EVALUATION OF CULTURE MEDIA, TEMPERATURE AND P^{H} FOR MAXIMUM GROWTH OF SCLEROTINIA SCLEROTIORUM (LIB.) DE BARY CAUSING WHITE MOLD OF RAPESEED MUSTARD

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ABSTRACT

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A study was conducted at the laboratory of Oilseed Research Centre, BARI, Gazipur, during March to April 2013 to standardize suitable growth media with optimum pH level and temperature for maximum growth of the pathogen *Sclerotinia sclerotiorum* causing white mold of rapeseed-mustard. The pathogen *S. sclerotiorum*, isolated from .0infected rapeseed-mustard was maintained on PDA medium in the laboratory for study at the laboratory of Oilseed Research Centre of BARI during March to April 2013.Its growth was compared on different solid media viz., potato dextrose agar (PDA), cornmeal agar (CMA), nutrient agar (NA) and V8 juice agar (V8A). To know the optimum pH for growth, the pathogen was grown on potato dextrose agar adjusted to pH levels of 4, 5, 6, 7 and 8. To determine the optimum temperature for vegetative growth, the pathogen was grown on potato dextrose agar and incubated at 10ºC, 15ºC, 20ºC, 25ºC, 30ºC and 35ºC. Among the various solid media potato dextrose agar promoted maximum growth of the fungus. The optimum pH for maximum growth of the fungus was found 4. The highest vegetative growth was recorded at 25°C. Therefore, the pathogen *S. sclerotiorum* can grow in PDA medium with pH 4 at 25ºC.

Key words: *Sclerotinia sclerotioram*, culture media, temperature, pH

INTRODUCTION

There are eight different crops in Bangladesh which produce oils of variable quantity and quality for use as edible purposes. Rapeseed-mustard is a major oilseed crop in Bangladesh. It contributes a lion share to the total edible oil production in the country covering 78% of oilseed cropped area (BBS 2015). Production of oilseed crops has gone down due to several abiotic and biotic reasons. The major biotic constrains are the occurrence of diseases and insect pests in the field as well as in the storage (Rajendra *el al.*2003, Bakr *et.al*.2009, Khatun *et al.* 2010). Rapeseed-mustard suffers from at least 14 diseases in Bangladesh and White mold of mustard caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is an emerging threat to rapeseed mustard in the country (Anon. 2016, Bakr *et al.* 2009, Hossain *et al*. 2014).

White mold of mustard caused by *Sclerotinia sclerotiorum* is the most common in temperate regions. Earlier it was a minor disease in Bangladesh but now a day, it has become the serious disease of mustard (Hossain *et al*. 2014; Anon. 2016). It infects rapeseed mustard leading to a diseased condition called white mold or *Sclerotinia* rot in which rotting of pod, leaf and stem are observed. The rotted foliage exhibit cottony white mycelial growth on their surface. With advancement of the disease the mycelial growth becomes dense and numerous carbon black coloured bodies called sclerotiaare formed on the surface and inside then rotted stem (Hossain *et al.* 2014). Sclerotia formed on or within the host tissue are dislodged on the soil surface by wind or during harvesting and threshing which are distributed in the vertical soil profile during land preparation (Cook *et al.* 1975). It is a soil and seed borne disease and monocropping of mustard results in severe development of the disease in a specific geographical area. This disease has been more prevalent in environments with greater moisture and low temperature (Nguyen and Dohroo 2006, Sharma *et al*. 1999). In view of the above facts a study was undertaken to standardize the suitable media, optimum pH and temperature for maximum growth of the pathogen at the laboratory of Oilseed Research Centre, BARI, Gazipur, during March to April 2013.

MATERIALS AND METHODS

Isolation of Pathogen

Rapeseed mustard plants showing symptoms of white mold or Sclerotinia rot were collected from Jamalpur area. The age of the crop varied from 50 to 65 days. Sclerotia collected from infected mustard were surface sterilized with 0.1 per cent mercuric chloride and rinsed with three changes of sterile water. The surface sterilized sclerotia were plated on PDA in sterile petri dishes and kept in an incubator at 25±1°C for 5 days. The fungus was sub-cultured and maintained on Potato Dextrose agar (PDA) medium. The stock cultures were maintained in PDA slants for long time storage under refrigerated condition at 4°C.

Selection of culture media for rapid growth of *S. sclerotiorum*

The growth of *S. sclerotiorum* was compared on different solid media viz., potato dextrose agar (PDA), corn meal agar (CMA), nutrient agar (NA) and V8 juice agar (V8A). The sterilized warm medium was poured@ 15 ml in each sterilized petri dish of 90 mm diameter and the medium was allowed to solidify. The mycelial blocks of the pathogen, cut from the edge of the pure culture grown in potato dextrose agar (PDA) were placed at the centre of petri dishes containing different media and incubated at 28±1ºC.Five replications were maintained for each medium. The diameter of the colonies in petridishes was measured after 72 hours of incubation. The best culture medium for growth of *S. sclerotiorum* was then selected for the test in different pH level and temperatures regime.

Effect of different pH level on mycelial growth of *Sclerotinia sclerotiorum*

To determine the effect of pH levels on the growth of *S. sclerotiorum*, potato dextrose agar medium was prepared and distributed in 250 ml conical flasks @ 100ml/flask and the pH of the medium was adjusted to 4, 5, 6, 7 and 8 with 0.1N HCl or 0.1N NaOH and autoclaved at 1.4 kg cm^{-2} for 20 min. Fifteen ml of the medium from each pH level was poured onto each sterilized 90 mm diameter plate and allowed to solidify. The mycelial block of the pathogen, cut from the edge of the pure culture grown in potato dextrose agar, were placed at the centre of petri dishes and incubated at 28±1ºC. Five replications were maintained for each pH level. The diameter of the colonies in petri dishes was measured after 72 hours of incubation.

Effect of different temperature on mycelial growth of *Sclerotinia sclerotiorum*

To determine the effect of temperature on mycelial growth of the fungus 90 mm diameter Petri dish containing about 15 ml sterile PDA was used. The mycelial blocks of the pathogen, cut from the edge of the pure culture grown on potato dextrose agar (PDA), were placed at the centre of Petri dishes and incubated at 10ºC, 15ºC, 20ºC, 25ºC, 30ºC and 35ºC. Five plates for each temperature were used. The diameter of the colonies in Petri dish was measured after 72 hours of incubation.

RESULTS AND DISCUSSION

Selection of culture media for rapid growth of *S. sclerotiorum*

Out of four different culture media used to test the growth of the fungus, the mean mycelial growth was maximum (72.0 mm) on PDA medium followed by nutrient agar medium (34 mm). The pathogen did not grow on V8 juice agar media. Khan (1976) reported the PDA as suitable medium for growth of the fungus *S. sclerotiorum* as well as production of maximum number of sclerotia. Nguyen and Dohroo(2006) reported maximum mycelial growth and sclerotia formation of *S. sclerotiorum* was on PDA followed by Pea's seed agar. Monika *et al*. (2013) showed that the fungus grow best on Sabouroud dextrose agar, potato dextrose agar and potato dextrose broth. Similar results were also reported by Hossain *et al*. (2014) (Table 1, Fig. 1).

Table 1. Effect of different culture media on mycelial growth of *Sclerotinia sclerotiarum*.

Cultural media	Radial growth after 72 hrs of incubation (mm)	Growth rate/day(mm)
PDA	72	24
CMA	21	7
NA	34	11
V8	5*	0
LSD (5%)	12	

* 5mm diameter of block means no growth

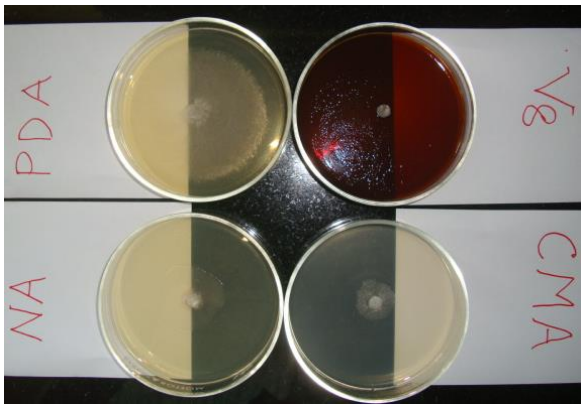

Fig. 1. Growth of *Sclerotinia sclerotiarum* at different culture media

Effect of pH levels on the growth of *S. sclerotiorum*

Maximum radial growth (57 mm) of the fungus was recorded at pH 4.0 followed by pH 5.0 (56 mm). Similar radial growth (52 mm) was recorded at pH 6.0 and pH 7.0. The lowest growth was recorded at pH 8 having colony diameter of 46 mm. These results are in conformity with that of Khan (1976) who reported the pH level of 4.5 as the best for the growth of the fungus. Willets and Wong (1980) observed pH below 5.0 as optimum for growth of *S. sclerotiarum* . Nguyen and Dohroo (2006) reported pH level of 5.0 as best for growth of S. sclerotiorum in liquid media. He found pH levels of 7.5 and 8.0 as unsuitable for growth of the pathogen. Monika *et al*. (2013) found the optimum pH level for maximum growth of *S. sclerotiorum* was 5.6 – 7.0(Table2, Fig. 2)

Table 2. Effect of different pH level on mycelial growth *of Sclerotinia sclerotiorum*

pH level	Radial growth after 64 hrs of incubation (mm)	Growth rate/day(mm)
4.0	57	28.5
5.0	56	28.0
6.0	52	26.0
7.0	52	26.0
8.0	46	23.0
LSD (5%)	4	

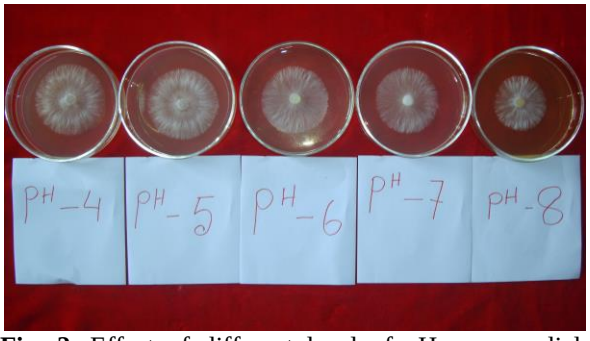

Fig. 2. Effect of different level of pH on mycelial growth of *S. sclerotiorum*

Effect of different temperature on the mycelial growth of *Sclerotinia sclerotiorum*

The highest radial growth of *S. sclerotiorum* occurred in plates incubated at 25ºC (Table 3, Fig. 3) followed by 20ºC. The growth rate per day was 33.4 mm at 25ºC and 29.4 mm at 20º C. Slight growth was observed at 15ºC but the pathogen did not grow at 10º C and at 30º C. Hossain *et al*. (2014) reported the highest radial growth of *S. sclerotiorum* at 25ºC followed by 20ºC in PDA. They also observed the highest vegetative growth per day (33.4 mm) at 25ºC. Domingues *et al*. (2016) reported that the mycelia growth of *S. sclerotiorum* occurred at temperature ranging 7 - 32ºC and showed maximum growth rate at 22ºC in PDA. According to Monika *et al*. (2013) 25 - 30ºC was optimum for the highest growth of *S. sclerotiorum.*

Table 3. Effect of different temperature on mycelial growth of *S.sclerotiarum*

Incubation temperature (ºC)	Radial growth after 64 hrs of incubation (mm)	Growth rate/day(mm)
35	0	0
30	0	0
25	89	33.4
20	78	29.4
15	5	2.0
10	2	0.75
LSD (5%)	7	

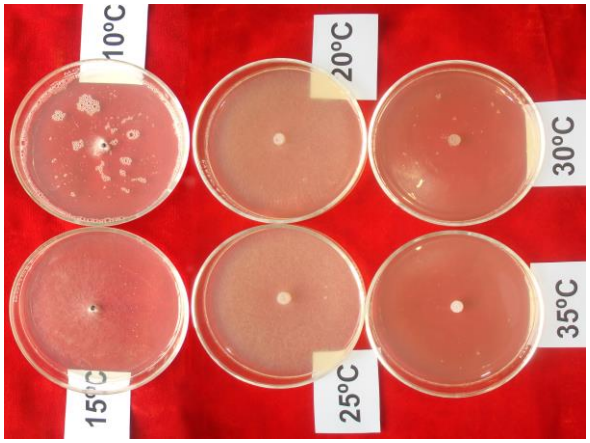

Fig. 3. Mycelial growth of *S. sclerotiarum* at different temperature

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