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# Assessment on antifungal activity of teak and beetel leaves extract against *Colletotrichum gloeosporioides* (penz.) Sacc. Causing leaf spot on *murraya koenigii* (l.) Spreng

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## Abstract

*Murraya koenigii* is considered as a medicinal plant as well as leafy vegetable. Leaf spot disease of *Murraya koenigii* caused by *Colletotrichum gloeosporioides* appears to be a destructive epidemic in many regions of India. Successful pathogenicity of the fungus on curry leaf was proved by Koch's Postulate method. The study reveals the isolation of this pathogen from the curry leaves on PDA medium and showing their inhibitory effect on teak and beetel leaves extract. The petroleum ether leaf extracts of these two plants were eluted using column chromatography to evaluate the antifungal property against *Colletotrichum gloeosporioides*. Antifungal activity of these leaf extracts were studied with four concentrations using food poison method. The data revealed that significant reduction in the growth of *Colletotrichum gloeosporioides* was observed with leaf extract of these plants and the extract showed significant difference in their efficacy. Among four concentrations, the highest percentage of inhibition shown at 20 µl followed by 15 µl, 10 µl and lowest inhibition was recorded at 5µl as compared to control. Statistically significant decrease in the radial mycelia growth of *Colletotrichum gloeosporioides* was observed with increase in concentration of diffusates. Thus the results suggest that the average increase of 10% inhibition in every concentration supports to prevent the complete mycelia growth at a particular concentration.

**Keywords:** *Murraya koenigii*, *Colletotrichum gloeosporioides,* PDA medium, petroleum ether

## Introduction

*Murraya koenigii* (L.) Spreng. is a medicinal plant, belongs to the family Rutaceae, commonly called "curry leaf" (Zachariah et al., 2009). Curry leaf is an important plant which is known for its culinary and medicinal value. Leaves are widely used for flavoring food stuffs (Joseph and Peter, 1985). But it is highly susceptible to fungal pathogen exhibiting leaf spot disease. Epidemics of these fungal leaf spot disease with characteristic necrotic spots were evident during the onset of monsoon season every year. Preliminary diagnosis of the disease revealed that the pathogen is fungus *Colletotrichum* with the formation of fruiting bodies visible as black globose bodies in necrotized tissues of the leaves and

the twigs, seen often with the presence of setae (Midhila Padman *et al*., 2011). Diseases caused by *Colletotrichum* species occur on a wide range of plant species and have been recorded worldwide as both pre and post harvest causes of crop loss (Jeffries, *et al*., 1990). To control these pathogen, frequent application of fungicides is required to the developing crops leading to chemical resistance by the fungi and environmental contamination by the chemicals (Griffee, 1973).Extracts isolated from several plants have been reported to have biological activity, such as anti-microbial, anti-fungal, anti-inflammatory and anti-oxidant activity (Sexena, 1983). Hence an attempt has been made to test some of commonly available botanical agents against the pathogen isolated from the leaf spot of *Murraya koenigii*. As a result the isolation, identification and colony growth of fungus *Colletotrichum* species has been carried out which were assessed for antifungal property of petroleum extract of teak and beetel leaves.

## Materials and methods

### Plant collection:

The samples of diseased curry leaves showing the symptoms of leaf spot were collected from different localities of Thrissur.

### Isolation of plant pathogenic fungus from diseased plant material:

Select infected plant parts. Cut into small pieces containing both diseased and healthy tissue and keep in sterile Petri dishes. For surface sterilization, dip the sample tissues in 1% mercuric chloride for about one minute and wash thoroughly using sterile water to free them from the chemicals if any. PDA media is used for the colony growth of fungus. Place four sterilized pieces of diseased leaf materials at different distance in single PDA plate. The inoculated plates were wrapped with high quality parafilm and kept in an inverted position and examine for 3-5 days. After incubation, colonies appeared are subcultured (Johnson and Chandra Sekhar, 2012).Slide culture technique has also been used for culturing fungal colonies.

### Identification of fungus

The isolated fungi were studied by using both simple and compound microscope. To confirm the fungi, isolated culture was sent to Kerala Agriculture University Vellanikkara for identification.

### Pathogenicity test

In order to prove Koch's postulates, pathogenicity test was carried out by inoculating the pathogen into fresh leaves the infection was recorded after 15 days. Leaf spots regions from diseased plants were surface sterilized, sectioned, and plated on Potato Dextrose Agar (PDA) for re-isolation and confirmation of the pathogen.

### Antifungal Screening

Column chromatographic method is used for the preparation of teak and betel leaves extracts. Petroleum ether extracts of these plant extracts were screened for antifungal activity by Poisoned food technique (Schmitz, 1930). For this various concentrations of selected crude leaf extracts were prepared (5µl, 10µl, 15µl, 20µl). 20 ml PDA mixed with requisite amount of extracts and poured into Petri dish. After the solidification of media, a mycelial plug (5mm) taken from the edges of 9 days old culture were put in the center of the PDA. Three Petri dishes were prepared for each concentration. PDA plates mixed with sterile distilled water are served as negative controls. The inoculated plates were incubated at room temperature for 9 days and the diameters of fungal colonies were measured. Inhibition of mycelial growth was calculated using the following formula:

Percentage of mycelial growth inhibition = **((a-b)/ a) × 100**

a = Mean diameter of fungal colony in control.
b =Mean diameter of fungal colony in plant extracts.

**Statistical analysis**
All data subjected to analysis of variance (ANOVA) using the general linear models procedure. The data of diameter of fungal colony at different concentrations compared and determined using POSTHOC=LSD test at $P \leq 0.05$ using SPSS software.

**Results and discussion**
The result of the experiments on isolation, identification and proving pathogenicity of fungus causing leaf spots in curry leaves are presented here:

**Antifungal assay:**
The antifungal activity of teak and betel leaf extracts was assayed, at four concentrations in the laboratory for their efficacy against the C. gloeosporioides using Food poison technique.

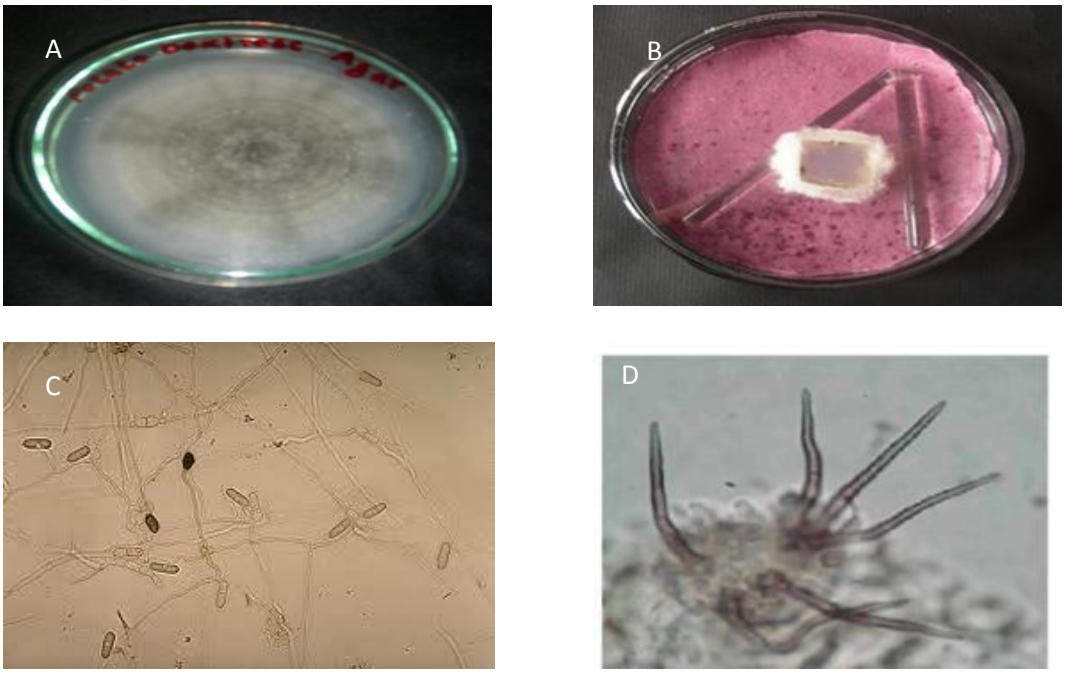



**Fig. 1: Identification of pathogen-A: Pure culture of *C. gloeosporioides* on PDA, B: Conidia of *Colletotrichum gloeosporioides*, C: Slide culture method, D: Acervuli**

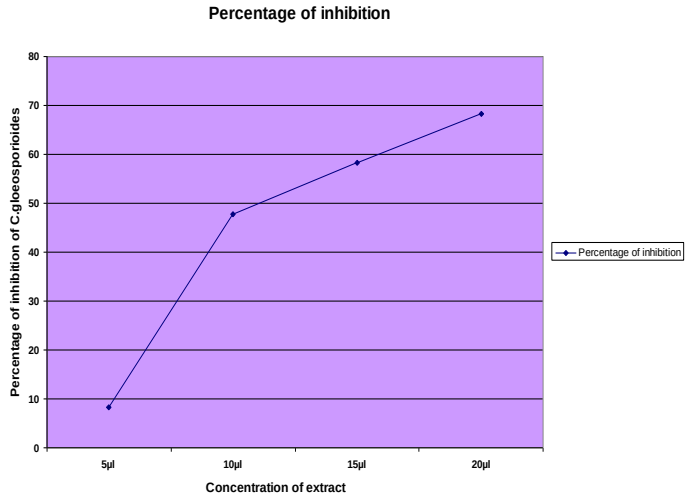
**Table 1: Percentage of inhibition of *C. gloeosporioides* by Teak leaf extract.**

| Sl No | Concentration of leaf extract | Experiment 1 | Experiment 2 | Experiment 3 | Mean diameter | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 5 µl | 55mm | 55mm | 55mm | 55mm | 8.33 |
| 2 | 10 µl | 34mm | 30mm | 30mm | 31.3 mm | 47.83 |
| 3 | 15 µl | 25mm | 25mm | 25mm | 25mm | 58.33 |
| 4 | 20 µl | 19mm | 19mm | 19mm | 19mm | 68.33 |
| 5 | Control | 60mm | 60mm | 60mm | 60mm | -- |

**Table 2: Percentage of inhibition of *C. gloeosporioides* by Betel leaf extract.**

| Sl No | Concentration of leaf extract | Experiment 1 | Experiment 2 | Experiment 3 | Mean diameter | % of inhibition |
|---|---|---|---|---|---|---|
| 1 | 5 µl | 58mm | 56mm | 56mm | 57.3mm | 4.5 |
| 2 | 10 µl | 40mm | 40mm | 40mm | 40mm | 33.33 |
| 3 | 15 µl | 35mm | 36mm | 36mm | 36mm | 40 |
| 4 | 20 µl | 29mm | 29mm | 29mm | 29mm | 51.66 |
| 5 | Control | 60mm | 60mm | 60mm | 60mm | -- |

**Graph 1: Percentage of inhibition of *C. gloeosporioides* by Teak (*Tectonagrandis*) leaf extract.**




**Graph 2: Percentage of inhibition of C. gloeosporioides by Betel (*Piper betle*) leaf extract.**

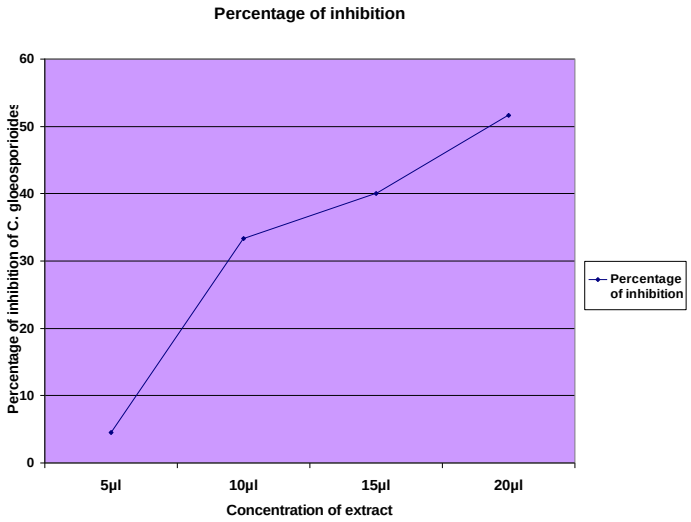


**Table 3: Statistical result of Teak leaf extracts-ANOVA.**

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 4042.267 | 4 | 1010.567 | 947.406 | .000 |
| Within Groups | 10.667 | 10 | 1.067 | | |
| Total | 4052.933 | 14 | | | |

Post Hoc Test - LSD

Multiple Comparisons

| (I) VAR00002 | (J) VAR00002 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 5 µl | 10µl | 23.66667* | .84327 | .000 | 21.7877 | 25.5456 |
| | 15 µl | 30.00000* | .84327 | .000 | 28.1211 | 31.8789 |
| | 20 µl | 36.00000* | .84327 | .000 | 34.1211 | 37.8789 |
| | control | -5.00000* | .84327 | .000 | -6.8789 | -3.1211 |
| 10µl | 5 µl | -23.66667* | .84327 | .000 | -25.5456 | -21.7877 |
| | 15µl | 6.33333* | .84327 | .000 | 4.4544 | 8.2123 |
| | 20 µl | 12.33333* | .84327 | .000 | 10.4544 | 14.2123 |
| | control | -28.66667* | .84327 | .000 | -30.5456 | -26.7877 |
| 15µl | 5 µl | -30.00000* | .84327 | .000 | -31.8789 | -28.1211 |
| | 10 µl | -6.33333* | .84327 | .000 | -8.2123 | -4.4544 |
| | 20µl | 6.00000* | .84327 | .000 | 4.1211 | 7.8789 |
| | control | -35.00000* | .84327 | .000 | -36.8789 | -33.1211 |
| 20 µl | 5µl | -36.00000* | .84327 | .000 | -37.8789 | -34.1211 |
| | 10 µl | -12.33333* | .84327 | .000 | -14.2123 | -10.4544 |
| | 15 µl | -6.00000* | .84327 | .000 | -7.8789 | -4.1211 |
| | control | -41.00000* | .84327 | .000 | -42.8789 | -39.1211 |
| control | 5 µl | 5.00000* | .84327 | .000 | 3.1211 | 6.8789 |
| | 10 µl | 28.66667* | .84327 | .000 | 26.7877 | 30.5456 |
| | 15µl | 35.00000* | .84327 | .000 | 33.1211 | 36.8789 |
| | 20µl | 41.00000* | .84327 | .000 | 39.1211 | 42.8789 |

***The mean difference is significant at the 0.05 level

**Table 4: Statistical result of Betel leaf extracts- ANOVA.**

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2230.267 | 4 | 557.567 | 1672.700 | .000 |
| Within Groups | 3.333 | 10 | .333 | | |
| Total | 2233.600 | 14 | | | |

Post Hoc Tests-LSD

Multiple Comparisons

| (I) VAR00002 | (J) VAR00002 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 5 µl | 10 µl | 17.33333* | .47140 | .000 | 16.2830 | 18.3837 |
| | 15 µl | 21.66667* | .47140 | .000 | 20.6163 | 22.7170 |
| | 20 µl | 28.33333* | .47140 | .000 | 27.2830 | 29.3837 |
| | control | -2.66667* | .47140 | .000 | -3.7170 | -1.6163 |
| 10µl | 5l µl | -17.33333* | .47140 | .000 | -18.3837 | -16.2830 |
| | 15 µl | 4.33333* | .47140 | .000 | 3.2830 | 5.3837 |
| | 20µl | 11.00000* | .47140 | .000 | 9.9496 | 12.0504 |
| | control | -20.00000* | .47140 | .000 | -21.0504 | -18.9496 |
| 15 µl | 5 µl | -21.66667* | .47140 | .000 | -22.7170 | -20.6163 |
| | 10 µl | -4.33333* | .47140 | .000 | -5.3837 | -3.2830 |
| | 20 µl | 6.66667* | .47140 | .000 | 5.6163 | 7.7170 |
| | control | -24.33333* | .47140 | .000 | -25.3837 | -23.2830 |
| 20 µl | 5 µl | -28.33333* | .47140 | .000 | -29.3837 | -27.2830 |
| | 10 µl | -11.00000* | .47140 | .000 | -12.0504 | -9.9496 |
| | 15 µl | -6.66667* | .47140 | .000 | -7.7170 | -5.6163 |
| | control | -31.00000* | .47140 | .000 | -32.0504 | -29.9496 |
| control | 5 µl | 2.66667* | .47140 | .000 | 1.6163 | 3.7170 |
| | 10 µl | 20.00000* | .47140 | .000 | 18.9496 | 21.0504 |
| | 15 µl | 24.33333* | .47140 | .000 | 23.2830 | 25.3837 |
| | 20 µl | 31.00000* | .47140 | .000 | 29.9496 | 32.0504 |

*. The mean difference is significant at the 0.05 level.

Statistical analysis done by multiple comparison of concentration of plant extracts and colony diameter of *C. gloeosporioides* which proved that diameter of the fungal colony decreased by increasing the concentration of plant extracts, when compared with control. The petroleum ether leaf extracts of these plants at 20 µl concentration showed higher mean differences. It indicates that at this concentration the growth of pathogen *C. gloeosporioides* lesser, compared with other concentrations.

## Discussion

The present investigation revealed that the petroleum ether extracts of four plants showed the antifungal property against *Colletotrichum gloeosporioides* causing leaf spots on curry leaf. The antifungal efficiency has been determined by repeating the experiment with different concentration of leaf extract using food poison technique. As a result, we found that the increase in concentration of plant extract increases the percentage of fungal growth inhibition. The maximum, percentage of inhibition is exhibited by 20µl of Teak (68.33%) followed by Betel (51.66%).Statistical analysis done by multiple comparison of diameter of fungal growth and concentration of plant extracts revealed that radial mycelial growth of fungus gradually decreased with an increase in concentration. The increase in concentration of plant extracts showed gradual decrease of fungal mycelial growth. So at a particular concentration of extracts we can prevent the complete mycelial growth in the medium. This concentration of the extract will be approximately below the concentration of 50µl and thereby the extract of these plants can used as a natural fungicide component for the growth of *Colletotrichum gloeosporioides* in curry leaf.

## Future scope

The study suggests that petroleum ether extracts of screened plants would be helpful in treating the fungal diseases in plants caused by *Colletotrichum gloeosporioides.* A further study is needed to isolate and identify the active compounds that are responsible for antifungal activity against *Colletotrichum gloeosporioides.*

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