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In vitro Evaluation of *Cymbopogon nardus* Essential Oil against Leaf Disease Fungus of Narra (*Pterocarpus indicus* Wild)

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Abstract

The efficacy of the essential oil from Cymbopogon nardus as antifungal agent of Pestalotia sp. which causes leaf spot disease of narra (Pterocarpus indicus wild*) was tested in vitro. Mycelial growth was evaluated in different concentrations of essential oils (0.02%, 0. 1%, 1%, 2%, and 5% v/v). Fungal growth inhibition was significantly observed in all concentrations. However, in the absence of the essential oil, the pathogenic fungi had a normal development in 10 days of incubation. The present study revealed that essential oil of C. nardus is a promising natural means of controlling the development of the pathogenic fungi of P. indicus.*

Keywords: *Pestalotia* sp., essential oil, antifungal, leaf disease, *Pterocarpus indicus.*

Introduction

Leaf spot caused by *Pestalotia* sp. is a common disease of Narra (*Pterocarpus indicus* Wild), an endemic tree species in the Philippines. The pathogen is also associated with other diseases of forest trees such as seed disease in *Agathis philippinensis*, *Eucalyptus deglupta* and *Acacia mangium*[1]. The pathogenicity of *Pestalotia* sp. in *P. indicus* is high showing a germ tube directly penetrated through the epidermis and infection hyphae in the mesophyll cells[2]. The genus *Pestalotia* is also known to cause leaf disease in *Vitellaria paradoxa*, *Nauclea latifolia*, *Daniellia oliveri,* and *Cocos nucifera*[3,4].

Synthetic fungicides were used in the past to control, if not eradicate or prevent, the occurrence of the pathogen in *P. indicus*. However, there has been a growing concern on the effects of these chemical substances to the health of organisms and the environment[5,6]. This has led to alternative means of pathogen management to minimize the use of synthetic fungicides. These include the use of biocontrol agents, essential oils with antifungal properties, and resistance genes for genetic engineering.

Essential oils of *Cymbopogon* species are known to possess antifungal properties. A number of studies have already been conducted showing inhibition of growth and development of several pathogenic fungi such as *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Candida albicans*, *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Helminthosporium* sp., *Trichoderma harzianum, Fusarium oxysporum*, *Phytophthora* sp. through essential oil from the species[7-13]. Chemical composition and biological activities of *Cymbopogon* essential oils were discussed in several studies[14,15]. However, there is limited information on the effectiveness of these essential oils to control the leaf spot disease-causing fungi of *P. indicus*. This present study aims to evaluate *in vitro* the efficacy of essential oil from *Cymbopogon nardus* at different concentrations against *Pestalotia* sp. causing leaf spot disease in *P. indicus*.

Material and Methods

The experiment was conducted at the Forest Pathology Laboratory of the College of Forestry and Natural Resources, University of the Philippines Los Baños, College, Laguna, Philippines. The fungus *Pestalotia* sp. from infected leaves of *P. indicus* showing typical symptoms of leaf spot disease was cultured using potato dextrose agar (PDA) medium for three to five days. Re-isolation was done to acquire pure cultures of the fungus. These isolates served as aseptically maintained cultures of the pathogen that was used in the study.

The essential oil from citronella (*Cymbopogon nardus* (L.) Rendle) of the Gold in Grass Corporation was used in the study. The antifungal activity of essential oils was tested through dilution to PDA. The essential oil was first added with 0.1 mL (1%) Tween 20 and then mixed with 9.9 mL of PDA in each Petri dish before solidification to produce five concentrations (0.02%, 0.1%, 1%, 2%, and 5% v/v). A PDA disc of 0.7 cm diameter with the mycelium of *Pestalotia* sp. was placed in the center of the Petri dish (5 cm diameter) and incubated at room temperature (27°C ± 1) for 10 days. For the control, the mycelium was placed in a Petri dish with PDA mixed with 0.1 mL Tween 20. Radial growth of the mycelium was then recorded and percent inhibition of growth (antifungal index) was computed following the formula[16]:

$$\text{Growth inhibition (\%)} = \left(\frac{\text{DC} - \text{DT}}{\text{DC}}\right) * 100$$

where DC is the diameter of growth zone in the control plate and DT is the diameter of the growth zone of the test plate.

The essential oil experiment was laid out in a completely randomized design with five replications. Analysis of Variance (ANOVA) was conducted through SAS statistical software.

Results and Discussion

The leaf spot of *P. indicus* is characterized by brown lesions in the leaf blade. Black fruiting bodies are also visible in some of the lesions. Sometimes, two or more leaf spots coalesce to form anthracnose. Yellowing also eventually occurs until death of leaves. When grown in the culture media, the fungi produced a white cottony mycelium (figure -1). Under the microscope, the species identified as *Pestalotia* sp. has fusiform and septated conidia with branched appendages (figure - 2). Spots can occur in leaves of both seedling and mature stages of the host plant *P. indicus.*

Figure-1
The leaf spot disease of narra (*Pterocarpus indicus*)

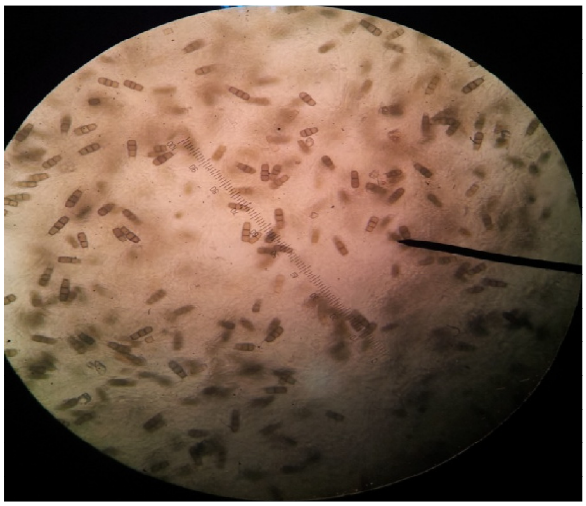

Figure-2
The conidia of *Pestalotia* sp. taken from the leaf spot of *Pterocarpus indicus*. (400 X magnification)

The essential oil effectively inhibited the mycelial growth of *Pestalotia* sp. In the control plates, the fungi grew well and had a normal development for 10 days. On the other hand, mycelial growth was significantly inhibited in all concentrations of the essential oil after 10 days of incubation (figure - 3). Growth inhibitions were insignificantly different among 1% to 5% concentrations of the essential oil (table - 1). After 10 days of incubation, the lowest concentration to completely inhibit the mycelial growth of *Pestalotia* sp. was 1% v/v. In a previous trial experiment (not shown), complete growth inhibition of the fungi was observed in larger volumes of the essential oil (25% and 50% v/v).

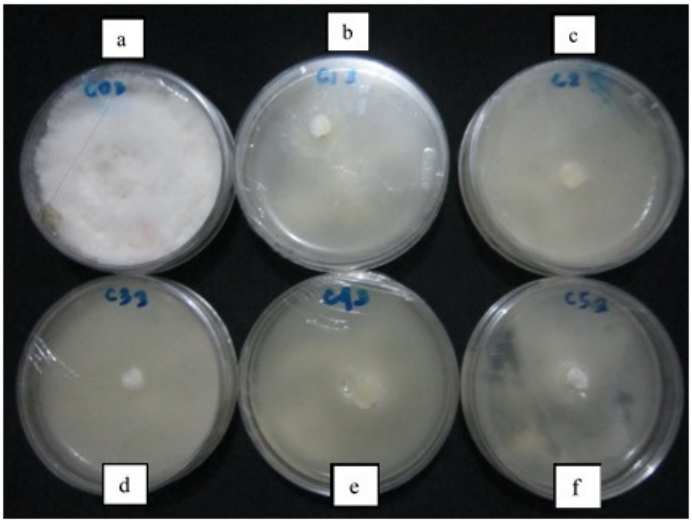

Figure-3
Mycelial growth of *Pestalotia* sp. of *Cymbopogon nardus* essential oil at different concentrations: (a) 0% (control), (b) 1%, (c) 2%, (d) 5%, (e) 0.1%, and (f) 0.02% v/v after 10 days

Table-1
Effect of *Cymbopogon nardus* on growth of *Pestalotia* sp. isolated from *Pterocarpus indicus* after 10 days of incubation

Concentration (% v/v)	Growth inhibition percentage*
Control	0.00 c
5	100.00 a
2	100.00 a
1	100.00 a
0.1	99.56 ab
0.02	99.00 b

*Growth inhibition percentage means with the same letter are not significantly different

Essential oil of *C. nardus* contains antibacterial and antifungal properties which make the oil an effective inhibitor of pathogenic microorganisms. While several studies have shown differences in chemical compositions and concentrations, all have agreed that citronellal is the major component of the oil[17]. The citronellal and linalool, followed by α- and β- pinenes were found to be effective in the growth inhibition of fungi[18]. Crude

extracts of *C. nardus* had also shown effectiveness in reducing fungal growth[19, 20].

Essential oils from *Cymbopogon* species have exhibited differences in fungitoxicity at varied concentrations. Sporulation and germination of three fungal species namely *Pyricularia grisea*, *Aspergillus* spp., and *Colletotrichum musae* were affected at 0.15 mg/mL of *C. nardus*[21]. Even small concentrations of *C. citratus* essential oil (0.125%) had efficiently inhibited the growth and spore formation of *A. niger*[22]. This present study shows that even at low concentrations, *C. nardus* has a huge potential to inhibit leaf disease of narra caused by *Pestalotia* sp. Fungitoxicity also was retained for longer periods (210 days) at varied temperature conditions[23]. The ability of these oils to control pathogens at the lowest possible concentration and at longer duration makes it an economically efficient means in controlling pathogen development.

Cymbopogon essential oils are known for its biological and pharmacological importance. The mechanisms of essential oils generally include disruption of cellular structure, damage cellular macromolecules, deterioration of important organelles, and disturbance in the metabolic pathway[14,15]. These mechanisms provide an advantage of essential oils in the management and control of several plant diseases. Several studies have also shown the efficiency of essential oil of *Cymbopogon* species against other microorganisms such as systematic bacteria from aquatic animals and other health-related microorganisms[17,22]. Essential oils from other species have also exhibited antibacterial properties. *Cinnamomum zeylanicum* essential oil was effective in inhibiting *Escherichia coli* and *Staphylococcus aureus*[24].

The natural means of controlling pathogenic fungi have gained attention as environmental and health awareness increase. Essential oils improved the germination of *Oryza sativa* seeds while controlling infection of seed-borne fungi[25]. Essential oils with antifungal properties also have the potential to protect the preservation of foods. Essential oils have the potential to inhibit growth and development of pathogen without damaging effects to the host. Moulds from traditional cheese wagashi had shown sensitivity to *Ocimum gratissimum* and *Cinnamomum zeylanicum* essential oils while fermentation and adulteration of a local drink was prevented through addition of *C. citratus* essential oil[26, 27, 28]. Duration of storage of smoked mackerel was also increased with application of essential oil from *Syzigium aromaticum*[29]. Foods were also protected against stored food fungi for a long duration after treatment with *Cymbopogon* essential oil without causing any harm after being fed to animals[23].

Conclusion

The essential oil of *C. nardus* is effective in inhibiting the mycelial growth of *Pestalotia* sp. which causes leaf spot disease in *Pterocarpus indicus*. This present study shows the potential of *C. nardus* essential oil as a natural alternative means in controlling diseases in forest trees. The oil has also an economic potential for antifungal use. Further study is needed to investigate its application *in vivo*.

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