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IN VIVO AND IN VITRO STUDIES ON MORPHOLOGICAL CHARACTERIZATION OF *SEPTORIA LYCOPERSICI* CAUSING LEAF SPOT DISEASE IN TOMATO

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ABSTRACT

Septoria lycopersici responsible for Septoria leaf spot disease was observed on the leaves of tomato. *Septoria lycopersici* was isolated and completion of Koch's postulates confirmed that the fungus was causal agent of the leaf spot disease. The fungus was cultured on potato dextrose agar medium. The fungus was very slow growing with 8-12 mm radial growth as recorded after 30 days of incubation. The fungus produced off white, irregular, hardened blackish mycelial growth oozing spore mass from pycnidia. Pycnidia were dark brown to black, globose to sub globose, ostiolated and thick walled. Pycnidiospores were filiform, straight with pointed to rounded ends.

Keywords: characterization, *in vivo, in vitro*, leaf spot, morphological , *Septoria lycopersici* tomato.

INTRODUCTION

Septoria leaf spot is one of the most destructive foliar diseases observed in temperate regions causing spoliage of foliage, reduction in plant vigour, crop yield and market value (Gul *et al.,* 2016). The disease chiefly affects the leaves and may also attack stems but rarely on fruits (Lopes *et al.,* 2005). The peculiar symptoms observed on the infected older leaves are the circular to elliptical lesions with grey centres and dark brown margins surrounded by yellow halo (Zhang *et al.,* 2018). The pathogen responsible for Septoria leaf spot of tomato has been identified on the basis of morphological characters as *Septoria lycopersici* Speg.The present investigation were attempted to explore the morphological characters of *Septoria lycopersici* isolated from tomato crop affected with Septoria leaf spot.

MATERIALS AND METHODS

Microscopic examination of the fungus on the host

Fresh tomato leaves with typical Septoria spots were collected in perforated polythene bags from Wadura campus of SKUAST-K. The leaves were critically examined for symptom expression and later used for isolation of pathogen. For microscopic examination, the leaves were kept in moist chamber for 48 hours to get pycnidia bulged. These bulged pycnidia were lifted with the help of teasing needle under stereoscopic microscope and mounted in cotton blue in lactophenol. Fifty pycnidiospores and thirty pycnidia were examined for their shape, size, septation and color.

Isolation

The isolation of causal agent was done by tissue bit transfer method. Leaves of tomato plants showing typical symptoms were collected from the fields of division of Vegetable Science, SKUAST-K, Wadura. The infected parts of the leaves were cut into small bits of size 2-5 mm with a sharp sterilized blade so that each diseased bit contained a portion of healthy tissue along with it. These bits were subjected to surface sterilization with 1 per cent sodium hypochloride solution for 30 seconds followed by three rinses with distilled sterilized water to remove the last trace of mercuric chloride solution. These bits were then placed on moist filter paper in a sterilized petriplate and incubated at 25°C for 24 hours to enhance symptoms of possible pathogen. The bits were later transferred aseptically to potato dextrose agar (PDA) medium in sterilized petri-plates and incubated at25±1ºC for periodic observations vis-à-vis, colony colour, texture and sporulation. Single spore isolation as given by Jhonston and Booth (1983) was applied to obtain axenic culture of the pathogen. The pathogenic isolate on tomato plants was

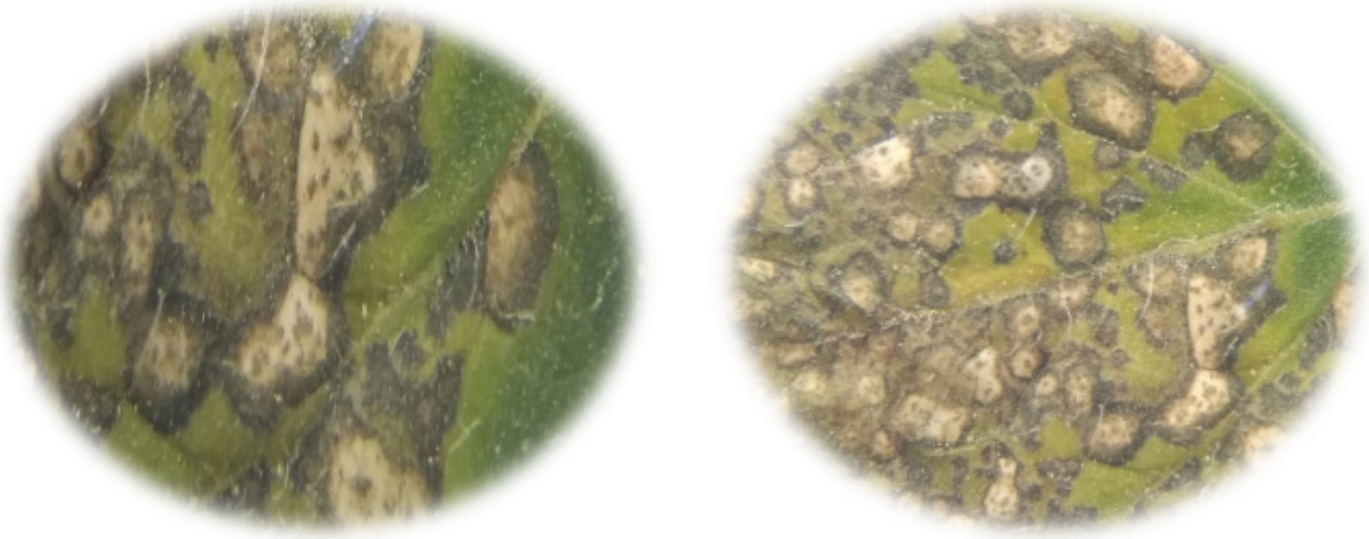
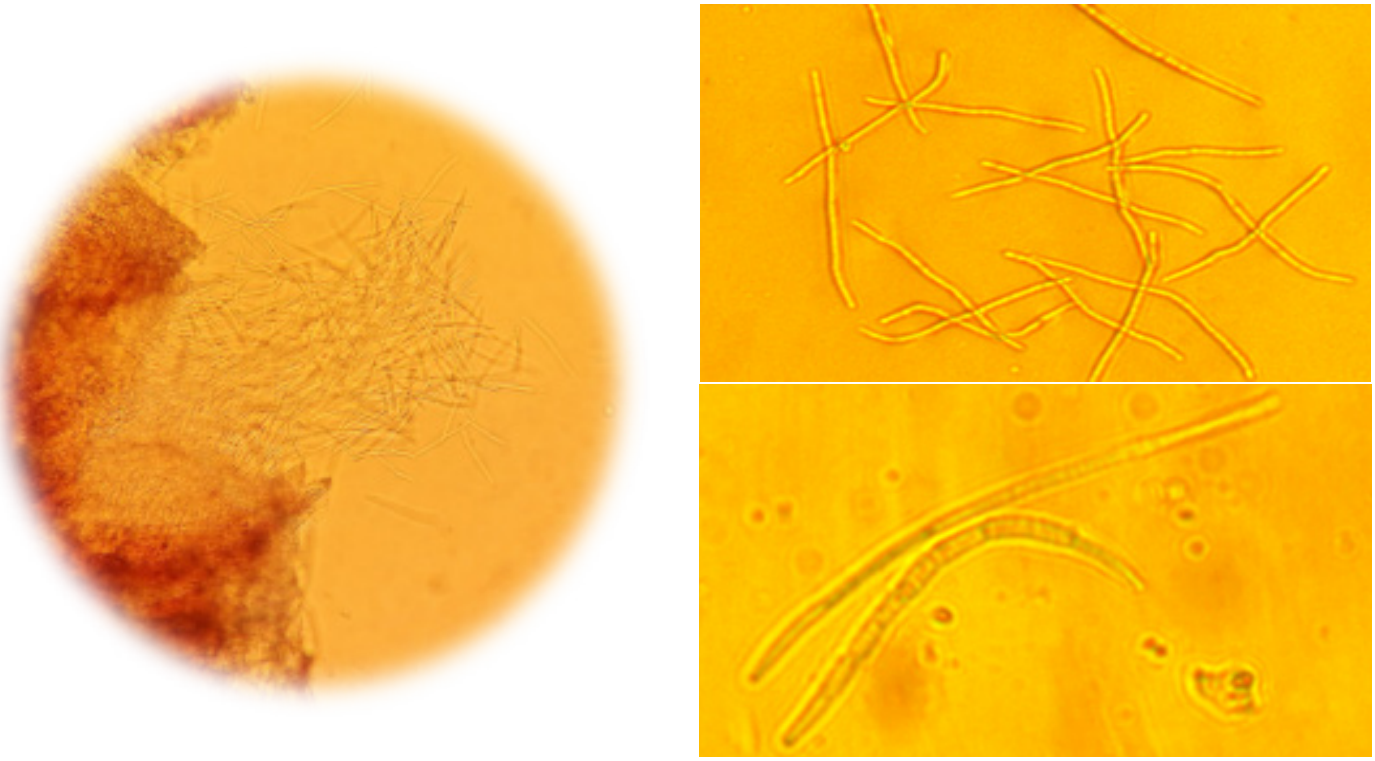


Plate 1: Pycnidial distribution on host



2a 2b

Plate 2: Pycnidia and pycnidiospores of *Septoria lycopersici* on host

identified on the basis of morphological characteristics of somatic and reproductive structures. The pure culture of *Septoria lycopersici* was maintained on PDA slants at 5±1°C in the refrigerator and cultured periodically at an interval of 30 days during the course of this study.

Morphological characterization

The morphological characters of the pathogen were studied on fungal culture of *Septoria lycopersici* in the laboratory. Semi- permanent slides were prepared from 14 days old culture stained with cotton blue in lacto phenol. With a view to identify the pathogen various morphological characters of the isolated pathogen were critically studied. The dimensions of 30 pycnidia and 50 pycnidiospores were measured and the observations were recorded by using 10x × 40x magnification.

RESULTS AND DISCUSSIONS

Morphological characteristics of the pathogen on host

Table 1: Morphological characteristics of *Septoria lycopersici* causing Septoria leaf spot of Tomato *in vivo*

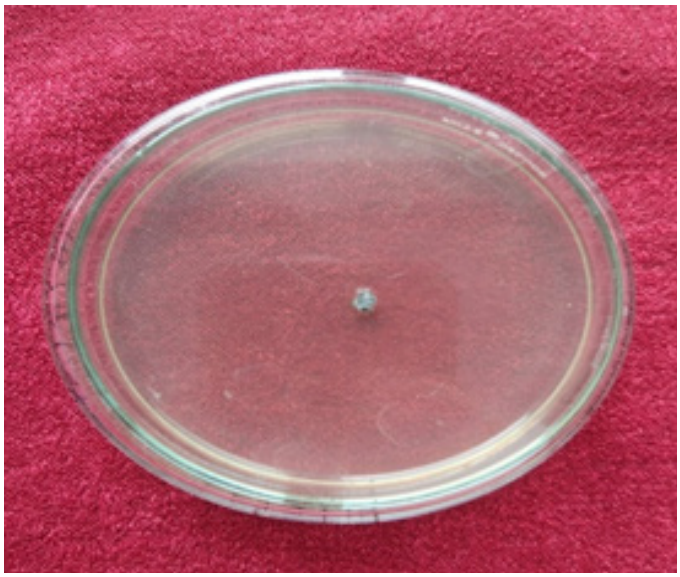
Propagule type	Size (µm)	Colour	Shape	Septation
Pycnidium	80-250 (av. 128.04)	Dark brown to black	Glucose to sub-globose, thick walled, embedded in the host tissue and ostiolate	-
Pycnidiospores	115.91-234.55 ×6-9.23 (av. 158.27× 7.78)	Hyaline	Filiform, straight with pointed to rounded ends. Some are slightly curved	2-9

Table 2: Cultural characteristics of *Septoria lycopersici* causing Septoria leaf spot of tomato.

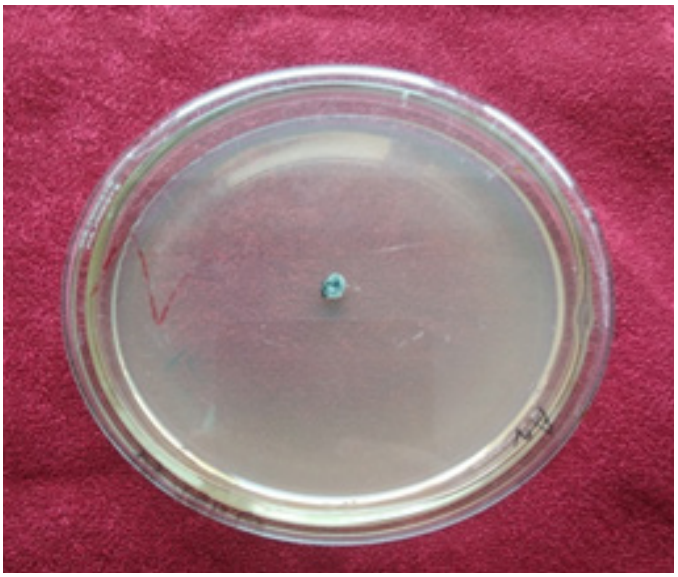
Growth period*	Colour	Size (mm)	Shape
10 days	Off white	2-3	Off white, compact, Irregular mycelial growth
20 days	Greyish to greyish black	4-6	Compact, irregular, raised, greyish to greyish black mycelial growth with pycnidial initiation.
30 days	Black	8-12	Compact, irregular, hardened blackish mycelial growth, oozing spore tendrils from the pycnidium

Table 3: Morphological characteristics of *Septoria lycopersici* causing Septoria leaf spot of tomato *in vitro*

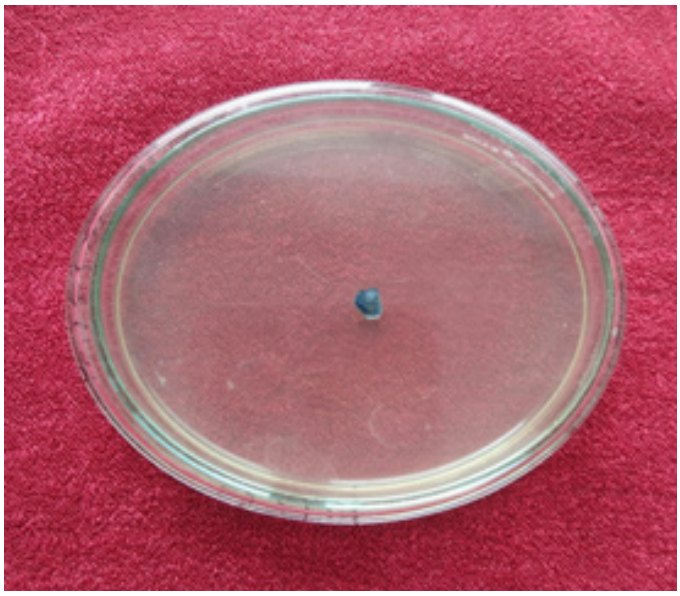
Propagule type	Colour	Size (µm)	Shape	Septation
Hyphae	Hyaline to light brown	2.36-5.84* (av. 2.24)	Branched, slightly bulged at septation	Septate
Pycnidium	Dark brown to black	75-224 (av. 120.40)	Globose to sub globose, erumpent, ostiolate and thick walled	-
Pycnidiospores	Hyaline	68.40-117.09× 2.28-3.74 (av. 92.98× 3.01)	Filiform, straight with pointed to rounded ends. Some are slightly curved	3-9



10 days old



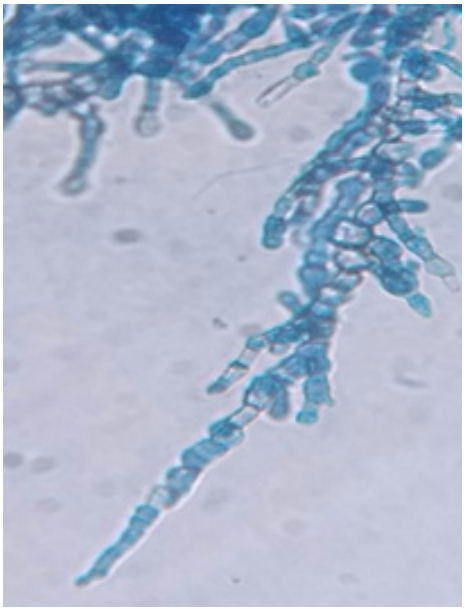
20 days old



30 days old

oozes

Plate 3: Colony characteristics of *Septoria lycopersici* causing Septoria leaf spot of tomato.



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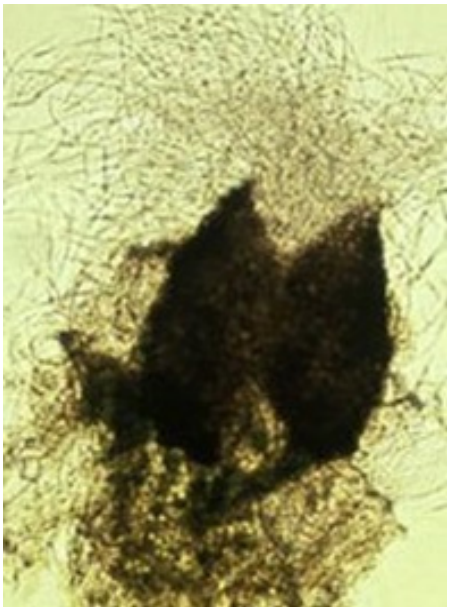

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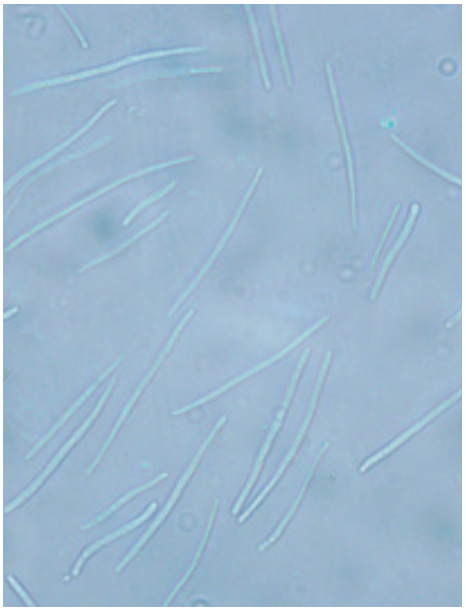

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Plate 4: Mycelium, pycnidia and pycnidiospores of *Septoria lycopersici*

The stereoscopic examination of the spots revealed the presence of abundant pycnidia on the upper side of the spot. The pycnidia were dark brown to black, scattered and partially submerged in the leaf tissue (Plate 1).Microscopic examination of these pycnidia revealed that they were globose to sub-globose, thick walled, ostiolated and ranged from 80-250µm in diameter with an average of 128.04 µm (Table 1, Plate 2a)

Under the moist chamber conditions pycnidia oozed a large number of pycnidiospores in the form of muciligenousciri. These pycnidiospores were hyaline, filiform, multiseptate 2-9 septa with pointed or rounded ends. The spore size ranged from 115.91-234.55 × 6-9.23 µm with an average size of 158.27 × 7.78 µm (Table 2, Plate 2b).

Morpho-cultural characteristics of the pathogen

The pathogen grown on potato dextrose agar medium produced visual growth after 5-6 days of inoculation. 10 days old colonies appeared as off white mycelial mat measuring 2-3 mm in diameter which later showed compact, submerged, raised mycelial growth with pycnidial initiation after 20 days of incubation. Off white colour of the mycelium changed to greyish black and finally imparted black colour to the medium when viewed from the bottom. Mycelium hardened and oozed spore tendrils from the pycnidium. The pathogen was very slow growing with 8-12 mm radial growth as recorded after 30 days of incubation (Table 2, Plate 3). Microscopic examination revealed that the pathogen produced hyaline mycelium composed of septate, branched thin walled hyphae measuring 2.36-5.84 µm in width with a mean of 2.24 µm. Later hyphae became comparatively thick walled, brownish in colour with slight bulging at each septa (Table 3, plate 4a). The pycnidia were observed after 20 days of incubation in the form of black dots. These pycnidia were globose to sub-globose, erumpent, ostiolatedand dark brown to black in colour measuring 75-224 um in diameter with a mean of 120.40 um. These pycnidia oozed pycnidiospores in the form of whitish mucilaginous cirri (Table 3, Plate 4b).Similar inferences were drawn by Singh (2018) regarding the morphology of *Septoria lycopersici,* that it produces epiphyllous, erumpent, sub-globose to globose, thick walled, honey yellow to dark brown colour and ostiolatedpycnidia. The pycnidiospores produced were hyaline, filiform, septate 2-6 with acute apex and truncate to obtuse base. The size of pycnidiospores varied from 52-95 × 2 µm and 60-120 × 2-4 µm. Carrera and Orellana (2001) perceived that the pycnidia were black, globose to sub-globose, ostiolated, 100-150 µm in diameter. Gul *et al.,* (2016) identified that the conidia were filiform, sub-straight to slightly curved, septate having 4-8 septa. The conidial size ranged from 70-150 × 2-3 **µ**m (Sohi, and Sokhi, 1973)

CONCLUSION

The fungus produced white to greyish black mycelium composed of hyaline, septate and branched hyphae turning brownish and becoming comparatively thick walled in later stages. The pycnidia produced were globose to sub-globose, thick walled, ostiolated and dark brown to black in colour measuring 75-224 µm in diameter. Pycnidiospores were hyaline, filiform and straight or slightly curved with pointed or rounded ends, 2-9 septate and measuring about 68.40-117.09 × 2.28-3.74 µm.

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COMPETING INTEREST

The authors declare that there is no competing interest in the publication of this manuscript.

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