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Research Article

Characterization of *Phytophthora capsici* Foot Rot Disease of Black Pepper in Ethiopia

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ABSTRACT

Ethiopia is one of the largest producers of black pepper (*Piper nigrum* L.) in Africa, but the production of this crop is in the decline, because of the foot rot disease caused by *Phytophthora capsici*. The objective of this study was to determine the morphological characteristics of the *Phytophthora capsici* the causal agent of foot rot disease of black pepper in southwestern Ethiopia. Thirteen major black pepper growing areas were surveyed and confirmed for the incidence of foot rot disease. Gizmeret farmers field had the highest disease incidence (75%) followed by Bebeka coffee state farm (70%) and the lowest incidence at Shosha (5%). The highest disease severity was at Gizmeret (70%) followed by Bebeka (62%) and the lowest at Shosha (4%). Based on morphological characterization, the foot rot pathogen exhibited globose oogonia with paragynous antheridia, chlamydospore, torulose hyphae and lemon shaped sporangia with long pedicel confirmed that the causal agent of foot rot disease of black pepper in southwestern Ethiopia was *Phytophthora capsici*.

Keywords: Foot rot disease; Morphological characterization; Disease incidence; *Phytophthora capsici*

INTRODUCTION

Black pepper (*Piper nigrum* L.), the "king of spices" is a traditional and historic spice crop, which has been used as spice since 4th century B.C. It was first brought into Ethiopia between 1979 and 1980 from potential producing countries [1]. Black pepper is a wood climber and is mainly produced in India, Brazil, Malaysia, Thailand, Sri Lanka and Vietnam. In Ethiopia Black pepper was widely cultivated in southwestern parts of the country mainly, Tepi, Bebeka, Kabo, and Gemadiro on small and large scale private farms. It is high value spice crop, since it earns significant foreign exchange for a country [2]. For instance, in 2012 around 45,000 kg of dry black pepper was exported from Ethiopia (Bebeka Horizon Plantation PLC report un published data, 2012). The demand for black pepper and its product is increasing year by year in the world market, but production is limited to few countries. Recently, black pepper has performed well in the southwestern part of Ethiopia, particularly, at Tepi, Gamadro and Bebeka large scale private farms [1]. Due to its promising performance in these areas, large scale black pepper production and value addition are started. However, disease infestation in fields are the biggest problem in black pepper cultivation in major producing areas of southwestern Ethiopia.

Foot rot is one of destructive disease of black pepper in Ethiopia. Foot rot is a devastating disease that effected plants to survive for several years and death of plants occur slowly over a period of 4 years [3]. This disease is more severe in the fields if nematodes such as *Radopholus similis* and *Meloidogyne incognita* are present together with the causal fungus [4]. This disease has been reported all over the world where the black pepper is grown [5].

Phytophthora foot rot is a highly destructive disease and always leads to low productivity of black pepper production in Ethiopia. In Ethiopia, there are no convincing reports available on the effect of the disease in terms of economic loss or yield loss. However, the disease has been reported to affect up to 95% of the vines in individual farms in southwestern parts of the country i.e., Bebeka Horizon plantation PLC.

Accurate identification of a pathogen is necessary for the appropriate management of the disease [6]. Morphological characteristics are key for the identification and taxonomy of many fungal species such as *Fusarium* and *Phytophthora* [7]. The *Phytophthora* taxonomic system has been based on morphological characteristics of the globose oogonia with paragynous antheridia, chlamydospore, torulose hyphae and lemon shape of sporangia [8].

Therefore, a study on the disease incidence and morphology of foot rot disease in Ethiopia was valuable to gain new information used in the planning of disease management practice and guide to researchers in prioritization of research to manage this disease in southwestern parts of Ethiopia.

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MATERIALS AND METHODS

Study sites

Thirteen fields in five areas (Bebeka, Gizmeret, Gamadiro, Kabo and Shosha) of the southwestern Ethiopia were surveyed for foot rot disease incidence and severity between November 2017 and December 2018 (Table 1). Black pepper of Gacheb variety had been planted in most of the commercial plantations, small-scale farms and backyard orchards were evaluated.

Crop age ranged from 2 to 6 years. Thirteen points were assessed and at each location, one out of three sites was selected and the mean disease severity for the location was calculated.

Sampling and data collection

The methods of detection and delimiting surveys were used to determine the presence of foot rot disease in commercial and small scale in in southwestern Ethiopia. The status of foot rot disease present was assessed randomly, at least 10% of the black pepper plants at a site in the field by means of disease intensity, which was measured through observation of disease incidence and severity of overall field symptoms of foot rot disease. Disease severity was determined using descriptive type assessment key with a 0-4 score scale represents; 0=0%, 1=1-30%, 2=31-50%, 3=51-75%, 4=76-100% [9].

The incidence and severity of foot rot disease is also measured based on leaf defoliation symptom on black pepper plant. Through this method, 10 plants in every surveyed location were examined by random sampling. Disease severity was determined using descriptive type assessment key with a 0-4 score scale represented by 0=0%, 1=1-25%, 2=26-50%, 3 =51-75%, 4=76-100%. The score scales were then converted to disease severity index for non-parametric measurements [10] and expressed as percentages:

$$DSI = \frac{\sum(ab)}{N \times K} * 100$$

DSI = Disease Severity Index.

Σab = Sum of the product of assessed plants with their corresponding score scale.

N = Total number of assessed plants.

K = Highest score scale.

Isolation and morphological characterization of *Phytophtora capsici*

Phytophtora capsici was isolated from infected black pepper roots collected from black pepper fields in Bebeka, Gezmeret, Gamadiro, Kabo and Shosha. The root samples were placed in a beaker and washed gently using running tap water. Subsequently, the roots were cut into small sections of advancing root lesions (0.5-1.0 cm), followed by surface sterilization using 10% sodium hypochlorite for 30-60 sec. The roots were then rinsed in sterile distilled water in a beaker for 1 min. The roots were blotted to dry using sterile filter paper. The roots were then transferred to Rose Bengal Agar (RBA) plates. The Rose Bengal Agar plates were incubated in an incubator at 28 ± 2°C for 72 h of continuous darkness. *Phytophtora capsici* colonies appeared on the plates were isolated and sub-cultured in Potato Dextrose Agar (PDA) plates. Morphological characteristics observation such as mycelia structure and types of conidia produced were performed using compound microscope. Images were captured using camera.

Table 1: Locations covered during *Phytophthora* foot rot disease surveys in in Southwestern Ethiopia.

Location	Number of fields observed
Bebeka	4
Gezmeret	3
Gamadiro	2
Kabo	2
Shosha	2
Total	13

Data analysis

Disease incidence, severity data on foot rot and leaf defoliation data was randomly recorded in field. The recorded data was tested using analysis of variance (ANOVA) were arcsine transformed prior to analysis by descriptive statistics using SAS 9.3. Means were compared using Duncan"s Multiple Range Test [11].

RESULTS

Incidence and severity of foot rot disease of black pepper in Southwestern Ethiopia

Foot rot disease symptoms were observed in all of the 13 fields surveyed. Overall, disease incidence of foot rot disease occurred in the Bebeka, Gezmeret, Gamadiro, Kabo and Shosha areas. The mean on disease incidence was 46.31% and severity was 40.91%. Mean incidence on defoliation was 68% and severity was 30% (Table 2). Leaves yellowing symptom was observed on both the upper and lower leaf canopies at all sites surveyed. Subsequently, high disease incidence on foot rot symptom was recorded in Gezmeret (75%) followed by Bebeka (70%), Shosha (65%) and Gamadiro (65%). While the least incidence was recorded on Gamadiro (5%) and Shosha (5%). The characteristic of foot rot disease during survey was indicating the symptoms of foliar yellowing, defoliation and collar rot (Figures 1a, 1b and 1c).

The disease severity on foot rot symptoms at Gezmeret was 70%, moderate severity was recorded at Bebeka (62%) and Shosha (62%) while the lowest disease severity was recorded at Gamadiro (4%) and Shosha (4%).

Morphological identification of *Phytophtora capsici*

Identification of *Phytophtora capsici* is mainly based on the morphology of sporangia. In the present study, *Phytophtora capsici* was successfully isolated and identified based on morphological characteristics. Figures 2a, 2b, 2c and 2d shows the morphological characteristics of a pure fungal isolate.

The characteristics of this isolate was typical of *Phytophtora capsici* such as globose oogonia with paragynous antheridia, chlamydospore, torulose hyphae and lemon shape of sporangia with long pedicels as described by Mchau and Coffey [8].

Table 2: Distribution and severity of *Phytophthora* foot rot disease on black pepper in in Southwestern Ethiopia in 2018.

Location	Fields	Plant age (year)	Incidence (%) Mean*	Severity(%) mean*
Bebeka	1	5	55^{c}	48^{c}
	2	6	62^{bc}	54^{bc}
	3	4	70^{ab}	62^{ab}
	4	4	35^{d}	26^{d}
Gezmeret	1	2	75^{a}	70^{a}
	2	3	35^{d}	28^{d}
	3	3	55^{c}	50^{c}
Gamadiro	1	4	65^{abc}	58^{bc}
	2	5	5^{e}	4^{e}
Kabo	1	5	55^{c}	48^{c}
	2	6	20^{d}	18^{d}
Shosha	1	6	65^{bc}	62^{ab}
	2	7	5^{e}	4^{e}
	Mean		46.31	40.92
	CV (5%)		30.57	31.98

Means within a column followed by the same letters are not significantly different at $p<0.05$
*Data was arcsine transformation

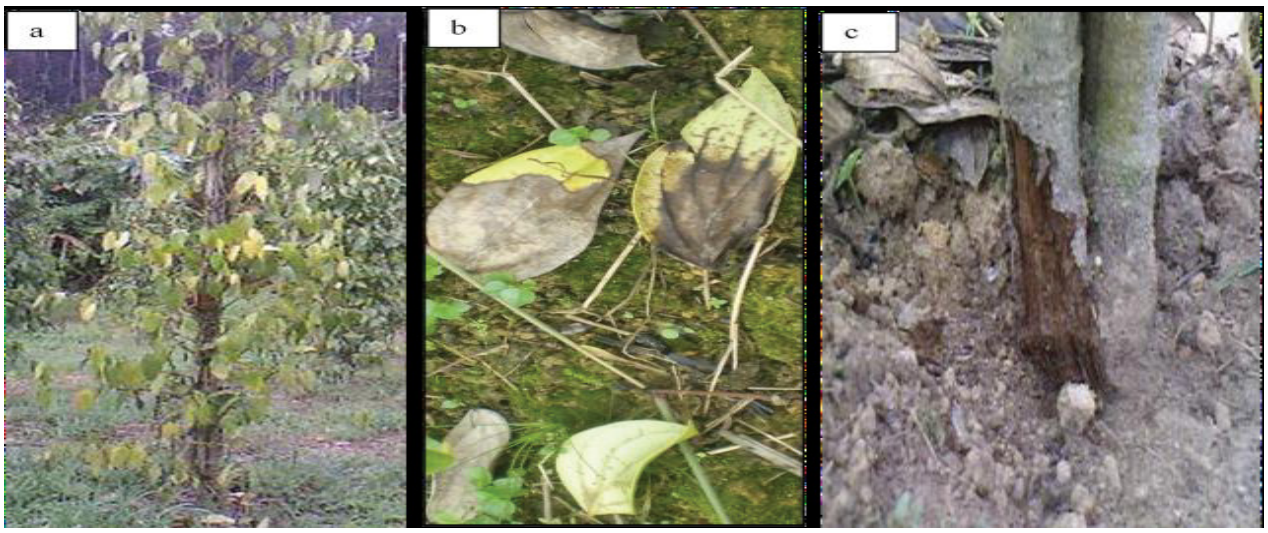

Figure 1: Field symptoms of *Phytophthora* foot rot disease observed on infected black pepper in in different areas of Southwestern Ethiopia (a) Leaves yellowing (b) Leaves defoliation and (c) Collar rot symptoms.

DISCUSSION

Phytophthora capsici is a diverse fungal species causing disease on a broad range of both temperate and tropical crops. In tropical countries as well as Ethiopia, this fungal pathogen has been reported to cause destructive disease on a variety of hosts, such as cacao, sweet orange, chili, papaya, black pepper, and others. In this study, we conducted field surveys in southwestern Ethiopia to determine the disease incidence and severity of foot rot disease in black pepper. The foot rot disease was detected in five major growing areas of black pepper in southwestern i.e., Bebeka, Gizmeret, Gamadiro, Kabo and Shosha. It is difficult to confirm that the black pepper arising areas will be free from the disease. Efficient application of disease management program is urgently required together with precise information concerning the quantitative measurement of the disease.

Based on the present findings, the highest percentage of disease incidence was recorded in Gizmeret (75%) followed by Bebeka (70%). These major growing areas of black pepper also had high percentage of disease severity 62-70%. High percentage of disease incidence and severity in the surveyed areas could possibly due to co-infection by other pathogens such as nematodes and *Fusarium solani*. The previous report of different countries showed that the foot rot disease has been speculated to be a nematode-fungus complex involving either *R. similis* or *Meloidogyne* spp. and *Fusarium spp.* and *P. capsici* [12].

The correlation between disease severity and surveyed location suggests the possibility of plantations being infested with pathogenic soil borne fungus long before they are replanted. Favorable microclimate conditions in the soil could promote the establishment of soil pathogens. This can be related to the significant correlation between temperature and rainfall in the surveyed areas. Relatively high rainfall during the summer with high soil moisture (>25%) and conducive temperature (22°C to 29°C) and suitable relative humidity (80%) are favourable for rapid multiplication of the fungus [4]. Bong and Saad [13] reported that

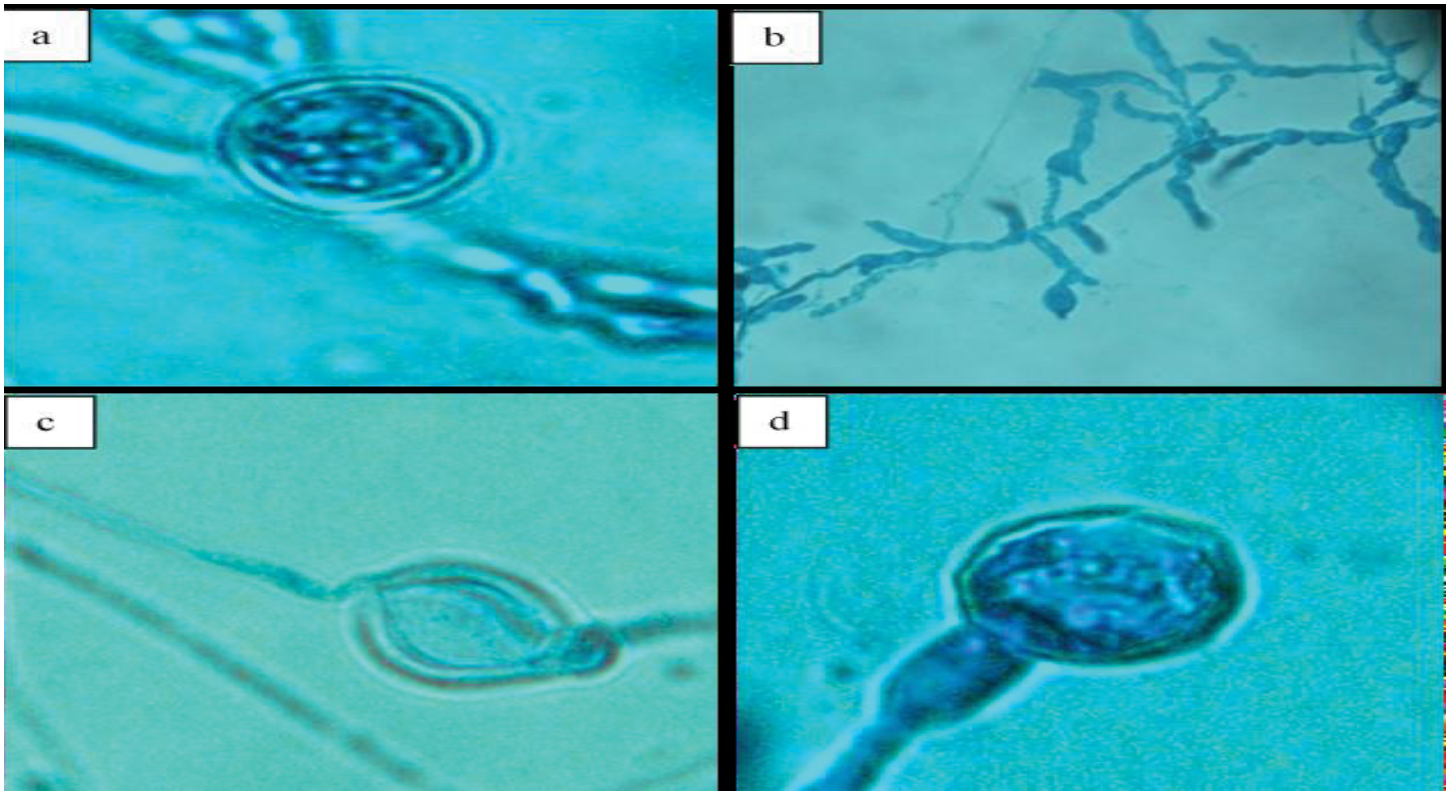

Figure 2: Typical morphological characteristics of *Phytophthora capsici* isolated from infected black pepper root: (a) Chlamysdospore (b) Torulose hyphae (b) Lemon shape sporangium with long pedicel and (d) Globose oogonia with paragynous antheridia.

black pepper *Phytophthora* is moisture loving and it will establish fast and reproduce rapidly in places where the water is plentiful. Moreover, occurrence of parasite nematode such as *R. similis,* which can complete their cycle within 25-30 days at temperature 21°C to 23°C [14] and *Meloidogyne spp.* which is widely distributed in tropical, subtropical and warm temperate regions. This could explain why foot rot disease of black pepper occurs more rapidly in this region based on the rainfall and temperature records.

In Ethiopia, foot rot disease of black pepper is caused by *Phytophtora capsici* was also shown the symptoms of decline on the black pepper plant. Based on field inspection, the collar rot infection occurs either at the collar or just above or below the soil level. Collar and root infection go unnoticed until the foliar yellowing symptom is recognized. The infection at initial stage starts as water soaked, which is the same as leaf infection. The lesions later turn to brown to dark brown in color within two to three days and later it appear as slimy dark patches. Young leaves become flaccid followed by yellowing and defoliation. The affected portion is wet discolored slimy emitting foul smell. Vascular discolorations observed in many cases but not consistently [15]. During the advanced stage of infection, the cortex gets disintegrated and peeled off. The infection of the collar gradually progresses downwards and spreads to the root system. This results in rotting of the root [16].

Root infections of the vines go unnoticed without any visible at aerial symptoms. The root infection starts at finer feeder roots [15], later it spreads to the main roots and the collar. The black pepper vines remain healthy until large portions of the roots are damaged. In the advanced stages of the root rot, foliar yellowing of the vine, and shedding of leaves, spikes and lateral branches are noticed. The amount of defoliation due to root rot infection is equal to root damage. The root loss to regeneration determines the spread of the decline and death of the vine. During the winter season with depletion of soil moisture, the remaining root system is unable to support the vine, so the entire vine collapses with wilting and drying of leaves. Foliar yellowing, flaccidity, defoliation, breaking of the stems at nodal regions and spike shedding are the characteristic aerial symptoms of root rot and collar rot infections [16].

CONCLUSION AND FUTURE PROSPECTS

Strengthening the future study on the management program of foot rot disease is truly recommended to find the best way on how to overcome the disease in the fields as this major disease would cause major loss on black pepper production in Ethiopia. Some recommendation from other research was avoiding planting black pepper in heavily or poorly drained areas and mulching black pepper plants which help in providing extra nutrition and at the same time increasing soil aeration. Practice sanitation in nurseries and fields also one of the best ways to control disease by removing severely infected leaves and clean up fallen plant debris. Meanwhile, planting leguminous ground cover in black pepper farms could provide nitrogen and prevent splashing of pathogen-infested soil onto foliage. Application of chemical pesticides to be kept at minimal if possible while green manures and organic matter should be employed to control soil-borne pathogens.

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