Journal of AgriSearch, 9 (2): 149-152
An Open Access International Peer Reviewed Quarterly

ISSN : 2348-8808 (Print), 2348-8867 (Online)
https://doi.org/10.21921/jas.v9i02.10121

Cultural, Morphological and Physiological studies of Sclerotinia sclerotiorum

NK UPADHYAY[*], VEDRATAN[1], VK YADAV[2], AJAY KUMAR[3]*, RAHUL KUMAR[4] AND ABHIMANYU[5]

ABSTRACT

Sclerotinia sclerotiorum is a soil borne pathogen which causes symptoms on ripe fruits. Pathogen isolated from infected fruits and grows on different solid culture media. Maximum growth recorded on potato dextrose agar medium. Morphological characters of fungus studied in detail and the causative fungus was identified as *Sclerotinia sclerotiorum* (Lib.) de Bary. Out of seven natural, semi-synthetic and synthetic media, potato dextrose medium supported the maximum growth and excellent growth of sclerotial production. The pathogen grew within a temperature between, 10°C to 40°C and has maximum growth at 25°C. Preliminary studies were undertaken on the germination of sclerotia by using different, which revealed that sclerotia germinated with the formation of one or more stripes without forming apothecia when incubated at 20±1°C in most Petri dishes containing filter papers. When sclerotia were floated in distilled water in Petri dishes and kept at 20±1°C, they started germination after 21 days with the formation of stripes which produce apothecia measuring up to 5.0 mm, on soil (1 part of soil + 5 part of sand) under pot condition, initially the sclerotia produced stripes after 32 days of sowing which later in most of the cases produced brownish yellow saucer shaped apothecia about 6-9 mm in diameter.

Keywords: Cultural, Tomato, Physiological, Sclerotinia, Morphological

ARTICLE INFO

Received on	:	23.04. 2022
Accepted	:	05.06.2022
Published online	:	16.06.2022

INTRODUCTION

Among the fungal diseases, fruit rot also known as target spot disease incited by *Sclerotinia sclerotiorum*, is world's most destructive disease. *Sclerotinia sclerotiorum* (Lib.) is a serious fungus affecting yield and product quality of many susceptible hosts. The fungus is worldwide in distribution and pathogen to more than 400 plant species. Thisdisease causes significant yield losses of various important crops including tomato(Lu, 2003). The capability of sclerotia to survive for more than 4 years becomes very difficult to manage the crop from theinfection of white mold fungus(Fernando *et al*., 2004).*S. sclerotiorum* is responsible for more than 60 plant diseases(Purdy, 1979). The pathogen produces sclerotia, which survive for long periods and attack roots of growing and mature plants, resulting in root rot, basal stem canker and wilt (Duncan*et al*., 2006). Sclerotinia Stem Rot (also known as white mold or Sclerotinia Stem and Root Rot) is one of the most important tomato soil borne diseases. Plant infection occurs either by myceliogenic germination of sclerotia or by ascospores released from apothecia during carpogenic germination of sclerotia. The myceliogenically germinating sclerotia are the main source of infection on processing tomato crops leading to rotting of aerial parts of the plant in contact with soil (Purdy, 1979). However, continuous use of same chemicals is not advisable for management of plant diseases, because of development of resistance strains among the pathogens. It has also hazardous to human health and environment. Not much attention has been given on this pathogen; thorough knowledge on the survivability of the pathogen is needed to evolve suitable control measures.Therefore, there is a need to search for management of this disease for sustainable improvement in quantity and quality production of fruit. Keeping in view, the importance of disease a study was undertaken to find out the cultural, morphological and physiological characters of *Sclerotinia sclerotiorum*.

MATERIALS AND METHODS

Morphological, cultural and physiological studies of *Sclerotinia sclerotiorum*

Pathogen was identified by its morphological and cultural characteristics. The following characters of the pathogen were studied to identify the pathogen.

Mycelial colony, sclerotial production, cultural and sclerotialcharacters

For the study of mycelial colony and sclerotial characteristics of the pathogen, Petri plates containing two per cent potato dextrose agar medium were inoculated with pure culture of the pathogen and incubated at 20 ± 1°C for 7 days and the observations on size, shape and colour were recorded.The

No. of sclerotia produced per 90 mm Petriplate	Intensity of sclerotia production
0	Nil
1-10	Poor
11-20	Fair
21-30	Good
31 and above	Excellent

[*1] Department of Plant Pathology, C.S. Azad University of Agriculture and Technology, Kanpur-222131, Uttar Pradesh, India
[2] Department of Agriculture, Invertis University Bareilly, 243123, Uttar Pradesh, India
[3] Department of Plant Pathology, Amar Singh College (CCS University Meerut) Lakhaoti, Bulandshahr-203407 222131, Uttar Pradesh, India
[4] Department of Plant Pathology, Birsa Agricultural University, Ranchi-834006, Jharkhand, India
[5] Krishi Vigyan Kendra, Farrukhabad (CSAUAT Kanpur) 222131,Uttar Pradesh, India
*Corresponding Author E-mail: kaushal4980@gmail.com

pathogen was grown on natural, synthetic and semi synthetic media to study thegrowth and cultural characteristics of the Pathogen. For competitive studies, production of sclerotia was observed according to the following grade.

Physiological studies of the pathogen

Effect of temperatures on the growth of the pathogen, was studied at six different temperatures (5, 10, 15, 20, 25 and 30°C). Potato dextroseagar medium was used as basal medium. Petri plates were filled with10 ml of medium. These Petri plates were inoculated with 5mm culture disc and incubated at 20°±l ºC. Three replications were used for each treatment. After 10 days of incubation, data were recorded and analyzed statistically. Sclerotia of the fungus were collected from the stems of diseased brinjal plant. The sclerotia were dipped in 0.1% mercuric chloride solution for 60 second and then washed thoroughly with sterilized water for 2-3 times to remove excess of mercuric chloride. Later on sclerotia were put in between two folds of sterilized blotting paper under aseptic condition to remove excess of moisture. These sclerotia were transferred to moist Petri dishes and kept in incubator at 20±l°C for 20 Days. Regular 0bservations were made till sclerotial germination took place.

Production of apothecia in distilled water

For production of apothecia, the sclerotia were floated in Petri dishes containing distilled water and were covered with their lids to prevent dust and other contaminants from entering in the Petri dishes. Toprovide adequate aeration for the floating sclerotia during germination, the Petri dishes were filled only about one third with water. The Petri dishes were placed at 20±1°C with sufficient light. Apothecia formation was observed regularly.

Germination of sclerotia in soil

An attempt was made to study about germination of sclerotia of the pathogen in soil. The soil and sand were 'mixed in the ratio of 1:5 and sterilized in autoclave at 15psi for one hour. After autoclaving, the mixture of soil and, sand was filled in earthen pots (30 cm diameter). Fifteen surface sterilized sclerotia 'were sown in each pot at depth of 2.0 cm and, the pots wereirrigated regularly to provide required humidity at an interval of two days for a month. These pots were kept at room temperature.The observations on the germination of the sclerotia were recorded.

RESULTS AND DISCUSSION

Cultural characters of the pathogen

The fungus was grown on seven different natural, semi synthetic and synthetic solid media in order to ascertain the best medium for the growth of the pathogen. Each medium was replicated thrice in 90mm Petri dishes. Data on linear growth and colony characters were recorded after 7 days of incubation at 20±1^{0}C.Aridsc1erotial development was recorded after 15 days of incubation at the same temperature.Average radial growth of the mycelium was taken separately for each treatment and data were analysed.The results are presented in Table , Plate 1 and Fig.1, is its corresponding bar diagram revealed that radialgrowth of the fungus on Potato dextrose agar was maximum (81.33mm) and significantly superior to other testedmedia. Next to Potato dextrose agar, Potato maltose agar supported the excellent growth of mycelium followed by good support of Corn meal agar medium but statistically both were at par. The fair growth, in order to merit was recorded on Water agar, Dextrose agar, Czapexdox agar medium followed by poor radial growth on Nutrient agar with significant difference.In respect to sclerotialproduction, the excellent performance was recorded on Potato dextrose agar and Potato maltose agar medium while good on' Corn meal agar medium, Fair on Water agar, Dextrose agar, Czapexdox agar medium and, poor onNutrient agar medium.Our results are in agreement with the previous studies where the *S. Sclerotiorum was* isolated on some vegetables (El-Gali, 2010).Several study used PSA culture media because of its simple formulation and ability to support mycelial growth of a wide range of fungi (Alem *et al*., 2001;Jeyalakshmi, 2001; El-Gali, 2008).

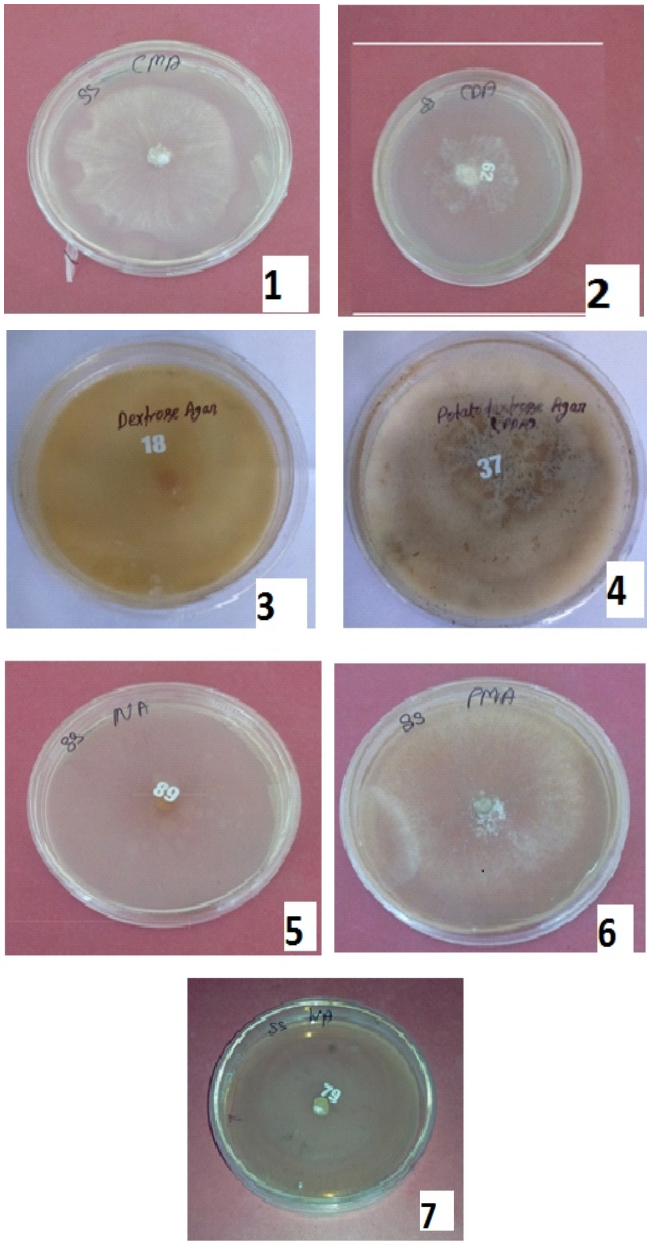

Plate 1. Growth of pathogen on different solid medium
T_1- Corn Meal Agar, T_2- Czapek'sDox Agar, T_3- Dextrose Agar, T_4- Potato Dextrose Agar, T_5- Nutrient Agar, T_6- Potato Maltose Agar, T_7- Water Agar

Table 1: Growth and sclerotialproduction of *S. sclerotiorum*on different solid media *in vitro.*

Medium	Av.Diameter of colony after 7 days (in mm)	Type of sclerotial production
Potato Dextrose agar	81.33	Excellent
Potato maltose agar	75.63	Excellent
Corn meal agar	71.20	good
Water agar	53.60	Fair
Dextrose agar	47.50	Fair
Czpek'sdox agar	42.26	Fair
Nutrient agar	00	poor

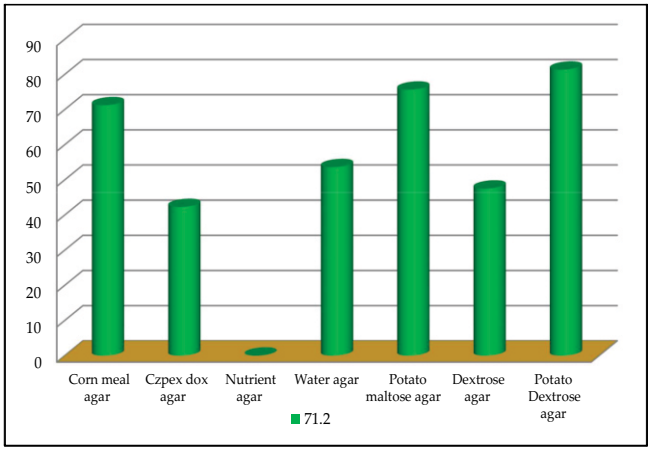

Fig. 1. Growth of fungus on different solid media

Morphological characters

The pathogen under study was identified on the basis of its morphological, physiological and culturalcharacters. Among morphological characters, fungal colony, mycelial and sclerotial characters were recorded on potato dextrose agar medium whereas, apothecia were recorded from germinating sclerotia in sterilized water and they are described as white, compact, uniform, circular with irregular margins forming numerous sclerotia on the surface. Initially the mycelium was hyaline, later turned light brown, 10 to 19 µm in size, septet and branched. Numerous lateral hyphae of smaller size were borne on the main hyphae. Mycelial growth on solid agar media was fast and formedmoderateto abundant amount of aerial mycelium. Sclerotia were grayish white to black in colour, round or semi-spherical in shape and measured 6.4 x 4.8 mm in size. These sclerotia were formed terminally and produced one or more concentric rings on agar culture media.

Table 2: Average radial growth and sclerotia productionof *S. sclerotiorum*

Treatments	Temperature (°C)	Average growth of colony(mm)	Type of sclerotial production
T_1- Nutrient agar	10	21.5	Nill
T_2- Dextrose agar	15	32.6	Nill
T_3- Water agar	20	42.6	Nill
T_4- Potato Dextrose agar	25	67.0	Excellent
T_5- Potato maltose agar	30	62.7	Good
T_6- Corn meal agar	35	50.3	Fair
T_7- zpek'sdox agar	40	25.3	Nill

Physiological studies

The pathogen was grown at seven different temperatures as described under "Material and Methods" and the average diameter of mycelial growth of the pathogen along with sclerotial production was recorded below in table 2.It is evident from the (Fig. 2) that the fungus could grow over a wide range of temperature from 10-40 °C through significant difference were observed in the growth of pathogen at each temperature. Maximum mycelialgrowth was observed at 25ºC followed by 20ºC and 35ºC temperature. However, 25 ºC was found statistically superior to other. Minimum growth was noticed at 10ºC. Excellent Sclerotial production was recorded at 25 ºC and fair at 35 ºC. However, the fungus *S. sclerotiorum* failed to produce sclerotia at10, 15, 20 and 40ºC.

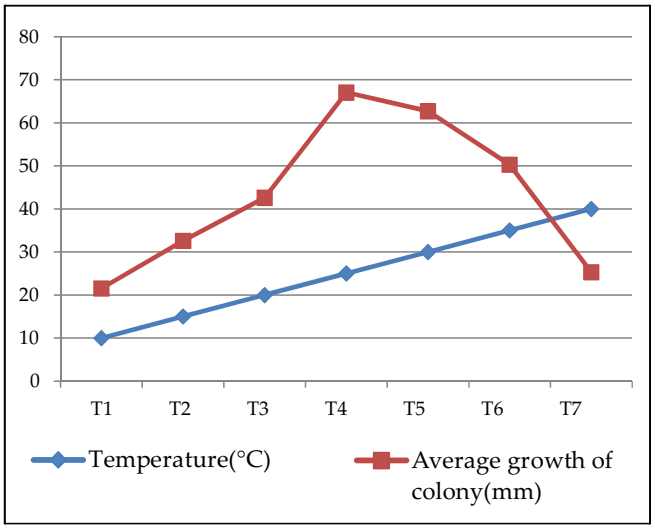

Fig. 2. Average radial growth and Sclerotia production of Sclerotinia sclerotiorum at different temperature

Sclerotial Germination of the fungus

Attempts were made to know the mode of germination of sclerotia of *S. sclerotiorum* under laboratory conditions using different methods. In Petri dishes, sclerotia of the fungus were incubated on moist filter paper at 25±1ºC.It was observed that the sclerotia started germination after 20 days of incubation with one or more stripes without forming apothecia.Germination in distilled water, sclerotia was floated in 1/3 part of the distilled water poured in Petri dishes and incubated at 21 ± 1°C.They germinated after 21days with the formation of stripes and later on these stripes also produced apothecia apically after 30 days of incubation. The apothecia were measured up to 5 mm in size. For sclerotial germination in soil, sclerotia were sown in pots according to the method

described in "Materials and Methods". Germination of sclerotia was initiated after 30 days of sowing in the pots consisting of soil and sand in the ratio of 1:5 and gave rise to several stripes. After 7 days the stripes borne saucer shaped, brownish yellow apothecia at its apex which emerged out from the soil surface. Diameter of apothecia varied from 6-9 mm.The present studies are in conformity with previous studies where themorphological, cultural and physiological characters of *Sclerotinia sclerotiorum* causing stalk rot of cauliflower (Cuong and Dohroo, 2006; El-Gali, 2010; Naema, 2016).

CONCLUSION

It can be concluded that the maximum growth of the pathogen was recorded on potato dextrose agar medium. Morphological characters of fungus studied in detail and the causative fungus was identified as *Sclerotinia sclerotiorum* (Lib.) de Bary. Out of seven natural, semi-synthetic and synthetic media, potato dextrose medium supported the maximum growth and excellent growth of sclerotial production.

REFERENCES

Alem MS, Begum MF, Sarker MA and Islam MR.2001. Effect of temperature, light and media on growth, sporulation, formation of pigments and pycnidia of *Botyodiplodia theobromae* Pat. *Pakistan J. Biol. Sci*. **4**: 1224-1227.

Duncan RW, Dilantha Fernandoa WG andRashid KY.2006. Time and burial depth influencing the viability and bacterial colonization of *Sclerotinia sclerotiorum*. *Soil BiolBioche*.**38**: 275-284.

El-Gali ZI.2008. A study of strawberry leaf spot in Al-Jabal El-Akhdar area, Libya. *Arab. J. Plant. Prot*. **26**: 160-162.

El-Gali ZI. 2010. Cultural, morphological and Physiological studies on some isolates of *Sclerotinia sclerotiorum*. *Libyan J. Plant Prot. Res*.**1**:11-16.

FAOSTAT.2016. Statistics division. FAO.http://faostat.fao.org.

Fernando, W. G., Nakkeeran S. and Zhang Y. 2004. Ecofriendly methods in combating *Sclerotinia sclerotiorum* Lib.) deBary. *Recent Res. Devel. Environ. Biol*.**1**: 329-347.

Jeyalakshmi C, DoraiswamyS,Valluvaparidasan V, Rabindran RH, Evens C and Harvey JO. 2001. Effect of substrate media on virulence of *Fusarium pallidoroseum:* a potential bioherbicide for *Parthenium* management. *Indian Phytopath*.**54**: 453-456.

Lu, G. 2003.Engineering *Sclerotinia sclerotiorum* resistance in oilseed crops.*African journal of biotechnology*,**2**: 509-516.

Naema AG, Mahdy AMM, Fawzy RN and Ahmed GA. 2016.Integrated Management of Tomato White Mold Disease Caused by *Sclerotinia sclerotiorum* using the Combined Treatments of Compost, Chemical Inducers and Fungicides.*Middle East J. Agric. Res*.,**5**: 479-486.

NHB. 2017. Hand book of Indian horticulture database. National Horticulture Board, Gurgaon. 177-185.

Purdy, L. H. 1979.*Sclerotiniasclerotiorum*: History, diseases and symptomatology, host range, geographic distribution, and impact. *Phytopathology*.**69**: 875-880.

Citation:

Upadhyay NK, Vedratan, Yadav VK, Kumar A, Kumar R and Abhimanyu. 2022. Cultural, morphological and physiological studies of Sclerotinia sclerotiorum. *Journal of AgriSearch* **9**(2):149-152