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Assessment of different culture media on growth and sclerotia formation of *Sclerotinia sclerotiorum* (Lib) de Bary

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Abstract
Sclerotinia stem rot incited by *Sclerotinia sclerotiorum* is a serious threat to oilseed production in Northern of Madhya Pradesh. The present investigation was conducted at Department of Plant pathology, College of Agriculture, Gwalior, (M.P.) with ten solid culture media *viz.*, French bean seed agar, Lettuce leaf agar, Pea seed agar, Mustard stem agar, Mustard seed agar, Mustard leaf agar, Mustard stem extract 10%+ sucrose 2% agar, Mustard seed extract 10%+ sucrose 2% agar, Glucose sodium chloride agar and Potato dextrose agar medium were evaluated against *S. sclerotiorum.* The data revealed that the maximum growth was recorded in Potato dextrose agar (85.33 mm) followed by glucose sodium chloride agar (82.33 mm), mustard leaf agar (72.67 mm) and mustard seed extract 10%+ sucrose 2% agar (69.67 mm), while minimum growth was recorded in lettuce leaf agar (44.33 mm) followed by French bean seed agar (51.67 mm) and mustard stem agar (53.33 mm) respectively. The maximum number of sclerotia per plate was recorded in Potato dextrose agar (10.33/plate) while the minimum number of sclerotia per plate was recorded in lettuce leaf agar (3.67/plate). The maximum size of sclerotia was recorded in Potato dextrose agar (4.67 mm) while the minimum size of sclerotia was recorded in lettuce leaf agar (2.67 mm). The highest weight of sclerotia per plate was recorded in Potato dextrose agar (725.00 mg) while lowest weight of sclerotia per plate was recorded in lettuce leaf agar (230.00 mg). Among culture media, PDA medium was found as most suitable media for mycelia growth and sclerotia formation of the *Sclerotinia sclerotiorum*.

Keywords: *Sclerotinia sclerotiorum*, culture, PDA, sclerotia

Introduction
Rapeseed-mustard group of crop are the major rabi oilseed crops of India. This group is mainly constituted by *Brassica juncea, B. napas, B. rapa, B. carinata* and *Eruca sativa.* In India, rapeseed-mustard crops are cultivated on an area of 6.69 million ha with a production of 10.11 million tones and productivity 1511kg/ha. (Anon, 2021) [1]. Rajasthan, Madhya Pradesh, Haryana, Uttar Pradesh, West Bengal, Gujarat, Jharkhand and Assam etc. are major mustard growing states of the country.In Madhya Pradesh rapeseed-mustard crops are cultivated in an area about 0.77 million hectares with the production of 1.31 million tones and productivity 1713kg/ha. (Anon, 2021) [1]. Chambal and Gwalior division of Madhya Pradesh are the major rapeseed-mustard growing area of state as these two divisions jointly contribute more than 70 and 80 per cent share in area and production of these crops in the state respectively.
Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* in rapeseed-mustard is a soil borne, neurotropic pathogen with worldwide distribution known to infect over 500 species of plants (Sharma *et al*., 2015) [12]. Yield losses vary with the percentage of plants infected, and the growth stage of the crop at the time of infection. Plants infected at the early flowering stage produce little or no seeds, and those infected at the late flowering stage will set and may suffer little yield reduction. About 72.8 to 80 per cent disease incidence was recorded in some districts of Haryana and Punjab (Kang and Chahal, 2000) [10]. This fungus can cause systemic and aerial infection by myceliogenic and cariogenic germination. Large numbers of sclerotia are formed in soil on organic matter, on roots, on and inside the pith of stem in rapeseed-mustard crop, and serve as source of primary inoculum for the next season. Symptoms appear on stem, pod and on decayed leaves as elongated water soaked spots. Small white structure appears on the stem which later on covered by whitish cottony mycelia growth of the fungus which later turned black colour as hard sclerotia. The present study aimed to study the effect of some factors on morphological and physiological characters of *S. sclerotiorum*.

Material and Methods

Isolation of the pathogen

Plant samples with blighted stems, branches and with sclerotia were arbitrarily sampled from the affected field. The infected plant samples were transferred to Plant Pathology laboratory of the department for further processing. Four sclerotia were collected and sclerotia were surface sterilized with 0.1% HgCl2 for 1 minute and wash with water three times after that sclerotia cut into two pieces with help of sterilized bled and placed on solidifying medium, there after allow to grow for five days. It repeated three to four times re-culture till obtain pure culture. These Petri plates were inoculated with 5 mm. mycelia disc of five day old young vigorous grown pure culture cut with help of corn borer and placed in the center of the plate. The inoculated plates were incubated at 25± 1°C. Each treatment was replicated thrice.

In this study, the ten solid medium were evaluated for obtaining maximum mycelia growth and sclerotia formation of the *S. sclerotiorum*. The experiment was carried out under complete randomized design with replicated three times. The culture media *viz.*, French bean seed agar, Lettuce leaf agar, Pea seed agar, Mustard stem agar, Mustard seed agar, Mustard leaf agar, Mustard stem extract 10%+ sucrose 2% agar, Mustard seed extract 10%+ sucrose 2% agar, Glucose sodium chloride agar and Potato dextrose agar medium were used to compare the growth rate of *S. sclerotiorum*. The culture medium were prepared by the standardized method and autoclaved at 121.6 °C, 15 psi pressure for twenty minutes. Uniform quantities (20 ml) of each medium were poured in 90 mm Petri plates. Each Petri plate was inoculated separately with uniform mycelia culture bits (5 mm) cut with the help of corn borer from young (5 days) vigorously growing culture were placed on the middle of the each pre poured medium and incubated at 25±2 °C (Dela Paz *et al.*, 2006) [4]. The diameter of the mycelia growth of the fungus was measured at 3, 5 and 7 days after inoculation and the number of sclerotia/ Plate, size per sclerotia (mm) and weight of sclerotia/ Plate (mg) were recorded at 15th day after inoculation.

Results and Discussion

Nutrition plays an important role in growth of the fungus, the differential support of ten different culture media on growth of *Sclerotinia sclerotiorum* are presented. Results of the experiment conducted during 2020-21 revealed that all the media were found effective against growth and sclerotia production of *Sclerotinia sclerotiorum*. Significant difference in the fungal growth was observed in the tested media at 3, 5 and 7 days after inoculation. The data presented in (Table-1and Fig.-1 and 2) revealed that maximum mycelium growth was recorded in Potato dextrose agar (41.33 mm and 62.67 mm) at 3 and 5 DAI followed by glucose sodium chloride agar (38.67 mm and 57.33 mm), mustard leaf agar (35.33 mm and 56.67 mm), mustard seed extract 10%+ sucrose 2% agar (32.67 mm and 43.67 mm), while minimum growth was recorded in lettuce leaf agar (17.67 mm and 28.67 mm) followed by French bean seed agar (19.33 mm and 34.33 mm) at 3 and 5 DAI. Similarly at 7 days after inoculation the maximum growth was recorded in Potato dextrose agar (85.33 mm), followed by glucose sodium chloride agar (82.33 mm), mustard leaf agar (72.67 mm) and mustard seed extract 10%+ sucrose 2% agar (69.67 mm), while minimum growth was recorded in lettuce leaf agar (44.33 mm) followed by French bean seed agar (51.67 mm) and mustard stem agar (53.33 mm) respectively. The maximum number of sclerotia per plate was recorded in Potato dextrose agar (10.33/Plate) followed by glucose sodium chloride agar (7.67/Plate), mustard leaf agar (7.33/Plate), pea seed agar (6.67/Plate), while the minimum number of sclerotia per plate was recorded in lettuce leaf agar (3.67/Plate). The maximum size of sclerotia was recorded in Potato dextrose agar (4.67 mm) followed by mustard leaf agar (4.33 mm), mustard seed extract 10%+ sucrose 2% agar (4.33 mm), glucose sodium chloride agar (4.00 mm), while the minimum size of sclerotia was recorded in lettuce leaf agar (2.67 mm). The highest weight of sclerotia per plate was recorded in Potato dextrose agar (725.00 mg) followed by mustard leaf agar (715.00 mg), glucose sodium chloride agar (710.00 mg), pea seed agar (620.33 mg), while lowest weight of sclerotia per plate was recorded in lettuce leaf agar (230.00 mg). The present study ten culture media showed that PDA medium was found as most suitable media for mycelia growth and sclerotia formation of *Sclerotinia sclerotiorum.* Similarly results supported by Cuong and Dohroo (2006) [3]; Elgorban *et al.* (2012) [5]; Singh *et al.* (2013) [14]; Bharti *et al.* (2015) [2]; Fatehpuria *et al.* (2016) [6]; Husain *et al*. (2018) [7] and Sharma (2021) [13] also reported PDA most suitable media for the growth and sclerotia formation of the *Sclerotinia sclerotiorum*. Khan (1976) [8] and Sharma (1979) [11] who also found potato dextrose agar medium suitable for growth of the fungus and production of maximum number of sclerotia. While, lettuce leaf agar was not found suitable for growth of the fungus, which required 7 days for full growth in the Petri plates, with average radial growth of 30.65 mm and 1.5 average number of sclerotia per Petri plate. The medium was tried for the first time and there was no conformity with previous study though, the fungus infected lettuce plants as per earlier reports. However, Khan (1976) [8] and Kaith (1977) [9] also reported poor growth on some other natural media such as sunflower seed extract and rapeseed extract, which might be due to presence of some growth inhibitory substances in their extract.

Table 1: Evaluation of crop based locally developed media for the growth and sclerotial formation of *Sclerotinia sclerotiorum.*

Culture medium	Mycelium growth (mm)			Number of Sclerotia / Plate	Average diameter (Size) of Sclerotia (mm)	Weight of Sclerotia / Plate (mg)	Colour of Sclerotia
	3DAI	5DAI	7DAI				
French bean seed agar	19.33	34.33	51.67	4.00	3.00	310.00	Light Black
Lettuce leaf agar	17.67	28.67	44.33	3.67	2.67	230.00	Light Black
Pea seed agar	21.33	40.33	67.67	6.67	3.33	620.33	Dark Black
Mustard stem agar	24.00	33.67	53.33	4.67	3.00	453.67	Light Black
Mustard seed agar	28.67	41.00	66.33	5.33	3.67	490.00	Light Black
Mustard leaf agar	35.33	56.67	72.67	7.33	4.33	715.00	Dark Black
Mustard stem extract 10%+ sucrose 2% agar	27.33	37.67	58.67	4.33	3.33	355.00	Light Brown
Mustard seed extract 10%+ sucrose 2% agar	32.67	43.67	69.67	5.67	4.33	530.00	Light Black
Glucose sodium chloride agar	38.67	57.33	82.33	7.67	4.00	710.00	Light Black
Potato dextrose agar	41.33	62.67	85.33	10.33	4.67	725.00	Light Black

Sem±	1.51	1.62	1.54	1.25	0.30	2.16	NS
CD at 5%	4.50	4.82	4.58	3.71	0.89	6.43	NS

*Data are the mean of three replication
*DAI: Days after inoculation
*Sclerotial observation were carried out at 15th days after inoculation

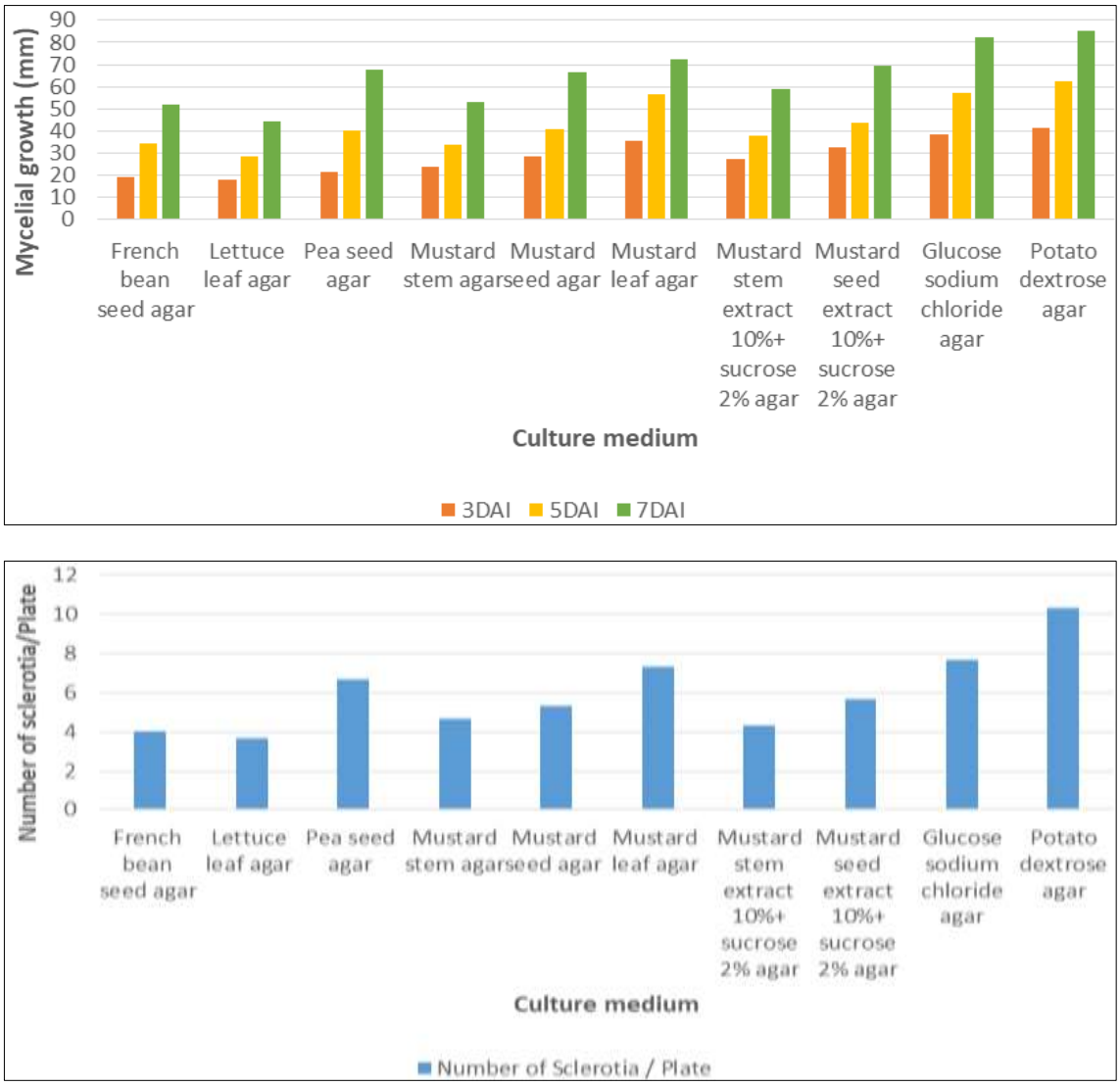
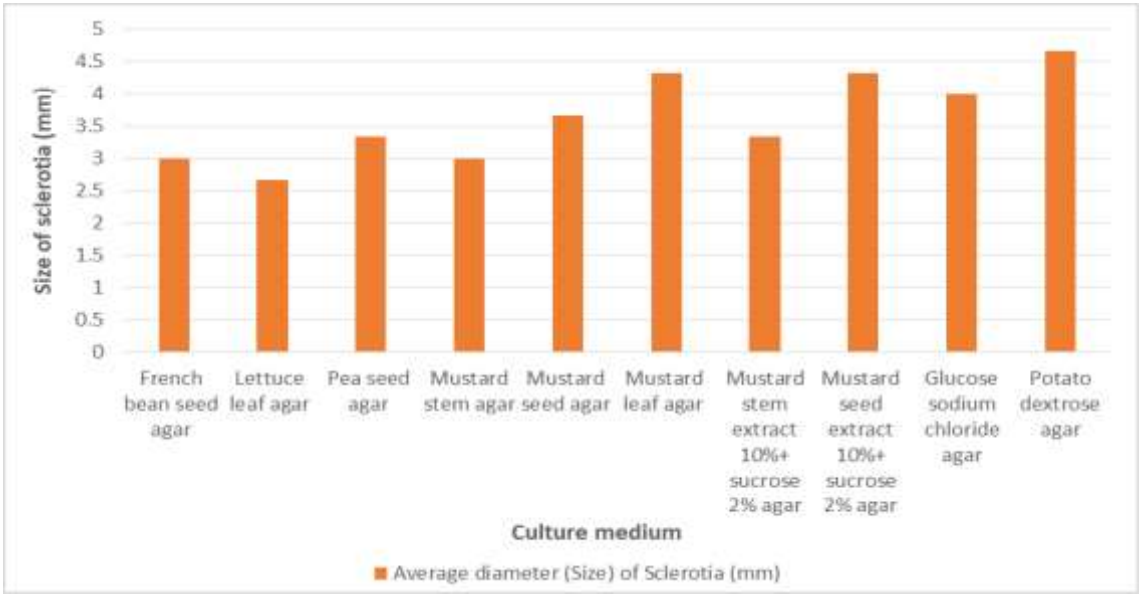


Fig 1: Evaluation of crop based locally developed media for the growth and sclerotial formation of *Sclerotinia sclerotiorum.*



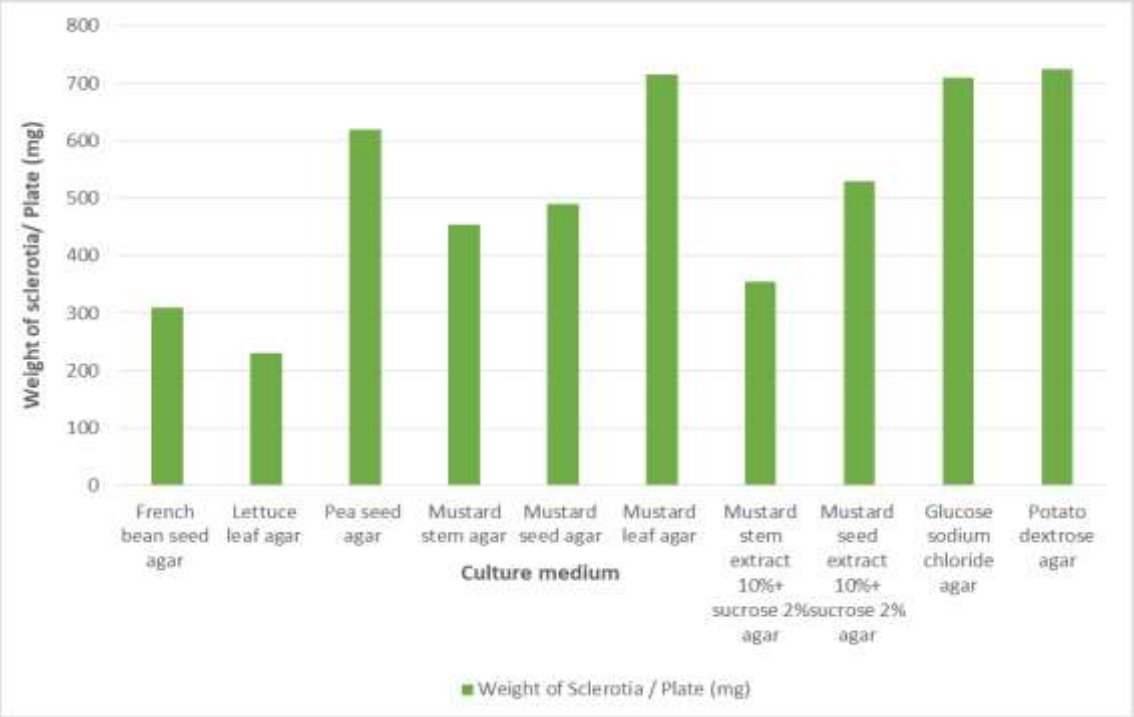


Fig 2: Evaluation of crop based locally developed media for the growth and sclerotial formation of *Sclerotinia sclerotiorum.*

Conclusion

Evaluation of all the culture medium, Potato dextrose agar medium was recorded maximum mycelia growth and sclerotia formation rather than, rest medium. It is also reported that low relative humidity does not support the mycelia growth and sclerotia formation of *S. sclerotiorum* under *In-vitro* studies; hence PDA is most suitable media for mycelia growth and sclerotia formation of the *Sclerotinia sclerotiorum*.

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