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Effect of light and aeration on the growth of *Sclerotium rolfsii in vitro*

Muthukumar, A.* and Venkatesh, A.

Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar-608 002, Chidambaram, Tamil Nadu, India.

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Sclerotium rolfsii **is one of the devastating soil-borne plant pathogens which cause severe loss at the time of seedling development. It also causes leaf spots in several crops and wild plants. In this experiment, exposure of pathogen to different light period and aeration in order to assess the mycelial growth, biomass production, weight and number of sclerotia of *S. rolfsii* was done. Three-fourth area of three plates, 50% area of three plates and 100% area of three plates were sealed with cellophane tape. The other three plates were not sealed. Two sets of such plates were prepared. All the plates were incubated at 28±2°C. One set was incubated in light whereas the other set was incubated in the dark. The results reveal that there was no significant difference in mycelial growth and number of sclerotia among them but significant difference was observed when compared with the control, that is, the plates which were not sealed. Sclerotial formations were directly influenced by air as completely sealed plates failed to produce sclerotia. Generally, the light condition induces the production of more number of sclerotia than dark condition. In another study, the exposure of pathogen to different light periods revealed that alternative cycles of 12 h light and 12 h darkness for ten days resulted in the maximum mycelial growth and dry weight, more number of sclerotia and weight of sclerotia was also seen when compared with other treatments.**

Key words: *Sclerotium rolfsii*, aeration, light, peppermint.

INTRODUCTION

Peppermint (*Mentha piperita L.*) is an important aromatic perennial herb grown throughout the world; it belongs to the family Lamiaceae. It is extensively cultivated in India and about 70% of the international annual requirement is met from crops raised in the central region of the Indo-Gangetic plains (Singh et al., 1999). *Mentha* is cultivated in Himalaya-hills, Haryana, Uttar Pradesh, Punjab and Bihar. Of these, Uttar Pradesh is the largest producing state in the country contributing 80-90% of the total production followed by Punjab, Haryana, Bihar and Himachal Pradesh. Cultivated peppermint, serves as a source of menthol, menthone, isomenthone, menthofuran, linanool, linalyl acetate, methyl acetate, terpenes, carvone, piperitenone oxide and other aromatic compounds. In India, peppermint is grown throughout the year (Shukla et al., 1998) and it is affected by several fungal diseases caused by *Rhizoctonia solani* (Kumar et al., 1997; Merin, 2002), *Verticillium dahliae* (Johnson and Santo, 2001), *Collectotrichum cocodes* (Johnson et al., 2002) and *Sclerotium rolfsii* (Anand and Harikesh Bahadur Bahadur, 2004) of which, collar rot caused by *S. rolfsii* is a major constraint in the peppermint cultivation in Tamil Nadu. *S. rolfsii* is a soil borne plant pathogen causing root rot, stem rot, collar rot, wilt and foot rot diseases on more than 500 plant species of agricultural and horticultural crops throughout the world (Aycock, 1966). The pathogen causes

*Corresponding author. E-mail: muthu78ap@yahoo.co.in.

a great economic loss in various crops. It has been reported that *S. rolfsii* caused about 25% seedling mortality in the groundnut cultivar JL-24 (Ingale and Mayee, 1986). In tomato, this pathogen was responsible for a crop loss of 30% (Thiribhuvanamala et al., 1999). Its occurrence on crossandra has been observed to be about 40-50% mortality of plants. In peppermint, this pathogen caused about 5 to 20% of crop loss under field condition (Anand and Harikesh, 2004). Diseases caused by *S. rolfsii* are initiated either directly from soil-borne sclerotia which germinate to form fine cottony hyphae infecting the collar region of host plants or sclerotia sticking on the lower/upper surfaces of the leaves by rain splashes where they germinate and cause leaf spots (Singh and Pavgi, 1965). Soil temperature of 25-30°C and soil moisture 90% play significant role in disease development (Gupta et al., 2002). Various biotic and abiotic factors which directly or indirectly influence the development of sclerotia were discussed in literature (Ellil, 1999; Sarma, 2002). The objectives of the present study were i) to isolate and identify the pathogen ii) to study the pathogenicity test iii) to study the role of air in the growth of *S. rolfsii* and influence of light on the growth of pathogen.

MATERIALS AND METHODS

Isolation, identification and maintenance of pathogen

The collar rot symptoms were collected from the Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India. The infected plant materials brought back from the field were washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface sterilized in 0.5% sodium hypochlorite solution for 5 min. and rinsed in three changes of sterile distilled water. The segments were separately dried in between sheets of sterile filter paper and placed (3 pieces per plate) on fresh potato dextrose agar (PDA) medium (Ainsworth, 1961) impregnated with streptomycin, and incubated for seven days at 28 ± 2°C. The fungal growth on 5^{th} day, which arose through the sclerotial bodies was cut by inoculation loop and transferred aseptically to the PDA slants and allowed to grow at room (28 ± 2°C) temperature to obtain the pure culture of the fungus. The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub cultured periodically. The purified isolate was identified as *S. rolfsii* based on morphological and colony characteristics (Punja and Damini, 1996; Sarma et al., 2002; Watanabe, 2002).

Assessing the pathogenicity of *S. rolfsii* isolate

The pot mixture was prepared by thoroughly mixing clay loam soil, sand and farm yard manure at the ratio of 1:1:1. The inoculum of *S. rolfsii* isolate was grown on sand-corn meal medium (twenty days old) mixed thoroughly at five percent (w/w basis) level, and applied to top two centimeter of the soil (Abeygunawardena and Wood, 1957). Then, apparently healthy surface sterilized mint cuttings were planted in inoculated pots. The cuttings planted in pots without inoculum served as control. Soil moisture was maintained at moisture holding capacity of soil by adding sterilized water on weight basis throughout the period. After 20 days of inoculation, the plants showing the typical wilting symptoms were observed. Re-isolation was made from such affected portion of the plant tissue and compared with that of original isolate for conformity.

Effect of air on sclerotial development of *S. rolfsii* in potato dextrose agar medium

Fifteen milliliters of molten PDA medium was dispensed into 12 sterile Petri plate. Mycelial discs taken from the advancing margins of seven days old culture of respective *S. rolfsii* isolate by the aid of cork borer were separately placed at the centre of the plate containing PDA medium. Three-fourth area of three plates, 50% area of three plates and 100% area of three plates were sealed with cellophane tape. The other three plates were not sealed. Two sets of such plates were prepared. All the plates were incubated at 28±2°C. One set was incubated in light whereas the other set was incubated in the dark. In this experiment, there were four treatments and each treatment consists of three plates and each treatment is repeated three times. The inoculated plates were sealed with the help of lab seal in the following manner, that is, no sealing (control), half sealed, $3/4^{th}$ and complete sealing. Each set contained three plates. After inoculation and sealing, Petri plates were incubated at 28 ± 2°C (light and dark) and the other sealed plates were wrapped with black paper and incubated as above. Visual observations were periodically made for sclerotial initiation, sclerotial development and number of sclerotia per plate.

Effect of light on the growth of *S. rolfsii*

Potato dextrose broth and agar were used in this experiment. Conical flasks of 250 ml capacity and each containing 100 ml of liquid broth were inoculated and exposed to different length of light hours viz., alternate cycles of twelve hours light and twelve hours darkness, continuous light and continuous darkness in an environmental conditions. Flasks were inoculated with 6 mm mycelial disc obtained from the periphery of seven days old culture of *S. rolfsii* and incubated at different light intensities. All the inoculated plates were incubated for ten days under different length of light hours. The number of sclerotia/flask and weight of sclerotia was recorded at the end of the incubation period. There were three treatments and each treatment consists of three plates and each treatment was repeated three times. Then the mycelial mat was filtered through Whatman No. 41 filter paper discs of 12.50 cm diameter dried to a constant weight at 60°C prior to filtration. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with the mycelium and dried to a constant weight in an electrical oven at 60°C, cooled in a dessicator and weighed immediately on an analytical electrical balance. The weight of dry mycelium was recorded and the data were statistically analyzed.

To carryout study on solid media, 15 ml of potato dextrose agar was poured in 90 mm sterile Petri plate. Such plates were inoculated with six mm mycelial disc obtained from the periphery of seven days old culture of *S. rolfsii* and incubated at different light intensities. Each treatment consists of three plates and each treatment was repeated three times. All the inoculated plates were incubated for ten days under different length of light hours. The mycelial growth, number of sclerotia/plate and weight of sclerotia was recorded at the end of the incubation period.

Statistical analysis

The data on effect of the treatments on the growth of pathogens was analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test (DMRT) and by least significance difference (LSD) at P = 0.05. The package used for analysis was IRRISTAT version 92-1 developed by the

Table 1. Effect of air on sclerotial development of *S. rolfsii* in potato dextrose agar medium.

Treatment	Observation									
	In dark visual observation after (days)				Average number of sclerotia/plate	In light visual observation after (days)				Average number of sclerotia/plate
	6	8	10	12		6	8	10	12	
No sealing (control)	+	++	++	+++	213[a]*	+	++	++	+++	276[a]*
1/2 sealing	+[F]	++	+++	+++	168[b]	+[F]	++	+++	+++	185[b]
3/4 sealing	+[F]	++	+++	+++	157[c]	+[F]	++	+++	+++	178[c]
Complete sealing	-	-	-	-	0[d]	-	-	-	-	0[d]

+- Sclerotial initial; ++- white sclerotia; +[F] -fewer sclerotia initials; +++ - dark brown sclerotia; - = no sclerotial initials; *Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05).

Table 2. Effect of light on the growth of *S. rolfsii* in potato dextrose agar medium and potato dextrose broth.

Treatments	Mycelial growth (mm)	Mycelial dry weight (mg)	Average number of sclerotia/plate	Weight (mg)/100 sclerotia
Continuous light	68.66[b]*	280.33[b]*	225[b]*	74[b]*
Continuous dark	47.33[c]	130.00[c]	156[c]	54[c]
Alternate cycle of 12 h light and 12 h darkness	89.66[a]	382.66[a]	263[a]	85[a]

*Values in each column followed by the same letter are not significantly different according to the DMRT method (P = 0.05).

Biometrics Unit of the International Rice Research Institute, The Philippines (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results of the present study reveal that the number of sclerotia in 3/4 and 1/2 sealed plates placed in light and darkness affected the mycelial growth and number of sclerotia significantly as compared to the control (unwrapped plates). In the control plates, sclerotia initials were observed after 6 days of inoculation as whitish, tiny, pinhead-like structures and after 6-8 days exudation commenced. In completely sealed plates, the fungal growth was relatively very slow, compact and profusely growing mycelium was observed after 6 to 8 days as compared to the control. In all completely sealed plates, there was no sclerotium formation even after 12 days after inoculation. In 3/4 and 1/2 sealed plates, the number of sclerotia were less but they were bigger in size as compared to the control. In control plates, mature sclerotia became brownish at 3/4th day after inoculation but in 1/2 and 3/4 sealed plates, such sclerotia were seen after 10 days (Table 1). Sclerotia are the asexual structures formed due to the aggregation of fungal mycelium. Several biotic and abiotic factors influence the aggregation of fungal hyphae in the culture medium. Punja and Damini (1996) and Singh et al. (2002) reported that sclerotial exudates directly influenced the development and maturation of sclerotia. The number and sclerotial weight were affected drastically due to improper aeration as average numbers of sclerotia were more in unsealed plates (Sudarshan et al., 2010). Bhoraniya et al. (2002) reported that due to pathogenesis, the level of oxalic acid increases in the infected plants and the increase of oxalic acid induces formation of sclerotial initiation at the collar region. It was reported that depletion of exudate inhibits the development of sclerotia of *S. sclerotiorum* (Singh et al., unpublished observation).

The exposure of the pathogen to alternative cycles of 12 h light and 12 h darkness for ten days resulted in the maximum mycelial growth and dry weight (89.66 mm; 382.66 mg, respectively) with more number of sclerotia/plate and weight of sclerotia of *S. rolfsii* which was significantly superior over other treatments tested (Table 2). The mycelial growth of pathogen exposed to continuous light resulted in moderate growth (68.66 mm; 280.33 mg) and continuous darkness resulted in minimum mycelial growth and dry weight of *S. rolfsii* (47.33 mm; 130.00 mg) and less number of sclerotia/plate and weight of sclerotia was also very less (156; 54, respectively). Similarly, Basamma (2008) reported that, *S. rolfsii* was exposed to alternate cycles of 12 h light and 12 h darkness recorded more number of sclerotia of *S. rolfsii.* This is in agreement with the findings of Chung and Kim (1977) and Punja (1985).

In the present experiment, we found that a proper aeration and light is essential for the mycelial growth anddevelopment of sclerotia of *S. rolfsii.*

REFERENCES

Abeygunawardena DVW, Wood RKS (1957). Factors affecting the germination of sclerotia and mycelial growth of *Sclerotium rolfsii* Sacc. Trans. Br. Mycol. Soc. 40:221-231.

Ainsworth GC (1961). Dictionary of fungi. Common Wealth Mycological Institute., Kew Burrey, England. p. 547.
Anand S, Harikesh Bahadur S (2004). Control of collar rot in mint (*Mentha* spp.) caused by *Sclerotium rolfsii* using biological means. Curr. Sci. 87:362-366.
Aycock R (1966). Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. N.C. Agric. Exp. Stn. Tech. Bull. 174.
Basamma (2008). Integrated management of *Sclerotium* wilt of potato caused by *Sclerotium rolfsii* Sacc. M.Sc. Thesis, University of Agricultural Sciences, Dharwad.
Bhoraniya MF, Khandar RR, Khunti JP (2002). Estimation of oxalic acid in chillies infected with *Sclerotium rolfsii*. Plant Dis. Res. 17:325.
Chung HS Kim HK (1977). Effect of light on *Sclerotium* formation of *Sclerotium rolfsii.* Sacc. on agar media. Korean J. Mycol. 5:21-23.
Ellil AHAA (1999). Oxidative stress in relation to lipid peroxidation, sclerotial development and melanin production by *S. rolfsii*. J. Phytopathol.147:561-566.
Gomez KA, Gomez AA (1984). Statistical Procedure for Agricultural Research. John Wiley Sons, New York, NY, USA. p. 680 .
Gupta SK, Sharma A, Shyam KR, Sharma JC (2002). Role of soil temperature and moisture on the development of crown rot (*Sclerotium rolfsii*) of French bean. Plant Dis. Res. 17:366-368.
Ingale RV, Mayee CD (1986). Efficacy and economics of some management practices of fungal diseases of groundnut. J. Oilseeds Res. 3: 201-204.
Johnson DA, Douhan LI Greary B (2002). Report of *Colletotrichum cocodes* associated with mentha. Plant Dis. 86:695.
Johnson DA, Santo GS (2001). Development of wilt in mint in response to infection by two pathotypes of *Verticillium dahliae* and co infection by *Prataylenchus penetrans*. Plant Dis. 85: 1189-1192.
Kumar S, Khol AP, Pata DD, Rar TM, Sinqii S Tyagi BR (1997). Cultivation of menthol mint in India. Farm Bull. 4:13-20.
Merin B (2002). Studies on epidemiology and stolon rot of mentha. M.Sc. Thesis, Department of Plant Pathology, Tamil Nadu Agri-cultural University, Coimbatore.
Punja Z (1985). The biology, ecology and control of *Sclerotium rolfsii*. Ann. Rev. Phytopathol. 23: 97-127.
Punja ZK, Damini A (1996). Comparative growth, morphology and physiology of three *Sclerotium* species. Mycologia 88:694-706.
Sarma BK (2002). Studies of variability, sexual sage production and control of *S. rolfsii* Sacc., the causal agent of collar rot of chickpea (*Cicer arietinum*). Ph.D., Thesis, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.
Sarma BK, Singh UP, Singh KP (2002). Variability in Indian isolates of *Sclerotium rolfsii*. Mycologia 94:1051-1058.
Shukla PK, Haseeb AS Sharma S (1998). Soil texture, root lesion nematodes and yield of peppermint (*Mentha piperita*). J. Herbs Spices Med. Plants 6:1-8.
Singh AK, Srivastav RK Kumar S (1999). Production and trade of menthol mint in India. Curr. Sci. 87.
Singh UP, Pavgi MS (1965). Spotted leaf rots of plants- a new sclerotial disease. Plant Dis. Rep. 49:58–59.
Singh UP, Sarma BK, Singh DP, Bahadur A (2002). Studies on exudate-depleted sclerotial development in *Sclerotium rolfsii* and the effect of oxalic acid sclerotial exudates and culture filtrate of phenolic acid induction in chickpea (*Cicer arietinum*). Can. J. Microbiol. 48:443-448.
Sudarshan Maurya Udai Pratap S, Rashmi S, Amitabh S, Harikesh BS (2010). Role of air and light in sclerotial development and basidiospore formation in *Sclerotium rolfsii*. J. Plant Prot. Res. 50:206-209.
Thiribhuvanamala G, Rajeswar, E, Sabitha Doraiswamy (1999). Inoculum levels of *Sclerotium rolfsii* on the incidence of stem rot in tomato. Madras Agric. J. 86: 334.
Watanabe T (2002). *Sclerotium* sp. morphologies of cultured fungi and key species: Pictorial Atlas of Soil and Seed fungi. 2nd Edn., CRC Press, New York.