



RESEARCH ARTICLE

**SCREENING OF *PIPER NIGRUM* L. VARIETIES/CULTIVARS AGAINST QUICK WILT CAUSED BY
PHYTOPHTHORA CAPSICI LEON. UNDER GREEN HOUSE CONDITION**

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ARTICLE INFO

Article History:

Received 7th, October, 2014

Received in revised form 16th, October, 2014

Accepted 10th, November, 2014

Published online 28th, November, 2014

Key words:

Quick Wilt, Black Pepper (*Piper nigrum* L.),
Phytophthora capsici Leon., Screening.

ABSTRACT

The fungal pathogen *Phytophthora capsici* Leon. is highly responsible for severe economic losses in Kodagu district of Karnataka, India due to 'Quick Wilt' disease of Black pepper (*Piper nigrum* L.). In this study nine varieties/cultivars of black pepper mainly cultivated in Kodagu region have been used for screening for disease severity to the pathogen under green house conditions. The cuttings of black pepper were grown in nursery bags and the zoospores of the pathogen were inoculated to the soil for each test plant. Among the tested varieties/cultivars Panniyur-5, Aribally and Karimunda were resistant (0%) to the pathogen, whereas Panniyur-1, Panniyur-3, Panniyur-4, Panniyur-6 and Panniyur-7 were highly susceptible showing 98.3% and 100% disease severity (SD) respectively. Among all these only Panniyur-2 showed tolerance to the pathogen with only 29.16% of SD even after the 50th day of inoculation.

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INTRODUCTION

The 'King of Spices'- Black pepper (*Piper nigrum* L.) is one of the economically important spice crops in India since ancient times. Black pepper is cultivated on a large scale in Kerala, Karnataka and Tamil Nadu and to a limited extent in Maharashtra, North Eastern states and Andaman and Nicobar Islands. Kerala and Karnataka account for 92% of black pepper production in the country (Ravindra, H *et al.*, 2014). Kodagu is one of the major Black pepper growing regions of Karnataka. Varieties/cultivars such as Panniyur-1, Panniyur-2, Panniyur-3, Panniyur-4, Panniyur-5, Panniyur-6, Panniyur-7, Aribally and Karimunda are commonly grown in Kodagu. Quick wilt caused by *Phytophthora capsici* Leon. is a major disease affecting Black pepper in Kodagu. *Phytophthora capsici* Leon. is a soil borne pathogen (Noveriza, R. and Quimio, T.H., 2004) and these mainly cause rots (root and crown rots) that affect belowground tissues and cause vascular wilts due to root infections (Koike, S.T. *et al.*, 2003) Soils that do not drain off water properly favors the survival and distribution of the pathogen and since Kodagu receives rainfall throughout June to November the proper drainage of water in the black pepper plantation cannot be achieved completely and this favors the development of the disease which incurs a heavy economic loss to the farmers. Hence, it is necessary to screen for Varieties/ cultivars resistant or tolerant to *Phytophthora capsici* propagules that persist in soil.

MATERIALS AND METHODS

Isolation of the pathogen

P. capsici was isolated from the soil of diseased vine collected from a plantation in Kanthoor village, Madikeri taluk of Kodagu district, Karnataka as per the method of Din *et al.*, (2012) and further cultured on Carrot Agar Medium for

multiplication at 27-28^oC (Devi and Smitha, 2013). The pathogen was identified and confirmed based on morphological and cultural studies. The pathogenicity of *P. capsici* was tested according to the method of Manohara (2002).

Collection of Black pepper for screening

Nine varieties/cultivars of Black pepper mainly grown in Kodagu were selected for screening.

The black pepper cuttings of 2-3 nodal stage collected were transferred to polythene bags of 16x25cm filled with unsterilized field soil (pH 6.40), Compost soil (pH 7.05) and sand in the ratio 1:1:1 on the last week of August, 2014 and maintained under green house. The cuttings were watered daily until new shoots and leaves developed. The field soil was collected from the Black pepper growing coffee estate of Ponnampet, Kodagu. The compost was prepared from all vegetable and plant waste.

Screening of Black pepper cuttings

The black pepper cuttings were screened by soil inoculation technique. For this *P. capsici* culture grown on Carrot Agar Media were cut into pieces and flooded with sterile distilled water and kept for incubation for 72 hour at 24^oC. Then they were placed at 5^oC for 1 hour and then incubated at 24^oC for 30-60 mins. (Larkin *et al.*, 1995). The suspension was then filtered through 2 layers of muslin cloth to remove hyphal and sporangial debris. The zoospore suspension containing approximately 10⁵ propagules /ml (Anith *et al.*, 2002) was counted using haemocytometer and then prepared in 100ml of sterile distilled water and then added to 10cm holes made in the soil containing the rooted black pepper vines. For each variety/cultivar 3 replicates were maintained along with one control for each. The experiment was carried out in green

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Table 1 Black pepper varieties/cultivars used for screening

Varieties/Cultivars	Place of collection
Aribally	Plantation Nursery, Suntikoppa, Kodagu
Panniyur-3, Panniyur-4, Panniyur-5 and Panniyur-7	Kerala Agricultural University
Panniyur-1 and Karimunda	Indian Institute of Spice Research, Khozikode, Kerala

house (25°C temp.). The vines were irrigated daily 2 times with tap water. The development of symptoms and wilting of cuttings were noted after regular checks.

Testing the disease severity of Black pepper cultivars/varieties against *P. capsici*

Each variety/cultivar of black pepper were checked for disease severity every week for 30 days on a scale from 0-4, where a score of 0 was given to healthy plants (0-24%), 1 was given to plants with only their leaves wilting (25-49%), 2 was given to plants which showed severe wilting symptoms (50-74%), 3 was given to plants with less wilting, but severely dry leaves (75-90%) and 4 was given to dead plants (91-100%).(Jimenez *et al.*, 2012).

The percentage of disease severity for each variety/cultivar was calculated using the formula as described by Mbayi *et al.*, (2014). Disease severity (SD) was evaluated using the formula

$$SD \% = \frac{\sum(\text{number of diseased plants in a rating category} \times \text{that rating category})}{(\text{total number of plants} \times \text{category maximum score})} \times 100$$

RESULTS

The varieties/cultivars of Black pepper used for screening showed differences in tolerance or resistance to *P. capsici*. When the susceptible vines were removed from the soil and washed with sterile distilled water rotting of the collar region of the stem was observed, this resulted in the wilting of the vines. The variety/cultivar Panniyur-5(P5), Aribally (AB) and Karimunda (KM) did not show any symptom (0%) and remained healthy even until the 50th day after inoculation with the zoospores of *P. capsici*. The vines remained to grow healthy without any future signs for disease development.

The variety/cultivar Panniyur-3(P3) and Panniyur-4(P4) died completely (100%) after 40 days of *P. capsici* zoospore inoculation. Whereas Panniyur-6 (P6) and Panniyur-7 (P7) died completely after 50 days of inoculation. Panniyur-1(P1) showed 98.3% mortality after 50 days of inoculation. This shows that P1, P3, P4, P6 and P7 are highly susceptible(HS) to the pathogen *P. capsici* Leon. Among all these varieties/cultivars Panniyur-2(P2) showed 29.16% of infection even after 50 days of inoculation. This suggests that P2 is a tolerant variety when compared to rest of the variety/cultivar of *P. nigrum* L. vines used for screening.

Table 2 Percentage of disease severity (SD %) determined for the different Varieties/Cultivars of *P. nigrum* L. that were artificially inoculated with *P. capsici* Leon. and maintained under green house condition.

Varieties/ Cultivars	% of disease severity days after inoculation										DiseaseResponse	
	0	5	10	15	20	25	30	35	40	45		50
Panniyur-1	0	0	0	0	0	53.3	67.5	72.5	75	87.5	98.3	HS
Panniyur-2	0	0	0	0	8.3	15.83	20	25	28.3	29.16	29.16	T
Panniyur-3	0	0	0	0	45	65.83	81.6	93.3	100	100	100	HS
Panniyur-4	0	0	0	0	50.83	70	80.83	94.16	100	100	100	HS
Panniyur-5	0	0	0	0	0	0	0	0	0	0	0	R
Panniyur-6	0	0	0	0	1.66	36.66	65	85	97.5	98.3	100	HS
Panniyur-7	0	0	0	0	57.5	69.16	85	93.3	97.5	98.3	100	HS
Aribally	0	0	0	0	0	0	0	0	0	0	0	R
Karimunda	0	0	0	0	0	0	0	0	0	0	0	R

(HS) highly susceptible; (R) resistant; (T) tolerant

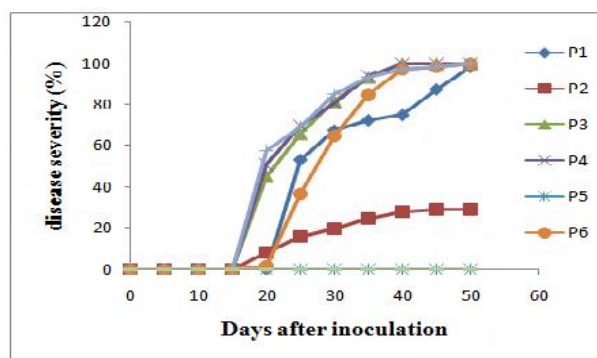


Figure 1 Disease severity (%) incited by *Phytophthora capsici* Leon. in nine different varieties/cultivars of *Piper nigrum* L. the severity of the disease can be observed from 20th to 50th day. Disease severity was scored as 100% for dead plants. Values are the mean of 10 replicates each at 0 to 50 days after inoculation with *P. capsici* Leon.

DISCUSSION

In this study, the difference in disease severity of 9 different varieties/cultivars of *Piper nigrum* L. to the disease ‘Quick Wilt’ caused by *Phytophthora capsici* Leon. have been investigated based on green house studies. 3 of the *P. nigrum* varieties/cultivars Panniyur-5, Aribally and Karimunda were found to be resistant to the disease, whereas Panniyur-1, Panniyur-3, Panniyur-4, Panniyur-6, and Panniyur-7, were highly susceptible and succumbed to the disease.

Mammooty *et al.*, (2008) showed that the Black pepper genotypes Karimunda I, Karimunda II, Panniyur-1 Panniyur-2, Panniyur-3, Panniyur-4, Panniyur-5, Panniyur-6 and Panniyur-7 showed 89.3%, 90%, 88.7%, 68%, 90%, 95.3%, 55%, 90.7% and 85.3% of leaf infection respectively and 73.7%, 62.2%, 67.2%, 67.8%, 67.3%, 73.7%, 31.2%, 60% and 59.3% mortality respectively by inoculation of zoospore and culture discs of *P. capsici* on leaves, stems and roots. Detached leaf pin prick in vitro tests by Archana and Rajan, (2013) revealed that Karimunda showed maximum infection after 48 hours, whereas Panniyur-1, Panniyur-2, and Panniyur-7, showed least infection. Stem inoculation with an inoculum disc of the pathogen at the 3rd internode showed that Karimunda was resistant to *P. capsici* (Bhai, *et al.*, 2007).

Since the pathogen *P. capsici* is mainly soil borne in this study we have adopted the soil inoculation technique with the zoospore suspension of the pathogen and the disease severity have been noted, which showed differences in susceptibility of the vines to the pathogen when compared to the above studies.

These varieties/cultivars have been assayed for disease susceptibility to the pathogen under green house and the experiments have to be carried out in the field.

Acknowledgement

The author is greatly thankful to University of Mysore, Mysore for sanctioning UGC NON-NET fellowship.

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