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Efficacy of different inoculation techniques for testing the pathogenicity of *Sclerotinia sclerotiorum* causing Sclerotinia blight of *Brassica juncea*.

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Abstract

Pathogenicity of isolated fungus *S. sclerotiorum* was tested using four techniques of inoculation viz., tooth pick injury method, Mycelial bit placement method, Sclerotia placement method and paraffin wax film method were evaluated to find out the most effective method for artificial screening of the disease at the experimental field of Department of Plant Pathology, College of Agriculture, RVSKVV Gwalior (M.P.) during Rabi 2014-15. Findings revealed that paraffin wax film method was most effective in causing successful stem rot infection (32.00%) followed by mycelia bit placement (25.74%) and tooth pick injury method (17.25%), while the inoculation with sclerotia was found as the least effective method. Paraffin wax film method was significantly superior over other methods. Mycelia bit placement was significantly superior over other two methods viz., tooth pick and sclerotia placement. All the methods including the least effective method that is sclerotia placement were superior over control.

Keywords: pathogenicity, *Sclerotinia sclerotiorum*, *Brassica juncea*

Introduction

Indian mustard (*Brassica juncea* (L.) Czernj. Cosson) is also known as Rai or Laha belong to *Brassica ceae* and Centre of origin Mideterian. Oilseed Brassicas, Rapeseed- Mustard are the world's third most important sources of vegetable edible oil. Rape seed and Mustard crops are being cultivated in 53 countries spreading over the six continents across the globe covered area. Madhya Pradesh contributed in rapeseed-mustard crops production area about 08.00 lakh hectares with production and productivity of 11.40 lakh tonnes and 1425 kg/ ha respectively during, (Farmer Welfare and Agriculture Development, Bhopal, Madhya Pradesh, 2013-14). Out of this Morena, Bhind, Gwalior and Sheopur jointly contribute >60% production of these crops in the state. Amongst the fungal diseases, Sclerotinia blight in mustard incited by *Sclerotinia sclerotiorum* (Lib.) de Bary earlier considered to be a minor problem in India but it has become a serious problem of rapeseed mustard over the years in some parts of country, (Shekhawat *et al*, 2012) [3]. Infection and symptoms of Sclerotinia blight are visible after flowering. Sclerotinia infection may be observed as individual plants scattered throughout the field, or in patches in the field where moisture was greatest. Lodged crops are more susceptible to Sclerotinia mold development.

Material & Methods

a. Isolation

The infected stem having sclerotia were collected from Zonal Agriculture Research Station Morena. The sclerotia were taken from infected plant for preparing culture of the pathogen. Sclerotia was cut with the help of blade into two parts and surface sterilized with mercuric chloride 0.01% for 1 minute and transferred to potato dextrose agar PDA culture by poisoned food technique (Schmitz, 1930) [4] and incubated at 25±2°C and examined at frequent intervals.

b. Purification

Purification of the isolated fungus was carried out using hyphal tip techniques as described by Dhingra and Sinclair (1985) [1].

c. Identification

Isolated Pure culture fungus was identified according to their morphological characters and the pure culture was maintained by sub culturing once in a fortnight on PDA slants. The medium will change from blue to yellow as colonies of *S. sclerotiorum* develop. The yellow halo is a result of oxalic acid production by the fungus.

Pathogenicity test

The pathogenicity of the isolated *Sclerotinia spp.* was tested using four methods of inoculation at the experimental field of

Department of Plant Pathology, College of Agriculture, RVSKVV Gwalior (M.P.).

Total 10 plants from each replication for each technique were evaluated under this experiment

(i) Mycelial bit placement method

In this method the stem of 45 days old mustard plant was scratched at the height of 10-12" from soil level. On the scratched portion the mycelial bits of *S.sclerotiorum* was placed with the help of cotton and it was covered with cello tape so that it remain in the contact with the stem.



Formation of hole in stem through cork borer



Hole are formed in stem



Placement of mycelial bit inside bordered stem



Wrapping of cotton on inoculated stem



Wrapping of tap in inoculated stem



Inoculated stem

(ii) Sclerotia placement method

The stem of 45 days old mustard plant was scratched at the height of 10-12" from soil level. On the scratched portion the

sclerotia was placed with the help of cotton and it was covered with cello tape so that it remain in the contact with the stem.



Formation of hole in stem through cork borer



Sclerotia in Petri disc



Sclerotia placed in bordered stem



Wrapping of cotton on inoculated stem



Wrapping of tap in inoculated stem



Inoculated stem

(iii) Tooth pick method

A mycelial disc of 7 mm diameter was taken from 7 days old culture with the help of sterilized cork borer grown on PDA

media, placed in broad hole made at the base of the plant by cork borer and covered with absorbent cotton.



(iv) Paraffin wax method

In this particular method surface of stem was rubbed with tooth pick stick and 7 days old culture was applied on rubbed surface. This was later covered by paraffin wax film and left for the appearance of symptoms.



(v) Control (without inoculation)

At the time of inoculation some of disease free plants were tagged and left uninoculated for comparison with inoculation methods. The percent disease incidence was calculated as per the following formulae:

$$\text{Disease incidence (\%)} = \frac{\text{Total Infected plants}}{\text{Total number of plants}} \times 100$$

Result and Discussion

Four inoculation technique viz. tooth pick, mycelia bit placement, sclerotia placement and paraffin wax film were evaluated for artificial screening of the disease. The paraffin wax film method was found most effective in causing successful stem rot infection followed by mycelial bit placement and tooth pick method, while the sclerotia placement was the least effective method. Paraffin wax film method was significantly superior over tooth pick and inoculation with sclerotia. All the methods including the least effective method that sclerotia placement was superior over control (table:1, fig:). present findings having similarity with the findings of Prasad *et.al* (2009) [2], they also evaluated four method to obtain best production of apothecia and recorded maximum infection (86.7%) under inoculums placement followed by tooth pick (55.6%) and ascospore inoculation method (53.3%).

Table 1: Efficacy of different methods for testing the pathogenicity of *Sclerotinia sclerotiorum* on *Brassica juncea*.

Inoculation Methods	Infected plants per cent
Mycelial bit placement method	25.74 (31.59)
Sclerotia placement method	11.25 (20.60)
Tooth pick method	17.25 (25.52)
Paraffin wax film method	32.00 (36.23)
Control (with out inoculation)	0.00 (0.00)
SE(m) ±	1.667(1.188)
CD at 5%	5.225(3.701)

The data are the mean of the four replications.

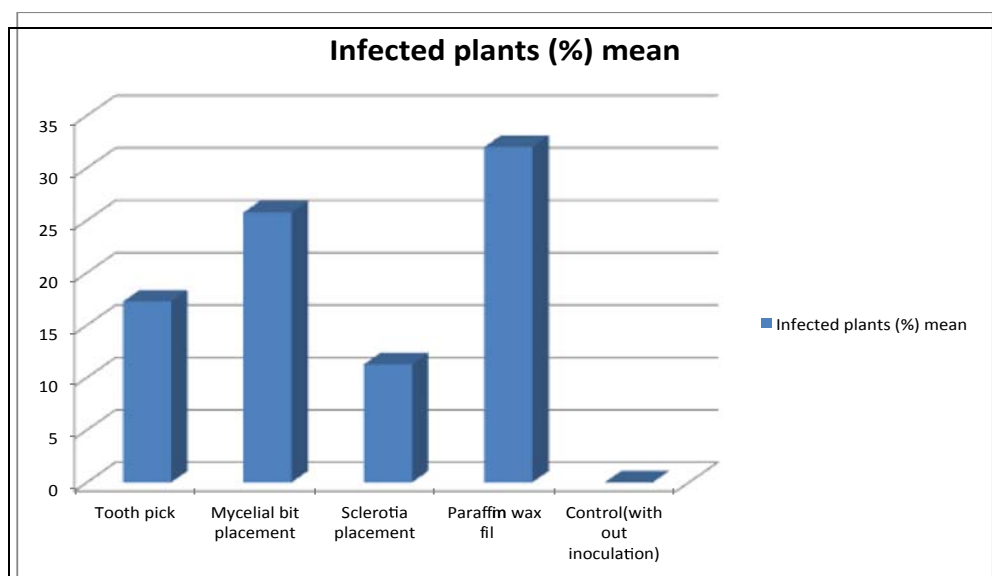


Fig 1: Efficacy of different method for testing the pathogenicity of *S. sclerotiorum* on mustard.

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