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Study on *Phytophthora capsici* Cause of Foot Rot Disease on Black Pepper *(Piper nigrum. L.)*, Tepi, Ethiopia

Habetewold kifelew[1], Girma Adugna (PhD)[2]

[1]*Tepi National Spices Research Center, Ethiopia*

[2]*Jimma University, Ethiopia*

Corresponding Author:** ***Habetewold kifelew, *Tepi National Spices Research Center, Ethiopia*

Abstract: *Although foot rot disease of black pepper is becoming a serious threat in cultivation of the crop, there is no research work have been done on the pathogen; The aim of this study was to assess and detect foot rot syndrome of black pepper and to isolate and identify its causal pathogen thereby determine the prevalence and Etiology of the disease. A total of 36 fields in two regions and three zones were surveyed. The average maximum leaf infection index (LII) 16.7% was recorded in Benchmaji zone while least LII 9.2% was observed in Mejenger zone. Among the different places surveyed, maximum LII 18.24% was recorded at Benchmaji zone while the minimum (6.7%) LII was recorded from Sheka zone estate-farms. Maximum 26.87% foliar yellowing index (FYI) was observed at Sheka zone and least 21.2% FYI at Benchmaji zone of SNNPRS. The mean maximum Leaf defoliation index (LDI) 17.9%, collar infection index (CII) 18.9% and vine wilt index (VWI) 21.4% was recorded in Mejenger zone and mean minimum LDI (10.9%), CII (5.5%) and VWI (14.5%) were recorded in Sheka zone. Rainfall, number of rainy days and relative humidity, had a positive significant correlation with the disease severity whereas; temperature and sunshine hours had a significant negative correlation to disease development under natural condition. From the total of 167 fungal isolates, Phytophthora species were the most abundant 107 isolates followed by Fusarium spp. and Phythium spp. The growth patterns of the isolates were cottony, rosaceous and stellet, with the occurrence of 50, 37, and 13% respectively. Faster colony growth was found on Potato dextrose agar followed by Tomato Agar and Carrot Agar. The length breadth ratio of sporangia was ranged from 1.2 to 2.0 µm. According to the current investigation all P. capsici isolates included in the test were pathogenic on black pepper. Pathogen of foot rot of black pepper was characterized as P. capsici on the basis of Etiology and by establishing its pathogenicity on black pepper rooted cuttings.*

Keywords: *Foot rot, P.capsici, Black pepper, Severity, and Incidence.*

1. INTRODUCTION

Black pepper (*Piper nigrum* L.) is an economically important spice crop that yields the 'black pepper' of commerce, which is profusely used as a culinary spice and condiment all over the world. Ethiopia has a long history of using and trading of black pepper since Aksumite kingdom. Spices are among the very few pioneer commodities traded internationally. "Berebere" is the general term for pepper in "Amharic", which includes black pepper, long pepper and capsicum. "kundo berbere", we would call this black pepper in Amharic, it is in the pepper species *Piper nigrum*, the improved cultivar was introduced to Ethiopia in between 1979 to 1980 from Brazil, Sirilanka, Panniyar, Kuching, and Costarica (Habetewold *et.al*, 2017). Production and productivities of black pepper increase ever since in Ethiopia. However, A new devastative disease were observed in the research center black pepper nursery in 2011, and the causal agent were found *Phytophthora* spp, subsequently a survey is conducted to see the distribution and the extent of damage on major producing areas of Ethiopia.

In the last decade the number of newly described *Phytophthora* spp. has more than doubled the number of known *Phytophthora* spp. (Brasier, 2008), but of these only two species descriptions originated from Africa (Maseko *et al*., 2007). Twenty species of *Phytophthora* reported from Africa, 18 species have been reported from South Africa and only two species are known from the rest of Africa. *P. megakarya* and *P. palmivora*, these two species causes a serious disease of Cacao (*Theobroma cacao*) in many cacao producing countries of African. The species occurring in The African continent includes *P. cactorum* (Boughalleb *et al*., 2006), *P. cinnamomi* (Huguenin et al.,

1975), *P. infestans* (Olanya *et al*., 2001) and *P. nicotianae* (Allagui and Tello-Marquina, 1996). In Ethiopia there are only two species of *Phytophthora* reported which is the notorious *P. infestans* on Potato (Bekele and Yaynu, 1996) and *P.colocasiae on Colocasia esculenta* (taro) (CABI/EPPO, 2014).

Foot rot diseases of black pepper referred to the pathogen *Phytophthora capsici* in many part of the world, different *Phytophthora* species have been attributed to be the causal agents of the disease; species *P. palmivora* var. *piperis* is the true causal agent for the disease in Java (Muller, 1937), later foot rot pathogen of pepper vines was attributed to *P. palmivora* in Malaysia (Holliday and Mowat, 1963). After a decade Zentmyer *et al*. (1977) reclassified the pathogen based on sporangial characteristics of isolates from Malaysia and Thailand as *P. palmivora* MF4 group. However, *P. capsici* of black pepper shows considerable variation in its morphology and ontogeny of sporangiophore (Sarma and Anandaraj, 1988). The aim of the study was to detect *Phytophthora* foot rot syndrome of black pepper and to isolate and identify causal pathogen thereby determine their prevalence.

2. MATERIALS AND METHODS

2.1. Descriptions of the Study Areas

The study was conducted at Jimma Agricultural Research Center (JARC) pathology laboratory, It is located 363 km away from Addis Ababa, at 7 0 46'N and 36 o 0'E. Survey was conducted at Sheka, Benchmaji and Majang zones of Ethiopia

2.2. Survey and Sampling

A survey was conducted from August 2011 to October 2011 in south west Ethiopia. Purposive stratified random sampling technique was employed in the study. From each field a single stand was sampled randomly by using "W" path. A total of 36 fields in two regions and three zones were surveyed. Scales were used to score severity for the parameter like percentage leaf infection (LII) 0-10 (Irfan *et al*., 2012), foliar yellowing (FYI), defoliation (DI) 0-3, wilting of vines (VWI) and collar infection (CII) 0-4 (Shahsidhara, 2007). The samples from root, collar, leaves and vine of infected black pepper stand and from the soil under symptomatic vine were collected for isolation of the causal agents.

2.3. Isolation and identification

Isolation of the pathogen from plant material was done by obtaining the crown tissue just below soil level, roots, leaf and vines of diseased black pepper. Isolation from soil underneath infected black pepper vines were done following castor seed baiting technique (Shashidhara, 2007). The semi selective medium for isolation of *Phytophthora capsici* was prepared as described by Zhijin *et al*., (2007) and Erwin *et al.*, (1987). For mass multiplication and sporangia production we were use carrot agar (Leslie and Summerell, 2006).

2.3.1. Cultural and Morphological Examination

Colony shape was determined after six days of growth in the dark at 25^0C on potato dextrose agar (PDA), carrot agar (CA) and tomato agar (TA). The plates were visually examined for colony texture and appearance. Morphological characteristics of the asexual structure assessed included sporangia morphology (shape, size and length–breadth ratio); papillation; papillate (apical thickening ≥ 4 µm), semi-papillate (Apical thickening < 4 µm), and non-papillate (Apical thickening not apparent); caducity (caduceus and non-caducity). Twenty arbitrarily chosen sporangia were examined and measured for each isolate using an ocular micrometer (Islam *et al*., 2004).

2.3.2. Pathogenicity Study

Pathogenicity of the isolates was tested on healthy young black pepper vines grown in Sterilized sandy soil. 10^5sporangia/ml for leaf inoculation and 2 ml, each containing 10^5 sporangia/ml for root inoculations were used (Shashidhara, 2007). Ten seedlings were prepared for root and leaf inoculation, the control plot was inoculated with sterilized distil water. Pots were maintained at 25~30^0C in humid growth chamber. Experimental plants were flooded and drained at daily intervals. Vines were observed regularly for the development of disease symptoms including wilting, defoliation and mortality (Truong *et al*., 2008).

3. RESULT AND DISCUSSIONS

3.1. Disease Incidence and Severity

The average maximum LII (16.7%) was recorded in Benchmaji zone while the least (9.2%) was observed in Mejenger zone. Among the different location surveyed, Benchmaji and Sheka zones estate-farms were indicated maximum (18.24%) and minimum (6.7%) LII. The difference in leaf infection severity may be due to high rainfall of the area, since rains splashing that favor disease spreading from the lower leaf to the upper as well as from the soil to the lower leaf (Ramachandran *et al*., 1988).

Maximum (26.87%) FYI was observed at Sheka zone, while the least (21.2%) was recorded at Benchmaji zone. During the survey it was observed in Sheka zone of most investor field Gravilia **(***Grevillea dielsiana)* support were used with no shade management practice where as farmers and estate farms of Sheka zone used Koarch (*Erythrina brucei*) support with good shade management practice. In the former case there is high leaf infection and foliar yellowing. This might be because of the conduciveness of microclimatic conditions that prevail under the unmanaged canopy. Since it create high humidity, low temperature and low sunshine expose that favor disease development under natural condition Anandaraj *et al*., (1994)

The mean maximum LDI (17.9%), CII (18.9%) and VWI (21.4%) was recorded in Mejenger zone where as mean minimum LDI (10.9%), CII (5.5%) and VWI (14.5%) were recorded in Sheka zone (table 1). The highest CII (35.8%) was recorded in Mejenger zone of Gambella region where some fields were abandoned. The disease was found widely distributed in black pepper plantation and all the commercial black pepper cultivars such as (Gacheb and Tato) were susceptible to the disease

Table1. *Severity of foot rot of black pepper during main rainy season (July to September) 2011*

Zones	Wereda	Farm type	LII (%)	FYI (%)	LDI (%)	CII (%)	VWI (%)
	Yeki and Andracha	Farmers field	10.7(5.911.2)	28.85(24.2-32.5)	7.2(0.0-20.0)	3.3(0.0-10.0)	17.3(0.0-34.5)
		Investor field	11.1(6.4-15.6)	23.43(22.5-25.0)	14.4(10.0-16.7)	5(0.0-10.0)	17.5(12.5-23.0)
Sheka		Estate farm	6.7(4.8-8.2)	28.33(21.7-37.2)	11.1(0.0-20.0)	8.3(5.0-12.5)	8.7(3.5-12.5)
		mean	9.5	26.87	10.9	5.53	14.5
	Bench and Guraferda	Farmers field	18.24(13.4-23.8)	32.8(26.7-40.5)	22.8(13.3-30.0)	7.5(0.0-17.5)	23.3(7.0-42.0)
		Investor field	15.45(10.6-18.8)	25.28(20.0-28.9)	17.8(3.3-33.3)	10(0.0-17.5)	19.0(15.0-24.0)
Benchmaji		Estate farm	16.33(12.3-20.0)	23.43(20.3-27.8)	11.1(3.3-20.0)	5.8(0.0-12.5)	17.2(11.5-23.5)
		Mean	16.7	21.2	17.2	7.8	19.8
	Godere	Farmers field	9.5(7.7-12.2)	31.34(22.5-39.7)	18.3(3.3-30.0)	13.3(0.0-30.0)	29.6(17.0-38.5)
Mejenger		Investors field	8.8(7.2-11)	21.20(16.1-26.4)	16.7(13.3-23.3)	37.5(17.5-60.0)	15.3(12.5-18.0)
		Estate farm	9.4(7.6-13.1)	26.39(21.1-29.4)	18.9(13.3-23.0)	5.8(0.0-10.0)	19.2(13.0-24.5)
		mean	9.2	26.3	17.9	18.9	21.4

3.2. Identification

3.2.1. Symptomatology

Leaf infection showed symptoms of gray centers surrounded by alternate dark and light brown zones with water soaked margins (Fig.1.a). The infected vine produced dark brown lesion on the lateral

branch and progress towards the main vine. Finally the vine was rot, wilted and defoliated severely (Fig.1.B & C).

(A), Infection on leaf (B), leaf defoliation (C), wilted vine

Fig1. *Symptom of foot rot diseases of black pepper*

3.2.2. Laboratory Diagnosis

A total of 167 fungal isolates were obtained from diseased root, root-zone soil, leaf and vine (Table 2). *Phytophthora* species were the most abundant (107 isolates) followed by *Fusarium* spp. and *Phythium spp*. Two species of *Phytophthora* i.e. *P. capsici* and *P. cinamomi* were identified based on morphological characteristics. Of the 107 isolates, 96 *P. capsici* isolates were obtained from diseased roots, vines, leaf and root-zone soil, while 11 isolates of *P. cinnamomi* were isolated from diseased roots and root-zone soil. All 96 *P. capsici* isolates were examined for morphological characteristics, but nine isolates were selected arbitrarily for specific measurements.

Table2. *Key fungal species isolated from diseased specimens and root-zone soil of black pepper and their isolation frequency*

Sn.	Species	Number of isolate				
		Leaf	Vine	Root	Soil	Total
1	*Phytophthora capsici*	14	15	19	48	96
2	*P. cinnamomi*	0	0	4	7	11
3	*Pythium species*	0	0	6	11	17
5	*Fusarium species*	0	0	9	17	26
6	*Rhizoctonia species*	0	0	3	6	9
7	*Diplodia species*	0	0	3	5	8
8	Total	14	15	44	94	167

Phytophthora capsici was recovered at high frequencies and this species was subsequently found to be pathogenic to black pepper, causing typical symptoms of foot rot at any growing stage. Although *P. cinnamomi*, were occasionally found to be associated with diseased root and soil, they were not shown to be pathogenic. This result is further supported by other earlier reports of *P. capsici* as the only pathogen of black pepper foot rot (Truong *et al*. 2002). *P. capsici* isolates were recognized as being pathogenic to black pepper (Holliday and Mowat 1963; Alconero *et al*. 1972).

Phytophthora species was determined as the pathogen of black pepper foot rot based on pathogenicity test with five fungal species recovered from diseased pepper vines which include; *Phytophthora* spp., *Fusarium solani*, *Diplodia* spp., *Pythium complectens* and *Rhizoctonia solani* (Long, 1991). *Pythium* spp. and *Fusarium* spp. have been isolated from black pepper tissue and root-zone soil associated with foot rot; however, they are not pathogenic to black pepper (Truong *et al*, 2008). More than 30 different fungal species, including *F. oxysporum*, *F. solani*, and *Pythium splendens*, have been frequently isolated from foot rot of black pepper (Watanabe *et al*. 1996); however, according to Truong et al., (2008) these fungal species did not produce any symptoms when inoculated artificially onto healthy plants.

3.2.3. Cultural Characteristics

The colony color were cottony white of PDA and TA where as white at the top and creamy yellow at the bottom on CA. the growth patterns of the isolates were cottony, rosaceous and stellet, with the occurrence of 50, 37, and 13% respectively.

Colony growth rate (mm/day) of *Phytophthora capsici* isolate was found significantly different on different medium (Table, 3). faster growth of *P. capsici* was obtained on PDA followed by TA and CA. Average daily growth of the tested isolates ranged between 7.6 to 10.1, 4.3 to 6.9 and 5.1 to 6.2mm/day on PDA, CA and TA respectively. All isolates were found significantly different $p < 0.05$ when they grow on three types of cultivation media. According to Islam *et al.*, (2005) *P. capsici* isolate obtained from processing pumpkin in Illinois differ in their growth rate and growth temperature on PDA. The difference in growth rate may be attributed to *P. capsici* strain diversity and aggressiveness of the isolate as well as difference in the medium.

Table3. *Colony characteristics of P. capsici isolate obtaining from black pepper growing area of Ethiopia*

Sr.no.	Isolate	Growth pattern	Colony growth (mm/day) $25^{O}C$		
			PDA	CA	TA
1	SFKoL	Rosacious	7.8^{BC}	4.7^{CD}	5.3^{BC}
2	SIBeV	Rosacious	10.1^{A}	4.3^{D}	6.2^{A}
3	SGShoL	Rosacious	7.6^{C}	4.8^{CD}	6.2^{A}
4	BFGe2R	Cottony	8.3^{BC}	5.1^{BCD}	6.0^{AB}
5	BISheS	Cottony	8.1^{BC}	4.4^{D}	5.8^{ABC}
6	BGBeb1R	Cottony	9.4^{ABC}	5.8^{ABC}	6.2^{A}
7	MGKa3V	Stellate	9.6^{AB}	5.8^{ABC}	5.3^{BC}
8	MFMe2L	Stellate	8.7^{ABC}	6.9^{A}	6.2^{A}
9	MIKa3V	Stellate	8.5^{ABC}	6.2^{AB}	5.1^{C}
	CV		12.0	12.0	7.9

Means with the same letter are not significantly different at LSD test P<0.05

3.2.4. Sporangial Morphology

The sporangia of nine isolates were found ellipsoid to ovoid in shape with light brown color; most of them produce papillate, deciduous sporangia on long pedicels. The mean length of sporangia among the isolates ranged from 37.6 to 46.3 µm, mean breadth of sporangia ranged from 18.8 to 33.7 µm, the length breadth ratio of sporangia ranged from 1.2 to 2.0 µm and mean pedicel length of sporangia ranged from 60.1 to 119.5 µm among the isolates.

All of the *Phytophthora* isolates identified as *P. capsici* had umbellate sporangial ontogeny with long caducous sporangial (up to 207 µm), broad sporangia, L/B ratio of more than 1.2, predominantly round sporangial bases and no chlamydospores production as well as being pathogenic to black pepper which resembled that of *P. capsici* with the morphological characteristics described by Aragaki and Uchida (2001).

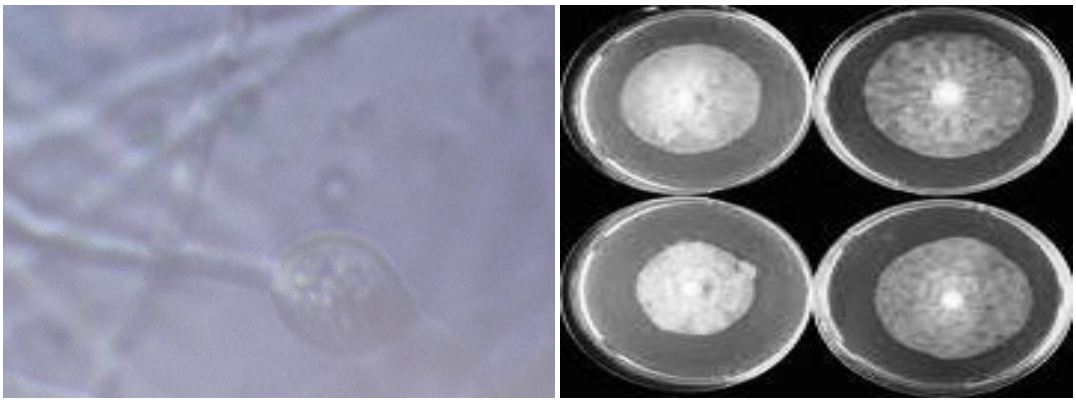


Fig1. (*A), lemon shaped sporangia, (B) colony on PDA*

Table4. *Morphological characteristics of isolates of P.capsici*

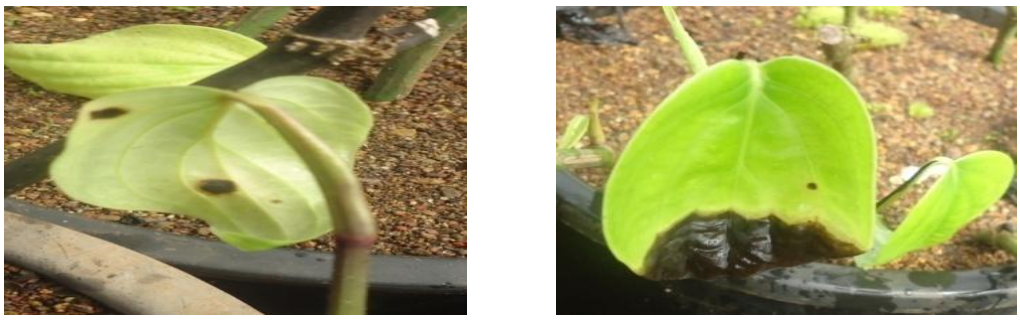
Sn.	Isolate	Sporangium		L/B ratio	Pedicel Length
		Length (µm)	Width (µm)		
1	SFKoL	37.6 (25.0-50.0)	22.7(15.0-30.0)	1.7 (1.6-1.7)	87.5 (25.0-150.0)
2	SIBeV	37.6 (20.0-55.0)	28.8 (15.0-42.5)	1.3 (1.2-1.3)	60.1 (21.0-99.0)
3	SGShoL	38 (20.0-56.0)	27.6 (15.0-40.0)	1.4 (1.1-1.3)	76.6 (24.0-129.0)

4	BFGe2R	38.7 (17.5-60.0)	18.8(17.5-20.0)	2.0 (1.0-3.0)	111.1 (36.0-186.0)
5	BISheS	43.5 (30.0-57)	21.3 (15.0-27.5)	2.0 (2.0-2.1)	88.96 (21.0-157.0)
6	BGBeb1R	46.3 (35.0-57.5)	23.2 (16.0-30.0)	2.0 (2.0-2.9)	108.5 (22.0-195.0)
7	MGKa3V	41 (27.0-55.0)	22.6 (15.0-30.0)	1.8 (1.8-2.0)	109.8 (26.0-192.0)
8	MFMe2L	41.2 (22.3-60.0)	33.7 (17.5-50.0)	1.2 (1.2-1.3)	113 (23.0-203.0)
9	MIKa3V	44.9 (31.0-59.0)	27.6 (15.0-40.0)	1.7 (1.4-2.0)	119.5 (32.0-207.0)

Range of sporangium length, width, L/R ratio and Pedicel length in parenthesis

3.3. Pathogenicity

All *P. capsici* isolates included in the test were pathogenic on black pepper. *P. capsici* was re-isolated from every vine that showed wilt symptoms. In contrast, *P.cinnamomi* was not re-isolated from the dead vine. Hence, Koch's Postulates for pathogenicity on black pepper were only demonstrated for *P. capsici*.



(A), symptom on back side of the leaf (B), symptom on front side of the leaf

Fig4. *Symptoms of foot rot diseases during pathogenecity test*

4. Conclusion and Recommendation

The study confirms that the causal agent of foot rot diseases of black pepper is *P. capsici*. It also gives good understanding and managing *Phytophthora* foot rot of black pepper in Ethiopia. Even if the pathogen is identified there is still little known about the disease cycle, genetic diversity and epidemiology. Studies are needed to test and evaluate different disease management practices.

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