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Pathological Studies of *Sclerotium rolfsii* causing Foot-rot disease of Brinjal (*Solanum melongena* Linn.)

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Abstract

During the regular survey of local brinjal growing field of Tikamgarh, it was observed that brinjal plants have been severely affected from a foot-rot disease caused by *Sclerotium rolfsii* sacc. It was also observed that the incidence of disease was much more in those areas where dead decaying plant debris and other organic matter accumulated near the plants. When disease plants uprooted and examined closely, it was seen that the basal portion of stem at foot region was infected and became dark brown in colour with superficial rotting. In some cases at collar region of stem on rotting tissues, superficial frosty growth of pathogen was observed which the characteristic symptoms of the pathogen are. In the experimental study the effect of four types of inoculum viz., sand oatmeal inoculum, infested wheat grains inoculum, seclerotia inoculum and mycelial suspension inoculum have been tested and investigated on the development of foot rot disease in sterilized and un-sterilized soil conditions. Sand oatmeal inoculum and infested wheat grains inoculum were proved to be the best as they cause 100% disease development and all the ten inoculated plants were collapsed in sterilized as well as in un-sterilized soil condition. In host range study of *Sclerotium rolfsii*, seedlings of ten different host species were tested in culture tubes against *Sclerotium rolfsii* by artificial inoculation method. Out of ten plant species, only five species namely chilli (*capsicum annum*), Fenugreek (*Trigonella foenum-graecum*), Gram (C*icer arietinum*), Tomato (*Lycopersicon esculentum*) and Wheat (*Triticum vulgare*) were found to be most susceptible, as degree of highly infection have been recorded in them and these plant species were completely collapsed within 10 days. Groundnut (*Arachis hypogaea*), Lady's finger (*Abelmoschus esculentus*), Linseed (*Linum usitatissimum*) and Sunnhemp (*Crotalaria juncea*) were found to be moderately susceptible. Ben (*Dolichos lablab*) was found to be resistant to some extent against *Sclerotium rolfsii* as a degree of a poor infection has recorded. It reveals that *Sclerotium rolfsii* has got a wide host range and the degree of infection differs from host to host.

Key-Words: *Sclerotium rolfsii*, Brinjal (*Solanum melongena*), Foot-rot, Tikamgarh

Introduction

Brinjal (*Solanum melongena* Linn.) is commonly called egg plant. It is an important summer vegetable crop grown all over India including Tikamgarh District of Madhya Pradesh. It is a small short lived perennial herb belonging to the family solanaceae of dicot angiosperm. It contains various chemicals such as Amino acids, Sugars and vitamins etc. (Prasad, 1977). The crop is attacked by a number of fungal species which put adverse effect on both the yield and the quality (Rao, 1969; Thakur, 1972; Vyas et. al., 1978; 1981).

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More then ten diseases have been reported on brinjal from this country (Rangaswami, 1972). Amongs them foot-rot of brinjal is most common disease caused by *Sclerotium rolfsii* sacc. This disease found to be serious in the tropical and sub-tropical regions, where temperature remains high which favour the growth and survival of the pathogen *Sclerotium rolfsii*.

It is well known that different types of inocula are mainly responsible for primary or secondary infection of host plant. Inocula of any pathogen may be in the form of vegetative hyphae, spores, conidia, zygote, oospore or any other resting resistant vegetative structure like sclerotia. When inoculum of pathogen came in contact with susceptible suitable host, in favourable conditions, after germination it will able to infect the plant and cause the disease. Horsfall (1932)

coined the term inoculum potential and he had the openion that the severety of disease is directly proportionate to mass (density) of inoculum. Mehrotra (1961) and Chaurasia (1976) conducted a number of experiments, which demonstrated that foot rot disease of pan (*Piper betle*) may be caused by different types of inocula such as zoospore suspension, infected host tissues etc. *Sclerotium rolfsii* is a well known soil borne plant pathogen. Cooper (1961) has termed *Sclerotium rolfsii* as a omnipathogenic fungus, as it possesses the ability to attack a large number of monocot and dicot plant species, belonging to about 100 families. According to Tu (1978) and Wydra (1996), *Sclerotium rolfsii* is especially severe on legums, solanaceous crops, cucurbits and other vegetables grown in rotation with beans. This pathogen is one of the most destructive and common pathogen of brinjal crop causing foot-rot disease in fields of Tikamgarh district of Madhya Pradesh. Due to this disease the local brinjal growers suffered a lot every year. Keeping the above facts in mind, in the present paper pathological studies of *Sclerotium rolfsii* sacc., a pathogen quite virulent on foot region of brinjal (*Solanum melongena*) plants at Tikamgarh district, have been investigated.

Material and Methods

Field survey

A general field survey of brinjal growing areas of Tikamgarh was made by frequently visiting the various fields of different localities. This was done mainly for the general observations with regard to foot rot disease of brinjal plants. The diseased brinjal plants were examined carefully in the field and their descriptions were recorded.

Collection of diseased material

The diseased material of brinjal plants were collected from the local field of Tikamgarh. These diseased materials were kept in sterilized polythene begs and brought to the laboratory for the purpose of isolation of the pathogen.

Isolation of pathogen

For isolation of pathogen, the diseased material of brinjal plants were cut with sterilized blade into small pieces of about 10-15 mm. The pieces were surface sterilized with 0.01% mercuric chloride solution for about 30 seconds. Soon after mercuric chloride treatment, these were repeatedly washed in sterilized distilled water to remove all the traces of chemicals. After surface sterilization, the isolation of pathogen was done by tissue segment and filter paper culture methods as adopted by Chaurasia (2000).

Purification of pathogen

The isolated pathogen was purified by hyphal tip method and single Sclerotium isolated method as given by Chaurasia (2000), Chaurasia (2001) and Chaurasia et.al. (2013).

Identification of pathogen

For identification of pathogen, morphological characters of isolated pathogen, compared with *Sclerotium rolfsii* (Sacc.).

Maintenance of pathogen culture

After obtaining the pure culture of *Sclerotium rolfsii*, the pathogen was transferred to agar slants of containing potato dextrose agar medium. After growing the culture of *Sclerotium rolfsii* the slants were kept at low temperature in refrigerator. After every 20-25 days, the pathogen was transferred to fresh slants for maintenance of pathogen culture.

Pathogenicity of pathogen

In order to investigate the pathogenicity of isolated pathogen, medium sized earthen pots were taken and filled 3/4 with field soil. These earthen pots were sterilized in autoclave at 20 lbs pressure for 30 minutes. After sterilization, the one seedling of healthy brinjal plant was transplanted and pots were-cared for few days. When all transplanted plants in earthen pots, got well established, five pots were selected for pathogenicity test. The inoculation of each healthy brinjal plant was done by placing infested wheat grains inoculum. Uninoculated pots were also kept as a control. Both inoculated and uninoculated pots were kept in a suitable place at room temperature in laboratory conditions. The inoculated plants were observed daily. As soon as the disease symptoms were evident, the pathogen was again re-isolated to confirm the infectivity of the isolated pathogen.

Effect of different types of inoculum on foot-rot disease development

To study the ability of infection and development of disease caused by *Sclerotium rolfsii*, in different soil conditions, the four types of inoculum prepared and inoculated as follows.

Preparation and inoculation of inoculum

(1) Sand-oat meal inoculum

For the preparation of sand-oat meal inoculum, white quartz sand was taken and sieved though a sieve having fine meshes. Sieved sand wash thoroughly in running water in order to remove the dust particles and other sticky substances and then dried. About 100 gms. of washed and dried sands were taken in each 250 ml. Erlenmeyer flask, then 5 gms. oat meal and 30 ml of water poured into each flask. These flasks were plugged tightly with non-absorbant cotton, then shake

vigorously to mix the sand and oat meal and autoclaved at 15 lbs. pressure for 20 minutes. After sterilization, each flask containing sand + oat meal was inoculated with 10 mm agar disc, which taken from the margin of freshly grown colony of pathogen *Sclerotium rolfsii*.

These inoculated flasks were incubated in incubator at 28°C for 15 days. During incubation, these flasks were shaken vigrously after every 3 days in order to obtain the uniform distribution of gorwing mycelium in sand-oat meal. After 15 days the homogenous mixture of sand oat meal inoculum was used for inoculation purpose. All pots with sterlized and un-sterlized soil were inoculated by placing the 5 gms. of freshly prepared oat meal inoculum near the foot region of growing brinjal plant.

(2) Infested wheat grains inoculum

For the preparation of wheat grain inoculum, clean & healthy wheat grains were selected and washed thoroughly in running water. About 50 gms. of moist wheat grains were taken and placed in 250 ml. Erlenmeyer flask. After plugging with non-absorbent cotton, these flasks were sterilized at 15 lbs. pressure for 20 minutes in autoclave. After proper sterilization, the moist wheat grains of the flasks were inoculated by transferring the 10 mm. agar disc taken from the advancing margin of the freshly grown culture of *Sclerotium rolfsii* inoculated flasks were incubated at 28°C for 25 days, during inoculation, these inoculated flasks were rotated & shake vigoroushly in order to obtain the uniform and homogenous inoculum. To study the effect of infested wheat grain inoculum on the disease development, all the taken earthen pots with sterilized and un-sterilized soil were inoculated by placing the five to ten heavily infested wheat grains near the foot region of growing brinjal plant and observed daily.

(3) Selerotial inoculum

The resting resistant structures " sclerotia" were used as the " Sclerotial inoculum". For the preparation of sclerotial inoculum, *Sclerotium rolfsii* was grown as aseptically, on the potato dextrose agar medium, at 30°C. for 25 days. After 25 days inoculation period, when sclerotia matured and abundantly developed on the medium then these were taken and used as inoculum.

During experimental investigation on the disease development, five mature sclerotia selected and placed near the foot region of growing brinjal plants in sterilized & un-sterilized soil and observed daily.

(4) Mycelial suspension Inoculum

To prepare the mycelial suspension inoculum, first of all, 20 ml. of sterilized liquid potato dextrose medium was taken in sterilized petridishes. These petriplates containing broth medium were inoculated by transferring the mycelial agar disc of 10 mm. diameter. The mycelial agar disc for inoculation purpose, cut with the help of sterilized cork borer from the advancing margin of freshly grown culture of *Sclerotium rolfsii*. Inoculated petridishes containing PDA broth medium were inoculated at 28°C. for about 9 days. After 9 days inoculation, the thick cottony mat of *Sclerotium rolfsii* was removed and washed thoroughly in sterilized distilled water. After washing, about 50 gms. of mycelial mat taken and placed in 1000 ml. conical flask which containing 250 ml. of distilled water and fine glass pieces. Then the mycelial mat, shake vigorously on the magnetic stirrer for obtaining the uniform mycelial suspension inoculum.

All the earthen pots containing sterilized and un-strerilized soil were inoculated by flooding the 50 ml. mycelial suspension inoculum near the base of stem portion which touches the soil, i.e., at the collar region of brinjal plant.

Preparation of Earthen Pots

50 small size of earthen pots were taken and 3/4 filled with air dried and sieved soil of vegetable fields. Half of the earthen pots were sterilized in autoclave at 20 lbs. pressure for 30 minutes. Two well developed brinjal seedling were planted in each pot containing sterilized and un-sterilized soil. To maintain the moisture content of soil, the sterilized water added regularly in to the pots. When transplanted plants were got well established in sterilized & un-sterilized soil (pots), the plants were inoculated with desired inoculum, as described earlier, to study the effect of inoculum on disease development. All the inoculated, pots were kept in suitable places and observed regularly for the development of disease for about one month. After one month of inoculation, the percentage of collapse plants were calculated with the help of following formula:

Collapsed Plant (%) = Total No. of Collapsed Plants/Total No. of PlantsX 100

Host range of *Sclerotium rolfsii*

In order to test the host range of *Sclerotium rolfsii*, seedlings of ten different host plants viz, Bean (*Dolichos lablab*), Chilli (*Capsicum annum*), Fenugreek (*Trigonella foenum-graecum*), Gram (*Cicer arietinum*), Groundnut (*Arachis hypogaea*), Lady's finger (*Abelmoschus esculentus*), Linseed (*Linum usitatissimum*), Sunnhemp (*Crotalaria juncea*), Tomato (*Lycopersicon esculentum*) and Wheat (*Triticum vulgare*) were raised under sterile conditions

in culture tubes of 20 mm. diameter. The detailed method was as follows"

(A) Collection and surface sterilization of the seeds

Seed of ten different host plants were purchased from the local seed supplier of Tikamgarh. All the seeds were first of all examined microscopically and only healthy seeds were selected. The selected seeds were first washed thoroughly in water and then were surface sterilized in 0.1% mercuric chloride solution for about three minutes. The seeds were washed thoroughly many times with sterilized distilled water in order to free them from the murcuric chloride.

(B) Raising the seedling in the culture tubes

Culture tubes of 20 mm. diameter were used for raising the seedlings. In each culture tube, 5 ml. of knop's solution was taken and a small piece of cotton was placed in bottom of tube. The tubes were plugged by non-absorbent cotton and autoclaved at 15 lbs. Pressure for 15 minutes. Tubes were cooled after sterilization. Surface sterilized seeds were now placed inside the tubes to obtain the seedlings. Only one surface sterilized healthy seed was sown and grown in one culture tube.

(C) Inoculation of culture tubes

The inoculation was made after 6 to 10 days of sowing the seeds, just after first two leaves appeared on the young seedling. Each tube was inoculated by placing the equal amount of inoculum at the base of seedling. The inoculum which was used a 8.0 mm. agar disc, taken from the margin of two days old colony of *Sclerotium rolfsii*. Simultaneously un-inoculated culture tubes of each host were also run parallel for purpose of control. All the inoculated and un-inoculated culture tubes were kept under the normal light and temperature conditions of the laboratory.

The culture tubes of each host were examined regularly upto 10 days and symptoms in the seedling were noted and compared with the un-inoculated seedling of control tube. Finally after 10 days, the degree of infection was categorized as given in following Table-1 according to the lesioning of the seedlings. :

Table 1: Degree of infection of Seedlings

Lesioning on basal part of seedling	Degree of infection	Symbol
Healthy seedling (no lesioning)	No infection	**0**
Initial lesioning	Poor infection	**1^+**
Heavy basal lesioning	Moderate infection	**2^+**
Collapsed seedling	**Highly infection**	**3^+**

Results and Discussion

Field Observations

A regular visit was paid to the local brinjal growing fields and during the survey, it was noticed that the brinjal plants in a patch have suffered from a severe disease known as "foot-rot of stem".

When disease plant was uprooted it was seen that basal portion of stem, near the soil level was infected and became dark brown in colour. In some severly infected plant besides dark brown discolouation at foot region, superficial rotting of tissues have also observed and in some cases rotten portion often frosty in appearance due to the superficial mycilial growth of pathogen. On severely infected portion, frosty appearance of mycelial growth is a charcteristic symtoms of the disease.

During the survey, sclerotia have not been found to be attached at infected portion on the superficial mycelium.

Roots of severely infected plant were developed feebly and became dark brown in colour. In early stage of growth, severely infected plant did not produce any fruit and showed wilting symptoms.

During field observation, it was also observed that the incidence of disease was much more in those fields in which plant debris and dead decaying organic matter accumulated near the plants. The decaying plant debris around the plants, favours the growth of pathogen and helping in builting of inoculum.

Isolation of pathogen

From the collected diseased samples, the isolation of pathogen was made successfully by routine pathological techniques of isolation, as described earlier. By tissue segment method, the pathogen was successfully isolated on the potato-dextrose agar medium within three days after inoculation. The growth of pathogen recorded around the bits of diseased samples was abundent, with its colony characteristic. While in case of "filter paper isolation method" growth of the pathogen obtained very late (i.e., after 8-10 days) around the diseased samples. Secondly, the isolated pathogen developed feebly on the moist filter paper sheets. Therefore, the transfer of isolated pathogen was also difficult to fresh petridish. From the above discussed results, it is concluded that the tissue segment method for the isolation of pathogen proved to be the best in comparison to "filter paper isolation method".

The isolated pathogen produced cottony white, superficial dense readiating hyhpae on the potato-dextrose agar medium (photo plate 1). On the mycelium, sclerotia were observed within 4-5 days, as a white pinkish, soft rounded bodies, which were with

the age became light brown to brown in color and ultimately changed in to dark brown and became hard. The development of mustard seed like "sclerotia" is the characteristic of pathogen Sclerotium (photo plate 2). The morphological characters of isolated pathogen was found to be identical with the *Sclerotium rolfsii* (Sacc.).

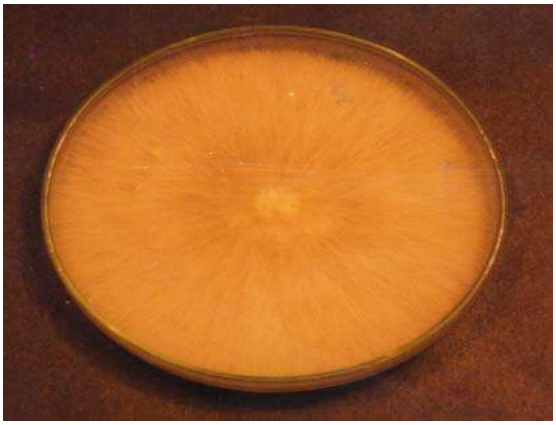

Photo Plate 1: Isolated *Sclerotium rolfsii*

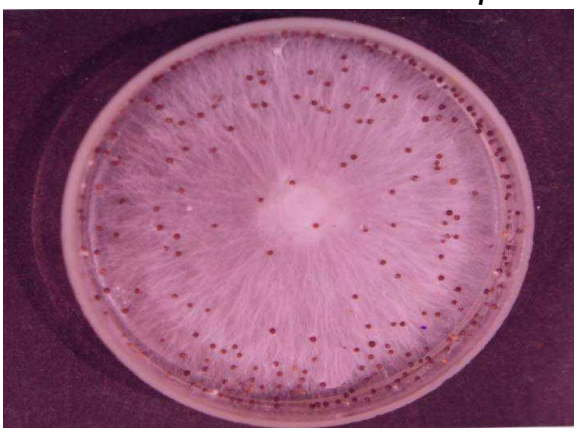

Photo Plate-2: Showing Sclerotia formation of *Sclerotium rolfsii*

Pathogenicity test

To observe and confirm the pathogenic ability of pathogen (*Sclerotium rolfsii*), trans planted healthy brinjal plants were inoculated by placing the inoculum of isolated pathogen, as described earlier. After few days of inoculation, it was observed that the inoculated brinjal plants were found to be infected and producing the typical disease symptoms of "foot-rot of stem", as observed in the field. After production of the disease, the pathogen (*Sclerotium rolfsii*) was successfully re-isolated from the artificially inoculated diseased plant. Then this re-isolated culture of pathogen compared with the original isolated culture. On comparison, re-isolated culture of pathogen was found to be identical with the previously isolated culture of pathogen, which confirms the kotch's postulates and pathogenic ability of isolated pathogen i.e., *Sclerotium rolfsii*.

Effect of different types of inoculum on foot-rot disease development

The effect of four different types of inoculum viz., sand oatmeal inoculum infested wheat grains inoculum, sclerotia inoculum and mycelial suspension inoculum have been successfully investigated on the development of disease "foot-rot of stem". The inoculated plants observed daily and final results are presented in table 2 and figure 1.

From the results it is revealed that oatmeal and infested wheat grains inoculum were proved to be the best as they cause 100% disease development and all the tested 10 plants were collapsed in sterilized and in un-sterilized soil conditions. From the daily observations, it has also noticed that in sterilized soil conditions both the inoculum proved to be more effective as disease appeared much early, in comparison to un-sterilized soil.

When mycelial suspension was used as a inoculum, all the tested plants remained healthy and no disease incidence was recorded in both sterilized and un-sterilized conditions of soil. This result suggests that mycelial suspension in soil did not multiply due to lack of plant debris and may destroyed.

In case of sclerotial inoculum, the 30% disease incidence was recorded only in those plants which were grown on sterilized soil. In un-sterilized soil sclerotia were unablde to infect the plants and it may due to antagonistic effect of soil micro organism. On germ tube, Sclerotia are hard and resistant structure and takes a long time for germination, therefore, infection process is very slow.

From the above results on the whole it is concluded that sand oat meal and infested wheat grain inoculum were found to be the best in both the soil conditions. Actually, the oat and wheat which are incorporated in the inoculum serves as food and faciliate the growth of pathogen in soil, thus the potentiality of inoculum increases which results in the more incidence of disease and the antagonistic effect of soil micro organism has also no pronounce effect on density of inoculums.

Host range of *clerotium rolfsii*

In order to study the host range of *Sclerotium rolfsii*, the seedling of ten different host plant viz., Bean (*Dolichos lablab*), Chilli (*Capsicum annum*), Fenugreek (*Trigonella foenum-graecum*), Gram (*Cicer arietinum*), Ground nut (*Arachis hypogaea*), Lady's finger (*Abelmoschus esculentus*), Linseed (*Linum*

usitatissimum), Sunnhemp (*Crotalaria juncea*), Tomato (*Lycopersicon esculentum*) and Wheat (*Triticum vulgare*) were raised and inoculated as described earlier. All the inoculated plant observed daily and final data was recorded in Table 3.

From the results presented in table 3 and figure 2, it is evident that all the 10 plant species were found to be susceptible to the pathogen *Sclerotium rolfsii*, as in all the tested plants, infection and disease development has recorded.

Table 2: Effect of different types of inoculum on foot-rot disease development

Inoculum	Un-sterilized soil				Sterilized soil			
	Number of pots	Number of plants	Number of collapsed plants	Percentage of collapsed plants	Number of pots	Number of plants	Number of collapsed plants	Percentage of collapsed plants
Sand oatmeal	5	10	10	100	5	10	10	100
Infested wheat grains	5	10	10	100	5	10	10	100
Sclerotia	5	10	0	0	5	10	3	30
Mycelial Suspension	5	10	0	0	5	10	0	0
Control	5	10	0	0	5	10	0	0

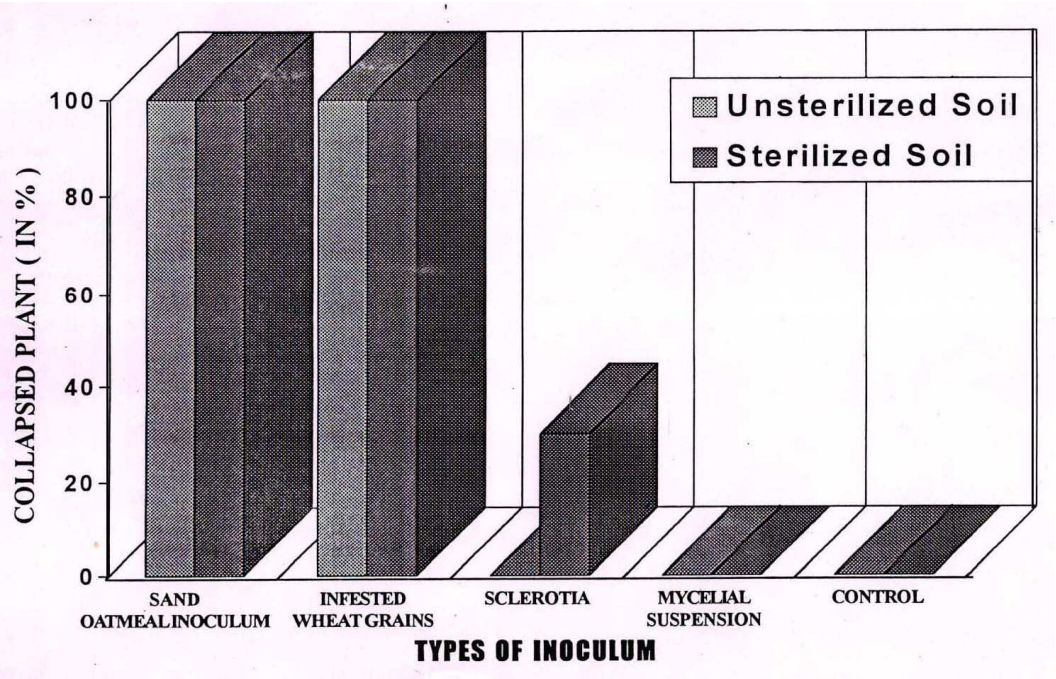

Fig. 1: Effect of different types of inoculum on foot-rot disease development

Table 3: Host range of *Sclerotium rolfsii* under artificial inoculation

Host	Degree of Infection
Bean (*Dolichos lablab*)	**1^+**
Chilli (*Capsicum annum*)	**3^+**
Fenugreek (*Trigonella foenum-graecum*)	**3^+**
Gram (*Cicer arietinum*)	**3^+**
Groundnut (*Arachis hypogaea*)	**2^+**
Lady's finger (*Abelmoschus esculentus*)	**2^+**
Linseed (*Linum usitatissimum*)	**2^+**
Sunnhemp (*Crotalaria juncea*)	**2^+**
Tomato (*Lycopersicon esculentum*)	**3^+**
Wheat (*Triticum vulgare*)	**3^+**

*** Degree of Infection**

0 (No Infection), 1^+ (Poor Infection), 2^+ (Moderate Infection), 3^+ (Highly Infection)

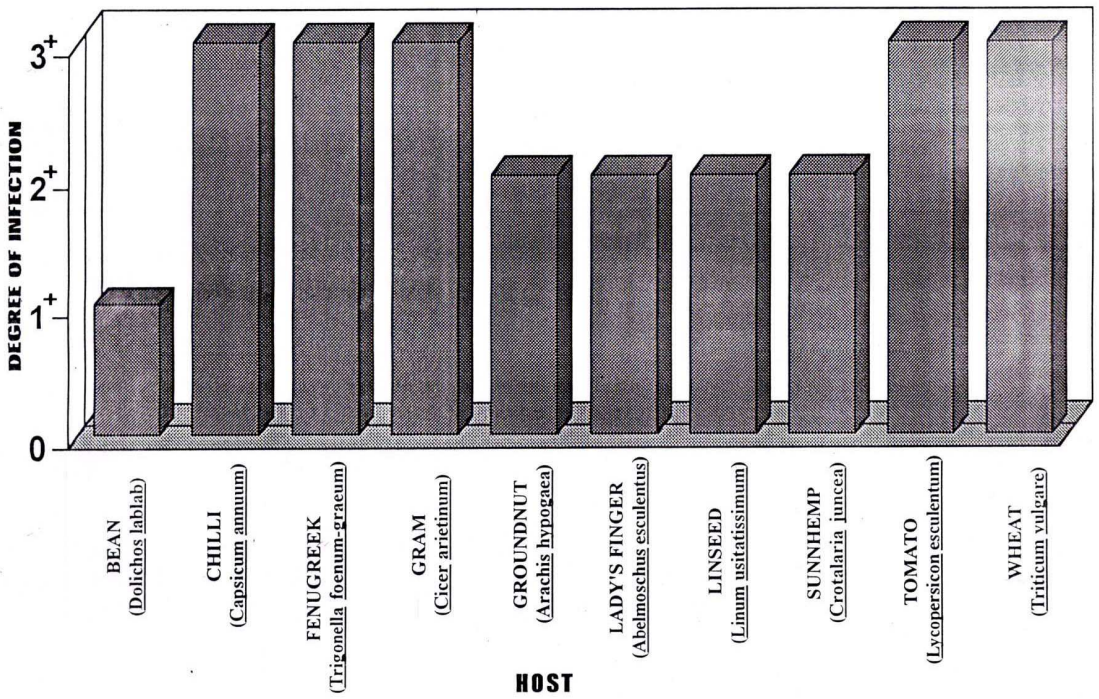

Fig. 2: Host range of *Sclerotium rolfsii* under artificial inoculation

Chilli (*Capsicum annum*), Gram (*Cicer arietinum*), Tomato (*Lycopersicon esculentum*), Fenugreek (*Trigonella foenum-graecum*) and wheat (*Triticum vulgare*) were found to be most susceptible for the disease, as maximum degree of infection have been recorded in them and these plants were completely collapsed within 10 days after inoculation. In these five plant species, the first sign of infection has appeared as browning of stem at foot region, which extended soon downward and after some time white frosty mycelial hypha growth was also observed on infected region. Ultimately these plants were collapsed within 10 days.

Ground nut (*Arachis hypogaea*), Lady's finger (*Abelmoschus esculentus*), Linseed (*Linum usitatissimum*) and Sunnhemp (*Crotalaria juncea*) were found to be moderately susceptible for pathogen *Sclerotium rolfsii*, as in these four plants species moderate degree of infections have been observe and at foot region about 50 to 60% area was infected and became dark brown in color. On infected portion, slight frosty growth of fungus has been observed.

Bean (*Dolichos lablab*) only plant species which was found to be resistant to some extent again *Sclerotium rolfsii*, as a very poor infection has recorded. Very small light brown colour lesion has observed very late at foot region and after 10 days, the growth of the plant has not showed any remarkable effect.

From the above result on the whole it can be concluded that chilli (*Capsicum annum*), Fenugreek (*Trigonella foenum-graecum*), Gram (*Cicer arietinum*), Tomato *(Lycopersicon esculentum*) and wheat (*Triticum vulgare*) have been found to be most susceptible while Ground nut (*Arachis hypogaea*), Lady's finger (*Abelmoschus esculentus*), Linseed (*Linum usitatissimum*) and Sunnhemp (*Crotalaria juncea*) have been observed as moderately susceptible. Bean (*Dolichos lablab*) has been found to be resistant to some extent against *Sclerotium rolfsii* and very poorly infected.

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