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EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS AGAINST *PHYTOPHTHORA MEADII* INFECTING ARECANUT

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ABSTRACT
Phytophthora diseases cause economic yield loss or death of the arecanut palm itself. Forty isolates of *Phytophthora* causing fruit rot disease of arecanut were purified from the infected arecanut samples collected from different arecanut growing regions of Goa, Karnataka and Kerala States. All the collected isolates were identified as *Phytophthora meadii* based on morphological characterization and also confirmed by sequencing ITS region. Forty isolates significantly varied in pathogenecity and the isolate P19 was found to be highly virulent. Eight fungicides and three native *Trichoderma* species, were screened against highly virulent isolate of *Phytophthora meadii*. Among the fungicides tested, Iprovalicarb 5.5 + Propineb 61.3 WP, Metalaxyl 8 + Mancozeb 64 WP, Mandipropamid 250 SC, Ametoctradin 300 + Dimethomorph 225 SC showed 100% mycelial growth inhibition at 0.012% and among three *Trichoderma* spp., *T. virens* exhibited highest mycelia growth inhibition (62.5%) of *P. meadii* both in simultaneous inoculation and inoculation of *Trichoderma* 48 hours after inoculation of *P. meadii*. Hence it is suggested that these effective fungicides and *T. virens* may be evaluated in field condition to develop an effective integrated management practices for *Phytophthora* diseases of arecanut.

INTRODUCTION

Arecanut (*Areca catechu* L.) palm is affected by a number of diseases and nutritional disorders during its developmental stages. Among them *Phytophthora* diseases such as fruit rot ('koleroga' or 'mahali'), bud rot and crown rot are very serious causing heavy economic loss. Fruit rot is a major yield limiting factor causing yield losses of up to 90% (Sarma *et al*., 2002). Bud rot and crown rot diseases occur frequently in fruit rot affected palms leading to death of palms. According to survey conducted in Dakshina Kannada district of Karnataka, yield loss of 15% is due to bud rot and crown rot diseases (Saraswathy, 1994). Prophylactic spraying of 1 % Bordeaux mixture has been recommended for the control of Phytophthora diseases of arecanut (Rao, 1960; Anandraj, 1985). In a mutlilocational trial on management of fruit rot disease using different fungicides revealed that Boredaux mixture 1 per cent spray still holds good in controlling fruit rot disease (Chowdappa *et al*., 2000). 1% Boredaux mixture with pH 7 is very effective against fruit rot disease, if it is properly prepared and applied. However, preparation of the Boredaux mixture with pH 7 every time is difficult and improper preparation and application makes it ineffective. Moreover effectiveness of spray lasts in 40-45 days and spraying again during rainy season is a difficult task. Hence the effectiveness of other new combi fungicides (combination of contact + systemic fungicides) available in the market and effective against Phytophthora diseases in other crops are needs to be tested against *Phytophthora meadii* infecting areacnut. Metalaxyl + Mancozeb 72WP and Acrobat MZ 90/600WP significantly reduced late blight disease severity on most of the potato varieties/lines (Khan, 2003). Thus, the present study was aimed at finding out the effective fungicides and also native biocontrol agents in order to develop appropriate integrated strategies for the management of *Phytophthora* diseases of arecanut.

MATERIALS AND METHODS

Isolation and identification of pathogen

Fruit rot infected arecanut samples were collected from disease endemic areas of Karnataka, Kerala and Goa states during monsoon seasons of 2012 and 2013. The pathogen was isolated from fruit rot disease infected arecanut samples by following direct plating and baiting methods. The pathogen was identified based on morphological traits (Erwin and Ribeiro, 2000) and further confirmed by sequencing DNA amplified through PCR with universal ITS primers. Two fungal specific ITS primers obtained from the conserved ITS region *viz*., ITS1 (5´TCCGTAGGTGAACCTTGCGG3´) and ITS4 (5´ TCCTCCGCTTATTGATATGC 3´) (White *et al*., 1990) were used for PCR.

Identification of highly virulent isolate

All *Phytophthora* isolates were tested for pathogenicity or aggressiveness on arecanuts. In order to induce sporulation carrot agar media containing petri plates were centrally inoculated separately with 5mm mycelial disc of each *Phytophthora* isolate and incubated in dark at 25 ± 1ºC for three days followed by incubation under continuous light for four days at 25 ± 1ºC. Subsequently each isolate containing plates were initially rinsed twice with sterile distilled water. Then, about 20 ml of sterile distilled water was added to each

plate and incubated at 15ºC for 15 minutes afterwards the plates were transferred to room temperature for about 20 minutes until the zoospores were released. Zoospore suspension from each isolate was filtered through a muslin cloth and the concentration adjusted to 5×10^5 ml^{-1} with sterile distilled water using a haemocytometer. Four months old arecanuts without any bruises and uniform in size were selected from Mangala variety, washed in running tap water and surface disinfected for 1 to 2 min using 70% ethanol. Then nuts were inoculated with 10μl of the zoospore suspension in an incision aseptically made with a surgical blade and inoculated part is covered with thin layer of wet cotton. The diameter of the infection lesion developed was recorded from three and five days of incubation in humid chamber at 25 ± 1ºC. The isolate which produced highest lesion size was considered as highly virulent.

Evlauation of fungicides and *Trichoderma* species against highly virulent isolate of *P. meadii*

Relative efficacy of eight fungicides *viz*, Iprovalicarb 5.5 + Propineb 61.3WP, Metalaxyl 8 + Mancozeb 64WP, Mandipropamid 250SC, Ametoctradin 300 + Dimethomorph 225SC, Cymaxanil 8 + Mancozb 64WP, Metalaxyl 63WP, Dimethomorph 50WP, Azoxystrobin 250SC and standard check 1% Bordeaux mixture were tested against highly virulent isolate of *P. meadii* (isolate P19) under *in vitro* condition. Suitable dilutions of each fungicide were prepared and incorporated into the molten carrot agar at 45ºC grading the concentration range of active ingredients from 0.012 to 0.1 percent (Nene and Thapliyal, 1971; Jain *et al.,* 2014). Each plate was inoculated with a mycelium agar disc (5mm) of *P. meadii* placed centrally onto the fungicide amended and fungicide-free media. Following inoculation, the plates were incubated at 27 ± 1 ºC in the dark and the colony diameter was recorded for 5 days. Percent inhibition of growth was calculated by applying formula given by Vincent (1927).

Percent inhibition (I) = (C-T) / T × 100

C = Growth of pathogen in control plate; T = Growth of pathogen in treated plates

Antagonistic activity of three native *Trichoderma* species such as *T. harzianum, T. viride, T. virens* were tested against highly virulent isolate of *P. meadii* by following dual culture technique in two methods *viz*., simultaneous inoculation of *P. meadii* + *Trichoderma* and inoculation of *Trichoderma* 48 hours after inoculation of *P. meadii*. Inoculated plates were incubated in room temperature for five days. Percent inhibition of growth was calculated using the following formula (Gade, 2012 and Reddy and Hynes, 1993).

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

R1 = Growth of pathogen in control, R2 = Growth of pathogen in dual culture

RESULTS AND DISCUSSION

Phytophthora infected arecanut samples were collected from Dakshina Kannada and Shivmogga districts of Karnataka, Kasaragod, Kannur and Thrissur districts of Kerala and also from Goa State during monsoon seasons of 2012 and 2013. In total 40 isolates of Phytophthora were purified after isolation. All the collected isolates were heterothallic, produced caducous, ovoid to ellipsoidal shape sporangia with distinct papilla and were identified as *P.meadii*. Further studies were conducted to confirm the identification of the *Phytophthora* species by molecular detection technique. Colony characters of the *Phytophthora* species were reported to differ in appearance, rate and manner of growth, amount of sporulation and sporangia size on various media (Brassier and Griffin, 1979). Among the 40 isolates, four representative isolates of *P. meadii* were amplified using ITS1 and ITS4, which yielded a product of approximately 900bp. The PCR product was eluted using Qiagen QIAquick gel extraction kit and sent to Scigenom, Cochin for sequencing. The sequences when run in BLASTn programme of NCBI showed homology to *P.*

Table 1 : Infection severity of *Phytophthora* isolates

Isolate ID	Lesion area (cm^2)
P1	10.10^{cd}
P2	7.70^{de}
P3	9.20^{d}
P4	9.60^{d}
P5	11.10^{cd}
P6	8.80^{d}
P7	9.40^{d}
P8	11.70^{c}
P9	10.30^{cd}
P10	17.00^{b}
P11	16.00^{b}
P12	17.40^{b}
P13	10.30^{cd}
P14	15.20^{bc}
P15	16.20^{b}
P16	15.30^{bc}
P17	16.70^{b}
P18	12.50^{c}
P19	19.40^{a}
P20	10.00^{cd}
P21	15.10^{bc}
P22	13.00^{c}
P23	12.00^{c}
P24	11.66^{c}
P25	13.00^{c}
P26	11.10^{cd}
P27	8.20^{d}
P28	15.60^{b}
P29	6.40^{e}
P30	12.50^{c}
P31	13.00^{c}
P32	16.6 ^{b}
P33	12.00^{c}
P34	9.30^{d}
P35	10.00^{cd}
P36	12.40^{c}
P37	11.10^{cd}
P38	13.20^{c}
P39	13.40^{c}
P40	15.20^{bc}
P41	15.00^{bc}

*Means with the same letter are not significantly different according to DMRT *(P = 0.05)*

Table 2 : In vitro inhibition of growth of *P. meadii* at different concentrations of fungicides

Fungicides	Concentration (%)	Mycelia growth inhibition (%)
Iprovalicarb 5.5 + Propineb 61.3WP	0.012	100[a]
	0.025	100[a]
	0.05	100[a]
	0.1	100[a]
Metalaxyl 8 + Mancozeb 64WP	0.012	100[a]
	0.025	100[a]
	0.05	100[a]
	0.1	100[a]
Mandipropamid 250SC	0.012	100[a]
	0.025	100[a]
	0.05	100[a]
	0.1	100[a]
Ametoctradin 300 + Dimethomorph 225SC	0.012	100[a]
	0.025	100[a]
	0.05	100[a]
	0.1	100[a]
Bordeaux mixture	1.0	100[a]
Cymaxanil8 + Mancozeb 64WP	0.012	63.3[d]
	0.025	79.5[b]
	0.05	100[a]
	0.1	100[a]
Metalaxyl 63WP	0.012	34.8[g]
	0.025	48.4[e]
	0.05	84.0[b]
	0.1	100[a]
Dimethomorph 50WP	0.012	62.0[d]
	0.025	76.1[bc]
	0.05	82.5[b]
	0.1	100[a]
Azoxystrobin 250SC	0.012	41.4[f]
	0.025	48.5[e]
	0.05	60.2[d]
	0.1	72.3[c]

*Means with the same letters are not significantly different according to DMRT *(P = 0.05)*

Table 3 : In vitro a antagonistic activity of three *Trichoderma spp*. against *P.meadii*

Biocontrol agents	Mycelia growth inhibition (%) Simeltaneous inoculation of *Trichoderma* + *P. meadii*	Inoculation of *Trichoderma* 48h after inoculation of *P. meadii*
T. virens	62.5[a]	35.2[a]
T. harzianum	50.0[b]	28.0[b]
T. viride	57.6[c]	33.5[c]

*Means with the same letters are not significantly different according to DMRT *(P = 0.05)*

meadii. The sequences were submitted to NCBI-GenBank and accession number obtained for respective isolates was P1 (LC076467), P28 (LC076469), P36 (LC076471) and P41 (LC076470).

Pathogenecity test revealed that all the isolates were produced typical symptoms of fruit rot disease on three days after inoculation. The lesion size ranged from 6.4 to 19.4 cm^2 (Table 1) and highest lesion size was produced by isolate No. P19 collected from Belthangadi taluk, Dakshina Kannada district of Karnataka and it was found highly virulent compared to other isolates.

All fungicides employed in *in vitro* screening significantly inhibited the growth of *P. meadii* (isolate P19). The relative growth rate of *P. meadii* at various concentrations of fungicides is summarized in Table 2. Out of eight fungicides tested at different concentrations (0.012 to 0.1 percent), Iprovalicarb 5.5 + Propineb 61.3WP, Metalaxyl 8 + Mancozeb 64WP, Mandipropamid 250SC, Ametoctradin 300 + Dimethomorph 225SC at 0.012% followed by Cymaxanil 8 + Mancozeb 64WP at 0.05% and Metalaxyl 63WP, Dimethomorph 50WP at 0.1% and 1% Bordeaux mixture exhibited 100% mycelia growth inhibition. Among three *Trichoderma* spp. tested, *Trichoderma virens* showed highest mycelial growth inhibition of *P. meadii,* which was significantly differed from other two *Trichoderma* species. Maximum of 62.5% inhibition was recorded in simultaneous inoculation of *P. meadii* + *T. virens* and 35.2% in inoculation of *T. virens* 48 hours after inoculation of *P. meadii* (Table 3).

In an earlier study, fungicides like Blitox, Captafol and Dithane M, 45 were found effective against the pathogen in the laboratory condition (Saraswathy, 1999). Among the systemic fungicides tested Fosetyl-Al and Metalaxyl at 0.5 per cent as spray gave good control of fruit rot (Sastry and Hegde, 1985; Anandraj and Saraswathy, 1986). In the present study

Iprovalicarb 5.5 + Propineb 61.3WP, Metalaxyl 8 + Mancozeb 64WP, Mandipropamid 250SC, Ametoctradin 300 + Dimethomorph 225SC at 0.012% were found very effective against *P. meadii*.

It is also clear from the results that among the fungicides tested, Iprovalicarb 5.5 + Propineb 61.3WP, Metalaxyl 8 + Mancozeb 64WP, Mandipropamid 250SC and Ametoctradin 300 + Dimethomorph 225SC showed 100% mycelia growth inhibition at very low concentration of 0.012% and among three *Trichoderma* spp. *T. virens* exhibited highest mycelia growth inhibition of *P. meadii* both in simultaneous inoculation and inoculation of *Trichoderm*a 48 hours after inoculation of *P. meadii*. Heller and Theiler-Hedtrich, (1994) also reported the effectiveness of *T.viride* and *Gliocladium virens* in the control of *Phytophthora* spp. causing cotton root disease. Moosa (2002) identified *T. viride* as potential antagonist against *P. palmivora* causing bud rot disease of coconut in India based on *in vitro* interaction studies. Hence it is suggested that these effective fungicides and *T. virens* may be evaluated in the field condition to develop an effective and ecofriendly management practices for *Phytophthora* diseases of arecanut.

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