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Influence of date of sowing and chickpea varieties on occurrence of collar rot and variability among isolates of *Sclerotium rolfsii*

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Abstract

The influence of three dates of sowing employing three chickpea varieties was studied on occurrence of collar rot and yield and variability among isolates of *S. rolfsii* on the basis of morphological and physiological characters. Variety JG-62 sown on 15 October showed maximum disease incidence and minimum in variety JAKI-9218 when sown on 1 November. Three isolates were grown on 6 culture media (broth) to study their colony characteristics. The growth was maximum on Czapeck's dox solution followed by Ashby's mannitol solution, Joffe's solution, Asthana and Hawkar's solution, Potato dextrose broth and least on Richard's solution. Indore isolate exhibited the maximum growth while minimum growth was observed in Sehore isolate. The temperature of 25 °C and 30 °C were found suitable for the growth of *S. rolfsii* and Khandwa isolate (39.0 mm) recorded the maximum growth. The pH of 8.5-9.5 was suitable for the growth of all the isolates and Khandwa isolate (76.53 mm) recorded the maximum growth. Among the nitrogen sources sodium nitrate was found as the best for the growth of *S. rolfsii* (62.83 mm) and the maximum growth was recorded in Khandwa isolate (65.25 mm). Among the carbon sources maximum growth of *S. rolfsii* was recorded on sucrose (63.83mm) and the Khandwa isolate (50.08 mm) recorded the maximum growth. The isolates produced a large number of sclerotia (>300 sclerotia/plate), while Sehore isolate produced fewer (<300 sclerotia/plate). The color of sclerotia was generally dark to reddishbrown at maturity.

Keywords: Sowing, chickpea varieties, occurrence, collar rot, *Sclerotium rolfsii*

Introduction

Chickpea (*Cicer arietinum* L.), a rich source of protein is the third important crop after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.). Collar rot caused by *Sclerotium rolfsii* Sacc. was first recorded from the United States by Peter Henery Rolfs in (1892) as a cause of tomato blight in Florida. Collar rot caused by *S. rolfsii* is an important disease in areas where seedling is exposed to high temperature and high moisture in the soil. Lower portion of stem of herbaceous plants decay with development of white mat of mycelium at the lesion site. This often spreads out on to the near by soil surface. Shortly after the mycelial mat develops, small (0.5-1mm), white round, fuzzy mycelial bodies begins to appear. These mustard grain sized light to dark brown sclerotia, serve as overwintering bodies and may be seen in the mycelium, on diseased tissues above or below ground, on soil surface, or in soil crevices. Foliage wilting and die-back develops as a consequence of rotting of the lower trunk or crown tissue. (Gupta and Sharma 2004)^[4] *S. rolfsii* is a ubiquitous and aerobic pathogen. The drying of plants in chickpea field is observed due to wilt and root/collar rot complex. Although integrated management employing resistant variety certainly will hold promise for this malady yet the date of planting and moisture level influence the collar rot to a great extent.

Materials and Methods

Studies were conducted on collar rot (*S. rolfsii*) on the influence of three dates of sowing i.e. 15 October, 1 Nov. and 15 Nov. employing three The incidence varieties namely JG-62, JAKI-9218 and JG-130. In the field in a split plot design with 3 replication in plots of 6 m² having row to row distance of 4 m. The incidence of collar rot number of pods/plant, yield (q/ha) and 100 grain weight (g) were recorded variability among isolates of *S. rolfsii* on the basis of morphological and physiological characters was also explored. Cultural and morphological characteristics of isolates potato dextrose agar medium was used during the course of in vitro investigation for Cultural and morphological characteristic of the isolates.

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Physiological studies on isolate of *S. rolf sii*.

Effect of variable temperatures on growth of different isolates of *S. rolf sii* in vitro.

A 6 mm disc of the pathogen grown on PDA for 7 days was cut and placed on the surface of the solidified medium (20 ml for each Petri plate) in a laminar air flow. The inoculated plates were incubated at 10, 15, 20, 25 and 30 °C \pm 1 for 5 days. Three replications were maintained for each treatment. Radial growth of the colony was recorded by measuring the colony diameter. Further the difference in the rate of growth was recorded and analyzed statistically.

Effect of variable pH on the growth of different isolates of *S. rolf sii* in vitro

A 6 mm mycelial disc from the actively growing culture of the pathogen isolates on PDA for 5 days were cut and placed on the surface of the medium (20 ml for each Petri plate). The effect of pH viz., 6.0, 7.0, 8.0, 8.5 and 9.0 were studied on the growth of the isolates which was adjusted by N/10 H₂SO₄ or N/10 KOH solution prior to sterilization. The inoculated plates were incubated at 28 \pm 1 °C for 5 days. Three replications were maintained for each treatment of pH for every isolate. The colony diameter was recorded by measuring the radial growth of the colony. Further, the difference in the rate of growth was recorded and data were analyzed statistically.

Utilization of various sources of nitrogen by different isolates of *S. rolf sii* in vitro

The growth of 3 different isolates of *S. rolf sii* on different nitrogen sources was studied by replacing standard nitrogen source i.e. NaNO₃ from the Czapek's medium with the desirable compound. Five sources of nitrogen, namely, potassium nitrate, ammonium nitrate, ammonium sulphate, methionine and tryptophane were substituted as five treatments having three replications. Twenty ml of the respective sterile medium was aseptically dispensed in sterilized Petri dishes under a laminar air flow which were inoculated with 6 mm discs of the culture of different isolates of *S. rolf sii* with the help of a sterile cork borer under aseptic conditions. The Petri dishes were incubated at 28 \pm 1 °C for 5 days in a BOD incubator. The growth in different treatments was measured as diameter of the colony.

Utilization of various sources of carbon by the different isolates of *S. rolf sii* in vitro.

Since Czapek's dox medium was found to be the best for the growth of isolates under consideration in cultural studies the

growth on different carbon sources was studied by replacing standard carbon source (sucrose) from this medium by the desired compound. Five sources of carbon as monosaccharide, disaccharides and polysaccharides, namely glucose, fructose, lactose, starch and mannitol were substituted as five treatments having three replications to study the growth of 3 isolates.

Twenty ml of respective sterile medium was aseptically dispensed in sterilized Petri dishes under a laminar air flow. They were inoculated with 6 mm discs of culture of different isolates of *S. rolf sii* with the help of a sterile cork borer under aseptic conditions. The Petri dishes were incubated at 28 \pm 1 °C for 5 days in a BOD incubator. There after the growth in different treatments three replication was measured as diameter of the colony in each Petri dish.

Results and Discussion

The effect of cultivars and dates of sowing on the basis of disease Incidence of collar rot was quite evident. The maximum Incidence of *S. rolf sii* was recorded in variety JG-62 (46.66%) and minimum on JG-130 (31.92%) showed low disease Incidence (30.95%) and the maximum disease Incidence developed in 15th October (46.71%) sowing. Interaction of varieties and dates of sowing variety JG-62 (51.42%) has sereved the maximum disease Incidence 15th October sowing and minimum in JG-130 (23.73%) under 1st November sown crop.

The effect of cultivars and dates of sowing affected the number of pods/ plant, the maximum was observed with varieties JAKI-9218 (32.33), JG-130 (26.22) and JG-62 (24.11). Maximum pods /plant were observed on 1st November (32.22), 15th November (28.44) and minimum in 15th October (22.00) sowing. Interaction of varieties and dates of sowing revealed maximum pods/plant on cultivar JAKI-9218 (39.00) under 1st November sown crop and minimum in JG-130 (18.33) under 15th October sown crop.

The effect of cultivars and dates of sowing showed that maximum seed yield (15qha⁻¹) recorded with JAKI-9218 followed JG-62(12.89) and JG- 130 (11.82) which was non significant. The highest seed yield (15.74qha⁻¹) was obtained under 1st November sown crop, seed yield (11.82qha⁻¹) was obtained under 15th November and seed yield (9.83 qha⁻¹) was obtained under 15th October sown crop which was significant. Interaction of varieties and dates of sowing showed the maximum yield on cultivar JAKI-9218 (19.16 qha⁻¹) under 1st November sown crop and minimum in JG-62 (8.41 qha⁻¹) under 15th October sown crop.

Table 1: Influence of varieties and date of sowing on occurrence of collar rot and yield and attributing parameters in chickpea

Treatment	Disease Incidence (%)	Number of pods/plant	yield (qha ⁻¹)	100 grain weight (g)
V1 D1	51.42	21.33	8.41	15.85
D2	41.30	26.67	15.27	16.83
D3	47.58	24.33	15.00	15.83
V2 D1	31.70	26.33	10.61	26.63
D2	27.83	39.00	19.16	26.13
D3	49.72	31.67	15.55	21.90
V3 D1	29.20	18.33	10.48	29.87
D2	23.73	31.00	12.77	26.93
D3	42.84	29.33	12.22	24.60
For interaction on between V X D				
CD (P=0.05)	18.37	NS	NS	NS
For comparison of varieties				
CD (P=0.05)	6.90	26.54	NS	1.42
For comparison of date of sowing				
CD (P=0.05)	10.60	4.84	3.25	2.80

Maximum 100 grain weight (24.12 g) was obtained under the 1st November date of sowing and minimum in 100 grain weight (20.78g) under 15th November sown crop. Interaction of varieties and dates of sowing showed maximum 100 grain weight (24.12 g) under the 1st November date of sowing and minimum in JG-62(15.83g) under 15th November sown crop. The studies carried out by Hussain *et al.* (2006) [5] in 2003 to assess the effect of age of the seedling and soil type on the incidence of collar rot in chickpea showed that increase in the inoculum load of the pathogen enhanced the disease incidence. Younger seedlings were more susceptible and the susceptibility decreased with the increase in age of seedlings. In the present studies also the incidence of collar rot showed a difference for early and late sown crop. The disease incidence was low (16%) sown on 1st October and increased with the passage of time. Thus delayed sowing results into early stage crop damage by collar rot where as wilt in this “wilt and root rot complex” effect the crop first prior to harvest.

All the 3 isolates of *S. rolfisii* exhibited variation in their cultural characteristics. The Colony growth of isolates was

fluffy. Mycelial colour of isolates was white in colour. The growth of 3 isolates of *S. rolfisii* after 5 days were also recorded. Among the isolates of *S. rolfisii*, the maximum number of sclerotia was recorded in Indore (423) followed by Sehore (293) and Khandwa (264). The diameter of sclerotia/plate of *S. rolfisii* the was recorded in isolate of Indore (39.8µm), Khandwa (39.6 µm) and Sehore (39µm) the in the decreasing order of size.

Indore isolate exhibited the maximum growth while minimum growth take place in Sehore isolate. The temperature of 25° to 30 °C was found suitable for the growth of *S. rolfisii* and Khandwa isolate exhibited the maximum growth. The pH of 7.5-8.5 was suitable for the growth of all the isolates and Khandwa isolate expressed the maximum radial growth. Among the nitrogen sources, sodium nitrate was found as the best for the growth of *S. rolfisii* and the maximum growth was recorded in Khandwa isolate. Among different sources of carbon the maximum growth of *S. rolfisii* was recorded on arabinose and the Khandwa isolate showed the maximum growth.

Table 2: Variability among isolates of *Sclerotium rolfisii* on the basis of morphological parameters and growth at variable pH and temperature

Isolate	Mycelium colour	Colony growth	No. of sclerotia/plate	Colony diameter (mm) at pH						Colony diameter (mm) at Temperature °c				
				4.5	5.5	6.5	7.5	8.5	9.5	15	25	30	35	45
Indore	White	Fluffy	423	51	58	79	72	51	62	12	77	84	6	6
Sehore	White	Fluffy	293	60	56	58	51	48	61	12	43	47	9	6
Khandwa	White	Fluffy	264	47	54	73	70	83	79	15	71	83	13	11

The isolates were grown on 6 culture media (broth) to study colony characteristics The growth was maximum on Czapeck's dox solution and lesser on 5 other media.

The effect of 6 variable sources of nitrogen were studied on the growth of 3 isolates of *S. rolfisii* as the constituents of Czapeck's dox agar media. The maximum growth of *S. rolfisii* was recorded on sodium nitrate (62.83 mm) and minimum growths in ammonium sulphate (40.00mm). Among the isolates, the maximum growth of *S. rolfisii* was recorded in

Khandwa isolate (65.25 mm) and minimum growths in Sehore isolate (45.41mm).

The influence of 6 variable carbon source maximum radial growth in sucrose (63.83 mm) and minimum in lactose (31.16 mm). Among the isolates the maximum radial growth of *S. rolfisii* on different carbon sources was recorded in Khandwa isolate (50.08 mm) and minimum growth in Sehore isolate (39.91 mm).

Table 3: Variability among isolates of *Sclerotium rolfisii* different nutrient media and sources of nitrogen and carbon

Isolate	dry mycelial weight (mg) on media						Colony diameter(mm) on nitrogen source			Colony diameter(mm) on carbon source								
	Czapeck's dox solution	Richard's solution	Ashby's mannitol	Ashana and hawkars	Joffee's solution	Potato dextrose broth	Ammonium nitrite	Ammonium sulphate	Sodium nitrite	Sodium nitrate	Urea	Methionine	Sucrose	Starch	Lactose	Mannitol	Glucose	Fructose
Indore	360	128	237	193	189	108	61	29	46	72	54	47	76	48	29	37	39	42
Sehore	214	120	339	326	334	140	58	43	43	33	39	55	43	63	24	36	34	38
Khandwa	363	110	343	324	354	148	63	48	70	82	59	67	72	62	39	31	42	53

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