiveness (SILVA et al., 2013). The characteristics aggressiveness and virulence of isolates are also correlated with their survival and inoculum production capacities, which are connected to the high variability of Fop isolates (DARIVA et al., 2015; SILVA et al., 2013).

The Fop mycelial inactivation in the organic substrate incorporated with cabbage can be attributed to volatile toxic products. Decreases in population of *Fusarium oxysporum* in soils grown with cucumber, after biofumigation with Brassicaceae species, was attributed to the toxic effect of isothiocyanates released after the hydrolysis of glucosinolate (MENG et al., 2018). Volatile products of biofumigation with Brassicaceae species, such as methyl sulfate and dimethyl disulfate, are directly associated with control of soil pathogens (WANG et al., 2009).

Regarding the chlamydospore production, the pathogen physiology is a factor that should be considered. In normal conditions, *F. oxysporum* f. sp. *passiflorae* presents vegetative activity (mycelial growth) and reproduction with production of spores (microconidia, macroconidia, and chlamydospores). Chlamydospores are essential for the infection (KATAN; SHLEVIN; KATAN, 1997) and dissemination of disease and are structures of resistance produced under environmental stress conditions in the soil to ensure the pathogen survival. In the present experiment, the initial inoculum (15 mycelium/spore discs) placed on the substrate was subjected to stress conditions by incorporation of organic materials with toxic activities; thus, the natural trend of the pathogen physiology was to produce chlamydospores from the initial inoculum in the substrate, decreasing the action of the treatments when compared to the control.

Regarding the chlamydospore germination, the effects of the treatments were similar to those found in the mycelial growth inhibition. The change in the organic substrate composition by incorporation of cabbage and bitter cassava was effective to significantly reduce the chlamydospore germination when compared to the other treatments (Figures 5a and 6). The five treatments were grouped into 3 different groups, according to the ABCGC (Figure 6).

There was no chlamydospore germination for 71% and 78% of the Fop isolates subjected to treatments with bitter cassava and cabbage, respectively (Figure 5b). None of the isolate presented total inhibition of chlamydospore germination when subjected to the sweet cassava, yellow passion fruit, and control treatments (Figure 5b); however, the treatment with sweet cassava

presented lower ABCGC than the yellow passion fruit and control treatments (Figure 6).

In the presence of moisture, the resistance structures of *F. oxysproum* germinate and the mycelium produces new spores. Considering that the resistance spore germination conditions were provided, according to the control treatment, the decrease in chlamydospore germination capacity was due to the toxic products released by the plant debris incorporated into the substrate. When subjected to stress conditions with thermal variation, *F. oxysporum* synthetizes proteins in the chlamydospores to ensure its survival (FREEMAN; GINZBURG; KATAN, 1989). The toxic products from the cabbage and bitter cassava probably interfered with the Fop capacity to synthesize proteins connected to the chlamydospore survival.

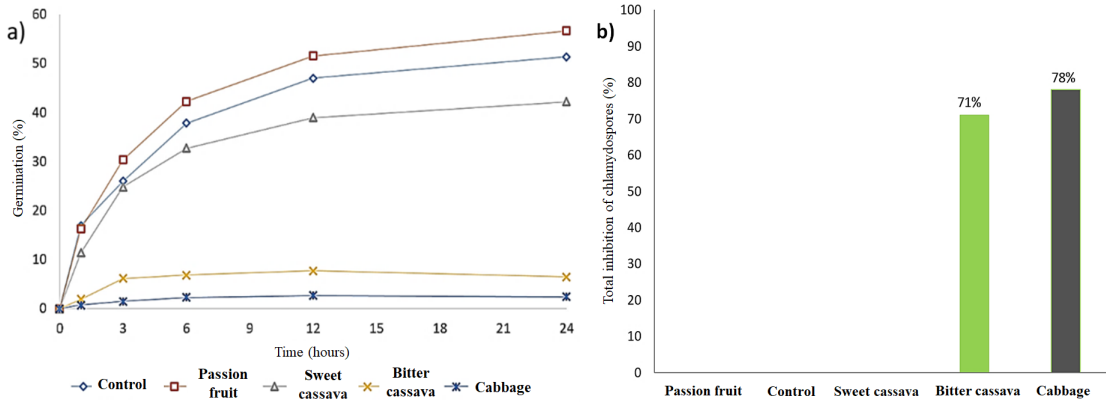

Figure 5. Percentage of chlamydospore germination of Fop subjected to different treatments with plant materials (a), and percentage of isolates with total inhibition of chlamydospore germination subjected to different plant materials (b).

Figure 6. Heatmap for the area below the chlamydospore germination curve (ABCGC) produced by isolates of *Fusarium oxysporum* f. sp. *passiflorae* subjected to treatments with different plant materials, under laboratory conditions.

The results obtained in this work contrast with previous studies that reported the efficiency of using Brassicaceae species materials for decreasing Fusarium wilt (BLOK et al., 2000; LARKIN; GRIFFIN, 2007; MENG et al., 2018). In addition to *Fusarium*, the effect of biofumigation has been reported for other pathogens, such as *Phytophthora* (WANG et al., 2014). The results show the potential of this approach to control several soil pathogens, mainly fusariosis. *F. oxysporum* f. sp. *passiflorae* survives in the soil through spores of resistance, making its control difficult and expensive, turning crop areas unfeasible, decreasing the useful life of plants, and generating high economic losses (CARVALHO et al., 2015; PREISIGKE et al., 2015; FREITAS et al., 2016). Thus, the effect of the plant debris evaluated in the present study on Fop survival mechanisms brings a perspective for development of technologies that can be used to control Fusarium in passion fruit plants, by the breeding of new agricultural processes with direct use of plant debris or by the development of formulated products.

Isolates representative of the main passion fruit state in Brazil are among the 14 isolates

analyzed in the present study. Therefore, the results denote that the biofumigation technique with cabbage and bitter cassava debris has potential for the management of the disease. This would be a double effect, both on the Fop saprophytic growth and on the viability of the primary inoculum of infection (the chlamydospores). The results obtained in the present work under laboratory conditions contribute to strategies for management and control of Fusarium wilt in passion fruit plants, and serve as a basis for further researches. These results also serve as a basis for the development of processes or products for biofumigation.

CONCLUSIONS

The incorporation of cabbage and bitter cassava debris into the substrate has a fungistatic effect, with inhibition of mycelial growth of Fop and affects the fungus survival by preventing or decreasing chlamydospore germination.

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