Biological Forum – An International Journal **14(1): 249-253(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Assessment of different Media on Growth and Sclerotia Formation of *Sclerotinia sclerotiorum* causing Sclerotinia Rot of Chilli under Laboratory condition

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(Received 07 October 2021, Accepted 11 December, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The efficacy of several solid and liquid media on *Sclerotinia sclerotiorun* growth (on the 7th day) and sclerotia formation (on the 15th day) was tested in the lab. The Potato Dextrose Agar medium had the highest mycelial growth (90.00 mm) and the most sclerotia formation (28.67), followed by Malt extract agar (76.68 mm and 17.33) and Oat meal medium (59.00 mm and 10.34), and Martins medium had the lowest mycelial growth (11.00 mm) and the fewest sclerotia formation (0.00). After 14 days of inoculation at 25°C, Potato Dextrose broth had the highest dry mycelial weight (170.16 mg) and number of sclerotia formation (21.75) of the five liquid media examined. Following that was Richard's medium (147.98 mg and 15.75). Asthana and Hawker's medium was recorded least dry mycelial weight and numbers of sclerotia formation.

Keywords: Solid and liquid media, mycelial, sclerotia, *Sclerotinia clerotiorun.*

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a soil-borne plant disease that is widely known as white mould and may live in the form of sclerotia for lengthy periods of time (Purdy, 1979; Willetts and Wong, 1980). The disease infects a wide range of succulent plants, including florals, shrubs, weeds, and crops such as chillies (Chupp and Sherf, 1960; Yanar and Miller, 2003; Hansda *et al*., 2014). This pathogen-caused ailment has been documented in several locations of India. Depending on the variety of afflicted crops, the disease was referred to as Sclerotinia rot, Sclerotinia fruit rot, fruit and stem rot, fruit rot, stem blight and rot, white mould, or pink joint (Farr *et al*., 1989 and Yousef *et al*., 2017). *Sclerotinia sclerotiorum* is a soil-borne necrotrophic and destructive fungus that is global and omnivorous (Purdy, 1979). In the globe, it was initially documented from sunflower in 1861 (Purdy 1979), and on chilli by Yanar *et al*. (1996), but in India, Sclerotinia rot was first reported on many hosts by Shaw and Ajerakar (1915), and on chilli by Srivastava and Divacor (1987). Culture media were played most important role in growth of pathogen and provide platform for isolation of pathogens from infected plant under *in vitro* condition. The fungus was able to grow on various solid and liquid media, but growth and number of sclerotia formation was varied on different media (Sharma *et al.*, 2016; Fagodiya *et al*., 2017). On culture medium, mycelial development was first uniform and sparse, but it eventually became fluffier and compact, with irregular margins (Goswami *et al*., 2012). *S. sclerotiorum* colonies were composed of globose to irregularly shaped black sclerotia that ranged in colour from white to grey (Kim and Cho, 2002). On PDA medium, S. sclerotiorum colonies developed quickly and were white in colour. After 8 to 12 days of development under 12 hours of light, a number of sclerotia appeared around the periphery of the dishes with these colonies (Reis and Nascimento, 2011). The sclerotia generated by the Sclerotinia fungus on culture medium were silvery white in the early stages of growth but darkened as the culture became older (Hansda *et al*., 2014).

Therefore, present study was carried out to see the effects of different solid and liquid media on growth and sclerotia formation of *Sclerotinia sclerotiorum* to know the effective media that provided maximum growth of pathogen at the Department of Plant Pathology, S.K.N. College of Agriculture, Jobner, Jaipur (Rajasthan- India).

MATERIAL AND METHODS

The laboratory experiments were conducted at the Department of Plant Pathology, S.K.N. College of Agriculture, Jobner, Jaipur (Rajasthan), India.

A. Effect of solid media on mycelial growth and sclerotia formation of Sclerotinia sclerotiorum

For maximal mycelial development of *S. sclerotiorum*, several solid media were tested. The study was set up in

a completely randomised fashion and was repeated four times. To assess the growth rate of *S. sclerotiorum* in vitro, six solid culture mediums were used: Potato Dextrose Agar medium (PDA), Czapeks-Dox Agar medium, Oat Meal medium, Corn Meal medium, Martins medium, and Malt Extract Agar Medium. The culture media were made using a standardized method that included weighing the various elements (Analytical grade) of each medium, adding distilled water to make up the volume 1000 ml, and autoclaving at 121.6°C for 20 minutes. In 90 mm diameter Petri plates, equal volumes (20 ml) of each medium were poured. Each Petri plate was infected separately with homogenous mycelial culture pieces (5 mm) cut with a sterilized cork borer from a young (7 days) briskly developing culture and put in the center of each pre-poured media. Three times each therapy was carried out. By eliminating the starting diameter (5 mm) of the bit, growth on solid media was assessed by measuring the colony diameter together with the two diagonals crossing through the centre of the colony. By the 7th day of incubation, mycelial growth was seen, and the number of sclerotia was counted at the 15th day of incubation.

B. Dry mycelial weight and sclerotia formation of Sclerotinia sclerotiorum on different liquid media

The various liquid culture media, such as Asthana and Hawker's medium, Brown's medium, Potato Dextrose broth medium, Czapek's Dox medium, and Richard's medium, were made according to a standardised technique and autoclaved for 20 minutes at 121.6°C. The pathogen was cultured in autoclaved Erlenmeyer flasks holding over 25 ml of liquid medium, infected with a 5 mm disc of fungus, and incubated at 25°C. After 15 days, the mycelium was removed, filtered using Whatsman filter paper No. 42, dried at 60°C for 24 hours, weighted, and the sclerotial count recorded. Four replications were kept in each experiment.

RESULTS AND DISCUSSION

A.Effect of solid media on mycelial growth and sclerotia formation of Sclerotinia sclerotiorum

Sclerotinia sclerotiorum mycelial growth was examined in vitro using six different solid media (Table 1, Fig. 1 and Plate 1). With 90.00 mm mycelial growth, Potato Dextrose Agar medium was shown to be considerably better to the other solid media in terms of mycelial growth and sclerotia production among the examined solid media. The second highest mycelial growth was recorded on Malt extract agar media (76.68 mm), followed by Oat meal medium (59.00 mm), and Corn meal medium (59.00 mm) (56.17 mm). Martin's medium had the smallest amount of mycelial development of *Sclerotinia sclerotiorum*. The largest number of sclerotia development was reported in Potato Dextrose Agar medium (28.67), followed by Malt extract agar medium (17.33), Oat meal medium (10.34), and Czapek's Dox agar medium (10.34). (5.33). Martin's medium had no evidence of sclerotia development. Several study workers have previously observed similar effects of diverse solid mediums on growth and sclerotia development. Cuong and Dohroo (2006) worked on cultural and morpho-physiological characteristics of *Sclerotinia sclerotiorum* causing stalk rot of cauliflower and reported that fungus growth was best on PDA, Krishnamoorthy *et al*. (2016); Fagodiya *et al*. (2017); Fatehpuria *et al*. (2017); Dhakad *et al*. (2018) also observed that maximum mycelial growth of pathogen was recorded on PDA. Similar findings on maximum mycelial growth and number of sclerotia formation on PDA were also reported by Panchal *et al*. (2012); Elgorban *et al*. (2013); Bharti *et al*. (2015); Husain and Choudhary (2018).

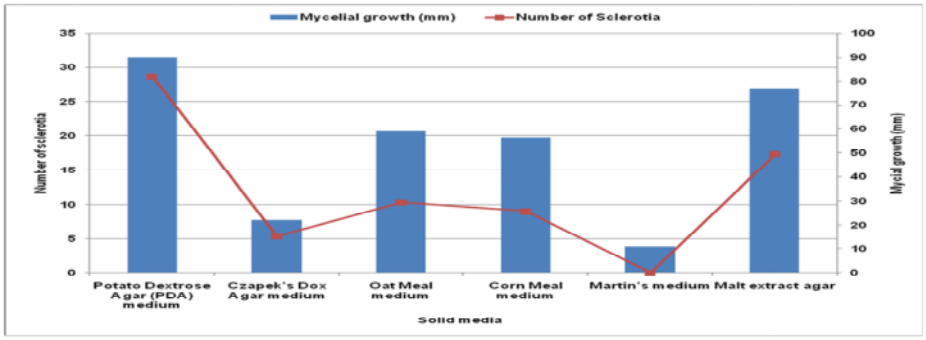

Fig. 1. Effect of solid media on mycelial growth and sclerotia formation of *S. sclerotiorum.*

Table 1: Influence of solid media on mycelial growth and sclerotia formation of *Sclerotinia sclerotiorum*.

Medium	Mycelial growth (mm)*	Number of Sclerotia
Potato Dextrose Agar (PDA) medium	90.00	28.67
Czapek's Dox Agar medium	22.16	5.33
Oat Meal medium	59.00	10.34
Corn Meal medium	56.17	9.00
Martin's medium	11.00	0.00
Malt extract agar	76.68	17.33
SEm±	1.39	0.27
CD (p=0.05)	4.28	0.84
CV	4.58	4.02

*Average of four replications

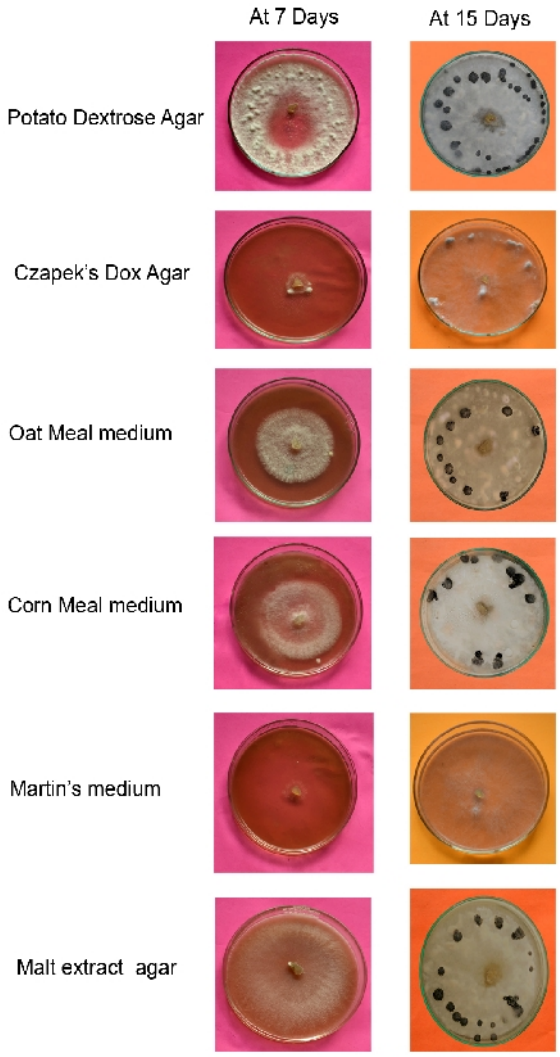

Plate 1. Effect of solid media on mycelial growth and sclerotia formation of *Sclerotinia sclerotiorum.*

B. Dry mycelial weight and sclerotia formation of Sclerotinia sclerotiorum on different liquid media

Table 2 and Fig. 2 show the results of the current investigation on the influence of different liquid media on dry mycelial weight and sclerotia development, with Potato Dextrose broth having the highest dry mycelial weight of 170.16 mg after 14 days of inoculation at 251°C. Richards medium came in second with 147.98 mg and was determined to be considerably superior than the other liquid media. Brown's medium had the lowest dry mycelial weight (65.21 mg) while Asthana and Hawker's medium had the highest (31.85 mg). In this investigation, the Potato Dextrose broth produced the maximum number of sclerotia (21.75) and was determined to be considerably superior to the other liquid media. Richard's medium (15.75) and Czapek's Dox were next (12.00). Minimum number of sclerotia (4.25) formation was observed in Asthana and Hawker's. Cuong and Dohroo (2006) observed that fungus was grow best on PDB and Richard's solution, Panchal *et al.* (2012); Elgorbon *et al*. (2013) reported that PDB was found good for dry mycelial weight and number of sclerotia, Sharma *et al.* (2016); Fagodiya *et al*. (2017); Husain and Choudhary (2018) also reported that PDB gave maximum dry mycelial weight and number of sclerotia formation.

Table 2: Dry mycelial weight and sclerotia formation of *S. sclerotiorum* on different liquid media.

Media	Average dry mycelial weight (mg)*	Number of Sclerotia
Asthana and Hawker's	31.85	4.25
Brown's medium	65.21	7.00
Potato Dextrose broth	170.16	21.75
Czapek's Dox	88.62	12.00
Richard's medium	147.98	15.75
SEm±	1.65	0.33
CD (p=0.05)	5.09	1.02
CV	3.28	5.44

*Average of four replications

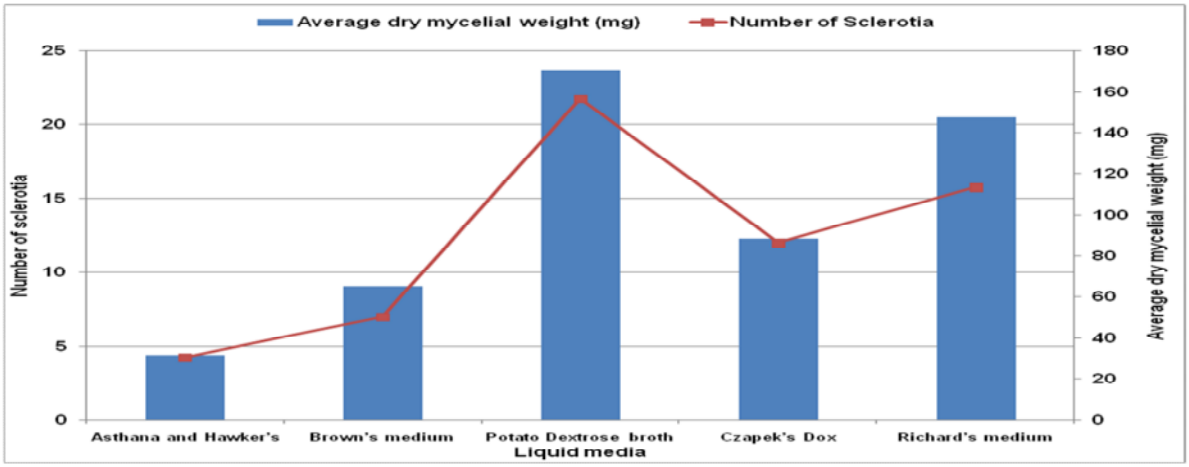

Fig. 2. Dry mycelial weight and sclerotia formation of *S. sclerotiorum* on different liquid media.

CONCLUSION

Nutrition is critical for improved development and sclerotia formation. Potato Dextrose Agar Medium, followed by Malt Extract Agar Medium, was shown to be the best for maximal growth and sclerotia production after studies on the influence of six different solid media on growth and sclerotia formation. Martin's medium had the least amount of growth and no sclerotia. After 14 days of inoculation, studies on the influence of several liquid media on dry mycelial weight and sclerotia development of *Sclerotinia sclerotiorum* in vitro revealed that Potato dextrose broth had the highest dry mycelial weight and sclerotia formation, followed by Richards medium. The medium Asthana and Hawker species, on the other hand, had the lowest dry mycelial weight and sclerotia production.

FUTURE SCOPE

Various media has been used in this study and result indicated that Potato Dextrose Agar Medium, followed by Malt Extract Agar Medium, was shown to be the best for maximal growth and sclerotia production. These results may be useful for further studies of this pathogen at molecular level i.e. to assess the molecular diversity among different isolates of *Sclerotinia sclerotiorum*, or molecular characterization of pathogen.

Acknowledgment. Authors are highly thankful to Head, Department of Plant Pathology, S.K.N College of Agriculture Jobner, Jaipur (Rajasthan) to provide existing facilities, guidance and support for completion of this experiment.
Conflict of Interest. None.

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How to cite this article: Satyadev Prajapati, Naresh Kumar, Shailesh Godika, Lalita Lakhran, Shivam Maurya and Sunil Kumar (2022). Assessment of Different Media on Growth and Sclerotia Formation of *Sclerotinia sclerotiorum* causing Sclerotinia Rot of Chilli under Laboratory condition. *Biological Forum – An International Journal*, *14*(1): 249-253.