

ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST *GANODERMA LUCIDUM* (CURTIS EX. FR.) KARST., CAUSING BASAL STEM ROT DISEASE IN ARECANUT

RANJANA CHAKRABARTY*, G. C. ACHARYA AND T. C. SARMA¹

Central Plantation Crops Research Institute, Kahikuchi, Guwahati -781 017, Assam

¹Department of Botany, Gauhati University, Ghy - 781 014, Assam

e-mail: ranjana74@yahoo.co.in

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***Corresponding author**

ABSTRACT

Basal stem rot disease caused by the fungus, *Ganoderma lucidum* (Curtis Ex. Fr.) Karst, is the most dreaded disease of arecanut in Assam. The experiment was carried out to test the antifungal activity of aqueous extract of thirty locally available plant species against the test fungus. Pure culture of *Ganoderma* sp. was isolated from fruiting body of infected arecanut palm. Among the plant species tested, *Allium sativum* corm extract exerted maximum inhibition with 90.89, 100 and 100 per cent inhibition over control at 5, 10 and 20 per cent conc. respectively, followed by extract of *Solanum nigrum* with 77.00, 84.11 and 100 per cent inhibition over control at 5, 10 and 20 per cent conc. respectively after 144h of incubation. Leaf extract of *Clerodendron infortunatum*, *Bidens pilosa*, *Leucas aspera*, *Spilanthes paniculata*, *Lawsonia inermis* and *Cinnamomum verum* showed strong inhibitory effect at 20 per cent conc. with 84.00, 83.88, 79.22, 77.22, 76.11 and 72.78 per cent inhibition over control respectively.

INTRODUCTION

The arecanut palm (*Areca catechu* L.) is one of the most important plantation crop in Assam grown in an area of 70,000 ha with an annual production of 62.7 thousand tonnes and a productivity of 896 kg chali/ha (India Horticulture Database, 2010). In recent years, arecanut cultivation is beset with recurring problems due to reduced productivity, delayed commercial yield, soil fertility depletion, small holding size, price fluctuation and pests and diseases. Among the various diseases, basal stem rot, a slow decline disease, caused by the fungus, *Ganoderma lucidum* (Curtis ex. Fr.) Karst is the most dreaded that has not only affected productivity but has also wiped out arecanut plantation in certain localities. It is a polyporoid fungus of the order Polyporales, family *Ganodermataceae*. The fungus has attracted the attention of many mycologist since they are considered both as plant pathogen (Hepting, 1971; Adaskaveg and Ogawa, 1990; Adaskaveg et al., 1993) and also as useful medicinal herb (Mizuno et al., 1995). The fungus, *Ganoderma* is cosmopolitan and causes white rot of woody plants by decomposing lignin, cellulose and related polysaccharides. The occurrence of the disease was recorded as early as 1807 from Karnataka (Buchanan, 1807). Butler, 1906 & 1909 reported the disease on cash crops such as coconut, betelnut and other plantation crops like *Casuarina*, *Acacia*, *Dalbergia sisso* and *Toona ciliata* in the North Eastern States. Venkatarayan, 1936 recorded and studied the biology of *Ganoderma* in arecanut and coconut. The disease is also reported from Kerala, Assam, West Bengal

(Sharpley, 1928), Nicobar Islands (Sangal et al., 1961), the present Karnataka (Coleman, 1911) and Mettupalayam areas of Tamil Nadu (Anonymous, 1960). It is a soil borne disease but secondary spread occurs through air borne spores formed on matured fruiting bodies, through irrigation water, repeated ploughing and other cultural operations. The disease is severe in neglected, ill drained and over-crowded gardens.

Crop loss due to the disease is not systematically documented. It is found to be a devastating disease in the North Eastern Region of India affecting arecanut plantation, with high incidence in Goalpara district (78%) followed by Kamrup (11%) and Morigaon (2.8%). Incidence is also reported in the palms of Bongaigaon district (CPCRI Annual Report, 2012-13). Despite extensive damage caused by the pathogen, scanty works has been done for its management. Although control measures using fungicides are reported to be effective (Nambiar and Nair, 1973; Kumar and Nambiar, 1990), it becomes very difficult for large scale adoption. Also, the indiscriminate use of synthetic chemicals for the control of pests and diseases of crops has resulted in serious threat to human health and environment leading to disturbed biodiversity, outbreak of secondary pests, reappearance of resistance in the pathogens and contamination of food chain in the ecosystem. But the present scientists are optimistic in developing alternatives to chemical fungicides. Currently studies pertaining to the use of botanicals in management of diseases are highly emphasized (Koche, 2013; Toppo, 2013; Mathad, 2013; Mahapatra, 2013; Bisht, 2013). However, actual use of plant extracts in managing soil borne diseases in particular is still limited. Keeping in

view the destructive nature of the disease and economic loss, the present investigation was undertaken to evaluate the efficacy of aqueous plant extracts, against *G. lucidum* under *in vitro* conditions.

MATERIALS AND METHODS

Isolation of the fungus

The fungus, *Ganoderma lucidum* was isolated from the fruiting body of basal stem rot affected arecanut palm. The samples were cut into small convenient pieces, sterilized in 0.1% HgCl₂ for one minute, then washed thrice in sterile distilled water and plated on Ganoderma Selective Media (Ariffin and Idris, 1991). Pure cultures were transferred into slants and maintained on Potato Dextrose Agar media for further study.

In-vitro assay of botanicals

Thirty locally available botanicals were tested for their antifungal property against *G. lucidum* by poisoned food technique (Nene and Thapliyal, 1982) under *in vitro* condition. Fresh leaves of test plants were taken for preparing crude extracts. The leaves were thoroughly washed with water and fine slurry was prepared by taking 100g leaves with 100 ml of sterilized distilled water (1:1w/v). The resultant slurry was filtered through three layer of muslin cloth and then through Whatman No.1 filter paper. Finally the filtrate thus obtained was used as stock solution. From the stock solution, 5, 10 and 20mL extract were added to 95, 90 and 80 mL of PDA medium, respectively, to make 5, 10 and 20 per cent concentration. The medium

was thoroughly shaken for uniform mixing of extract. Twenty ml of each medium was poured into 90 mm sterile Petri plates. Mycelium of seven mm size discs from periphery of actively growing culture were cut out by sterilized cork borer and one such disc was placed at the centre of each agar plate. Control was also maintained by growing the pathogen on only PDA. Plates were incubated at 28 ± 2°C for 144h and radial growth was measured. The efficacy of plant products or botanicals was expressed as per cent of radial growth over the control, which was calculated by using the formula (Vincent, 1927).

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

Where,

I = Per cent inhibition over control

C = Radial growth in control

T = Radial growth in treatment

The values obtained in different categories are transformed, wherever necessary and subjected to statistical analysis (Panse and Sukhatme, 1995) for treatment comparison.

Compatibility study of botanicals with bio-agent, *Trichoderma viride* T16 (CPCRI, RC, Kahikuchi) was carried out by poisoned food technique with same procedure as above.

RESULTS AND DISCUSSION

Efficacy of botanicals on mycelial growth of *G. lucidum*

Table1: List of plant species used in the experiment

Sl. No.	Plant species	Family	English name
1	<i>Ageratum haustonianum</i> Mill.	Asteraceae	Floss flower, blue mink, blueweed, pussy foot
2	<i>Allium cepa</i> L.	Amaryllidaceae	Onion
3	<i>Allium sativum</i> L.	Amaryllidaceae	Garlic
4	<i>Amaranthus viridis</i> L.	Amaranthaceae	Amaranth or pigweed
5	<i>Azadirachta indica</i> A.Juss	Meliaceae	Neem
6	<i>Bidens pilosa</i> L.	Asteraceae	Cobbler's pegs or Spanish needle
7	<i>Carica papaya</i> L	Caricaceae	Papaya
8	<i>Catharanthus roseus</i> (L.) G.Don	Asteraceae	Toothache plant
9	<i>Centella asiatica</i> (L.) Urban	Mackinlayaceae	Indian pennywort
10	<i>Chromolaena odorata</i> (L.) King & H E. Robins	Asteraceae	Siam weed
11	<i>Cinnamomum verum</i> J.Presl	Lauraceae	Cinnamon
12	<i>Clerodendron infortunatum</i> L.	Lamiaceae	Hill glory bower
13	<i>Lantana camara</i> L.	Verbenaceae	Lantana
14	<i>Lawsonia inermis</i> L.	Lythraceae	Henna
15	<i>Leucas aspera</i> (Willd)L.	Lamiaceae	Thumbai
16	<i>Lippia alba</i> (Mill.)N.E.ex Britton & P.Wilson	Verbenaceae	Bushy matgrass/Bushy lippia
17	<i>Melastoma malabathricum</i> L.	Melastomataceae	Indian rhododendron
18	<i>Mimosa pudica</i> L.	Fabaceae	Shy, bashfull or shrinking or Touch me not
19	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Tulsi, Holy basil
20	<i>Oxalis corniculata</i> L.	Oxalidaceae	Creeping wood sorel
21	<i>Pavonia odorata</i> Willd	Malvaceae	Fragrant swamp mallow, Pavonia, Fragrant pavonia
22	<i>Peperomia pellucida</i> Kunth.	Piperaceae	Shiny Bush, Slate pencil plant, silverbush
23	<i>Piper betle</i> L.	Piperaceae	Betelvine
24	<i>Psidium guajava</i> L.	Myrtaceae	Guava
25	<i>Rauvolfia serpentina</i> (L.) Benth ex. Kurz	Apocynaceae	Snakeroot
26	<i>Senna tora</i> (L.)Roxb.	Caeselpinaceae	Sickle pod senna
27	<i>Solanum nigrum</i> L.	Solanaceae	Black night shade
28	<i>Spilanthes paniculata</i> Wall.ex.DC	Apocynaceae	Periwinkle
29	<i>Vitex negundo</i> L.	Lamiaceae	Five leaved chaste tree
30	<i>Zingiber officinalis</i> Roscoe.	Zingiberaceae	Ginger

Table 2: Efficacy of aqueous plant extracts on radial growth of *Ganoderma lucidum*.

S. No.	Botanicals	Plant part used	5% Mycelial growth (mm)	Per cent inhibition over control	10% Mycelial growth (mm)	Per cent inhibition over control	20% Mycelial growth (mm)	Per cent inhibition over control
1	<i>Ageratum haustonianum</i> Mill.	Leaf	44.5	50.55(45.32)	41.0	54.44(47.55)	32.2	64.22(53.26)
2	<i>Allium cepa</i> L.	Bulb	61.9	31.22(33.97)	55.4	38.44(38.32)	50.4	44.00(41.55)
3	<i>Allium sativum</i> L.	Corm	8.2	90.89(72.43)	00.0	100.00(89.48)	00.0	100.00(89.48)
4	<i>Amaranthus viridis</i> L.	Leaf	39.7	55.89(48.38)	34.6	61.55(51.68)	30.3	66.33(54.53)
5	<i>Azadirachta indica</i> A.Juss	Leaf	39.5	56.11(48.51)	34.6	61.55(51.68)	34.2	62.00(51.94)
6	<i>Bidens pilosa</i> L.	Leaf	69.6	22.67(28.43)	33.5	62.78(52.41)	14.5	83.88(66.33)
7	<i>Carica papaya</i> L.	Leaf	39.4	56.22(48.57)	34.9	61.22(51.48)	30.5	66.11(54.40)
8	<i>Catharanthus roseus</i> (L.) Don	Leaf	41.8	53.55(47.04)	39.4	56.22(48.57)	33.7	62.55(52.27)
9	<i>Centella asiatica</i> (L.)Urban	Leaf	49.5	45.00(42.13)	45.5	49.44(44.68)	45.3	49.67(44.81)
10	<i>Chromolaena odorata</i> (L.) King & H E. Robins	Leaf	60.5	32.78(34.93)	57.3	36.33(37.07)	53.5	40.55(39.55)
11	<i>Cinnamomum verum</i> J.Presl	Leaf	40.7	54.78(47.74)	34.7	61.44(51.61)	24.5	72.78(58.55)
12	<i>Clerodendron infortunatum</i> L.	Leaf	31.0	65.55(54.06)	20.6	77.11(61.42)	14.4	84.00(66.42)
13	<i>Lantana camara</i> L.	Leaf	61.8	31.33(34.04)	59.7	33.67(35.47)	50.5	43.89(41.49)
14	<i>Lawsonia inermis</i> L.	Leaf	30.5	66.11(54.40)	24.5	72.78(58.55)	21.5	76.11(60.94)
15	<i>Leucas aspera</i> (Willd) L.	Leaf	19	78.89(62.65)	18.7	79.22(62.88)	18.7	79.22(62.88)
16	<i>Lippia alba</i> (Mill.)N.E.ex Britton & P.Wilson	Leaf	54.4	39.55(38.97)	51.6	42.67(40.79)	51.5	42.78(40.85)
17	<i>Melastoma malabathricum</i> L.	Leaf	50.7	43.67(41.36)	48.0	46.67(43.09)	46.4	48.44(44.11)
18	<i>Mimosa pudica</i> L.	Leaf	43.5	51.67(45.96)	39.5	56.11(48.51)	32.4	64.00(53.13)
19	<i>Ocimum tenuiflorum</i> L.	Leaf	70.1	22.11(28.05)	69.5	22.78(28.51)	34.7	61.44(51.61)
20	<i>Oxalis corniculata</i> L.	Leaf	43.5	51.67(45.96)	42.6	52.67(46.53)	40.6	54.89(47.81)
21	<i>Pavonia odorata</i> Willd	Leaf	58.5	35.00(36.27)	55.2	38.67(38.45)	52.6	41.55(40.14)
22	<i>Peperomia pellucida</i> Kunth.	Leaf	55.4	38.44(38.32)	54.4	39.55(38.97)	53.3	40.78(39.69)
23	<i>Piper betle</i> L.	Leaf	38.2	57.55(49.34)	35.2	60.89(51.29)	30.7	65.89(54.27)
24	<i>Psidium guajava</i> L.	Leaf	87.5	2.78(1.60)	84.5	6.11(4.31)	80.5	10.55(18.95)
25	<i>Rauvolfia serpentina</i> (L.) Benth ex. Kurz	Leaf	39.5	56.11(48.51)	29.5	67.22(55.01)	29.5	67.22(55.07)
26	<i>Senna tora</i> (L.) Roxb.	Leaf	51.4	42.89(40.91)	49.6	44.89(42.07)	32.7	63.67(52.93)
27	<i>Solanum nigrum</i> L.	Leaf	20.7	77.00(61.34)	14.3	84.11(66.51)	00.0	100.00(89.48)
28	<i>Spilanthes paniculata</i> Wall.ex.DC	Leaf	22.6	74.89(59.93)	21.5	76.11(60.74)	20.5	77.22(61.49)
29	<i>Vitex negundo</i> L.	Leaf	43.6	51.55(45.89)	47.5	47.22(43.41)	48.5	46.11(42.77)
30	<i>Zingiber officinalis</i> Roscoe.	Rhizome	64.7	28.11(32.02)	59.7	33.67(35.47)	44.7	50.33(45.19)
31	Control		90.00	0.00	90.00	0.00	90.00	0.00
	SED(±)		-	0.26	-	0.20	-	0.19
	CD(0.05)		-	0.52	-	0.40	-	0.38

The data are the mean of 3 replications; Data within parentheses are the angular transformed values

Efficacy of botanicals on mycelial growth of *G. lucidum* varied with different botanicals at different concentrations viz., 5, 10 and 20 per cent (Table 2). At 5 per cent concentration, *Allium sativum* recorded maximum inhibition (90.89%) in mycelial growth, followed by *Solanum nigrum* (77.00%) and *Leucas aspera* (78.89%) and minimum inhibition was recorded in *Psidium guajava* (2.78%). Similar trend was recorded at 10 per cent concentration. *A. sativum* recorded maximum inhibition (100.00%) in mycelial growth, followed by *S. nigrum* (84.11%) and *L. aspera* (79.22%) and minimum inhibition was recorded in *P. guajava* (6.11%). At 20 per cent concentration, *A. sativum* and *S. nigrum* recorded maximum inhibition (100 %) in mycelial growth, followed by *Clerodendron infortunatum* (84.00%) and minimum inhibition was recorded in *P. guajava* (10.55%). At all the concentration, there is significant difference between the treatments. However, there is no significant difference between *A. sativum* and *S. nigrum* at 20 per cent concentration. As a whole, extract of *A. sativum* and *S. nigrum* at all the concentration were highly effective in inhibiting the mycelial growth of the test fungus. Garlic was found to be fungitoxic to a number of plant pathogen (Iyer *et al.*, 2004, Gowda and Nambiar, 2006, Chakrabarty *et al.*, 2013). Crude extract of different plant parts of *Solanum nigrum* obtained using solvents viz., petroleum ether, chloroform, acetone, ethanol and methanol showed that leaf aqueous extract was more active against all the microbes tested (Ramya *et al.*, 2012).

The plants and its derivatives are of great use in agriculture, public health, medicines, cosmetics, etc. Plant extracts are effective against plant pathogens as they have unique antimicrobial properties that act in a holistic manure due to presence of certain secondary metabolites, viz., alkaloids, terpenoids, glycosides and phenolic acids (Srivastava *et al.*, 1994; Singh *et al.*, 1999). Kharkwal *et al.* (2012) determined the antifungal activity of the dealcoholized extract of the leaves of *Clerodendron infortunatum* Retz. against four fungal organisms i.e. *A.niger*, *P. frequentance*, *P. notataum* and *B. cinera*. Bhardwaj (2012) carried out test of aqueous extract of twenty plants for their antifungal activity against *Fusarium solani*, the causal organism of dry rot disease of potato. The combined leaf extracts of *Lawsonia alba* and stem extracts of *Acacia catechu* in general showed a strong enhancement in activities over the individual extract of each against the mycelial growth of the fungus.

The compatibility study of *Trichoderma viride* with plant extracts showed that except the extract of *Allium sativum*, all the tested plant extracts are compatible with *T. viride*. Thus, except *A. sativum* all the plant extracts that were tested can be integrated in IDM package.

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