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Screening black pepper (*Piper nigrum* L.) genotypes against Phytophthora foot rot disease

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Abstract
Fifty genotypes of black pepper including five varieties *viz*., Panniyur 1, Panniyur 5, Vijay, IISR Malabar Excel and Pornami were screened against Phytophthora foot rot disease by artificial inoculation of culture disc of *Phytophthora capsici* in detached leaves. None of the genotypes were found immune to *P. capsici*. Black pepper variety IISR Malabar Excel, cultivars such as ICP 102, Kulathurpuzha, Mundi, Padarppan, Vellanamban 1 and Veluthanamban 5 recorded lowest lesion development after 48, 72 and 96 hours of inoculation with *P. capsici.* Cultivars, ICP 102, Vellanamban 1, Angamaly and Arakkalamunda 4 came in the lowest classes of disease incidence. Among the different genotypes screened against foot rot disease, ICP 102 was found to be the most tolerant followed by Vellanamban 1, which showed lowest lesion development and came in the lowest classes of disease incidence.

Keywords: *Phytophthora capsici*, vellanamban 1, angamaly and arakkalamunda

Introduction
Black pepper is one of the most important export oriented commodity and foreign exchange earner among the Indian spices. In addition to the use as spice, it has several medicinal uses such as anti-inflammatory (Kunnumakkara *et al*., 2018) [7], anticancerous (Do *et al*., 2013) [3] properties, regulate cholesterol level (Duangjai *et al*., 2013) [4] and sugar content in blood (Maeda *et al*., 2018) [8]. Often the production of black pepper is dwindled by heavy crop losses caused by the epidemic disease, Phytophthora foot rot also known as quick wilt caused by *Phytophthora capsici* (Vandana *et al.*, 2014) [17]. In India, Kerala alone accounts for more than 70 per cent of the area under cultivation of black pepper, but the productivity is very low (280 kg / ha) due to various reasons of which *Phytophthora* is the major constraint (Nybe *et al*., 2007) [10]. The disease spreads very quickly in the field during the rainy season and is difficult to control. Plants affected by this disease die within two to three weeks and adjacent plants may also get infected (Anh *et al.*, 2018) [1].

Plant protection measures using fungicides have resulted only in partial success. Moreover, there is huge demand for clean spices free from residues of chemicals. All the cultivated black pepper cultivars/varieties are susceptible to this disease. Conventional breeding programmes to develop black pepper lines having resistance to foot rot have not been successful since high degree of resistance is lacking in the existing germplasm resources. However, *Piper colubrinum* Link., a wild relative of black pepper was found immune to foot rot disease (Sarma *et al.*, 1991) [12]. Interspecific hybrids of *P. nigrum* and *P. colubrinum* have been reported (Vanaja *et al*., 2008) [16] but it is not repeatable. Hence identification of resistant sources of Phytophthora foot rot is of utmost importance in disease resistance breeding of black pepper. Present investigations of screening germplasm of black pepper against *Phytophthora* foot rot disease were taken up with the objective of identifying resistant/tolerant genotypes which can be used in further breeding programmes for the development of foot rot resistant/tolerant varieties.

Materials and Methods
Phytophthora capsici, the causal organism of Phytophthora foot rot in black pepper was isolated from naturally infected pepper plants by standard techniques. The fungus was cultured in Potato Dextrose Agar (PDA) medium and the Koch's postulates were proved. Based on morphological and cultural characters, the organism was identified. The isolated fungus was maintained by periodic subculturing in PDA medium.

Technique for screening and scoring for Phytophthora foot rot in black pepper was followed as per the procedures reported by Kueh and Khew (1980) [6] and Shylaja *et al*. (1996) [13].

Healthy leaf samples of same maturity were collected from 50 black pepper genotypes including five varieties *viz*., Panniyur 1, Panniyur 5 and Vijay (released by Kerala Agricultural University), IISR Malabar Excel and Pornami ((released by Indian Institute of Spices Research, Kozhikode) conserved in the germplasm of Pepper Research Station, Panniyur. The leaf samples were thoroughly washed with water to remove the dust and other remnants and then wiped with 70 per cent alcohol. Pin-pricks were made on the leaves. Culture discs of *P. capsici* measuring 5 mm diameter was cut out from the petri dish using cork borer and placed on the pin-pricked area of the leaf. The disc was moistened by keeping a piece of moistened cotton over it. The inoculated leaf was bagged using moistened polypropylene cover, tied and incubated at room temperature. The experiment was laid out in a completely randomized design.

The average diameter of the lesions developed on the leaf was observed at 48 h, 72 h and 96 h after inoculation of *P. capsici*. Depending upon the average diameter of the lesion formed, the different genotypes were grouped into five classes as shown below.

Class	Diameter of disease incidence (cm)
1	< 0.50
2	0.50 – 1.00
3	1.00 – 1.50
4	1.50 – 2.00
5	> 2.00

Data were analyzed using one-way ANOVA using GRAPES software (Gopinath *et al*., 2021) [5].

Results and Discussion

Tolerance level of the genotypes to the disease was determined based on the intensity of lesion development (Table 1). Significant variation was observed among the genotypes of black pepper for symptom expression.

Diameters of lesion development ranged from 0.36 cm to 1.60 cm after 48 hours of inoculation. Among the genotypes, maximum lesion development was recorded in HP 105 (1.60 cm) which was on par with Elampara 2 (1.43 cm), ICP 51 (1.40 cm), ICP 79 (1.26 cm), P-24 (1.25 cm) and Elampara 1 (1.20 cm). Lesion development was lowest in ICP 102 (0.36 cm). It was on par with Vellanamban 1 (0.50 cm), Malabar excel (0.50 cm), Alakkodan (0.60 cm), Arakkalamunda 4 (0.63 cm), Mundi (0.66 cm), Kulathurpuzha (0.66 cm), Culture 1041 (0.66 cm), Veluthanamban 5 (0.70 cm), Padarppan (0.73 cm), Chalakkudi (0.73 cm), Neelgiri (0.75 cm) and Josegiri 1 (0.76 cm).

Increase in lesion development was noticed after 72 hours of inoculation and it ranged from 0.80 cm to 2.66 cm. Among the genotypes studied, highest lesion development was observed for Ottamundi (2.66 cm) which was on par with Elampara 2 (2.46 cm), ICP 51 (2.43 cm), Kalluvally 2 (2.43 cm), Arikuttanadan (2.40 cm), HP 105 (2.40 cm), Elampara 1 (2.33 cm), Ottanadan (2.30 cm), Aayiram Ekkar Bukkayi (2.26 cm), ICP 79 (2.16 cm), Poonjarmunda 1 (2.16 cm), Pournami (2.16 cm), P-24 (2.15 cm), Karimunda Chittarikkal (2.13 cm) and Vattamundi (2.06 cm). The lowest value of lesion diameter was observed for ICP 102 (0.83 cm) which was on par with Vellanamban 1 (1.07 cm), Panniyur 5 (1.20 cm), IISR Malabar Excel (1.23 cm), Karimthakara (1.26 cm), Veluthanamban 5 (1.33 cm), Mundi (1.40 cm), Kulathurpuzha (1.40 cm), Padarppan (1.43 cm) and HP728 (1.43 cm).

Further increase in lesion development was noticed after 96 hours of inoculation and it varied from 1.86 cm (Arakkalamunda 4) to 6.50 cm (Ottanadan) (Plate 1, 2, 3 and 4). The lowest lesion development was observed in Arakkalamunda 4 (1.86 cm), which was on par with ICP 102 (1.90 cm), Vellanamban 1 (1.93 cm), Angamaly (1.96 cm), Malabar Excel (2.03 cm), Kulathurpuzha (2.33 cm), Karimthakara (2.30 cm), HP728 (2.33 cm), Chalakkudi (2.43 cm), Kurachimundi (2.50 cm), Kiriyath (2.53 cm), Panniyur 5 (2.55 cm), Aimpiriyan (2.56 cm), Veluthanamban 5 (2.66 cm), P-24 (2.75 cm), Kalluvally 2 (2.76 cm), Panniyur 1 (2.83 cm), ICP 79 (2.83 cm), Arakkalamunda 2 (2.83 cm), Kottanadan 2 (2.86 cm) and Karimunda 7 (2.86 cm).

After 48 h of inoculation, two per cent of genotypes (ICP 102) came in class 1 (<0.5 cm) with an average lesion diameter of less than 0.5 cm (Table 2). Majority of the genotypes (58 %) were grouped in the second class with an average lesion diameter of 0.5 to 1.0 cm. Thirty-eight percent of genotypes came in class 3 (1 – 1.5 cm) with an average lesion diameter of 1.00 to 1.50 cm.

In lesion development after 72 hours of inoculation, none of the genotypes came in class 1, two per cent of genotypes were grouped in class 2 (ICP 102), 18 per cent under class 3, 44 per cent in class 4 and 36 per cent in class 5.

After 96 hours of inoculation, none of the genotypes came in class 1, 2 and 3. Eight per cent of genotypes *viz.* ICP 102, Vellanamban 1, Angamaly and Arakkalamunda 4 came in class 4 while ninety two percent of genotypes were grouped in class 5. Similar difference between cultivars of black pepper in the tolerance level of Phytophthora foot rot disease has been reported by many workers such as Shylaja *et al.* (1996) [13], Sarma *et al.* (1997) [12], Rajagopalan *et al.* (1998) [11], Suseela *et al*. (2007) [15], Mammootty *et al.* (2008) [9] and Sinoj *et.al*. (2014) [14]. Dagde (1999) [2] reported that anatomical and biochemical differences exist between the moderately foot rot tolerant black pepper cultivar 'Kalluvally' and the susceptible variety 'Panniyur 1'. The variation in tolerance reaction of the different genotypes in the present study can be due to anatomical and biochemical differences between them.

Among the genotypes tested, none were immune to *P. capsici*. Genotype ICP 102, variety IISR Malabar Excel, cultivars such as Kulathurpuzha, Mundi, Padarppan, Vellanamban 1 and Veluthanamban 5 recorded lowest lesion development after 48, 72 and 96 hours of inoculation with *P. capsici.* ICP 102 was grouped under class 1 at 48 and 72 h of inoculation and under class 4 at 96 h of inoculation. Vellanamban 1 was grouped under class 2, class 3 and class 4 at 48, 72 and 96 h of inoculation respectively. Angamaly and Arakkalamunda 4 were grouped under class 2 at 48 h of inoculation and class 4 at 72 and 96 h of inoculation. ICP 102, Vellanamban 1, Angamaly and Arakkalamunda 4 came in the lowest classes of disease incidence after 48, 72 and 96 hours after inoculation. In the present study, ICP 102 was found to be most tolerant genotype to Phytophthora foot rot disease. Vellanamban 1 also showed lowest lesion development and came in the lowest classes of disease incidence after inoculation.

Table 1: Response of black pepper genotypes to lesion development after 48, 72 and 96 hours of inoculation of *P. capsici*

Sl. no.	Genotypes	Diameter of lesion (cm) after inoculation of *P. capsici*		
		48 h	72 h	96 h
1.	Panniyur 1	0.93^{cdefghij}	1.83^{bcdefghijkl}	2.83^{efghijkl}
2.	Panniyur 5	0.85^{defghijk}	1.20^{klm}	2.55^{ghijkl}
3.	Pournami	0.96^{bcdefghij}	2.17^{abcdefg}	3.33^{cdefghi}
4.	Malabar excel	0.50^{ijk}	1.23^{jklm}	2.03^{jkl}
5.	Aayiramekkarbukkayi	1.17^{abcdefg}	2.27^{abcdef}	3.50^{cdefg}
6.	Aimpiriyan	0.90^{cdefghij}	1.67^{cdefghijkl}	2.57^{ghijkl}
7.	Alakkodan	0.60^{ijk}	1.53^{fghijklm}	2.93^{defghij}
8.	Angamaly	0.86^{defghij}	1.60^{efghijkl}	1.97^{kl}
9.	Arakkalamunda 2	0.86^{defghij}	1.70^{bcdefghijkl}	2.83^{efghijkl}
10.	Arakkalamunda 1	0.83^{defghijk}	1.97^{abcdefghijk}	3.73^{bcde}
11.	Arakkalamunda 4	0.63^{hijk}	1.67^{cdefghijkl}	1.87^{l}
12.	Arikuttanadan	1.16^{abcdefg}	2.40^{abcd}	3.90^{bcd}
13.	Ceylon	0.90^{cdefghij}	1.88^{bcdefghijk}	3.05^{defghi}
14.	Chalakkudi	0.73^{fghijk}	1.70^{bcdefghijkl}	2.43^{hijkl}
15.	Culture 1041	0.67^{ghijk}	1.60^{efghijkl}	2.93^{defghijk}
16.	Elampara 1	1.20^{abcdef}	2.33^{abcde}	3.47^{cdefg}
17.	Elampara 2	1.43^{ab}	2.47^{ab}	3.67^{bcdef}
18.	HP105	1.60^{a}	2.40^{abcd}	3.33^{cdefghi}
19.	HP728	0.80^{defghijk}	1.43^{ghijklm}	2.33^{ijkl}
20.	ICP 51	1.40^{k}	2.43^{m}	3.63^{kl}
21.	ICP102	0.36^{cdefghij}	0.83^{abcdefghijk}	1.90^{cdefghi}
22.	ICP-48	0.90^{abc}	1.93^{abc}	3.13^{cdef}
23.	ICP79	1.26^{abcd}	2.17^{abcdefg}	2.83^{efghijkl}
24.	Josegiri 1	0.76^{defghijk}	1.87^{bcdefghijk}	4.67^{b}
25.	Kalluvally	0.93^{cdefghij}	2.00^{abcdefghij}	3.00^{defghij}
26.	Kalluvally 1	0.76^{defghijk}	2.03^{abcdefghi}	3.07^{cdefghi}
27.	Kalluvally 2	1.16^{abcdefg}	2.43^{abc}	2.77^{efghijkl}
28.	Karimthakara	0.96^{bcdefghij}	1.27^{ijklm}	2.33^{ijkl}
29.	Karimunda 7	1.03^{cdefghij}	1.97^{abcdefg}	2.87^{cdefghi}
30.	Karimundachittarikkal	0.90^{bcdefghij}	2.13^{abcdefghij}	3.13^{cdefghi}
31.	Karimundakuttiatoor	0.96^{bcdefghi}	2.00^{abcdefghijk}	3.10^{efghijkl}
32.	Kiriyath	0.93^{cdefghij}	1.63^{defghijkl}	2.53^{ghijkl}
33.	Kottanadan 2	1.00^{bcdefghij}	1.97^{abcdefghijk}	2.87^{efghijkl}
34.	Kottaram	0.80^{defghijk}	1.73^{bcdefghijkl}	4.07^{bc}
35.	Kulathurpuzha	0.66^{ghijk}	1.40^{ghijklm}	2.33^{ijkl}
36.	Kurachimundi	1.03^{bcdefghi}	1.70^{bcdefghijkl}	2.50^{ghijkl}
37.	Mundi	0.67^{ghijk}	1.40^{ghijklm}	3.73^{bcde}
38.	Neelgiri	0.75^{efghijk}	1.70^{bcdefghijkl}	3.00^{defghijk}
39.	Ottamundi	1.00^{bcdefghij}	2.67^{a}	3.60^{cdef}
40.	Ottanadan	1.03^{bcdefghi}	2.30^{abcdef}	6.50^{a}
41.	P-24	1.25^{abcde}	2.15^{abcdefg}	2.75^{efghijkl}
42.	Padarppan	0.73^{fghijk}	1.43^{ghijklm}	2.93^{defghijk}
43.	Palulutta	1.03^{bcdefghi}	1.80^{bcdefghijkl}	3.40^{cdefgh}
44.	Poonjarmunda 1	1.10^{bcdefghi}	2.17^{abcdefg}	2.93^{defghijk}
45.	TMB 2	1.00^{bcdefghij}	1.93^{bcdefghijk}	2.93^{defghijk}
46.	TMB10	1.00^{bcdefghij}	1.87^{abcdefghijk}	2.93^{defghijk}
47.	Vattamundi	1.13^{abcdefgh}	2.07^{abcdefgh}	3.00^{defghijk}
48.	Vellanamban 1	0.50^{ijk}	1.07^{lm}	1.93^{kl}
49.	Veluthanamban 5	0.70^{fghijk}	1.33^{hijklm}	2.67^{fghijkl}
50.	Vijay	1.13^{abcdefgh}	1.97^{abcdefghijk}	3.70^{bcde}
	CV (%)	26.28	20.56	20.14

Table 2: Grouping of black pepper genotypes based on lesion development after 48, 72 and 96 hours of inoculation of *P. capsici*

Class based on lesion diameter (cm)	Diameters of disease incidence (cm)	Percentage of plants		
		48 h	72 h	96 h
1	<0.50	2	0	0
2	0.50 – 1.00	58	2	0
3	1.00 – 1.50	38	18	0
4	1.50 – 2.00	2	44	8
5	>2.00	0	36	92

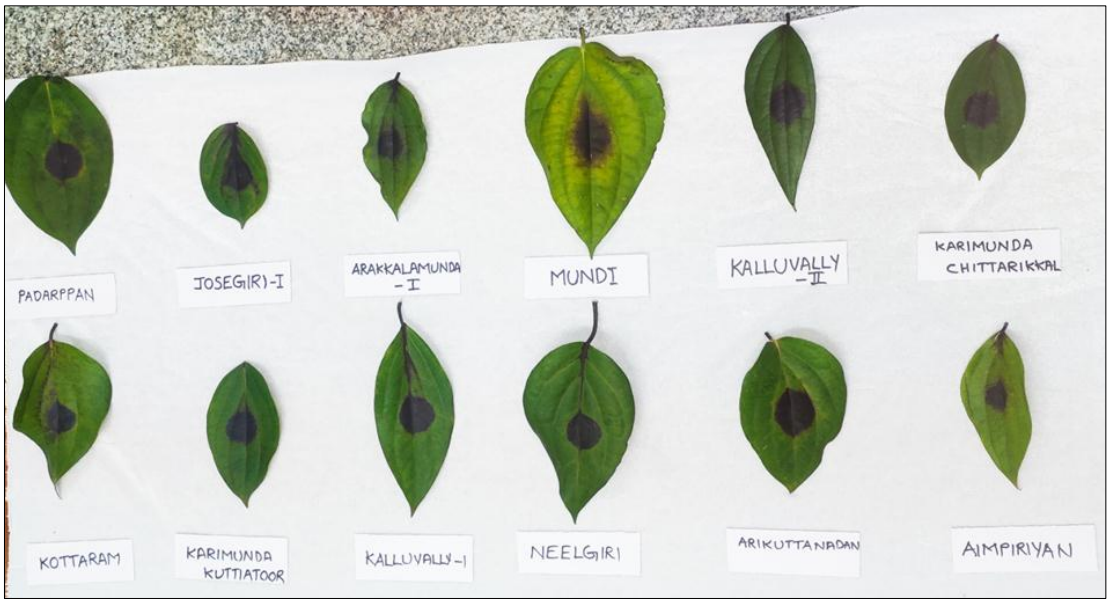

Plate 1: Lesion development in leaves after 96 hours of inoculation with culture disc of *P. capsici*– Set 1

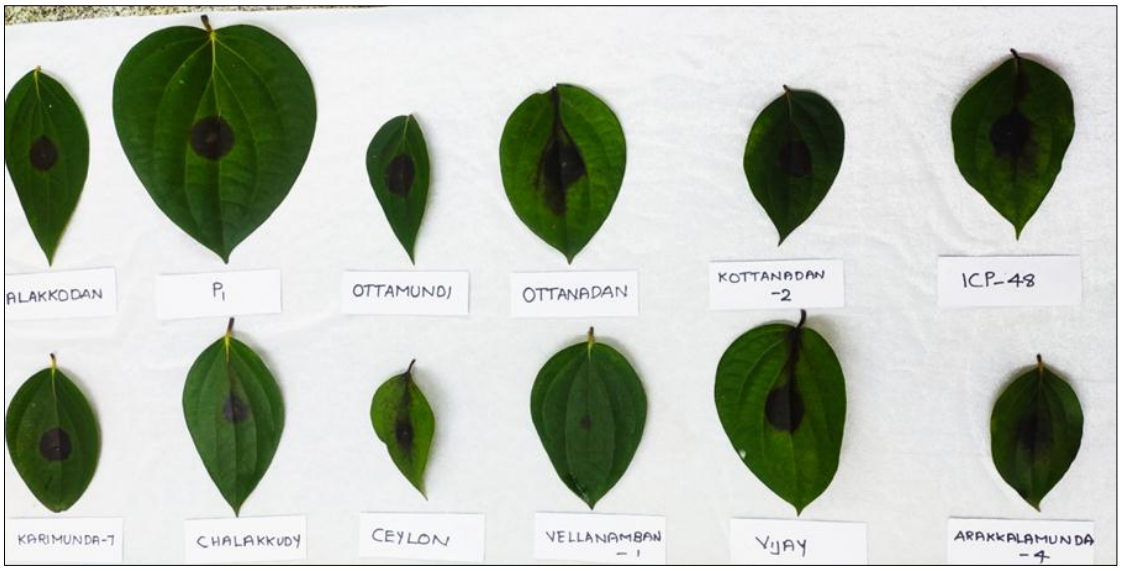

Plate 2: Lesion development in leaves after 96 hours of inoculation with culture disc of *P. capsici* – Set 2

Plate 3: Lesion development in leaves after 96 hours of inoculation with culture disc of *P. capsici*– Set 3

Plate 4: Lesion development in leaves after 96 hours of inoculation with culture disc of *P. capsici*– Set 4

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