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Antifungal activity of garlic (*Allium sativum* Linn.) extracts on *Thielaviopsis paradoxa* (de Seynes) von Hohnel, the pathogen of stem bleeding disease 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 (*Cocos nucifera* Linn.) reported from all the coconut growing countries of the world. Antifungal effect of *Allium sativum* Linn. against the pathogen was studied under *in vitro* and *in vivo* conditions. Potculture experiment was also done to evaluate the fungitoxic effect of garlic against the pathogen. Garlic extract at 5 per cent concentration was found to be fungitoxic to the pathogen under *in vitro* conditions inhibiting its mycelial growth completely. Germination of both endoconidia and chlamydospores of the pathogen was completely inhibited (100 per cent) by one per cent concentration of garlic extract. Under *in vivo* conditions, garlic extract exerted 62 per cent inhibition of lesion formation by the pathogen in detached coconut leaf petioles after 15 days of inoculation, when the pathogen and bulb extract were inoculated 4 cm apart from each other. Simultaneous inoculation of the pathogen inoculum and bulb extract to the same "well" in detached leaf petiole exerted 89 per cent inhibition of lesion formation after 15 days of inoculation. Potculture experiment showed 100 per cent destruction of the pathogen inoculum after one month of inoculation in autoclaved / unautoclaved soils treated with sliced garlic bulb material.

Key words : Coconut, stem bleeding, *Thielaviopsis paradoxa*, endoconidia, chlamydospore, *Allium sativum*, antifungal effect

Introduction

Stem bleeding disease of coconut (*Cocos nucifera* Linn.) is found in all the tropical countries where it is grown. The disease is found in all soil types throughout the year.

Nambiar *et al.* (1986) proved the pathogenesity of the fungus by reproducing the stem bleeding disease symptoms by inoculating the healthy trees with the fungus. Effective management of the disease using systemic fungicide, application of neem cake in coconut basins etc. had been reported (Nambiar and Sastry, 1918; Radhakrishnan, 1990; Ramanujam *et al.*, 1993). Use of toxic chemicals or pesticides will lead to environmental pollution and health hazards. No work has been done so far on the biological control of this disease using botanical pesticides or phytochemicals. There were reports where garlic (*Allium sativum* Linn.) was found effective as a biocontrol agent of plant diseases (Alice and Rao, 1986 ; Shetty *et al.*, 1989; Lakshmanan *et al.*, 1990). Hence in the present study garlic bulb extract was tested for its antifungal activity against *T. paradoxa*.

Materials and Methods

a) In vitro studies

In vitro evaluation of fungitoxic effect of acetone extract of garlic bulb 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.

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Using acetone extract of garlic (one, five, and ten per cent) concentrated PDA media were taken in 90 mm sterile petriplates and mycelial discs of 5mm diameter taken from the periphery of two-day old culture of *T. paradoxa* on PDA plate were kept in the center 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. Four replicates were maintained for each concentration and plates without plant extract served as control. The plates were incubated at 26 ± 2 °C and colony diameter in each plate was recorded at 24 hrs. and 48 hrs. after inoculation. Percentage of growth inhibition was recorded using the equation given by Vincent (1927):

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

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 inoculation, transferred to fresh PDA plates without any extract and fungal growth was studied.

Germination of endoconidia and chlamydospores of *T. paradoxa* was studied on poisoned sugarcane juice agar plates (1, 5 and 10 per cent concentrations) and sugarcane juice agar plate without bulb extract served as the control. Four replicates were maintained. Germination of endoconidia and chlamydospores was recorded four and six hrs. after inoculation respectively. Germination counts were taken by observing ten occular fields and percentage of inhibition of spore germination was calculated in each case.

b) In vivo studies

Acetone extract of garlic bulb was used for *in vivo* testing using detached coconut leaf petioles against *T. paradoxa* infection. Healthy coconut leaf petioles of West Coast Tall (WCT) palms, free from injuries were collected and cut into pieces of 30cm length. After thorough washing, they were surface sterilized with 0.1 per cent mercuric chloride solution and 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 through cut ends.

Two types of inoculations were adopted. In one set, two cavities or "wells" at a distance of four cm apart were made at the centre of the dorsal surface of the detached petiole piece by removing tissue plugs using a 5mm diameter sterile cork borer. One "well" was filled with 3 ml of acetone extract of garlic bulb and the other "well" with *T. paradoxa* inoculum. The "well" filled with fungal inoculum was covered with moist sterile cotton. Both the "wells" were then covered with pure transparent gum tape. Suitable controls were maintained by filling one "well" with three ml of sterile distilled water and the other "well" with *T. paradoxa* inoculum. One more set of control was maintained where both the "wells" were kept empty (without plant extract 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 method of inoculation, only one "well" was made at the centre of the petiole piece using 5mm diameter sterile cork borer. The "well" was filled with 3 ml of acetone extract of garlic bulb. After the extract was absorbed by the tissues, *T. paradoxa* inoculum disc was inoculated in the same "well" and was covered with moist sterile cotton and then with clean gum tape. Control was maintained by first filling the "well" with sterile water and then with the fungal inoculum. One more set of control was maintained where the "well" was not filled by the extract or fungal inoculum. Four replicates were maintained for each set as in the earlier method.

The experimental materials were incubated at 26 ± 2°C for fifteen days. Then these petioles were split longitudinally through the inoculated site. The lesion length and breadth were measured in two directions at right angles 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.

c) Pot culture experiment to evaluate the fungitoxic effect of garlic against *T. paradoxa*:

Clean dry earthern pots of 15 inches diameter were filled each with 3 kg of autoclaved soil and mixed up with 300 gm of chopped pieces of garlic bulb. *T. paradoxa* inoculum was prepared by growing the fungus on sterilized coconut inflorescence rachis bits of 2cm length (Nambiar *et al.*, 1986) to each one of which a sterilized nylon thread was tied. In each pot 40 inoculum bits were added to the soil mixture. The same type of experiment was repeated with unautoclaved soil. Four replicates were maintained and were kept in green house for four months viz from June to September under rainfed condition. When there was no rain, the pots with autoclaved soil were watered with sterilized water and pots with

unautoclaved soil were irrigated with tap water for every two days. At the end of every month, ten inoculum bits from each pot were removed and tested for the survival of *T. paradoxa* by plating them on PDA plates.

Results and Discussion

a) In vitro studies

In vitro studies on the effect of acetone extract of garlic bulb on *T. paradoxa* revealed that the garlic extract was inhibitory to the mycelial growth of the fungus. Garlic extract was reported to be antifungal to *Macrophomina*

Table 1. Effect of garlic extract on mycelial growth and spore germination of *T. paradoxa*

	Percentage of inhibition of *T. paradoxa*		
	1%	5%	10%
1. Growth inhibition			
a) 24 hr after inoculation	100	100	100
b) 48 hr after inoculation	74.42	100	100
2. Spore germination inhibition			
a) Endoconidia	100	100	100
b) Chlamydospores	100	100	100

phaseolina (Tassi.) Goid (Ahmed and Sultana, 1984); *Alternaria zinnae*. Pape. (Chalfoun and Carvalho, 1987); *Drechslera oryzae* (Brede de Hann.) Subram and Jain (Alice and Rao, 1986);

Fusarium oxysporum Schlecht.ex.Fr. and *F.accuminatum* Ell. and Ev. (Assadi and Behroozin, 1987); *Sclerotium rolfsii* Sacc. (Singh and Dwivedi, 1987); *Sarcocladium oryzae* (Kanagarajan, 1988); *Phytophthora nicotiana* Brede de Hann. and *P. infestans* (Mont.) de Bary (Paik, 1989); *P. palmivora* (Raghu, 1990); *Thanatephorus cucumeris* (Fr.) Donk, (Lakshmanan *et al.*, 1990); *Curvularia lunata* (Wakker) Boed.(Upadhyaya and Gupta, 1990);

Aspergillus flavus Link. and *A. parasiticus* Spheare (Kshemkalyani *et al.*, 1990) and *A. fumigatus* Fres. (Rees *et al.*, 1993). In the present study 5 per cent to ten per cent bulb extracts of *A. sativum* completely inhibited *in vitro* mycelial growth of *T. paradoxa* on PDA plates even after 24 hr. of inoculation (Table 1). When the fungal inoculum discs of *T. paradoxa* from 5 per cent and 10 per cent PDA plates were transferred after 3 days of inoculation to fresh PDA plates without the extract, *T. paradoxa* did not grow on the fresh plates. Hence garlic extract at 5 per cent and 10 per cent concentration was found to be fungitoxic to *T. paradoxa.*

In the present study garlic extract completely inhibited (100 per cent) the germination of both endoconidia and chlamydospores of *T. paradoxa* at one per cent concentration itself (Table 1). Thus garlic extract will be quite beneficial for use in the biocontrol of *T. paradoxa.* Extract of garlic was reported to be inhibitory to the spore germination of many fungi like *Alternaria alternata* (Fr.) Keissler, (Kumar *et al.*, 1979); *A. solani* Jones and Grout and *A. temuissima*(kze.ex. pers.) Wilshire (Singh *et al.*, 1990); *Botryodiploidia theobromae* (Ahmed and Sultana, 1984); *Corynespora cassicola* (Berk. and Curt.) Wi., (Kumar *et al.*, 1979); *Drechslera oryzae* (Brede de Hann.) Subram and Jain. (Ramanujam *et al.*, 1988); *D. oryzae* (Brede de Hann.) Subram and Jain (Narasimhan and Pillai, 1992). *D. rostrata* (Drechs.) Richardson and Fraser., (Kumar *et al.*, 1979); *Fusarium oxysporum* Schlecht.ex. Fr. f. sp. *niveum.* (Kumar *et al.*, 1979); *F. oxysporum* Schlecht. ex. Fr. f. sp. *lycopersici* (Tariq and Magee, 1990); *Macrophomina phaseolina* (Tassi.) Goid, (Ahmed and Sultana, 1984); *R. solani* Kuhn. (Lakshmanan *et al.*, 1990);

b) In vivo studies

In vivo experiment was done with two types of inoculations. When the pathogen and garlic extract were inoculated 4 cm apart from each other, the extract exerted 62 per cent inhibition of lesion formation by the pathogen in detached coconut leaf petioles after 15 days of inoculation. Simultaneous inoculation of the pathogen inoculum and garlic extract to the same "well" in detached leaf petiole exerted 89 per cent inhibition of lesion formation after 15 days of inoculation. There are reports where garlic extract of *A. sativum* was used as a good biocontrol agent against pathogens like *D. oryzae* (Alice and Rao, 1986) and *Trichoconiella padwickii.* Ganguly (Shetty *et al.*, 1989). In the present study also garlic extract was found to be effective in reducing the lesion size due to *T. paradoxa* in the detached petioles of coconut. Hence garlic can be used beneficially in the biocontrol of *T. paradoxa.*

c) Pot culture experiment

Studies on the survival of *T. paradoxa* in autoclaved and unautoclaved (natural) soil mixed separately with garlic bulb slices were carried out in earthern pots under green house condition. Complete (100 per cent) destruction of *T. paradoxa* inoculum was observed after one month of inoculation in both autoclaved and unautoclaved soils amended with sliced garlic bulb material (Table 2). When *T. paradoxa* inoculum on rachilla bits from pot soil after one month were transferred to fresh PDA plates, no growth of *T. paradoxa* was found. Lakshmanan *et al.* (1990) reported significant reduction of collar rot disease incidence of *Phaseolus aureus* seedlings due to *Thanatephorus cucumeris* (Fr.) Donk. when 25% aqueous garlic bulb extract was used as soil drench in his pot culture experiments. Thus, garlic bulb

Table 2. Pot culture experiment on survival and growth inhibition of *T. paradoxa* inoculum in soils amended with garlic bulb slices.

Type of soil	Time of observation after inoculation of *T. paradoxa*							
	One month		Two months		Threemonths		Four months	
	Sur-vival %	Inhi-bition%	Sur-vival%	Inhi-bition%	Sur-vival%	Inhi-bition%	Sur-vival%	Inhi-bition%
Autoclaved soil	0	100	0	100	0	100	0	100
Unautoclaved soil	0	100	0	100	0	100	0	100

which was found to be fungicidal to *T. paradoxa* in the soil can be used as a good biocontrol agent against *T. paradoxa* in garden soil.

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