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Cultural and morphological variability of *Sclerotinia sclerotiorum* in Rapeseed-Mustard

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The stem blight disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary earlier was of minor importance. But with changing scenario of climatic parameters it has become important in India causing considerable damage to the crop. In the present studies sclerotia have been collected from different species of diseased *Brassica* plants. A total of five different isolates were collected. The growth rate, colony characters, mycelial growth, number, shape and size of sclerotia of different isolates were examined. The isolate 2 isolated from *Brassica napus* was found to be fast growing followed by isolate 1 and the least aggressive was found to be isolate 3 isolated from *Brassica carinata*. Isolate 2 yielded as high as 70 sclerotia after 28 days of inoculation followed by isolate 1 with 60 sclerotia after 28 days. Size of sclerotia differed significantly from one isolate to another ranging from 2mm (Bharatpur) to 7mm (*Brassica carinata*) in length and 2mm (Bharatpur) to 3mm (*Brassica juncea*) in width. The mycelium colour in all isolates was white, fluffy and forming abundant aerial masses. But the mycelium of isolate 3 (*Brassica carinata*) was slightly greyish in colour and produced sclerotia of maximum size among all the isolates. The difference in such cultural characteristics will be helpful for identification and adoption of effective management practices for the pathogen.

Key words: Rapeseed-mustard, Sclerotinia sclerotiorum, variability

INTRODUCTION

The stem blight disease caused by Sclerotinia sclerotiorum (Lib.) de Bary was first recorded in India. It is a soil-borne pathogen with a wide host range. Earlier this disease was of minor importance but with due course of time it has become important in India causing considerable damage to the crop. The states which have been hit by the fungus are UP, Bihar, Rajasthan, Haryana, Punjab and other mustard growing areas. Of late the pathogen has gained the status of major disease. The pathogen affects all above ground parts of the mustard crop. Initially the disease appears in the form of water-soaked lesions on the plant stem. Later in the season these water-soaked lesions are smothered by white cottony mycelial growth of the fungus. Eventually the lesions increase in size and under favourable environmental conditions girdle the stem completely resulting in wilting and toppling down of the plant. However in many cases the infection does not spread much and is confined to only a smaller portion in the pith area. Hence resulting in stunting and premature ripening of the crop. In such cases there is no sudden collapse and death of plants. The infected portion turns white and tends to shred off. Scores of greyish white to black sclerotia of diverse forms and sizes appear on the surface of the lesions and also inside the pith. Moreover mycelial growth of the fungus tends to form various sizes of sclerotia on the surface of the pods. Mustard [Brassica juncea (L.) czern & Coss.] belonging to family Cruciferae has different species which are differently affected by the pathogen. Thus its suspected that there is some difference in the pathogens due to which such effect is produced. There certainly must be some morphological and cultural variability in among the pathogens. The study of such characters hence becomes

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an important tool in identification and better identification leads enhanced management practices.

MATERIALS AND METHODS

The sclerotia were collected from different species of diseased Brassica plants from different parts of Uttar Pradesh with characteristics symptoms alongwith an isolate from Bharatpur, Rajasthan. The sclerotia were isolated from different parts of plants viz. Stem or leaf. The sclerotia were first surface sterilised with the help of 0.1% mercuric chloride. Then these sclerotia were cut into small pieces with sterilized scalpel. These small pieces were again decontaminated by dipping into 0.1% mercuric chloride solution for 30 seconds. Later these pieces were taken out and 3 subsequent washes were done with sterilised water. Sterilised sclerotia were pressed between 2 folds of sterilized blotting paper so as to remove excess moisture. The sclerotia were then transferred to Petri dishes containing 20ml Potato Dextrose Agar medium with the help of sterilised forceps in inoculation chamber. The sclerotia were placed right in the centre of the Petri dishes. The inoculated Petri dishes were incubated at 25 + 1°C. Daily observations of the Petri dishes for mycelia growth of the fungus were conducted thereafter. The cut sclerotia gives rise to a mycelia which grows radialy in the plate. The growth rate, colony characters, mycelia growth, number, shape and size of sclerotia of different isolates were examined. The plates were viewed every day. Growth after seven days was noted down. The growth of mycelia was measured with help of a ruler by noting down the radius of the mycelial growth from all sides of the pertiplate. The day of initiation and formation of sclerotia was observed. The total number of sclerotia formed are counted for each plate. The length and breadth of sclerotia was measured with the help of graph paper.

RESULTS AND DISCUSSION

The different cultural and morphological characteristics of various isolates were observed on PDA. The different characters were noted as described below-

Colony

Colour-white; texture- fluffy; shape- circular and others- irregular margin forming scores of greyish to black sclerotia.

Mycelium

Colour- hyaline but later became light brown; diameter- 10-19 μ m; septation- hyphae is septate; branching- branched hyphae of smaller size borne on the main hyphae and Others- mycelia growth on PDA was fast and formed moderate to abundant amount of aerial mycelium.

Sclerotia

Colour- Ash grey to black; texture-rough; shaperound to spherical mostly but diversity in shape is observed; size- 10mm x 4.8-6.4mm and otherssclerotia were formed terminally and usually appeared in concentric rings in Petri plate culture.



Fig. 1 : Culturing and morphological characteristics of different isolates of *Sclerotinia sclerotiorum*

Mycelial growth and sclerotial characteristics of different isolates of *S. sclerotiorum* were studied on Potato Dextrose Agar (PDA). Mycelial and sclerotial characteristics of the isolates are presented in Table 1, Fig. 1, 2, 3 and 4. It is evident from the results that isolate 2 isolated from *Brassica napus* was found to be fast growing, covering entire Petri plate in just 4 days with a radial growth of 90 mm in 7 days followed by isolate 1 isolated from *Brassica juncea*, covering entire Petri plate



Fig. 2 : Time required for growth in different isolates

in 5 days with radial growth of 88.3 mm in 7 days. Least growth of pathogen was recorded in isolate 3 isolated from *Brassica carinata*, covering entire Petri plate in 8 days with radial growth of 70mm in

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Isolates	Full plate growth (days)	Mycelial Growth (mm) in 7 days	Initiation of sclerotia (days)	No. of of sclerotia	Formation of sclerotia (days)	Length of sclerotia (mm)	Width of sclerotia (mm)
Isolate 1 (<i>Brassica juncea)</i>	5	88.3	7	60	9	4	3
lsolate 2 (<i>Brassica napus</i>)	4	90	6	70	8	5	2
Isolate 3 (Brassica carinata)	8	70	10	27	12	7	3
Isolate 4 (Bharatpur)	7	76.6	8	32	11	2	2
Isolate 5 (<i>Brassica rapa)</i>	6	73.4	9	23	11	3	2
C.D. (P=0.05)	0.81	5.70	1.22	4.88	1.30	0.40	0.32

Table 1	1 :	: Culturing	and	morphologi	cal cha	racteristics	of	different	isolates	of	Sclerotinia	sclerotiorum
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7 days. Maximum number of sclerotia was harvested from isolate 2 with 70 sclerotia after 28 days followed by isolate 1 with 60 sclerotia after 28 days. Size of sclerotia differed significantly from one isolate to another ranging from 2mm (Bharatpur) to 7mm (*Brassica carinata*) in length and 2mm (Bharatpur) to 3mm (*Brassica juncea*) in width. Mycelium in all isolate was white, fluffy and forming abundant aerial mycelium but the mycelium of isolate 3 (*Brassica carinata*) was slightly greyish in colour and produced sclerotia of largest size among all the isolates.



Fig. 3: Culturing and morphological characteristics of different isolates of *Sclerotinia sclerotiorum*

The morphological and cultural characters of the fungus studied on Potato dextrose agar medium (in culture) and host (in nature) for the identification of fungus revealed that the colonies in culture were white, compact, circular, fast growing and formed numerous sclerotia. Mycelium septate, branched, hyaline to light brown in colour and measure 10-19 μ m in size. Sclerotia- superficial, formed in concentric rings, greyish white to black in colour, spherical to cylindrical or irregular in shape measuring 6.4-4.8 mm in size with rough and

wrinkled surface. Similar morphological characters were also described by Ahmed and Akhond (2015) and Goswami *et al.*, (2012). Cultural, morphological, pathogenic variability and mycelia



Fig. 4: Sclerotia size and number of sclerotia of different isolates of *Sclerotinia sclerotiorum*

compability among isolates of *Sclerotivia sclerotiorum* were also studied by Garg *et al* .(2010), Hidayah Baiq Nurul *et al.* (2014) Sharma *et al.* (2013), Manjunath *et al.* (2014), Gill *et al.* (2015), Sharma *et al.* (2015) and Upadhyay *et al.* (2015).

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