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Chickpea (*Cicer arietinum* L.) collar rot disease: Prevalence, symptomatology, isolation, identification and pathogenicity

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Abstract
The present study was undertaken in order to find out the prevalence and incidence of the collar rot in chickpea in seven districts of Marathwada region of Maharashtra caused by *Sclerotium rolfsii*, a roving survey done in Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad and Parbhani districts district during *Rabi* season of 2014-15. Collar rot is often seen in the seedling stage, particularly under high moist condition. Maximum incidence was recorded in the field of Marathwada region, the collar rot incidence was recorded in the range of 6.5 to 27 per cent, the highest incidence of collar rot (27%) was noticed at Vasmat (District Hingoli) and minimum disease incidence (6.5%) was recorded at Matrewadi, Tahsil Badnapur (District Jalna). For the laboratory experimental studies, standard potato dextrose agar (PDA) medium was used for isolation and culturing *S. rolfsii*. Standard tissue isolation technique was followed for the isolation of the fungus. Later, the bit of hyphal tip of fungal growth was transferred to PDA slants for obtained pure culture. The pathogen was inoculated through soil inoculation, seed inoculation and seedling inoculation for testing pathogenicity.

Keywords: *Cicer arietinum*, wet root rot, *Sclerotium rolfsii*, Pathogenicity, soil inoculation, seed inoculation

1. Introduction
Chickpea (*Cicer arietinum* L.) is a member of the Fabaceae subfamily and genus Cicer tribe *Cicereae* (Bentham and Hooker, 1972) [1]. With chromosome 2n=16, it is a diploid organism. Moreover, it goes by several other names, including gramme, Bengal gramme, garbanzo, garbanzo bean, Egyptian pea, Chana, and chhole. It was first domesticated in South West Asia (Turkey), and it has since been grown in Asia and Europe. The world's largest pulse crop, chickpea, which is cultivated extensively in India, accounts for about 75% of all pulse production (Keote *et al*., 2019) [2]. It is cultivated during the Rabi season and plays a significant role in the arid and semi-arid farming system. It is grown under irrigation and rainfed circumstances.

The chickpea is a vital grain legume that is a significant source of minerals, fibre, and proteins for both people and animals (Varol *et al*., 2020) [3]. High levels of phosphorus (340 mg/100 g), calcium (190 mg/100 g), magnesium (140 mg/100 g), iron (7 mg/100 g), and zinc (3 mg/100 g) have been found in chickpea seeds. On average, they also contain 23% protein, 64% total carbohydrates (47% starch, 6% soluble sugar), 5% fat, 6% crude fibre, and 3% ash (Anonymous, 2015) [4]. It is sometimes referred to as the "poor man's meat" because it provides a source of inexpensive protein for all cereal-based diets, particularly for vegetarians, and because its protein is high in lysine and contains few amino acids that include sulphur. It is widely regarded as a healthy diet because of its high unsaturated fatty acid content in the lipid ratio. On the other hand, by fixing atmospheric nitrogen, chickpeas contribute significantly to the improvement of soil fertility. It can fix up to 140 Kg N/ha from air and provides a significant quantity of residual nitrogen for succeeding crops, meeting 80% of the nitrogen requirement. Also, a lot of organic matter is added, which raises the fertility and health of the soil. Because of its thick taproot structure, it can withstand drought by drawing water from the soil's deeper layers (Kashiwagi *et al*., 2005; Krishnamurthy *et al*., 2003) [5, 6].

India is the country with the highest levels of consumption and production of chickpeas worldwide. During Rabi 2017–18, chickpeas were planted on 10.57 million hectares of land in India, producing 11.15 million tonnes and yielding 1055 kilogrammes per hectare, while they were grown on 1.38 million hectares of land in Rajasthan, producing 1.47 million tonnes and

yielding 1065 kilogrammes per hectare (Anonymus, 2019) [7]. Madhya Pradesh (41%), Maharashtra (16%), Rajasthan (13%), Karnataka (8%), Andhra Pradesh (6%), Uttar Pradesh (6%), and the other states produced the least amount of goods. India is the major chickpea growing country of the world and in India the major producing states are Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh together contributing to 90 per cent of the area and 91 per cent of the production in the country (Singh, 2010) [8]. Despite the high total production and more nutritive value, productivity of chickpea was low due to many biotic and abiotic constraints. Among the biotic constraints Chickpea crop is attacked by 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and mycoplasma) from entire the world (Nene *et al.,* 1996) [9]. Among all, only a few of them have the potential to destroy crops. Wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*), collar rot (*Sclerotium rolfsii*), wet root rot (*Rhizoctonia solani*), and Ascochyta blight are some of the worst illnesses (*Ascochyta rabiei*). The main issues affecting chickpea output include soil-borne illnesses including Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*), and collar rot (*Sclerotium rolfsii*). Depending on the timing of the infection, chickpea illnesses can reduce output by up to 100%. Due to rapid climate change, dry root rot and collar rot are becoming a significant danger to the production of chickpeas (Pande *et al*. 2010) [10]. *Sclerotium rolfsii*, a biotic cause of collar rot, is one of these. Sacc. is a significant disease that affects seedlings in locations with high soil moisture levels and temperatures. Affected seedlings turn yellow and are simple to pull out. Due to its ability to produce distinct sclerotia and sterile mycelia, Saccardo (1913) [11] classified the fungus as belonging to the genus *Sclerotium rolfsii*. Herbaceous plants' lower stems deteriorate, and a white mycelium mat forms at the site of the damage. It frequently spreads to the nearby soil surface. Mycelial bodies that are small (0.5-1mm), white, spherical, and fuzzy start to appear shortly after the mycelial mat forms.

Sclerotia, which are light to dark brown structures the size of mustard grains that are used as overwintering bodies, have further developed from these. They can be found in the mycelium, on sick tissues above or below ground, on the soil surface, or in soil fissures. Infection sites near the crown can also generate the distinctive white mycelial mats and sclerotia under the right circumstances. As a result of the lower trunk or crown tissue rolling, foliage withering and die-back ensue. A common and aerobic pathogen is *S. rolfsii*. Wilt and a root/collar rot complex in the chickpea field are causing the plants to dry out. *Sclerotium rolfsii* Sacc. caused collar rot is one of these diseases that is growing more dangerous at the seedling stage, particularly in areas where paddy or soybean-based cropping systems are practised. *Sclerotium rolfsii*, an important soil-borne and quickly spreading fungal disease, produces chickpea collar rot, which significantly damages the plant stand. According to reports, *S. rolfsii* can cause seedling mortality in chickpea to range from 54.7 to 95.00 percent (Shrivastava *et al*., 1984) [12] *Sclerotium rolfsii*, an important soil-borne and quickly spreading fungal disease, produces chickpea collar rot, which significantly damages the plant stand. According to reports, *S. rolfsii* can cause seedling mortality in chickpea to range from 54.7 to 95.00 percent (Shrivastava *et al*., 1984) [12]. According to reports, *S. rolfsii* can reduce chickpea output by up to 50% in field settings. The pathogen significantly reduced the population of plants with a diverse host range (Aycock, 1966; Punja, 1988) [13, 14].

2. Materials and Methods

2.1 Collection of diseased samples

Infected plants showing collar rot symptoms were collected during the month of October to December 2014 from chickpea fields. Samples were brought into the laboratory of Plant Pathology for isolation and further studies. The rapid roving survey of chickpea fields of Marathwada region (Maharashtra State) in Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad and Parbhani districts. Thus, total of 7 localities were selected and in each locality 3 fields (minimum area 1 acre) were randomly selected. To know the disease incidence, an area of 1m x 1m was marked diagonally across the field at five spots on each field and per cent disease incidence (PDI) was calculated with the help of following formula: Percent disease incidence equal to number of plants exhibited collar rot divided by total number of plant observed under X100.

The localities wise incidence of collar rot was worked out by calculating the mean of three fields of respective locality. During the disease appearance, growth of the plant is checked, initially the leaves of infected chickpea plants turn light pale in colour, plant start drying and finally die, seedling pulled up very easily. The seedling was found to collapse and showed rotting at collar region. White mycelial growth adhered to collar region along with mustard like sclerotia were observed. During survey, personal discussions were also held with the cultivators regarding incidence and severity of the disease *viz*., agronomic practices and field history.

2.2 Symptomatology

Visual and microscopic examination

Visual observations were undertaken for manifestation of the collar rotting symptoms induced by *Sclerotium rolfsii* in chickpea under natural epiphytotic conditions cum artificial soil inoculated condition (pathogenicity test) and pot culture experiments. Temporary mounts of the pure culture of *Sclerotium rolfsii* obtained from the collar rot affected chickpea plants were prepared on clean glass slide in lactophenol cotton blue, covered with cover slip and observed under compound microscope (Make: Labomed Vison 2000). Two hundred gram of cleaned, washed and peeled potato tuber was chopped into pieces. Later these pieces were boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose and agar-agar 20 g of each were dissolved in the potato extract and the volume was made up to 1000 ml by adding distilled water.

2.3 Sterilization

The glasswares were sterilized in hot air oven at 180 ºC for 2 hours whereas media was sterilized in an autoclave at 15 lbs pressure per square inch (15 lbs psi) for 20 min at 121.6 ºC. Soil used for pot culture experiments was sterilized at 2.10 kg pressure per square inch (15 lbs psi) for two hours for two consecutive days.

2.4 Isolation of fungal pathogen

Naturally infected plants showing typical collar rot symptoms were collected from the fields, washed thoroughly with distilled water, blot dried and cut with sharp sterilized blade into small bits (5 mm). Plant pieces taken from the lower

hypocotyls and upper tap root were then surface sterilized with 0.1 per cent aqueous solution of Sodium Hypo Chloride (NaOCl), for two minutes. Then, the root bits were washed thoroughly by three sequential changes with sterile distilled water to remove the traces of Sodium Hypo Chloride if any, blot dried and aseptically transferred. PDA medium poured in sterile glass Petri plates (90 mm) under, Laminar air flow cabinet (make: ACS, Bangalore) and diseased sample placed in PDA containing Petri plates, incubated in BOD incubator (make: MAC, Delhi) at 27 ± 2 °C temperature. Within 3-4 days of incubation, *Sclerotium rolfsii* exhibited whitish mycelial mat along with, numerous brown black sclerotia with in 10 to 12 days of incubation, *Sclerotium rolfsii* exhibited white cottony mycelial growth, along with micro sclerotia was developed. Applying hyphal tip and single spore isolation technique, the test pathogens were transferred aseptically on the PDA slant in test tubes, sub cultured, purified the cultures and pure cultures thus obtained of the test pathogens were maintained on PDA slant tubes in refrigerator for further studies.

2.5 Purification and identification of associated pathogen

The fungal growth which across through the infected tissue was taken by inoculation needle and transferred aseptically to Petri plates 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 after purification, *Sclerotium rolfsii* was identified by observing the colony character and sclerotia formation. The objective material was grown on potato dextrose agar medium and maintained at 25±1 °C temperature.

2.6 Maintenance of the cultures

The fungus was sub-cultured on the PDA slants and allowed to grow at 27±1 °C temperature. The cultures so obtained were stored in refrigerator at 5 °C for further studies.

2.7 Mass multiplication

In order to get maximum growth of the pathogen, for mass multiplication of inoculum, sand corn meal medium was used. The sand corn meal medium was prepared in the proportion of 90:10 in order to get maximum inoculum of fungus. Four hundred grams of corn meal sand medium was taken in 1000 ml flask and watered to 20 per cent of its weight and sterilized at 1.1 kg / cm^2 pressure for 20 minutes. The pure culture of *Sclerotium rolfsii* (two or four bit) was inoculated to different flasks under aseptic conditions and incubated at 27±1 °C for 30 days. These flasks were shaken periodically to get uniform growth. The giant culture so obtained was used for further studies.

2.8 Pathogenicity Test

2.8.1 Soil infestation

Inoculum was thoroughly mixed in sterilized sand + soil (1:1) @ 100 g / 2 kg soil. The sterilized soil mixed with inoculum multiplied on chickpea straw was placed in earthen pot. Seeds of JG 62 were surface sterilized with 0.1% Sodium Hypochloride (NaOCl) for one minute. Ten seeds were placed in one polythene bags and three replications were maintained. These bags were kept in a net house. Proper isolation was maintained to avoid other pathogens. Observations on germination, pre and post emergence mortality were recorded. No soil treatment with test fungus was done in control.

2.8.2 Seedling inoculation

Sterilized soil was taken in polythene bags. Surface sterilized JG 62 seeds were sown on polythene bags in net house. The test fungus inoculated on seven days old seedlings at collar region. The test fungus was not inoculated in proper control. Seven days after inoculation, observations on the mortality of seedlings were recorded.

3. Result and Discussion

3.1 Survey on incidence of chickpea collar rot under Marathwada region

A survey was carried out for recording the incidence of chickpea collar rot caused by *S. rolfsii* during *Rabi* 2014-15 in Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad and Parbhani districts of Marathwada region (Maharashtra State) and the results obtained are presented in (Table1, 2,3, Fig.1). Incidence of collar rot chickpea was noticed in all the places surveyed on commonly grown varieties as mentioned in table 1. In Marathwada region, the collar rot incidence was recorded in the range of 6.5 to 27 per cent, the highest incidence of collar rot (27%) was noticed at Vasmat (District Hingoli) and minimum disease incidence (6.5%) was recorded at Mathrewadi, Tahsil Badnapur (District Jalna). In Aurangabad district, collar rot incidence was recorded in the range of 14 to 20.5 per cent, the highest incidence of collar rot (20.5%) was noticed at Kannad and minimum disease incidence (14%) was recorded at Vaijapur. The average collar rot incidence was 17.37 percent at Aurangabad district.

In Beed district, collar rot incidence was recorded in the range of 9.5 to 18 per cent, the highest incidence of collar rot (18%) was noticed at Shelgaon (Gevrai Tahsil) and minimum disease incidence (9.5%) was recorded at Dhamangaon. The average collar rot incidence was 14.25 percent at Beed district. In Hingoli district, collar rot incidence was recorded in the range of 17 to 27 per cent, the highest incidence of collar rot (27%) was noticed at Kannad and minimum disease incidences (17%) were recorded at Kapadsingi (Sengaon Tahsil) and Hingoli itself. The average collar rot incidence was 21 percent at Hingoli district. In Jalna district, collar rot incidence was recorded in the range of 6.5 to 12 per cent, the highest incidence of collar rot (12%) was noticed at Pirsawangi and minimum disease incidence (6.5%) was recorded at Matrewadi (Badnapur Tahsil). The average collar rot incidence was 9.31 percent at Jalna district. In Latur district, collar rot incidence was recorded in the range of 7.5 to 12.5 per cent, the highest incidence of collar rot (12.5%) was noticed at Devni and minimum disease incidence (7.5%) was recorded at Ansur (Jalkot Tahsil). The average collar rot incidence was 9.87 per cent at Latur district. In Nanded district, collar rot incidence was recorded in the range of 12 to 24 per cent, the highest incidence of collar rot (24%) was noticed at Loni and minimum disease incidence (12%) was recorded at Tambi and both locations situated at same Tahsil (Himayatnagar). The average collar rot incidence was 18.62 percent at Nanded district.

In Osmanabad district, collar rot incidence was recorded in the range of 10.5 to 14.5 per cent, the highest incidence of collar rot (14.5%) was noticed at Murum (Umerga Tahsil) and minimum disease incidences (10.5%) were recorded at Mankeshwar (Bhoom Tahsil) and Kaudgaon (Osmanabad Tahsil). The average collar rot incidence was 12 percent at

Osmanabad district. In Parbhani district, collar rot incidence was recorded in the range of 12 to 16 per cent, the highest incidence of collar rot (16%) was noticed at Dastapur and minimum disease incidence (12%) was recorded at Manvat. The average collar rot incidence was 14.64 percent at Parbhani district.

3.2 Variety wise disease incidence

During *Rabi* 2014-15 (Table 3), amongst different 62 field location of Marathwada region, Vijay variety shown 14.06% average incidence of collar rot disease, Virat (14.75%), Vishal (14.76%), Digvijay (14.00%) and Local varieties were shown the highest average collar rot incidence i.e. 15.96 per cent. It indicated that, in occurrence of disease and its incidences were not shown much more difference amongst currently cultivated varieties including local. Results of the present study on collar rot of chickpea incidence are in consonance with the earlier reports of various workers (Weber *et al.*, 1931; Singh *et al.*, 2011 and Ghosh *et al.*, 2013) [15, 16, 17].

3.3 Symptomatology

Visual observation of infected plants was made to assess the disease incidence at different stage of crop growth. Collar rot infected chickpea plants were observed in the field at seedling stage (20-45 days). The affected plants turned yellow and pulled very easily. The seedling was found to collapse and showed rotting at the collar region. White mycelial growth adhered to collar region along with mustard like sclerotia were observed. On the basis of visual observations recorded at different crop growth stages revealed that the severity of the disease was more pronounced at seedling stage *i.e.* up to 45 days after sowing and gradually decrease with the age of crop. Several workers have previously reported seeing the same chickpea collar rot symptoms caused by *S. rolfsii* and the same species on numerous other crops (Weber, 1931; Punja and Rahe *et al*., 1992; Ansari *et al*., 2005; Prasad and Naik *et al*., 2008) [15, 18, 20, 19]. Chickpea plants showing symptoms of collar rot were collected in the month of November and December 2014 from chickpea field, Badnapur. Samples were brought into the Department of Plant Pathology for isolation and further studies.

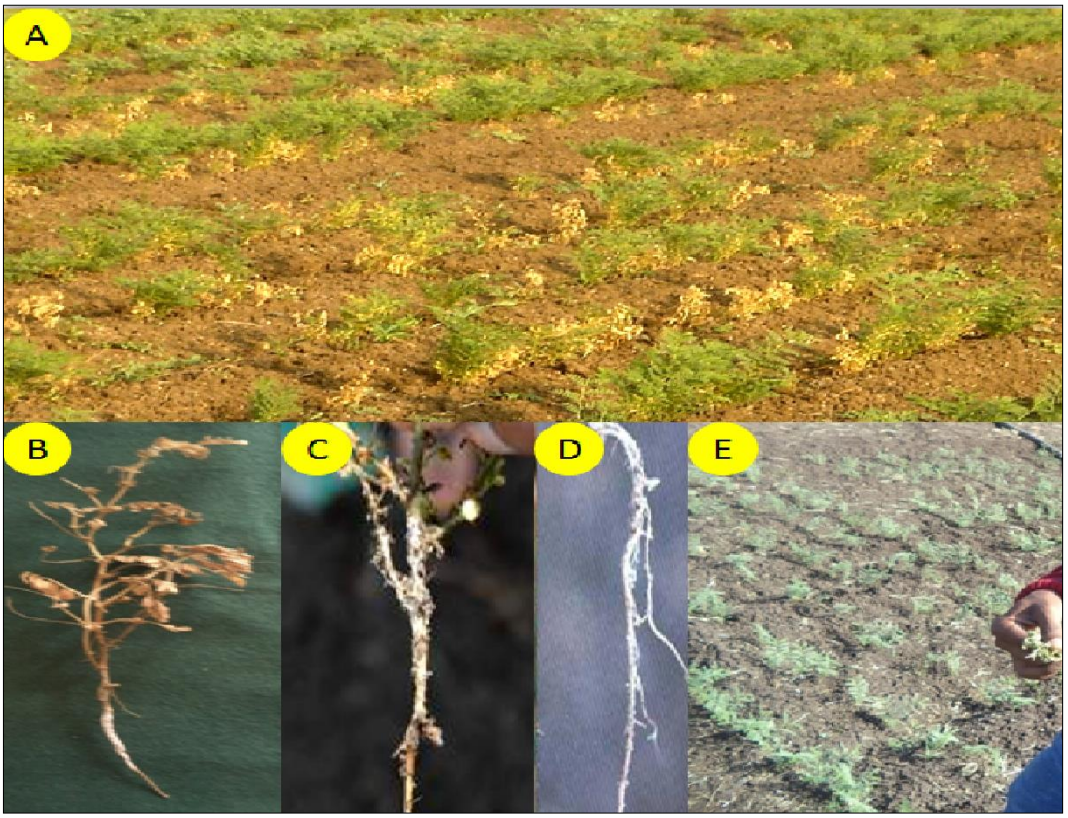

Fig 1: Symptoms of WRR (A) Seedling mortality symptoms of chickpea collar rot caused by *S. rolfsii*, (B) White mycelial growth of *S. rolfsii* on collar region of crop (B, C & D) and collar rot incidence on chickpea crop in field (E).

Table 1: Disease incidence of chickpea collar rot of different locations of Marathwada region

Sr. No.	Name of Farmer	Village	Tahsil	Variety	Soil Type	Previous crop	Stage of Crop	Area (acres)	Disease Incidence (%)
I. Aurangabad									
1	Sandip Shinde	Aurangabad	Aurangabad	Vishal	Heavy Black soil	Maize	Seedling	2	18.0
2	Gopinath Wagh	Wakla	Aurangabad	Local	Light soil	Maize	Seedling	3	17.0
3	Gajanan Vitekar	Murshidabad	Fulumbri	Virat	Black soil	Bajra	Seedling	3	16.0
4	Tukaram Darwante	Babultel	Vaijapur	Digvijay	Black soil	Soybean	Seedling	5	14.0
5	Vasant Patil	Kannad	Kannad	Local	Heavy Black soil	Maize	Seedling	3	20.5
6	Anil Nikam	Vita	Kannad	Vishal	Black soil	Soybean	Seedling	6	19.0
7	Kadoba Dapke	Lihakhedi	Sillod	Virat	Black soil	Jawar	Seedling	2	18.5
8	Rashid Tadvi	Soyegaon	Soyegaon	Local	Black cotton soil	Soybean	Seedling	5	16.0

Average mean of disease incidence of Aurangabad district									17.37%
II. Beed									
9	Satish Dhandge	Shendri	Ashti	Local	Heavy Black soil	Bajra	Seedling	3	16.0
10	Shivaji Zinzurke	Dhamangaon	Beed	Vijay	Heavy black soil	Soybean	Seedling	5	09.5
11	Vitthal Sanap	Gevrai	Beed	Vijay	Black soil	Maize	Seedling	4	17.5
12	Ramrao Pawar	Mirkala	Beed	Virat	Heavy Black soil	Soybean	Seedling	6	15.0
13	Sudam Dhok	Wadgaon	Beed	Local	Medium soil	Bajra	Seedling	5	13.5
14	Mahadev Jagtap	Shirur	Ambejogai	Virat	Medium soil	Soybean	Seedling	3	13.0
15	Dhondiba Dhormare	Savargaon	Ambejogai	Local	Black soil	Sorghum	Seedling	5	11.5
16	Pandurang Dhakne	Shelgaon	Gevrai	Vijay	Black soil	Sorghum	Seedling	3	18.0
Average mean of disease incidence of Beed district									14.25%
III. Hingoli									
17	Janardhan Gaikwad	Sengaon	Sengaon	Vijay	Heavy Black soil	Soybean	Seedling	5	23.0
18	Tulshiram Phupate	Vatkali	Sengaon	Vijay	Heavy black soil	Soybean	Seedling	3	19.0
19	Manik Sable	Kapadsingi	Sengaon	Virat	Black soil	Jawar	Seedling	5	17.0
20	Vilas Chandne	Vasmat	Vasmat	Local	Black soil	Soybean	Seedling	6	27.0
21	Kishanrao Manohare	Babulgaon	Vasmat	Vijay	Medium soil	Soybean	Seedling	3	21.5
22	Shankar Dhotre	Loni	Hingoli	Virat	Medium soil	Jawar	Seedling	4	24.5
23	Gangaram Chavan	Borkhedi	Hingoli	Vishal	Black soil	Soybean	Seedling	3	19.0
24	Shankar Solanke	Hingoli	Hingoli	Vijay	Light soil	Soybean	Seedling	4	17.0
Average mean of disease incidence of Hingoli district									21.00%
IV. Jalna									
25	Shivaji Rasal	Rajewadi	Jalna	Vijay	Heavy black soil.	Maize	Seedling	3	10.0
26	Vishnu Mote	Mapegaon	Jalna	Vijay	Medium	Soybean	Seedling	5	08.5
27	Dadarao Dugane	Golapangri	Jalna	Vijay	Medium soil	Bajra	Seedling	6	10.0
28	Baliram Khedekar	Pirsawangi	Jalna	Vijay	Light soil	Bajra	Seedling	5	12.0
29	Kailash Pawar	Mathrewadi	Badnapur	Vishal	Heavy Black soil	maize	Seedling	6	06.5
30	Yogesh Kolte	Shelgaon	Badnapur	Virat	Light soil	Jawar	Seedling	4	12.0
31	Ramkisan Kunde	Somthana	Badnapur	Vijay	Medium soil	Soybean	Seedling	3	07.0
32	Panditrao Hinge	Davalwadi	Badnapur	Virat	Black soil	Soybean	Seedling	3	08.5
Average mean of disease incidence of Jalna district									09.31%
V. Latur									
33	Ananthrao More	Chakur	Chakur	Local	Medium soil	Maize	Seedling	5	11.0
34	Ashok Jadhav	Jalkot	Jalkot	Vijay	Black soil	Bajra	Seedling	4	08.5
35	Bhimrao Pawar	Ansur	Jalkot	Virat	Black soil	maize	Seedling	3	07.5
36	Rameshwar Chalwad	Ausa	Latur	Vijay	Black soil	Soybean	Seedling	3	09.5
37	Madhave Ghuge	Atola	Latur	Vijay	Black soil	Soybean	Seedling	6	12.0
38	Anurag Kamble	Devni	Latur	Local	Black soil	Bajra	Seedling	5	12.5
39	Dattu Jadhav	Karepur	Latur	Virat	Medium soil	Soybean	Seedling	3	08.0
40	Ganpath Biradar	Adalgaon	Latur	Vijay	Black soil	maize	Seedling	4	10.0
Average mean of disease incidence of Latur district									9.87%
VI. Nanded									
41	Kishan Mungale	Phulwad	Kandhar	Virat	Heavy Black soil	Jawar	Seedling	3	22.0
42	Kashinath Phulwale	Naigaon	Nanded	Vishal	Black soil	Soybean	Seedling	3	18.5
43	Shankar Jadhav	Loni	Himayatnagar	Vishal	Medium soil	Soybean	Seedling	5	24.0
44	Digambar Akkalwad	Tambi	Himayatnagar	Vijay	Medium soil	Soybean	Seedling	3	12.0
45	Mohan Karamunge	Tamsi	Naigaon	Vijay	Heavy Black soil	Bajra	Seedling	6	17.0
46	Pandurang Jangilwad	Chenapur	Ardhapur	Vishal	Black soil	Soybean	Seedling	4	15.5
47	Gangadhar Malvankar	Divshi	Bhokar	Vishal	Black soil	Maize	Seedling	3	19.0
48	Kishan Sawant	Gorta	Umbri	Virat	Heavy Black soil	Soybean	Seedling	3	21.0
Average mean of disease incidence of Nanded district									18.62%
VII. Osmanabad									
49	Prakash Dhage	Kathi	Tuljapur	Vishal	Black soil	Mung	Seedling	5	13.0
50	Achut Shinde	Tuljapur	Tuljapur	Local	Light soil	Soybean	Seedling	6	12.0
51	Bhimrao Gugle	Kaudgaon	Osmanabad	Virat	Red soil	Maize	Seedling	3	10.5
52	Bharat Apune	Sonari	Paranda	Virat	Medium soil	Sorghum	Seedling	3	11.5
53	Pandurang Mote	Bhoom	Bhoom	Vishal	Medium soil	Bajra	Seedling	5	12.0
54	Shivaji Gore	Mankeshwar	Bhoom	Vishal	Light soil	Soybean	Seedling	3	10.5
55	Ramesh Ghabne	Murum	Umarga	Vijay	Black soil	Jawar	Seedling	3	14.5
Average mean of disease incidence of Osmanabad district									12.00%
VIII. Parbhani									
56	Manik Singare	Dastapur	Parbhani	Virat	Black soil	Soybean	Seedling	3	16.0
57	Vachist Chaukhat	Manvat	Manvat	Vijay	Light soil	Soybean	Seedling	6	12.0
58	Balasaheb Khanpate	Jintur	Jintur	Local	Medium soil	Jawar	Seedling	5	13.5
59	Kisanrao Narwade	Shakh	Shelu	Vijay	Heavy Black soil	Bajra	Seedling	5	17.5
60	Dadarao Ekkar	Tandulwadi	Shelu	Vishal	Black soil	Soybean	Seedling	3	13.5
61	Dinkar Bachewad	Mantha	Mantha	Virat	Black soil	Soybean	Seedling	4	15.0
62	Dadarao Chavan	Pathri	Pathri	Vijay	Black soil	Soybean	Seedling	3	15.0
Average mean of disease incidence of Parbhani district									14.64%

Table 2: District wise disease incidence and its range of chickpea collar rot

Sr. No.	Districts	Average collar rot disease incidence (%)	Range (%)
1	Aurangabad	17.37	14.0 to 20.5
2	Beed	14.25	09.5 to 18.0
3	Hingoli	21.00	17.0 to 27.0
4	Jalna.	09.31	06.5 to 12.0
5	Latur	09.87	07.5 to 12.5
6	Nanded	18.62	12.0 to 24.0
7	Osmanabad	12.00	10.5 to 14.5
8	Parbhani	14.64	12.0 to 16.0

Table 3: Variety wise chickpea collar rot disease incidence

Sr. No.	Variety→ ↓Districts	Vijay	Virat	Vishal	Digvijay	Local
1	Aurangabad	--	17.25 (2)	18.50 (2)	14.00 (1)	17.86 (3)
2	Beed	15.00 (3)	14.00 (2)	--	--	13.66 (3)
3	Hingoli	20.12 (4)	20.75 (2)	19.00 (1)	--	27.00 (1)
4	Jalna.	09.50 (5)	10.25 (2)	06.50 (1)	--	--
5	Latur	10.00 (4)	07.75 (2)	--	--	11.75(2)
6	Nanded	14.50 (2)	21.50 (2)	19.25 (4)	--	--
7	Osmanabad	14.50 (1)	11.00 (2)	11.83 (3)	--	12.00 (1)
8	Parbhani	14.83 (3)	15.50 (2)	13.50 (1)	--	13.50 (1)
Mean Incidence		14.06 (22)	4.75(16)	14.76 (12)	14.00(1)	5.96(11)

Figures in parenthesis are total locations surveyed of particular variety in each district.

3.4 Collection of diseased sample and Isolation

Chickpea plants showing symptoms of collar rot (Fig. 1) were collected in the month of November and December 2014 from chickpea field, Badnapur. Samples were brought into the Department of Plant Pathology for isolation, washed thoroughly with distilled water, blot dried and cut with sharp sterilized blade into small bits (5 mm). Plant pieces taken from the lower hypocotyls and upper tap root were then surface sterilized with 0.1 per cent aqueous solution of Sodium Hypo Chloride (NaOCl), for two minutes. Then, the root bits were washed thoroughly by three sequential changes with sterile distilled water to remove the traces of Sodium Hypo Chloride if any, blot dried and aseptically transferred. PDA medium poured in sterile glass petriplates (90 mm) under, Laminar air flow cabinet and diseased samples placed in petriplates, incubated in BOD incubator at 27 $\pm$1 °C temperature. Within 3-4 days of incubation, *Sclerotium rolfsii* exhibited whitish mycelial mat along with, numerous brown black sclerotia within 10 to 12 days of incubation, *Sclerotium rolfsii* exhibited white cottony mycelial growth, along with micro sclerotia was developed.

The fungus developed as cottony white to brownish white, lustrous, fluffy and fast growing round colony with filamentous margin and fan shaped appearance. Applying hyphal tip and single spore isolation technique, the test pathogens were transferred aseptically on the PDA slant in test tubes, sub-cultured, purified the cultures and pure cultures thus obtained of the test pathogens were maintained on PDA slant tubes in refrigerator. The aforesaid isolation and identification of *S. rolfsii* attempted in the present study are in consonance with the earlier reports of several workers (Grover and Chona *et al.*, 1960; Subramanian *et al.*, 1971; Mordue *et al.*, 1974; Hussain *et al.* 2003; Shinde and Reddy *et al.* 2009 and Singh *et al.* 2011) [21, 22, 23, 24, 26, 16].

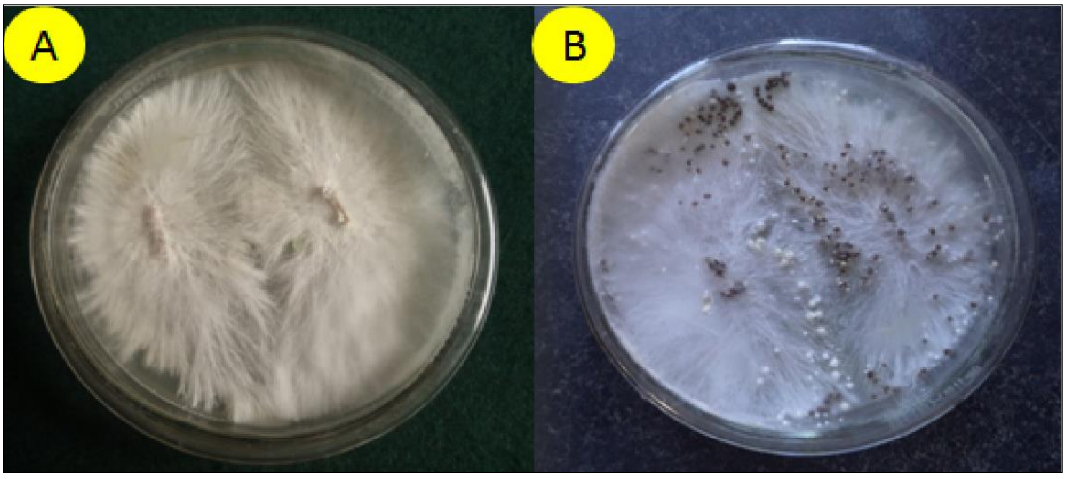

Fig 2: Photograph showing growth of *Sclerotium rolfsii* A) Radial growth of mycelium of *S.rolfsii* B) Matured sclerotial bodies C) Growth of *S. rolsfsii* on sand corn meal D) Mass multiplication

3.4 Pathogenicity test

Pathogenicity test was conducted by using different inoculation techniques *viz*., soil inoculation and seedling inoculation. In both techniques, one variety *i.e.*, JG 62 was used for testing. After critically going through data, it revealed that, sown chickpea seed of susceptible varieties *i.e.*, JG 62 in sick soil containing polythene bags, took minimum 20 days for express the symptoms on seedling. Similarly, the maximum seedling mortality after initiation of disease ranged from 20 to 45 days. Collar rot incidence decreased with increasing age of crop. In seedling inoculation technique (100%) and in soil inoculation technique (85.10%) maximum Collar rot incidence recorded. In control (UN inoculated) pots all the plants remained healthy. The test pathogen was confirmed as *S. rolfsii* and pathogenicity of *S. rolfsii* was proved by applying Koch's postulates.

Earlier reports from a number of research workers described the pathogenicity of *S. rolfsii*-induced chickpea collar rot as being similar (Sengupta and Das *et al.*, 1970; Mathur and Sorbhoy *et al*., 1976; Punja and Damiani *et al*., 1996; Chen *et al*., 1998; Ansari and Agnihotri *et al*., 2000 and Abida *et al*., 2007) [27, 29, 28, 30, 31].

Fig 2: Pathogenicity of chickpea collar rot incited by *S. rolfsii*

3.5 Purification and Identification of Pathogen *Sclerotium rolfsii*

Isolation from infected chickpea roots showing symptoms of collar rot were made on potato dextrose agar medium. *Sclerotium rolfsii* was found to be associated with the infected roots of the entire sample collected from the field. Culture of *Sclerotium rolfsii* produced thick white to dirty white colony, profusely branched, fluffy fan shaped mycelial growth. Sclerotia are at first white, becoming light brown to dark brown at maturity. They are sub spherical, the surface being finely wrinkled or pitted, sometimes flattened, commonly 0.5-1.5 mm in diameter. On the basis of morphological characters, the pathogen was identified as *Sclerotium rolfsii*. The culture was purified by single hyphal tip technique and maintained on PDA slants at 25ºC temperature for further investigation.

Isolation, pathogenicity and identification of *S. rolfsii* infecting chickpea and other crops were attempted earlier by several workers (Shinde and Ready, 2009; Sultana *et al*., 2012) [24, 33].

4. Conclusion

Sclerotium rolfsii (Sacc.) is one of the most destructive pathogens of chickpea, putting the growers in considerable economic losses. Chickpea plants shows symptoms of collar rot were collected in the month of November and December probably. The pathogen (*S. rolfsii*) could grow and proliferate better on PDA media, which encouraged abundant mycelial growth as well as sclerotial production of *S. rolfsii.*

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