academicJournals

Vol. 12(49), pp. 6837-6842, 4 December, 2013
DOI: 10.5897/AJB2013.13201
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Physiological studies of *Sclerotium rolfsii* Sacc. causing collar rot of peppermint

Muthukumar, A*. and Venkatesh, A.

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

Accepted 29 October, 2013

***In vitro* studies were conducted on the effect of temperature, pH levels, carbon, nitrogen and amino acids on the mycelial growth and biomass production of *Sclerotium rofsii* Sacc. causing collar rot of mint. The results reveal that the growth of *S. rolfsii* was maximum at 30°C which was reduced significantly below 20°C and above 35°C. Of the pH levels tested, acetic pH (5.0) produced maximum mycelial dry weight which was followed by exposing the pathogen to pH 6.0. Among the nine carbon sources tested, sucrose recorded the maximum mycelial growth and dry weight of *S. rolfsii*, while peptone was the best among the nitrogen sources and tryptophane and phenylalanine was the best amino acids on the mycelial growth and biomass production of *S. rolfsii*.**

Key words: *Sclerotium rolfsii*, pH, mint, collar rot, nutritional studies, temperature.

INTRODUCTION

Peppermint (*Mentha piperita* L.) is an important aromatic perennial herb grown throughout the world, belonging 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 to 90% of the total production followed by Punjab, Haryana, Bihar and Himachal Pradesh. Cultivated peppermint mint, serves as a source of menthol, menthone, isomenthone, mentho-furan, linanool, linalyl acetate, methyl acetate, terpenes, carvone, piperitenone oxide and other aromatic com-pounds.

In India, peppermint is grown throughout the year (Shukla et al., 1998) and it is affected by several fungal diseases; of which, collar rot caused by *Sclerotium 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 horti-cultural crops throughout the world (Aycock, 1966). The pathogen causes a great economic loss on 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 respon-sible for a crop loss of 30% (Thiribhuvanamala et al., 1999). Its occurrence on crossandra has been observed about 40 to 50% mortality of plants.

In peppermint, this pathogen caused about 5 to 20% of crop loss was observed under field condition (Anand and Harikesh Bahadur, 2004). These pathogens exhibit varia-tion in their morphological biological and immunological characteristics and pathogenicity or resistance against harmful environment. Morphogenic and pathogenic varia-tions are known in many fungal pathogens and as such detailed investigation was carried out on the variations with regards to pH, temperature, nutritional factors on the mycelia growth and biomass production of *S. rolfsii*.

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

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 5th 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 based on morphological and colony characteristics (Punja and Damini, 1996; Sarma et al., 2002; Watanabe, 2002).

Effect of different temperature levels on the mycelial growth of *S. rolfsii in vitro*

Solid medium

A quantity of 15 ml of the sterilized potato dextrose agar medium was poured into 90 mm sterile Petri plates. The plates were inoculated with 6 mm mycelial disc of the pathogen obtained from 7 days old grown on PDA and incubated in BOD at different temperature (5, 10, 15, 20, 25, 30 and 35°C) for 7 days in an incubator. The mycelial growth of the pathogen was measured in mm at the end of incubation period.

Liquid medium

Erlenmeyer flasks (250 ml) containing 50 ml of potato dextrose broth were sterilized, inoculated and incubated at different temperatures namely: 5, 10, 15, 20, 25, 30 and 35°C for 10 days in BOD incubator.

At the end of the incubation period the mycelial mat was filtered through Whatman No. 41 filter paper of known weight. The filter paper with mycelial mat was dried in hot air oven at 105°C for 48 h and the mycelial dry weight was calculated. In both the methods, three replications were maintained for each treatment.

Effect of certain pH levels on the mycelial dry weight of *S. rolfsii in vitro*

Potato dextrose broth of different pH levels (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) were prepared and sterilized. They were inoculated with 6 mm mycelial disc of the pathogen obtained from 7 days old grown on PDA.

The flasks were incubated for 10 days at 28 ± 2°C in BOD incubator. After incubation, the fungal biomass was separated through filtration in a previously dried and weighed filter paper (Whatman No. 41). Then the mycelial dry weight was calculated. Three replications were maintained for each treatment.

Effect of certain nutritional factors on the mycelial growth of *S. rolfsii in vitro*

Linear growth of S. rolfsii on solid medium

Czapek's agar medium was amended with various carbon sources, nitrogen sources and amino acids on equivalent weight basis and were dispensed in sterile Petri plate at 15 ml quantities. After cooling, they were inoculated with 6 mm mycelial disc of the pathogen obtained from 7 days old culture grown on PDA in Petri plates and incubated for 7 days. The linear growth of the pathogen was measured in mm at the end of the incubation period.

Carbon source

Growth on liquid medium

The *in vitro* growth of the fungus was tested with nine different carbon sources (cellulose, dextrose, fructose, glucose, lactose, maltose, mannitol, starch and sucrose). Czapek's broth was taken as the basal medium for the study. In the czapek's broth, sucrose was replaced with various carbon sources on equivalent weight basis and autoclaved at 15 lb pressure for 20 min.

Nitrogen source

Growth on liquid medium

The *in vitro* growth of the fungus was tested with nine different nitrogen sources (ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, peptone, potassium nitrate, sodium nitrite, sodium nitrate and urea). Czapek's broth was taken as the basal medium for the study. In the czapek's broth, sodium nitrate was replaced with various nitrogen sources on equivalent weight basis and autoclaved at 15 lb pressure for 20 min.

Amino acids

Growth on liquid medium

The *in vitro* growth of the fungus was tested with eight different sources of amino acids namely: alanine, asparagine, cysteine, glutamic acid, phenylalanine, tryptophan, tyrosine and valine. Czapek's broth was taken as the basal medium for the study. In the czapek's broth, the nitrogen source was replaced with various amino acids on equivalent weight basis and autoclaved at 15 lb pressure for 20 min. In all the cases, the final pH of the medium was adjusted to 7.0.

After that, the medium was inoculated with 6 mm mycelial disc of pathogen obtained from 7 days old culture grown on PDA. The inoculated media were incubated for 10 days at room temperature (28 ± 2°C). At the end of the incubation period, the mycelial mats were filtered through previously dried and weighed filter paper (Whatman No. 41) and dried in hot air oven at 105°C for 48 h and the mycelial dry weight was calculated. Three replications were maintained for each treatment.

RESULTS AND DISCUSSION

Effect of different temperature levels

Among the temperature levels (6, 10, 15, 20, 25, 30 and 35°C) tested, 30°C was found to be more conducive for the mycelial growth of *S. rolfsii* (89.00 mm) under *in vitro* recording the highest mycelial dry weight of 610.66 mg, which was followed by 25° (Figures 1 and 2). However, the exposure of *S. rolfsii* to high temperature that is, 35°C was found to be highly detrimental to the growth of *S. rolfsii*. Each pathogen has got its own cardinal temperature and understanding the temperature requirement of the pathogen will help to standardize the management

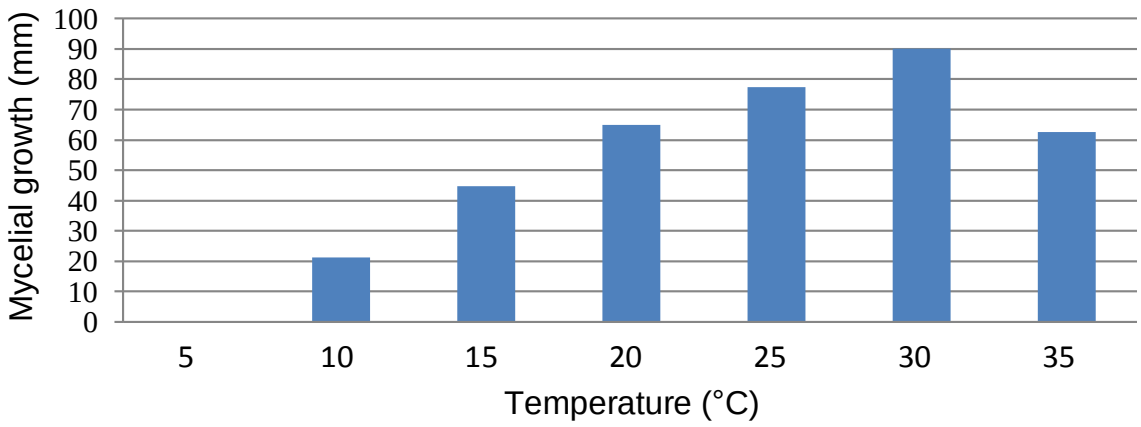

Figure 1. Effect of different temperature levels on the mycelial growth of *Sclerotium rofsii*.

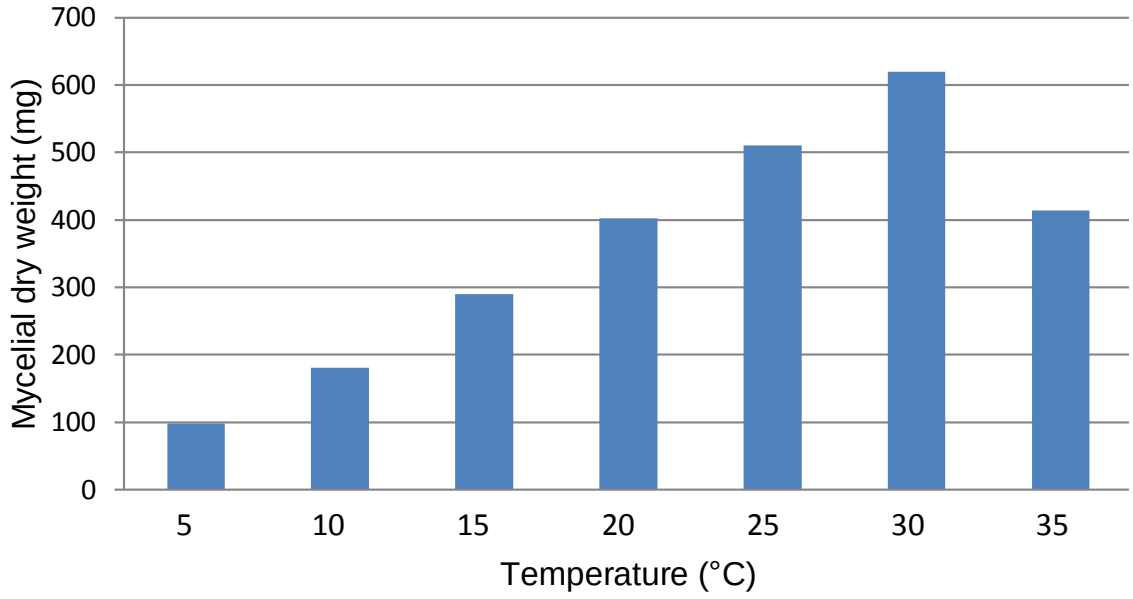

Figure 2. Effect of different temperature levels on the mycelial dry weight of *Sclerotium rolfsii*.

practices. It plays an important role in the growth and reproduction of fungi. Earlier published reports also clearly indicated that the optimum temperature for the *in vitro* growth of *S. rolfsii* is in the range of 25 to 30°C. Mahen et al. (1995), Tripathi and Khare (2006), Basamma (2008) and Lin et al. (2009) reported that 25 to 30°C was more conducive for the vegetative growth of *S. rolfsii*. Recently, Zape et al. (2013) reported that the *S. rolfsii* showed rapid mycelial growth at 30°C and maximum sclerotial production was recorded at 25°C.

Effect of different pH levels

The results presented in Figure 3 showed that among the pH levels (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) tested, exposure of pathogen to acetic pH (5.0) produced maximum mycelial dry weight recording 514.66 mg which was followed by exposing the pathogen to pH 6.0 under *in vitro*. Increase (or) decrease in pH beyond 5.0 and 6.0 was not conducive for the growth of pathogen.

This finding was inline with the earlier reports made by Dey et al. (1992), Mahen et al. (1995) and Basamma (2008). Recently, Zape et al. (2013) reported that the optimum pH for the mycelial growth and sclerotial formation of *S. rolfsii* was pH 5.5 to 7.5. The fungi generally utilize substrate in the form of solution only if the reaction of the solution is conductive to fungal growth and metabolism. This brings the importance of hydrogen ion concentration for the better fungal growth. In general, the pathogen showed preference for pH level towards acidic side.

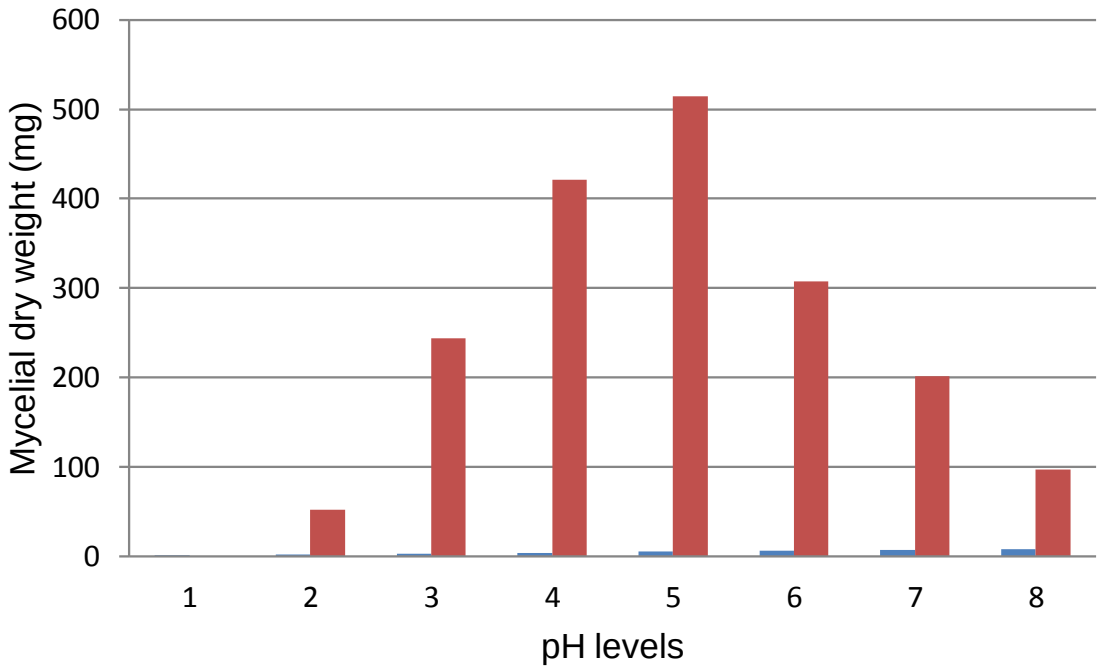

Figure 3. Effect of different pH levels on the mycelial dry weight of *Scelrotium rolfsii.*

Table 1. Effect of different carbon sources on the mycelial growth and dry weight of *S. rolfsii.*

Carbon source	Mycelial growth (mm)	Mycelial dry weight (mg)
Cellulose	50.66[e]*	210.00[f]*
Dextrose	80.66[b]	610.00[b]
Fructose	63.66[d]	250.00[e]
Glucose	75.00[c]	533.66[c]
Lactose	45.66[g]	100.66[h]
Maltose	53.00[e]	230.33[f]
Mannitol	47.00[f]	170.33[g]
Starch	74.33[c]	495.66[d]
Sucrose	89.33[a]	650.66[a]
Control	52.00[e]	70.33[i]

* Value within a column with same letter do not differ significantly according to the DMRT method (P = 0.05).

Effect of different carbon sources

Among the nine carbon sources tested, sucrose recorded the maximum mycelial growth of 89.33 mm and dry weight of 650.66 mg (Table 1). It was succeeded by dextrose and glucose. However, mycelial growth and dry weight was minimum with lactose as carbon source. This was similar to the findings of Prasad et al. (1986), Chun et al. (2003), Survase et al. (2006) and Xiao et al. (2012). Almost half of the mycelial dry weight of the fungal cell consists of carbon which is the main structural element (Lilly and Barnett, 1951). The utilization of various carbon compounds may depend on either of the activity of the fungus to utilize simpler forms or on its power to convert the complex carbon compounds into simpler forms, which may be easily utilized. Sucrose is generally utilized as a good source by most of the plant pathogenic fungi.

Effect of different nitrogen sources

The data presented in Table 2 revealed that all the nitrogen sources namely, ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, peptone, potassium nitrate, sodium nitrite, sodium nitrate and urea favoured the growth of *S. rolfsii.* Among the nine nitrogen sources tested, peptone recorded maximum mycelial growth (89.66 mm) and dry weight (790.33 mg) of *S. rolfsii*. Culturing of *S. rolfsii* in calcium nitrate and ammonium

Table 2. Effect of different nitrogen sources on the mycelial growth and dry weight of *S. rolfsii.*

Nitrogen source	Mycelial growth (mm)	Mycelial dry weight (mg)
Ammonium chloride	67.33[e]*	410.00[g]*
Ammonium nitrate	73.66[d]	620.33[e]
Ammonium sulphate	70.00[d]	590.66[f]
Calcium nitrate	65.33[e]	350.00[h]
Peptone	89.66[a]	790.33[a]
Potassium nitrate	85.00[b]	750.00[b]
Sodium nitrite	84.33[b]	730.66[c]
Sodium nitrate	80.00[c]	690.66[d]
Urea	82.00[c]	700.00[d]
Control	52.66[f]	71.66[i]

* Value within a column with same letter do not differ significantly according to the DMRT method (P = 0.05).

Table 3. Effect of different amino acids on the mycelial growth and dry weight of *S. rolfsii.*

Amino acid	Mycelial growth (mm)	Mycelial dry weight (mg)
Alanine	70.00c*	600.00d*
Asparagine	67.33d	520.33e
Cysteine	72.66c	670.00c
Glutamic acid	65.00d	405.66f
Phenylalanine	89.33a	785.00a
Tryptophan	89.66a	780.66a
Tyrosine	60.00e	320.00g
Valine	77.33b	698.33b
Control	53.00f	72.00h

* Value within a column with same letter do not differ significantly according to the DMRT method (P = 0.05).

chloride was found to be recording minimum mycelial growth and dry weight of *S. rolfsii*. Similarly, Azhar Hussain et al. (2003), Khattabia et al. (2004) and Basamma (2008) reported that potassium nitrate and peptone recorded the maximum mycelial growth of *S. rolfsii*. Nitrogen being a component of protein is an essential element and like carbon, it is used by fungi for functional as well as structural purposes. But all the sources of nitrogen are not equally good for the growth of fungi.

Effect of various amino acids

The effect of various amino acids on the mycelial growth and dry weight of *S. rolfsii* are summarized in Table 3. Among the eight amino acids (alanine, asparagine, cysteine, glutamic acid, phenylalanine, tryptophan, tyrosine and valine) tested, phenylalanine and tryptophane supported maximum mycelial growth and dry weight of *S. rolfsii* when compared to control which was followed by valine. The minimum mycelial growth and dry weight was registered in tyrosine. Similarly, Muthukumar and Eswaran (2008a) who explained that maximum mycelial growth and dry weight of *Pythium aphanidermatum* was recorded in phenylalanine and tryptophan amended medium. So far no report is available on this area. A large number of amino acids are recorded as good nutritional sources for many fungi. However, the nutritive capacity of individual amino acids varies highly with organisms.

REFERENCES

Ainsworth GC (1961). Dictionary of fungi. Common Wealth Mycological Institute., Kew Burrey, England. p. 547.

Anand S, Harikesh Bahadu 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.

Azhar Hussain SH, Muhammad IN, Abdul Majeed H (2003). Physiological study of *Sclerotium rolfsii* Sacc. Pakistan J. Plant Pathol. 2:102-106.

Basamma (2008). Integrated management of *Sclerotium* wilt of potato caused by *Sclerotium rolfsii* Sacc. M.Sc. Thesis, University of Agricultural Sciences, Dharwad.

Chun SC, Schneider, RW, Chung IM (2003). Determination of carbon source utilization of *Bacillus* and *Pythium* sp. by biological and microplate assay. J. Microbiol. 41: 252-258.
Dey TK, Ali, MS, Goswami, BK, Siddique AM (1992). Collar rot (*Sclerotium rolfsii* Sacc.) of taro a new disease in Bangladesh. J. Root Crops 18:108-112.
Ingale RV, Mayee CD (1986). Efficacy and economics of some management practices of fungal diseases of groundnut. J. Oilseeds Res. 3: 201-204.
Khattabia N, Brahim E, Latifer L, Abdullah O (2004). Effect of nitrogen fertilizers on *Trichoderma harzianum* and *Sclerotium rolfsii.* Agronomia 24: 281-288.
Lilly VG, Barnett HL (1951). Physiology of the fungi. Mc Graw Hill Book Co., New York. p. 439.
Lin MA, Michael C, Malin (2009). Biological characteristics of *Sclerotium rolfsii* Sacc. in *Coptis chinensis* and screening on its controlling fungicides. J. Anhui Agric. Sci. 34: 240-245.
Mahen VK, Mayer CD, Brennemen TB, Mcdonald D (1995). Stem and pod rot of groundnut. Info. Bull. 4:28.
Muthukumar A, Eswaran A (2008) . Effect of certain epidemiological factors on the *in vitro* growth of *Pythium aphanidermatum* (Edson) Fitz. Pl. Arc. 8:297-299.
Prasad BK, Thakur SP, Sinha, Prasad A (1986). Influence of nutritional factor, pH and temperature on growth of *Sclerotium rolfsii* Sacc. isolated from tomato fruit. Indian J. Mycol. Plant Pathol. 16:209-212.
Punja ZK, Damini A (1996). Comparative growth, morphology and physiology of three *Sclerotium* species. Mycologia 88: 694-706.
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, Srivastava RK, Kumar S (1999). Production and trade of menthol mint in India. Curr. Sci. 87.
Survase SA, Saudagar PS, Singhal RS (2006). Production of scleroglucan from *Sclerotium rolfsii*. Bioresour. Technol. 97:989-993.
Thiribhuvanamala G, Rajeswar, E, Sabitha Doraiswamy (1999). Inoculum levels *Sclerotium rolfsii* on the incidence of stem rot in tomato. Madras Agric. J. 86: 334.
Tripathi BP, Khare N (2006). Growth of *Sclerotium rolfsii* of chick pea as influenced by bioagents. Ann. Plant Prot. Sci. 13:492-493.
Watanabe T (2002). *Sclerotium* sp. morphologies of cultured fungi and key species: Pictorial Atlas of Soil and Seed fungi. 2nd Ed., CRC Press, New York.
Xiao Z, Xiaoxia LI, Shao Y, Zhang S (2012). Effects of different carbon and nitrogen sources on *Sclerotium rolfsii* in various growing areas of *Atractylodes macrocephala* in Guizhou province. Guizhou Agric. Sci. 10:310-314.
Zape AS, Gade RM, Ravindra Singh (2013). Physiological studies on different media, pH and temperature on *Sclerotium rolfsii* isolates of soybean. Sch. J. Agric. Sci. 2:238-241.