Inhibitory effect of *Caesalpinia spinosa* leaflets crude extract on *Fusarium solani* and *Phoma tarda*

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ABSTRACT. In order to evaluate the plant extract effect on the *in vitro* growth of *Fusarium solani* and *Phoma tarda*, hexane crude extract from spiny holdback (*Caesalpinia spinosa*) leaflets was obtained and incorporated into potato-dextrose-agar (PDA) at 2204 mg L^{-1}, 4460 mg L^{-1}, 6370 mg L^{-1}, 7644 mg L^{-1} and 16179 mg L^{-1} concentrations. The hexane crude extract inhibited mycelial growth at the range of 3,95% to 32,20% of *P. tarda* and 7,29% to 33,83% of *F. solani*, according to the extract concentration. It was demonstrated that the extract has antifungal activity and might be an alternative to physical or chemical control methods of fusariosis disease in several cultivations and of *Phoma* spot on coffee plant leaf.

Key words: fungistasis, *Coffea arabica*, fusariosis, *Phoma* leaf spot.

RESUMO. Efeito inibitório do extrato hexânico dos folíolos de *Caesalpinia spinosa* em *Fusarium solani* e *Phoma tarda*. Extrato hexânico foi obtido dos folíolos do falso pau-brasil (*Caesalpinia spinosa*) e incorporado em BDA (batata-dextrose-ágar), obtendo-se as concentrações de 2204 mg L^{-1}, 4460 mg L^{-1}, 6370 mg L^{-1}, 7644 mg L^{-1} e 16179 mg L^{-1}. Foi avaliado o crescimento micelial de *Fusarium solani* e *Phoma tarda*. Os resultados mostraram o efeito inibitório do extrato em porcentagens variáveis de 3,95% a 32,20% para *P. tarda* e de 7,29% a 33,83% para *F. solani*, conforme as doses crescentes do extrato, cuja fungitoxidade evidencia seu potencial alternativo aos métodos físicos e químicos de controle da fusariose em vários cultivos e mancha de *Phoma* no cafeeiro.

Palavras-chave: fungistase, *Coffea arabica*, fusariose, mancha de *Phoma*.

Introduction

Biological control has been widely and successfully used to control plant diseases. This study focuses biotechnology for plant diseases control.

Wilson (1998) defines "biological control of plant diseases" as the control of a disease through a natural biological process or with the product of a natural biological process. According to this broad definition, chemicals used in biocontrol could be extracted from living biological organisms or produced by living organisms. Biological control under this definition would be clearly distinguishable from physical control and from synthetic chemical control of plant diseases. These diseases cause great losses in important agricultural crops. Those caused by fungi that harm the plants during growth and after the crop deserve our attention. Their consequences are losses in either productivity or quality, or in both. Biological, physical and chemical methods have been used for controlling those diseases, in order to minimize the damages (Eckert and Ogawa, 1985; Sitton and Patterson, 1992; Wilson and Wisniewski, 1994). Physical and biological methods are interesting alternatives to chemical treatment because toxic residues do not remain in the products. So, the use of natural fungicides is shown as a plausible alternative to the use of synthetic fungicides, in terms of fungal diseases control (Wilson and Wisniewski, 1994). Data in literature reveal that the use of extracts of several plant species is being researched in order to explore their fungicidal activities (Kurita *et al.,* 1981; Wilson *et al.,* 1997). Several crude plant extracts with biological activity have been studied. Among these, it has also been reported that plant extracts

from peel, fruits and leaves of *Caesalpinia spinosa* (Molina) O. Kuntze (spiny holdback) have inhibitory effect on the activity of some bacteria. Liu *et al.* (2003) report the evidences that those extracts have bactericidal properties, as they act selectively in some gram positive bacteria, like *Staphylococcus aureus* and *Bacillus subtilis.* However, there is no data in the literature referring to the association of spiny holdback extracts in plant pathogenic fungi control. Therefore, this work aimed to evaluate the *in vitro* effect of spiny holdback leaflets hexane extract on the mycelial growth of the fungi *Fusarium solani* and *Phoma tarda*, as a possible alternative to control both fusariosis, which attacks several crops, and *Phoma* spot on coffee plant (*Coffea arabica*) leaves.

Material and methods

Plant material

The leaflets of *Caesalpinia spinosa* (spiny holdback) were colleted in Campanha, State of Minas Gerais, Brazil. A voucher specimen was deposited in the Esal Herbarium, at the Federal University of Lavras, Minas Gerais, under the number 18983.

Culture of fungi

Isolates of *Fusarium solani* and *Phoma tarda* from the Fungi Collection of the Phytopathology Department, Federal University of Lavras, were used. The cultures were transplanted every ten days to obtain new colonies. For the mycelia growth, pathogens were cultivated in PDA (potato, dextrose and agar) culture media. This culture medium was chosen for promoting the best production of spores for each phytopathogen. The pathogens were grown in a previously melted medium, placed into a 10-cm diameter Petri dish, in a laminar flux chamber. The incubation was done in a germination chamber, under NUV (near ultra-violet), at a temperature range of 21ºC ± 2ºC and a 12-hour photoperiod.

Extraction

The plant material was oven-dried at 45ºC and crushed. For the extracting process, 195.07 g of dried and crushed spiny holdback leaflets were used. The extraction was promoted through hexane refluxing for a 24-hour period. The liquid obtained was filtered in a Bückner vacuum filter. The solvent present in the filtered solution was removed in a Büchi rotary evaporator, at 40ºC and under reduced pressure. The residue obtained was placed in an oven at 32 ±1ºC, for solvent evaporation.

Antimicrobial assay and experimental model

The hexane extract was then melted in a microwave, in medium potency, for three minutes, and added to the melted PDA medium (45ºC), to obtain the following concentrations: 2204 mg L^{-1}, 4460 mg L^{-1}, 6370 mg L^{-1}, 7644 mg L^{-1} and 16179 mg L^{-1}, referring to the dosages of 0.173 g, 0.350 g, 0.500 g, 0.600 g and 1.270 g, respectively. Each concentration represented a treatment (T). For control, a standard Petri dish containing only PDA (T6) was used. For the experiments, starting from 10-day-old colonies, grown in Petri dishes containing BDA, under NUV and a 12-hour photoperiod, mycelial disks (Ø = 5mm) of colonies of *F. solani* and *P. tarda* were placed in the center of the Petri dish, containing the culture medium obtained from the hexane extract, already melted. The incubation was accomplished in germination chambers (21ºC ± 2ºC, under NUV; 12-hour photoperiod), for eight days. This procedure was repeated four times for each concentration and two evaluations were made. The experimental design was totally randomized subdivided parcels. The parcels and subparcels were represented by the two evaluations and by the dosages of the plant extract and mycelial growth, totaling 48 experimental units. The results were submitted to variance analysis and to linear regression, being considered the averages of each treatment in each evaluation. Tukey's test for mean comparison was used at the 5% probability level. The crude extract efficiency was verified by calculating the percentage of inhibition in the different treatments, compared to the control, applying the following formula: PIC = [(cm test - cm trat) / cm test] x 100, where: PIC = percentage of mycelial growth inhibition; cm test = mycelial growth in the control; cm trat = mycelial growth in each treatment; 100 = factor of percentage calculation.

Evaluation of Mycelial growth

The first evaluation was made 8 days after the begining of the control experiment, (T6), showing 2/3 of colony growth. The second evaluation was made after the complete covering of control the surfaces. The average mycelial growth was obtained by measuring the radial growth of each colony in two orthogonal axes.

Results and discussion

The mean results obtained from the colonies of fungi isolates, submitted to six concentrations of hexane plant extract are in Table 1.

Table 1. Mycelial Growth (MG): average of *F. solani* and *Phoma tarda* submitted to six concentrations of hexane extract from spiny holdback leaflets.

	Concentrations (mg L^{-1})					
	Control (0)	2204	4460	6370	7644	16179
F. solani	25.06 d	22.93 c	23.21 cd	21.53 bc	20.56 b	16.56 a
P. tarda	39.18 d	37.62 d	33.46 c	32.56 c	30.53 b	26.81 a

Means followed by the same letter do not differ significantly from each other at 5% by the Tukey test.

It was observed that the hexane extract had partial inhibitory effect against *F. solani* and *P. tarda* (Table 1). There was significant difference among the mycelial growth averages of *F. solani* obtained with different dosages of hexane extract.

The maximum inhibitory effect of the hexane extract was observed against *F. solani*, when the inhibition zone was 16.56 mm, compared to the control (25.06 mm).

It was also observed that there was no significant difference between the mycelial growth averages of *P. tarda* obtained in the control treatment and in the presence of 0.173 g dosage. The dosages of 0.350 and 0.500 g did not differ significantly from each other, but, according to Pimentel (1987), differences that do not reach the statistical level of significance are not rarely important and should be checked by other methods.

At the dosages of 0.350 g, 0.500 g, 0.600 g and 1.270 g, the mycelial growth averages were significantly shorter than the control.

The minimum inhibitory activity was observed against *P. tarda* (33.46 mm), compared to the control (39.18 mm). There was a trend of decreasing averages of *F. solani* and *P. tarda* colony growth with the increasing of the hexane extract concentrations, up to 0.173 g (Table 1).

There was an inverse correlation among the hexane extract concentrations and the mycelial growth for the two fungi species in the experiment.

By means of response functions adjustment, using regression techniques (Little, 1981; Dawkins, 1983), the plant extract demonstrated fungitoxic properties in all treatments, promoting mycelial growth reduction for *F. solani* and *P. tarda*.

The mycelial growth reduction increased proportionally to the increase of the plant extract dosages, for both fungi.

It is known that hexane extracts preferentially resins, oils and waxes and therefore, some of these metabolites classes may be responsible for the inhibitory action.

The variance analysis showed highly significant effect ($p < 0.05$) of the treatments on the fungi mycelial growth. The response functions of the mycelial growth of the fungi submitted to different treatments with plant extract followed highly significant linear models. Figures 1 and 2 show the adjustment of the linear regression models of plant extract dosages upon the mycelial growth of *Phoma tarda* and *Fusarium solani* isolates.

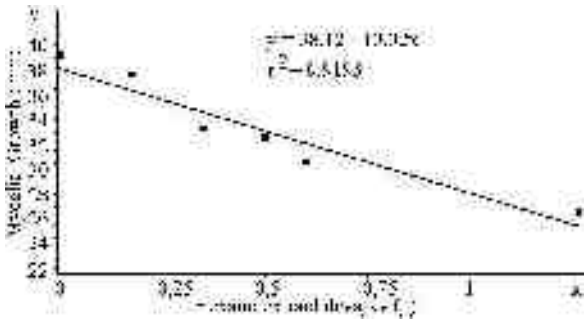

Figure 1. Effect of hexane extract dosages of spiny holdback (*Caesalpinia spinosa*) leaflets in *Phoma tarda* mycelial growth.

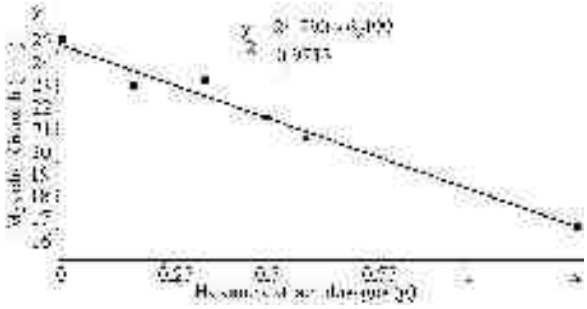

Figure 2. Effect of hexane extract dosages of spiny holdback (*Caesalpinia spinosa*) leaflets in *Fusarium solani* mycelial growth.

Comparing Figures 1 and 2, a partial inhibition for both fungi is found, by utilizing the hexane extract. Increasing dosages of the plant extract reduced the mycelial growth of *P. tarda* and *F. solani*. The observed averages showed negative correlation and, therefore, variables dosage and MG varied linearly in opposite directions. The estimate of the determination coefficients (r^2) indicated a very good data adjustment to the linear regression equations, which means that the variable MG in relation with the extract dosages was explained by the regression line in 91.93% of the cases for *P. tarda* and in 97.13% for *F. solani*. The inhibition degree of the phytopathogens MG increased as the extract dosages (or concentrations) increased.

Regarding the extract efficiency, starting from the dosage of 0.173 g, the extract inhibited *P. tarda* growth in 3.95%, 15.10%, 16.90%, 22.10% and 32.20%, respectively. For *F. solani*, the values of 8.50%, 7.29%, 14.08%, 17.87% and 33.83% of inhibition were obtained, respectively, when compared to the control.

In this experiment, germination and sporulation

were not considered. However, considering that the hexane extract of spiny holdback leaflets reduced the mycelial growth of the phytopathogens, it may be concluded that it also reduced the total number of spores produced by then.

The partial reduction of the fungi mycelial growth in all treatments, when compared to the control, suggests that the hexane extract of spiny holdback leaflets may contain active substances in low concentrations. So, it may be inferred that the extract consists of a group of substances that the hexane can extract from the sample and that does not volatilize with the solvent evaporation. Those substances, soluble in hexane, include preferentially resins, oils and waxes, among others. For that reason, some of those metabolites classes may be responsible for the inhibitory action of fungi mycelial growth. Some classes of secondary metabolites have been widely employed for phytopathogens biological control, such as benzoquinones, saponins, alkaloids, amines, amides, fatty acids, phenolic compounds, flavonoids, xanthones, long alkene chains and terpenoids (Harbone, 1994). Wedge *et al.* (1999) utilized several natural and synthetic lactonic sesquiterpenes against pathogenic fungi such as *Fusarium oxysporum*, *Colletotrichum gloeosporoides*, among others, with promising results. Gali-Muhtasib *et al.* (1998) investigated the growth inhibitory and cytotoxic effects of tannin extracted from *Caesalpinia spinosa* on a mouse fibroblast cell line; results of cell cycle analysis indicated that tannin arrested cells in the G0-G1 stage of the cell cycle and induced apoptosis. Interestingly, Tarapod tannic acid (TA) was also found to protect against UV-induced cell damage when used at low dosages.

Research studies with plant extracts are wide and promising. However, bibliographical references about biological activity of spiny holdback extracts in phytopathogenic fungi were not found. Therefore, this experiment concluded that hexane extract of the spiny holdback leaflets may be pointed out as potentially useful as an alternative for fusariosis disease control and *Phoma* leaf spot.

Conclusion

The hexane extract of spiny holdback (*Caesalpinia spinosa*) leaflets promoted partial inhibition of the mycelial growth of *Fusarium solani* and *Phoma tarda* and the inhibition was directly proportional to the dosages tested.

Acknowledgements

The authors thank to CNPq and Fapemig for the financial support.

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Received on January 26, 2005.
Accepted on June 21, 2005.