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INOCULATION TECHNIQUES AND LIQUID MEDIA STUDIES ON *SCLEROTINIA SCLEROTIORUM* (LIB) DE BARY CAUSING SCLEROTINIA ROT OF INDIAN MUSTARD

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ABSTRACT

Indian mustard [*Brassica juncea* (L.), Czern & Coss] is the second important oilseed crop of India. Sclerotinia rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major disease of mustard. The present research work aimed to study the inoculation techniques, morphological and cultural characters of *S. sclerotiorum.* Isolations were made and culture was purified by adopting hyphal tip method from Sclerotinia rot infected plants. Small mycelia tufts and black semi spherical to round or irregular shape sclerotia observed in the culture growth and which measured 2-10 mm × 13-15 mm in size. Highest per cent disease incidence (82.00 %) was observed in seed + soil inoculation technique followed by soil inoculation (70.00%). Among liquid media tested, maximum dry mycelia weight 75.10 mg and excellent sclerotial formation was recorded on Potato Dextrose broth. It was followed by Richard's medium resulted 68.60 mg dry mycelia weight and good sclerotia formation.

KEYWORDS: Mustard, *Sclerotina sclerotiorum*, Pathogenicity, Liquid Media

INTRODUCTION

Indian mustard [*Brassica juncea* (L.), Czern & Coss], belongs to family *Crucifereae* (*Brassicaceae*). The oilseed *Brassica* crops generally grouped as rapeseed and mustard, commonly cultivated species are *B. campestris* var. sarson Prain: yellow and brown sarson; *B. campestris* var. toria; *B. juncea* Czern and Coss; rai and *Eruca sativa* Mill. taramira. Indian mustard [*Brassica juncea* (L.), Czern & Coss] is the second most important oilseed crop of India. The black and brown seeds possess about 40 per cent oil content. The oil is mainly used for direct human consumption. Indian contribution in global rapeseed and mustard production is 7.8 million tonnes with an area of 6.5 million hectares and average productivity of 1208 kg/ha (Anonymous, 2013-14a). Rapeseed and mustard crops are extensively grown in northern and western parts of India viz., Rajasthan, Uttar Pradesh, Madhya Pradesh, Haryana, Gujarat, West Bengal, Assam, Bihar, Punjab and Jammu & Kashmir. Among these, Rajasthan state ranks first both in area and production i.e. 2.78 million ha and 3.62 million tonnes, respectively with an average productivity of 1301 kg / ha (Anonymous, 2013-14b). In Rajasthan, Tonk district ranks first both in area and production (2.66 lakh ha and 3.85 lakh tonnes, respectively) followed by Sriganganagar, Alwar, Bharatpur and Hanumangarh. The attack of diseases and pests is the most important factor causing yield instability in rapeseed and mustard. The severe attack of many diseases and insects not only deteriorates the quality of the seed, but also reduces the oil content considerably. Out of more than thirty diseases reported on *Brassica* in India, the Sclerotinia rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is major disease. *S. sclerotiorum*

is a necrotropic pathogen, it damage to the plant tissue followed by cell death and development of soft rot or white mould. Yadav *et al.* (2013) reported 17.4 per cent diseases incidence from six districts of Rajasthan and among these districts maximum per cent disease incidence was reported in Dausa (29.2%). The present study aimed to study the inoculation technique for pathogenicity and morphological and cultural characters of *S. sclerotiorum*.

MATERIALS & METHODS

Collection & Isolation of Pathogen

Sclerotinia rot affected plants of Indian mustard were collected from farmer's field of Jaipur district of Rajasthan. Diseased stem portion 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 0.1 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 20 ± 2 ˚C. Similarly, isolation was also made from black sclerotia present inside 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 0.1 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 slant culture tubes then incubated for 4 days at 20 ± 2 ˚C. The fungus was purified by hyphal tip method (Riker and Riker, 1936). The culture was maintained in refrigerator at 10 ˚C and renewed after every fifteen days.

Inoculation Techniques for Pathogenicity

Soil was sterilized at 1.045 kg/cm^2 pressure for one and half hour on two successive days. 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 different inoculation techniques such as seed inoculation, soil inoculation and seed + soil inoculation techniques. Seeds were inoculated by smothering with 7 days old culture of test fungus. The pathogen multiplied on sterilized sorghum grains and mixing at the rate of 20 g per pot (30 cm dia.) in the upper layer of the each pot. The soil in pots moistened and covered with polythene bags and left for 24 hours in cage house. On next day, apparently fifteen healthy sterilized seeds of Indian mustard variety Varuna (T-59) were sown in each pot. Ten seedlings in each pot were maintained after removing the excess seedlings on 10th day of germination. Five replications of each treatment were maintained; reisolation from infected plants yielded the same fungus.

Liquid Media Studies

Glassware were washed with Potassium dichromate sulphuric acid solution, washed with sterilized water, sterilized in hot air oven at 180 ˚C for two hours. For liquid media studies, five broth media viz. Asthana and Hawker's medium, Brown's medium, Czapek's Dox broth (CDB), Potato Dextrose broth (PDB) and Richards' medium were used. The media were prepared with single distilled water. The pathogen was grown on above 25 ml each liquid media containing in Erlenmeyer flasks, autoclaved, inoculated with 5 mm disc of fungus and incubated at room temperature ranging from 25 ± 1 ˚C. Mycelium was harvested after 15 days and filtered through Whatsman filter paper No. 42, dried at 60 ˚C for 24 hours and weighted and sclerotial size noticed. In each experiment four replication were maintained.

RESULTS & DISCUSSIONS

Isolation of Pathogen

Isolations were made from diseased portions of plant. Uniformly one type of fungus colony with whitish growth started appearing after 24 hours. Later on, the growth of fungus was very fast which covered the entire Petri plate within 72 hours. After 5 days of growth, small mycelial tufts started to develop at the periphery of the Petri plates and later such growth covered the entire Petri plate Shiny water droplets were seen frequently in culture plates around the mycelial tufts. Later on, these mycelial tufts were converted into hard black coloured sclerotia. Individual sclerotium was seen embaded in white mycelium net. Semispherical to round or irregular shape sclerotia measured 2-10 mm x 13-15 mm in size. On carpogenic germination these sclerotia give rise to 2-5 columnar structures (stipes). The exposed portion of stipe was brown coloured. Apothecia were produced after 50 days of burial at 2-3 cm depth in soil. Apothecia were cup-shaped with disc concave, light yellowish brown in colour and varied from 2-10 mm (average being 5-6 mm) in diameter (Plate 3). Apothecium is formed on a slender stalk of 15-55 mm in length called stipe. Asci are arranged on periphery of ascocarp. They are hyaline, cylindrical and produced in tightly packed mass with filiform paraphyses at the upper surface of apothecium. Ascospores are elliptical to oval, one celled, hyaline, numbering eight in each ascus.

Inoculation Techniques for Pathogenicity

Observations of pathogenicity of *S. sclerotiorum* are presented in Table 1. The pathogenicity of the isolated fungus was proved by seed inoculation, soil inoculation and seed + soil inoculation techniques. After 25-30 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. Highest per cent disease incidence (82.00 %) was observed in seed + soil inoculation technique followed by soil inoculation (70.00%). Wang and Rang (2013) and Gill *et al.* (2014) also isolated and proved pathogenicity of *S. Sclerotiorum* on *Brassica sp.*

Liquid Media Studies

To study the effect of 5 liquid media on growth and sclerotial formation, inoculated flasks were incubated at temperature 25 ± 1 ˚C for 15 days and mycelial mats were harvested, dried and weighed. Result presented (Table 2) maximum dry mycelia weight 75.10 mg and excellent sclerotia formation was recorded on Potato Dextrose broth. It was followed by Richard's medium resulted 68.60 mg dry mycelia weight and good sclerotia formation. Subramanyam *et al.* (1993) and Elgorban *et al.* (2013) also proved that Potato Dextrose broth medium is good for growth of plant pathogenic fungi including *S. sclerotiorum.*

Table: 1: Screeing of Inoculation Techniques for Pathogenicity of *Sclerotinia sclerotiorum*

Technique	Percent Disease Incidence*
Soil inoculation	70.00 (56.79)
Seed inoculation	58.00 (49.60)
Seed + soil inoculation	82.00 (64.90)
Healthy control	0.0 (0.00)
SEm+	0.86
CD (P=0.05)	2.64

*Average of five replications

Table: 2: Dry Mycelial Weight, Sclerotial Formation and Sclerotia Size of *S. sclerotiorum* in Liquid Media

Media	Average Dry* Mycelial Weight (Mg)	Sclerotial* Formation	Size of* Sclerotia (Mm)
Asthana and Hawker's	20.40 (26.85)	+	1-3
Brown's	45.20 (42.25)	++	1-3
Czapek's dox	57.00 (49.02)	+++	1-4
Potato Dextrose broth	75.10 (60.07)	++++	1-6
Richard's	68.60 (55.92)	+++	1-5
SEm±	0.92		
CD (p=0.05)	2.83		

* Average of four replications

Figures given in parentheses are angular transformed values

+ = poor (1-5), ++ = Fair (6-10), +++ = good (11-15), ++++ = Excellent (16-20)

CONCLUSIONS

Indian mustard [Brassica juncea (L.), Czern & Coss] has gained importance in different parts of Rajasthan as potential oilseed crop and is grown in almost every district of Rajasthan. This crop suffers from many diseases among which Sclerotina rot caused by Sclerotinia sclerotiorum (Lib.) de Bary, has become a serious problem in recent years in Rajasthan and in other mustard growing parts of India. The diseased plant samples collected from farmer's field in Jaipur district and fungus associated was isolated and multiplied on sorghum grains. Pathogenicity was proved using Varuna (T-59) as a susceptible cultivar. After 25-30 days of sowing, water soaked tiny specks appear on collar region. These water soaked soft specks enlarge rapidly in size, girdling the stem and advancing up and downwards. The pith is found completely filled with hard, black and irregular sclerotia similar to those formed in culture. At later stages, the white necrotic lesions formed on stem and leaves. The fungus was found to be pathogenic to Indian mustard producing typical symptoms in 25-30 days of inoculation. Highest per cent disease incidence was recorded in seed + soil inoculation technique. The fungus was capable of growing on different liquid media, but variation in growth and amount of sclerotial formation was observed on five liquid media. Results showed that Potato Dextrose broth was good for mycelium growth and sclerotial formation followed by Richard's medium and Czapek's dox medium.

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