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Effect of physical parameters on the growth and sclerotia formation of *Sclerotinia sclerotiorum* (Lib.) de Bary, causing stem rot of coriander

RAJ KUMAR FAGODIYA, S. GODIKA AND BABU LAL FAGODIA*

Department of Plant Pathology, S.K.N. College of Agriculture (SKNAU), Jobner, Rajasthan, 303329

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ABSTRACT

An incubation study was carried out in laboratories of Department of Plant Pathology, S.K.N. College of Agriculture, Jobner (Rajasthan) to study the effect of physical parameters on the growth and sclerotia formation of S. sclerotiorum causing stem rot of coriander during rabi season 2015-16. Results revealed that the potato dextrose agar medium was found maximum supporter for mycelial growth followed by oat meal medium. Effect of physical parameters showed that maximum mycelial growth and sclerotia formation of fungus was observed at 25ºC temperature, 90 and 100 per cent relative humidity, 6.0 pH and potato dextrose agar medium. Potato dextrose broth was found significantly superior in mycelial growth and sclerotia formation among the liquid media under study.

Keywords: Sclerotia, seed, temperature, relativehumidity, potato dextrose agar, coriander

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is a major seed spices crop of the family *Umbelliferae*. Dry seed of corianders are used in preparation of curry powder, garam masala and seed oil used in industries for flavouring the products, like pastry, cookies, cakes, buns, candy, cocoa, chocolate, confectionery, cordial, soda, syrups, preservers, gelatin dessert, tobacco and alcoholic beverages particularly the 'gin'. The coriander seeds and leaves also have high medicinal value and are used to flavouring purgatives and to prevent gripping. Coriander has been used as a folk medicine for the relief of anxiety and insomnia. It is also used as a carminative, diuretic, stomachachic and digestive aid. Coriander has been documented as a traditional treatment for diabetes, as eye wash for preservation of eyesight in smallpox and also in conjunctivitis. Roasted seeds are useful in dyspepsia. Chemicals derived from coriander leaves were found to have antibacterial activity against *Salmonela choleraenuis sp. choleraesuis*.

In India, it is mainly cultivated in the states of Rajasthan, Andra Pradesh, Madhya Pradesh, Gujarat and Tamil Nadu covering an area of 447140 ha with annual production of 313640 tonnes and average productivity 701 kg/ha (Anonymous, 2015). In Rajasthan coriander is largely cultivated in the districts of Jhalawar, Baran, Kota, Chittorgarh, Bundi, Tonk, Sawai Madhopur, Jodhpur and Ajmer covering an area of 212725 ha with an annual production of 227203 tonnes and average productivity 1068 kg/ha (Anonymous, 2015-2016). If we look at productivity of this crop we will realize that it is very low being only 701 kg/ha. Some of the very obvious reasons for such low yield are that this crop is often cultivated on marginal lands with poor management of soil fertility, irrigation fertilizers, attack of diseases and the pests. Coriander crop suffers from various diseases caused by fungi and other microorganisms. Important diseases incited by fungi are stem galls or tumours (*Protomyces macrosporus*) (Bhardwaj and Shrestha, 1985), wilt (*Fusarium oxysporum f. sp. coriandri*) (Koike, 2005), powdery mildew (Erysiphe polygoni), root and stem rot (*Rhizoctonia solani and Macrophomina phaseolina)* (Godara *et al.* 2010) and Alternaria blight by *Alternaria poonensis* (Raghunath, 1963) and *Alternaria alternata* (Khan *et al.* 1984). Reis and Nascimento (2011) first time reported the occurrence of white mold disease caused by *S. sclerotiorum* in coriander (*Coriandrum sativum*) from Brazil. Amongst the major fungal diseases of coriander the stem rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is becoming a measure threat to the coriander cultivation. *Sclerotinia sclerotiorum* (Lib.) de Bary, causal organism of stem rot is the most ubiquitous, omnivorous, soil-borne and

**Department of Plant Pathology, Rajasthan College of Agriculture (MPUAT), Udaipur, Rajasthan, 313001 Email*- blfagodia25@gmail.com, Mobile no. -9461249010*

destructive plant pathogen, inciting disease on more than 500 plant species (Saharan and Mehta, 2008 and Sharma, 2014). Stem rot is more common and severe in temperate and subtropical regions of cool and wet seasons (Saharan and Mehta, 2008). Disease caused by *S. sclerotiorum* is initiated either directly from soil borne sclerotia which germinate to form hyphae that can penetrate the roots of nearby plants or by air borne ascospores (Purdy, 1979). The disease appears in field after on 60-65 days of sowing. Sudden drooping of leaves followed by drying of plants are characteristic features of the disease. In the pith of the infected stem, large cavities lined by fluffy mycelium and numerous black sclerotia of the fungus are seen.

MATERIALS AND METHODS

Different synthetic and semi-synthetic media were prepared by weighing the different constituents (Analytical grade) of each medium and then adding the distilled water to make up the volume 1000 ml and autoclaved at 1.045 kg/cm^2 for 20 minutes. Inoculation was done with 5 mm disc of mycelial mat taken from 7 days old fungal culture and incubated at 25+1^{0}C (except temperature study) for 10 days. Each experiment of physical parameter studies was arranged in completely randomized design (CRD) with three replications.

Mycelial growth and sclerotia formation of S. sclerotiorum on different solid media

Growth on solid media was determined by measuring the colony diameter along with the two diagonals passing through the centre of colony by excluding initial diameter (5 mm) of bit. Five solid media (potato dextrose agar, Czapeck's dox agar, oat meal, corn meal and Martin's medium) were taken for *In vitro* experiment. Petri plates having sterilized medium were inoculated with 5 mm disc of mycelial growth with the help of sterilized cork borer and incubated at 25+1^{0}C in incubator for 7 days. Observation on mycelial growth (radial growth) was taken after 7 days of incubation.

Dry mycelial weight and sclerotia formation on different liquid media

Glasswares were washed with potassium dichromate sulphuric acid solution, washed with sterilized water, sterilized in hot air oven at 180 ˚C for two hours. The liquid (Asthana and Hawker's, Brown's, potato dextrose broth and Richards' medium) 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 25 ˚C. Mycelium was harvested after 15 days and filtered through Whatman filter paper No. 42, dried at 60 ˚C for 24 hours and weighted and sclerotial size noticed. In each experiment four replications were maintained (Sharma, 1989). The media have highest dry mycelial weight and excellent formation of sclerotia, used for further studies.

Effect of physical parameters on mycelial growth

Temperature

Effect of temperatures on mycelial growth of *S. sclerotiorum* was studied *In vitro*. Twenty ml of sterilized PDA medium was poured in each sterilized Petri plates. Inoculation was made with 5 mm disc of 7 days old culture of *S. sclerotiorum* with the help of sterilized cork borer and incubated at 5 different levels of temperature viz., 10, 15, 20, 25 and 30^0 C for 7 days. Observation on mycelial growth was recorded after 7 days of incubation.

Relative humidity

To study the effect of relative humidity on mycelial growth of *S. sclerotiorum,* five different levels of relative humidity *i.e.* 60, 70, 80, 90 and 100 per cent were maintained by using the concentrate sulphuric acid and sterilized distilled water in different proportions in glass desiccators by the method suggested by Buxton and Mellanby (1934). The composition of the acid solution was used as follow

Relative humidity (%)	Stock solution (ml)*	Distilled water (ml)
60	374.0	396.0
70	348.0	510.3
80	294.0	640.0
90	161.0	712.0
100	-	Only DW

** 50% v/v solution of concentrate sulphuric acid*

Petri plates containing PDA medium were inoculated with 5 mm disc of 7 days old culture of *S. sclerotiorum* with the help of sterilized cork borer. Inoculated Petri plates were immediately accommodated in glass desiccators containing mixture of sulphuric acid and distilled water in required proportion and incubated at 25±1^{0}C for 10 days. Observation on mycelial growth was recorded after 7 days of incubation.

pH

To study the effect of different levels of pH on mycelial growth, the pH of medium (broth) was adjusted at 6.0, 6.5, 7.0, 7.5 and 8.0 using citrate phosphate buffer before sterilization with the help of pH meter. Flasks having liquid medium of each pH level were inoculated with 5 mm disc of seven days old fungus culture. Flasks were incubated at 25±1°C and the observations on mycelial growth (on dry weight basis) were recorded after 14 day of inoculation.

RESULTS AND DISCUSSION

To find out a suitable medium for mycelial growth of *S. sclrotiorum*, five different media were tested. Perusal of data (Table 1) revealed that potato dextrose agar medium was significantly superior in supporting maximum mycelial growth (90.00 mm). This was followed by oat meal medium (79.20 mm), corn meal agar (60 mm) and Czapeck's dox agar (21.33 mm). Mycelial growth of the fungus was not recorded on Martins media.

Table 1: Effect of solid media on mycelial growth of *S. sclerotiorum* at 25±1°C

Medium	Mycelial growth (mm)*	No. of Sclerotia
Potato dextrose agar media	90.00	16.4
Czapeck's Dox Agar	21.33	5.8
Oat meal media	79.20	11.4
Corn meal media	60.00	8.2
Martins media	0.00	0.0
SEm±	0.78	
CD (p=0.05)	2.55	

** Average of four replication*

To study the effect of 4 liquid media on growth and sclerotia formation, inoculated flasks were incubated at room temperature (18 - 25 ˚C) for 14 days and mycelial mats were harvested, dried and weighed. Observations on growth and sclerotia formation on different media recorded after 14 days are presented in (Table-2).Maximum dry mycelia weight 74.80 mg and excellent sclerotia formation was recorded on potato dextrose broth. It was followed by Richard's media resulted 59.87 mg dry mycelia weight and good sclerotia formation. Singh *et al.* (2013) reported different culture media for growth of *S. sclerotiorum* (Lib.) de Bary causing white mould of chick pea. The fungus was found to grow best on Sabouraud dextrose agar medium, PDA and potato dextrose broth.

Table 2: Average dry mycelial weight and sclerotia formation of *S. sclerotiorum* on different liquid media

Media	Average dry* mycelial weight (mg)	Sclerotia formation
Asthana and Hawker's media	20.60 (41.96)	2.1
Brown's media	44.70 (41.96)	4.5
Potato Dextrose broth media	74.80 (59.87)	18.3
Richard's media	67.20 (55.06)	14.7
SEm+	1.09	
CD (p=0.05)	3.77	

**Average of four replications*
Figures given in parentheses are angular transformed values

The entire microorganisms grow under certain range of temperature within which a minimum, optimum and maximum temperature could be located. It is evident from the data (Table-3) that the fungus grew at all the temperature range of 10 ^{0}C to 30 ^{0}C. Maximum mycelial growth of the fungus was observed at 25 ^{0}C (90 mm) and found at par with 20 ºC (89.50 mm). Minimum mycelial growth (20.00 mm) of the fungus was observed at 30 ^{0}C. Panchal *et al.* (2012) reported maximum growth of *S. sclerotiorum* causing stem rot of fennel was recorded at 25 ^{0}C temperature followed by 20 ^{0}C after three days of incubation. Poor growth of the

fungus was recorded at 5 ^{0}C temperature and no growth at 30 ^{0}C and 35 ^{0}C temperatures, whereas maximum sclerotia were recorded at 15 ^{0}C temperature after seven days of incubation.

Table: 3. Effect of temperature on mycelial growth of S. sclerotiorum

Temperature (^{O}C)	Mycelial growth (mm)*
10	64.33
15	84.66
20	89.50
25	90.00
30	20.00
SEm±	1.19
CD (p = 0.05)	3.88

** Average of four replications*

To evaluate the effect of atmospheric moisture, the fungus was exposed directly to different levels of relative humidity viz. 60, 70, 80, 90, 100 per cent and incubated at 25±1^{0}C for 7 days. It was observed that all the five humidity levels favoured the growth of *S. sclerotiorum*. Perusal of data (Table-4) showed that maximum mycelial growth (90 mm) of *S. sclerotiorum* was observed at 100 and 90 per cent relative humidity and found at par with 80 per cent (84.62 mm) relative humidity. A significant decrease in mycelial growth was observed at 70 per cent (83.50 mm) relative humidity. Minimum mycelial growth (77.62 mm) was observed at 60 per cent relative humidity. Huang *et al.* (1998) investigated using 2 isolates of *S. sclerotiorum* causing seed rot and seedling wilt of sunflowers. In the absence of exogenous nutrients, sclerotia germinated more readily at 100% RH than at 95% RH or lower.

Table: 4. Effect of relative humidity on mycelial growth of S. sclerotiorum at 25±1^{0}C

Relative humidity (%)	Mycelial growth (mm)*
60	77.62
70	83.50
80	84.62
90	90.00
100	90.00
SEm±	1.90
CD (p = 0.05)	6.22

** Average of four replications*

In general, fungi are capable of growing within a wide range of hydrogen ion concentrations of the medium while, most of them grow best in neutral or slightly acidic medium. The pH preference of most of the pathogens ranges 6.0 to 8.0 which obviously favour establishment of the pathogen in their host. Hydrogen ion concentration governs several physiological and metabolic processes of microorganisms. The relationship of pH to the mycelial growth of *S. sclerotiorum* was determined at different pH levels *viz*. 6.0 to 8.0 at 25±1 ºC for 14 days (Table 5).

Table: 5. Effect of pH on mycelial growth and sclerotia formation of S. sclerotiorum in PDB medium

pH level	Dry weight of Mycelial growth (mg)*	Number of sclerotia
6.0	155.8	11.0
6.5	124.0	5.25
7.0	72.70	1.75
7.5	51.0	1.50
8.0	29.9	0.50
SEm±	01.9	
CD (p = 0.05)	06.4	

** Average of four replications*

Of all the five pH levels, pH 6.0 was found to be ideal and produced the maximum dry mycelial weight of 155.8 mg and number of sclerotia formation 11.0 followed by pH 6.5 (124 mg) and sclerotia formation 5.25 which was at par to each other. The dry mycelial weight was lowest at pH 8 which recorded 29.9 mg and number of sclerotia formation 0.50. Kumar *et al.* (2004) observed that pH range of 4.0 to 6.0 was found optimum for the growth and formation of sclerotia of *S. sclerotiorum* causing stem and root rot of broccoli. Panchal *et al.* (2012) reported that the fungus *S. sclerotiorum* casual agent of stem rot of fennel was differ in their requirements and Sclerotia formation was recorded at pH 6.0, while higher dry mycelium of sclerotia was recorded at pH 5.0. Sclerotial formation did not occur at pH 2.0, 8.0 and 9.0.

REFERENCES

Anonymous (2015) Paradism in Seed Spices Research, ICAR- All India Coordinated Research Project on Spices, Jobner pp. 7.

Anonymous (2015-16) Rajasthan Agriculture Statistics. Directorate of Agriculture, Pant Krishi Bhawan, Jaipur, *Rabi* crops pp. 2-6.

Bhardwaj, L.N. and Shrestha, S. N. (1985) Efficacy of fungicides application in the control of stem gall of coriander. *Ag. Ecosystem and environment* **13**: 319-323.

Buxton, P.A. and Mellanby, B.M. (1934) The measurement and control of humidity. *Bulletin of Entomology Research* **25**:171-175.

Godara, S.L., Kapoor, B.B.S. and Rathore, B.S. (2010) Disease management of spice crops. Madhu publications, Bikaner (Raj.) pp. 28-32.

Huang, H.C., Chang, C. and Kozub, G.C. (1998) Effect of temperature during sclerotial formation, sclerotial dryness, and relative humidity on myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. *Canadian Journal of Botany* **76** (3): 494-499.

Khan, A.J., Sattar, A. and Husain, A. (1984) *Alternaria* blight of *Coriandrum sativum* L. The occurrence of *A. alternata* on this host is newly recorded for India. *Indian Journal of Plant Pathology* **2** (2): pp. 201.

Koike, S. T. (2005) First Report of Fusarium Wilt of Cilantro Caused by *Fusarium oxysporum* in California. University of California Cooperative Extension, Salinas **89** (10): 11301.

Kumar B, Pandey, R and Verma, AK. (2004) Effect of temperature and pH on growth and sclerotial formation of *Sclerotinia sclerotiorum* causing stem and root rot of broccoli. *Progressive Agriculture* **4** (1): 89-90.

Panchal, J.A., Patel, K. DL., Jaiman, R.K. and Patel, N.R. (2012) Physiological studies of *Sclerotinia sclerotiorum* causing stem rot of fennel (*Foeniculum vulgare* Mill.). *International Journal of Seed Spices* **2** (2): 46-53.

Purdy, L.H. (1979) *Sclerotinia sclerotiorum* history, disease and symptomatology, host range, geographic distribution and impact. *Phytopathology* **69**: 875-880.

Raghunath, T. (1963) A new species of *Alternaria* causing blight of *Coriandrum sativum* L. *Mycopathol.* **21**: 315-317.

Reis, A. and Nascimento, W.M. (2011) New apiaceous hosts of *Sclerotinia sclerotiorum* in the Cerrado region of Brazil. *Horticultura Brasileira* **29**: 122-124.

Saharan, G.S. and Mehta, N. (2008) Sclerotinia diseases of crop plants: Biology, ecology and disease management. *Springer Science + Busines Media B.V. The Netherlands,* pp. 485.

Sharma, P. (2014) Worldwide new host record of *Sclerotinia sclerotiorum. Sarson News* **18**: 9.

Sharma, S.K. (1989) Studies on stem rot of mustard (*Brassica juncea* var. sarson) caused by *S. sclerotiorum* (Lib.) de Bary. pp. 11-14.

Singh, M., Sharma, O.P., Bhagat, S. and Pandey, N. (2013) Effect of systemic fungicides, culture media, temperature and pH on growth of *Sclerotinia sclerotiorum* (Lib.) de Bary, causing white mold of chick pea. *Annals of Plant Protection Sciences* **21** (1): 136-139.