*Research Article* *Journal of Plantation Crops, 32 (Suppl.), December 2004 : 321-329*

# Evaluation of botanicals against major pathogens of coconut leaf rot disease and their antagonistic organisms

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**Abstract**

The leaf rot, caused by a group of fungi (*Colletotrichum gloeosporioides*, *Exserohilum rostratum*, *Fusarium solani* etc.), is a part of coconut root (wilt) disease complex. Extracts of seventeen plants were evaluated *in vitro* against the major pathogens of leaf rot, and also their effect on two bacterial (*Bacillus subtilis* and *Pseudomonas fluorescens*) and a fungal (*Trichoderma harzianum*) antagonists by poisoned food technique in various experiments. All plants tested inhibited the major pathogens of leaf rot to various extents. Extracts of *Adenocalymma allicea*, *Lawsonia inermis*, *Curcuma longa*, *Cinnamomum zeylanicum*, *Ocimum sanctum*, *Azadirachta indica* and *Allium sativum* inhibited the pathogens. Acetone extraction of the plants expressed higher inhibition of the pathogens over alcohol and aqueous extractions. Fungicidal and fungistatic effects of plant extracts on the pathogens were also observed. The inhibitory effect of plant extracts on antagonists was generally less than that on the pathogens. *B. subtilis* expressed relatively more tolerance to plant extracts. It is the first report on inhibition of leaf rot pathogens by plant extracts.

*Key words* : Coconut, root (wilt), leaf rot, disease complex, pathogens, antagonists, disease control, plant extracts

## Introduction

Lear rot is a part of coconut root (wilt) disease complex, endemically prevalent in southern districts of Kerala state. Its incidence in Northern districts of Kerala and in certain districts of Tamil Nadu, bordering Kerala, is also of concern (Srinivasan, 2002). Leaf rot is due to a group of fungi wherein *Colletotrichum gloeosporioides*, *Exserohilum rostratum* and *Fusarium solani* are the major pathogens (Srinivasan and Gunasekaran, 2000a). The leaf rot contributes significantly in the deterioration of root (wilt) affected palms and thus the control of leaf rot forms an integral part in root (wilt) management strategies. Though fungicidal control of leaf rot has been recommended, the necessity of eco-friendly measures in the disease management has been widely recognized in view of environmental considerations (Srinivasan and Gunasekaran, 1998, 2000b; Srinivasan, 2003; Gunasekaran *et al.*, 2003; Joseph *et al.*, 2003). Various plant extracts have shown to inhibit the growth of different groups of plant pathogens (Dhaliwal *et al.*, 2002; Muralidharan *et al.*, 2003). Information on efficacy of botanicals against the pathogens of leaf rot disease in coconut is lacking. The results of the evaluation of botanicals against the major pathogens of leaf rot disease and also their effect on certain bacterial and fungal antagonistic organisms are presented in this paper.

## Materials and Methods

Fresh cultures of *C. gloeosporioides*, *E. rostratum* and *F. solani*, isolated from leaf rot affected palms and grown in potato dextrose agar (PDA) medium, and standard cultures of two bacterial antagonists (*Bacillus subtilis* and *Pseudomonas fluorescens*) and a fungal antagonist (*Trichoderma harzianum*) were utilized in the study. The plant species and their parts used in different experiments are given in Table 1.

The plant species were selected based on their known efficacy of inhibiting the fungal growth and their availability in the locality of study. Fresh and healthy bulbs of *A. sativum*, rhizomes of *C. longa* and *Z. officinale*, and oil of *A. indica* (neem oil) were obtained from the local market and utilized in the study. To study

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the effect of plant extracts on growth of leaf rot fungi and antagonists poisoned food technique was employed (Srinivasan and Gunasekaran, 1998).

Table 1. List of plants and their parts used in assay against major pathogens of leaf rot disease and their antagonistic organisms

| S. No. | Botanical name of plant | Common name | Part used |
|---|---|---|---|
| 01 | *Clerodendron infortunatum* | Peruvilum | Leaf |
| 02 | *Phyllanthus nururi* | Kizhanelli | Entire plant (without root) |
| 03 | *Adenocalymma allicea* | Garlic climber plant | Leaf |
| 04 | *Lawsonia inermis* | Henna plant | Leaf |
| 05 | *Leucas aspera* | Leucas | Entire plant (without root) |
| 06 | *Glycosmis pentaphylla* | Panad | Leaf |
| 07 | *Azadirachta indica* | Neem | Leaf/Neem oil |
| 08 | *Ocimum sanctum* | Sacred basil | Leaf |
| 09 | *Andrographis paniculata* | Kiriyathu | Entire plant (without root) |
| 10 | *Claoxyloan mercurialis* | Kunukittati | Entire plant (without root) |
| 11 | *Aegle marmelos* | Indian quine | Leaf |
| 12 | *Cinnamomum zeylanicum* | Cinnamon | Leaf |
| 13 | *Allium sativum* | Garlic | Bulb |
| 14 | *Curcuma longa* | Turmeric | Rhizome |
| 15 | *Annona squamosa* | Custard apple | Leaf |
| 16 | *Zingiber officinale* | Ginger | Rhizome |
| 17 | *Mimosa pudica* | Touch me not plant | Leaf |

In the first experiment, 14 species of plants besides neem oil (Tables 2-5) were used to study their effect on *C. gloeosporioides*, *E. rostratum* and *F. solani*. All plant extracts were made in acetone as extraction medium. Plant parts (leaves, bulbs, rhizomes or entire plant without roots, as the case may be) were thoroughly washed with sterile distilled water and air dried. One gram of plant tissue was ground and macerated with one ml of acetone using a pestle and mortar. The macerate was squeezed through a muslin cloth and filtered through Whatman No. 1 filter paper. The acetone was evaporated by keeping the extract in the laminar flow chambers. Each plant extract at 1%, 2% and 4% concentrations was tested for its efficacy. Each plant extract was added separately to sterilized and cooled PDA medium in such a way to arrive at required concentration. The medium was gently shaken for uniform distribution of the plant extract, poured into 90 mm size sterile petri plates (15 ml/petri plate) and solidified.

Mycelial discs (5 mm diameter) were cut from the periphery of five day old cultures of each fungus and inoculated onto centre of the plates containing the medium with plant extracts. Three replications were maintained for each concentration for each fungus. The plates inoculated with the fungus but without plant extracts in the medium served as control. The inoculated plates were incubated at 30±2°C. The radial growth of the fungal colony in each plate was recorded each day for five days by measuring the diameter of the colony in two directions at right angles and average was taken as the colony diameter. The fungal growth in plant extract treated plates was compared with control and the inhibition of growth of fungi particularly after 5 days of incubation was computed using the following formula.

$$I = \frac{C-T}{C} \times 100$$

(where I = Percent inhibition of growth, C = diameter of fungus in control and T = diameter of fungus in treatment). Mean inhibition effect of the concentrations of plant extracts after 5 days of incubation was analyzed.

In the second experiment ten plant species were used (Table 6). Plant extracts were made in three solvents/ extraction media *viz.*, acetone, alcohol and distilled water following the procedure described earlier. Acetone and alcohol were evaporated by keeping the extracts in laminar flow chambers. The extract prepared with distilled water was used directly. The plant extracts at 1%, 2%, 3% and 4% concentrations were tested for inhibitory effect on leaf rot fungi. Three replications were maintained for each extract and each concentration. The inoculated plates including control were incubated. The fungal growth in plant extract treated plates was compared with control at 24 h. interval for successive five days as described earlier. Mean inhibition effect of the concentrations of plant extracts particularly after 5 days of incubation was analyzed after computation of per cent inhibitions of growth of the fungi.

The effect of these extracts on growth of antagonistic bacteria and fungus was also studied (Table 7). In the case of antagonistic bacteria nutrient agar was used as basal medium. *B. subtilis* and *P. fluorescens* were individually streaked onto the medium, previously mixed with extracts in four different concentrations, in petri plates. Three replications were maintained for each treatment and control. The inoculated petri plates were incubated at 30±2°C. Observations on growth of bacteria upto 5th day was qualitatively recorded in comparison with relevant control and grouped as (-): No growth, (+): Low growth, (++): Moderate growth, (+++): Good growth and (++++): Abundant growth. For the antagonistic fungus, *T. harzianum*, PDA was used as basal medium. The five day old mycelial discs of the antagonist (5 mm diameter) were inoculated onto centre of petri plates containing the medium, previously mixed with plant extracts in four different concentrations. The inoculated plates (three replications) including control

Table 2. Effect of acetone based plant extracts in various concentrations on growth of *Colletotrichum gloeosporioides* in different days of incubation in preliminary experiment*

| S. No. | Plant species | Conc. of extract | Colony diameter in different days after incubation (mm) | | | | | Per cent inhibition after 5 days of incubation |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | |
| 1 | *Clerodendron infortunatum* | 1 | 13.00 | 23.00 | 42.50 | 52.00 | 59.00 | 28.05 |
| | | 2 | 13.00 | 26.00 | 40.50 | 53.00 | 59.00 | 28.05 |
| | | 4 | 12.00 | 23.50 | 40.00 | 48.50 | 59.00 | 28.05 |
| 2 | *Phyllanthus niruri* | 1 | 12.00 | 25.50 | 41.50 | 62.00 | 72.50 | 11.58 |
| | | 2 | 12.00 | 24.00 | 39.50 | 57.83 | 70.00 | 14.63 |
| | | 4 | 12.00 | 23.50 | 38.00 | 57.00 | 69.50 | 15.24 |
| 3 | *Adenocalymma allicea* | 1 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 4 | *Lawsonia inermis* | 1 | 09.00 | 14.00 | 18.00 | 25.00 | 35.00 | 57.31 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 5 | *Leucas aspera* | 1 | 17.50 | 33.00 | 45.00 | 54.00 | 65.00 | 20.73 |
| | | 2 | 16.00 | 30.00 | 42.50 | 51.00 | 60.00 | 26.82 |
| | | 4 | 14.00 | 26.00 | 36.50 | 45.00 | 53.00 | 35.37 |
| 6 | *Glycosmis pentaphylla* | 1 | 15.00 | 32.00 | 48.00 | 57.50 | 57.50 | 30.48 |
| | | 2 | 13.50 | 28.50 | 45.00 | 53.50 | 56.50 | 31.70 |
| | | 4 | 10.00 | 25.00 | 37.00 | 45.00 | 50.00 | 39.02 |
| 7 | *Azadirachta indica* | 1 | 10.00 | 18.00 | 26.00 | 35.00 | 44.50 | 45.73 |
| | | 2 | 08.00 | 16.00 | 25.50 | 34.50 | 42.00 | 48.78 |
| | | 4 | 06.00 | 14.50 | 24.00 | 31.00 | 38.00 | 53.65 |
| 8 | *Ocimum sanctum* | 1 | 10.00 | 20.00 | 32.00 | 40.00 | 47.00 | 42.08 |
| | | 2 | 05.00 | 15.00 | 26.00 | 33.00 | 40.00 | 51.21 |
| | | 4 | 0 | 10.00 | 18.00 | 24.00 | 32.00 | 60.97 |
| 9 | *Andrographis paniculata* | 1 | 16.00 | 28.50 | 39.50 | 56.00 | 68.00 | 17.07 |
| | | 2 | 15.00 | 27.00 | 38.50 | 56.00 | 67.00 | 18.29 |
| | | 4 | 14.50 | 27.00 | 34.00 | 53.00 | 60.50 | 26.21 |
| 10 | *Claoxyloan mercurialis* | 1 | 15.50 | 33.00 | 50.00 | 61.00 | 68.00 | 17.07 |
| | | 2 | 15.50 | 32.00 | 47.00 | 60.00 | 67.50 | 17.68 |
| | | 4 | 15.00 | 30.00 | 41.00 | 57.50 | 63.00 | 23.17 |
| 11 | *Aegle marmelos* | 1 | 20.00 | 32.00 | 50.00 | 65.00 | 75.00 | 08.53 |
| | | 2 | 17.00 | 30.00 | 49.00 | 63.00 | 70.00 | 14.63 |
| | | 4 | 10.00 | 30.00 | 34.00 | 39.00 | 48.00 | 41.46 |
| 12 | *Cinnamomum zeylanicum* | 1 | 0 | 19.00 | 29.00 | 40.00 | 48.00 | 41.46 |
| | | 2 | 0 | 08.00 | 15.00 | 27.00 | 38.50 | 53.05 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 13 | *Allium sativum* | 1 | 09.00 | 15.00 | 35.00 | 45.00 | 56.50 | 31.10 |
| | | 2 | 06.00 | 12.50 | 34.00 | 44.00 | 52.50 | 35.10 |
| | | 4 | 0 | 12.00 | 30.00 | 40.00 | 52.00 | 36.59 |
| 14 | *Curcuma longa* | 1 | 06.00 | 12.00 | 20.00 | 26.00 | 30.00 | 63.14 |
| | | 2 | 0 | 08.00 | 16.00 | 20.00 | 24.00 | 70.73 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 15 | Neem oil | 1 | 11.00 | 20.00 | 29.00 | 38.00 | 48.00 | 41.46 |
| | | 2 | 11.00 | 19.00 | 29.00 | 38.00 | 48.00 | 41.60 |
| | | 3 | 09.00 | 17.00 | 26.00 | 36.00 | 39.50 | 51.82 |
| | Control | | 15.00 | 28.00 | 43.00 | 60.00 | 82.00 | |

*Mean of three replications

were incubated and percent inhibition of the fungus computed as described earlier.

## Results and Discussion

The results of the evaluation of plant extracts on leaf rot pathogens in the first experiment are given in Tables 2-5. Extracts of all plant species and neem oil tested resulted in the growth inhibition of all the three pathogens at differential levels. Among these plants *A. allicea* extract was found to inhibit the fungi up to 100%. The extracts of *L. inermis*, *C. longa*, *C. zeylanicum*, *O. sanctum* and *A. sativum* were also very effective in

Table 3. Effect of acetone based plant extracts in various concentrations on growth of Exserohilum rostratum in different days of incubation in preliminary experiment*

| S. No. | Plant species | Conc. of extract | Colony diameter in different days after incubation (mm) | | | | | Per cent inhibition after 5 days of incubation |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | |
| 1 | *Clerodendron infortunatum* | 1 | 16.00 | 35.00 | 42.00 | 52.00 | 72.00 | 04.00 |
| | | 2 | 13.00 | 30.00 | 38.00 | 44.50 | 59.00 | 21.33 |
| | | 4 | 13.00 | 26.50 | 36.00 | 43.00 | 51.00 | 32.00 |
| 2 | *Phyllanthus niruri* | 1 | 15.50 | 28.50 | 41.50 | 47.00 | 60.50 | 19.33 |
| | | 2 | 15.00 | 23.00 | 31.00 | 45.00 | 49.00 | 34.66 |
| | | 4 | 13.00 | 20.50 | 28.00 | 39.50 | 44.50 | 30.50 |
| 3 | *Adenocalymma allicea* | 1 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 4 | *Lawsonia inermis* | 1 | 0 | 13.00 | 18.00 | 24.50 | 30.50 | 59.33 |
| | | 2 | 0 | 10.00 | 15.00 | 20.00 | 25.00 | 66.66 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 5 | *Leucas aspera* | 1 | 18.00 | 37.50 | 40.00 | 57.50 | 66.50 | 11.33 |
| | | 2 | 15.50 | 32.00 | 46.50 | 56.50 | 60.00 | 20.00 |
| | | 4 | 12.00 | 25.50 | 38.00 | 52.50 | 61.50 | 13.50 |
| 6 | *Glycosmis pentaphylla* | 1 | 15.00 | 30.00 | 40.00 | 50.50 | 67.50 | 10.66 |
| | | 2 | 14.00 | 28.00 | 35.50 | 50.00 | 54.00 | 18.66 |
| | | 4 | 13.00 | 30.00 | 39.00 | 42.50 | 54.00 | 18.66 |
| 7 | *Azadirachta indica* | 1 | 12.00 | 24.00 | 28.00 | 38.00 | 47.50 | 37.33 |
| | | 2 | 10.00 | 19.50 | 25.00 | 35.00 | 45.00 | 40.00 |
| | | 4 | 09.00 | 18.00 | 24.00 | 34.00 | 45.00 | 40.00 |
| 8 | *Ocimum sanctum* | 1 | 09.00 | 20.00 | 32.00 | 40.50 | 44.00 | 41.33 |
| | | 2 | 06.00 | 13.00 | 19.00 | 27.00 | 34.00 | 54.66 |
| | | 4 | 0 | 0 | 08.00 | 13.00 | 18.00 | 76.00 |
| 9 | *Andrographis paniculata* | 1 | 19.50 | 31.50 | 33.00 | 49.50 | 52.00 | 30.66 |
| | | 2 | 19.50 | 31.00 | 35.00 | 48.50 | 51.00 | 32.00 |
| | | 4 | 17.00 | 30.00 | 34.00 | 45.00 | 48.00 | 36.00 |
| 10 | *Claoxyloan mercurialis* | 1 | 18.00 | 32.00 | 40.50 | 48.50 | 52.00 | 30.66 |
| | | 2 | 17.50 | 29.00 | 39.00 | 48.00 | 54.00 | 28.00 |
| | | 4 | 16.50 | 28.00 | 36.00 | 40.50 | 48.00 | 36.00 |
| 11 | *Aegle marmelos* | 1 | 13.00 | 32.00 | 45.00 | 57.00 | 61.50 | 18.66 |
| | | 2 | 15.00 | 28.00 | 39.00 | 49.50 | 53.00 | 29.33 |
| | | 4 | 10.00 | 20.00 | 30.00 | 35.50 | 36.50 | 51.60 |
| 12 | *Cinnamomum zeylanicum* | 1 | 0 | 20.00 | 29.50 | 33.00 | 55.00 | 26.00 |
| | | 2 | 0 | 0 | 08.00 | 11.50 | 18.00 | 76.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 13 | *Allium sativum* | 1 | 0 | 06.00 | 18.00 | 22.00 | 25.00 | 14.20 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 14 | *Curcuma longa* | 1 | 10.00 | 18.00 | 24.00 | 36.00 | 40.00 | 46.66 |
| | | 2 | 08.00 | 16.00 | 20.00 | 26.00 | 30.00 | 60.00 |
| | | 4 | 0 | 0 | 0 | 06.00 | 10.00 | 86.66 |
| 15 | Neem oil | 1 | 18.00 | 24.50 | 33.00 | 45.00 | 50.50 | 33.20 |
| | | 2 | 14.00 | 23.50 | 30.00 | 42.00 | 47.00 | 37.33 |
| | | 3 | 12.50 | 22.00 | 29.00 | 38.50 | 46.00 | 38.66 |
| | Control | | 23.00 | 40.00 | 57.00 | 68.00 | 75.00 | |

*Mean of three replications

inhibiting the mycelial growth of the fungi. Moderate inhibitory effect has been observed also in cases of other plant species and in neem oil. As such very high inhibition (more than 50%) in mean fungal growth has been noticed with six species of plants. The ability of these plant species in inhibiting all the three major pathogens of leaf rot in a higher order is a point of importance (Table 5). Concentrations of the extracts of some plants also influenced the extent of inhibition of fungi (Tables 2-4). For example, at the level of 4% concentration of *L. inermis* and *C. zeylanicum* all the three fungi were inhibited upto 100%; similarly *C. longa* on *C.*

Table 4. Effect of acetone based plant extracts in various concentrations on growth of Fusarium solani in different days of incubation in preliminary experiment*

| S. No. | Plant species | Conc. of extract | Colony diameter in different days after incubation (mm) | | | | | Per cent inhibition after 5 days of incubation |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | |
| 1 | *Clerodendron infortunatum* | 1 | 22.00 | 39.50 | 52.00 | 53.50 | 55.50 | 00.00 |
| | | 2 | 21.00 | 36.00 | 44.50 | 48.50 | 52.00 | 03.80 |
| | | 4 | 23.00 | 29.00 | 34.00 | 42.50 | 50.00 | 07.40 |
| 2 | *Phyllanthus niruri* | 1 | 07.00 | 14.50 | 30.00 | 40.00 | 51.00 | 05.55 |
| | | 2 | 0 | 13.00 | 28.00 | 40.00 | 50.00 | 07.40 |
| | | 4 | 0 | 11.00 | 22.00 | 38.00 | 42.50 | 21.59 |
| 3 | *Adenocalymma allicea* | 1 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 4 | *Lawsonia inermis* | 1 | 0 | 10.00 | 13.00 | 16.50 | 30.00 | 44.44 |
| | | 2 | 0 | 08.00 | 10.00 | 12.00 | 24.00 | 55.55 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 5 | *Leucas aspera* | 1 | 13.00 | 20.00 | 28.50 | 37.50 | 43.50 | 20.37 |
| | | 2 | 11.00 | 20.00 | 27.00 | 33.00 | 41.00 | 24.07 |
| | | 4 | 06.00 | 08.50 | 27.00 | 32.50 | 38.00 | 29.96 |
| 6 | *Glycosmis pentaphylla* | 1 | 09.50 | 18.00 | 28.00 | 34.50 | 38.00 | 29.96 |
| | | 2 | 09.50 | 18.00 | 27.50 | 32.00 | 36.00 | 33.33 |
| | | 4 | 08.00 | 17.00 | 26.00 | 30.00 | 34.50 | 36.11 |
| 7 | *Azadirachta indica* | 1 | 08.00 | 15.50 | 19.00 | 25.67 | 32.00 | 40.74 |
| | | 2 | 07.00 | 12.00 | 16.50 | 22.50 | 27.50 | 49.07 |
| | | 4 | 05.50 | 10.50 | 12.50 | 18.50 | 24.00 | 55.55 |
| 8 | *Ocimum sanctum* | 1 | 09.00 | 20.00 | 27.00 | 33.50 | 38.00 | 29.62 |
| | | 2 | 07.50 | 17.50 | 23.16 | 31.00 | 30.00 | 44.44 |
| | | 4 | 0 | 11.00 | 17.50 | 22.00 | 22.50 | 58.33 |
| 9 | *Andrographis paniculata* | 1 | 10.00 | 16.50 | 26.00 | 34.67 | 59.00 | 00.00 |
| | | 2 | 10.00 | 17.00 | 24.00 | 32.00 | 40.00 | 25.92 |
| | | 4 | 08.00 | 16.50 | 20.00 | 27.00 | 35.00 | 35.18 |
| 10 | *Claoxyloan mercurialis* | 1 | 11.50 | 21.50 | 29.50 | 33.50 | 40.50 | 13.50 |
| | | 2 | 10.00 | 18.00 | 26.00 | 28.00 | 41.50 | 23.15 |
| | | 4 | 07.50 | 17.50 | 23.50 | 23.50 | 23.50 | 56.48 |
| 11 | *Aegle marmelos* | 1 | 12.00 | 28.00 | 32.00 | 42.00 | 45.00 | 16.66 |
| | | 2 | 08.00 | 20.50 | 30.00 | 36.00 | 38.50 | 28.70 |
| | | 4 | 0 | 12.00 | 21.00 | 23.00 | 23.00 | 57.40 |
| 12 | *Cinnamomum zeylanicum* | 1 | 0 | 15.00 | 23.00 | 32.00 | 44.00 | 18.50 |
| | | 2 | 0 | 06.00 | 12.00 | 20.00 | 29.00 | 46.29 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 100.00 |
| 13 | *Allium sativum* | 1 | 10.00 | 32.00 | 41.00 | 48.00 | 54.00 | 100.00 |
| | | 2 | 16.00 | 29.50 | 34.00 | 40.00 | 46.50 | 13.88 |
| | | 4 | 06.00 | 28.00 | 30.00 | 36.00 | 41.00 | 24.07 |
| 14 | *Curcuma longa* | 1 | 0 | 10.00 | 20.00 | 26.00 | 30.00 | 44.44 |
| | | 2 | 0 | 08.00 | 16.00 | 21.00 | 26.00 | 51.85 |
| | | 4 | 0 | 0 | 0 | 6.00 | 10.00 | 81.48 |
| 15 | Neem oil | 1 | 12.00 | 20.00 | 29.00 | 41.50 | 49.00 | 09.25 |
| | | 2 | 11.00 | 18.00 | 28.50 | 40.00 | 46.00 | 14.81 |
| | | 3 | 10.00 | 15.00 | 26.00 | 35.00 | 39.00 | 27.77 |
| | Control | | 12.00 | 21.00 | 30.00 | 40.00 | 54.00 | |

*Mean of three replications

*gloeosporioides* and *A. sativum* on *E. rostratum* as compared to lesser effect of extracts in lower concentrations. The nature of fungi toxicity of plant extracts was also tested in certain cases where no fungal growth was observed. For this the inoculum discs were retrieved at the completion of required observations from the poisoned medium and placed on normal PDA followed by re-incubation, and recovery of the fungus, if any, recorded. Leaf extracts of A. *allicea* and *L. inermis* at 4% concentration were fungicidal to *C. gloeosporioides* whereas *C. zeylanicum* and *C. longa* were fungistatic. Like wise the leaf extracts of *A. allicea, A. sativum* and *C. zeylanicum* at 4% concentration were found to be fungicidal to *E. rostratum*, while the extract

Table 5. Mean effect of acetone based plant extracts on growth of major pathogens of leaf rot in preliminary experiment (Mean of three replications)

| S. No. | Plant species | Mean percent inhibition in growth on 5th day of incubation* | | | Mean (C.D. at 1%: 17.2) |
|---|---|---|---|---|---|
| | | *Colletotrichum gloeosporioides* | *Exserohilum rostratum* | *Fusarium solani* | |
| 1 | *Clerodendron infortunatum* | 28.1 | 19.1 | 03.7 | 16.9 |
| 2 | *Phyllanthus niruri* | 13.8 | 28.2 | 11.5 | 17.8 |
| 3 | *Adenocalymma allicea* | 100.0 | 100.0 | 100.0 | 100.0 |
| 4 | *Lawsonia inermis* | 85.8 | 75.3 | 66.7 | 75.9 |
| 5 | *Leucas aspera* | 27.6 | 14.9 | 24.8 | 22.4 |
| 6 | *Glycosmis pentaphylla* | 33.7 | 15.9 | 33.1 | 27.6 |
| 7 | *Azadirachta indica* | 49.4 | 39.1 | 48.5 | 45.7 |
| 8 | *Ocimum sanctum* | 51.4 | 57.3 | 44.1 | 50.9 |
| 9 | *Andrographis paniculata* | 20.5 | 32.9 | 20.4 | 24.6 |
| 10 | *Claoxyloan mercurialis* | 19.3 | 31.6 | 31.0 | 27.3 |
| 11 | *Aegle marmelos* | 21.5 | 33.2 | 34.3 | 29.7 |
| 12 | *Cinnamomum zeylanicum* | 64.8 | 67.3 | 54.9 | 62.3 |
| 13 | *Allium sativum* | 34.3 | 71.4 | 45.9 | 50.5 |
| 14 | *Curcuma longa* | 77.9 | 64.4 | 59.3 | 67.2 |
| 15 | Neem oil | 44.9 | 36.4 | 17.3 | 32.9 |
| | Mean | 44.9 | 45.8 | 39.7 | |

*Mean of three concentrations (1%, 2% and 4%).

of *L. inermis* was fungistatic. The extract of *A. allicea* was fungicidal to *F. solani* at 2%-4% concentration, but the extract of *L. inermis* and *C. zeylanicum* expressed fungistatic effect on the fungus even at 4% concentration.

The effect of plant extracts in different concentrations, made out in different solvents on growth of the pathogens, was recorded in different days of incubation (data not shown). The mean effect of concentrations of the plant extracts computed for 5th day of incubation is given in Table 6. Inhibitory effects of all plant extracts under different medium of extraction were consistently observed. However, the relative efficacy of plant extracts depended on the species and also solvent involved in the extraction of the probable inhibitory principle. All plants tested and whose extracts made out in acetone medium generally lead to higher levels of inhibition of fungi, followed by alcohol and distilled water medium of extractions. Among the plants the mean percent inhibitions of fungi were higher with acetone or alcohol based extracts of *A. allicea* and *L. inermis*, followed by that of *C. zeylanicum*, *O. sanctum*, *M. pudica*, *A. indica* etc. Except *L. aspera* all other plants in acetone extractions effected more than 50% inhibition of fungi. In alcohol-based extractions the level of inhibition was relatively lesser than that in acetone based extractions. In water based extraction of *A. indica* also higher mean inhibition of the fungi was observed, an interesting feature. This was followed by *L. inermis*, *A. allicea* and *O. sanctum*. Moderate inhibition could be observed in water-based extractions of other plant species. In acetone and alcohol based extracts of *A. allicea* or *L. inermis* only cent percent inhibition of the fungi could be observed at least in their higher concentrations as compared to water extractions of various plants.

The results of the *in vitro* effect of plant extracts on antagonistic organisms are given in Table 7. The effect of plant extracts on the antagonists was less than their inhibitions of pathogens. The extent of inhibition of antagonists also depended on the medium of extraction, as relatively a higher inhibition with acetone followed

Table 6. Mean effect of plant extracts under different solvents of extraction on growth of major pathogens of leaf rot (Mean of three replications)

| S. No. | Plant species | Mean percent inhibition in growth on 5th day of incubation under different solvents of extraction* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Acetone | | | | Alcohol | | | | Water | | | |
| | | *Cg* | *Er* | *Fs* | Mean (C.D. at 1%: 10.7) | *Cg* | *Er* | *Fs* | Mean (C.D. at 1%: 10.3) | *Cg* | *Er* | *Fs* | Mean (C.D. at 1%: 8.4) |
| 1 | *Adenocalymma allicea* | 64.7 | 89.8 | 88.3 | **80.9** | 62.8 | 82.4 | 85.6 | **76.9** | 52.8 | 61.7 | 57.9 | **57.5** |
| 2 | *Azadirachta indica* | 56.2 | 63.5 | 59.2 | **59.6** | 53.4 | 55.9 | 57.9 | **55.7** | 73.6 | 68.9 | 68.2 | **70.2** |
| 3 | *Cinnamomum zeylanicum* | 60.3 | 71.9 | 73.6 | **68.6** | 57.2 | 68.6 | 67.1 | **64.3** | 49.8 | 63.5 | 51.9 | **55.1** |
| 4 | *Lawsonia inermis* | 72.0 | 94.4 | 69.4 | **78.6** | 70.3 | 71.4 | 66.7 | **69.5** | 67.9 | 57.4 | 52.1 | **59.1** |
| 5 | *Ocimum sanctum* | 66.3 | 71.4 | 64.4 | **67.4** | 65.8 | 67.1 | 68.1 | **67.0** | 58.9 | 64.3 | 53.5 | **58.9** |
| 6 | *Annona squamosa* | 39.9 | 53.5 | 68.5 | **53.9** | 37.6 | 40.8 | 54.8 | **44.4** | 35.3 | 31.1 | 41.8 | **36.1** |
| 7 | *Zingiber officinale* | 48.4 | 64.3 | 72.8 | **61.8** | 47.2 | 57.1 | 55.5 | **53.3** | 53.9 | 45.9 | 50.3 | **50.0** |
| 8 | *Curcuma longa* | 52.1 | 53.8 | 63.5 | **56.5** | 50.5 | 39.8 | 46.5 | **45.6** | 43.3 | 31.2 | 39.4 | **37.9** |
| 9 | *Mimosa pudica* | 52.5 | 63.3 | 82.2 | **66.0** | 51.7 | 60.9 | 63.6 | **58.7** | 44.0 | 49.2 | 44.8 | **46.0** |
| 10 | *Leucas aspera* | 33.0 | 43.6 | 59.5 | **45.4** | 31.8 | 38.7 | 55.9 | **42.1** | 24.8 | 24.9 | 49.2 | **32.9** |
| | Mean | **54.5** | **66.9** | **70.1** | - | **52.8** | **58.3** | **62.2** | - | **50.4** | **49.8** | **50.9** | - |
| | | C.D. at 1%: 05.9 | | | - | C.D. at 5%: 05.7 | | | - | | - | | - |

*Mean of four concentrations (1%, 2%, 3% and 4%); *Cg* - *Colletotrichum gloeosporioides*, *Er* - *Exserohilum rostratum*, *Fs* - *Fusarium solani*.

Table 7. Effect of plant extracts on growth of antagonistic organisms under different solvents of extraction on 5th day of incubation (Mean of three replications)

| S. No. | Plant species | Conc. of extract | Level of bacterial growth * | | | | | | Mean percent inhibition in growth *of T. harzianum* ** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acetone | | Alcohol | | Water | | Acetone | Alcohol | Water | Mean (C.D. at 1%: 10.3) |
| | | | *Bs* | *Pf* | *Bs* | *Pf* | *Bs* | *Pf* | | | | |
| 1 | *Adenocalymma allicea* | 1 | +++ | ++ | +++ | ++ | ++++ | +++ | | | | |
| | | 2 | ++ | + | ++ | + | +++ | ++ | 72.8 | 63.5 | 44.0 | **60.1** |
| | | 3 | + | - | - | - | +++ | + | | | | |
| | | 4 | - | - | - | - | + | + | | | | |
| 2 | *Azadirachta indica* | 1 | +++ | ++ | ++ | ++ | ++++ | +++ | | | | |
| | | 2 | ++ | + | + | + | +++ | ++ | 63.3 | 49.2 | 35.1 | **49.2** |
| | | 3 | - | - | + | + | ++ | + | | | | |
| | | 4 | - | - | - | - | + | + | | | | |
| 3 | *Cinnamomum zeylanicum* | 1 | +++ | +++ | +++ | ++ | ++++ | +++ | | | | |
| | | 2 | +++ | + | + | + | ++++ | ++ | 53.8 | 54.9 | 43.1 | **50.6** |
| | | 3 | +++ | - | + | - | +++ | + | | | | |
| | | 4 | + | - | - | - | +++ | ++ | | | | |
| 4 | *Lawsonia inermis* | 1 | +++ | ++ | +++ | +++ | ++++ | ++++ | | | | |
| | | 2 | ++ | ++ | +++ | + | +++ | +++ | 73.6 | 62.1 | 49.6 | **61.8** |
| | | 3 | + | + | ++ | + | ++ | ++ | | | | |
| | | 4 | + | + | ++ | + | + | ++ | | | | |
| 5 | *Ocimum sanctum* | 1 | ++++ | +++ | +++ | +++ | ++++ | +++ | | | | |
| | | 2 | +++ | ++ | ++ | ++ | +++ | +++ | 58.9 | 54.3 | 44.2 | **52.5** |
| | | 3 | ++ | + | + | + | +++ | ++ | | | | |
| | | 4 | + | + | + | - | ++ | ++ | | | | |
| 6 | *Annona squamosa* | 1 | +++ | +++ | +++ | ++ | ++++ | +++ | | | | |
| | | 2 | +++ | ++ | +++ | + | ++++ | ++ | 56.4 | 46.6 | 38.3 | **47.1** |
| | | 3 | +++ | + | ++ | + | +++ | ++ | | | | |
| | | 4 | + | - | + | + | ++ | + | | | | |
| 7 | *Zingiber officinale* | 1 | +++ | ++ | +++ | +++ | ++ | +++ | | | | |
| | | 2 | ++ | + | ++ | + | ++ | ++ | 49.7 | 41.3 | 38.1 | **43.0** |
| | | 3 | ++ | + | ++ | + | + | + | | | | |
| | | 4 | + | + | ++ | - | + | + | | | | |
| 8 | *Curcuma longa* | 1 | +++ | ++ | +++ | ++ | +++ | +++ | | | | |
| | | 2 | ++ | + | ++ | + | ++ | +++ | 64.3 | 59.1 | 53.2 | **58.9** |
| | | 3 | ++ | + | ++ | + | ++ | ++ | | | | |
| | | 4 | + | - | + | + | ++ | ++ | | | | |
| 9 | *Mimosa pudica* | 1 | +++ | ++ | +++ | ++ | +++ | +++ | | | | |
| | | 2 | ++ | + | ++ | + | ++ | +++ | 45.4 | 42.8 | 40.3 | **42.8** |
| | | 3 | ++ | + | ++ | + | ++ | ++ | | | | |
| | | 4 | + | - | + | + | ++ | ++ | | | | |
| 10 | *Leucas aspera* | 1 | +++ | ++ | +++ | +++ | ++++ | +++ | | | | |
| | | 2 | +++ | + | +++ | ++ | +++ | ++ | 69.8 | 52.8 | 49.6 | **57.4** |
| | | 3 | + | + | ++ | + | +++ | + | | | | |
| | | 4 | + | - | ++ | + | ++ | + | **60.8** | **52.7** | **43.6** | **{Mean (C. D. at 1%: 05.)}** |

*Bs - Bacillus subtilis, Pf - Pseudomonas fluorescens*; *(-): No growth, (+): Low growth, (++): Moderate growth, (+++): Good growth, (++++): Abundant growth, {(Growth in controls: (++++)}; **Mean of four concentrations (1%, 2%, 3% and 4%).

by alcohol and distilled water. In water extracts, no complete inhibition of bacterial and fungal antagonists was seen, even as their growth generally decreased with increase in concentration of extracts. *T. harzianum* was not completely inhibited in alcohol extracts in a similar manner, irrespective of plant species, but at 4% concentration of acetone extracts of *A. allicea* and *L. inermis* it was completely inhibited. The mean inhibitory effect of plant species/ medium of extraction on *T. harzianum* were significant. Both *B. subtilis* and *P. fluorescens* survived in alcohol extracts of *L. inermis*, *A. squamosa*, *C. longa*, *M. pudica* and *L. aspera* (in decreasing order with increase in concentration). A similar trend could be observed in acetone extracts of *L. inermis*, *O. sanctum* and *Z. officinale*. Higher concentration of acetone and alcohol extracts of *A. allicea* and *A. indica* completely inhibited both the bacterial antagonists. While *B. subtilis* could relatively grow in different concentrations of acetone extracts of *C. zeylanicum*, *A. squamosa*, *C. longa*, *M. pudica* and *L. aspera*, the growth of *P. fluorescens* was less in such extracts amended medium. Further, *P. fluorescens* lost its viability in higher concentrations of these extracts. A similar trend was seen in alcohol extracts of *C.*

*zeylanicum*, *O. sanctum* and *Z. officinale* on these bacterial species. Hence *B. subtilis* was less affected by plant extracts than *P. fluorescens* and consequently relative tolerance of *B. subtilis* to plant extracts could be inferred.

It is evident that all plants tested in the study inhibited major pathogens of leaf rot. The inhibitory potential among the plants differed and variations were observed in terms of their effect on species of pathogenic fungus, medium of extraction and concentration of extract. The differences in activity among plant extracts may be due to differential antifungal compounds of the plants and the concentration effect as a consequence of the level of inhibitory principle involved. Acetone extractions of plants lead to higher inhibitions over alcohol and aqueous extractions. A vast number of reports have emerged out in literature about the inhibitory effect of various plant extracts on fungal pathogens of crop plants. Ethanol extract of the leaves of *A. indica* inhibited *Sclerotinia sclerotiorum* (Qais. K. Zewain *et al.*, 2004) and application of even the aqueous extract of the plant leaves lead to reduced level of onion blight caused by *Stemphylium botryosum* (Prasad and Baranwal, 2004). Pramila Tripathi and Dubey (2003) reported fungi toxic effect of the extracts of *A. allicea*, *A. marmelos*, *O. sanctum* etc. in different solvents against *Penicillium italicum*. Ancy. P. George *et al.* (2003) found inhibitory effect of *A. sativum*, *A. cepa* etc. in checking the *in vitro* growth of *C. capsici*. Water extracts of the bulbs of *A. sativum* effectively inhibited the growth of *Botryodiplodia theobromae* (Deepa *et al.*, 2003) and *C. capsici* (Chidananda Swamy and Srikant Kulkarni, 2003). Naik *et al.* (2003) found *O. sanctum* as an effective inhibitor of *Alternaria sesame*. Inhibitory effect of different plant species on a number of plant pathogenic fungi (e.g., *L. inermis* on *Rhizoctonia solani*; *O. sanctum* on sugarcane fungal pathogens; *C. infortunatum* on *Ganoderma lucidum*; *A. allicea* and *A. marmelos* on *Penicillium* spp. and *Aspergillus* spp.), as observed by various other workers, also have been reported elsewhere. Higher inhibitory potential of *A. allicea*, *L. inermis* etc. as observed currently is illustrative of the inhibitory trend of various species of plants on the pathogens and this study forms the first report of inhibitory role of plant extracts on leaf rot pathogens.

Scant information is available about the sensitivity of antagonistic organisms to botanicals. However, relatively lesser sensitivity, particularly the bacterial antagonists to plant extracts (aqueous extracts), as observed in the current study lends scope for combining plant derivatives with antagonists as a possible potential consortium for the disease control. Inhibitory role of *A. indica* on leaf rot pathogens, but insensitivity or relatively low sensitivity of antagonistic bacteria to the plant extract may be cited as a point of importance. Toxic effect of neem or its formulations on plant pathogenic fungi have been widely documented. Hence plant products derived through minimal processing, common organics (neem cake, marotti cake) etc. could be availed as base/carrier for antagonists' preparation. Importance of evolving induced systemic resistance against root (wilt)-leaf rot complex has been suggested, there lies a scope for application of biocontrol technology (Srinivasan, 2004; Srinivasan and Rohini Iyer, 2004). The plant growth promoting rhizobacteria (PGPR) having the potential to induce systemic resistance against diseases has received attention in recent years. Bio pesticides bearing such potential disease control mechanism are likely to be more useful in eco-friendly manner for the integrated management of the disease complex. Further work is progressing on such lines. As plant extracts, besides bacterial antagonists, are also inhibitory to leaf rot pathogens strategic techniques are also being evolved for combining these biocontrol measures to gain synergism.

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