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Research Article

# MANAGEMENT STRATEGIES FOR GANODERMA WILT DISEASE OF COCONUT

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**Abstract:** Basal stem rot or Ganoderma wilt caused by *Ganoderma* spp. is the most destructive and lethal disease in coconut. Refinement of management practices is imperative for effective management of Ganoderma disease in endemic areas. Thus, in the present investigation five new fungicides and biocontrol agents were tested against *G. lucidum* under *in vitro* condition in order to select effective ones to test against basal stem rot disease under field condition. Subsequently, effective fungicide such as Hexaconazole 5EC and bioagent, *Trichoderma harzianum* were evaluated under field condition for three years from 2014 to 2017. Results revealed that root feeding with 1%Hexaconazole 5EC+ soil application of *T. harzianum* enriched neem cake @ 5kg/palm at four months interval and soil application of *T. harzianum* enriched neem cake @ 5kg/palm at three months interval were effective in reducing the disease index from 32.2 to 17.1 and 36 to 20% respectively during first year and recorded complete recovery of the infected palms during third year.

**Keywords:** *Ganoderma, Basal stem rot, Coconut, Hexaconazole*

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## Introduction

Coconut (*Cocos nucifera* L.) is an important plantation crop and popularly known as 'Kalpavriksha'. It plays a vital role in export potential as well as domestic requirements and in employment generation. Also provides livelihood security to several millions of people across the world and capacity of coconut in providing improved nutrition, employment and income generation are well known. Coconut is extensively grown in 94 countries in the world and India accounts for 31.46 percent of the world's coconut production. India is also the highest domestic consumer, at 1494.4 million nuts annually. Four southern states such as Kerala, Karnataka, Tamil Nadu and Andhra Pradesh account for 90% of the coconut production in India [1].

Even though coconut palm is hardy in nature and adaptable to varied climatic conditions, it is counteracted by many pest and diseases [2,3]. Coconut production is affected by many diseases, some of which are lethal and others are debilitating in nature. Among the fungal diseases that affect coconut production in India, basal stem rot (*Ganoderma* wilt) caused by *Ganoderma applanatum* (Pers) Pat. and *G. lucidum* (Leys) Karst. is the most destructive disease other than bud rot [4,5]. Ganoderma has a broad host range including palms such as coconut, arecanut and oilpalm [6-8], tree species [9], tea, cocoa and rubber [10]. Basal stem rot disease is also referred as Thanjavur wilt in Tamil Nadu or Ganoderma wilt in Andhra Pradesh and foot rot or Anabe roga in [11-13].

This disease was first time noticed from Karnataka state by Butler [14] and it was reported from Thanjavur district of Tamil Nadu after 1952 and 1956 cyclones. Subsequently, similar type of disease has been noticed in Andhra Pradesh by Papa Rao and Govinda Rao (1966) [15] and in Kerala by Wilson *et al*. (1987) [16]. This disease was noticed in Thanjavur district in 1952 and it was confined to the coastal districts of Tamil Nadu till 1966 [17]. In 1978 the Ganoderma disease was noticed in all over the districts of Tamil Nadu and incidence ranged from 0.6 to 4.9 percent [17]. According to survey conducted by Snehalatharani *et al*. (2014) [18], maximum disease incidence (13.8%) was recorded in East Godavari District of Andhra Pradesh, followed by Hassan district of Karnataka (7.5%) and in Thanjavur district (6.5%) of Tamil Nadu.

In Kasaragod district of Kerala highest incidence of disease was observed in Pilicode (4%) panchayath followed by Pallikkara [19]. Gradually the disease was spread and presently the disease is prevalent in all most all coconut growing areas. Earlier this disease was effectively controlled by soil drenching with 0.1% carboxin or tridemorph [20]. Also using aureofungin solution and Tridemorph. By employing talc-based formulation of only bioagents *Trichoderma reesei* and *Pseudomonas fluorescens* [21]. Presently the disease is prevalent in most of the coconut growing areas. Thus, there is a need to develop integrated disease management strategies by using effective fungicide and potential native biocontrol agents. The objective of present study was to refine integrated disease management strategies for effective management of basal stem rot disease.

## Materials and Methods

### Isolation and identification of pathogen

Root samples, soil and sporocarps were collected from palms showing Ganoderma wilt type symptoms from Kasaragod and Dakshina Kannada district of Karnataka. Pathogen was isolated from root samples, soil and sporocarps by following standard tissue isolation technique using Ganoderma specific medium. The pathogen was identified by sequencing ITS region.

### Screening of fungicides and biocontrol agents against *Ganoderma lucidum*

The relative efficacy of six fungicides *viz*, Hexaconazole 5EC, Hexaconazole 2SC, Difenconazole 250EC, Tebuconazole 25.9EC, Propiconazole 25EC, Fenpropimorph 79.5%EC at different concentrations (125ppm to 1000ppm) were evaluated against *Ganoderma lucidum* by following poisoned food technique. Measured quantity of fungicide solution prepared in sterilized distilled water was incorporated into sterilized carrot agar medium to get the required concentration. The poisoned medium was then poured into sterilize petriplates @ 15 ml/plate. After solidification of the medium each plate was inoculated centrally with 5mm mycelial disc of five days old culture and the plates were incubated at room temperature for five days.

The percent inhibition of growth of *Ganoderma* was calculated by using formula given by Vincent (1927) [22].

I=C-T/C×100

I=Inhibition of fungal growth, C= Growth in control, T= Growth in Treatment

Antagonistic activity of three *Trichoderma* spp. (*T. harzianum, T. viride and T. virens*,) and two bacterial bioagents (*Bacillus subtilis, Pseudomonas fluorescens*) were tested against *Ganoderma lucidum* following dual culture technique (Skidmore and Dickinson, 1976). The observations were recorded on the radial growth of the pathogen after every 24 hours till the colony of the pathogen was completely overgrown. The diameter of the pathogen was also recorded in dual culture till the growth of the pathogen ceased. Percent inhibition of growth was calculated using the following formula [23].

*Percentage inhibition of radial growth=R1-R2/R1×100*

R1= Radial growth of pathogen in control

R2= Radial growth of pathogen in dual culture

**Evaluation of effective fungicide and biocontrol agent in field condition**

Effective fungicide such as Hexaconazole 5EC and potential native isolate from coconut based cropping system, *Trichoderma harzianum* (CPTD28 GenBank No. LC155111) were tested against Ganoderma wilt disease. Field management trial was conducted in disease prevalent coconut gardens at Neeleshwar block, Kasaragod district of Kerala from 2014 to 2017. Coconut trees showing basal stem rot disease symptoms were selected and 30 infected trees were selected for each treatment. *Trichoderma* (*T. harzianum*, LC155111) talc powder formulation developed by ICAR-CPCRI was mixed with neem cake in the ratio of 1:100 and used for field evaluation. After soil application of *T. harzianum* enriched neem cake @ 5kg/palm irrigation was provided at 5 days interval followed by mulching with dried leaves. The treatments were imposed from April 2014 to March 2017 at three- and four-months interval after recording pre-treatment disease index. Ganoderma disease index was calculated using formula refined by Bhaskaran and Karthikeyan, (1994). Disease index= 23.6+17.4h+36.6r-0.6l, where h is height of bleeding patch, 'r' is reduction in leaf size (0-4 scale), 'l' is number of functional leaves. The disease index at the beginning and at quarterly interval was recorded in individual replication and the average was calculated. Data on post treatment disease index, *Trichoderma* population were recorded from the treated coconut palms at regular intervals. The data was analyzed statistically using SAS software.

**Results and discussion**

**Isolation and identification of pathogen**

The pathogen was isolated from root samples and sporocarps using Ganoderma specific medium and it was identified as Ganoderma lucidum based on sequencing of ITS region.

**Screening of fungicides and biocontrol agents against *Ganoderma lucidum***

Relative growth rate of *Ganoderma lucidum* at various concentrations of fungicides is summarised in [Table-1]. Among the fungicides evaluated at different concentrations (125ppm to1000ppm) against *Ganoderma lucidum*, Hexaconazole 5EC at 125 ppm followed by Difenconazole 250EC, Tebuconazole 25.9EC at 500 ppm and Propiconazole 25EC at 1000 ppm exhibited 100% mycelial growth inhibition [Table-1], [Fig-1]. Effectiveness of different fungicides against Ganoderma under *in vitro* was demonstrated by Lim *et al*., (1990) [24] (penconzole and tridemorph), Srinivasulu *et al*. (2002) [25] (Triademifon, Tridemorph, Bitertenol, Copper Oxy Chloride and Hexaconazole). Tridemorph 80EC @ 0.3% completely inhibited the growth of *Ganoderma lucidum* under *in vitro* condition followed by Carbendazin, 50WP @ 0.3% with 91.33% inhibition over control [26]. The present study also proved efficacy of Hexaconazole5EC@ 125ppm against *G. lucidum*.

Several organisms are known to be antagonistic against plant pathogens. Among the biocontrol agents, *Trichoderma* is being effectively utilized for the management of many economically important diseases in coconut ecosystem. Out of three *Trichoderma* spp. and two bacterial bioagents tested against *G. lucidum*, *T. harzianum* (CPTD 28, GenBank No. LC155111) exhibited highest mycelial growth inhibition of *Ganoderma lucidum* upto 81.4% followed by *T. viride and T. virens* [Table-2], [Fig-2]. These results are in confirmation with previous researchers, who reported potentiality of *Trichoderma harzianum* against *Ganoderma lucidum* [27-29]. *Trichoderma* and Pseudomonas effective in inhibiting mycelial growth of Ganoderma [30].

Table-1 *Efficacy of fungicides against Ganoderma lucidum in in vitro*

| Fungicides | Concentration (ppm) | Percent growth inhibition |
|---|---|---|
| Hexaconazole 5EC | 125 | 100[a] |
| | 250 | 100[a] |
| | 500 | 100[a] |
| Difenconazole 250EC | 500 | 100[a] |
| Tebuconazole 25.9EC | 500 | 100[a] |
| Propiconazole 25EC | 1000 | 100[a] |
| Propiconazole | 500 | 80.5b |
| Tebuconazole | 250 | 78.5b |
| Difenconazole | 250 | 77.3b |
| Propiconazole | 250 | 75.0b |
| Fenpropimorph 79.5EC | 1000 | 71.2b |
| Tebuconazole | 125 | 56.5c |
| Difenconazole | 125 | 53.2c |
| Fenpropimorph | 500 | 53.5cd |
| | 250 | 50d |
| | 125 | 40e |

**Means with the same letter are not significantly different according to DMRT (P=0.005)*

Table-2 *Efficacy of biocontrol agents against Ganoderma lucidum in in vitro*

| Biocontrol agents | Percent growth inhibition |
|---|---|
| *1. T. harzianum* | 81.4a |
| *2. T. viride* | 66.5b |
| *3. T. virens* | 63.3b |
| *4. Bacillus subtilis* | 24.2c |
| *5. Pseudomonas fluorescens* | 22.6c |

**Means with the same letter are not significantly different according to DMRT (P=0.005)*

Effective fungicide such as Hexaconazole 5EC and potential biocontrol agent, *Trichoderma harzianum* (CPTD28) were evaluated for their efficacy against Basal stem rot disease of coconut under field condition. Out of six treatments imposed against the disease, significant reduction in disease index was recorded in T5 (root feeding with Hexaconazole 5EC 1% + soil application of *T. harzianum* enriched neem cake @ 5kg/palm at four months interval) and T1 (soil application of *T. harzianum* (CPTD 28) enriched neem cake @ 5kg/palm at three months interval) during first year. During 2016 three treatments, T1, T2 and T5 were on par in reducing disease index and in third year complete recovery of palms were observed T1, T2, and T5. While in control palms, significant increase in disease index was noticed and also death of one infected palm [Table-3]. Several management trials suggested vast number of chemicals *viz*., 1% Bordeaux, Aureofungin-sol (0.2%) alone or in combination effective in controlling Ganoderma wilt disease [31]. Field trial conducted at Palghat (Kerala) by CPCRI, Kasaragod showed less disease in Tridemorph80EC and aureofungin solution treated palms [32]. The lowest-disease index was achieved with Tridemorph80EC root feeding (2%) + soil drenching @ 0.3% [33]. Chemical control approach has been widely practiced for the management of basal stem rot disease but non-judicial usage of chemical pesticides leads to soil pollution and also detrimental effects in animals and humans. In order to overcome harmful effects of pesticide, the integrated disease management system involving biocontrol agent is imperative for effective management of the disease. The present study also demonstrated root feeding with Hexaconazole 5EC 1% + soil application of *T. harzianum* enriched neem cake @ 5kg/palm at four months interval is very effective in reducing the disease in first year itself compare to root feeding or drenching alone with Hexaconazole 5EC 1%. Among the biocontrol agents, *Trichoderma* is of immense importance in the plant disease management because of their bio-control potential against an array of phytopathogens through several modes of action. In the current study soil application of *T. harzianum* (CPTD 28) enriched neem cake @ 5kg/palm at three months interval was found effective during initial year and this treatment was on par with other treatments with *Trichoderma* application at four months interval on third year. The disease suppression in *Trichoderma* treatment was achieved because of increased population of *Trichoderma* in treatment with application of *Trichoderma* enriched neem cake @ 5kg/palm at three months interval followed by application at four months interval [Table-4].

Table-3 *Efficacy of Hexaconazole 5EC and T. harzianum against Ganoderma wilt disease*

| Treatments | Pre-treatment disease index | Pos-treatment disease index 2015 | Post-treatment disease index 2016 | Post-treatment disease index 2017 |
|---|---|---|---|---|
| T1- Application of *T. harzianum* enriched neem cake @ 5kg/palm at three months interval | 36.0 | 20. 0a | 5.0[a] | 0.0[a] |
| T2- Application of *T. harzianum* enriched neem cake @ 5kg/palm at four months interval | 40.0 | 24.5ab | 6.0[a] | 0.0[a] |
| T3- Root feeding with Hexaconazole 5EC @ 1% at four months interval | 31.1 | 25.4ab | 15.5[b] | 8.0[b] |
| T4- Drenching with Hexaconzole 5EC @ 0.1% at four months interval | 37.0 | 27.0b | 18.0[b] | 10.0[b] |
| T5- Root feeding with Hexaconazole 1% + application of *T.harzianum* enriched neem cake @ 5kg/palm at four months interval | 32.2 | 17.1a | 5.5[a] | 0.0[a] |
| T6-Control* | 34.6 | 46.0c | 55.3[c] | 67.3[c] |

*Means followed by same letters are not significantly different according to DMRT (P<0.01), * one palm dead*

Table-4 *Trichoderma population estimated in different treatments*

| Treatments | Colony forming units $\times 10^8$/g |
|---|---|
| T1- Application of *T. harzianum* enriched neem cake @ 5kg/palm at three months interval | 150.3[a] |
| T2- Application of *T. harzianum* enriched neem cake @ 5kg/palm at four months interval | 120.8[b] |
| T5- Root feeding with Hexaconazole 1% + application of *T.harzianum* enriched neem cake @ 5kg/palm at four months interval | 125.2[b] |

*Means followed by same letters are not significantly different according to DMRT (P<0.05)*

These results are in corroborating with previous researchers who reported frequency of application of bioagents should be at three months interval based on the rhizosphere populations of the bioagents [34]. Bhaskaran *et al*., 1994 [35] recorded enhanced population of *Trichoderma* sp. due to application of neem cake into the soil.

## Conclusion

The present study concluded that Ganoderma wilt disease of coconut could be effectively and eco-friendly managed either by root feeding with Hexaconazole 5EC @1% + soil application of *T. 0* enriched neem cake @ 5kg/palm at four months interval or soil application of *T. harzianum* enriched neem cake @ 5kg/palm at three months interval.

**Application of research:** Management strategies developed in the present study could be used for effective and eco-friendly management of basal stem rot disease of coconut.

**Research Category:** Crop Protection

**Abbreviations:** ITS- Internal transcribed spacer
CPCRI- Central Plantation Crops Research Institute
CDB-Coconut Development Board

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Research project name or number: Integrated management of Ganoderma wilt disease of Coconut

**Author Contributions:** All authors equally contributed

**Author statement:** All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

**Study area / Sample Collection:** Neeleshwar, Kasaragod district of Kerala

**Cultivar / Variety / Breed name:** Coconut (*Cocos nucifera* L.)

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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