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Pathogenic variability of *Phytophthora* spp. inciting Bud Rot of Coconut in Kerala

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ABSTRACT: Bud rot caused by *Phytophthora* spp. is one of the most destructive diseases of coconut. The management of the disease is a major challenge due to the frequent development of pathogenic variability within the pathogens. It is necessary to comprehend the pathogenic heterogeneity among various pathogen isolates in order to design appropriate management strategies. Hence, the present study evaluated the variability in pathogenicity of thirteen different isolates of *Phytophthora* spp. isolated from coconut bud rot samples. Ten isolates from three different agroecological units of Kerala *viz*., AEU 8 (southern laterites), AEU 9 (south central laterites) and AEU 11 (northern laterites) and three from Central Plantation Crops Research Institute, Kasaragod were tested for their pathogenicity on detached spear leaves and nuts of West Coast Tall variety of coconut under *in vitro*. Two different methods *viz*., inoculation of mycelium as culture disc and zoospore suspension were employed and lesion size developed on nuts and leaves were recorded. The *Phytophthora* isolates recorded significant difference in the lesion size at regular intervals. The isolate 8A (*P. palmivora*) obtained from southern laterites was the most virulent one. The species *P. palmivora* and *P. nicotianae* were found to be almost similar in aggressiveness on coconut. The comparison of virulence between the isolates from different agroecological units indicated that, isolates from southern region of Kerala were found to be more pathogenic than northern region. The current investigation confirmed the presence of highly virulent *Phytophthora* spp. isolates in Kerala, which can pose an imminent risk to the coconut cultivation of the state.

Keywords: *Phytophthora*, pathogenicity, coconut, bud rot, variability.

INTRODUCTION

Major challenges facing in coconut farming is the incidence of variety of disease which remarkably reduces the yield. Bud rot caused by *Phytophthora* spp. is a fatal disease of coconut which is prevalent in southern region of India especially in Kerala. The development of disease is profoundly favoured by the south-west monsoon. The disease has an irregular distribution in the field, but the highest disease incidence seems to correlate with the wettest period and areas. Several scientists studied the symptomatology of the disease in detail and reported that, spear leaf wither initially which is followed by spreading of the infection to the inner portion and complete rotting of the bud in advanced stage (Briton-Jones, 1940; Quillec *et al*., 1984; Nambiar, 1994; Thamban *et al*., 2016). This accompanied by a foul horrible smell which is due to the secondary bacterial infection. Upon advancing the symptoms, complete leaves shed and result in death of the palm (Rajeswari *et al*., 2020).

The fungus, *Phytophthora* spp. is an oomycete plant pathogen. Major species causing bud rot of coconut is *Phytophthora palmivora.* But the species such as *P. nicotianae* and *P. meadii* are also associated with the disease in some localities (Sharadraj, 2013). Kerala is one the major coconut producing states in India and is consistently vulnerable to the threat of bud rot disease. Management of the disease is still a challenge due to the devastating nature of the pathogen, well developed survival mechanisms and frequent development of pathogenic variability within the causal organism. Even though, various chemical management strategies are there, the intensive and indiscriminate use of fungicides may lead to the development of resistance towards the pathogen and also affect the functioning of ecosystem, thus reduce the agriculture sustainability. Since, a very few data are available on the pathogenic variability of *Phytophthora* on coconut, it is very important to understand the variability in pathogenicity of the fungi at regular intervals for developing accurate management strategies. Hence, the present study evaluates the pathogenicity of different *Phytophthora* isolates on detached spear leaves and nuts of coconut.

MATERIAL AND METHODS

A. Isolation and culture maintenance of Phytophthora spp

Bud rot affected coconut palms were identified from farmer's fields of three different agroecological units of Kerala *viz*., AEU 8 (southern laterites), AEU 9 (south central laterites) and AEU 11 (northern laterites). The spear leaf samples with exact symptoms of bud rot were brought to the laboratory. Initially, the surface debris on the leaves were removed by washing in running tap

water. After surface sterilization, the tissues with progressing symptoms were placed on the Petri plate containing potato carrot agar medium amended with antibiotic. Incubation of the Petri plates were done at room temperature (27°C ± 2) for 4-5 days. After the incubation the mycelial bits from *Phytophthora* colonies were taken and placed on potato dextrose agar medium. For obtaining the pure cultures successive subculturing was done by hyphal tip method using potato dextrose agar medium and were stored in refrigerated condition. For maintaining the pure cultures for long term, the mycelial bits were kept in sterile distilled water inside small vials.

B. Pathogenicity test of Phytophthora spp.

Plant materials: For studying the pathogenic variability among the isolates, artificial inoculation of the pathogen was done on both detached spear leaves and nuts (7-8 months old) of West Coast Tall variety. Two different methods were employed for the test.

Inoculation of mycelium as culture disc: Initially, leaves and nuts were surface sterilized with 70 per cent ethanol and kept inside Laminar Air Flow chamber. Both leaves and nuts were given with pin pricks using a sterile needle. Mycelial discs of 5 mm diameter were excised from the margins of actively growing cultures of *Phytophthora* and placed on nuts (perianth region) and leaf. Then mycelial discs were covered with moist cotton wetted with sterile water and kept inside the polythene cover for incubation.

Inoculation of zoospore suspension: For zoospore suspension inoculation, the mycelial bits were excised from actively growing cultures of *Phytophthora* and kept in sterile water for two days. After the development of sufficient mycelium and sporangia, a cold shock was given on third day to release the zoospores into the water. This suspension was inoculated onto the spear leaves and nuts after giving pin pricks. Inoculated leaves and nuts were kept inside polythene cover and sterile water was sprinkled onto the nuts and leaves every day for maintaining the moisture.

Both the nuts and leaves were observed for symptom development at regular intervals. The lesion size was recorded at 3, 5, 7, 10 and 15 days after inoculation (DAI). Average of the lesion size at 7 DAI on both nuts and leaves was taken and based on that isolates were separated into different groups *viz*., group 1 (1-3 cm), group 2 (3-5 cm), group 3(5-7 cm), group 4 (7-10 cm) and group 5 (10-15cm).

RESULTS AND DISCUSSION

Isolation of *Phytophthora* spp. A total of 13 isolates of *Phytophthora* spp. were obtained from the bud rot affected samples, out of these three were collected directly from Central Plantation Crops Research Institute (CPCRI), Kasaragod. Isolates were named according the agroecological units from where they were collected (Table 1).

Pathogenic variability of different *Phytophthora* isolates. Pathogenic variability among the isolates was studied on the basis of nature of symptom and size of lesions produced on inoculated leaves and nuts.

Mycelial bit inoculation onto the detached leaves: All the isolates produced characteristic rot symptoms on detached leaves. There was significant variation in the lesion size produced by the 13 different isolates (Table 2). The isolate 8A obtained from AEU8 was found to be the most virulent among these, which took only one day to produce the symptom and the complete rotting of the leaf was observed at 10 days after inoculation. The symptom initially started as water-soaked lesion with light brown margin. This gradually spread and turned to dark brown lesion with centre region remained light brown in colour (Fig. 1). The similar kind of symptoms were produced by the isolates 9A, 9B, 9C, 11A and 11C. The isolates 8B, 11B and PP1 produced dark brown coloured lesions from the initial stage itself and later spread to the whole leaf area. The isolates 8C and PP2 produced light brown coloured lesions with dark brown margin. Whereas inoculation of 8D and PP3 resulted in light brown lesions in the whole leaf area. The second most virulent isolate was 9A which produced a lesion size of 9.30 ± 0.50 cm at 10 DAI. The complete rotting of the leaf could be observed by 15 DAI for all the isolates. Even though the isolates PP1 and 11B produced symptom on the second day of inoculation the lesion spread was slow compared to 9A.

Mycelial bit inoculation onto the nuts: all the isolates were able to cause infection on detached nuts when inoculated at the perianth region. Most of the isolates took only one day to develop the symptom (Table 3). The isolate 8A was found to be more virulent in infecting the nut, which produced a lesion size of 27.65 cm at 15 DAI. Isolate 9A was the second most virulent one (23.5 cm). The least virulent isolate was PP3 which developed a lesion of size 6 cm at 15 DAI. The symptom initially developed as water-soaked lesions, later turned into brown in colour (Fig. 2). This kind of symptom was produced by 8A, 9A, 11C and 11A. The isolates 8B, 9B, 9C, 11B, PP1 and PP2 produced reddish brown coloured lesions with dark margins. Whereas the 8C and 8D produced a blackish-brown lesions at the initial stage itself. The white mycelial growth of the fungus on the lesions could be observed for isolates 8A, 9A, 9C and 11C.

Zoospore inoculation onto the detached leaves: The symptom initiated as water- soaked lesions. These lesions gradually appeared brown in colour and enlarged in size (Fig. 3). Definite dark coloured margin could not be observed in case of lesions which developed after inoculation with zoospore suspension. Significant difference in the lesion size was noticed among the isolates and complete rotting of the leaves was observed at 10 DAI for most of the isolates (Table 4). The isolate 8A was found to be the most virulent which initiated symptom on next day of inoculation. Progression of the lesion was also quick in subsequent days. The isolate 8C was the second most virulent one. The least virulent isolate was PP2 which produced lesion of size 8.50± 0.95 cm at 15 DAI.

Zoospore inoculation onto the nuts: Initial symptom was the development of water-soaked lesions at the perianth region of nuts. Then gradually tissues started rotting and appeared light brown in colour (Fig. 4). At the later stage turned to dark brown in colour. The

isolates 9A, 11C and PP1 developed lesions with reddish brown colour. Whereas, 11A and 11B produced black coloured lesions. The progression of the lesion was irregular and water-soaked lesions spread quickly on the husk of the nuts. The white mycelial growth of fungus on nut surface was also noticed for some isolates. The isolate 9A was found to be the most virulent which developed a lesion of size 14.25 cm at 15 DAI (Table 5). This was followed by 11A (14 cm), PP1 (12 cm) and 8A (11.75 cm). A foul smell was noticed on nuts at the later stage of infection.

Based on the lesion size, five different groups were formed (Table 6). Among the different pathogenicity methods tried, isolate 8A was found to be the most virulent. All the isolates developed typical rot symptoms on leaves and nuts with slight variations in colour and nature of symptoms. The fast lesion progression in nuts indicates the aggressive nature of isolates. In case of leaf inoculation, the symptom initiated as water- soaked lesions which gradually spread to whole leaf area and turned brown in colour. The mycelial bit inoculation developed lesions with dark brown margin in case of some of the isolates. The variation in symptom development could only be observed at the initial stage. Whereas at the advanced stage, all the leaves were rotten completely and remained same in appearance. Similar brown coloured lesions were observed by Maizatul-Suriza *et al.* (2019) in detached white unopened spear leaves of oil palm when inoculated with *Phytophthora palmivora*. In case of nut, the reddish-brown lesions were characteristic for some isolates and which remained as such for long time. These variation in symptoms was irrespective of the location and agroecological unit from where pathogen has been isolated. This suggests that each isolate expresses the illness in a unique way. The plant materials (leaves and nuts) utilized in this investigation were all harvested from the same variety of coconut palm at the same stage of growth, which supports the theory that any difference in symptom expression is simply the result of a pathogen isolate.

There were noticeable differences in virulence between isolates, as evidenced by a comparison of lesion sizes caused by distinct isolates. A gradient in virulence from less aggressive to very aggressive was observed when the isolates were grouped based on the extent of the lesion. The differences in virulence across isolates belonging to the same agroecological units were readily apparent. Sharadraj (2013) also noticed the inter and intra specific pathogenic variability among the various *Phytophthora* isolates when inoculated to three months old (fistula size) nuts. This kind of difference in aggressiveness among *Phytophthora palmivora* isolates has been noticed by various scientists in cocoa (Nyasse *et al*., 1995; Iwaro *et al*., 1998; Coulibaly *et al*., 2018) and *Piper nigrum* (Turner, 1973). Latifah *et al.* (2017) also reported the variation in length of lesion developed by different isolates of *Phytophthora palmivora* in oil palm leaflets.

Considering the pathogenic virulence between the isolates of different agroecological units, the isolates from southern laterites and southcentral laterites were highly pathogenic compared to isolates from northern laterites. Reports by earlier workers state that, northern region of Kerala is having high incidence of bud rot compared to south (Rohini Iyer and Reshmi 2005: Sharadraj and Chandramohanan 2013). But from the present study it can be concluded that southern region of Kerala may also falls in the risk of high incidence of bud rot which results in a substantial drop in the region's coconut production. Wael Alsultan *et al.* (2022) also reported the variability in virulence between isolates of *P. palmivora* in cocoa pods and suggested that pathogenic diversity of Malaysian isolates of *P. palmivora* is related to their geographical origins. Each pathogen isolate is unique and pathogenic variability among the isolates may also be due to the genetic makeup of that isolate. Scientists suggests that, the existence of distinct RXLR effectors may account for the observed variations in aggressiveness across the isolates, perhaps contributing to their various functional and co-evolutionary characteristics (Mohamed Azni *et al*., 2019).

Among the different species of *Phytophthora, P. palmivora* and *P. nicotianae* have almost similar aggressiveness on coconut. *P. meadii* was moderately pathogenic. Whereas according to Latifah *et al.* (2017) *P. palmivora* were more aggressive than *P. nicotianae* and produced comparatively large lesions in oil palm leaflets. Since in the present study, the number *P. nicotianae* and *P. meadii* isolates obtained were very less compared to *P. palmivora,* the level of virulence among the species should be confirmed with more number of isolates.

Table 1: *Phytophthora* isolates obtained from different locations.

Agroecological unit	Isolate	Identification
AEU8	8A	*Phytophthora palmivora*
	8B	*Phytophthora palmivora*
	8C	*Phytophthora palmivora*
	8D	*Phytophthora palmivora*
AEU9	9A	*Phytophthora nicotianae*
	9B	*Phytophthora palmivora*
	9C	*Phytophthora palmivora*
AEU11	11A	*Phytophthora nicotianae*
	11B	*Phytophthora meadii*
	11C	*Phytophthora palmivora*
CPCRI, Kasargod	PP1	*Phytophthora palmivora*
	PP2	*Phytophthora palmivora*
	PP3	*Phytophthora palmivora*

Table 2: Lesion size at different days after inoculation of mycelial bit of *Phytophthora* spp. on spear leaves of coconut.

Isolate	DTSD	Lesion size (cm) at different days after inoculation				
		3 DAI	5 DAI	7 DAI	10 DAI	15 DAI
8A	1	1.30± 0.15^{a}	5.00± 0.27^{a}	10.91± 0.76^{a}	Complete rotting	Complete rotting
8B	3	0.43± 0.02^{ef}	1.73± 0.17^{gh}	6.16± 0.30^{d}	8.46± 1.32	Complete rotting
8C	3	0.32± 0.24^{ef}	1.95± 0.20^{efg}	5.18± 0.57^{f}	8.18± 0.50	Complete rotting
8D	3	0.26± 0.02^{f}	2.16± 0.07^{e}	5.16± 0.14^{f}	7.80± 0.42	Complete rotting
9A	3	0.78± 0.12^{cd}	4.20± 0.08^{b}	7.35± 0.56^{b}	9.30± 0.50	Complete rotting
9B	3	0.56± 0.12^{de}	2.08± 0.07^{e}	5.95± 0.26^{de}	7.10± 0.30	Complete rotting
9C	3	0.73± 0.29^{cd}	2.96± 0.27^{d}	7.06± 0.48^{bc}	8.98± 0.80	Complete rotting
11A	3	0.36± 0.25^{ef}	2.05± 0.08^{ef}	4.05± 0.25^{g}	6.08± 0.32	Complete rotting
11B	2	1.16± 0.07^{ab}	3.85± 0.27^{c}	6.53± 0.22^{cd}	7.83± 0.61	Complete rotting
11C	3	0.56± 0.27^{de}	2.05± 0.05^{ef}	5.28± 0.22^{ef}	7.56± 0.59	Complete rotting
PP1	2	0.93± 0.07^{bc}	1.65± 0.05^{h}	6.36± 0.30^{d}	7.43± 0.61	Complete rotting
PP2	3	0.38± 0.12^{ef}	1.80± 0.13^{fgh}	4.98± 0.35^{f}	7.00± 0.88	Complete rotting
PP3	3	0.45± 0.30^{de}	1.91± 0.10^{efgh}	5.11± 0.15^{f}	7.26± 1.09	Complete rotting
CD (0.05)		0.246	0.280	0.668	-	-

DTSD- Days taken for symptom development; Mean ± SD of three replication, Values followed by superscripts are not significantly different at 5% level

Table 3: Lesion size at different days after inoculation of mycelial bit of *Phytophthora* spp. on nut.

Isolate	DTSD	Lesion size (cm) at different days after inoculation				
		3 DAI	5 DAI	7 DAI	10 DAI	15 DAI
8A	1	3.20	7.25	10.00	22.00	27.65
8B	1	2.35	5.40	7.25	10.10	16.75
8C	2	1.20	2.85	6.00	9.35	13.00
8D	2	1.25	3.00	6.75	7.80	11.50
9A	1	2.50	6.75	11.25	19.00	23.50
9B	2	1.95	4.50	7.85	11.00	13.50
9C	3	0.85	3.35	8.75	9.65	12.50
11A	3	0.75	2.30	4.15	7.65	11.25
11B	3	0.55	2.95	4.95	9.25	12.50
11C	1	2.55	6.00	9.65	11.25	14.50
PP1	2	1.15	2.80	4.85	7.95	10.25
PP2	2	1.20	2.50	6.25	8.25	11.75
PP3	3	0.85	1.25	3.25	5.25	6.00

DTSD- Days taken for symptom development

Table 4: Lesion size at different days after inoculation of zoospore suspension of *Phytophthora* spp. on spear leaves of coconut.

Isolate	DTSD	Lesion size (cm) at different days after inoculation				
		3 DAI	5 DAI	7 DAI	10 DAI	15 DAI
8A	1	2.11± 0.16^{a}	8.13± 0.37^{a}	11.30± 0.57^{a}	Complete rotting	Complete rotting
8B	2	1.38± 0.15^{bcd}	3.40± 0.40^{cd}	8.76± 0.46^{de}	Complete rotting	Complete rotting
8C	2	1.46± 0.05^{bc}	4.15± 0.40^{c}	10.71± 0.65^{ab}	Complete rotting	Complete rotting
8D	2	1.21± 0.07^{bcde}	2.83± 0.38^{de}	3.71± 0.47^{f}	6.41± 0.52	Complete rotting
9A	3	0.05± 0.08^{h}	3.91± 0.53^{c}	9.33± 0.52^{cd}	Complete rotting	Complete rotting
9B	3	0.18± 0.20^{gh}	1.06± 0.15^{f}	4.41± 0.38^{f}	7.10± 0.65	Complete rotting
9C	3	0.70± 0.13^{efg}	7.18± 0.51^{b}	9.45± 1.08^{cd}	Complete rotting	Complete rotting
11A	2	1.35± 1.20^{bcd}	2.98± 0.89^{de}	4.80± 1.20^{f}	5.23± 0.41	Complete rotting
11B	2	0.80± 0.05^{def}	3.56± 0.16^{cd}	10.00± 0.66^{bc}	Complete rotting	Complete rotting
11C	4	-	0.93± 0.25^{f}	3.90± 0.42^{f}	5.90± 0.32	Complete rotting
PP1	2	1.78± 0.23^{ab}	6.71± 0.75^{b}	7.76± 0.70^{e}	Complete rotting	Complete rotting
PP2	3	0.55± 0.10^{fgh}	2.21± 0.37^{e}	4.48± 0.59^{f}	6.28± 0.80	Complete rotting
PP3	2	1.13± 0.33c^{def}	2.40± 0.13^{e}	4.26± 0.88^{f}	6.35± 1.23	Complete rotting
CD (0.05)		0.615	0.781	1.184	-	-

DTSD- Days taken for symptom development; Mean ± SD of three replication, Values followed by superscripts are not significantly different at 5% level

Table 5: Lesion size at different days after inoculation of zoospore suspension of *Phytophthora* spp. on 7-8 months old nut of coconut.

Isolate	DTSD	Lesion size (cm) at different days after inoculation				
		3 DAI	5 DAI	7 DAI	10 DAI	15 DAI
8A	2	1.6	7.1	9.00	9.35	11.75
8B	3	0.95	6.05	6.50	6.75	7.65
8C	2	1.35	5.50	7.00	7.60	10.75
8D	2	1.40	6.50	7.10	8.00	10.00
9A	3	0.75	5.15	6.75	9.25	14.25
9B	2	1.50	7.10	7.45	8.15	9.90
9C	3	0.85	5.15	5.85	6.20	7.75
11A	2	1.75	5.50	6.55	9.10	14.00
11B	2	1.80	4.90	6.10	8.55	10.75
11C	2	1.5	6.55	6.90	7.40	8.15
PP1	2	1.9	5.25	6.75	8.75	12.00
PP2	2	1.45	4.50	6.50	6.75	8.25
PP3	2	1.00	3.75	5.45	6.95	7.70

DTSD- Days taken for symptom development

Table 6: Grouping of isolates according to lesion size at 7 DAI.

Group	Lesion size	*Phytophthora* isolates
1	1-3 cm	—
2	3-5 cm	11A, PP3
3	5-7 cm	8D, 9B, 11B, 11C, PP1, PP2
4	7-10 cm	8B, 8C, 9A, 9C
5	10-15 cm	8A

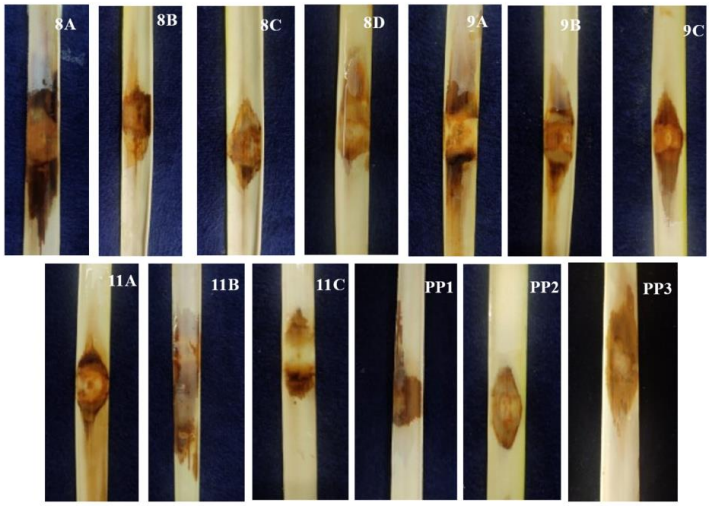

Fig. 1. Symptom developed on spear leaf by inoculation with mycelial bit of different isolates of *Phytophthora* spp. at 5th DAI.

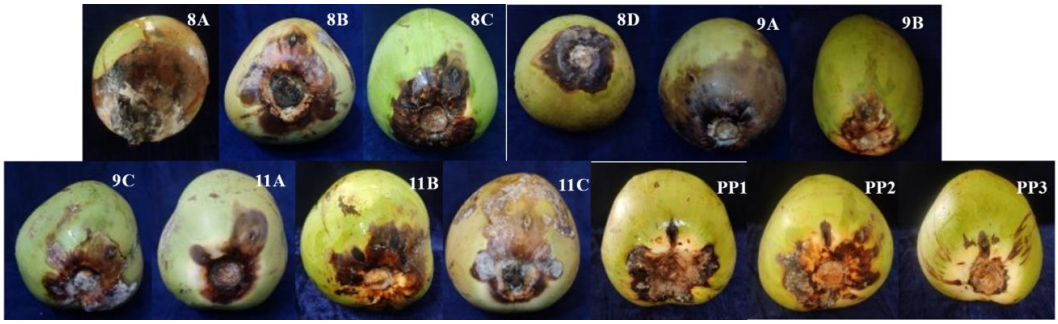

Fig. 2. Symptom developed on nuts by inoculation with mycelial bit of different isolates of *Phytophthora* spp. at 15th DAI.

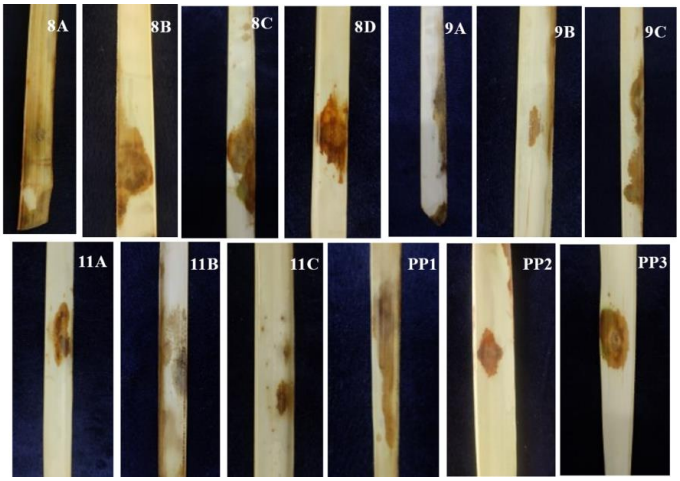

Fig. 3. Symptom developed on spear leaf by inoculation with zoospore suspension of different isolates of *Phytophthora* spp. at 5th DAI.

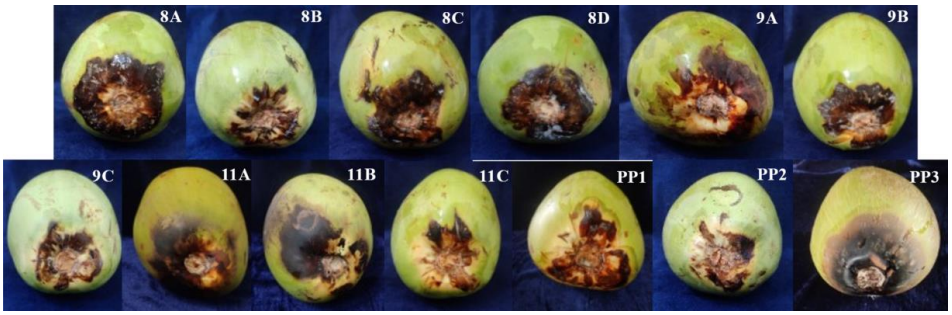

Fig. 4. Symptom developed on nut by inoculation with zoospore suspension of different isolates of *Phytophthora* spp. at 15th DAI.

CONCLUSIONS

Hence, the present study suggest that isolates of *Phytophthora* spp. differ in their ability to cause the bud rot disease in coconut. Considerable pathogenic variability was observable among the isolates from same agroecological units of the state. Being the primary focus of breeding for resistance, the information on presence of pathogenic diversity within the species is significant. The highly heterogeneous nature of the isolates in their pathogenicity can pose a challenge in adopting the management practices and finally in the cultivation of coconut. Even though the isolates used in the study are less in number, the results with these isolates can be considered as a valuable basic information on the pathogenic variability among the *Phytophthora* isolates of Kerala.

FUTURE SCOPE

The results from the present study are the relevant data for understanding pathogenic variability of *Phytophthora* isolates and can used for adopting proper management practices.

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Conflict of Interest. None.

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