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Isolation, identification and pathogenicity of *Sclerotinia sclerotiorum* causing *Sclerotinia* rot of chilli

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Abstract
Sclerotinia rot caused by *Sclerotinia sclerotiorum* is one of the most devastating soil borne pathogen, which is threatening the production of chilli crop. In this study associated fungus was isolated on PDA medium and purified by using hyphal tip method from infected chilli plant samples were collected from farmer's fields. On PDA, uniformly one type growth of fungus colony started without zonation with whitish to gray growth in colour and fungus rapidly covered the entire Petri plate within 72 hours. Fungal mycelium aggregate to form small mycelial tufts which was converted into hard black sclerotia with Oval to irregular shape at the periphery of the Petri plates. Microscopic observation showed, hyphae were hyaline and branched and asci arranged on periphery of ascocarp. Ascus was hyaline, barrel shaped and produced in tightly compact mass with filiform paraphyses at the upper surface of apothecium. Ascospores were elliptical to oval, single celled, hyaline and posses eight numbers in each ascus. Pathogenicity was proved on chilli plants using seed, soil and seed + soil inoculation method. Among the different inoculation methods, seed + soil inoculation recorded maximum disease incidence (72.66%) followed by soil inoculation method (64.00%). On the basis of cultural, morphological and pathogenicity test, isolated fungus was identified as *Sclerotinia sclerotiorum*.

Keywords: Chilli, cultural, morphological, sclerotia, pathogenicity, *Sclerotinia sclerotiorum*

Introduction
Chilli (*Capsicum annuum* L), a member of *Solanaceae* is a major vegetable cum spice crop with considerable economic importance grown in tropical and sub-tropical regions of the world. Chilli, the native of New World of tropics and sub-tropics was introduced into India from Brazil in 16th century by the Portuguese. It has 24 chromosomes (2n) and may be herb or sub-shrub of height up to 2.5 m with extensively branched stem having hairy growth with purplish spots near the nodes. The tap root is strong with numerous lateral roots. Chilli fruits are considered vegetable and are botanically berries (Saxena *et al.*, 2016) [15].
Chilli are grown in almost all states of the country and the major growing states in terms of production share are Andhra Pradesh (49%), Karnataka (15%), Orissa (8%), Maharashtra (6%), West Bengal (5%), Rajasthan (4%) and Tamil Nadu (3%). The most popularly cultivated chilli varieties in the country are, Sannam, LC334, Byadagi, Wonderhot, Pusa Jwala etc. (Kumara *et al.*, 2014) [11].
Chilli crop suffers with many fungal, bacterial, viral and nematode diseases resulting in huge yield losses (Kalmesh and Gurjar, 2001) [9]. Among the fungal diseases *Sclerotinia* rot caused by *Sclerotinia sclerotiorum* is one of the most devastating soil borne pathogen, which is threatening the production of chilli crop (Yanar *et al*., 1996) [18]. The pathogen attacks nearly all kinds of succulent plants including flowers, shrubs, weeds and vegetables including chilli (Chupp and Sherf, 1960) [2]. On PDA, colonies of *S. sclerotiorum* consisted of white to gray mycelia and globose to irregular and black sclerotia (Kim and Cho, 2003) [10]. Pones *et al.* (1979) [13] were isolated *Sclerotinia sclerotiorum* from lettuce, cabbage and bean and proved their pathogenicity by using sterilized lettuce seed, colonized by the fungus inoculums. The research was therefore undertaken to study on isolation, purification and pathogenicity of *Sclerotinia sclerotiorum* with an aim to give more information for disease management strategy.

Collection and isolation of pathogen
Sclerotinia rot affected diseased samples of chilli plants were collected from the farmer's field grown under protected cultivation of Jaipur district of Rajasthan and brought to the laboratory for isolation. Diseased portions were washed with sterilized water and cut into small pieces of 4-5 mm size with the help of sterilized blade.

Each piece was surface sterilized with 1 per cent sodium hypochlorite solution for one minute followed by three consecutive washing with sterilized water and dried on sterilized blotter paper. One bit was placed aseptically in 2 per cent PDA (Potato Dextrose Agar) slant culture tubes then incubated for 4 days at 25 ± 1 ºC.
Similarly, isolation was also made from black sclerotia present inside and on the diseased stem tissues. Sclerotia after surface sterilization were cut into small pieces with the help of sterilized blade. Each piece was surface sterilized with 1per cent sodium hypochlorite solution for one minute followed by three consecutive washing with sterilized water and dried on sterilized blotter paper. One bit was placed aseptically in 2 per cent PDA slant culture tubes then incubated for 4 days at 25 ± 1 ºC.

Identification
The fungus was purified by hyphal tip method (Riker and Riker, 1936) [14]. The culture was maintained in refrigerator at 4 ºC and renewed after every fifteen days. Identification of isolated associated fungi was identified as *Sclerotinia sclerotiorum* on the basis of morphological and colony characters.

Pathogenicity of isolated pathogen on chilli plant
Multiplication of the pathogen inoculum
Sorghum grains were used to multiply the test isolates of *Sclerotinia sclerotiorum*. Sorghum grains were initially soaked in water (1-2 hrs.), then drained off the excess water, filled 100 g sterilized sorghum grain in 500 ml Erlenmeyer flasks, plugged with non-absorbent cotton and autoclaved at 15 psi at 121°C for 30 minutes. After cooling at room temperature, these Erlenmeyer flasks were inoculated separately with mycelial discs of 7 days old culture of *Sclerotinia sclerotiorum* under aseptic condition (Laminar-airflow Cabinet) and inoculated at 25 ± 1 ˚C temperature for one week or until the sorghum grains completely covered with *S. sclerotiorum* growth.

Inoculation techniques for Pathogenicity
Soil used in the present studies was sterilized at 1.045 kg/ pressure for one and half hour. Pots were surface sterilized by dipping them in 10 per cent formaldehyde solution for 5 minutes.
The pathogenicity of the isolated fungus was proved by seed inoculation, soil inoculation and seed + soil inoculation techniques.

Seed inoculation methods
For seed inoculation, seeds of chilli were first surface sterilized with 1 per cent sodium hypochlorite solution and then sterilized seeds were smothering with 7 days old culture of fungus grown on PDA. The inoculated seeds were shown in plastic pots containing sterilized soil. Surface sterilized seeds were shown in untreated pot served as a check.

Soil inoculation
For soil inoculation sterilized pots were filled with sterilized soil and pots were inoculated with pathogen inoculums, multiplied on sorghum grains @ 20 gram/pot before seed sowing and kept in polyhouse. Surface sterilized seeds were shown in untreated (without inoculums) pot served as a check.

Seed + soil inoculation methods
For this method first inoculum of pathogen was applied in soil and then seeds were smothering with 7 days old culture of the pathogen grown on PDA. Inoculated seeds were shown in pots contain inoculated soil with pathogen inoculum. Surface sterilized uninoculated seeds were shown in untreated (without inoculum) pot served as a check.
Five replication of each treatment were maintained using completely randomized design under polyhouse condition. Seedlings were maintained in each pots after removing the excess seedling on ten day of germination.

Results
Collection and isolation
Plants of chilli affected with *Sclerotinia* rot pathogen showing partial or total wilting of plant with rotting of stems and branches (plate 1), were collected from farmer's field of the chilli growing areas of Jaipur district and diseased plants were brought to the laboratory. Isolations of associated pathogen were made from infected diseased portions of chilli plant on Potato Dextrose Agar medium.

Identification of pathogen
The isolated fungal culture was purified by using hyphal tip method. Identification of the *Sclerotinia sclerotiorum* was done on the basis of cultural and morphological characteristics (Table 1). After twenty four hours of inoculation on PDA, uniformly one type growth of fungus colony started without zonation with whitish to gray growth in colour. Later on, the growth of fungus rapidly covered the entire Petri plate within 72 hours. After five days of fungal growth, fungal mycelium aggregate to form small mycelial tufts which were developed at the periphery of the Petri plates. Later, mycelial tufts covered the entire Petri plate. Glossy water droplets were appeared frequently around the mycelial tuft in culture plates. Later on, hard black coloured sclerotia were developed from converted mycelial tufts and single sclerotium was fixed and deeply surrounded by white mycelium net. Oval to irregular shape of sclerotia were formed and measured 2.5-8.5mm (length) x 2-5.5 mm (width) in size (Plate 2).
Under microscope observation, the hyphae were hyaline and branched. Asci are arranged on periphery of ascocarp. They are hyaline, barrel shaped and produced in tightly compact mass with filiform paraphyses at the upper surface of apothecium. Ascospores are elliptical to oval, single celled, hyaline and each ascus posses eight numbers of ascospores (Plate 3).

Pathogenicity of isolated pathogen on chilli plant
The test fungus was found pathogenic on chilli seedling and produced typical symptoms of disease. After 40 days of sowing young seedlings showed visible small water soaked lesion on the stem at the ground level, these water soaked lesions enlarge rapidly in size and girdled the entire base of young stem at the collar region. At later stage, whitish mycelial growth was observed on infected stems. Wilted symptoms were seen above the stem portion and later seedlings were died. Black sclerotia were formed on infected portion of stems. Observations of pathogenicity of *S. sclerotiorum* are presented in Table 2.
Pathogenicity of *Sclerotinia sclerotiorum* on chilli seedlings was done using three methods of inoculation, in which seed + soil inoculation method results maximum per cent disease incidence with 72.66 per cent followed by soil inoculation method with 64.00 per cent disease incidence. Lowest per cent disease incidence was recorded in seed inoculation

method with 56.30 per cent disease incidence. The pathogen was reisolated from these infected seedlings on PDA under aseptic condition and culture of the reisolated fungus was found identical to original one

Discussion

Isolations of *Sclerotinia sclerotiorum* were made from naturally infected diseased portions of chilli plant on PDA. After isolation of the fungus, the isolated fungal culture was purified by using hyphal tip method. Identification of the *Sclerotinia sclerotiorum* was done on the basis of cultural and morphological characteristics. On PDA, uniformly one type growth of fungus colony started without zonation with whitish to gray growth in colour and covered the entire Petri plate within 72 hours. After five days of fungal growth, small mycelial tufts which were developed at the periphery of the Petri plates. Later on, hard black coloured sclerotia were developed from converted mycelial tufts and single sclerotium was fixed and deeply surrounded by white mycelium net. Oval to irregular shape of sclerotia were formed and measured 2.5-8.5mm (length) x 2-5.5 mm (width) in size. Under microscopic observation, the hyphae were hyaline and branched. Ascospores are elliptical to oval, single celled, hyaline and each ascus posses eight numbers of ascospores. These results are in conformity to the findings of several earlier workers. Pones *et al*. (1979) [13] isolated fungus from lettuce, cabbage and bean and identified on the basis of cultural characteristics, Kim and Cho (2003) [10] observed that colonies of *Sclerotinia scleotiorum* on PDA were white to grey and formed globose to irregular black sclerotia, Abdel-Kader *et al.* (2012) [1] isolated *Sclerotinia sclerotiarum* from cucumber and pepper, Goswami *et al*. (2012) [3] were isolated from mustard and pathogen produce white to light brown colony on PDA with uniform mycelial growth, black sclerotia were formed at the periphery of plates, Hansda *et al*. (2014) [4] pathogen was isolated on brinjal and observed that white mycelium with hyaline, branched with septate hyphae, Husain and Choudhary (2018) [5] isolated from oilseed brassica and identified *Sclerotinia sclerotiorum* on the basis of cultural and morphological characteristics similar result also reported by Mahalingam *et al*. (2018) [12], Prova *et al*. (2018) [8] and Islam *et al*. (2020) [7].

The tested fungus was found pathogenic on chilli seedling and produced typical symptoms of disease. Seedlings showed visible small water soaked lesion on the stem at the ground level, these water soaked lesions enlarge rapidly in size and girdled the entire base of young stem at the collar region. Whitish mycelial growth of the pathogen and sclerotia formation was observed on infected stems. Seedlings showed wilted symptoms and later seedlings were died.

Among the methods, seed + soil inoculation method results maximum per cent disease incidence with 72.66 per cent followed by soil inoculation method with 64.00 per cent disease incidence. These studies were supported by Pones *et al.* (1979) [13] were proved pathogenicity on lettuce by seed inoculation method, Iqbal *et al.* (2003) [6] proved pathogenicity on brinjal and Hansda *et al*. (2014) [4] were proved pathogenicity on brinjal and chilli by using artificial inoculation and soft rot symptoms were observed on brinjal and chilli which died after 10-15 days of inoculation. Similar findings were also observed by Kim and Cho (2003) [10], Yanar and Miller (2003) [17], Abdal-Kader *et al*. (2012) [1] on chilli plant using soil inoculation. Sharma *et al*. (2016) [16] proved pathogenicity of *Sclerotinia sclerotiorum* causing *Sclerotinia* rot of Indian mustard using seed, soil and seed cum soil inoculation method on Indian mustard and found that highest per cent disease incidence was observed in seed cum soil inoculation followed by soil inoculation method.

Table 1: Cultural and morphological characters of *Sclerotinia sclerotiorum*

S. No.	Fungal characters	Colour	Shape
1.	Colony	Whitish to gray	Uniformly fluffy regular
2.	Hyphae	Hyaline	Branched, cottony and septate
3.	Sclerotia	Blackish	Oval to irregular
4.	Ascus	Hyaline	Barrel shaped
5.	Ascospore	Hyaline	Elliptical to oval, single celled

Table: 2: Pathogenicity test of isolated pathogen on chilli plant

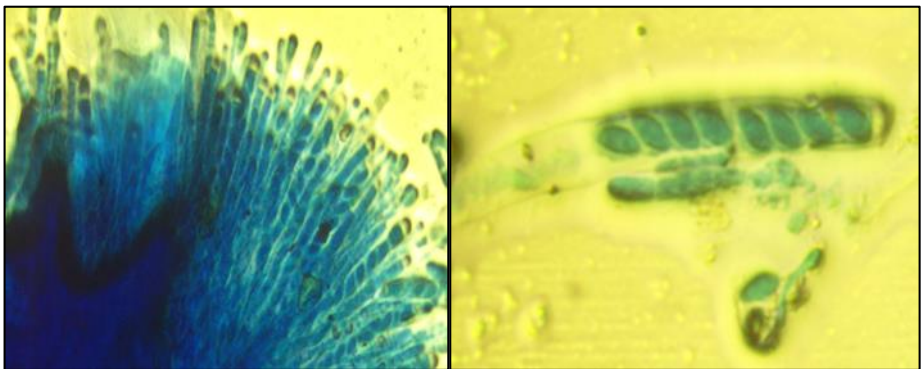
S. No.	Inoculation method	Disease incidence*
1.	Seed inoculation	56.30 (48.62)
2.	Soil inoculation	64.00 (53.13)
3.	Seed + Soil inoculation	72.66 (58.47)
4.	Uninoculated (Control)	0.00 (0.00)
	SEm±	1.52
	CD (P=0.05)	4.67

* Average of five replications

Plate 1: Diseased plant

Colony growth on PDA Sclerotia formation

Plate 2: Cultural characters of *Sclerotinia*



Arrangement of asci on periphery of ascocarp Ascus with eight ascospores

Plate 3: Morphological characters of *Sclerotinia sclerotiorum*

Conclusion

Isolation of associated fungus was done on basal medium (PDA) by infected plant samples were collected from farmer's field in Jaipur district. After isolation of the fungus, fungal was purified by using hyphal tip method and pathogen was identify as a *Sclerotinia sclerotiorum* on the basis of cultural and morphological characteristics. Pathogenicity of isolated *Sclerotinia sclerotiorum* was also proved through Koch postulates techniques under pot condition with seed, soil and seed cum soil inoculation methods. The tested fungus was found pathogenic on chilli seedling and produced typical symptoms of disease. Among the different methods of pathogen inoculation, seed cum soil inoculation was recorded maximum disease incidence.

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