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Screening and *in-vitro* comparative evaluation of different isolates of *Sclerotinia sclerotiorum* under five selective media

Pramod Kumar Fatehpuria, RK Pandya, RS Sasode, JK Patidar and Reeti Singh

Abstract
The aim was to find out best media to support the maximum mycelial growth of *Sclerotinia sclerotiorum*, with keeping this view in mind present experiment was conducted at Department of Plant Pathology, College of Agriculture, RVSKVV, and Gwalior Madhya. Pradesh. The five selected culture media were tested *viz*., Potato dextrose agar, Glucose sodium chloride agar, Beetroot dextrose agar, Richard's agar, and Carrot dextrose agar, Corn meal agar, were evaluated against seven *Sclerotinia sclerotiorum* isolate of grid zone of M.P. The radial fungal growth recorded from 7th days after inoculation. The experiment was laid out in Complete Randomize Block Design with three replications. The maximum growth of all the isolates at 7 days after inoculation was recorded. In Glucose agar media, in which the maximum growth was recorded in Morena isolates (84.67 mm) followed by Guna (79.67 mm), Bhind (78.00 mm), Gwalior (77.67 mm), Sheopur (68.33 mm) and Ashoknagar (67.33 mm), while minimum growth was recorded in Shivpuri (64.00mm) as for as the mean performance of all the isolate concerned against the five selective media. The glucose media support the maximum growth pf Sclerotinia sclerotiorum. The average growth of pathogen was also compared on different media, the results revels that the average maximum growth was recorded in Morena isolate, while minimum growth in Shivpuri.

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

Introduction
Rapeseed-mustard group of crops are the major *Rabi* oilseed crops of India. The group is mainly constituted by *Brassica juncea B. napus B. rapa and B. carinata*. The area, production and productivity of rapeseed-mustard in the country was 7.06 million ha, 7.23 million tonnes and 1025 kg/ha, respectively (Anon 2016-17) [1]. Among rapeseed-mustard genotypes Indian mustard is the major crop of the India, occupying more than 80% of the total rapeseed-mustard. Major mustard growing states are Rajasthan, Gujarat, Maharashtra, Madhya Pradesh, Karnataka and Andhra Pradesh are the major rapeseed-mustard growing states of the country. *B. juncea* is the most important oilseed crop of grid zone of Madhya Pradesh comprising Morena, Bhind, Gwalior, Sheopur, Guna, Shivpuri and Ashoknagar districts contributing more than 80% share in the production of this crop in the state.

Sclerotinia sclerotiorum (Lib) De Bary is the causal fungus of Sclerotinia stem rot of rapeseed-mustard is a soil borne necrotrophic pathogen with worldwide distribution known to infect over 400 species of plants (Boland and Hall, 1994) [3]. This fungus can cause systemic and aerial infection by myceliogenic and carpogenic 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 appear on the stem which later on covered by whitish cottony mycelial growth of the fungus while later turn to black colour as hardened sclerotia. Moreover, no specific management practices have been recommended for Sclerotinia stem rot except some cultural practices and chemical control which are less effective because of soil borne nature and wide host range of the pathogen. Therefore, a comprehensive management approach has to be evolved to manage this disease and its rapid spread.

Method and Materials

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. mycelial disc of five day old young vigorous grown pure culture cut with help of corn borer and placed in the centre of the plate. The inoculated plates were incubated at 25± 1°C.Each treatment was replicated thrice.

Radial fungal growth of pathogen in five selective culture medium.

The different culture media were evaluated for obtaining maximum fungal growth of *S. sclerotiorum*. The experiment was laid out in complete randomized block design with three replication. Five selective culture media are use in study to test virulence *viz.*, Richard agar, Carrot dextrose agar, Beetroot dextrose agar, Glucose sodium chloride agar and Potato dextrose agar used as a Control to compare the fungal mycelia growth of *S. sclerotiorum*. The culture medium was prepared by the standardized method and autoclaved at 121.6 °C, 15 psi pressure for twenty minutes. The twenty milliliters quantities of each medium were poured in 90 mm diameter of Petri dish. Each Petri dish was inoculated separately with uniform mycelium disc of 7 mm diameter cut with the help of Cork borer from young 5 days vigorously growing pure culture of seven different *Sclerotinia sclerotiorum* isolate of grid zone of M.P. were placed on the middle of the each pre poured medium and incubated at 25±1oC (Dela Paz *et al*., 2006) [4]. Each treatment was replicated in three times. The diameter of the growth of the fungus was measured in 7 days after inoculation and radial growth of mycelium was calculated in mm with the help of measuring scale and data were recorded.

Results

Seven isolates of *Sclerotinia sclerotiorum* were collected from seven district of grid zone of M.P. *viz.*, Ashoknagar, Bhind, Gwalior, Sheopur, Guna, Shivpuri and Morena. To study in five selective media *viz.*, Carrot dextrose agar media, Beetroot dextrose agar media, Richard agar media, Glucose agar media and Potato dextrose agar media. The data summarized in (Table-1) revels that the maximum growth of all the isolates at 7 days after inoculation was recorded. In Glucose agar media, in which the maximum growth was recorded in Morena isolates (84.67 mm) followed by Guna (79.67 mm), Bhind (78.00 mm), Gwalior (77.67 mm), Sheopur (68.33 mm) and Ashoknagar (67.33 mm), while minimum growth was recorded in Shivpuri (64.00mm). In Glucose agar media, the Morena isolates was significantly superior over the remaining isolates Guna isolate was significantly superior over Sheopur, Ashoknagar and Shivpuri isolate, while it was statically at par with Bhind and Gwalior isolate. Bhind and Gwalior isolates where significantly superior over Sheopur, Ashoknagar and Shivpuri isolate. The isolates of Sheopur and Ashoknagar where significantly superior over Shivpuri.

On mean bases the next effective medium was Potato dextrose agar medium followed by Richard's agar medium and Beetroot dextrose agar medium, whereas the Carrot dextrose agar was found least effective. The Morena isolate also showed maximum growth in Potato dextrose agar medium (75.00 mm) followed by Gwalior (72.67 mm), Ashoknagar (65.33 mm), Bhind (64.67 mm),Sheopur (62.00 mm), Guna (60.33 mm), while minimum growth was recorded in Shivpuri (55.67 mm) isolate. In Richard's agar medium again Morena isolates showed maximum growth (77.00 mm) followed by Bhind (64.67 mm), Gwalior (64.33 mm), Sheopur (62.67 mm), Guna (56.00 mm) and Shivpuri (54.00 mm), while the maximum growth was recorded in Ashoknagar (53.00 mm).

Table 1: Evaluation of selective culture media for the growth of seven isolate of *Sclerotinia sclerotiorum*

Districts	Fungal diameter (in mm)					
	Carrot dextrose agar	Beetroot dextrose agar	Richards agar	Glucose agar	Potato dextrose agar	Mean
Ashoknagar	32.67	42.33	53.00	67.33	65.33	52.13
Bhind	41.67	58.33	64.67	78.00	64.67	61.46
Gwalior	45.33	59.67	64.33	77.67	72.67	63.93
Sheopur	42.00	52.67	62.67	68.33	62.00	57.53
Guna	31.33	43.33	56.00	79.67	60.33	54.13
Shivpuri	37.67	49.33	54.00	64.00	55.67	52.13
Morena	51.67	58.67	77.00	84.67	75.00	69.40
Mean	40.33	52.04	61.66	74.23	65.09	58.67
Sem±	1.15	2.44	2.03	1.52	2.98	
CD at 5%	3.56	7.48	6.23	4.66	9.13	

* Mean of three replications.

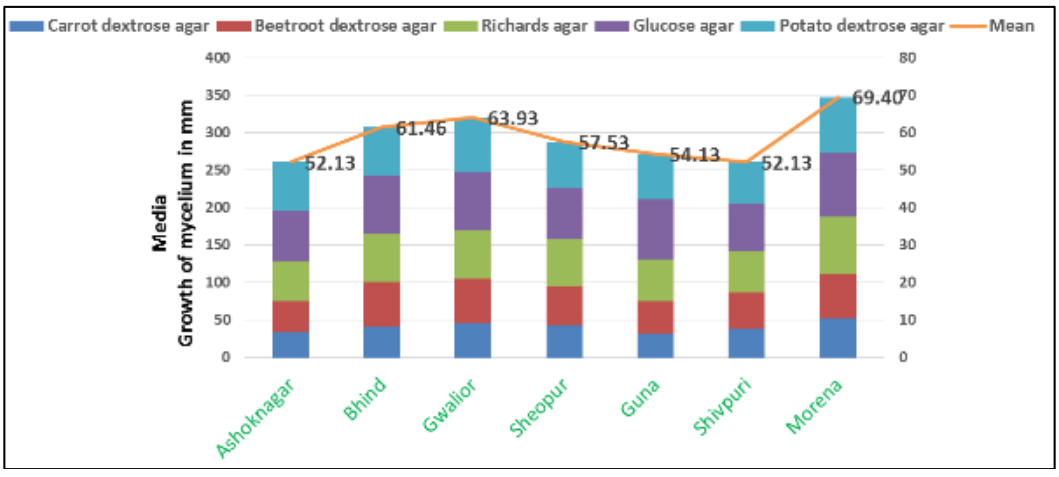

Fig 1: Evaluation of selective culture media for the growth of seven isolate of *Sclerotinia sclerotiorum*

the mean performance of all the seven isolates of *Sclerotinia sclerotiorum* in the five selective media was analyzed and present in (Table-2) which reveals that the average maximum growth was found in Glucose agar (73.99 mm) followed by Potato dextrose agar (65.05 mm), Richards agar (61.73 mm) and Beetroot dextrose agar (52.09 mm), while the carrot dextrose agar media was found least suitable as it give only (40.33 mm) growth. The Glucose agar medium was significantly superior over all the remaining four tested media. Potato dextrose agar medium was significantly superior over carrot dextrose and beetroot dextrose agar medium, while it was statistically at pat with Richards agar media. Richards's agar medium was also significantly superior over carrot and Beetroot dextrose agar. Through the Beetroot dextrose agar medium was found less suitable for the culture of Sclerotinia sclerotiorum but it was significantly superior over carrot dextrose agar medium.

Table 2: Comparative performance of selective media for the growth of *Sclerotinia sclerotiorum*

S.no	Culture media	Media designated	Mean
1	Carrot Dextrose agar	M1	40.33
2	Beetroot Dextrose agar	M2	52.09
3	Richard's agar	M3	61.73
4	Glucose agar	M4	73.99
5	Potato Dextrose agar	M5	65.05
	Sem±		1.085
	CD at 5%		3.465

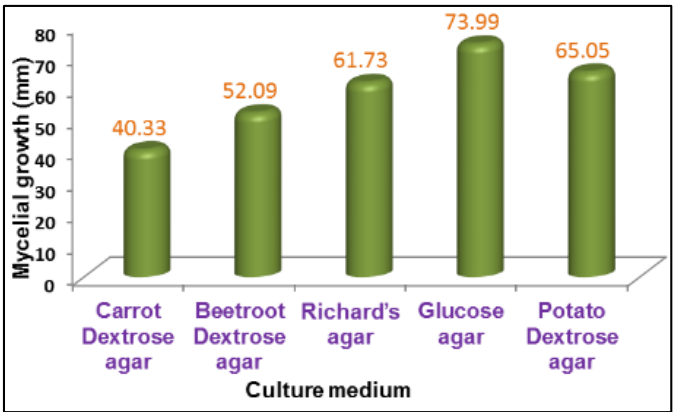

Fig 2: Performance of selective media for the growth of *Sclerotinia sclerotiorum*

Seven isolates of *Sclerotinia sclerotiorum viz.*, Ashoknagar, Bhind, Gwalior, Sheopur, Guna, Shivpuri and Morena were grown in five selective culture media *viz.*, Carrot dextrose agar, Beetroot dextrose agar, Richards agar, glucose agar and Potato dextrose agar and where compared on the bases of average growth in all the five media at 7 days after inoculation. The data summarized in (Table-3) revels that the average maximum growth was obtained in Morena isolate (69.40 mm) followed by Gwalior (63.93 mm), Bhind (61.07 mm), Sheopur (58.00 mm), Guna (54.13 mm) and Ashoknagar (52.13 mm), while the minimum growth was recorded in Shivpuri (52.00 mm) in respect of growth the Morena isolate was significantly superior over all the remaining isolate. The Gwalior and Bhind isolate where statistically at par to each other but where significantly superior over Shivpuri, Ashoknagar, Guna and Sheopur isolates. Sheopur isolate was significantly superior over Shivpuri Ashoknagar and Guna isolates. Shivpuri, Ashoknagar and Guna isolate where statistically at par at each other.

Table 3: Comparative growth performance of *Sclerotinia sclerotiorum* isolates in five selective media

S.no	Districts	Isolate designated	Mean
1.	Ashoknagar	SS_1	52.13
2.	Bhind	SS_2	61.07
3.	Gwalior	SS_3	63.93
4.	Sheopur	SS_4	58.00
5.	Guna	SS_5	54.13
6.	Shivpuri	SS_6	52.00
7.	Morena	SS_7	69.40
	Sem±		1.004
	CD at 5%		3.075

* Mean of three replications.

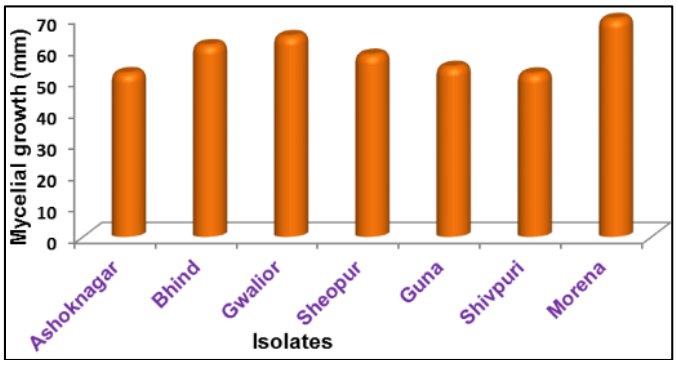

Fig 3: Performance of *Sclerotinia sclerotiorum* isolates in five selective media

Discussion

Among the five selected culture media for the evaluation of *Sclerotinia sclerotiorum*. The maximum fungal growth after 7DAI was recorded in glucose agar media in which the maximum growth was recorded in Morena isolate followed by Guna, Bhind, Gwalior, Sheopur and Ashoknagar while the least fungal growth was recorded in Shivpuri. The next effective media was potato dextrose agar followed by Richards's agar and beetroot dextrose agar media whereas carrot dextrose agars are least effective. In potato dextrose agar media again the Morena isolate show the maximum growth followed by Gwalior, Ashoknagar, Bhind, Sheopur and Guna with the minimum growth was recorded in Shivpuri isolate.

The analysis of mean performance of all seven isolate again the five selective media revels that the average maximum growth was recorded in glucose agar followed by potato dextrose agar, Richards agar, beetroot dextrose agar and carrot dextrose agar with the minimum growth in carrot dextrose agar media.

The comparative performance of selective media for the growth of *Sclerotinia sclerotiorum* shows that average growth was maximum in Morena isolate followed by Gwalior, Bhind, Sheopur, Guna and Ashoknagar with the minimum in Shivpuri.

Similar result found by Panchal *et.al*, (2012) [8] who also found glucose sodium chloride agar media supported the maximum growth of the fungus. Khan 1976 and Sharma 1979 also found potato dextrose agar media suitable for growth of Sclerotinia sclerotiorum and production maximum number of sclerotia. The results are in accordance the finding of Fatehpuria *et.al.* 2017 reported that potato dextrose as a semi solid media gave maximum radial mycelia growth. Kirsanov, G.P. *et.al*. 1974 supported that solid and semi-solid liquid media having hydrolysate with 5% Nacl and 3% glucose gave best growth of *Sclerotinia sclerotiorum*. Similarly Jani (1990)

[7]. Stated the best growth and sclerotial formation of fungus was found glucose sodium chloride agar and Richards's agar liquid medium. Bharti *et.al*. (2016), Elgorban et.al. (2012), reported the maximum growth of fungus in potato dextrose agar media thus the present finding are in agreement with the finding of above research worker.

Summary

The maximum growth of *Sclerotinia sclerotiorum* was found in glucose agar media. The maximum average growth was obtained in Morena isolate with was significantly superior over all the isolates.

Conclusion

The evaluated all the selective culture media, glucose agar media was recorded maximum radial growth rather than the other medium. On the basis of mean performance of isolate also the glucose agar media was proved to be best for the growth of tested fungus culture of *Sclerotinia sclerotiorum*. Similarly on the basis of mean performance of different isolate against the selective media the Morena isolate gave the better growth performance.

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