EC AGRICULTURE
Research Article

Eucalyptus Rust can not be Controlled with the Application of Ethanolic Extract of Banana "Heart"

Nivea Patrícia Ribeiro Reges[1], Marcos Paulo dos Santos[2]*, Eliane Vieria Rosa[3] and Monica Lau da Silva Marques[4]

[1]*Agronomic Engineer, Soil Laboratory Technique, Catalão, Brazil*

[2]*Plant Production Sector, School of Agronomy, Federal University of Goiás, Goiânia, Brazil*

[3]*Department of Cell Biology, Federal Institute Goiano, Campus Ceres, Ceres, Brazil*

[4]*Department of Phytopathology, Federal Institute Goiano, Campus Ceres, Ceres, Brazil*

***Corresponding Author**: Marcos Paulo dos Santos, Plant Production Sector, School of Agronomy, Federal University of Goiás, Goiânia, Brazil.

Received: September 05, 2018; **Published:** November 30, 2018

Abstract

In the search for less aggressive alternatives, plant extracts have been studied for the control of phytopathogenic fungi. The objective of this study was to evaluate the effect of banana "heart" extract in the control of rust disease (*Puccinia psidii* Winter) on eucalyptus plants. Prior to the test, the seedlings underwent a period of acclimatization and inoculation with the respective pathogen. Thirty days after inoculation, 224 seedlings presenting severity symptoms S1 and S2 were selected for the execution of the test. The experiment was carried out in a greenhouse in a randomized block design in the 4 x 2 factorial arrangement, with four extracts concentrations (0, 250, 500 and 1000 $mg.L^{-1}$) and two eucalyptus species (*Eucalyptus urograndis* and *E. citriodora*). Thirty days after the application of the extract was evaluated the effect of the banana extract and the species on rust severity. The application of crude ethanolic extract of banana heart was not a viable tool for the control and mitigation of rust symptoms in eucalyptus seedlings. The *E. urograndis* species is more tolerant to disease than seedlings of *E. citriodora*, being an option for planting in places where cases of the disease have been reported.

Keywords: *Alternative Control; Natural Fungicide; Plant Diseases; Severity; Myrtaceae*

Abbreviation

CEEBH: Crude Ethanolic Extract of Banana Heart

Introduction

Eucalyptus plantations in Brazil were considered disease-free until the 1970s. However, the advance of reforested areas to warmer and wetter regions, the planting of susceptible species and the use of successive crops in the same area created favorable conditions for occurrence of diseases [1]. Among these diseases, rust caused by *Puccinia psidii* Winter has been considered since the 1990s, with one of the most limiting factors in the establishment of new plantations and in the management of shoots of some species and provenances of eucalyptus [2,3].

In Brazil the fungus *Puccinia psidii* was described in 1944, focusing on seedlings of *Corymbia citriodora* [4]. However, since the 1930s there were reports of the fungus on *Psidium pomiferum* L. and *P. guajava* L. [5]. Concern about the infestation of this phytopathogenic agent from the commercial point of view occurred in the 1980s, when more than 300 hectares of six-month-old *Eucalyptus grandis* in the Rio Doce Valley of Minas Gerais were severely infected by fungus [6,7].

The disease occurs in clonal gardens and mini-gardens, new plantings up to two years of age, and sprouts in the field after cutting. The pathogen affects developing organs such as leaves, floral buds, fruits and branches and causes deformations, mini cancers, loss of apical dominance and reduction of growth, being the presence of intense yellow-colored pustules the distinguishing feature for diagnosis of the disease [8]. In addition to eucalyptus, *P. psidii* infects species of various genera of Myrtaceae, including jabuticaba, guava, cagaita, murici, jambo and other species of agricultural interest.

In the control of eucalyptus rust, triadimenol is the most used fungicide since the 80's. However, in view of the concern about the continuous use of the same active principle, together with the restrictions of the forest certification bodies on the use of products that do not contain chlorine in its molecule, as this, if present, can persist in the environment, becomes necessary to the search for other fungicides efficient in the control of the disease [9]. In addition, the use of chlorine in its molecule, if present, may persist in the environment, is necessary to find other fungicides that are efficient in disease control. Azoxystrobin, tebuconazole, and trifloxystrobin can also be used as fungicides, since they have also been effective in inhibiting *P. psidii* infection in eucalyptus seedlings [9].

The use of natural products is shown as an alternative to the use of conventional fungicides, which often present problems with fungus resistance to its active principles [10]. In the soybean crop for example the use of banana extract allowed the 50% inhibition of the germination of uredymospores of *Phakopsora pachyrhizi* [11]. The use of plant extracts to control plant diseases has been studied, but in practice it has been used more frequently by farmers practicing organic agriculture. As a cause of the low use of this technique, we can highlight the difficulty of obtaining effective plant tissues in the control of diseases, as well as protocols for preparation of the extract, since there is a culture of acquiring the finished product [12]. Due to the environmental problems caused by the synthetic fungicides used in agriculture, there has been an increase in the search for alternative methods, safe, viable and efficient in the control of phytopathogenic fungi [2].

Objective of the Study

The objective of this study was to evaluate the effect of foliar application of ethanolic extract of banana "heart" on the control of rust of Myrtaceae (*Puccinia psidii* Winter) on seedlings of two eucalyptus species.

Materials and Methods

Experimental data

The experiment was carried in a greenhouse at the Federal Institute Goiano, Campus Ceres, Goiás, Brazil from January to March 2014. The study site presents an average annual temperature of 25°C, with the possibility of maximums of up to 40°C in the spring. The minimum value of temperature can reach values of up to 10ºC, in the months of May, June and July. The climate according to Köppen classification is Aw (savanna climate or tropical climate of wet and dry seasons - Tropical Seasonal, dry winter) [13]. During the experimental period, relative humidity in the greenhouse was around 67% and the minimum and maximum average temperatures were 17 and 36°C, respectively.

Acclimation of seedlings, obtaining, identifying and inoculating the pathogen

Seedlings purchased from a certified nursery with an age of approximately four months were transplanted into polyethylene plastic bags with a capacity of two kg of substrate, where they underwent a four week acclimation period. The growth vessel was filled with substrate in the ratio of 1: 2: 1 soil: sand: tanned bovine manure.

The inoculum of the fungus *P. psidii* Winter was obtained from the experimental area of fruit growing of the Federal Institute Goiano - Campus Urutaí, Goiás. Leaves of yellow Jambo (*Syzygium jambo*s) were collected with circular necrotic spots, a typical symptom of myrtaceae rust. After the collect, these leaves were sent to the microbiology laboratory of the Federal Institute Goiano - Campus Ceres, where the fungus causing rust of the Myrtaceae (*P. psidii*) was identified with stereoscopic loupes and optical microscope.

The preparation of the isolates was carried out from infected leaves, from which pustules were removed and allowed to immersion in distilled water until total suspension of the inoculum occurred [14]. The isolates were then inoculated into 300 healthy eucalyptus seedlings, 150 of which were *Eucalyptus citriodora* and 150 *E. urograndis* clones (*E. grandis* x *E. urophylla*), respectively. The inoculation was carried out in all leaves of the plants at the end of the afternoon, a period of low solar incidence, with the aid of a manual compression sprayer (Tramontina®), with a capacity of 2L.

At 30 days after inoculation the seedlings were characterized using the diagrammatic severity scale of eucalyptus rust (Figure 1) proposed by Junghans., *et al* [15].

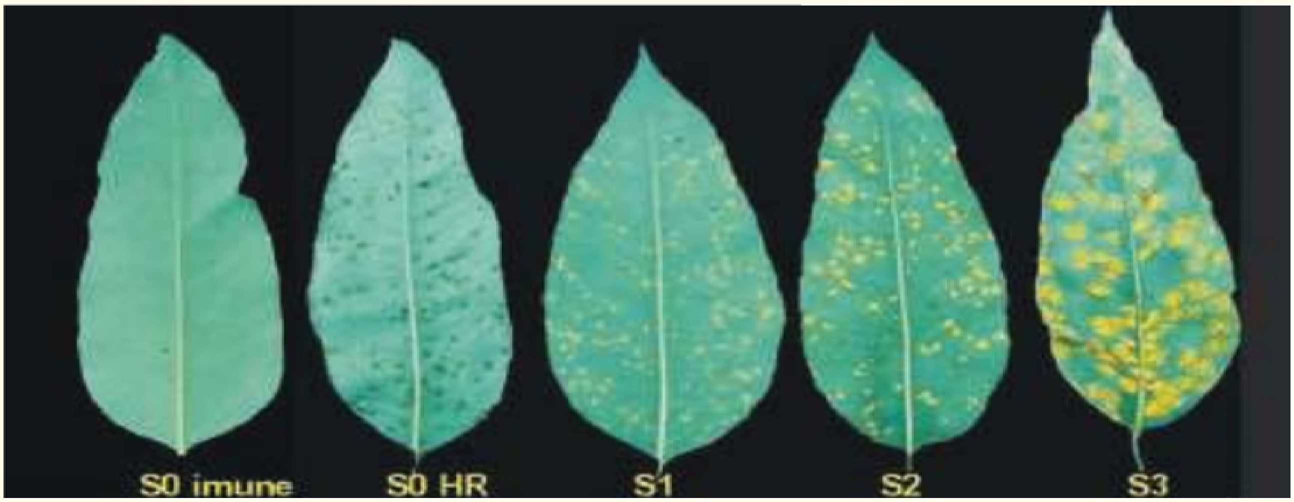

Figure 1: *Scale of notes for evaluation of eucalyptus rust resistance (Eucalyptus sp.), with four severity classes: S0 = hypersensitivity or "fleck" or necrotic hypersensitivity reaction; S1 = Pustules < 0.8 mm of diameter; S2 = Pustules of 0.8 to 1.6 mm diameter; and S3 = Pustules > 1.6 mm of diameter. Plants in severity classes S0 and S1 are considered resistant, while S2 and S3 are susceptible.*

Source: Junghans., et al [15].

Obtaining and applying the crude banana extract

"Hearts" of *Musa acuminata* cv. Grand Naine grown in the fruit growing sector of the Federal Institute Goiano - Campus Ceres were collected and taken to the biology laboratory for selection of healthy parts and fragmentation. After fragmentation, the plant material was dried in a forced ventilation oven at 60°C. The dry material was immersed in 92.8% ethanol and stored in dark bottles in order to avoid photodegradation of its chemical compounds.

Before the installation of the test, the dry material was immersed in ethanol 96% during seven days, in dark environment, with daily agitation. After this period, the obtained sample was filtered in gauze and the solvent was evaporated in a waterbath to obtain the crude ethanolic extract of banana heart (CEEBH), following the methodology detailed in Rosa [16]. The CEEBH, obtained after evaporation, was stored in a desiccator for seven days. After obtaining the CEEBH the following concentrations (250, 500 and 1000 mg L^{-1}) were prepared (dilution of the extract was performed in 10% alcohol solution, due to the low solubility of the extract in distilled water). The diluted solution was then filtered and sprayed on seedlings of two eucalyptus species.

Experimental design

Of the 300 inoculated seedlings were selected 224, being 112 of each species (Table 1). The experiment was conducted in a greenhouse covered with polyethylene under intermittent misting at 4 hour intervals and eight minute wetting period. The experimental design was a randomized complete block design in a factorial scheme 4 x 2, with four concentrations of CEEBH (0 [water only], 250, 500 and 1000 mg L-1) and two eucalyptus species (*Eucalyptus citriodora* and *E. urograndis*), with four replicates and seven plants in each experimental unit.

Eucalyptus citriodora		*Eucalyptus urograndis*	
S1*	S2	S1	S2
56	56	56	56
112		112	

Table 1: *Amount of seedlings distributed in rust severity classes in eucalyptus seedlings 30 days after inoculation with Puccinia psidii Winter. Ceres - GO, 2014.*

**: Diagrammatic scale of rust severity in eucalyptus seedlings proposed by Junghans., et al. [15], detailed in figure 1.*

Variables analyzed

The efficiency of the CEEBH in the control of eucalyptus rust was evaluated thirty days after its application. For this, the frequency of disease symptoms (scales varying from S1 to S3), survival and cure of the plants that were characterized as infected previously to the application of CEEBH were quantified (Table 1). The frequencies of each response variable were transformed in percentage (%) of occurrence.

Statistical analysis

Because they did not present normal distribution, and yet, heterogeneity of variances the data were transformed by the expression: $\sqrt{(x+1)}$ and then subjected to the variance analysis (Test F) using software Sisvar Inc., Brazil [17]. When there was a significant effect of the factors, the means were compared by the t test for paired samples at the 5% level of error probability.

Results and Discussion

The interaction of the genotypes (*E. urograndis* and *E. citriodora*) with the concentrations of crude ethanolic extract of banana heart (CEEBH) was not significant ($p > 0.05$) for any of the variables analyzed (Table 2). This implies that the factors acted in isolation, so that the eucalyptus species did not potentiate the effect of the concentration of CEEBH, at the proposed levels.

Source of Variation	S1 (%)	S2 (%)	S3 (%)	Cured (%)
Species (S)	2,66^{NS}	12,02*	3,75^{NS}	56,25*
Concentrations of CEEBH (C)	1,32^{NS}	1,20^{NS}	0,71^{NS}	0,65^{NS}
S x C	0,38^{NS}	0,64^{NS}	0,50^{NS}	1,82^{NS}
CV (%)	54,25	66,82	40,39	40,98

Table 2: *F values of the symptomatic behavior of rust (Puccinia psidii Winter) on eucalyptus seedlings 30 days after application of crude ethanolic extract of banana heart (CEEBH) in previously infected seedlings. Ceres-GO, 2014.*

**: F value significant at 5% probability of error. NS: Not Significant.*

The use of CEEBH has not been shown to be efficient in the control of rust in eucalyptus seedlings, regardless of the species in which it was applied (Table 2). These results open possibilities for investigation of other forms of application and even different methods of preparation of CEEBH, as well as the use of doses higher than those proposed, since *P. psidii* is a pathogen difficult to control even by chemicals with high concentration of active ingredient. Silva [18] emphasizes that the efficiency of plant extracts is associated with the processes involved in their production and manipulation, as well as the infected plant species and the type of pathogen to be controlled.

Other studies have demonstrated that plant extracts present direct antifungal properties [18-20], inhibiting mycelial growth and germination of spores. It has also been observed indirect action of the extract by induction in the production of phytoalexins, indicating the presence of compounds with characteristics of elicitors, triggering a series of alterations in plant cell metabolism that together, contribute to the formation of a network of defense mechanisms of the plant [20]. However, these properties can be affected by factors inherent to the plants, such as organ used to obtain the extract, age, vegetative stage and harvest season. In addition, environmental factors, such as soil pH, season, and temperature can also affect the efficiency of these products.

The use of plant extract in the control of plant diseases has been studied, but in practice it has been used more frequently by farmers practicing organic agriculture. One of the reasons for its low use lies in the difficulty in obtaining the vegetable tissues to be processed and in preparing the extract, because there is a culture of acquiring the finished product. The alternative control of phytopathogenic fungi has been widely discussed in the current context. Many natural products, including extracts and essential oils of medicinal plant, spice and aromatic, have potential for the management of plant diseases. In a study carried by Androcioli [21], with ethanolic propolis extract promising results were found in the alternative control of coffee rust (*Cercospora coffeicola*), although it did not differ from chemical control, indicating the need for compensation in the price of the final product, by consumers to keep the system competitive.

Unfortunately results of field research proving the effect of plant extracts on the rust of Myrtaceae are still incipient, especially for rust in eucalyptus. In this crop, rust damages are of great magnitude, due to the extensive cultivated areas in Brazil and mainly because the incidence and severity vary with geographic location, season of year and existence of juvenile organs in the plant [8]. Due to these limitations and the wide variability inter and intra-specific, rust has been controlled by the planting of resistant materials [9,22], being these restricted. Chemical control has been done only in nurseries, banks and clonal gardens and, exceptionally in the field, for materials of high value silvicultural and technological.

The genetic material differed as to the symptomatic behavior of rust. The *E. urograndis* species was more tolerant to rust (Figure 2), showing a considerable recovery 34.1% (rate of conversion of infected plants in cured plants) at 60 days after inoculation of the pathogen, while *E. citriodora* presented only 2.99%. Silva., *et al.* [23] comparing ten species of eucalyptus to partial resistance to rust also verified that the *E. urograndis* species had lower severity, higher mean latent period and lower values of the area below the disease progression curve in relation to *E. citriodora*, thereby having greater partial resistance to rust.

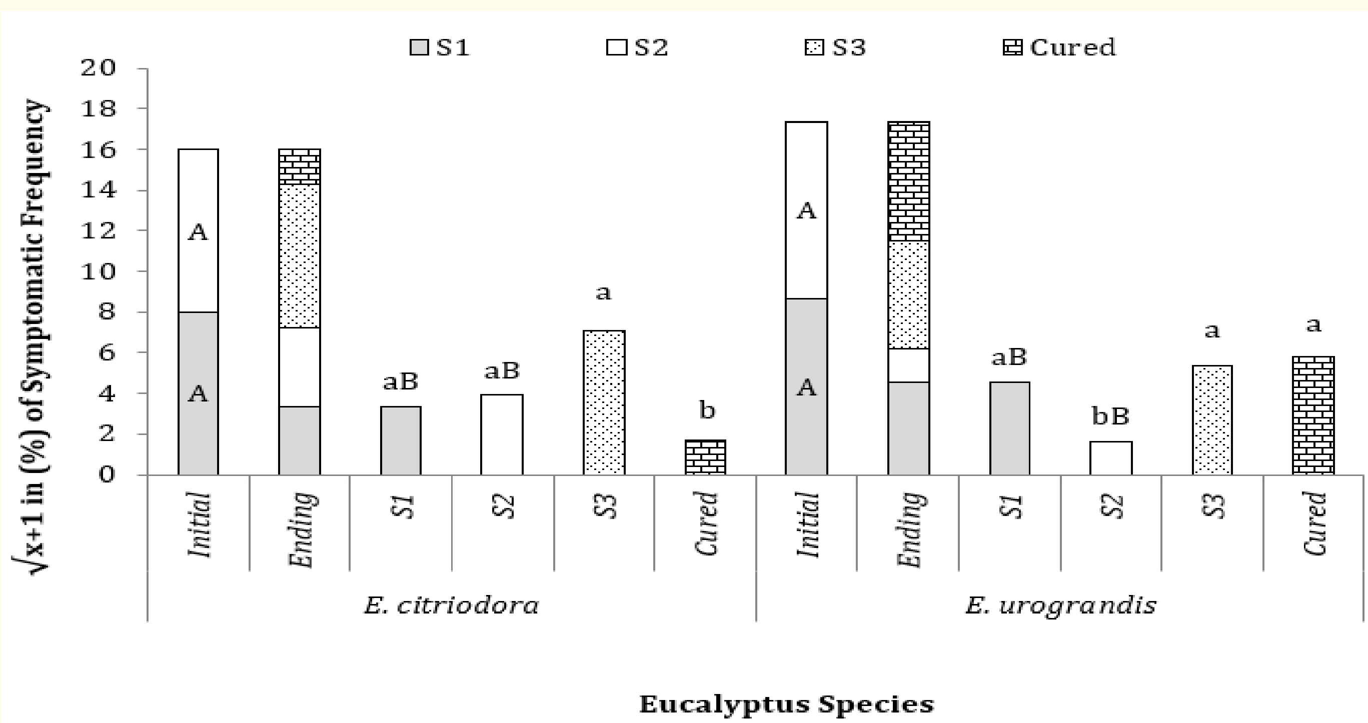

Figure 2: *Symptomatological behavior of mirtaceae rust (Puccinia psidii Winter) on seedlings of two eucalyptus species. Ceres-GO, 2014. Upper case letters compare initial vs. final condition (30 days after application of CEEBH) for symptoms S1 and S2 within each species, while lower case letters compare the final symptomatology among species at the 5% probability level by t-test for paired samples.*

The tolerance and resistance of plants against phytopathogens can occur through structural mechanisms, such as cuticles and sclerenchyma's (which act as physical barriers and inhibit the entry and spread of the pathogen) and biochemical (occurring in plant cells and tissues and produce substances that are either toxic to the pathogen or create conditions that inhibit the development of the pathogen in the plant). However, these mechanisms are genetically determined and their efficiency is dependent on the expression of the same in place and at appropriate times, and should occur after the contact of the pathogen with the host [24]. Studies that elucidate these factors may help to advance the knowledge of resistance to rust conferred by some genotypes, such as *E. urograndis.*

Conclusion

The use of CEEBH is not a viable tool for the control and mitigation of the symptoms of rust of the Myrtaceae on Eucalyptus seedlings.

Eucalyptus seedlings of the species *E. urograndis* are more tolerant to Myrtaceae rust disease than *E. citriodo*ra seedlings, being a favorable option for planting in places where cases of the disease are reported.

Bibliography

1. Furtado EL., *et al*. "Impacto potencial das mudanças sobre a ferrugem do eucalipto no Estado de São Paulo". In: Ghini R and Hamada E (ed) Mudanças climáticas: impactos sobre doenças de plantas no Brasil. Embrapa Informação Tecnológica - Brasília (2008): 273-286.
2. Silva MB., *et al*. "Uso de princípios bioativos de plantas no controle de fitopatógenos e pragas". *Informe Agropecuário* 31.255 (2010): 70-77.
3. Masson MV. "Ferrugem do eucalipto: planejamento evasivo, estimativa de dano e análise da viabilidade do controle químico". Universidade Estadual Paulista "Júlio de Mesquita Filho" Faculdade de Ciências Agronômicas - Botucatu (2009).
4. Joffily J. "Ferrugem do eucalipto". *Bragantia* 4.8 (1944): 475-487.
5. Maclachlan JDA. "Rust of pimento tree in Jamaica". *Phytopatology* 28.3 (1938): 157-170.
6. Ferreira FA and Silva AR. "Comportamento de procedências de *Eucalyptus grandis* e de *Eucalyptus saligna* a ferrugem (*Puccinia psidii*)". *Fitopatologia Brasileira* 7.1 (1982): 23-28.
7. Zauza EAV., *et al*. "Myrtaceae species resistance to rust caused by *Puccinia psidii*". *Australasian Plant Pathology* 39.5 (2010): 406-411.
8. Alfenas AC., *et al.* "Clonagem e doenças do eucalipto". Editora UFV - Viçosa (2004).
9. Zauza EAV., *et al*. "Eficiência de fungicidas sistêmicos no controle da ferrugem do eucalyptus". *Revista Árvore* 32.5 (2008): 829-835.
10. Lorenzetti ER. "Controle de doenças do morangueiro com óleos essenciais e *Trichoderma ssp*". Universidade Federal de Lavras (2012).
11. Borges DI., et al. "Efeito de extratos e óleos essenciais de plantas na germinação de urediniósporos de *Phakopsora pachyrhizi*". *Revista Brasileira de Plantas Medicinais* 15.3 (2013): 325-331.
12. Bettiol W and Ghini R. "Proteção de plantas em sistemas agrícolas alternativos". In: Campanhola C and Bettiol W (ed) "Métodos alternativos de controle fitossanitário. Embrapa Meio Ambiente - Jaguariúna (2003): 80-96.
13. Alvares CA., *et al*. "Köppen's climate classification map for Brazil". *Meteorologische Zeitschrift* 22.6 (2014): 711-728.
14. Alfenas AC and Mafia RG. "Métodos em fitopatologia". Editora UFV - Viçosa (2007).
15. Junghans DT., *et al*. "Escala de notas para quantificação da ferrugem em Eucalyptus". *Fitopatologia Brasileira* 28.2 (2003): 184-188.
16. Rosa EV. "Investigação de processos de proliferação celular do epitélio branquial de Poecilia vivipara exposto a extratos etanólicos de Caryocar brasiliensis". Dissertation, Universidade Federal de Goiás - Instituto de Ciências Biológicas (2004).
17. Ferreira DF. "Sisvar: a guide for its bootstrap procedures in multiple comparisons". *Ciência e Agrotecnologia* 38.2 (2014): 109-112.
18. Silva MB. "Desenvolvimento de produtos à base de extratos de plantas para o controle de doenças de plantas". In: Venezon M., *et al*. (eds.) Controle alternativo de pragas e doenças". EPAIG/CTZM - Viçosa (2005): 221-246.
19. Brum RBCS. "Efeito de óleos essenciais no controle de fungos Fitopatogênicos". Dissertation, Universidade Federal do Tocantins - Gurupi (2012).
20. Schwan-Estrada KRF., *et al*. "Uso de extratos vegetais no controle de fungos fitopatogênicos". *Floresta* 30.1-2 (2000): 129-137.

21. Androcioli HG., *et al*. "Produtos alternativos no controle da *Hemileia vastarix* (Berkeley and Broome) e *Cercospora coffeicola* (Berkeley and Cooke) em cafeeiros". *Coffee Science* 7.2 (2012): 187-197.

22. Kimati H., *et al*. "Manual de fitopatologia: doenças das plantas cultivadas". 4th edition. São Paulo, Agronômica *Ceres* (2005).

23. Silva DEM., *et al*. "Avaliação da resistência de espécies de eucalipto à ferrugem (Puccinia psidii Winter)". *Summa Phytopathologica* 40.1 (2014): 54-62.

24. Pascholati SF. "Potencial de Saccharomyces cerevisiae e outros agentes bióticos na proteção de plantas contra patógenos". Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo - Piracicaba (1998).

Volume 4 Issue 2 December 2018