RESEARCH ARTICLE

***In vitro* evaluation of bio-control agents and fungicides against *Ganoderma lucidum* causing basal stem rot of arecanut**

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Abstract: Basal stem rot or *Ganoderma* wilt caused by *Ganoderma lucidum* (Curtis Ex.Fr.) Karst is one of the major constraints in arecanut cultivation in Assam, India. Pure culture of *Ganoderma* was isolated from fruiting bodies of diseased arecanut palms. Twenty-two *Trichoderma* spp. and twelve *Pseudomonas fluorescens* isolates were prepared from rhizosphere of healthy arecanut palms from different locations. The study was carried out during 2012-13 and 2013-14. Dual culture study revealed that among the *Trichoderma* spp., *T. viride* Tv-16 isolate, produced maximum reduction in radial growth of *G. lucidum* (88.56%) followed by Ta-7 and Ta-10 with 88.26 and 84.07 per cent inhibition over control after 144 hrs of incubation respectively. Among the twelve isolates of *P. fluorescens*, Pf6 was most effective, followed by Pf10 with 77.55 and 77.33 per cent inhibition over control, respectively. Seven fungicides were assayed for their efficacy against the test pathogen at 0.1, 0.2 and 0.3 per cent concentration. Among the systemic fungicides, hexaconazole 5EC and tridemorph 80EC completely inhibited the growth of *G. lucidum* at all the concentrations. Among the contact fungicides, mancozeb 75%WP (86.11% inhibition over control) gave maximum control against the pathogen. Compatibility study of fungicides with *T. viride* Tv-16 revealed that the systemic fungicides, carbendazim 50%WP, hexaconazole 5%EC and tridemorph 80%EC were highly sensitive to *T. viride* with 100 per cent inhibition at all the tested concentration. Non-systemic fungicide mancozeb 75%WP was found to be compatible, whereas copper oxychloride 50%WP and captan 50%WP showed low to moderate degree of compatibility with *T. viride* Tv16.

Keywords: Arecanut, basal stem rot, fungicides, *Pseudomonas fluorescens*, *Trichoderma* spp.

Basal stem rot or *Ganoderma* wilt caused by *Ganoderma lucidum* (Curtis Ex. Fr.) Karst is the most dreaded disease of arecanut in Assam since it causes damage to the product as well as the crop. Butler (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 of India. It is a cosmopolitan fungus and causes white rot of woody plants by decomposing lignin, cellulose and polysaccharides. The disease has not only affected the productivity but also wiped out arecanut plantations in certain localities. The disease is severe in neglected, ill drained and over-crowded orchards. Disease incidence was reported as high as 77 per cent in Goalpara district of Assam. Although control measures using fungicides are reported to be effective, it becomes very difficult for large scale application. Moreover, with the increased awareness on toxic hazards of chemicals to crops, consumers and the environment, exploration of some innovative techniques for the management of the disease has become imperative. Biological control holds promise as a strategy for disease management and it is environment friendly too. Antagonistic fungi especially

Trichoderma species and bacteria *Bacillus subtilis* and fluorescent *Pseudomonads* have been widely used against a number of phytopathogens. Currently studies pertaining to the use of bio-control agents in management of diseases are highly emphasized (Leelavathi *et al.*, 2014). Bhaskaran *et al.* (1988) studied the antagonistic potentiality of *T. harzianum* and *T. hamatum* against *G. lucidum* and found that application of neem cake encouraged the saprophytic soil microflora especially *Trichoderma* spp. in coconut basins and was effective in the control of the disease. In addition, the compatibility of fungicides has been considered to be the most important criterion with regards to integrated disease management (IDM). The compatibility of fungicides with bio-control agents is desirable with a view to reduce their cost of application. A number of fungicides had been tested for their compatibility with most commonly used fungal antagonists, *Trichoderma* spp., many of which have been reported to be compatible with *T. harzianum* (Sharma *et al.*, 2001).

Considering the economic loss of the crop, the present study was undertaken to elucidate the efficacy of bio-control agents and fungicides and their compatibility under *in vitro* condition with an objective of developing an IDM strategy.

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MATERIALS AND METHOD

Isolation of the test fungus

A total of thirty samples were collected (sporophores, bark and roots) during survey from different districts of Assam. The samples were collected in fresh polythene bags and brought in to the laboratory for isolation. The diseased samples were cut into small convenient pieces, sterilized in 0.1% HgCl_2 for one minute, then washed thrice in sterile distilled water and plated on *Ganoderma* selective media. Pure culture was transferred into slants and maintained on Potato Dextrose Agar media for further study.

Isolation and *in vitro* efficacy study of biocontrol agents

The isolation of the bio-control agents was done from the rhizosphere of healthy arecanut palms from different districts of Assam using serial dilution plate technique. Bio-control agents, *Trichoderma* spp. and *P. fluorescens* were isolated on *Trichoderma* selective media (Elad and Chet, 1983) and on King's B media, respectively. *Trichoderma* spp. and *P. fluorescens* were tested against *G. lucidum* on PDA media by using dual culture technique (Dhingra and Sinclair, 1985). Twenty ml of sterilized and cooled PDA was poured into sterile petridishes and allowed to solidify. In case of *Trichoderma* spp., mycelial discs of test fungus were inoculated at one end of petriplates and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of *P. fluorescens*, the bacterium was streaked at both ends of petriplates and mycelial disc of the fungus was placed at the centre. A petriplate was inoculated only with *G. lucidum* that served as control. Each experiment was replicated three times and incubated at $28 \pm 2^\circ\text{C}$ and radial growth of the pathogen was recorded in both treated and control plates. The per cent inhibition over control was calculated.

The values obtained in different categories were transformed, wherever necessary and subjected to statistical analysis for comparison.

In vitro efficacy study of fungicides

Seven fungicides (four systemic and three non-systemic) were assayed for their efficacy against the test pathogen at 0.1, 0.2 and 0.3% concentration by using poisoned food technique. The required quantities of fungicides were thoroughly mixed in the medium before pouring into petridishes so as to get the desired concentration of active ingredient of each fungicide separately. Twenty ml of fungicide amended medium was poured in each of 90mm sterilized petridishes and allowed to solidify. The plates were inoculated centrally with 7mm disc of six days old culture of *G. lucidum*. The control without fungicides was maintained. The experiment was conducted in completely randomized design (CRD) with three replications. Petriplates were incubated at $28 \pm 2^\circ\text{C}$. The radial growth was measured up to six days and per cent inhibition of growth over control was calculated.

In vitro compatibility study of fungicides with bio-control agent

In vitro study was undertaken to determine the compatibility of both systemic and non-systemic fungicides with a bio-control agent (*Trichoderma viride* Tv-16, a fungal antagonist) to determine the suitability of these fungicides in IDM system following poisoned food technique. The PDA medium amended with a series of concentrations (0.1, 0.2 and 0.3%) of test fungicides in petriplates with replicated treatments were inoculated at their centers with 7 mm diameter mycelial discs of freshly growing cultures of *T. viride* taken from the periphery of culture plate. The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$. Observations were recorded on the radial growth after 144hrs of incubation. Control was maintained with PDA medium without fungicides.

RESULTS AND DISCUSSION

The pathogen, *G. lucidum* was confirmed according to the identification key (Seo and Kirk, 2000) based on morphological and cultural characteristics. The antagonistic fungi viz., *Trichoderma* spp., isolated from the rhizosphere of arecanut palms were confirmed according to the identification key (Rifai, 1969) based on branching of conidiophores, shapes of phialides, emergence of phialospores and shape of phialospores. *Pseudomonas fluorescens* isolates were confirmed based on the identification key given by Holt *et al.* (1994).

In vitro evaluation of antagonistic fungi against the test pathogen

Study on antagonistic potential of fungi revealed that *T. viride* Tv-16 was the most effective in inhibiting the growth of *G. lucidum* *in vitro*. After 48 hrs of incubation, the highest inhibition (76.26%) was recorded in *T. asperellum* (Ta-7, Ta-9, Ta-10) and *T. viride* (Tv-16 and Tv-17). The isolates, Ta-13, Tv-21, Ta-2, Ta-3, Ta-4, Ta-5, Ta-6, Ta-8, and Tv-15 showed above 60% inhibition over control. The isolates, Tv-22, Ta-14, Tv-20, *T. harzianum* (Th-24 and Th-23) were found to be least effective with 58.46, 58.46, 55.49, 52.52 and 40.65% inhibition. Similarly, after 96hrs of incubation, Tv-16 recorded the highest inhibition (85.71%) closely followed by Ta-7 (84.71%). However, isolates Ta-9, Ta-10, Tv-17 and Ta-12 showed above 80% inhibition (83.25, 83.25, 83.25 and 80.63%, respectively). The isolates, Ta-11, Ta-13, Tv-21, Ta-3, Ta-4, Ta-5, Ta-6, Ta-8 and Tv-15 showed 79.46, 75.54, 74.10, 70.97, 70.97, 70.97, 70.97 and 70.52% inhibition, respectively. Inhibitory effect of *Trichoderma* spp. may be due to the fact that they are known to produce large quantities of fungistatic metabolites viz., trichodermin, dermin, trichiridin, trichobrachin, etc. which are active against many soil borne plant pathogens. *In vitro* studies had shown that *Trichoderma* spp., *Aspergillus* spp., and *Penicillium* spp. were having antagonistic effect on *Ganoderma*. Among the bio-agents, three fungal cultures viz., *T. harzianum* (63.99%), *T. viride* (66.55%) and *Gliocladium virens* (62.12%) had shown inhibitory effect on mycelial growth of *G. lucidum* after 96hrs of incubation (Chakrabarty and Ray, 2007).

Table 1. Inhibitory effect of different isolates of *Trichoderma* spp. on radial growth of *G. lucidum* G9 after varying hours of incubation

Isolates	48 hrs		96 hrs		144 hrs	
	Radial growth (mm)	% inhibition over control	Radial growth (mm)	% inhibition over control	Radial growth (mm)	% inhibition over control
<i>T. asperellum</i> (Ta-1)	12.00	64.39(53.36)	18.00	69.85(56.70)	22.23	75.29(60.17)
<i>T. asperellum</i> (Ta-2)	13.00	61.42(51.60)	19.23	67.79(56.70)	24.57	72.71(58.48)
<i>T. asperellum</i> (Ta-3)	11.00	61.42(51.60)	17.35	70.97(57.40)	20.23	77.52(61.67)
<i>T. asperellum</i> (Ta-4)	11.00	61.42(51.60)	17.33	70.97(57.40)	20.23	77.52(61.67)
<i>T. asperellum</i> (Ta-5)	11.00	61.42(51.60)	17.33	70.97(57.40)	20.23	77.52 (61.67)
<i>T. asperellum</i> (Ta-6)	11.00	61.42(51.60)	17.33	70.97(57.40)	20.23	77.52 (61.67)
<i>T. asperellum</i> (Ta-7)	8.00	76.26(60.84)	8.53	84.92(67.15)	10.57	88.26(69.93)
<i>T. asperellum</i> (Ta-8)	12.00	64.39(53.36)	17.33	70.97(57.40)	20.33	77.41 (61.59)
<i>T. asperellum</i> (Ta-9)	8.00	76.26(60.84)	10.00	83.25(65.84)	14.90	83.45(65.60)
<i>T. asperellum</i> (Ta-10)	8.00	76.26(60.84)	10.00	83.25(65.84)	14.33	84.07(66.45)
<i>T. asperellum</i> (Ta-11)	8.00	76.26(60.84)	12.26	79.46(63.05)	14.53	83.85(66.28)
<i>T. asperellum</i> (Ta-12)	8.00	76.26(60.84)	11.56	80.63(63.89)	14.60	83.78(66.28)
<i>T. asperellum</i> (Ta-13)	10.00	70.32(56.99)	14.60	75.54(60.36)	17.40	80.66(63.89)
<i>T. asperellum</i> (Ta-14)	14.00	58.46(49.87)	19.53	67.29(55.12)	24.57	72.70(58.48)
<i>T. viride</i> (Tv-15)	13.00	61.42(51.60)	17.60	70.52(57.12)	23.37	74.04(59.34)
<i>T. viride</i> (Tv-16)	8.00	76.26(60.84)	9.00	85.71(67.79)	10.30	88.56(70.20)
<i>T. viride</i> (Tv-17)	8.00	76.26(60.84)	10.00	83.25(65.84)	14.73	83.63(66.11)
<i>T. viride</i> (Tv-20)	15.00	55.49(48.15)	20.21	66.15(54.42)	24.63	72.63(58.63)
<i>T. viride</i> (Tv-21)	11.00	67.36(55.16)	15.46	74.10(59.41)	18.57	79.37(62.96)
<i>T. viride</i> (Tv-22)	14.00	58.46(49.87)	19.60	67.17(55.00)	24.57	72.71(58.48)
<i>T. harzianum</i> (Th-23)	20.00	40.65(39.61)	25.33	57.57(55.04)	29.23	67.52(55.23)
<i>T. harzianum</i> (Th-24)	16.00	52.52(46.44)	21.43	64.10(49.35)	24.57	72.71(58.48)
Control	33.70	0.00(0.31)	59.70	0.00(0.31)	90.00	0.00(0.31)
CD at 5%	1.58	0.010	6.30	5.06	0.41	0.32

Data are the mean of four replications, Data within parentheses are angular transformed values

Among the twelve isolates of *P. fluorescens*, Pf6 was most effective (77.55%), followed by Pf10 with 77.33% inhibition (Table 2). Biological control by using

Table 2. Inhibition of radial growth of *G. lucidum* by isolates of *P. fluorescens* after 144hrs of incubation

Isolates	Radial growth (mm)	% inhibition
Pf1	20.8	76.89(61.24)
Pf2	20.5	77.22(61.22)
Pf3	20.6	77.11(61.39)
Pf4	21.4	76.22(60.79)
Pf5	21.4	76.22(60.79)
Pf6	20.2	77.55(61.69)
Pf7	22.1	75.44(60.27)
Pf8	21.4	76.22(60.79)
Pf9	20.5	77.22(61.47)
Pf10	20.4	77.33(61.54)
Pf11	20.7	77.00(61.32)
Pf12	20.8	76.89(61.24)
control	90.0	0.00(0.57)
CD at 5%	0.20	0.31

Data within parentheses are the angular transformed values

antagonistic micro-organisms viz., *Trichoderma* spp., *Bacillus* spp., *Streptomyces* sp., *Mucor* sp., *P. fluorescens*, etc. were found to be effective against *G. lucidum* and *Fusarium oxysporum* f. sp. *ciceri* (Wavare *et al.*, 2017).

Effect of fungicides on mycelial growth of *G. lucidum*

In vitro evaluation of fungicides provided useful information regarding its efficacy against the test pathogen (Table 3). Among the systemic fungicides, hexaconazole 5EC and tridemorph 80EC completely inhibited the growth of the test fungus at all the concentrations, whereas, carbendazim 50WP (88.44, 88.67 and 90.89% inhibition over control) and metalaxyl 8% + mancozeb 64 WP (82.00, 83.33 and 86.33%) showed higher degree of inhibition at 0.1, 0.2 and 0.3% concentration, respectively. Among the contact fungicides, mancozeb 75WP (81.89, 83.22 and 86.11%) gave maximum control against the pathogen, followed by copper oxychloride 50WP (81.67, 82.67 and 86.00%) and captan 50WP (84.00, 85.89 and 85.89%) at 0.1, 0.2 and 0.3% concentrations. Fungicides - thiram, difolatan, benomyl, plantavax, vitavax and aureofungin sol + copper sulphate were found to be inhibitory to *G. lucidum* (Wavare *et al.*, 2017).

Table 3. Efficacy of fungicides against *G. lucidum* after 144hrs of incubation

Fungicides	Radial growth (mm) in 0.1%	%inhibition	Radial growth (mm) in 0.2%	% inhibition	Radial growth (mm) in 0.3%	%inhibition
Carbendazim 50%WP (bavistin)	10.4(3.30)*	88.44(70.09)**	10.2(3.27)*	88.67(70.30)**	8.20(2.95)*	90.89(72.40)**
Captan 50% WP (Captaf)	14.4(3.86)	84.00(66.40)	12.70(3.68)	85.89(67.91)	12.70(3.63)	85.89(67.91)
Copper Oxychloride 50% WP (Blitox 50W)	16.5(4.12)	81.67(64.62)	15.6(4.01)	82.67(65.37)	12.60(3.62)	86.00(68.01)
Hexaconazole 5%EC(Contaf)	00.0(0.71)	100.00(89.39)	00.0(0.71)	100.00(89.39)	00.0(0.71)	100.00(89.39)
Mancozeb 75%WP(DithaneM-45)	16.2(4.09)	81.89(64.79)	15.00(3.94)	83.22(65.79)	12.50(3.61)	86.11(68.09)
Metalaxyl 8% + Mancozeb 64% WP (Ridomil MZ-72WP)	16.3(4.10)	82.00(84.88)	15.10(3.95)	83.33(65.88)	12.30(3.58)	86.33(68.27)
Tridemorph 80EC (Calixin)	00.0(0.71)	100.00(89.39)	00.0(0.71)	100.00(89.39)	00.0(0.71)	100.00(89.39)
Control	90.0(9.51)	0.00(0.71)	90.0(9.51)	0.00(0.57)	90.00(9.51)	0.00(0.57)
CD at 5%	0.018	0.65	0.018	0.01	0.019	0.50

*Data within parentheses are the square root transformed values, **Data within parentheses are the angular transformed values

Fig. 1. Radial growth inhibition of *T. viride* Tv-16 by fungicides at various concentrations

Compatibility study of fungicides with *T. viride* Tv-16

Compatibility study of fungicides with *T. viride* Tv-16 (Fig. 1) showed that systemic fungicides, carbendazim 50WP, hexaconazole 5%EC and tridemorph 80EC were highly sensitive to it with 100% inhibition at all the tested concentrations. Metalaxyl 8% + mancozeb 64WP (0% inhibition) was found to be compatible at 0.1 and 0.2% concentrations, whereas, partially compatible (10.30%) at 0.3%. Non-systemic fungicides showed varying degree of sensitivity with *T. viride*. At 0.1 and 0.2% concentrations, mancozeb 75WP was found to be highly compatible, whereas, at 0.3%, it was partially compatible (8.78% inhibition on radial growth). At 0.1, 0.2 and 0.3%, captan 50WP recorded 60.59, 61.59 and 72.81% inhibition on radial growth, whereas, copper oxychloride 50%WP, recorded 11.22, 39.18 and 39.31% inhibition, respectively. The differential response of antagonistic microorganisms to various fungicides might be attributed to varying degree of inherent resistance to most of the

fungicides and ability to degrade chemicals. Bagwan (2010) reported that thiram, copper oxychloride and mancozeb @ 0.2% were compatible with *T. harzianum* and *T. viride*, whereas, most sensitive to captan, tebuconazole, vitavax, propiconazole and chlorothalonil.

It can be summarized that among the *Trichoderma* spp., *T. viride* Tv-16 isolate, produced maximum reduction in radial growth of *G. lucidum* followed by Ta-7 and Ta-10. The isolate of *P. fluorescens*, Pf6 was most effective in reducing radial growth of the fungus. The systemic fungicides, hexaconazole 5EC and tridemorph 80EC completely inhibited the growth of *G. lucidum*. Carbendazim 50%WP, hexaconazole 5%EC and tridemorph 80%EC were highly sensitive to *T. viride* Tv-16 with 100% inhibition. The contact fungicides, mancozeb 75%WP gave maximum control against the pathogen. Mancozeb 75%WP was compatible, whereas copper oxychloride 50%WP and captan 50%WP showed low to moderate degree of compatibility with Tv-16.

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