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Cultural and morphological variability among the isolates of *Sclerotium rolfsii* Sacc. Causing collar rot of soybean

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Abstract
Sixteen different pathogenic isolates of *Sclerotium rolfsii* collected from five different major soybean growing regions of the country were subjected for variability studies. The cultural and morphological variation was observed among the sixteen isolates of the pathogen. The results revealed that all the isolates showed variation with respect to time taken for complete growth *i.e*., some isolates were fast growing and some were slow growing, colour, texture and margin of the colony. Morphological variations with respect to sclerotial characters also varied among the isolates *viz*., time take for sclerotial production, size, shape, colour, number and test weight of sclerotial bodies and among the ten solid media tested, potato dextrose agar was the best media for good growth ad sclerotial production.

Keywords: Soybean, variability, *Sclerotium rolfsii*, collar rot

Introduction
Glycine max (L.) Merrill commonly known as "Soybean" is one of the important oilseed and pulse crops of India. Major soybean producing countries are USA, Brazil, Argentina, China and India. In India the crop covers an area of 12.20 m.ha with a production of 11.90 m.t and a productivity of 991 kg/ha. The state Madhya Pradesh, has the lions share in soybean production, is often referred as “Soya State” followed by Maharashtra, Rajasthan, Karnataka and Telangana. In Karnataka, soybean is grown over an area of 3.80 lakh hectare with a production of 3.82 lakh tonnes and productivity of about 1005 kg/ha (Anon., 2022) [1].
Despite of having a large area under soybean cultivation, India still suffers in productivity mark when compared to world productivity. A loss of more than seven million tonnes of soybean was reported in the world due to diseases alone (Sinclair, 1988) [7]. Diseases are the main constraints hindering production and productivity reports of soybean in India. Soil-borne plant pathogens are the potential threats that affect soybean yield and quality as it knocks down the plant in all the stages of crop growth. Among the soil borne pathogens, collar rot caused by *Sclerotium rolfsii* Sacc. is gaining importance in recent years due to climate change. *Sclerotium rolfsii* survives in soil as a Saprotrophic and attack living plants. It is a soil-borne, facultative parasite and Omni pathogenic organism which occurs worldwide and infects more than 500 plant species. There was no systematic study on cultural and morphological variability of *Sclerotium rolfsii* in major soybean growing regions of the country. Hence, the present study was carried out to understand the variability in cultural morphology, sclerotium formation, their shape, size and test weight of the sclerotial bodies. This study also concentrates to know the better media for the growth and sclerotial production of the pathogen.

Material and Methods
Collection of infected samples and isolation of the fungus
The symptoms showing collar rot portion o host were collected from sixteen different regions and infected plant parts were subjected for standard tissue isolation. Infected plant parts from the field were cut into several bits of 5 mm including the advancing margins of infection. The segments were surface disinfected in 0.1% sodium hypochlorite solution for 1 min and rinsed separately three times changes in sterile water to remove the traces of chemicals present on the infected portion and such bits taken out separately, dried on sterile tissue paper and transferred to sterile Petri plates containing sterilized solidified Potato Dextrose Agar (PDA) and incubated for 7 days at 28±1 ºC.

The initiated growth from the incubated plates was observed and separated on to other Petri dishes contain PDA and again such plates were incubated for 7 days at 28±1 ^{0}C and maintained them in slants, which were stored in refrigerator at 4 ^{0}C and they were sub-cultured periodically once in a month for further studies.

Cultural and morphological characteristics of *Sclerotium rolfsii* on different solid media

Cultural and morphological characteristics of *Sclerotium rolfsii* on ten different solid media *viz*., potato dextrose agar, potato carrot agar, V8 juice agar, Czapeck's Dox agar, tryptic soya agar, oat meal agar, nutrient agar, starch casein agar, Richard's agar and Sabouraud's dextrose agar was studied. To carry out the study, all the media were prepared and sterilized at 1.1 kg cm^{-2} pressure and 121 ^{0}C for 15 min. Twenty ml of each of the medium was poured in 90 mm Petri plates. Such Petri plates were inoculated with five mm disc which was taken from periphery of actively growing seven days old culture and incubated at 28 ±1 ^{0}C. Each treatment was replicated thrice. Observations were taken when the fungus covered Petri plate completely in any one of the media. Observations on colony diameter, colour, texture, growth nature and days taken for complete growth were recorded. The data on radial growth was analysed statistically (Gawande *et al*., 2013) [2].

Cultural and morphological variability

To study the cultural and morphological characters of the isolates, a five millimetre disc from margins of actively growing, five day old colony of each isolate was inoculated in the centre of Petri plate (85 mm diameter) containing PDA and kept for incubation at 28±1 ^{0}C. Each isolate was replicated three times. The different cultural and morphological parameters like mean colony diameter, colony colour and margin of the colony were recorded at seven Days After incubation (DAI) and the sclerotial characters such as days taken for sclerotia initiation, colour and number of sclerotia per plate was recorded (Manu *et al*., 2018) [4].

Results and Discussion

Sclerotium rolfsii from infected collar portion of soybean crop was isolated and designated as per the place of their collection. The isolated fungus was identified as *Sclerotium rolfsii* based on the cultural and morphological characters and were subjected for variability studies. The results obtained are presented in Table 1-4.

Cultural characteristics of *Sclerotium rolfsii* on different solid media

The maximum mean radial growth of the colony was observed on potato dextrose agar (85.00 mm) and the minimum was observed in Sabouraud's dextrose agar (31.00 mm). Days taken for complete growth (85 mm diameter) varied from 3-6. Margin of the colony was regular in all the media except in Richard's agar. Sabouraud's dextrose agar and Nutrient agar where the margin was irregular. The results are in confirmation with Sangeetha and Shamarao Jahagirdar (2013) [6], Zape *et al*. (2013) [9] and Sivakumar *et al*. (2016) [8] who reported that the most suitable media for the better growth and sclerotial production of the pathogen were potato dextrose agar and Czapeck's Dox agar (Table 1).

The variability among the isolates of *S. rolfsii* was studied with respect to cultural and morphological characters. Days taken for complete growth (85 mm diameter) varied from 3-5. Among the sixteen isolates, four isolates (KADh 7, KABe 9, KABe 10 and KaBe 11) took three days, ten isolates (MPSe 1, MPSe 2, MHSa 3, AMJo 4, KADh 5, KADh 8, KaBa 12, KaHa 13, KADh 14 and KABi 16) took four days and two isolates (KADh 6 and KABe 15) took five days for complete growth (85 mm diameter). Colour of the colony was categorised in to two groups *viz*., pure white and dull white. Eleven isolates (MPSe 1, MPSe 2, AMJo 4, KADh 5, KADh 6, KADh 8, KABe 9, KaBe 11, KaBa 12, KABe 15 and KABi 16) were pure white in colour and five isolates (MHSa 3, KADh 7, KABe 10, KaHa 13 and KADh 14) were dull white in colour. Raised type of colony growth was observed in thirteen isolates, whereas flat type of growth was observed in remaining three isolates. Margin of the colony was regular in thirteen isolates (MHSa 3, AMJo 4, KADh 5, KADh 6, KADh 7, KADh 8, KABe 9, KABe 10, KaBe 11, KaHa 13, KaBa 12, KABe 15 and KABi 16) and irregular in three isolates (MPSe 1, MPSe 2 and KADh 14). Raised type of colony growth was observed in thirteen isolates (MPSe 1, MPSe 2, MHSa 3, AMJo 4, KADh 5, KADh 6, KADh 7, KADh 8, KaBe 11, KaHa 13, KADh 14, KABe 15 and KABi 16), whereas flat type of growth was observed in remaining three isolates (Table 2, 4 and Fig. 1).

Table 1: Cultural and morphological characteristics of *Sclerotium rolfsii* on different growth media

Sl. No.	Media	Morphological characters					
		Radial growth of mycelium (mm)	Colour of colony	Texture of colony	Margin of colony	Growth nature	Days taken for complete growth (90 mm diameter)
1	Potato dextrose agar	85.00 (67.21)*	Pure white	Smooth	Regular	Raised	3
2	Potato carrot agar	82.00 (64.90)	Dull white	Coarse	Regular	Raised	3
3	V8 juice agar	80.00 (63.43)	Pure white	Smooth	Regular	Raised	3
4	Czapeck's Dox agar	75.00 (60.00)	Dull white	Coarse	Regular	Raised	4
5	Tryptic soya agar	71.00 (57.42)	Pure white	Smooth	Regular	Raised	4
6	Oat meal agar	61.00 (51.35)	Pure white	Smooth	Regular	Raised	4
7	Nutrient agar	54.00 (47.29)	Dull white	Coarse	Irregular	Flat	5
8	Starch casein agar	39.00 (38.65)	Dull white	Coarse	Regular	Flat	6
9	Richard's agar	37.00 (37.46)	Pure white	Smooth	Irregular	Flat	6
10	Sabouraud's dextrose agar	31.00 (33.83)	Dull white	Coarse	Irregular	Flat	6
S. Em. ±		0.54					
C.D. @ 1%		2.17					

*Angular transformed values

Table 2: Cultural and morphological variability among the isolates of *Sclerotium rolfsii* Sacc.

Sl. No.	Place of collection	State	Isolates designation	Colony characters				
				Days taken for complete growth (85 mm diameter)	Colour	Growth nature	Margin	Texture
1	Sehore	Madhya Pradesh	MPSe 1	4	Pure white	Raised	Irregular	Smooth
2	Sehore	Madhya Pradesh	MPSe 2	4	Pure white	Raised	Irregular	Coarse
3	Sangli	Maharashthra	MHSa 3	4	Dull white	Raised	Regular	Smooth
4	Jorhat	Assam	AMJo 4	4	Pure white	Raised	Regular	Smooth
5	MARS, Dharwad (Dharwad)	Karnataka	KADh 5	4	Pure white	Raised	Regular	Smooth
6	Naredra (Dharwad)	Karnataka	KADh 6	5	Pure white	Raised	Regular	Coarse
7	Garag (Dharwad)	Karnataka	KADh 7	3	Dull white	Raised	Regular	Smooth
8	Kalaghatagi (Dharwad)	Karnataka	KADh 8	4	Pure white	Raised	Regular	Smooth
9	Kittur (Belagavi)	Karnataka	KABe 9	3	Pure white	Flat	Regular	Coarse
10	Chikkodi (Belagavi)	Karnataka	KABe 10	3	Dull white	Flat	Regular	Smooth
11	Ugar Khurd (Belagavi)	Karnataka	KABe 11	3	Pure white	Raised	Regular	Coarse
12	Bagalkote	Karnataka	KABa 12	4	Pure white	Flat	Regular	Smooth
13	Haveri	Karnataka	KAHa 13	4	Dull white	Raised	Regular	Smooth
14	MARS, Dharwad (Dharwad)	Karnataka	KADh 14	4	Dull white	Raised	Irregular	Smooth
15	Hirebagewadi (Belagavi)	Karnataka	KABe 15	5	Pure white	Raised	Regular	Smooth
16	Bidar	Karnataka	KABi 16	4	Pure white	Raised	Regular	Smooth

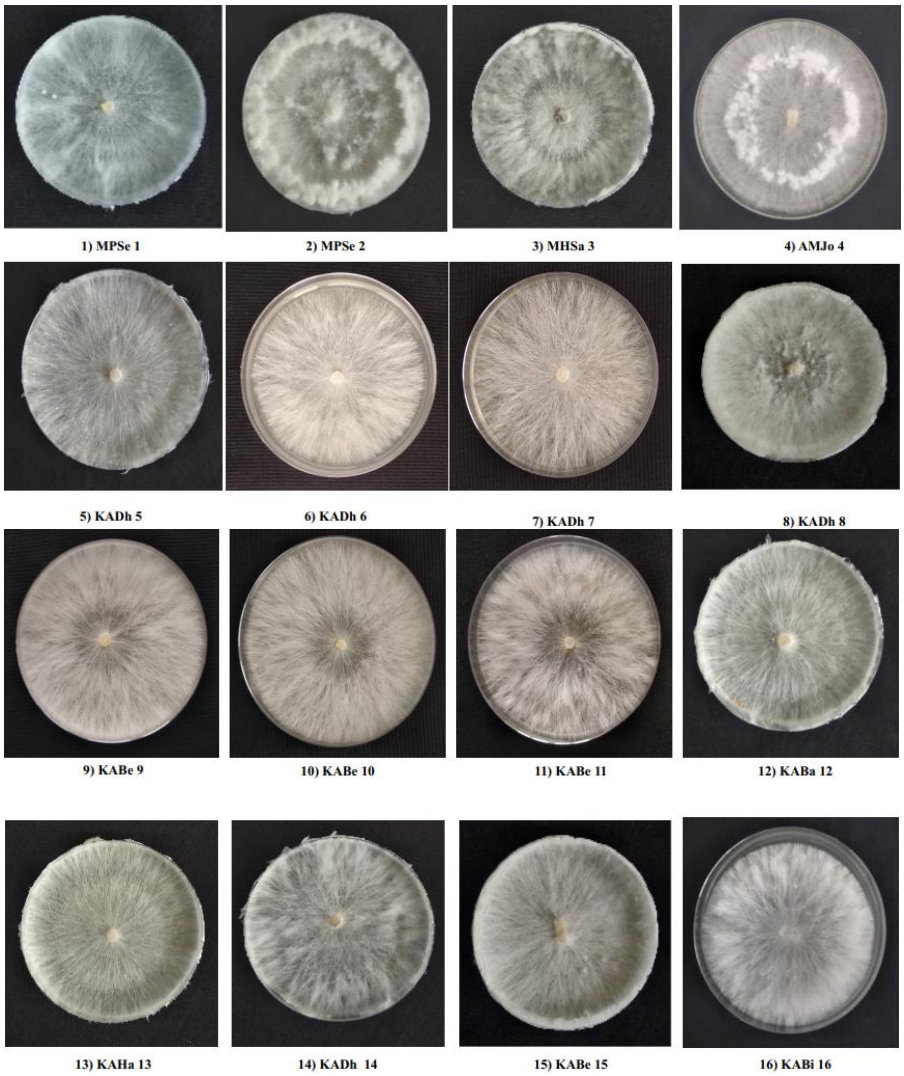

Fig 1: Cultural and morphological variability among the isolates of *Sclerotium rolfsii* Sacc

Morphological variability

Variation in morphology of sclerotial bodies among the isolates of *Sclerotium rolfsii viz*., shape, size, colour, diameter, test weight, number of sclerotial bodies/cm^2 and days taken to form sclerotial bodies were recorded. The isolates showed variation with respect to time taken for sclerotial formation. Days taken to form sclerotial bodies varied from 9 to15. Out of sixteen isolates, nine isolates (MHSa 3, KADh 5, KADh 6, KADh 8, KABe 9, KaBe 11, KaHa 13, KADh 14 and KABi 16) showed scattered arrangement of sclerotial bodies, three isolates (KABe 10, KaBa 12 and KABe 15) showed peripheral arrangement and

four isolates (MPSe 1, MPSe 2, AMJo 4 and KADh 7) showed sclerotial arrangement both at the centre and periphery. The shape of the sclerotial bodies varied from round to oval and colour varied from light brown to dark brown. The size of sclerotial bodies was considerably varied and they were grouped into three categories based on mean diameter of 25 sclerotial bodies from each isolate. Five isolate (KADh 5, KADh 6, KADh 7, KABe 10 and KaHa 13) were having less than 1.00 mm in diameter and grouped as small, eight isolates (MHSa 3, KADh 8, KABe 9, KaBe 11, KaBa 12, KADh 14, KABe 15 and KABi 16) were having 1.00-1.20 mm diameter whereas three isolates (MPSe 1, MPSe 2 and AMJo 4) were having more than 1.2 mm diameter and grouped as large size (Table 3, 4 and Fig. 2).

These results are in accordance with the work of Prabhu (2003) [5], Jyothi (2006) [3] and Manu *et al*. (2018) [4]. Prabhu (2003) [5] reported that the growth rate was between 65 to 85 mm at 3rd day of inoculation among the isolates. Number of sclerotia per cm^2 of culture was in the range of 3.20 to 9.90. Manu *et al*. (2018) [4] observed variation with respect to colony diameter, colour of the mycelium, shape, size and colour of sclerotia among the isolates of *Sclerotium rolfsii* isolated from collar/stem portions of soybean. By these results it was concluded that variations was there in different *Sclerotium* isolates and these variations depends on the geographical location of the isolate from where it has collected.

Table 3: Variability among the isolates of *Sclerotium rolfsii* Sacc. for sclerotial characters

Sl. No.	Isolates	Sclerotial characters						
		Days taken to produce sclerotial bodies	Distribution pattern	Colour	Shape	Diameter (mm)	Number of sclerotial bodies/cm^2	Weight of 100 sclerotial bodies (mg)
1	MPSe 1	14	Centre and periphery	Light brown	Round	1.25	4.90	155.20
2	MPSe 2	11	Centre and periphery	Light brown	Round	1.21	7.67	175.20
3	MHSa 3	10	Scattered	Dark brown	Round	1.19	7.82	90.03
4	AMJo 4	9	Centre and periphery	Light brown	Round	1.32	2.43	188.30
5	KADh 5	11	Scattered	Light brown	Round	0.98	3.95	140.25
6	KADh 6	12	Scattered	Dark brown	Round	0.66	9.21	63.00
7	KADh 7	8	Centre and periphery	Dark brown	Round	0.76	8.82	108.40
8	KADh 8	15	Scattered	Dark brown	Round	1.16	2.97	145.78
9	KABe 9	13	Scattered	Light brown	Oval	1.01	5.20	129.50
10	KABe 10	12	Periphery	Light brown	Round	0.85	7.32	88.45
11	KABe 11	14	Scattered	Dark brown	Round	1.04	4.31	56.81
12	KABa 12	12	Periphery	Light brown	Round	1.01	5.16	142.33
13	KAHa 13	14	Scattered	Light brown	Oval	0.96	4.22	136.50
14	KADh 14	10	Scattered	Light brown	Round	1.02	8.14	82.26
15	KABe 15	15	Periphery	Light brown	Round	1.11	7.52	133.05
16	KABi 16	13	Scattered	Dark brown	Round	1.12	8.11	118.70

Table 4: Grouping of isolates based on cultural and morphological characters of *Sclerotium rolfsii* Sacc.

Sl. No.	Characters		Number of isolates	Percentage of isolates
1.	Days taken for complete growth (85 mm diameter)	3	04	25.00
		4	10	62.50
		5	02	12.50
2.	Colony colour	Pure white	11	68.75
		Dull white	05	31.25
3.	Growth nature	Raised	13	81.25
		Flat	03	18.75
4.	Margin of colony	Regular	13	81.25
		Irregular	03	18.75
5.	Texture of colony	Smooth	12	75.00
		Coarse	04	25.00
6.	Days taken to produce sclerotial bodies	8-10	04	25.00
		11-13	07	43.75
		14-16	05	31.25
7.	Growth pattern of sclerotial bodies	Periphery	03	18.75
		Centre and periphery	04	25.00
		Scattered	09	56.25
8.	Shape of sclerotial bodies	Round	14	87.50
		Oval	02	12.50
9.	Colour of sclerotial bodies	Light brown	10	62.50
		Dark brown	06	37.50
10.	Size of sclerotial bodies	Small (less than 1.00 mm)	05	31.25
		Medium (1.00 to 1.20 mm)	08	50.00
		Large (more than 1.20 mm)	03	18.75
11.	Test weight of sclerotial bodies	50-100 mg	05	31.25
		100.1-200 mg	11	68.75

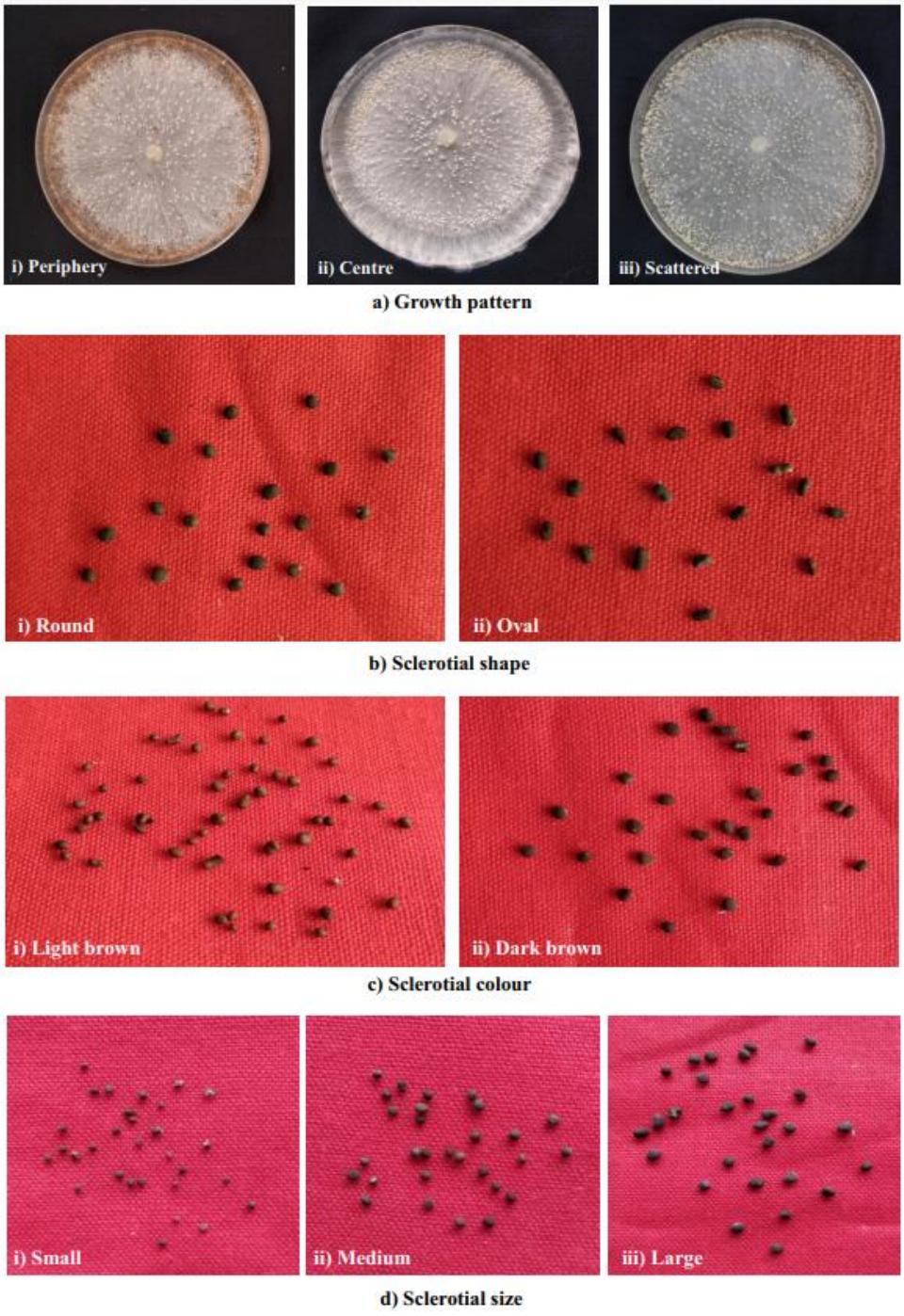

Fig 2: Variability in morphology of sclerotia among the isolates of *Sclerotium rolfsii* Sacc

Conclusion

The study clearly identified cultural variability among the isolates with respect to time taken for complete growth, colour, margin and texture of the colony. While morphological variability was with respect to time taken to form sclerotial bodies, colour, size, shape and test weight of sclerotial bodies.

References

1. Anonymous. Annual Report, All India Coordinated Research Project on Soybean. ICAR - IISR, Indore; 2021-22, p. 2-3.
2. Gawande SP, Borkar SG, Chimote VP. Variation in growth and oxalic acid production by different crop isolates of *Sclerotium rolfsii* Sacc. Journal of Mycopathological research. 2013;51(1):95-100.
3. Jyothi KC. Morphological and molecular variability among the isolates of *Sclerotium rolfsii* Sacc. From different host plant. M. Sc. (Agri.) Thesis, Univ. of Agric. Sci., Dharwad, Karnataka, India, 2006.
4. Manu TG, Nagaraja A, Manjunatha SV. Morphological and cultural variability among the *Sclerotium rolfsii* isolates. Journal of pharmacognosy and phytochemistry. 2018;7(1):904-907.
5. Prabhu HV. Studies on collar rot of soybean caused by *Sclerotium rolfsii* Sacc. M.Sc. (Agri.) Thesis, Univ. of Agric. Sci., Dharwad, Karnataka, India, 2003.
6. Sangeetha TV, Shamarao Jahagirdar. Variability of pathogens associated in causing root rot complex of soybean in Karnataka. Indian journal of plant protection.

2013;6:229-235.
7. Sinclair JB. Anthracnose of soybean P: 92-149. In: Soybean diseases of North Central Region (Eds. Wyllie, T. D. and Scott, D. H.). American Phytopathological Society, St. Paul, Minnesota, USA, 1988, P:104.
8. Sivakumar T, Sanjeevkumar K, Balabaskar P. Variability in *Sclerotium rolfsii* Sacc. Causing Stem rot of groundnut. Bulletin of Environment, Pharmacology and Life Sciences. 2016;2:92-99.
9. Zape AS, Gade AM, Singh R. Physiological studies on different media, pH and temperature on *Sclerotium rolfsii* isolates of soybean. Scholarly journal of agricultural science; c2013. p. 238-241.