Management of *Phytophthora* leaf fall disease of nutmeg (*Myristica fragrans* Houtt.)

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Abstract

Nutmeg trees are proned to various fungal diseases. Recently, a leaf fall disease caused by *Phytophthora* sp. has become a serious problem in Kerala, during South - West monsoon period resulting in poor crop stand and yield. *In vitro* and *in vivo* studies were conducted to find out the efficacy of different fungicides and bioagents against this pathogen. *In vitro* evaluation of different fungicides showed, complete inhibition of the pathogen with 1% Bordeaux mixture, copper hydroxide (2 g l^{-1}), copper oxychloride (2.5 g l^{-1}), potassium phosphonate (3ml l^{-1}) and the combination fungicides iprovalicarb + propineb (1.5 and 2.0 g l^{-1}) and cymoxanil + mancozeb (2 g l^{-1}) and the antagonists *viz*. *Trichoderma viride* -1, *T. harzianum* and *T. viride* -2, the isolates from nutmeg rhizosphere soil and the reference cultures, *T. viride* (KAU), *T. harzianum* (IISR). Of the various treatments tested in *in vivo* experiment, spraying of 1%Bordeaux mixture + soil drenching of copper hydroxide (2 g l^{-1}) and spraying of 1% Bordeaux mixture + soil application of *T. viride* showed maximum reduction of the disease. All copper fungicides tested under study were effective, of which, Bordeaux mixture was the most promising one followed by copper hydroxide and copper oxychloride. In addition, either spraying or drenching with systemic fungicides *viz.* iprovalicarb + propineb and cymoxanil + mancozeb also showed good results. Soil application of *Trichoderma* alone was found to be effective however, prophylactic spraying of 2% *Psuedomonas fluorescens* and soil application of *Trichoderma* showed better result.

Key words: Copper fungicides, Leaf fall disease, Nutmeg, *Phytophthora*, *Psuedomonas fluorescens*, *Trichoderma*

Introduction

Nutmeg is unique among the tree spices as it is the donor of the two distinct spices, kernel and mace. Nutmeg trees are proned to various fungal diseases. Recently, a leaf fall disease caused by *Phytophthora* sp. has become a serious problem in Kerala during South - West monsoon period (Mathew and Beena, 2012). A severe outbreak of this disease has been reported from major nutmeg growing areas of Thrissur, Ernakulam and Kottayam districts of Kerala in 2013 (Mathew and Miniraj, 2013). Experts and farmer's organisations estimated about 35 per cent dip in the total yield during that year. The impact of the attack can adversely affect the economy for the next three to four years if the problem is not tackled properly (Villatt, 2013).

The causative organism, *Phytophthora*, is a serious pathogen, causing diseases in several economically important spices and plantation crops in Kerala *viz.* black pepper, cardamom, vanilla, rubber, cocoa, coconut and arecanut. These diseases are very serious either causing heavy yield loss or complete death of the plants. Therefore, management of this leaf fall disease is very much imperative. Copper fungicides have been used since early 1900s to control *Phytophthora* diseases. Ramachandran (2011) reported the effectiveness of the microbial inoculants like *Trichoderma* and *Psuedomonas* in the reduction of *Phytophthora* infection in many horticultural crops. The present study was taken up to find out the efficacy of different fungicides and bioagents against *Phytophthora* leaf fall of nutmeg.

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Materials and Methods

Efficacy of the selected fungicides and antagonists were tested against *Phytophthora* sp. under *in vitro* and *in vivo* conditions.

In vitro evaluation of fungicides

In vitro evaluation of seven different fungicides *viz*. Bordeaux mixture (1%),copper hydroxide 77 WP (1.5 & 2g l^{-1}), copper oxychloride 50 WP (2 & 2.5g l^{-1}), cymoxanil 8% + mancozeb 64% WP (1.5 & 2g l^{-1}), iprovalicarb 5.5% + propineb 61.3% WP (1& 2g l^{-1}), carbendazim12 + mancozeb 63 % WP (2g l^{-1}) and potassium phosphonate (3ml l^{-1}) were studied by the poisoned food technique (Zentmyer, 1955). Medium without fungicides served as control. Three replications were maintained for each fungicide. Observations were recorded, till the full growth of the pathogen was attained in control plates. Per cent inhibition of the pathogen was calculated using the formula suggested by Vincent (1927).

$$\text{Per cent inhibition of the pathogen} = \frac{C-T}{C} X\ 100$$

C = Growth of the pathogen in control
T = Growth of the pathogen in treatment

In vitro evaluation of antagonists

Three *Trichoderma* sp. (*T.viride*-1, *T.viride*-2 and *T. harzianum*) isolated from nutmeg rhizosphere soil and three reference cultures *viz. T. viride* (KAU), *T. harzianum* (IISR) and *Pseudomonas fluorescens* (KAU) were screened for their antagonistic activity against the pathogen, *Phytophthora* by adopting dual culture technique (Johnson and Curl, 1972). Antagonistic activity of *Trichoderma* sp. were tested by employing deferred antagonism and bacterial antagonist by simultaneous antagonism methods. Monoculture of the pathogen served as control. Three replications were kept for each antagonist. Observations were recorded daily till control plates attained full growth of the pathogen and the per cent inhibition of the pathogen was calculated.

Management of leaf fall disease of nutmeg under in vivo condition

Seven fungicides, which showed cent per cent inhibition of the pathogen under *in vitro* condition as well as one efficient isolate of *Trichoderma* sp. from nutmeg along with an efficient standard antagonist were tested against the pathogen under *in vivo* condition on three month old nutmeg seedlings. The experiment was laid out in CRD design with three replications and a total of 30 plants per treatment. The treatments consisted of fungicidal spraying alone [Bordeaux mixture (1%), copper oxychloride (2.5 g l^{-1}), copper hydroxide (2 g l^{-1}), cymoxanil + mancozeb (2 g l^{-1}), iprovalicarb + propineb (1.5 g l^{-1}), potassium phosphonate (3 ml l^{-1}), carbendazim + mancozeb (2 g l^{-1})], soil drenching/ application of fungicides/ bioagents alone [copper oxychloride (2.5 g l^{-1}), copper hydroxide (2 g l^{-1}), cymoxanil + mancozeb (2 g l^{-1}), iprovalicarb + propineb (2 g l^{-1}), *T. viride* – 1 of nutmeg, *T. viride* (reference culture)], both spraying and drenching of fungicides/bioagents [Bordeaux mixture (1%) + copper oxychloride (2.5 g l^{-1}), Bordeaux mixture (1%) + copper hydroxide (2 g l^{-1}), copper oxychloride (2.5 g l^{-1}), copper hydroxide (2 g l^{-1}), Bordeaux mixture (1%) spray + *T. viride* (reference culture) soil application, *P. fluorescens* (2%) spray + *T. viride* (reference culture) soil application] and two control for inoculation of pathogen on leaves and soil application of pathogen.

Two methods of inoculations were adopted based on the method of treatments. In spraying treatments, culture disc of the pathogen from five day old culture was inoculated on the midrib region of lower surfaces of the injured leaves @ five leaves/ plant, with injury. In soil application/ drenching seven day old inoculum grown in sterilized carrot bits was applied @ 10 g per plant, to the collar region of the seedlings after wounding. Whereas in spraying + soil application treatments, inoculum was applied to foliage and soil. Inoculated plants were covered with the moistened polythene bags to provide humidity. Antagonists were applied ten

days before the challenge inoculation of the pathogen and chemical treatments were given on symptom appearance (2DAI). Observations on disease severity and leaf fall were recorded at 10, 15 and 20 days of inoculation.

Statistical analysis

Analysis of variance was performed on the data collected in the experiment using the statistical package MSTAT (Freed, 1986). Multiple comparisons among treatment means were done using DMRT.

Results and Discussion

In vitro evaluation of fungicides against Phytophthora isolate of nutmeg

Data presented in Table 1 revealed that, all chemicals tested under the study were found effective against the pathogen, however, the efficiency varied with the chemicals. Bordeaux mixture (1%), potassium phosphonate (3 ml l^{-1}) and the combination fungicide, iprovalicarb + propineb at lower (1.5 g l^{-1}) and higher (2 g l^{-1}) concentrations showed cent per cent inhibition of the pathogen. Lower doses of copper hydroxide (1.5 g l^{-1}), copper oxychloride (2 g l^{-1}) and cymoxanil + mancozeb (1.5 g l^{-1}) showed about 90 per cent inhibition, however, complete inhibition was observed with the higher doses of 2.0, 2.5 and 2.0 g l^{-1} of these fungicides respectively. The combination fungicide,carbendazim +mancozeb, was least effective and recorded only 70.55 per cent inhibition. These findings are in agreement with Prem (1995), who also observed complete inhibition of *P. palmivora* with 1% Bordeaux mixture and 0.2% copper oxychloride. Kirk et al. (2005) noticed reduction in the growth of *P. infestans* with cuprous oxide, copper sulphate, copper hydroxide and copper carbonate under *in vitro* condition and the effectiveness of iprovalicarb fungicide against *Phytophthora* sp. was reported by Thind (2011).

In vitro evaluation of antagonists

All the six antagonists showed antagonistic activity against the pathogen and all *Trichoderma* sp. showed cent per cent inhibition at 4 DAI by the overgrowth mechanism of antagonism, causing complete disintegration of the pathogen (Table 2). However, the bacterial antagonist, *P. fluorescens*, showed only 61.11 per cent inhibition. Of the five *Trichoderma* isolates, *T. viride* (reference culture) and *T. viride* -1 of nutmeg showed faster inhibition and growth with mean colony diameter of 6.8 and 6.3 cm respectively at 3 DAI. Efficacy of *Trichoderma* against *Phytophthora* spp. have been reported by many workers (Vijayaraghavan, 2003; Hernandez et al*.,* 2011; Ambuse, 2015).

In vivo experiment for disease management

Seven fungicides and two antagonists which

Table 1. In vitro evaluation of selected fungicides against *Phytophthora* of nutmeg

Chemical name	Concentration	Mean colony diameter (cm)	Per cent inhibition
Bordeaux mixture	1%	0	100
Copper hydroxide 77 WP	1.5g l^{-1}	0.85	90.5
	2g l^{-1}	0	100
Copper oxychloride 50 WP	2g l^{-1}	0.86	90.4
	2.5g l^{-1}	0	100
Potassium phosphonate 50%	3ml l^{-1}	0	100
Cymoxanil 8% + mancozeb 64% WP	1.5g l^{-1}	0.82	90.9
	2g l^{-1}	0	100
Iprovalicarb 5.5% + propineb 61.3% WP	1.5g l^{-1}	0	100
	2g l^{-1}	0	100
Carbendazim 12%+mancozeb 63 %WP	2g l^{-1}	2.65	70.55

Table 2. In vitro screening of antagonists against pathogen

Antagonists	Mean colony diameter (cm) Days after incubation										Per cent inhibition of the pathogen
	1		2		3		4		5		
	A	P	A	P	A	P	A	P	A	P	
Trichoderma viride -1	1.3	2.1	3.8	3.4	6.3	2.7	9	-	9	-	100
Trichoderma harzianum	1.0	1.8	3.5	3.1	6.1	2.9	9	-	9	-	100
Trichoderma viride -2	1.3	1.7	3.7	3.3	6.1	2.9	9	-	9	-	100
Trichoderma viride (Reference culture KAU)	1.4	2.0	3.8	3.2	6.8	2.2	9	-	9	-	100
Trichoderma harzianum (Reference culture IISR)	1.1	1.7	3.6	2.8	6.2	2.8	9	-	9	-	100
Pseudomonas fluorescens (Reference culture KAU)	-	1.0	-	2.4		3.5	-	3.5	-	3.5	61.11
Control (Pathogen alone)	-	2.5	-	4.3	-	6.4	-	8.1	-	9.0	-

showed higher efficiency in *in vitro* screening were selected for *in vivo* experiment. Observations on disease severity was recorded and the results are summerised in Table 3.

All treatments were superior to control, with 66.1 - 79.4 per cent disease reduction over control and significant difference was noticed among the treatments at all intervals of observations. Among the treatments, spraying of 1% Bordeaux mixture + soil drenching of copper hydroxide showed lowest disease severity at all intervals of observations, recording 17.87, 17.87 and 19.2 per cent and spraying of 1% Bordeaux mixture + soil application of *T. viride* with 18.23, 18.67 and 19.73 per cent against 100 and 43.33, 100 and 66.67 and 100 and 80.67 in control at 10, 15 and 20 DAI respectively and showed 79.43 and 78.86 per cent reduction over control. In addition, spraying of 1% Bordeaux mixture +soil drenching of copper oxychloride, spraying and drenching of copper hydroxide or copper oxychloride and even spraying of Bordeaux mixture alone were found equally effective, recording only 20.0 to 20.6 per cent severity, with 78.6 – 77.9 per cent disease reduction at 20 DAI. Among the treatments, minimum disease reduction over control (66.1 per cent) was noticed in soil drenching of copper oxychloride alone. It is also noted that, statistical analysis of the data on disease severity at 15 and 20 DAI revealed no significant difference from the observations at 10 DAI except in control which indicate the positive effect of treatments on the spread of infection. Sashidhara (2010) reported the efficacy of 1% Bordeaux mixture spray and soil drenching of copper hydroxide (2 g l^{-1}) against *Phytophthora* disease of black pepper. Similarly, Rama (2012) observed the effective management of *Phytophthora* disease of black pepper with 1% Bordeaux mixture spray and soil application of *Trichoderma*. Likewise, a spate of literature suggest the effectiveness of copper fungicides against *Phytophthora* diseases (Bhai and Sarma, 2005 and Foster and Hausbeck, 2010).

Data presented in Table 3 also indicated that, per cent severity varied significantly with method of application of fungicide and antagonists. Among the fungicidal spraying treatments, minimum severity (20.6 per cent) was recorded with 1% Bordeaux mixture followed by iprovalicarb + propineb (21.33 per cent) and cymoxanil + mancozeb (21.87 per cent) against cent per cent in control at 20 DAI. In soil drenching method, lowest severity (21.2 per cent) was noticed in drenching of iprovalicarb + propineb which was on par with cymoxanil + mancozeb (23.33 per cent) and copper hydroxide (25.33 per cent) while the maximum

Table 3. Effect of treatments on per cent severity of *Phytophthora*leaf fall of nutmeg

Treatment. No	Treatments	*Per cent disease severity Days after inoculation			Per cent reduction over control	Progress of infection (%)	Per cent leaf fall
		10	15	20		10 – 20 DAI	10- 20 DAI
T_1	1% Bordeaux mixture spray	19.73(4.44)^{efg}	20.00(4.47)^{hijkl}	20.60(4.54)^{ghij}	77.93	0.87	1.33
T_2	Copper oxychloride (2.5 g l^{-1}) spray	20.80(4.56)^{cdefg}	22.67(4.76)^{defgh}	23.20(4.82)^{def}	75.14	2.40	2.00
T_3	Copper hydroxide (2 g l^{-1}) spray	20.67(4.55)^{cdefg}	22.13(4.70)^{efghi}	22.93(4.79)^{ef}	75.43	2.26	1.33
T_4	Cymoxanil + mancozeb (2 g l^{-1}) spray	20.00(4.47)^{efg}	20.80(4.56)^{fghijk}	21.87(4.67)^{fghi}	76.57	1.87	-
T_5	Iprovalicarb +propineb (1.5 g l^{-1}) spray	20.00(4.47)^{efg}	20.67(4.55) ^{fghijk}	21.33(4.62)^{fghij}	77.15	1.33	-
T_6	Potassium phosphonate (3 ml l^{-1}) spray	22.67(4.76)^{cde}	23.33(4.83)^{def}	24.53(4.95)^{de}	73.72	1.86	2.00
T_7	Carbendazim +mancozeb (2 g l^{-1}) spray	20.67(4.76)^{cde}	23.33(4.83)^{def}	23.47(4.84)^{def}	74.85	2.8	2.66
T_8	Copper oxychloride(2.5 g l^{-1}) soil drenching	24.00(4.89)^{c}	26.67(5.16)^{c}	27.33(5.23)^{c}	66.12	3.33	2.00
T_9	Copper hydroxide (2 g l^{-1}) soil drenching	22.67(4.76)^{cde}	25.33(5.03)^{cd}	25.33(5.032)^{cd}	68.61	2.66	1.33
T_{10}	Cymoxanil + mancozeb (2 g l^{-1}) soil drenching	21.60(4.65)^{cdef}	22.67(4.76)^{defgh}	23.33(4.83)^{def}	71.08	1.73	-
T_{11}	Iprovalicarb +propineb (2 g l^{-1}) soil drenching	20.00(4.47)^{efg}	20.27(4.450^{ghijkl}	21.20(4.60)^{fghij}	73.72	1.2	-
T_{12}	1% Bordeaux mixture spray + copper oxychloride (2.5 g l^{-1}) soil drenching	18.67(4.32)^{fg}	19.20(4.38)^{defgh}	20.26(4.501)^{hij}	78.29	1.59	1.33
T_{13}	1% Bordeaux mixture spray+ copper hydroxide (2 g l^{-1}) soil drenching	17.87(4.23)^{g}	17.87(4.23)^{l}	19.20(4.38)^{j}	79.43	1.33	-
T_{14}	Copper oxychloride (2.5 g l^{-1}) spray and soil drenching	18.67(4.32)^{fg}	19.47(4.41)^{ijkl}	20.53(4.53)^{ghij}	78.00	1.86	-
T_{15}	Copper hydroxide (2 g l^{-1}) spray and soil drenching	18.67(4.32)^{fg}	19.20(4.38)^{jkl}	20.00(4.47)^{ij}	78.57	1.33	-
T_{16}	1% Bordeaux mixture spray and T. *viride* (KAU) soil application	18.23(4.27)^{g}	18.67(4.32)^{kl}	19.73(4.44)^{ij}	78.86	1.5	1.33
T_{17}	2% *P. fluorescens*spray and *T. viride* (KAU) soil application	20.27(4.50)^{defg}	21.6(4.65)^{efghij}	22.67(4.76)^{efg}	71.90	2.4	-
T_{18}	*Trichoderma viride* of nutmeg soil application	23.33(4.83)^{cd}	24.00(4.90)^{cde}	24.67(4.97)^{de}	69.42	1.34	-
T_{19}	*T. viride*(KAU) soil application	21.60(4.65)^{cdef}	22.93(4.79)^{defg}	24.33(5.03)^{cd}	68.60	2.73	-
T_{20}	Control (inoculation of pathogen on leaves)	100.0(10.0)^{a}	100.0(10.0)^{a}	100.0(10.0)^{a}	-	Full infection	100
T_{21}	Control (soil application of pathogen)	43.33(6.58)^{b}	66.67(8.162)^{b}	80.67(8.98)^{b}	-	37.33	-
	CD (0.05)	0.343	0.290	0.243	-	-	-

*Mean of three replications Figures in parenthesis are transformed values DAI- Days after inoculation

severity (27.33 per cent) was with copper oxychloride. This is in agreement with the findings of Boughalleb et al. (2006) who alsoreported the effectiveness of soil drenching of cymoxanil + mancozeb and iprovalicarb + propineb in the management of *P. cactorum* of apple trees.

Application of chemical by both spraying and drenching showed minimum severity of 19.47 per cent with 1% Bordeaux mixture spray + copper hydroxide drenching and the other three treatments, spraying of 1 % Bordeaux mixture and drenching with copper oxychloride, spraying and drenching of copper hydroxide / copper oxychloride were statistically on par.

Among the bioagents, prophylactic spraying of 2 % *P. fluorescens* and soil application of *T. viride* recorded the minimum severity of 20.27 per cent and this was on par with soil application of *T. viride* of nutmeg and *T. viride* (reference culture). This is line with the observation recorded by Zegeye (2011) who noticed that, spraying of *P. fluorescens* along with soil application of *T. viride* provided better management of *P. infestans* causing late blight of potato.

It is also observed from the data that, disease progress was very less in all treatments which ranged from 0.87 – 3.33 per cent against 37.33 per cent and full infection in control, at different intervals of observation. Analysis of data on per cent disease progress revealed that, the progress of infection was minimum (0.87 per cent) in seedlings treated with 1% Bordeaux mixture spray followed by application of iprovalicarb + propineb as soil drench (1.2 per cent) or spray (1.33 per cent). The maximum progress was noticed in soil drenching of copper oxychloride 2.5 g l^{-1} with 3.33 per cent. Control treatments recorded cent per cent infection and 37.33 per cent for inoculation of pathogen on leaves and soil respectively.

Similarly, while analyzing the per cent leaf fall in different treatments, it is noted that, the leaf fall symptom was noticed only with the inoculation of the pathogen on the foliage. However, no leaf fall symptom was observed in treatments consisted of spraying of cymoxanil + mancozeb, spraying of iprovalicarb + propineb, 1% Bordeaux mixture spray + soil drenching of copper hydroxide, spraying and soil drenching of copper oxychloride or copper hydroxide and the leaf fall per cent was very less in other treatments also, which ranged only from 1.33 to 2.66 per cent against cent per cent in control. Application of inoculum to the soil showed only die back symptom.

Thus the present study highlighted that, spraying of 1% Bordeaux mixture + soil drenching of copper hydroxide and spraying of 1% Bordeaux mixture and soil application of *T. viride* showed maximum reduction of the disease. It is also noticed that, all copper fungicides tested under study were effective against leaf fall disease of nutmeg, of which, Bordeaux mixture was the most promising one followed by copper hydroxide and copper oxychloride.

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