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Effect of bio-control agents and botanicals on *in vitro* growth and development of *Ganoderma applanatum*

K.B. Palanna*, B. Boraiah[1], M.S. Nagarj[2], N.E. Thyagaraj[2] and G.R. Ramaswamy[3]

Agricultural Research Station, UAS (B), Konehally, Tiptur - 572202, Tumkur Dist., Karnataka
[1]Agricultural Research Station, UAS (B), Arsikere - 573103, Hassan Dist., Karnataka
[2]College of Agriculture, UAS (B), Karekere - 573225, Hassan Dist., Karnataka
[3]Zonal Agricultural Research Station, Babur Farm, Hiriyur - 572143, Chitradurga Dist., Karnataka

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Abstract

Efficacy of bio-control agents and botanicals against *Ganoderma applanatum*, a fungi causing basal stem rot of coconut was studied at Agricultural Research Station, Arsikere during the year 2008-09. Among the 17 bio-control agents screened, native *Trichoderma* sp. (V_2) recorded minimum radial growth of 1.72 cm by exerting 81 per cent reduction over control, which was followed by *Trichoderma* sp. (12a) by accounting 2.30 cm radial growth with 74 per cent reduction over control. Among the six bio-control agents, tested for biomass production, native *Trichoderma* sp. (B_4) recorded maximum biomass (0.76 g) followed by *Trichoderma* sp. (C_4) which accounted for 0.7 g 100 ml^{-1} potato dextrose broth. Among 10 botanicals tested, only *Glyricidia* was found to be inhibitory against *G. applanatum*, by recording radial growth of 5.4 cm as against 9.0 cm in control.

Keywords: Basal stem rot, bio-control agents, botanicals, coconut, *Ganoderma applanatum*

Introduction

In India coconut palms are grown in an extent of 1.94 million hectares with an annual production of 14811.4 million nuts and a productivity of 7608 nuts ha^{-1}, predominantly in southern states. Kerala ranks first in terms of area and production followed by Tamil Nadu, Karnataka and Andhra Pradesh. Tamil Nadu ranks first in productivity followed by Andhra Pradesh and Kerala. Coconut palms are normally affected by various biotic and abiotic stresses resulting in leading to drastic yield reduction. Among the various biotic stresses that affect coconut production in India, basal stem rot (BSR) caused by *Ganoderma applanatum*, Pers. and *G. lucidum* Leys. Karst, is one of the major constraints in coconut production, especially in dry tracts of southern Karnataka. The disease is also reported from Tamil Nadu (Tanjavur wilt), Andhra Pradesh (basal stem rot), Kerala, Maharastra, Gujarat and Orissa (Bhaskaran *et al.*, 1994; Wilson *et al.*, 1987). *Ganoderma* sp. has a wide host range, attacking a variety of palms and several forest, avenue and fruit trees (Naidu *et al.*, 1966; Govindu *et al.*, 1983; Bhaskaran *et al*., 1994). The fungus usually attacks old or weak palms growing under unfavorable conditions. It is a soil dweller inhabiting dead as well as living plant material in the soil, enters through the wounds and disease spreads mainly through soil. Basal stem rot disease incidence ranged from 6.06 to 36.15 per cent in Arsikere Taluk of Karnataka (Naik *et al.*, 2000). Its incidence was observed maximum (up to 62.50%) in coconut gardens raised in sandy and red soils in coastal district of Andhra Pradesh (Srinivasulu *et al*., 2003). Though, several researchers (Bhaskaran *et al.*, 1994; Radhakrishnan, 1990; Srinivasalu *et al*., 2001) have reported different practices for the management of the disease, the results are inconsistent and not much

**Corresponding Author: kbpalanna@gmail.com*

work has been done relating to the aspects of ecological sustainability. Biological control of soil borne diseases and control of diseases by botanicals is gaining importance in perennial crops owing to the ill effects and non-consistent results of the chemical management practices (Bhaskaran *et al.*, 1994). In this context, *in vitro* screening of native bio-control agents (BCAs) and botanicals is a prerequisite for determining their efficacy and for further field studies in controlling these diseases. Hence, the present investigation was taken up to congregate information on these lines to exploit native BCAs and botanicals in integrated disease management of basal stem rot (BSR) of coconut.

Materials and methods

Collection and isolation of bio-agents

Soil samples were collected from rhizosphere and non-rhizosphere regions of coconut from various places to isolate native *Trichoderma* spp. Isolation was done by serial dilution technique using *Trichoderma* selective medium (TSM) developed by Elad and Chet (1983). *Trichoderma* spp. were identified based on the characters described by Rifai (1969). Fungal and bacterial bio-control agents were also collected from Project Directorate of Biological Control (PDBC), ICAR, Bangalore for *in vitro* screening and identification of potential bio-control agents against *Ganoderma applanatum*.

Screening of potential bio-control agents against *G. applanatum* (G_{14})

Fungal and bacterial bio-control agents were screened against BSR by dual culture technique using potato dextrose agar medium. Dual cultures of the fungal antagonists and test pathogen were prepared by inoculating potato dextrose agar mycelial discs from the growing margins of the respective cultures on to petri dishes containing PDA (Gams *et al.*, 1980) and incubated at room temperature (28 ± 2 °C) for seven days. The mycelial growth of test pathogen was measured at three and seven days after inoculation.

In case of bacterial antagonists, 9 mm mycelial discs of the test pathogen were placed individually at one corner of plates and bacterial strain was streaked on opposite side of Petri plate with PDA medium and incubated. The mycelial growth of test pathogen was measured at 3 and 7 days after incubation. The dual cultures were observed for antibiosis, mycoparasitism and other typical interactions under light microscope from the regions where the colonies were merged.

Table 1. List of fungal and bacterial bio-control agents screened against *G. applanatum* (G_{14}) in vitro

Sl. No	BCAs	Source
1	*T. viride* (Tv-1)	PDBC, Bangalore
2	*T. harzianum* (Th-10)	PDBC, Bangalore
3	*T virense* (Tvs-12)	PDBC, Bangalore
4	*T. hamatum*	PDBC, Bangalore
5	*T. koningii*	PDBC, Bangalore
6	*T.* sp (12b)	ARS, Arsikere
7	*T.* sp (V_1)	Vitlapura
8	*T.* sp (C_4)	ARS, C4 Block
9	*T.* sp (B_4)	ARS, B4 Block
10	*T.* sp (12a)	Haranahally
11	*T.* sp (T_8)	Karagundha
12	*T.* sp (V_2)	Vitlapura
13	*T.* sp (Y)	Yalavare
14	*Chaetomium globossum*	PDBC, Bangalore
15	*Pseudomonas fluoresences* (Pf-1)	PDBC, Bangalore
16	*Pseudomonas fluoresences* (Pf-2)	PDBC, Bangalore
17	*Bacillus subtilis*	PDBC, Bangalore

Studies on biomass production of bio-control agents

Potential bio-agents identified by *in vitro* screening were tested for biomass production. The flasks containing 100 ml potato dextrose broth were inoculated with 7 day old 9 mm culture disc of *Trichoderma* spp. Three replications were maintained for each treatment. The inoculated flasks were incubated at room temperature (28 ± 2 °C) for 10 days, and then mycelial mat was harvested on a previously weighed Whatman No.4 filter paper and dried at 105 °C in a hot air oven till constant weight was obtained. The dry mycelial weight was recorded and expressed in mg 100 ml^{-1} broth.

In vitro evaluation of botanicals against *G. applanatum* (G_{14})

Ten per cent (wet wt per volume) leaf extract of locally available ten plant species (Table 2) were evaluated against *G. applanatum* by poisoned food technique. Three replications were maintained for each treatment. The petri plates containing PDA with 10 per cent leaf extract of

Table 2. List of plants evaluated *in vitro* against *G. applanatum* (G_{14}) (10% leaf extracts)

Sl No.	Botanicals used	
	Common name	Botanical name
1	Neem	*Azadirachta indica*
2	Pongamia	*Pongamia pinnata*
3	Tamarind	*Tamarindus indica*
4	Curry leaf	*Murraya koenigii*
5	Glyricidia	*Glyrecidia sepium*
6	Communist weed	*Euphorbia communnis*
7	Lantana	*Lantana camera*
8	Thonde	*Coccinia grandis*
9	Cowhage	*Mucuna pruriens*
10	Parthenium	*Parthenium hysterophorus*

different plants were inoculated with 9 mm culture discs of 7 days old test pathogen and incubated at room temperature (28 ± 2 °C) for 7 days. The radial growth of test pathogen was measured after seven days of incubation and per cent inhibition was calculated.

$$\% \text{ Inhibition} = \frac{\text{(Mean radial growth in control - Mean radial growth in treatment)}}{\text{Mean radial growth in control}} \times 100$$

Results and discussion

Collection and isolation of bio-agents

Seventeen native *Trichoderma* spp. were isolated from rhizosphere and non-rhizosphere soil samples collected from various places. *Trichoderma* spp. are known to be ubiquitous and it occurs in almost all soil types and other natural habitats. The population of *Trichoderma* spp. vary greatly with the physiological parameters of the soil (Palanna *et al.*, 2005d). Individual species aggregates may be restricted in their geographic distribution (Danielson and Davey, 1973). Nagamani and Mew (1987) reported that there was a wide distribution and variation in dominance of different species of *Trichoderma*. Presence of *T. harzianum* was found to vary significantly from month to month, but no regular pattern of seasonal variation was observed (Eastburn and Butler, 1988). Seasonal variation of fungal population was more pronounced in the upper layers of soil with maximum population during January and minimum during May (Behera *et al*., 1991).

Screening of potential BCAs against *G. applanatum* (G_{14})

Seventeen bio-control agents (fungal and bacterial) were screened against *G. applanatum* by dual culture technique. Among seventeen BCAs, (Table 4 and Fig. 1) native *Trichoderma* sp. (V_2) recorded minimum radial growth of 1.72 cm by exerting 81 per cent reduction over control, which was followed by *Trichoderma* sp. (12a) and

Table 3. *In vitro* screening of potential BCAs against *G. applanatum* (G_{14})*

Sl. No	BCAs	Radial growth (cm)	% reduction over control	Over growth (mm)	Microscopic and other observations
1	*T.* sp (12b)	4.10	54	0.00	Inhibition zone/antibiosis
2	*T.* sp (12a)	2.30	74	0.00	Inhibition zone
3	*T.* sp (T_8)	3.30	63	11.77	Mycoparasitism, antibiosis and lysis
4	*T.* sp (V_2)	1.72	81	0.00	Inhibition zone
5	*T.* sp (V_1)	2.65	71	0.00	Antibiosis and lysis
6	*T.* sp (C_4)	4.65	48	0.00	Antibiosis and lysis
7	*T.* sp (B_4)	2.70	70	12.50	Partial mycoparasitism, antibiosis and coiling
8	*T.* sp (Y)	3.45	62	0.00	Antibiosis lysis and mycelial deformation
9	*T.V*-1	3.00	67	8.33	Partial mycoparasitism, antibiosis and coiling
10	*TH*-10	4.55	49	0.00	Antibiosis and lysis
11	*TVS*-12	3.50	61	35.00	Complete mycoparasitism, antibiosis and lysis
12	*T. hamatum*	3.80	58	0.00	Antibiosis, lysis, coiling and mycelial deformation
13	*T. koningii*	4.60	49	0.00	Antibiosis and lysis
14	*C. globossum*	7.78	14	0.00	---
15	Pf-1	4.60	49	--	Lysis and mycelial deformation
16	Pf-2	4.00	56	--	Lysis, mycelial deformation and reddish pigmentation
17	Bs-1	4.95	45	--	Lysis and mycelial deformation
18	Control	9.00	--	--	

SE(m) ± 0.173; CD(5%) 0.354; C.V(%) 3.89; *Mean of three replications

Table 4. Biomass production (on dry weight basis) of BCAs*

Sl. No	BCAs	Biomass (g per 100ml)
1	*T.* sp (V1)	0.54
2	*T.* sp (C4)	0.70
3	*T.* sp (B4)	0.76
4	*T.* sp (Y)	0.54
5	*T.* sp (V2)	0.72
6	*T.* sp (12a)	0.74
7	*T.V*-1 (PDBC)	0.33
8	*TH*-10 (PDBC)	0.51
9	TVS-12 (PDBC)	0.68
10	*T. koningii* (PDBC)	0.57
11	*T. hamatum* (PDBC)	0.60

SE (±) 0.112; CD (0.05) 0.270; C.V (%)18.67
*Mean of three replications

Trichoderma sp. (V1) by accounting 2.30 and 2.65 cm radial growth with 74 and 71 per cent reduction over control respectively. Among 14 fungal bio-agents screened for growth/mycoparasitism (growth of BCAs on pathogen) was observed only in four *Trichoderma* sp. *T. virens* (Tvs-12) exerted 100 per cent mycoparasitism followed by *Trichoderma* sp. (B_4) and *Trichoderma* sp. (T_8).

Among bacterial BCAs, *Pseudomonas fluorescens* (Pf-2, PDBC) recorded minimum (4 cm) radial growth which accounted for 56 per cent reduction over control (9 cm radial growth) in potato dextrose agar medium. *P. fluorescens* (Pf-2) produced strong reddish pink pigmentation and it was not noticed in other bacterial bio-control agents used in the study. Srinivasalu *et al.* (2004) reported that native BCAs (*T. viride, T. harzianum* and *T. hamatum)* were effective in controlling basal stem

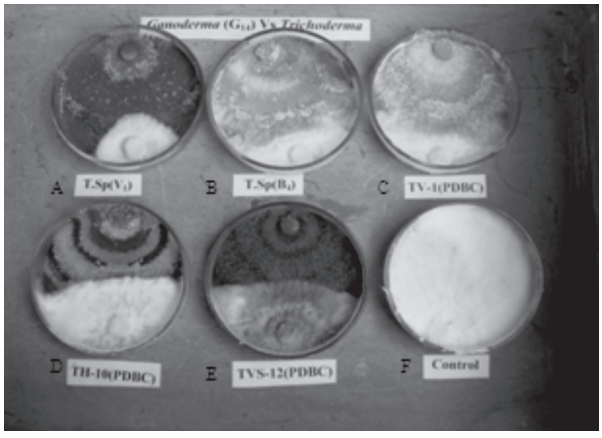

Fig. 1. ***In vitro*** **antagonism of bio-control agents against** ***G. Applanatum*****. (A)** ***T.*** **spp (V1); (B)** ***T.*** **spp (B4); (C)** ***T. viride*** **(Tv-1); (D)** ***T. harzianum*** **(TH-10); (E)** ***T. virens*** **(TVS-12); (F) control**

rot pathogens (*G. lucidum* and *G. applanatum*) *in vitro. T. harzianum* and *T. viride* were reported to be antagonistic to *G. lucidum* (Gunasekaran *et al.*, 1986; Bhaskaran, 1990).

Studies on biomass production of BCAs

Potential bio-agents identified by *in vitro* screening were tested for biomass production. The results revealed that, among 11 BCAs, native *Trichoderma* sp. (B_4) recorded maximum biomass (0.76 g) followed by *Trichoderma* sp. (12a) and *Trichoderma* sp. (V_2) which accounted 0.74 and 0.72 g per 100 ml potato dextrose broth respectively and are on par with each other. Native bio-control agents recorded higher biomass (0.54 to 0.76 g per 100 ml) compare to collected *Trichoderma* sp. used under study, which accounted 0.33 to 0.68 g per 100ml PDB. The results revealed that all the isolates used in this study showed great variability (Table 6) in biomass production on PDB. Palanna *et al.* (2005a, 2005b and 2005c) reported that biomass production of *T. viride* was influenced by pH, addition of NPK complex fertilizers, secondary and micro-nutrients to the medium.

Table 5. ***In vitro*** **evaluation of botanicals (10% leaf extract) against** ***G. applanatum*** **(G_{14})**

Sl. No.	Botanicals	Radial growth (cm)	% inhibition after 7 days
1	Azadirachta indica	9.0	0
2	Pongamia pinnata	9.0	0
3	Tamarindus indica	9.0	0
4	Murraya koenigii	9.0	0
5	Glyricidia sepium	5.4	40
6	Euphorbia communnis	9.0	0
7	Lantana camera	9.0	0
8	Coccinia grandis	9.0	0
9	Mucuna pruriens	9.0	0
10	Parthenium hysterophorus	9.0	0
11	Control (PDA)	9.0	-

SE (±) 0.021; CD(0.05) 0.047; C.V(%) 0.25

In vitro evaluation of botanicals against *Ganoderma applanatum* (G_{14})

Locally available ten plant species were evaluated against *G. applanatum* by poison food technique with 10 per cent leaf extract (Table 7). Among the ten plants, *Glyricidia* was found to be inhibitory against *G. applanatum*, which accounted 40 per cent reduction over control by recording radial

growth inhibition by 5.4 cm as against 9.0 cm in control (Fig. 2). Garlic extract of 10 per cent concentration completely arrested the growth of *T. viride*, *T. harzianum* and *T. hamatum* and both the species of *G. lucidum* and *G. applanatum* (Srinivasalu *et al.*, 2002). Neem cake extract, banana rhizome extract and *Tephrosia purpurea* extract gave 100, 86 and 54 per cent inhibition respectively (Bhaskaran *et al*., 1988).

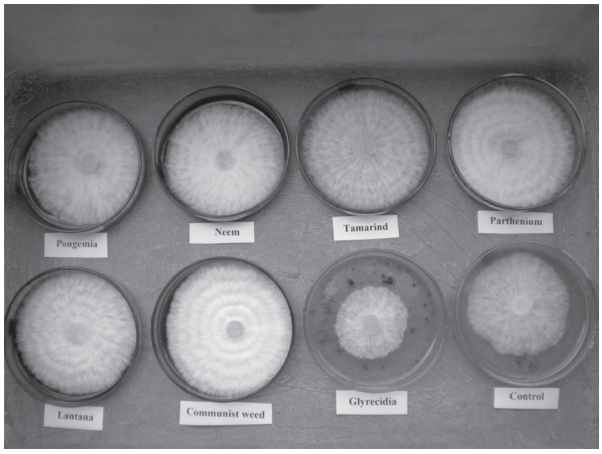

Fig. 2. *In vitro* evaluation of 10% leaf extract of botanicals against *G. applanatum* (A) *Pongamia*, (B) *Azadirachta*, (C) *Tamarindus*, (D) *Parthenium*, (E) *Lantana*, (F) *Euphorbia*, (G) *Glyricidia*, (H) Control

The present investigation under *in vitro* revealed that *Trichoderma* spp. showed great variability in controlling *G. applanatum* causing basal stem rot of coconut. Among various bio-control agents, native isolates were found to be most effective in controlling *G. applanatum.* Among 10 botanicals tested, Glyricidia (10% leaf extract) was found inhibitory for *G. applanatum in vitro*, further, its inhibitory effect on pathogen can be tested under field condition for eco-friendly disease management.

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