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RESEARCH ARTICLE :

Pathogenicity test through artificial inoculation techniques for stem rot in mustard

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SUMMARY : Stem rot disease has emerged as a potential threat in most mustard growing area at globally. It is being continuously serious and devastative disease year after year. The pathogenicity done by different techniques *viz.*, Mycelial bit placement, Mycelial bit placement in inside tender bark, Mycelial bit placement on scratched stem, Mycelial suspension spray, Mycelium slurry inoculation, Paraffin wax film or two sided tape, Placement of inoculum through brushing scratched stem, Sclerotial placement, Sclerotial powder dusting and Tooth pick technique. The results revealed that six techniques were proved successfully infection of *Sclerotinia sclerotiorum*. Paraffin wax film technique was found statistically superior over other inoculation techniques followed by tooth pick, mycelial bit, sclerotial placement, mycelial bit placement inside tender bark and mycelial bit placement on scratched stem. Paraffin wax film technique put secure place for early successfully infection, while the inoculation with sclerotia was found as the late establishment of infection. However, successfully establishment of the infection within 5-8 days after inoculation in the favorable environmental condition in 45-65 days old plants.

KEY WORDS :
Pathogenicity, Inoculation Technique, Stem rot, Infection, Mustard

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BACKGROUND AND OBJECTIVES

Stem rot in mustard incited by *Sclerotinia sclerotiorum* (Lib.) de Bary is the most important serious disease among the mustard diseases. Earlier, it was considered a minor problem in India but it has emerged a serious problem over the years in most mustard growing area of the country. Now, Stem rot is a threat to cultivation of mustard cultivation in Madhya Pradesh and other growing areas of India (Agarwal *et al.*, 1997). Sclerotia are serving as source of primary inoculum for the next season (Willett and Wong, 1971). Sclerotia survive with seed as a contaminant as well as soil-borne pathogen even under adverse conditions up to 5 years. The initial infection and symptoms of stem rot are visible 40-55 days after sowing (natural condition) and a week after artificial inoculation with favourable environment. For the artificial inoculation following techniques were performed in the field condition.

RESOURCES AND METHODS

The experiment was conducted in experimental field at College Of Agriculture,

Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.) during 2014-15 and 2015-16. The experiment was laid out in randomized block design and replicated four times. The isolation of pathogen from infected stem having sclerotia were used for isolation in the laboratory. The sclerotia were surface sterilized with 0.1 % mercuric chloride for one minute (Clarkson *et al.*, 2003). This is followed by three washings in distilled water to remove traces of $HgCl_2$. The sterilized sclerotium was cut into two pieces with the help of sterilized blade in sterilized petri plate and transferred to potato dextrose agar PDA culture and incubated at 25 ± 1°C. The pathogen (*S. sclerotiorum*) mycelium disc (5mm) were taken from five days old young vigorous growth of pathogen grown on PDA medium to transfer to sterilized petri plates containing PDA medium for purification of the isolated fungus was carried out by using hyphal tip technique as described by Dhingra and Sinclair (1985). Purified test pathogen was used for examination at frequent intervals for identification based on its morphological and taxonomical characters (Plate-2 and 3).The pathogenicity of *S. sclerotiorum* on susceptible variety (Rohini) was tested by following methods for establishing better infection and inoculation was done at 55 days after sowing in all the techniques. The girdled length on infected stem was also recorded.

Mycelial bit placement :

A mycelial disc of 7 mm diameter was taken from 5 days old growing vigorous young culture, cut with the help of sterilized cork borer and taken with help of forcep then placed in bored hole made on stem 10-12 cm above at the base of the plant by cork bored and covered by absorbent cotton and tied with paper tap. The inoculated area was kept moist with water for seven days for establishing the infection of *S. sclerotiorum*.

Tooth-pick Technique :

Tooth pick covered with moist cotton used to cock mycelial disc inoculated area on stem.

Sclerotial placement :

In this technique, fifty five days old mustard plants were inoculated with sclerotia, before inoculation, sclerotia were cut into two pieces and are piece was placed in bored hole on stem and cocked with cotton then wrapped by paper tape.

Paraffin wax film :

Five days old vigorous young mycelial disc kept inside the bored hole on stem. This was later covered by paraffin wax film tape and left for the appearance of symptoms.

Mycelium suspension spray :

The pure culture of *S. sclerotiorum* obtained from liquid broth medium. The mycelial suspension was diluted @ 106 cfu/g and spray over plants as a treatment for symptoms of the disease.

Mycelium slurry inoculation :

To perform this technique, the mycelium mat was prepared in liquid medium (250 ml) conical flasks. The mass multiplication of the pathogen was done on pea, potato, corn meal, oat meal and sand mix (1:1) and allowed to grow for ten days. The mycelial mat and mass multiplied culture were collected in a bucket for preparing the mycelium slurry which was on 55 days old plants.

Sclerotial powder dusting :

Sclerotia were obtained from broth medium. The sclerotia were collected and dried in light avoiding contamination then crushed and powdered for dusting on the plants as a treatment for symptoms of the pathogen.

Mycelial bit placement inside tender bark :

A mycelial disc of 7 mm diameter was inserted inside the flap of bark on the stem 10-12 cm above the base of the plant and covered by absorbent cotton and tied with paper tap.

Mycelial bit placement on scratched stem :

The tagged plants stem were slightly scratched with the help of sterilized blade. A mycelial disc of 7 mm diameter was taken from 5 days old young culture and was put on the scratched stem. The stem was covered with cotton and tied with paper tape.

Placement of inoculum through brushing over scratching stem :

In this technique, seven days old mycelium was used. The stem was scratched with the help of sterilized blade and the inoculum was applied on scratched stem with help of brush. The inoculated stem was covered with cotton and tied with paper tape. Control (Un-

Inoculated) plant was not inoculated. All the techniques were comparison with was made with inoculated plants of different techniques for the symptom appearance.

OBSERVATIONS AND ANALYSIS

The result revealed that the ten artificial inoculation techniques were tested for pathogenicity of the *S. sclerotiorum* (Plate 3). Out of ten inoculation techniques, Paraffin wax film was found best and significantly superior over other techniques and control. With the view of Stem rot disease scope, study was made on the different artificial inoculation that which artificial inoculation techniques would infection earliest. From the all studied techniques, six artificial inoculation techniques was evolved first time by me for noticing earliest infection and maximum incidence of this disease. These six artificial techniques were not mentioned by earlier workers on Stem rot.

Girdled length on plant stem :

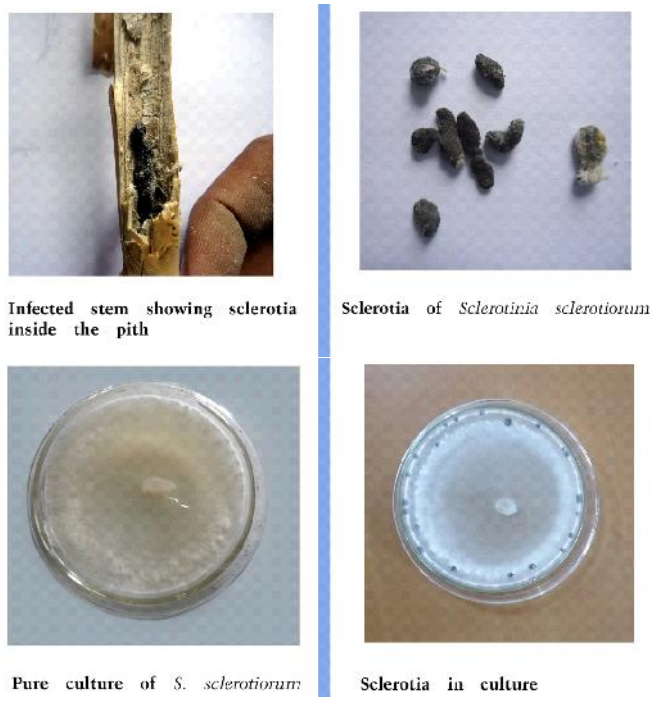
Since, the pathogen has systemic and aerial infection at the both stages by myceliogenic and carpogenic

Table 1 : Girdled length (cm) on stem in inoculation techniques

Technique	15 DAI			30 DAI			45 DAI			60 DAI		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
Mycelial bit placement	9.65	9.25	9.45	15.80	15.60	15.70	23.40	23.80	23.60	30.95	32.80	31.88
Mycelial bit placement in inside tender bark	6.65	6.80	6.73	6.65	15.55	11.10	21.95	21.90	21.93	28.85	30.15	29.50
Mycelial bit placement on scratched stem	7.05	7.55	7.30	7.05	15.15	11.10	22.05	22.10	22.08	27.00	29.00	28.00
Mycelial suspension spray	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mycelium slurry inoculation	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Paraffin wax film or two sided tape	11.30	11.55	11.43	11.30	20.05	15.68	25.45	26.50	25.98	32.00	34.60	33.30
Placement of inoculum through brushing scratched stem	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sclerotial placement	6.60	6.55	6.58	6.65	13.25	9.95	21.15	21.85	21.50	27.25	27.90	27.58
Sclerotial powder dusting	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tooth pick	7.20	8.00	7.60	15.40	15.35	15.38	22.00	22.10	22.05	29.30	32.05	30.68
Control	0.00	00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.E. ±	0.168	0.110	0.336	0.121	0.145	0.371	0.177	0.195	0.536	0.191	0.191	0.959
C.D. (P=0.05)	0.198	0.317	0.971	0.350	0.419	1.071	0.512	0.564	1.547	0.553	0.551	2.770

Table 2 : Morphological characteristics of Sclerotia in inoculation techniques

Technique	Number of sclerotia			Size per sclerotia in mm			Weight per sclerotia in mg		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
Mycelial bit placement	9.30	7.45	8.38	5.30	5.20	5.25	32.25	29.25	30.75
Mycelial bit placement in inside tender bark	9.55	8.00	8.78	5.80	5.40	5.60	31.65	30.90	31.28
Mycelial bit placement on scratched stem	10.05	8.55	9.30	5.50	5.35	5.43	30.35	29.50	29.93
Mycelial suspension spray	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mycelium slurry inoculation	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Paraffin wax film or two sided tape	11.85	10.80	11.33	5.25	5.05	5.15	33.65	33.25	33.45
Placement of inoculum through brushing scratched stem	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sclerotial placement	9.80	8.30	9.05	4.25	4.40	4.33	30.25	29.35	29.80
Sclerotial powder dusting	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tooth pick	11.35	9.80	10.58	4.80	5.10	4.95	33.45	32.15	32.80
Control	0.00	00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.E. ±	0.705	0.736	0.713	0.486	0.389	0.320	1.756	1.382	1.532
C.D. (P=0.05)	2.037	2.126	2.059	1.404	1.124	0.923	5.073	3.990	4.424



Infected stem showing sclerotia inside the pith

Sclerotia of *Sclerotinia sclerotiorum*

Pure culture of *S. sclerotiorum*

Sclerotia in culture

Plate 1 : Isolation and purification of *Sclerotinia sclerotiorum*

Materials for inoculation

To make hole on stem through cork borer

Picking mycelial disc with help of needle

Placement of mycelial disc inside the stem hole

Coverving of inoculated hole with absorbant Cotton

Wrapping of inoculated stem with help of tape

Plate 2 : Placement of mycelial bit in pith of the stem through hole and its packing through cotton and tap

Plate 3 : comparative performance of more effective inoculation techniques

germination of sclerotia, the symptoms of the disease appeared after 5-8 days after inoculation (Table-1). The disease initiates as water soaked lesion near the stem inoculated part. Out of 10 inoculation techniques, paraffin wax film technique was recorded maximum girdled length on stem at 15 DAI (11.43 cm), 30 DAI (15.68 cm), 45 DAI (25.98 cm) and 60 DAI (33.30 cm), followed by bit placement the girdle length, tooth pick, mycelial bit placement in inside tender bark and mycelial bit placement on scratched stem, while, minimum girdled length was recorded in sclerotia placement technique at 15 DAI (6.58 cm), 30 DAI (9.95 cm), 45 DAI (21.50 cm) and 60 DAI (27.58 cm). However, the length of girdle varies in different treatments. The mycelial suspension spray, sclerotial powder dusting, mycelium slurry inoculation, placement of inoculum through brushing on scratched stem, the plants were remained free from infection on stem.Present investigation is supported by Scott (1984) who described a method for inoculation of swede rape with *S. sclerotiorum* in the field and glass house using infected barley grains secured in leaf exil with Parafilm. The present findings are in agreement with the work of Prasad *et al*. (2009) tested four methods to obtain best production of apothecia. Inoculum placement proved to be the best method of inoculation and showed 86.7%

infection followed by tooth pick 55.6 and ascospores inoculation method 53.3%. Soil inoculation method appeared to be less effective as compared to other methods. Rahmanpour *et al.* (2011) reported that the two plant inoculation techniques (oxalic acid and fungal mycelium) resulted in significant differences between genotypes in reaction to the disease.

Morphological characteristics of the sclerotia :

Morphological characteristics of the sclerotial development inside the stem of mustard depended on the length of girdled and favourable environmental conditions (Temperature 15-25°C and RH >80. Present investigation on the morphological characteristics was statistically difference (Table-2). The formation of number sclerotia per plant depends on length formed on stem. The sclerotia formed was rangedinparaffin wax film (11.33) tomycelial bit placement (8.38) per plant were recorded. However, size (average diameter) per sclerotia in mm and weight per sclerotia in mg were not significantly different noticed which varied from4.33 mm to 5.60 mm and29.80 mm to 33.45 mg, respectively. The present findings are in agreement with the work of Zang *et al.* (2010) investigated the effect of *Sclerotinia sclerotiorum* mycelial culture time, concentration of mycelial suspension for inoculation, and time for keeping high relative humidity after inoculation on disease development in the inoculated rape plants, thereby established an effective spray inoculation technique system based on mycelial suspension for *S. sclerotiorum*.

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