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RESEARCH ARTICLE

Variability of pathogens associated in causing root rot/wilt of soybean in Northern Karnataka

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ABSTRACT

Soybean [*Glycine max* (L.) Merill.] is a protein rich oilseed crop. It is considered as a golden bean, miracle bean and wonder crop of the 20th century because of its characters and usage. In India, losses due to various diseases are estimated as 12 per cent of total production. The root trot/ wilt complex has become a major production constraint in Karnataka. Three pathogens are involved in causing root rot/wilt namely, *Sclerotium rolfsii*, *Rhizoctonia bataticola* and Fusarium sp.The association of these pathogens involving two or three varied from region to region. On the basis of morphological characters, the pathogens were identified as *Sclerotium rolfsii*. which produces the mustard sized sclerotial bodies, *Rhizoctonia bataticola* which produced brown to black, right angle branched mycelia with septa and *Fusarium* sp. which produced three kinds of spores *viz*., microconidia, macroconodia and chlamydospores. The results of physiological and morphological variations are discussed in this paper.

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INTRODUCTION

Soybean [*Glycine max* (L.) Merill.] is a protein rich oilseed crop. It is considered as a golden bean, miracle bean and wonder crop of the 20th century because of its characters and usage. In India, losses due to various diseases are estimated as 12 per cent of total production. The root trot/ wilt complex has become a major production constraint in Karnataka. Soybean is being attacked by more than 100 pathogens (Sinclair and Shurtleff, 1975). Plant pathogens exhibit variations in their morphological, biological and pathogenic characters. Although wilt complex disease has assumed economic importance in India and Karnataka, so far there is very limited information on these pathogens, etiology, epidemiology and management strategies of wilt complex. Looking into the magnitude of severity of this complex disease in Northern Karnataka we studied the associated causal agents and mapped out their association in different regions and also their role in penultimate death of the plant.The results are discussed in this paper.

MATERIAL AND METHODS

The investigations on isolation, identification and pathogenicity studies were conducted at Department of Plant Pathology, UAS, Dharwad during 2010. Soybean plants showing wilting symptoms were collected from different major soybean growing areas of Karnataka. The infected root portion was used for isolation. The isolation was made by following standard tissue isolation procedure. The infected specimens were cut into small bits and washed in running water. These bits were surface sterilized with 4 per cent of sodium hypochlorite solution for one minute, washed thoroughly with sterile distilled water for three times to remove the traces of sodium hypochlorite and then aseptically transferred to Petri plates containing the sterilized PDA medium. The plates were incubated at 27 ± 1^0 C three days for *Sclerotium* and *Rhizoctonia* and eight days for *Fusarium*. The fungal growth

which arose through the infected tissue was taken by inoculation loop and transferred aseptically to Pertiplates containing the sterilized PDA medium. The pure culture of the fungus was maintained by further growing the culture and following hyphal tip culture method under aseptic conditions (Rangaswami, 1972).For pathogenicity studies,the soybean seeds were sown in pots filled with sterile soil. Twenty days old seedlings were used for inoculation with one month old giant culture. Control treatment was maintained in which no inoculum was added. Treatments employed were : *S. rolfsii, Fusarium* sp., *Rhizoctonia* sp., *S. rolfsii* + *Fusarium* sp., *Fusarium* sp.+ *Rhizoctonia* sp, *S. rolfsii* + *Rhizoctonia* sp., *S. rolfsii* $_+$ *Fuarium* sp. + *Rhizoctonia* sp.Giant culture of *S. rolfsii, Rhizoctonia bataticola* and *Fusarium* sp. were inoculated separately and in combination in set of pots. The pots were maintained at 25 per cent moisture holding capacity. Observations were made every day on the development of wilt symptoms. When the plants showed wilt symptoms, such plants were carefully up rooted and the pathogens were reisolated by standard tissue isolation method. The pathogens were compared with original culture.

Growth characters of isolates on different solid media :

The growth characters of different isolates of *S.rolfsii, Rhizoctonia* sp. and *Fusarium* sp. were studied on different solid media.*viz.*,Potato dextrose agar,Czapek's -dox agar,Oat meal agar,Sabouraud's agar and Host extract agar.

All the media were sterilised at 1.1 kg/cm^2 pressure for 15 minutes. To carry out the study, 20 ml of each of the medium was poured in 90 mm Petriplates. Such Petriplate was inoculated with 5 mm disc cut from periphery of actively growing seven day old culture of the individual isolate grown on PDA in Petriplate and incubated at 27±1^0 C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete Petriplate in any one media. The colony diameter was recorded by averaging the radial growth of the colony in two directions. The data on radial growth were analyzed statistically.Sclerotium production measured as # + + + Excellent sclerotial production (>60 sclerotia / plate), + + Good sclerotial production (30 to 60 sclerotia / plate) and + Poor sclerotial production (<30 sclerotia / plate.

Growth phase :

The growth study was conducted on Potato dextrose broth .Twenty ml of the Potato dextrose broth was dispensed into each of 150 ml flasks. These flasks were sterilized at 1.1 kg/cm^2 pressure for 15 minutes. Each of the flasks was inoculated aseptically with five mm culture disc obtained from the growing periphery of seven day old culture of the fungus grown on PDA in petriplate. The flasks were incubated at 27±1°C. Each treatment was replicated thrice.

The culture was filtered through Whatman no.42 filter paper which was previously dried at 60^0 C in an electrical oven for three days to obtain constant weight. The mycelia mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with it. The filter paper along with the fungal mat was then dried in oven at 60^0 C for three days to obtain constant weight and weight of the fungal mat was recorded using analytical balance.

The above said procedure of harvesting and assessing the weight of the growth of the fungus was started from second day of incubation and continued with two days interval till 20th day of incubation since the weight of the fungal growth fall down after attaining the maximum growth on tenth day. Growth is expressed as the dry mycelia weight of the fungus.

Physiological studies :

Temperature requirement **:**

The spores of pathogenic agent were placed in cavity slides containing 2% sucrose solution and three replications were maintained. The different temperature regimes like 15, 20, 25, 30 and 40^0 C for 12 hours were used for inoculation. Further, sclerotia germinated per cavity slide were observed and per cent germination of sclerotia was calculated.

For this experiment Potato dextrose broth was used. The different temperatures tried for the growth of the fungi were 15, 20, 25, 30 and 40^0 C. For each treatment level, three replications were maintained. The flasks were inoculated at respective temperatures. The dry mycelial weight was recorded and results were analysed statistically. All the data were subjected for statistical analysis as per procedure of Sukhatme and Amble (1985).

RESULTS AND DISCUSSION

The results on various aspects of isolation, identification and morphological and physiological aspects are discussed in the following pages. Pathogenecity studies were conducted at two stages in order to ascertain the degree of association of all the three fungi in root rot complex.

Table 1 : Growth rates of fungi *Selerotium rolfsii, Rhizoctonia* sp. and *Fuarium* sp. cultured in different combinations on Potato dextrose agar

Incubation period	Colony diameter (mm)						
	S. rolfsii + *Rhizoctonia* sp.	*Rhizoctonia* sp. + *Fuarium* sp.	*S. rolfsii* + *Fuarium* sp.	*S. rolfsii* + *Rhizoctonia* sp. + *Fuarium* sp.	*S. rolfsii*	*Rhizoctonia* sp.	*Fuarium* sp.
4 days	30.00 + 46.66	43.33 + 30.66	48.66 + 33.33	26.33 + 38.66 + 14.66	81.60	86.00	58.33
6 days	36.00 + 55.66	55.55 + 35.33	54.44 + 31.00	30 + 42.33 + 18.66	90.00	90.00	74.66

Interaction of the pathogen on culture media :

The observations on interaction of three major pathogens are presented in Table 1. Initially no significant interaction was observed between the three fungi on media. However, after 4 days of incubation, 30 mm and 46.66 mm colony diameter was recorded in simultaneous inoculation of *S. rolfsii* + *Rhizoctonia* sp., respectively. A colony growth of 30.66 mm and 43.33 mm diameter was recorded when inoculated in combination of *Fusarium* sp. + *Rhizoctonia* sp., respectively. While in *S. rofsii* + *Fusarium* sp. colony growth of 33.33 mm and 48.66 mm was recorded, respectively and 38.66, 26.33, 14.66 mm diameter in simultaneous inoculation of *Rhizoctonia* sp. + *S. rolfsii*+ *Fusarium* sp. recorded, respectively when compared to 81.66, 86, 58.33 mm in *S. rolfsii, Rhizoctonia* sp and *Fusarium* sp. alone, respectively. While after 6 days of incubation, 36 mm and 55.66 mm colony diameter was recorded in simultaneous inoculation of *S. rolfsii* + *Rhizoctonia* sp. respectively. A colony growth of 35.33 mm and 55.55 mm diameter was recorded in when inoculated in combination of *Fusarium* sp. + *Rhizoctonia* sp., respectively. While in *Fusarium* sp. + *Rhizoctonia* sp. colony growth of 31 mm and 54.44 mm was recorded, respectively and 42.33, 30, 18.66mm diameter in simultaneous inoculation of *Rhizoctonia* sp.+ *S. rolfsii*+ *Fusarium* sp. recorded, respectively, when compared to 90, 90, 74.66 mm in *S. rolfsii, Rhizoctonia* sp. and *Fusarium* sp. alone, respectively. *Rhizoctonia* sp. overgrew *S. rolfsii* and *Fusarium* sp. to some extent.

Inoculation of pathogenic fungi on soybean seedlings :

The pathogenicity studies were conducted in glasshouse on susceptible cultivar JS-335. The data on percentage of diseased plants inoculated with the all the pathogens are presented in the Table 2. After 20 days of inoculation of the giant culture of the different pathogens in isolation and also in combination, revealed that maximum per cent disease incidence in case of dual inoculation of *S. rolfsii* + *Rhizoctonia* sp. (83.33%) and mixed inoculation of *S. rolfsii* + *Rhizoctonia* sp.+ *Fusarium* sp. (83.33%) followed by dual inoculation of *S. rolfsii* + *Fusarium* sp. and *Fusarium* sp.+ *Rhizoctonia* sp. (75 %). The pathogens alone also recorded more than 50 per cent disease incidence. Maximum per cent incidence in single inoculation of *S. rolfsii* and *Fusarium* sp. with 66.66 per cent. Least disease incidence was recorded in *Rhizoctonia* sp. (58.33 %). Root length (root rot index)

Table 2 : Interaction effect between *Selerotium rolfsii, Rhizoctonia* sp. and *Fusarium* sp. on inoculated soybean seedlings

Treatments	Per cent disease incidence	Root length rotted (cm)
S. rolfsii	66.66	3.08
Fusarium sp.	66.66	4.08
Rhizoctonia sp.	58.33	2.87
S. rolfsii + *Fusarium* sp.	75.00	4.33
Fusarium sp.+ *Rhizoctonia* sp.	75.00	4.15
S. rolfsii + *Rhizoctonia* sp.	83.33	5.00
S. rolfsii + *Fuarium* sp. + *Rhizoctonia* sp.	83.33	5.00
Control	0.00	0.00

Table 3 : Growth phase of *Selerotium rolfsii, Rhizoctonia* sp. and *Fusarium* sp. on Potato dextrose broth

Days after inoculation	Mean dry mycelial weight (mg)		
	Selerotium rolfsii	*Rhizoctonia* sp.	*Fusarium* sp.
2	31.70	35.00	24.30
4	37.30	57.50	41.30
6	60.00	93.00	65.00
8	206.70	142.00	112.30
10	287.30	196.70	130.00
12	243.30	233.00	152.30
14	223.30	219.70	174.00
16	193.30	198.00	206.00
18	167.70	193.00	202.30
20	143.30	189.70	192.00
SEm±	4.90	2.23	2.76
C.D. (0.01)	14.45	6.58	8.14

was maximum (5 cm) in treatment *S. rolfsii*+ *Rhizoctonia* sp.and dual inoculation of *S. rolfsii* + *Rhizoctonia* sp. and triple inoculation of *S. rolfsii* + *Rhizoctonia* sp. + *Fusarium* sp. followed by *S. rolfsii*+ *Fusarium* sp. (4.33 cm) and *Fusarium* sp. (4.08 cm) . The root rot index was minimum in *Rhizoctonia* sp. (2.87 cm).

Cultural, nutritional and physiological studies of fungal pathogens :

Growth phase **:**

The results are presented in Table 3.Every living organism has a definite growth pattern, in which it attains a maximum growth and declines thereafter. *S. rolfsii* attained maximum growth, after ten days of incubation in Potato dextrose broth and after 14 days of inoculation, a decline in dry mycelial weight was observed. The results of the study indicated that the sclerotial initiation started from 12th day after inoculation. In case of *Rhizoctonia* sp. the maximum dry mycelial weight of (233 mg) was recorded after 12 days of inoculation followed by (219 mg) after 14 days. The maximum dry mycelial weight differed significantly with rest of days of inoculation. After 14 days, dry mycelial weight reduced from 16 – 20 days (198 – 189.70 mg). As the maximum growth was observed on 12th day after inoculation, this period was used as maximum growth period for further studies.

Table 4 : Growth of different isolates of *Sclerotium rolfsii* on different solid media

	Growth in colony diameter (mm)					
Isolates	Potato dextrose agar	Oat meal agar	Host extract agar	Czapek's agar	Saburaud's agar	Mean
SrUKD	89.30	90.00	90.00	89.60	89.30	89.64
SrGKK	90.00	90.00	90.00	90.00	77.80	87.56
SrDWR	87.30	76.10	70.50	88.60	76.00	79.70
SrKGI	88.00	89.00	83.30	89.60	90.00	87.98
SrHTG	71.30	77.60	81.60	79.00	76.80	77.26
Mean	85.18	84.54	83.08	87.36	81.98	
		A (Isolate)	B (Media)	A × B		
	SEm±	0.35	0.35	0.80		
	C.D. (0.01)	1.36	1.36	3.04		

UKD-Ugarkhurd, GKK-Gokak, DWR-Dharwad, KGI-Khalgatgi, HTG-Hathargi

Table 5 : Growth of different isolates of *Rhizoctoni sp.* on different solid media

	Growth in colony diameter (mm)					
Isolates	Potato dextrose agar	Oat meal agar	Host extract agar	Czapek's agar	Sabouraud's agar	Mean
RbUKD	78.30	83.00	90.00	90.00	90.00	86.26
RbCKI	90.00	90.00	90.00	80.00	90.00	88.00
RbHVR	90.00	87.60	90.00	90.00	90.00	89.58
RbRBG	90.00	89.00	90.00	90.00	90.00	89.80
Mean	87.07	87.40	90.00	87.50	90.00	
	A (Isolate)		B (Media)		A × B	
SEm±	0.39		0.35		0.79	
C.D. (0.01)	1.51		1.35		3.03	

UKD-Ugarkhurd, GKK-Gokak, DWR-Dharwad, KGI-Khalgatgi, HTG-Hathargi

Table 6 : Growth of different isolates of *Fusarium* sp. on different media

	Growth in colony diameter (mm)					
Isolates	Potato dextrose agar	Oat meal agar	Host extract agar	Czapek's agar	Sabouraud's agar	Mean
FsKDL	90.00	63.33	54.00	71.50	54.00	66.56
FsBGL	90.00	61.66	47.33	80.00	45.00	64.78
FsUKD	90.00	63.50	68.00	82.60	51.60	71.14
Mean	90.00	62.82	56.44	78.03	50.20	
	A (Isolate)		B (Media)		A × B	
SEm±	0.44		0.34		0.76	
C.D. (0.01)	1.72		1.33		2.98	

KDL–Kundagol, BGL–Bailhongal, UKD–Ugarkhurd

In case of *Fusarium*, after two days of inoculation, the minimum growth of 24.30 mg gradually increased up to 6th day of inoculation. After 6th day there was significant increase in growth and attained maximum dry mycelial weight (206 mg) after 16 days of inoculation. There was no significant difference in dry mycelial weight between 16 and 18 days after inoculation. As the maximum growth was observed on 16th day after inoculation, this period was used as maximum growth period for further studies.

Similar observations were also made by Ramprasad (2005) and Ammajamma (2010) in coleus crop against *S. rolfsii*. *Rhizoctonia* sp. attained maximum growth after 12 days of incubation in Potato dextrose broth and thereafter a decline in dry mycelial weight was observed. The results of

Table 7 : Effect of temperature on growth of *Selerotium rolfsii*

	Dry mycelial weight (mg)					
Isolates	15º C	20 ºC	25º C	30ºC	40ºC	Mean
SrUKD	106.3	250.00	313.30	296.60	0	193.24
SrGKK	90.0	186.50	203.00	220.00	0	139.90
SrDWR	176.3	200.00	263.30	260.00	0	179.92
SrKGI	123.4	303.00	300.00	347.60	0	214.80
SrKTG	86.73	250.30	296.60	256.60	0	178.04
Mean	116.50	237.80	276.20	276.00	0	
	A (Isolate)		B (Media)		A × B	
SEm±	0.11		0.11		0.25	
C.D. (0.01)	0.43		0.43		0.98	

UKD–Ugarkhurd, GKK–Gokak, DWR–Dharwad, KGI–Khalgatgi, HTG–Hathargi

Table 8 : Effect of temperature on the germination of sclerotia

Temperature	% germination of sclerotial bodies	Mycelial growth on cavity slide (mm)
15º C	100	0.5
20º C	75	0.75
25º C	100	1.62
30º C	100	2.25
40º	0	0

Table 9 : Effect of temperature on the growth of *Rhizoctonia* sp.

	Dry mycelial weight (mg)					
Isolates	15 ºC	20 ºC	25 ºC	30 ºC	40 ºC	Mean
RbUKD	71.33	100.00	190.00	203.30	0	112.92
RbCKI	84.30	173.30	176.60	206.60	0	128.16
RbHVR	106.60	173.30	193.30	213.30	0	137.30
RbRBG	103.30	126.60	170.00	190.00	0	117.98
Mean	91.38	143.30	182.42	203.30	0	
	A (Isolate)		B (Media)		A × B	
SEm±	0.36		0.41		0.82	
C.D. (0.01)	1.40		1.57		3.15	

UKD–Ugarkhurd, CKI–Chikkodi, HVR–Haveri, RBG–Raibag

Table 10 : Effect of temperature on the growth of *Fusarium* sp.

Temperature	No of spore germinated/ per microscopic field	No of spore ungerminated/ per microscopic field	% germination of spores
15ºC	15.00	76.6	16.48
20 ºC	15.33	73.33	20.90
25 ºC	29.60	70.00	29.70
30 ºC	41.33	13.66	75.15
40 ºC	0.00	75.00	0.00

the study indicated that the autolysis stage of fungus started from 14th day after inoculation. Ramamurthy (1982) and Sachidananda (2005) also made the similar observations. In the present study, *Fusarium* sp. attained maximum growth after 16 days of incubation in Potato dextrose broth and there after a decline in dry mycelial weight was observed. The results of study indicated that the autolysis stage of fungus started from 18th day after inoculation. Similar results were also obtained by Ramprasad (2005) and Kulkarni (2006) in *Fusarium*.

Growth characters on different solid media :

The results are presented in Table 4 to 6.Every living being requires food for its growth and reproduction and the fungi are not an exception. Fungi derive the food from substrate upon which they grow. In order to culture the fungi artificially, it is necessary to supplement in the medium, those essential nutrients needed for their growth, different synthetic, non-synthetic media were tested. The radial growth of the fungus was used to determine growth on solid media.

S. rolfsii was grown on five different media and the results indicated that the best mycelial growth was made on Czapek's agar medium(87.36 mm) followed by (85.18 mm) in PDA. Least inhibition was observed in Host extract agar (83.08 mm). Similar observations were made by Hari *et al.* (1988); Basamma (2008) in potato affected by *S. rolfsii*. *S. rolfsii* took ten days for sclerotial germination on solid media. However, *S. rolfsii* also differed with regard to time taken for sclerotial initiation on solid media, it also differed with different isolates as stated by Ansari and Agnihotri(2000), in 40 different isolates of soybean. The earliest sclerotial initiation was observed on seventh day on potato dextrose agar.

Rhizoctonia sp. was grown on five different media. The results indicated that best growth (90 mm) was recorded in Host extract and Sabouraud's agar followed by 87.50 mm in Czapek's agar. The mean minimum colony diameter (87.07 mm) was recorded in PDA. Among the different isolates RbHVR and RbRBG have shown maximum growth 89.58 and 89.80 mm respectively. The findings are in line with earlier workers Seetharaman (2003). and Salunkhe *et al.* (2009) for Czapek's agar while for Potato dextrose results are against the present findings. *Rhizoctonia* sp. produced excellent microsclerotia in Czapek's agar and Potato dextrose agar. Similar observations were made by Vasudeva (1937); Lokesh (2003) and Salunkhe *et al.* (2009) in cow pea and soybean.

Fusarium sp. was grown on five different media. The results indicated that best mycelial growth was made on Potato dextrose agar (90 mm) followed by Czapek's agar (78.03 mm). Least growth was recorded in Host extract (56.44 mm) and Sabouraud's agar (50.20 mm). The results are in confirmation with the findings of Shyla (1998) and Sachidananda (2005) in case of *F. chlamydosporium*, Kulkarni (2006) in case of *Fusarium oxysporum* f.sp. *gladioli*. Growth also differed with respect to different isolates. Abundant sporulation was observed in PDA and Czepek's agar. Similar observations are made by Sachidananda (2005) and Ammajamma (2010) in *F. chlamydosporium* causing wilt of coleus.

Physiological studies :

Temperature requirement **:**

The results are presented in Table 7 to 10.Temperature plays an important role, among the external factors which influence the growth and reproduction of fungi. Each fungus has its own temperature requirement. In the present study, maximum growth of *S. rolfsii* (276 mg) was observed at 30^0 C and also cent per cent of sclerotial germination was also observed in 30^0 C, however temperature requirement differed with isolates. Irrespective of the temperature, SrKGI has given maximum dry mycelial weight (214.8 mg) but there was no growth of fungus at 40^0 C. In case of *Rhizoctonia* sp., maximum dry mycelial weight (213.3 mg) was recoded at 30^0 C in RbHVR isolate followed by RbCKI (206.6 mg). Irrespective of the isolates maximum growth was recorded at 30^0 C (203.3 mg). However, there was no growth of fungus at 40^0 C. In case of *Fusarium* sp. maximum per cent germination (75.15) of spores was observed at 30^0 C followed by 29.7 per cent at 25^0C, while at the 40^0 C there was no germination of spores. Where as optimum range was between 20^0 C to 30^0 C, 25^0 C to 30^0 C and 25^0 C to 30^0 C for *S. rolfsii*, *Rhizoctonia* sp. and *Fusarium* sp., respectively.

Togashi (1949) reported that a number of plant pathogenic fungi have optimum temperature range of 20^0 C to 30^0 C and about half of these have their optimum temperature between 25^0 C to 30^0 C. The present findings are in conformity with the results of Waseer *et al.* (1990); Salunke *et al.* (2009) in soybean for *Rhizoctonia* sp., Ramprasad (2005) in case of Species of *Fusarium*, Manjappa (1979); Basamma (2008) in *S. rolfsii*.

Conclusion :

The root rot/ wilt is caused by *S.rolfsii*, *Rhizoctonia* sp. and *Fusarium* sp. in northern Karnataka, either in combination of two or more than two pathogens. The investigations also proved the variability of isolates among different pathogens.

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