

## Studies on mycotoxic properties of *Eucalyptus* species on *Thielaviopsis paradoxa* (de Seynes) von Hohnel, the stem bleeding pathogen of coconut

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### Abstract

Stem bleeding disease caused by *Thielaviopsis paradoxa* (de Seynes) von Hohnel is one of the important diseases of coconut reported from all the coconut growing countries of the world. Antifungal effect of *Eucalyptus* against the pathogen was studied under *in vitro* conditions by poisoned food technique and under *in vivo* conditions in detached coconut leaf petioles. *Eucalyptus* oil (0.6%) completely inhibited both mycelial growth and spore germination of the pathogen under *in vitro* conditions. Ten per cent acetone leaf extract of *Eucalyptus* exerted 82.0 per cent growth inhibition of the pathogen at 24 hrs after inoculation. Germination of endoconidia was inhibited to the extent of 60 per cent by 10 per cent leaf extract while chlamydospore germination was completely inhibited by one per cent leaf extract under *in vitro* conditions. Under *in vivo* conditions, *Eucalyptus* oil exerted complete inhibition of lesion formation due to the pathogen in detached coconut leaf petioles after 15 days of inoculation.

**Key words:** Coconut, stem bleeding, *Thielaviopsis paradoxa*, endoconidia, chlamydospore, antifungal effect, *Eucalyptus*.

### Introduction

Stem bleeding disease of coconut (*Cocos nucifera* L.) is common in all the tropical countries where it is grown. All the varieties of coconut are susceptible to the disease. The disease is found in all the soil types throughout the year. *Thielaviopsis paradoxa* (de Seynes) von Hohnel, is a weak pathogen entering the coconut stem through growth cracks and causes stem bleeding disease. Application of systemic fungicides like Calixin through root feeding and / or drenching the basin, phytosanitation and application of Calixin 5 per cent on the chiselled surface on affected stem followed by the application of neem cake @ 5 kg per palm in the basin were found effective in managing the disease (Nambiar and Sastry, 1988; Radhakrishnan, 1990; Ramanujam *et al.*, 1993). Use of toxic chemicals, systemic fungicides or pesticides will lead to environmental pollution and health hazards. No work has been done on the biological control of the disease using botanical pesticides or phytochemicals. There are many reports, where species of *Eucalyptus*

was found effective against plant diseases (Babu and Reddy, 1986; Arun and Arya, 1988; Jacob *et al.*, 1988; Salama *et al.*, 1988). Hence in the present study, leaf extract and oil from *Eucalyptus globulus* L. has been tested for its antifungal activity against *T. paradoxa*, the stem bleeding pathogen of coconut.

### Materials and Methods

*In vitro* and *in vivo* experiments were conducted in the present study.

#### a) *In vitro* studies

*In vitro* evaluation of fungitoxic effect of acetone extract and oil from *Eucalyptus* leaf on the mycelial growth and spore germination of *T. paradoxa* was done by poisoned food technique (Nene, 1971) on PDA growth medium and sugarcane juice agar medium respectively. One, five and ten per cent concentrated PDA media with leaf extract and oil from *Eucalyptus globulus* L. separately, were taken in 90 mm sterile petriplates. Five mm diameter mycelial discs taken from the

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periphery of two days old *T. paradoxa* culture on PDA plate were kept at the centre of the plate containing poisoned medium at the rate of one disc per plate with the surface of the fungal disc in contact with the surface of the medium. In the case of Eucalyptus oil, the same experiment was repeated for 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 concentrations, as one percent of oil was found effective against *T. paradoxa*. Four replicates were made for each concentration and plates without plant extract or oil served as the control. All the plates were incubated at  $26 \pm 2^{\circ}$  C for 48 hrs. Colony diameter in each plate was recorded at 24 hrs. and 48 hrs after inoculation. Percentage of growth inhibition was calculated using the equation given by Vincent (1927) i.e.

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

where I = inhibition of fungal growth, c = Growth in culture plate and T = growth in treatment. To study the nature of fungitoxicity, inoculum discs were picked from poisoned media after three days of incubation and were transferred to fresh PDA plates without any extract or oil in it and the fungal growth was studied.

Endospore and Chlamydospore germination of *T. paradoxa* was studied on poisoned sugarcane juice agar plates of one, five and ten per cent concentrations of the extract and oil separately and the culture plate without the extract or oil served as the control. In the oil, spore germination study was also carried out at 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 per cent of oil, as the oil completely inhibited spore germination at its one percent concentration.

In each case, four replicates were maintained. Percentage of germination of endoconidia after 4 hrs. of inoculation and chlamydospore after 6 hrs. of inoculation were recorded. Germination counts were taken by observing ten ocular fields and percentage of inhibition of spore germination was calculated in each case.

#### b) *In vivo* studies

As the Eucalyptus oil was found more effective than the leaf extract inhibiting both mycelial growth and spore germination *in vitro* at less than one percent concentration, only oil was used for *in vivo* testing. Oil was tested against *T. paradoxa* infection in detached coconut leaf petioles. Healthy coconut leaf petioles of West Coast Tall (WCT) green variety palms free from any injuries were collected and cut into pieces of 30cm length. After thorough washing, they were surface sterilised with 0.1 per cent Mercuric Chloride solution

and then were again washed in three changes of sterile distilled water. The cut ends of the petiole stalks were smeared with pure petroleum jelly to prevent direct evaporation of water from tissues inside through the cut ends.

Two types of inoculations were made inside the laminar flow chamber. In one set, two cavities or 'wells' at a distance of four cm apart were made at the center of the dorsal surface of the detached petiole piece by removing the tissue plug using a 5 mm diameter sterile cork borer. One such hole was filled with about 3 ml of the oil and the other hole was filled with *T. paradoxa* inoculum. The hole filled with fungal inoculum was covered with moist sterile cotton. Both the holes were then covered with pure transparent gum tape. Suitable controls were maintained by filling one hole with three ml sterile distilled water and the other hole with the *T. paradoxa* inoculum. One more set of control was maintained where both the holes were kept empty without filled by oil or fungal inoculum. Four replicates were maintained. Each set was carefully kept inside a clean fresh polythene bag the mouth of which was closed with a rubber band to serve as a humidity chamber.

In the second set of inoculation, only one bore hole was made at the centre of the petiole piece using 5 mm diameter sterile cork borer. The cavity was filled drop by drop with 3 ml of Eucalyptus oil. After the oil was absorbed by the tissues, *T. paradoxa* inoculum disc was inoculated to the same hole and was covered with moist sterile cotton and then with clean gum tape. Control was maintained by first filling the hole with sterile water and then with the fungal inoculum. One more set of control was maintained where the hole was not filled by the oil or fungal inoculum. Four replicates were maintained for each set and were carefully kept inside the polythene bag which were then closed with rubber bands.

The above petiole pieces were incubated at  $26 \pm 2^{\circ}$  C for fifteen days and then petioles were split longitudinally through the inoculated sites. The lesion length and breadth were measured in two directions at right angle to each other and the percentage of inhibition of lesion formation due to *T. paradoxa* in each petiole was found out. The fungus was reisolated from the lesion wherever the infection had established.

## Results and Discussion

### (a) *In vitro* studies

Theoretical value at 1% level of significance for 6 df is 3.707

From the above analysis applying t-test, it is observed that as the concentration of leaf extract increases, the growth inhibition of *T. paradoxa* also increases at 1% level of significance. Also, referring to growth inhibition of *T. paradoxa* with respect to Eucalyptus oil, it is observed that at all concentrations it completely inhibits the fungal growth.

*In vitro* studies on the effect of acetone extract of *Eucalyptus* leaf and its oil revealed that Eucalyptus oil was very much inhibitory to the mycelial growth of the fungus than the leaf extract. Leaf extract was reported to be antifungal to *Pestalotiopsis mangiferae* (P.Henn) Stey (El – Sayed, *et al.*, 1980), *Aspergillus flavus* Link. ex.Fr. (Loksha *et al.*, 1986), *Sclerotium rolfsii* Sacc. (Singh and Dwivedi, 1987), *S. cepivorum* Berk, (Salama *et al.*, 1988 and Ismail *et al.*, 1989). In the present study ten per cent of the extract had showed 78.9 per cent growth inhibition of *T. paradoxa* after 48 hr. of inoculation (Table 1). Eucalyptus oil was found inhibitory to the mycelial growth of *S. rolfsii* Sacc. (Singh and Dwivedi, 1987). In the present study Eucalyptus oil was found very effective against the mycelial growth of *T. paradoxa* 0.6 per cent concentration of the oil completely (100.0 per cent) inhibited the mycelial growth of *T. paradoxa* (Table 2). When the fungal inoculum discs of *T. paradoxa* from 0.6 per cent PDA plates were transferred after 3 days of incubation to fresh PDA plates with out the oil, *T. paradoxa* did not show any growth. Hence Eucalyptus oil was found to be fungicidal to *T. paradoxa* at 0.6 per cent.

**Table 1. Percentage of mycelial growth inhibition of *T. paradoxa* in vitro by the acetone extracts of leaf and oil from *Eucalyptus globulus*.**

| Plant product used      | Time after fungal inoculation | Concentration of the extract / oil |       |       |
|-------------------------|-------------------------------|------------------------------------|-------|-------|
|                         |                               | 1%                                 | 5%    | 10%   |
| Acetone extract of leaf | 24 hr.                        | 36.0                               | 44.0  | 82.0  |
|                         | 48 hr.                        | 24.4                               | 37.7  | 78.9  |
| Eucalyptus oil          | 24 hr                         | 100.0                              | 100.0 | 100.0 |
|                         | 48 hr                         | 100.0                              | 100.0 | 100.0 |

#### Statistical Analysis:

| Plant product used      | Time after fungal inoculation | Per cent of concentration | t-value    | t-value     |
|-------------------------|-------------------------------|---------------------------|------------|-------------|
|                         |                               |                           | calculated | theoretical |
| Acetone extract of leaf | 24 hr.                        | Between 1% & 5%           | 5.172      | 3.707       |
|                         |                               | Between 1% & 10%          | 4.811      | 3.707       |
|                         | 48 hr.                        | Between 5% & 10%          | 4.92       | 3.707       |
|                         |                               | Between 1% & 5%           | 4.798      | 3.707       |
|                         |                               | Between 1% & 10%          | 6.656      | 3.707       |
|                         |                               | Between 5% & 10%          | 6.146      | 3.707       |

**Table 2. Percentage of mycelial growth inhibition of *T. paradoxa* in vitro by different concentrations of Eucalyptus oil.**

| Hour after inoculation | Concentration of the oil |      |      |      |      |     |     |     |     |     |
|------------------------|--------------------------|------|------|------|------|-----|-----|-----|-----|-----|
|                        | 0.1                      | 0.2  | 0.3  | 0.4  | 0.5  | 0.6 | 0.7 | 0.8 | 0.9 | 1.0 |
| 24                     | 21.2                     | 34.6 | 51.9 | 61.5 | 80.8 | 100 | 100 | 100 | 100 | 100 |
| 48                     | 11.1                     | 31.1 | 38.9 | 53.3 | 58.9 | 100 | 100 | 100 | 100 | 100 |

Eucalyptus leaf extract was reported as inhibitory to the spore germination of *S. cepivorum* Berk. (Salama *et al.*, 1988). In the present study, only 10 per cent leaf extract could show 60 per cent and 100 per cent inhibition of germination of endoconidia and chlamydo spores of *T. paradoxa* respectively. One per cent leaf extract did not show any sign of inhibition of spore germination (zero per cent) of *T. paradoxa* (Table 3). But Eucalyptus oil completely inhibited (100.0 per cent) the germination of both endoconidia and chlamydo spores of *T. paradoxa* at 0.6 per cent concentration (Table -4). Hence Eucalyptus oil will be quite beneficial for the use in the biocontrol of *T. paradoxa*.

**Table 3. Percentage of inhibition of spore germination of *T. paradoxa* by the leaf extract / oil of *Eucalyptus*.**

| Plant product           | Type of spore  | Concentration |     |     |
|-------------------------|----------------|---------------|-----|-----|
|                         |                | 1%            | 5%  | 10% |
| Eucalyptus leaf extract | Endoconidia    | 0             | 0   | 60  |
|                         | Chlamydo spore | 0             | 20  | 100 |
| Eucalyptus oil          | Endoconidia    | 100           | 100 | 100 |
|                         | Chlamydo spore | 100           | 100 | 100 |

**Table 4. Percentage of inhibition of spore germination of *T. paradoxa* by low concentrations of Eucalyptus oil.**

| Type of spore  | Concentration of the oil |      |      |      |      |      |      |      |      |      |
|----------------|--------------------------|------|------|------|------|------|------|------|------|------|
|                | 0.1%                     | 0.2% | 0.3% | 0.4% | 0.5% | 0.6% | 0.7% | 0.8% | 0.9% | 1.0% |
| Endoconidia    | 40                       | 45   | 80   | 90   | 95   | 100  | 100  | 100  | 100  | 100  |
| Chlamydo spore | 75                       | 80   | 85   | 90   | 95   | 100  | 100  | 100  | 100  | 100  |

#### (b) *In vivo* studies :

*In vivo* experiment was done with two types of inoculations. As the leaf extract was found not so effective as the oil, only the oil was used for *in vivo* studies. In both the types of inoculations as described in materials and methods, lesion formation by *T. paradoxa* in the detached coconut leaf petiole was studied after 15 days of incubation and it was found that lesion formation was completely (100 percent) inhibited by the oil.

There are many reports where *Eucalyptus* was used as a good biocontrol agent of plant pathogens. It was used against *Colletotrichum gloeosporioides* and *Botrydiploidia theobrome* Pat. (Babu and Reddy, 1986) causing fruit rot of lemon, *Phomosis sp.*, causing fruit

rot of *Vitis vinifera* and *Psidium guajava* L. (Arun and Arya, 1988), *Pythium aphanidermatum* (Eds.) Fitz causing pre-emergence damping off of *Solanum melongena* L. (Jacob *et al.*, 1988) and *S. cepivorum* Berk. causing white rot disease of *Allium cepa* L. (Salama *et al.*, 1988). From the present study, it may be concluded that Eucalyptus oil can be used for the biocontrol of *T.paradoxa* causing stem bleeding disease of coconut and a proper method has to be evolved for the correct administration of this oil into the palm.

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