 DOI: 10.1051/bioconf/20160701044

Antifungal activity of plant extracts on *Phaeomoniella chlamydospora*

Carine Rusin[1,a], Ires Cristina Ribeiro Oliari[1], Carla Daiane Leite[1], Cacilda Márcia Duarte Rios Faria[1], Renato Vasconcellos Botelho[1], and Marcus André Kurtz Almança[2]

[1] UNICENTRO, *Câmpus* CEDETEG, Guarapuava, PR, Brasil. Fellowship: CAPES
[2] IFRS, *Câmpus* Bento Gonçalves, RS, Brasil

Abstract. The decline and death of the vine has become an obstacle to world wine production. Among the causative agents highlights the *Phaeomoniella chlamydospora*, causal agent of Esca and Petri disease. Plant extracts may become a viable option control considering their fungistatic and/or fungicide substances. The objective of this work was to verify the potential of the aqueous extracts of plants on the control of *Phaeomoniella chlamydospora* comparing to other products. The following treatments were applied: sulfur, mancozeb, difeconazole, pyraclostrobin, tebuconazole, chitosan, *Bacillus subtilis, Trichoderma harzianum,* and extracts of india flowers, cinnamon bark, dried leaves of rosemary and lemon grass. The treatments were added in PDA culture medium, previously autoclaved at 120º for 20 min. Disc of 5 mm diameter of *P.chlamydospora* colony were transferred to the center of Petri dishes and kept at 20°C in the dark. The experimental design was completely randomized with five replications. It was evaluated the mycelial growth at five, eight and fourteen days after the installation of the experiment, obtaining the area under curve of the mycelial growth (AUCMG). The clove India extracts, cinnamon and rosemary, proved to be a control option considering their effect in the decrease of AUCMG compared to the control.

Introduction

The *Chlamydospora Phaeomoniella*, one of the causative agents of Esca's disease and Petri disease, along with several other stem funguses, has become an obstacle to world wine production. The decline of the vine is responsible for the increase of wine production cost, estimated over 1.5 billion dollars in the global replacement of diseased vineyards [1].

Fungi associated with the decline of the vine which is usually infected by pruning wounds and/or grafting, may lead to plant death. It can be manifested by a sudden defoliation, but usually starts with a slow decline that translates into a reduced growth and several other symptoms such as chlorosis, necrosis and withered leaves. Internal symptoms in adult plants can be characterized by white rot in the trunk, which the evolution can cause cracks. Furthermore, they can also be observed black dots, and brown or black streaks in xylem tissues [2].

Since the prohibition of sodium arsenite use in 2003 in all winegrowers countries, no effective treatment to these fungi was implemented [3]. In search of lower environmental impact methods, the plant extracts may contain fungitoxic or fungistatic substances that promote the control of various fungi.

For vine leaf diseases, garlic extract provided control of *Plasmopara viticola* [4] *Phomopsis viticola* and *Elsinoe ampelina* [5,6]. The garlic extract was also effective in controlling in vitro *Botryosphaeria dothidea, Diplodia seriata* and*Eutypa lata, Macrodidyma Ilyonectri* and *Phaeocremonium aleophilum* [7].

Clove extract proved effective for the control of *Colletotrichum* sp., *Cercospora Kikuchii*, *Fusarium solani* [8] and *Phomopsis phaseoli* var. *sojae* [9]. Another study showed the efficiency of lemongrass and rosemary extracts for the control of *Fusarium oxysporum* in flowers [10].

The objective of this work was to study the effects of aqueous extracts of plants on the control of *Phaeomoniella chlamydospora*, compared to other products.

Methodology

Three trials were carried out at the Phytopathology Laboratory of UNICENTRO. For the first experiment the following products were used: Sulphur 3 g L^{-1} (ai: 800 g kg^{-1}), mancozeb (3 gL^{-1}, ai: 800 g kg^{-1}) difeconazole (1 mL L^{-1}, ai: 250 g L^{-1}), pyraclostrobin (5.33 mL L^{-1} ai: 250 g L^{-1}) and tebuconazole (8.75 mL L^{-1} ai: 200 g L^{-1}). In the second experiment, commercial products containing the following components were tested: chitosan (16 mL L^{-1}, ai: 2%), *Bacillus subtilis* (80 mL L^{-1}, ai: 13.68 g L^{-1}) and *Trichoderma harzianum* (4 g L^{-1} was: 300 g kg^{-1}). In the third experiment were applied flower buds of clove (*Caryophyllus aromaticus* L.) bark cinnamon (*Cinnamomum zeylanicum*), fresh bulbs of garlic (*Allium sativum* L.), dried leaves of rosemary (*Rosmarinus officinalis*) and grasslemon (*Cymbopogon citratus*). Plant extracts were prepared using 30g of plant material in 100 ml of distilled water, placed in infusion at 70 °C for 10 minutes and subsequent filtering through cheesecloth.

[a] e-mail: carine.rusin@gmail.com

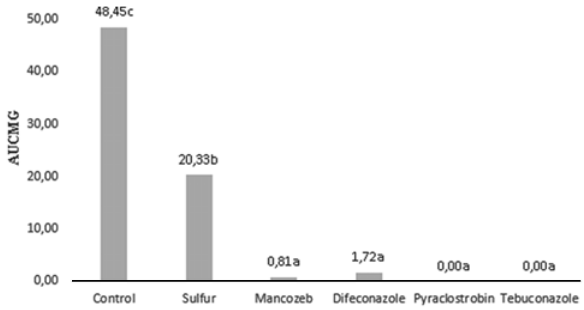

Figure 1. Area under curve of the mycelial growth of *Phaeomoniella chlamydospora* submitted to treatments with different fungicides (Guarapuava-PR, Brasil, 2016). Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$).

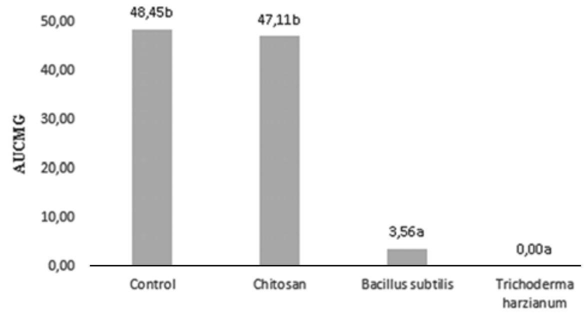

Figure 2. Area under curve of the mycelial growth of *Phaeomoniella chlamydospora* submitted to treatments of chitosan, *Bacillus subtilis* e *Trichoderma harzianum.* (Guarapuava-PR, Brasil, 2016). Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$).

For the first and second experiment, commercial products were added to PDA culture medium (potato dextrose agar). In the third trial, plant extracts was added to the melting PDA culture to give the final concentration of 10%. The experimental design was completely randomized, with five replications.

Mycelial discs of 5 mm diameter of an isolated of *Phaeomoniella chlamydospora* obtained in Bento Goncalves, State of Rio Grande do Sul (Brazil) were transferred at the center of Petri dishes containing culture medium with the treatments and incubated in growth of camera at 25^{o}C in the dark.

The evaluation of mycelial growth was performed daily by diameter measurements (cm) of the colony with the aid of digital caliper, and the plates marked externally in perpendicular direction, with the purpose of measuring the radial growth of the colony in two orthogonal axes, calculating the average values per plate. Growth was assessed at five, eight and fourteen days after the installation of the experiment.

From the evaluations of mycelial growth, it was estimated the area under mycelia growth curve (AUMGC) and the data were submitted to analysis of variance and mean comparison by Tukey test at 5%. The percentage inhibition of each treatment was calculated in relation to the control and mean comparison was performed by Scott-Knott test at 5%. For statistical analysis It used the statistical program SPSS version 22.0 [11].

Results and discussions

For the first treatment with the use of chemicals to control *P. Chlamydospora*, all treatments showed AUCMG significantly lower than the control (Fig. 1). The largest reductions were observed for Mancozeb, Difeconazol, Pyraclostrobin and Tebuconazole. The sulfur-based treatment was also effective, but had intermediate result compared to other fungicides.

Other studies have demonstrated effective *in vitro* control of *P. chlamydospora* with the use of chemical fungicides. The use of difeconazol and tebuconazole can reduce up to 100% mycelial growth of the pathogen [12–15].

The use of chitosan not reduced AACCM compared to the control. However, treatments with *B. subtilis* or *T. harzianum* were effective for *in vitro* control of *P. chlamydospora* (Fig. 2).

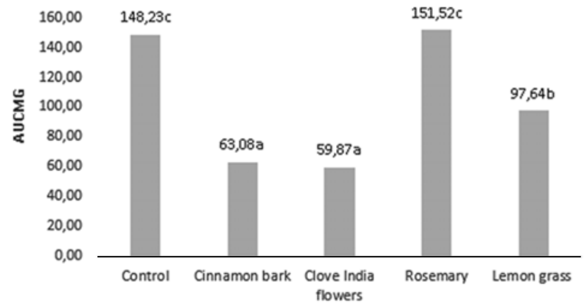

Figure 3. Area under curve of the mycelial growth of *Phaeomoniella chlamydospora* submitted to different plant extracts (Guarapuava-PR, Brasil, 2016). Means followed by the same letter do not differ significantly by the Tukey test ($p \leq 0.05$).

The results obtained with the addition of chitosan to the medium culture were opposed to other work that demonstrated that its use reduced mycelial growth by up to 100% [15,16]. Other authors also found antagonistic activity of *Bacillus* sp. in control of *P. chlamydospora* [17] and efficiency above 60% [18]. Similarly the use of 22 different strains of *Trichoderma* reduced by up to 100% mycelial growth of *P. chlamydospora* [19].

Treatments with cinnamon, clove india and lemongrass extracts decreased significantly AUCMG compared to the control. However, the rosemary extract did not get this effect on mycelial growth (Fig. 3).

These results were consistent with those of other authors. Rozwalka et al. (2008) found that the use of clove india extract controlled 100% growth of *Glomerella cingulata* and *Colletotrichum gloeosporioides* [20]. In another work, clove and cinnamon india extracts showed an inhibition rate above 50% when tested for *Aspergillus* sp., *Penicillium* sp., *Cercospora Kikuchii*, *Colletotrichum* sp., *Fusarium solani* and *Phomopsis* sp. [21].

Carli et al. (2010) did not found efficiency of cinnamon extract on the control of *Colletotrichum gloeosporioides* and *Penicillium* sp. [22]. Likewise, rosemary extracts and lemongrass were not efficient in the control of *Alternaria solani* [23].

Cobos et al. (2015) found that garlic extracts, green coffee, lemon peel, propolis, vanilla, chitosan and lichen *Evernia prunastri* were efficient *in vitro* control of *Botryosphaeria dothidea, Diplodia seriata, Eutypa lata, Ilyonectria macrodidyma, Phaeocremonium*

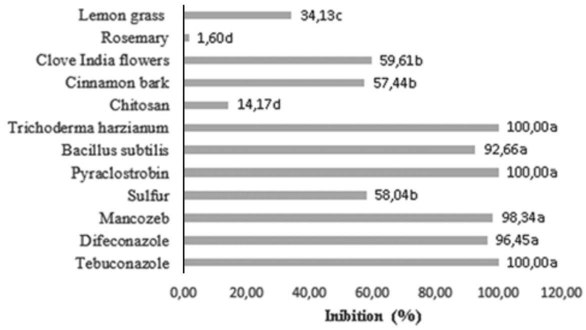

Figure 4. Percent inhibition of mycelial growth of *Phaeomoniella chlamydospora* submitted to the different treatments. (Guarapuava-PR, Brasil, 2016). Means followed by the same letter do not differ significantly by the Scott-Knott test ($p \leq 0.05$).

aleophilum, Phaeomoniella chlamydospora and *Plasmopara viticola* [7].

When comparing the percentage of inhibition of mycelial growth of *P. chlamydospora*, the treatments, *B. subtilis*, Pyraclostrobin, Mancozeb, Difeconazol, Tebuconazole obtained the highest percentage of control, followed by sulfur, clove india and cinnamon extract, which reduced more than 50% mycelial growth of the pathogen (Fig. 4).

High antifungal activity is attributed when the aqueous extracts provide inhibition equal or greater than 50% [21]. The data obtained for the clove cloves and cinnamon extracts obtained inhibition of 59.6% and 57.4%, respectively. The inhibition of mycelial growth of *Phaeomoniella chlamydospora* provided by these extracts did not differ significantly from the use of sulfur. These data suggest that aqueous extracts of clove and cinnamon india can be an alternative possibility to chemicals in controlling the pathogen.

Conclusion

The treatments with *B. subtilis*, Pyraclostrobin, Mancozeb, Difeconazol or Tebuconazole obtained the highest percentage control of *Phaeomoniella chlamydospora*. The aqueous extracts obtained high antifungal activity and may be an alternative control to agrochemicals.

References

[1] V. Hofstetter, B. Buyck, D. Croll, O. Viret, A. Couloux, K. Gindro. Fungal Diversity, **54**, 51 (2012)
[2] L. Mugnai, A. Graniti, G. Surico, Plant Dis, **83**, 404 (1999)
[3] M. de la Fuente. *OIV*, **1**, 3 (2016)
[4] C.D. Leite, R.V. Botelho, C.M.D.R. Faria, A.J. Maia, Rev. Bras. Frutic, **33**, 429 (2011)
[5] C.D. Leite, R.V. Botelho, C.M.D.R. Faria, A.J. Maia, Rev. Bras. Agroecol, **4**, 1409 (2009)
[6] R.V. Botelho, A.J. Maia, E.J. Paioli, M.M. Terra, Rev. Bras. Frutic, **31**, 096 (2009)
[7] R. Cobos, R.M. Mateos, J.M. Alvarez-Pérez, M.A. Olego, S. G. García, E. Garzón, J.J.R. Coque, Appl Environ Microbiol, **81**, 6474 (2015)
[8] L.R. Venturoso, L.M.A. Bacchi, W.L. Gavassoni, L.A. Conus, B.C.A. Pontim, F.R. Souza. Arq. Inst. Biol. **78**, 89 (2011)
[9] D.C. Pastro, L. Pascuali, D.O. Sandri, S.P. Zela, F.S. da Silva. Enciclopédia Biosfera. **8**, 389 (2012)
[10] V.C. Sartori, F.E. Magrini, L.B. Crippa, C. Marchett, L. Venturin, R.S. Ribeiro, Rev. Bras. Agroecol, **6**, 117 (2011)
[11] *IBM Corp Released* (2013)
[12] M. Groenewald, S. Denman, P.W. Crous, S. Afr. J. Enol. Vitic, **21**, 59 (2000)
[13] M. Jasper, Phytopathol Mediterr, **40**, 453 (2001)
[14] D. Gramaje, A. Aroca, R. Raposo, J. García-Jiménez, J. Armengol. Crop Protection. **28**, 1091 (2009)
[15] R.F. dos Santos, E. Blume, E. Junges, M.C.N.F. Rego, Enciclopédia Biosfera, **9**, 3724 (2013)
[16] T. Nascimento, C. Rego, H. Oliveira, Phytopathol. Mediterr, **46**, 218 (2007)
[17] A. Alfonzo, G. Conigliaro, L. Torta, S. Burruano, G. Moschetti. Phytopathol. Mediterr, **48**, 155 (2009)
[18] R. Haidar, J. Roudet, O. Bonnard, M.C. Dufour, M.F Corio-Costet, M. Fert, T. Gautier, A. Deschamps, M. Fermaud. Microbiological Research, **192**, 172 (2016)
[19] S. Di Marco, F. Osti, R. Roberti, F. Calzarano, A. Cesari. Atti Giornate Fitopatologiche, **2**, 419 (2002)
[20] L.C. Rozwalka, M.L.R.Z.D.C. Lima, L.L. May de Mio, T. Nakashima. Cienc. Rural. **38**, 301 (2008)
[21] L.R. Venturoso, L.M.A. Bacchi, W.L. Gavassoni, L.A. Conus, B.C.A. Pontim, A.C. Bergamin, Summa phytopathol, **37**, 18 (2011)
[22] M.D. Carli, C.M.D.R. Faria, I. Baldin, M.V. Faria, J.T.V. Resende, Hortic. bras, **28**, 1091 (2010)
[23] A.T. Itako, K.R.F. Schwan-Estrada, J.B. Tolentino Júnior, J.R. Stangarlin, A. E. da Silva Cruz, Trop Plant Pathol, **33**, 241 (2008)