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Bio-Fungicide Potential of *Araucaria Columnaris* (Cook Pine) Aqueous Resin Extract Against Major Phytopathogens

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Abstract: Use of chemical fungicide to control plant diseases causes several adverse effects such as, development of resistance in the pathogen, residual toxicity, pollution to the environment etc. So an alternative way to overcome the usage of dreadful chemicals is very important. The use of plant extracts as biofungicide is one of the popular and effective method. *Araucaria columnaris* is a commonly seen ornamental plant known as Christmas tree. It's a South African species, under the family Araucariaceae. Hence, in the present study, the plant resin extract was tested *in-vitro* against major plant pathogensby preliminary bioassay. It was found that up to 95% reduction of mycelium growth was observed against major phytopathogens such as *Fusarium oxysporyum*, *Rhizoctonia*sp, *Cylindrocladium*sp, *Alternaria*sp, and *Colletrotricum* sp., causing tomato wilt, damping off, foliage blight, and leaf blight diseases in economically important plants. Up to our knowledge it is the first report showing the antifungal activity of *Araucaria columnaris* resin as antifungal agent. By using this valuable tree, further work will be done to formulate commercial biofungicide.

Keyword: Biofungicide, *Fusariumoxysporum*, *Rhizoctonia*sp, *Cylindrocladium*sp, *Alternaria*sp, *Colletrotricum* sp.

INTRODUCTION

Auracaria columnaris is a commonly seen ornamental plant (local name Christmas tree). It comes under the family Araucariaceae, is divided into two genera, *Agatis* and *Araucaria*, including 38 species distributed throughout the southern hemisphere[1]. [2]Wilson *et al.* says that the natural products derived from plants (secondary metabolites) can control diseases and it is an economical and efficient alternative, since it does not affect environment and their residues are easy to degrade. The systematic search of higher plants for antifungal activity has shown that plant extracts have the ability to inhibit spore germination and mycelial growth in many fungal species[3, 4]. According to the survey made by the WHO, more than 50,000 people in developing countries are annually poisoned and 5,000 die as a result of the effects of toxic agents, used in agriculture. In India 35,000 – 40,000 tons of hazardous chemicals are sprayed on the crops every year, instead of helping the poor, these chemicals are causing cancer, sterility and death[5]. Keeping these problems in view, efforts are in progress to search cost-effective safe phytochemicals, which could be utilized for disease control. In the present study aqueous extract of plant resin was used to test for its antifungal activity in *in-vitro* condition. Resin is a hydrocarbon secreted by many plants, particularly coniferous trees, valued for its chemical constituents and is used in varnishes and adhesives. Plants produce resins for various reasons whose relative importance is debated. It is known that resins seal plant's wounds, kill insects and fungi, and also allow the plant to eliminate excess metabolites[6]. So the aim of the study is to formulate value added product from this ornamental plant.

MATERIALS AND METHODS

Collection of plant resin: Healthy disease free, mature plant resin of *Araucaria columnaris*(commonly known as Christmas tree) were collected form garden of Nehru Arts and Science College, Coimbatore, Tamil Nadu, India, and it was used for the preparation of aqueous extract. The plant *Araucaria columnaris* was identified taxonomically and authenticated by the Institute of forest genetics and tree breeding center (IFGTB), R.S. Puram, Coimbatore. The resin samples were shade dried, powdered and stored in polypropylene air-tight containers under proper conditions for further uses.

Test pathogens: Five major phytopathogens such as *Fusarium oxysporium*, (causal agent of tomato wilt) *Rhizoctonia*sp, (causual agent of damping off), *Cylindrocladium*sp,(causual agent of foliage blight), *Alternaria*sp,(causal agent of leaf blight) and *Colletrotricum*sp, (causual agent of anthracnose) were used for the present study. It was maintained in Potato dextrose agar slants at 4 ^{o}c.

Preparation of the resin extracts: The crude powdered resin samples were weighed and subjected to aqueous extraction for 8-10 hrs repeatedly using Soxhelt apparatus. The extract was then concentrated at 40-45°C and air dried. The dried samples were then stored in air tight bottles at 4°C for further analysis.

ANTIFUNGAL ASSAY

Mycelium Reduction Test: The extracts were screened for antifungal activity by agar well diffusion method with little modification. Potato dextrose agar (PDA) plates were prepared and fungal discs (7mm) were placed at the centre. Four wells of 5 mm were cut around the fungal disc using sterile cork borer. The wells were loaded with extracts and for each fungus separate negative control plates were kept. The plates were incubated at 28 ±2 ^{0}C for 2 to 9 days and fungal mycelial growth was recorded periodically at 2 day intervals. The mycelial growth was compared with negative control and growth reduction was calculated using the formula[7].

$$\% \text{ of mycelial reduction} = \frac{(C-T)}{T} X\,100$$

Where, C - Growth in control, T-growth in test.

Poisoned food technique: Antifungal assay was carried out using Poisoned food technique of Grover and Moore[8]. 2ml of plant extracts were poured in sterilized petri dish containing molten PDA medium. A positive and negative control was maintained for each fungi.7.0 mm of fungal disc was inoculated in the center of each assay plate which was then incubated at 28 ±2 ^{0}C for 7 days. The colony diameter in the treatments as well as in the control sets was measured and the percentage inhibition of mycelial growth was calculated. Each experiment contained three replicates and was repeated twice.

RESULT AND DISCUSSION

The plant fungal diseases are traditionally been controlled by chemical fungicides. The development of resistant strains of pathogens against various chemical fungicides and their toxic properties make the use of these chemicals limited[9]. The use of plants or plant material as fungicide is of great importance, which needs more attention[10].Various plant products like gums, oil, resin etc are used as fungicidal[11, 12]. In current study significant effect of *Araucaria columnaris* resin extracts was found to be on major action against phytopathogensin reducing their mycelial growth. The aqueous extract of resin showed the maximum mycelial reduction in case of *Fusarium oxysporum* followed by *Alternari*a sp (99% and 95%) and no inhibition of mycelial was observed in *Aspergillus*sp and it was shown in **Figure 1**.In case of Poisoned food technique maximum growth inhibition was found in *Fusarium oxysporum* and *Alternaria* sp compared with others. No growth inhibition was observed in *Aspergillus* sp and it was shown in **Table 1**. But compared with both the technique mycelium retardation was found to be maximum in well diffusion method, it may be due to the fine diffusion of resin aqueous extract into agar plate.

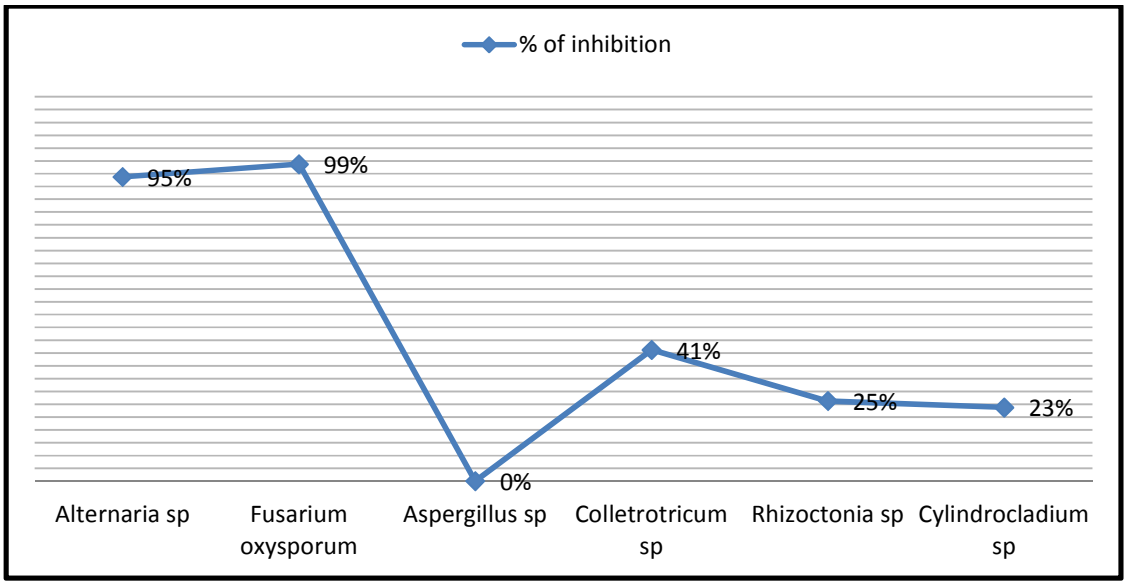

Figure 1: Percentage inhibition of aqueous resin extract of *A. columnaris* against phytopathogens.

Table-1: Antifungal assay using Poisoned plate technique.

Sl. No.	Test organisms	Average colony diameter in control plates (cm)	Average colony diameter in plates with extract (cm)
1	*Alternaria*sp	4	3
2	*Fusariumoxysporum*	3.9	2.8
3	*Aspergillus*sp	8	8
4	*Colletrotricum*sp	4.3	4
5	*Rhizoctonia*sp	4.4	4.1
6	*Cylindrocladium*sp	4	3.8

***Mean value of 3 replicates**

Carlos *et al.*[13]. reported that lignans found in the *Araucaria araucana* heart wood extractives act on white rotting and wood staining fungi and show antibacterial and antifungal activities. This could indicate that such metabolites can play an important role in the wood's natural preservation. The various effect of phytopathogen against different plant extracts is due to plant species and solvent used for phytochemicals extraction[14-16].Several higher plants and their constituents have shown success in controlling plant diseases while proving to be harmless and non-phytotoxic[17-19]. A variation in the antifungal effectiveness of different extracts against different fungi was most likely due to difference in the nature of the inhibitors materials they contained[20-22]. Hasson *et al.*[23].worked on antibacterial and antifungal activity of three *Boswellia* species, it was found that the methanolic extract of oleo gum resin showed maximum inhibition on bacterial and fungal strains. These results are clear indication for the possible use of crude aqueous extract from *Araucaria columnaris*resin to control fungal pathogens.

CONCLUSION

Plant products are attractive alternatives to synthetic products because of biocompatibility, low toxicity, environmental friendliness and low price compared to synthetic products. Our aim of the study is to investigate the importance of *Araucaria columnaris* resin extract for its biofungicide property. Further research on isolation, characterization of active constituents are required to identify the compounds responsible for its bioactivity, to be developed as best biocontrol agent, thus hoping for the formulation of bioproduct in the future.

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