

A comparison of symptom-development by different isolates of *Phellinus noxius*: the causal agent of brown root disease of rubber

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Abstract

Brown root disease caused by the pathogen *Phellinus noxius* is an emerging disease condition in the Sri Lankan rubber industry. The possibility of the development of variant pathogen isolates with more pathogenic forms can be considered as one of the factors influencing the increased frequency of its occurrence in the country during the recent past especially in certain parts of the country. The study was conducted to evaluate the variability in symptom development ability of 24 Sri Lankan isolates of *Phellinus noxius*. A pot trial was carried out by artificially inoculating three months-old rubber seedlings with an inoculated mixture of rice bran and saw dust. Forty seedlings were inoculated with each pathogen isolate, and another forty seedlings were kept as controls without inoculation. Starting after two weeks of inoculation, ten destructive samplings were carried out at two weeks intervals to observe the pathogenicity levels of the different isolates. Based on the below-ground signs and symptoms, a pathogenicity score was given to each uprooted plant. Then those ranks were subjected to Kruskal–Wallis analysis and subsequently to the Wilcoxon rank-sum test. A variation of pathogenicity was observed among the 24 *Phellinus noxius* isolates. As all the isolates showed a stabilized pathogenicity value at three and half months of the inoculation, a cluster analysis was performed for the mean score values of pathogenicity rank of different isolates at three and half months and the developed dendrogram showed that the test isolates were separated into two main clusters at the similarity level 0.8. It denotes that the studied pathogen population consists of variability and these results can be applied at the development of management strategies.

Key words: brown root disease, dendrogram, rubber, symptom development

Introduction

Brown root disease caused by *Phellinus noxius* is one of the important root pathogens of rubber plantations in Sri

Lanka (Silva *et al.*, 2013, 2017 and 2019). It is distributed in tropical and sub-tropical regions in Asia (including Southern Japan, Mainland China, Hong

Kong, Taiwan and Malaysia), Central America, Africa and Oceania (Larsen *et al.* 1990; CABI/EPPO, 1997; Chang *et al.* 1998; Ann *et al.*, 2002). Brown root disease has a very wide host range including economically important plantation and other crop species such as, *Camellia sinensis* (tea), *Coffea spp.* (coffee), *Artocarpus altilis* (breadfruit), *Cinnamomum spp.* (cinnamon), *Theobroma cacao* (cocoa), *Cocos nucifera* (coconut), *Garcinia mangostana* (mangosteen), *Citrus sp.* (citrus), *Mangifera indica* (mango), *Artocarpus heterophyllus* (jack), *Tectona grandis* (teak) and *Swietenia mahagoni* (mahogany). *Phellinus noxius* spreads by root contact and persists in roots and stumps of infected plants for more than 10 years after the death of the host (Chang 1996). The economic impact of *P. noxius* is highly variable and a loss of up to 60% can be caused in some other rubber-growing countries (Nandris *et al.*, 1987). Though the brown root disease has been reported in Sri Lankan rubber during the early phase, and has been stated probably the most common root disease of the rubber tree in the country at that time (Petch, 1911 and 1921), until the recent past its significance has not been well recognized.

The increased frequency of its occurrence in the country during the recent past have to be discussed especially in certain parts of the

country. The factors influencing may include the expansion of the cultivation to non-traditional areas in dry and intermediate parts of the country and the possibility of the pathogen to be mutated into more virulent forms. Considering the diversity of soil and climatic conditions in which the pathogen develops, and the large number of tree species they attack, it is necessary to have an understanding whether there is any variation in symptom development ability (pathogenicity) within the population of the fungus. Ultimately, this heterogeneity in the aggressiveness of the parasite would be helpful in the development of management strategies against the disease. Therefore, this study was carried out with the objective of reviewing the variability in pathogenicity among the Sri Lankan isolates of the *P. noxius* in order to facilitate the development of effective management strategies.

Materials and Methods

Collection of disease samples

Twenty four number of *P. noxius* isolates available in the Department of Plant Pathology and Microbiology, Rubber Research Institute of Sri Lanka, (collected from mid-2013 to mid-2016) were used for the study (Table 1). These isolates represented different agro-ecological regions of the country.

Table 1. Description of the isolates in the isolate collection

Name of the isolate	Host	Location	GPS locate	Agro-ecological Zone
1	<i>Hevea brasiliensis</i>	Galigamuwa, Sri Lanka	7° 14' 12.7104" N 80° 18' 33.714" E	WL2b
2	<i>Hevea brasiliensis</i>	Matugama, Sri Lanka	6° 31' 40.1124" N 80° 5' 39.1272" E	WL1a
3	<i>Hevea brasiliensis</i>	Agalawatta, Sri Lanka	6° 31' 48.432" N 80° 9' 24.7932" E	WL1a
4	<i>Hevea brasiliensis</i>	Galigamuwa, Sri Lanka	7° 14' 7.3968" N 80° 18' 28.1556" E	WL2b
5	<i>Hevea brasiliensis</i>	Moneragala, Sri Lanka	6° 50' 46.1004" N 81° 19' 29.4924" E	IL1c
6	<i>Hevea brasiliensis</i>	Galagedara, Sri Lanka	7° 22' 18.6564" N 80° 30' 37.854" E	IL1a
7	<i>Hevea brasiliensis</i>	Moneragala, Sri Lanka	6°53' 30.663 "N 81°18' 31.948"E	IL1c
8	<i>Hevea brasiliensis</i>	Badalkumbura, Sri Lanka	6°55'1.165"N 81°13'31.246"E	IM2b
9	<i>Hevea brasiliensis</i>	Kithulgala, Sri Lanka	6° 59' 40.0992" N 80° 24' 43.1064" E	WM1a
10	<i>Hevea brasiliensis</i>	Kithulgala, Sri Lanka	6° 59' 43.7784" N 80° 24' 40.6332" E	WM1a
11	<i>Hevea brasiliensis</i>	Dehiowita, Sri Lanka	6° 57' 55.9548" N 80° 15' 59.0364" E	WL1a
12	<i>Hevea brasiliensis</i>	Agalawatta, Sri Lanka	6° 31' 53.2092" N 80° 9' 19.8504" E	IL1c
13	<i>Hevea brasiliensis</i>	Deraniyagala, Sri Lanka	6° 55' 45.9048" N 80° 20' 21.498" E	WL1a
14	<i>Hevea brasiliensis</i>	Haldummulla, Sri Lanka	6° 45' 33.5556" N 80° 52' 40.4544" E	IM2a
15	<i>Hevea brasiliensis</i>	Hopton, Sri Lanka	6°59' 31.56"N 81°11'55.68"E	IL1c
16	<i>Hevea brasiliensis</i>	Lunugala, Sri Lanka	7°01'60.00"N 81°11'60.00" E	IM2b
17	<i>Hevea brasiliensis</i>	Moneragala, Sri Lanka	6° 51' 49.9104" N 81° 20' 43.6164" E	IL1c
AH 1	<i>Cereya arborea</i>	Warakapola, Sri Lanka	7°08'22.8"N 80°14'04.2"E	WL2b
AH 2	<i>Gmelina arborea</i>	Badalkumbura, Sri Lanka	6°55'1.165"N 81°13'31.246"E	IM2b
AH 3	<i>Bridelia retusa</i>	Bulathkohupitiya, Sri Lanka	7°05' 27.838"N 80°20' 9.449"E	WL1a

Symptom variation by different *Phellinus noxius* isolates

Name of the isolate	Host	Location	GPS locate	Agro-ecological Zone
AH 4	<i>Mangifera indica</i>	Moneragala, Sri Lanka	6°53' 30.663 "N 81°18' 31.948"E	IL1c
AH 5	<i>Artocarpus heterophyllus</i>	Gampaha, Sri Lanka	7°06' 2.652 "N 79°59'42.359"E	WL3
AH 6	<i>Tectona grandis</i>	Hopton, Sri Lanka	6°59' 31.56"N 81°11'55.68"E	IL1c
AH 7	<i>Cinnamomum zeylanicum</i>	Polgahawela, Sri Lanka	7°20' 26.967"N 80°16' 41.978"E	IL1a

Preparation of *P. noxius* inoculum

For the artificial inoculation, the method described by Bartz in 2007 was used with modifications. A medium comprising of rice bran and saw dust (1:2 w/w) with 15% (w/w) moisture was used as the carrier medium. The prepared medium was autoclaved for 45 minutes at 121°C in polythene bags. Two agar blocks of 30 cm² from the advancing margin of each test fungal culture grown on MEA was transferred aseptically into each bag of autoclaved medium and incubated for 12 weeks at RT (28 ± 2 °C) under dark conditions.

Inoculation of rubber seedlings

Three-month-old rubber seedlings raised under controlled greenhouse conditions at RRISL were used for the study. Polybags (30 cm x 30 cm) were filled with unsterilized soil collected from rubber rhizosphere and four seedlings were planted in each bag. The inoculation was carried out by incorporating 100 g of inoculated medium (with respective fungal isolate) into the potting medium in a way to ensure contact with the collar region of each seedling. The completely randomized block design was adopted

in the experiment. Forty seedlings were inoculated with each isolate, and another 40 seedlings without inoculation were kept as the control. Soil moisture level was checked every two days using a neutron moisture gauge and was kept constant.

Assessment on the pathogenicity level

Starting after two weeks of inoculation, ten destructive samplings were done (each time four seedlings inoculated with each isolate) at two weeks intervals to observe the pathogenicity levels of different pathogen isolates against rubber seedlings. Based on the signs and symptoms, a score was assigned for the foliar symptoms as; 0 (no infection), 1 (mycelial crust without root decay), 2 (mycelial crust with root decay) and 3 (plant death). For each uprooted plant a score was assigned for the root and collar signs and symptoms as; 0 (no infection), 1 (mycelial crust without root decay), 2 (mycelial crust with root decay) and 3 (plant death).

Statistical analysis

The pathogenicity levels recorded as ranks were subjected to Kruskal–Wallis analysis and subsequently to the

Wilcoxon rank-sum test, as the scores obtained for the different isolates were significant. Spearman's rank correlation coefficient was calculated to assess whether a correlation exists among the two sets of pathogenicity ranks: foliar and roots. A cluster analysis was performed for the mean score values of pathogenicity ranks and dendrograms were developed for the Mean Score Values of Pathogenicity rank for both foliar and root symptoms.

Results and Discussion

The spearman's rank correlation coefficient of the two sets of pathogenicity ranks: foliar and roots showed a correlation (Correlation 0.820, adjusted for ties 0.787 at the probability < 0.001). The mean score values of the pathogenicity rank assigned for foliar and collar symptoms among the 24 *Phellinus noxius* isolates are shown in Tables 1 and 2, respectively.

Table 1. The mean score values of the pathogenicity rank assigned for foliar symptoms

Isolate	Mean score values of the pathogenicity rank (foliar) at different incubation durations*									
	0.5 Months	1 Months	1.5 Months	2 Months	2.5 Months	3 Months	3.5 Months	4 Months	4.5 Months	5 Months
1	47	45.25	33	34.75	25.25	43.5	42.5	42.5	42.5	42.5
2	47	33	33	24.125	25.25	18.5	18	18	18	18
3	59	57.5	44.5	43.5	62	64.5	63.5	63.5	63.5	63.5
4	47	45.25	51.25	64.75	62	54	53	53	53	53
5	47	57.5	44.5	45.375	33.875	33	32	32	32	32
6	47	71.3	44.5	45.375	53.375	64.5	63.5	63.5	63.5	63.5
7	47	33	33	24.125	25.25	18.5	18	18	18	18
8	47	57.5	44.5	34.75	33.875	43.5	53	53	53	53
9	71	57.5	69.5	73.5	88.25	89.25	89	89	89	89
10	47	57.5	56	56	52.25	43.5	42.5	42.5	42.5	42.5
11	47	45.25	44.5	45.375	33.875	33	32	32	32	32
12	47	57.5	56	64.75	62	54	53	53	53	53
13	47	57.5	56	45.375	52.25	54	53	53	53	53
14	47	33	44.5	34.75	25.25	25.75	25	25	25	25
15	47	45.25	44.5	45.375	52.25	43.5	42.5	42.5	42.5	42.5
16	47	57.5	62.75	73.5	65.375	69.25	68.5	68.5	68.5	68.5
17	47	45.25	44.5	34.75	33.875	33	32	32	32	32
AH1	47	45.25	56	56	62	64.5	63.5	63.5	63.5	63.5
AH2	47	57.5	67.5	73.5	71.75	79.75	79	79	79	79
AH3	47	57.5	44.5	54.125	62	54	53	53	53	53

Symptom variation by different *Phellinus noxius* isolates

Isolate	Mean score values of the pathogenicity rank (foliar) at different incubation durations*									
	0.5 Months	1 Months	1.5 Months	2 Months	2.5 Months	3 Months	3.5 Months	4 Months	4.5 Months	5 Months
AH4	47	33	33	34.75	25.25	18.5	18	18	18	18
AH5	47	45.25	44.5	45.375	42.5	54	53	53	53	53
AH6	47	45.25	44.5	45.375	52.25	54	53	53	53	53
AH7	47	69.75	67.5	64.75	62	54	63.5	63.5	63.5	63.5

*Note that the critical difference is 4.56 to compare the values within the columns

Table 2. The mean score values of the pathogenicity rank assigned for root symptoms

Isolate	Mean score values of the pathogenicity rank (root) at different incubation durations									
	0.5 Months	1 Months	1.5 Months	2 Months	2.5 Months	3 Months	3.5 Months	4 Months	4.5 Months	5 Months
1	48.5	44.5	48.5	50	44.75	59.875	57.75	57.75	57.75	57.75
2	48.5	44.5	36.5	38	33.125	21.75	20	20	20	20
3	48.5	44.5	60.5	50	51.5	50.125	48.25	48.25	48.25	48.25
4	48.5	56.875	60.5	50	56.375	51	48.5	48.5	48.5	48.5
5	48.5	44.5	48.5	38	33.125	31.5	39	39	39	39
6	48.5	61	48.5	50	44.75	51	48.5	48.5	48.5	48.5
7	48.5	44.5	36.5	26	21.5	21.75	20	20	20	20
8	48.5	56.875	48.5	50	44.75	51	48.5	48.5	48.5	48.5
9	48.5	69.25	60.5	74	81.5	91	90.5	90.5	90.5	90.5
10	48.5	44.5	48.5	50	44.75	50.125	48.25	48.25	48.25	48.25
11	48.5	44.5	36.5	38	44.75	31.5	29.5	29.5	29.5	29.5
12	48.5	56.875	48.5	50	56.375	68.75	67	67	67	67
13	48.5	44.5	36.5	50	44.75	41.25	48.5	48.5	48.5	48.5
14	48.5	44.5	36.5	38	44.75	31.5	29.5	29.5	29.5	29.5
15	48.5	44.5	48.5	38	33.125	31.5	29.5	29.5	29.5	29.5
16	48.5	69.25	60.5	50	56.375	59.875	57.75	57.75	57.75	57.75
17	48.5	44.5	36.5	38	44.75	31.5	29.5	29.5	29.5	29.5
AH1	48.5	56.875	48.5	62	68	68.75	76.25	76.25	76.25	76.25
AH2	48.5	56.875	60.5	74	68	68.75	67	67	67	67
AH3	48.5	44.5	60.5	50	44.75	50.125	48.25	48.25	48.25	48.25
AH4	48.5	44.5	36.5	38	33.125	31.5	29.5	29.5	29.5	29.5

Mean score values of the pathogenicity rank (root) at different incubation durations*										
	0.5 Months	1 Months	1.5 Months	2 Months	2.5 Months	3 Months	3.5 Months	4 Months	4.5 Months	5 Months
AH5	48.5	44.5	48.5	50	44.75	41.25	48.5	48.5	48.5	48.5
AH6	48.5	56.875	48.5	50	56.375	59.875	67	67	67	67
AH7	48.5	44.5	60.5	62	68	68.75	67	67	67	67

* Note that the critical difference is 4.56 to compare the values within the columns

According to the results, no isolate has shown either foliar or root symptoms at 2 weeks of inoculation and all the isolates have initiated a stabilized pathogenicity value at three and half months of inoculation.

As a stabilized pathogenicity value has been initiated for all the isolates at three

and half months of inoculation, cluster analysis was performed for the mean score values of pathogenicity rank that time duration and the dendrograms were developed for the Mean Score Values of Pathogenicity rank for both foliar and root symptoms (Figs. 1 & 2).

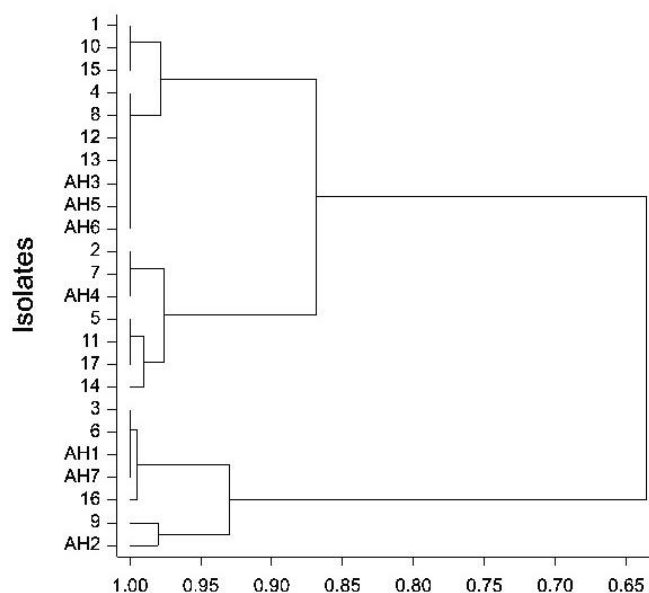


Fig. 1. Dendrogram for the Mean Score Values of Pathogenicity rank at 3.5 months of inoculation (Foliar)

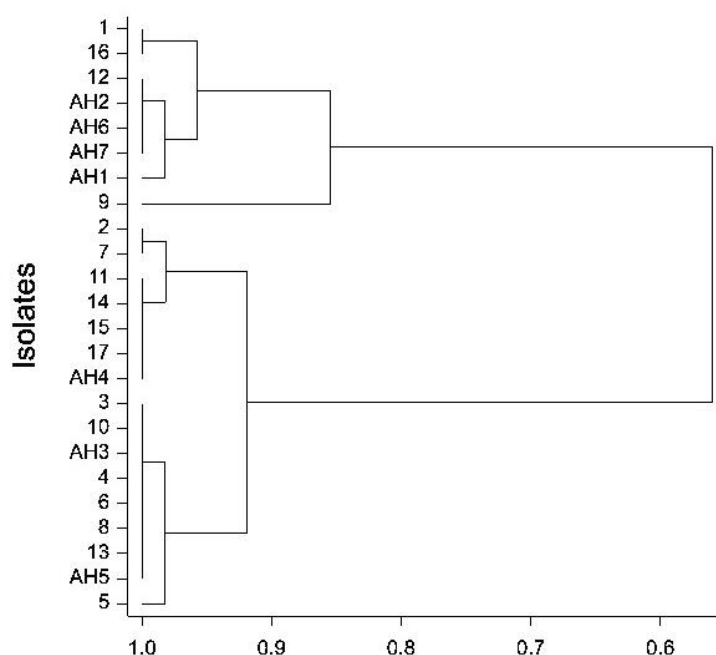


Fig. 2. Dendrogram for the Mean Score Values of Pathogenicity rank at 3.5 months of inoculation (Root)

According to the dendrograms for the Mean Score Values of pathogenicity rank for both foliar and root symptoms, it could be observed that the test isolates were separated into two main clusters at the similarity level 0.8. Isolate 9 which has been collected from Kithulgala, Sri Lanka (WM1a) was the most virulent, while the isolate 7 which has been collected from Moneragala, Sri Lanka (IL1c) was the least pathogenic.

In both foliar and root pathogenicity development, the isolates 16, (from rubber from Lunugala, Sri Lanka-

IM2b) AH1 (from *Cereya arborea* from Warakapola, Sri Lanka-WL2b) and AH2 (from *Gmelina arborea* from Badalkumbura, Sri Lanka - IM2b) have grouped with Isolate 9 in the cluster and they can be considered as more virulent isolates. Similar to this study, in a study carried out by Nandris *et al.* in 1987 on the variability in pathogenicity among several *P. noxius* isolates using segments of rubber tree branches, root penetration has occurred even with the least virulent strains used. Therefore, they have assumed that the differences in

pathogenicity would be revealed mainly during tap root colonization. On this point, the speed of development of root necrosis toward the collar seemed to play an important role in pathogenesis. Ectotrophic development of *P. noxius* on plant roots has been proven by Nandris in 1985, and Garrett (1970) has established the “ectotrophic infection habit” as the more rapidly a parasite progresses into the tap root, the less time the plant has to react. It appears that in this experiment, contact with the roots is asynchronous and accordingly the initial moment of infection had fluctuate as a result of the biology of the fungus which does not possess a mycelial structure comparable to rhizomorphs of *R. lignosus*. *P. noxius* contacts the roots by means of a crusty mycelial sleeve which has a slower rate of development and Nandris *et al.* in 1987 suggest that this fact may involve an underestimation of the pathogenic potential of this fungus. Moreover, they state that the failure to make contact with the host may be the weak point in the success of artificial inoculation with *P. noxius*. In the study carried out by Nandris *et al.* in 1987, the mortality rate has declined from the third month on, suggesting a reduction in fungal activity, while pathogenicity ranks:

both foliar and root has been stabilized at 3 had half months of the inoculation. This decline has been described in previous work with rubber seedlings artificially infected by *Rigidoporus lignosus* (Nicole *et al.*, 1983) and with *Eutcalyptus* infected by *Armillaria* (Leach, 1937) using two hypotheses: a decrease of the fungal activity due to the exhaustion of trophic reserves in the woody sticks of the inoculum (Geiger *et al.*, 1986 b) and a delay of the colonization of the roots by the fungus induced by the development of host resistance (Geiger *et al.*, 1986a; Nicole *et al.*, 1986) and has been confirmed by epidemiological observations in rubber plantation (Nandris, 1985). When the pathogenicity of the different isolates of *P. noxius* is concerned, the varying levels of pathogenicity, demonstrate an avenue to correlate it with the enzyme production capabilities of different strains. However, Nichol *et al.* 1985 states that the attempts to correlate pathogenicity with the capacity of different strains to degrade plant polymers in vitro had not furnished positive results. Nandris *et al.* in 1987 and Nichole *et al.* 1983 state that, the heterogeneity in pathogenicity among isolates demands testing of many isolates when a control method is developed.

Moreover, they state that, this fact must be taken into account particularly in the case of resistance breeding.

In this study, to distinguish differences in virulence among isolates, it was attempted to approach natural conditions and consequently the inoculum was placed close to the tap root instead of inserting it. The good association between the development of foliar symptoms and the damage to roots belowground in this study can be considered as a great advantage in future similar studies as it is generally difficult, costly and time consuming to excavate roots for observation of symptoms. In some other studies as well, foliar inspection was often relied upon identifying infected trees in the field (Nandris *et al.* 1987, Ann *et al.* 2002).

From a practical perspective, the heterogeneity in pathogenicity observed among isolates demands many isolates to be tested when control methods are developed *i.e.* at the screening of fungicides, they have to be screened against a set of fungal isolates representing the different clusters developed by the above dendrograms.

Conclusions

A variation of pathogenicity was observed among the *Phellinus*

noxius isolates and the set of test isolates separated into two main clusters at the similarity level 0.8. Moreover, this heterogeneity in the pathogenicity of the pathogen population has to be considered in the development of management strategies.

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