Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(2): 221-227
Received: 19-01-2021
Accepted: 21-02-2021

Rimamay Konjengbam
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Iroishemba, Imphal West, Manipur, India

Rajkumari Tombisana Devi
Department of Plant Pathology, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam, Barapani, Meghalaya, India

Corresponding Author:
Rimamay Konjengbam
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Iroishemba, Imphal West, Manipur, India

In-vitro assessment of biocontrol agents against *Sclerotium rolfsii* causing white rot of onion in Manipur

Rimamay Konjengbam and Rajkumari Tombisana Devi

Abstract
Antagonistic potential of five biocontrol agents were evaluated against *Sclerotium rolfsii* causing white rot of onion in Manipur. *Trichoderma hamatum* was most outstanding and effective against the test pathogen. The minimum mycelial growth of *S. rolfsii* was recorded in a dual culture with *T. hamatum* (2.0cm) followed successively by dual culture with *T .harzianum* (2.90cm) and *T. viride* (3.17cm) respectively. *Trichoderma hamatum, T. harzianum* and *T. viride* showed 78.88%, 67.77% and 64.77% inhibition on mycelial growth of the pathogen respectively. *T. harzianum* formed a translucent inhibition of 0.1cm at the interaction site but eventually overgrow the test pathogen and completely inhibited sclerotia formaton. Subsequently the test pathogen was also overgrown by *T. hamatum* and *T. viride* completely inhibiting sclerotia formation. *Penicillium glabrum* and *P. citrinum* were ineffective against *S. rolfsii* and both were overgrown by the pathogen.

Keywords: white rot, onion, *Sclerotium rolfsii*, biocontrol, sclerotia

Introduction
Onion (*Allium cepa* L.) is an important vegetable and spice crop in Manipur. It is mostly cultivated as a cool season crop. Onion suffers from many diseases which reduced crop yield considerably, alter the cropping pattern, lowers the quality of harvest produce, affects the local and export markets and cause significant economic losses (Mishra *et al*. 2014) [21]. White rot of onion is one of the prime diseases which reduced onion productivity. It is commonly observed in onion cultivating sites in the valley districts of Manipur and has traumatized onion cultivation in the state. *Sclerotium rolfsii* was first reported from Florida as a causal organism of tomato blight (Rolfs, 1892) [32]. The teleomorph of the fungus is *Athelia rolfsii* (Curzi) Tu and Kimbrough (Curzi, 1931; Tu and Kimbrough, 1978) [13, 39]. The symptoms commenced with the drying of leaves from the tips which extend downwards followed by blighting, drooping, wilting of leaves. The affected onion bulbs are watery, soft and rots accompanied by white cotton like mycelial growth of the fungus along with small white, brown to black colour sclerotia. The fungus attack many hosts since the fungus has an extensive host range (Aycock, 1966; Mordue, 1974; Punja, 1985; Punja, 1988) [4, 22, 26, 27]. The fungus produces oxalic acid and several enzymes including endo-polygalacturonase, endo-pectimethylpolygalacturonase, cellulase (Punja *et al.*, 1985) [30]. The fungus survives by producing sclerotia which remains viable for long period (Punja, 1985; Mullen, 2001; Marcuzzo and Schuller, 2014) [26, 23, 20]. Sclerotia comprises of three layers namely, inner medulla, middle cortex and outer rind (Punja and Damiani, 1996) [28]. Moreover, the wall of sclerotia contains melanin pigment, elevated amount of lipid, ash and non hydrolysable residue which are responsible for making sclerotia tolerance to biological and chemical degradation (Abbo Ellil, 1999; Chet *et al.*, 1967) [3, 12]. The fungus sometimes developed hymenial layers for survival and also survives as mycelium in infected plants, plant debris and on dead organic materials (Mullen, 2001) [20]. The isolates of *Sclerotium rolfsii* from different hosts and locations varies in cultural, morphological, physiological, pathogenicity and genetic characters (Punja and Damiani, 1996; Punja and Sun, 2001; Shukla and Pandey, 2008) [28, 29, 37]. The substantial use of fungicides confers numerous harmful effects including residual issue, phytotoxic effect, health hazard, environmental degradation, climate change and non target effects on unrelated organisms (Ma *et al.*, 2003; Boxall *et al.*, 2009; Dias, 2012; Bollmann *et al.*, 2014; Nettles *et al.*, 2016) [19, 11, 15, 9, 24]. Although fungicides are included in effective and efficient management strategies against fungus usually developed resistance against fungicides. *S. rolfsii* isolates resistant to tebuconazole, flutolanil and pentachloronitrobenzene (PCNB) were reported from peanut

et al., 1998) [36]. Hence, the potential and efficiency of fungicides are not constant since the effect varies from time to time. Thus, in recent years, biocontrol agents were tested for their effectiveness against several plant diseases. The utilization of biocontrol agents provides an alternative and appreciable strategy to manage the plant diseases as it does not degrade the environment and biodiversity. Therefore, the present investigation were carried out to evaluate five biocontrol agents that prevent both mycelial growth and sclerotia production of the fungus isolate causing white rot in onion under laboratory conditions.

Materials and Methods

Isolation, identification and maintenance of the pathogen

The diseased onion bulbs were collected. The cottony white mycelium from the infected bulb scales was inoculated on potato dextrose agar and was incubated at 28 ± 1^{0}C for 4 days. The fungus was purified by hyphal tip cut method and re-isolated on PDA. The fungus was identified by comparison with relevant monographs. The culture of the fungus was maintained on PDA and was sub cultured periodically every week to freshly prepared PDA throughout the research period.

Collection and maintenance of biocontrol agents

Biocontrol agents namely, *Trichoderma harzianum, Trichoderma hamatum, Trichoderma viride, Penicillium glabrum* and *Penicillium citrinum* were collected from the Department of Plant Pathology, College of Agriculture, Imphal. All the biocontrol agents were maintained on potato dextrose agar throughout the research period.

Assessment of biocontrol agents against *Sclerotium rolfsii*

Five biocontrol agents were evaluated under laboratory conditions by dual culture method on the mycelial growth and sclerotia production of the fungus. Potato dextrose agar (PDA) was prepared and sterilized at 121°C at 15lbs for 20 minutes. PDA was poured into sterilized petriplates and allowed to solidify. 5mm disc of all the biocontrol agents and the test pathogen were inoculated in opposite direction at 3 cm apart from each other. 5mm mycelial disc of the test pathogen alone were inoculated on the centre. This serves as a control. Three replications were maintained for each treatment. All the inoculated petriplates were incubated at 28 ± 1^{0}C in inverted position until the control plates were fully grown by the test fungus.

Bell's scale was used to compare the antagonistic activities of five biocontrol agents

Bell's scale (Bell *et al.*, 1982) [7] with slight modification (Class I-VI):

Class l = The antagonist completely overgrew the pathogen (100% or overgrowth).

Class ll = The antagonist overgrew at least ⅔rd (75% overgrowth) of the pathogen surface.

Class lll= The antagonist colonizes on half of the growth of the pathogen (50% overgrowth). Class lV = The pathogen and the antagonist locked at the point of contact.

Class V = The pathogen overgrew the mycoparasite.

Class Vl = Formation of inhibition zone between pathogen and antagonist.

The per cent inhibition of mycelial growth of test fungus over control was calculated by using the formula recommended by Dennis and Webster (1971) [14]

$$PI = \frac{C-T}{C} \times 100$$

Where PI= Percent inhibition
C = linear growth of the fungus in control
T = linear growth of the growth of the fungus in treatment

Per cent inhibition of sclerotia production was calculated after 35 days of incubation by
adopting the formula described by Vincent (1927) [40] as

$$PI = \frac{C-T}{C} \times 100$$

Where PI= Percent inhibition
C = number of sclerotia produced by the fungus in control
T = number of sclerotia produced by the fungus in treatment

Results and Discussion

The fungus was identified as *Sclerotium rolfsii* Saccardo based on morphological characteristics and taxonomic keys available in the literatures (Saccardo, 1913; Mordue, 1974; Punja, 1985) [22, 26, 33].

Bell's scale

All the evaluated biocontrol agents showed different antagonistic potential against *Sclerotium rolfsii.* Two *Trichoderma* species namely, *T. viride* and *T. hamatum* and two *Penicillium* species namely, *P. glabrum and P. citrinum* came in contact with *S. rolfsii* after 2 days of incubation. However, *T. harzianum* form a translucent inhibition zone of 0.1cm at interaction site. However, on the fourth day of incubation *T. harzianum* came in contact with *S. rolfsii* and subsequently overgrew the pathogen completely. The test pathogen *S. rolfsii* was completely overgrown by *T. harzianum, T. hamatum* and *T. viride* after 5 days of incubation while *P. glabrum* and *P. citrinum* were completely overgrown by *S. rolfsii* after 5 days of incubation. Bell's scale disclosed Class I by *T. hamatum* and *T. viride,* Class VI and subsequently Class I by *T. harzianum* and Class V by *P. glabrum* and *P. citrinum.* The present results are in corroboration with the findings of Amin *et al.* (2010) [2] who reported that Bell's scale revealed Class I category by two isolates (Th-1 and Th-2) of *T. harzianum* and three isolates of *T. viride* (Tv-1, Tv-2 and Tv-3) in dual culture with *S. rolfsii*. Similarly, Biswas and Sen (2000) [8] reported that T8 and T10 isolates of *T. harzianum* in a dual culture with *S. rolfsii* showed Class I of Bell's scale. Yaqub and Shahzad (2005) [41] reported that *T. harzianum* inhibited the growth of *S. rolfsii* and exhibits coiling around the mycelium of *S. rolfsii* on fourth day of incubation and showed B type of interaction in which the biocontrol agent and pathogen colony does not meet, however, the biocontrol agent produced coils around the hyphae of *S. rolfsii* which corroborates to Class VI of Bell's scale. The present results are also in agreement with the findings of Basumatry *et al.* (2015) [6] who reported that *Penicillium* species tested against *S. rolfsii* was ultimately overgrown by the pathogen which confirms Class V category of Bell's scale. Senthilkumaran *et al.* (2010) [34] also reported that *T. harzianum* and *T. viride* in dual culture with *S. rolfsii* showed Class I category of Bell's scale. Appearance of translucent inhibition zone in dual culture of *Trichoderma species* could be attributed to antibiosis caused by antibiotics, volatile and non volatile metabolites, hydrolytic enzymes and

parasitism (El- Katatny *et al*., 2001; Barakat *et al*., 2006; Raut *et al*., 2014; Ali and Javaid, 2015) [16, 5, 31, 1].

Efficacy of biocontrol agents on the mycelial growth of *Sclerotium rolfsii*

Amidst the biocontrol agents evaluated against the antagonist, three *Trichoderma* species
were effective in reducing the mycelial growth of *S. rolfsii.* The minimum mycelial growth of *S. rolfsii* was recorded in a dual culture with *T. hamatum* (2.0cm) followed successively by dual culture with *T .harzianum* (2.90cm) and *T. viride* (3.17cm) respectively. *T. hamatum* gave maximum inhibition of 78.88% on mycelial growth of *S. rolfsii* followed by *T. harzianum* giving 67.77% and *T. viride* giving 64.77% inhibition on mycelial growth of the test pathogen. Both *Penicillum glabrum* and *P. citrinum* were ineffective against *S. rolfsii* giving only 3.0% and 1.45% inhibition on mycelial growth of the test pathogen. The mycelial growth of *S. rolfsii* was maximum in a dual culture with *P. citrinum* (8.87cm) followed by dual culture with *P. glabrum* (8.73cm). Singh and Singh (1994) [38] reported that three isolates of *T. harzianum* (Th-1, Th-2 and Th-3) and two isolates of *T. viride* (Tv-1 and Tv-2) showed maximum inhibition of mycelial growth of *S. rolfsii* with Th-2 giving 73.40% , Th-1 giving 67.22%, Th-3 giving 57.23%, Tv-2 giving 59.62% and Tv-1 giving 53.53% inhibition of mycelial growth of the test pathogen after 96 hours of incubation. Barakat *et al.* (2006) [5] also reported that *T. harzianum* (J14) and *T. hamatum* (T36) were the most effective isolates against *S. rolfsii* at 25°C in dual culture showing 79% inhibition on mycelial growth of *S. rolfsii* and both *Trichoderma species* grew over the test pathogen and formed branches that coiled around the pathogen. He also stated that *T. harzianum* (J14), *T. hamatum* (T36) and *T. viride* (J8) grew over and parasitized the hyphae of *S. rolfsii.* Basumatry *et al.* (2015) [6] reported that *T. harzianum* and *T. viride* inhibited the mycelial growth of *S. rolfsii* producing a clear inhibition zone with *T. harzianum* giving 77.39% and *T. viride* giving 76.54% mycelial growth inhibition of the pathogen. He also stated that *Penicillium* species was ineffective and gave 29.05% mycelial growth inhibition of the test pathogen and was ultimately overgrown by the pathogen after 7 days of incubation. Similarly, Karthikeyan *et al.* (2006) [18] reported that Tv1 isolate of *T. viride* gave 69.40% inhibition on mycelial growth of *S. rolfsii* in dual culture while Tv2 and Tv3 isolates gave 56.35% and 56.90 % mycelial growth inhibition after 5 days of incubation. Bosah *et al.* (2010) [10] reported that *Penicillium* species failed to produce obvious significant inhibitory effect against *S. rolfsii* and the pathogen even outgrew the growth of *Penicillium* on the fourth day of incubation.

Efficacy of biocontrol agents on sclerotia poduction by *Sclerotium rolfsii*

All the three *Trichoderma* species completely inhibited sclerotia production of *S. rolfsii* giving 100% inhibition as the pathogen was completely overgrown and parasitized by the three *Trichoderma* species. However, the test pathogen could produced sclerotia in a dual culture with both the *Penicillium* species. *Penicillium glabrum* gave 66.02% inhibition on sclerotia production and *P. citrinum* gave 62.83% inhibition on sclerotia production of *S. rolfsii* over control. The present results corroborates to the findings of Shaigan *et al.* (2008) [35] who reported that *T. viride, T. harzianum* and *T. hamatum* parasitizes the hyphae of *S. rolfsii* and also destructs and lyses already produced sclerotia at 98.5, 86,5 and 85% respectively. Basumatry *et al.* (2015) [6] reported that maximum number of sclerotia were produced in a dual culture with *Penicillium* species. Patel (2018) [25] reported that sclerotia production of *S. rolfsii* was completely inhibited by liquid culture filtrate of *T. harzianum* and *T. viride.* The present results are in conformity with the findings of Yenis *et al.* (1983) [42] and Ali and Javaid (2015) [1].

The present investigation disclosed that all the biocontrol agents had different bioefficacy against *Sclerotium rolfsii* causing onion white rot in Manipur. The effective biocontrol agents should also be tested for its efficacy under field conditions in natural epiphytotic situation. Therefore, further research work is considered very essential as biocontrol agents can be employed in organic farming and integrated disease management strategies.

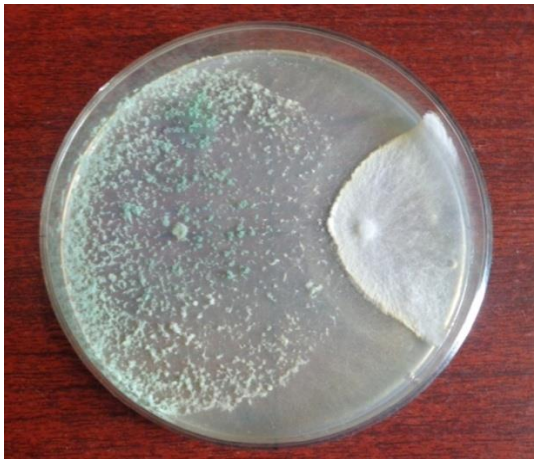

Plate I: Dual culture of *T. harzianum* and *S. rolfsii*

Plate II: Dual culture of *T. hamatum* and *S. rolfsii*

Plate III: Dual culture of *T. viride* and *S. rolfsii*

Plate IV: Dual culture of *P. glabrum* and *S. rolfsii*

Plate V: Dual culture of *P. citrinum* and *S. rolfsi*

Plate VI: Culture of *S. rolfsii* (Control)

Table 1: Comparision of antagonistic effect of biocontrol agents by Bell's scale

Biocontrol agent	Duration of point of contact between biocontrol agent and pathogen	Bell's scale
Trichoderma harzianum	No contact (Inhibition zone) up to 3th day and contact on 4th day	VI & I
Trichoderma hamatum	2th day	I
Trichoderma viride	2th day	I
Penicillium glabrum	2th day	V
Penicillium citrinum	2th day	V

Table 2: Efficacy of biocontrol agents against *Sclerotium rolfsii*

Biocontrol agent	Mycelial growth (cm)*	Inhibition (%) of mycelial growth	Sclerotia production*	Inhibition (%) on sclerotia production
Trichoderma harzianum	2.90 (1.84)	67.77	-	100
Trichoderma hamatum	2.00 (1.58)	78.88	-	100
Trichoderma viride	3.17 (1.91)	64.77	-	100
Penicillium glabrum	8.73 (3.04)	3.00	331	66.02
Penicillium citrinum	8.87 (3.06)	1.45	362	62.83
Control	9.00 (3.08)	-	974	
SE(d) ±	(0.04) (0.01)			
$CD_{(0.05)}$	0.1 (0.03)			

*Mean of three replications, Figures in parenthesis are (√x+0.5) transformed values

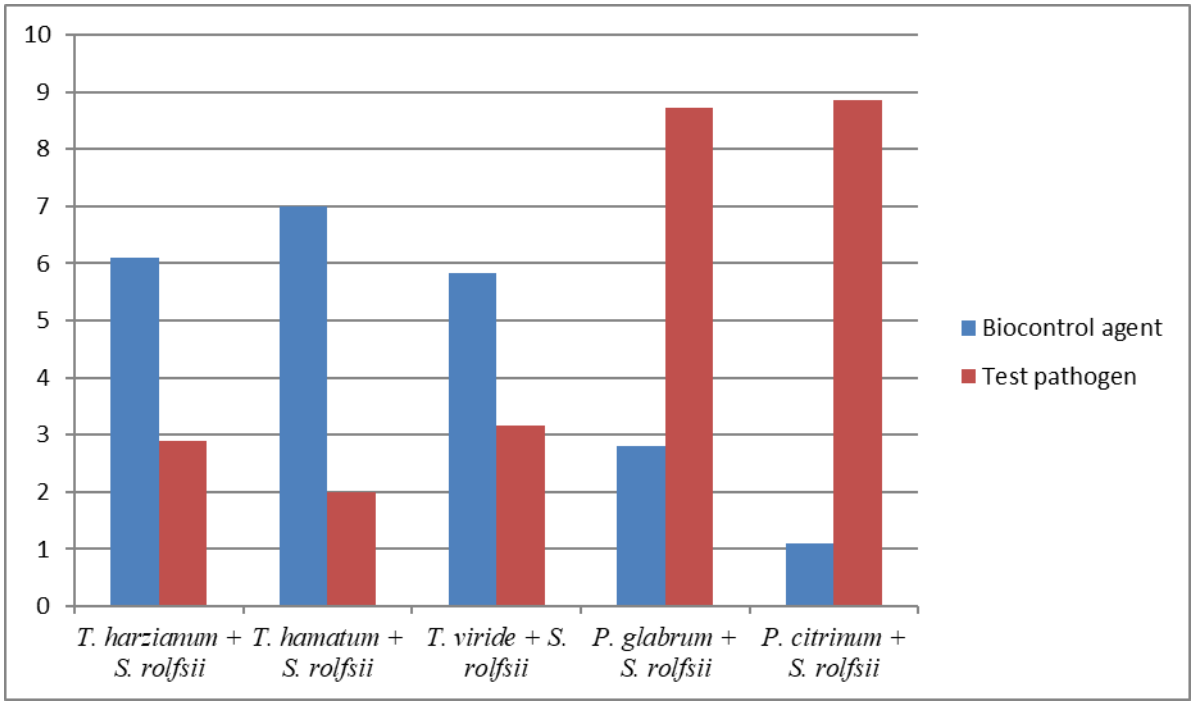

Fig 1: Growth rate of biocontrol agents and *S. rolfsii* in dual culture

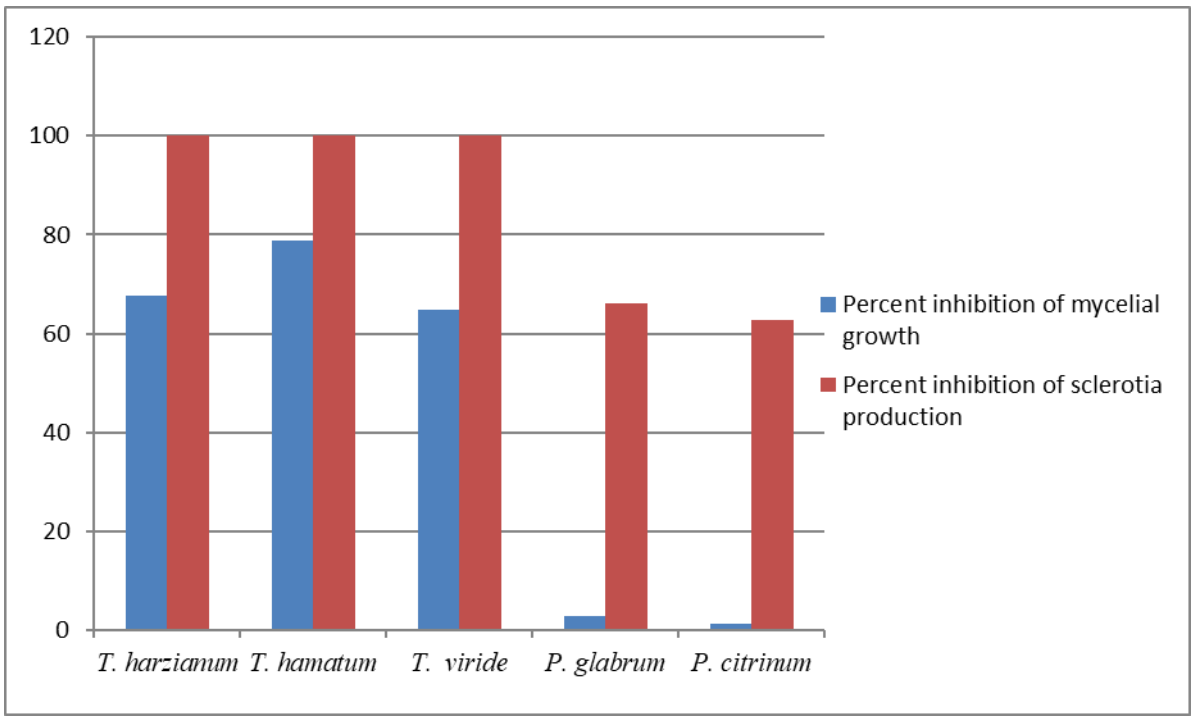

Fig 2: Antagonistic effect of biocontrol agents against *S. rolfsii* in dual culture

Acknowledgment
I would like to extend deep gratitude to my late mother Nongmaithem Madhuri Devi for her endless support, kindness, guidance, and encouragement while conducting this research.

References

1. Ali A, Javaid A. Screening of Trichoderma species for their biological control potential against *Sclerotium rolfsii*, the cause of collar rot disease of chickpea. Mycopath 2015;13(2):93-96.
2. Amin F, Razdan VK, Mohiddin FA, Bhat KA, Banday S. Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. Phytology 2010;2(10):38-41.
3. Abbo Ellil ABA. Sclerotial development, melanin production and lipid peroxidation by *Sclerotium rolfsii*. Folia Microbiologica 1999;44:181-186.
4. Aycock R. Stem rot and other diseases caused by Sclerotium rolfsii. N.C State Univ. Tech. Bull 1996;174:202.
5. Barakat RM, Al-Mahareeq F, Al-Masri M. Biological Control of *Sclerotium rolfsii* by Using Indigenous *Trichoderma* spp. Isolates from Palestine. Hebron University Research Journal 2006;2(2):27-47.
6. Basumatary M, Dutta BK, Singha DM, Das N. Some *in vitro* Observations on the Biological Control of

Sclerotium rolfsii, a Serious Pathogen of Various Agricultural Crop Plants. IOSR Journal of Agriculture and Veterinary Science 2015;8(2):87-94.
7. Bell DK, Wells HD, Markham CR. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 1982;72(4):379-382.
8. Biswas KK, Sen C. Management of stem rot of groundnut caused by *Sclerotium rolfsii* through *Trichoderma harzianum*. Indian Phytopathology 2000;53(3):290-295.8.
9. Bollmann UE, Tang C, Eriksson E, Jönsson K, Vollertsen J, Bester K. Biocides in urban wastewater treatment plant influent at dry and wet weather: concentrations, mass flows and possible sources. Water Research 2014;60:64-74.
10. Bosah O, Igeleke CA, Omorusi VI. *In Vitro* Microbial Control of Pathogenic *Sclerotium rolfsii*. International Journal of Agriculture & Biology 2010;12(3):474-476.
11. Boxall AB, Hardy A, Beulke S, Boucard T, Burgin L, Falloon PD *et al.* Impacts of climate change on indirect human exposure to pathogens and chemicals from agriculture. Environmental Health Perspectives 2009;117(4):508-514.
12. Chet I, Henis Y, Mitchell R. Chemical composition of hyphal and sclerotial walls of *Sclerotium rolfsii* Sacc. Canadian Journal of Microbiology 1967;13(2):137-141.
13. Curzi M. Studi su lo "*Sclerotium rolfsii*". Boll. Staz. Patol. Veg. N. S. 1931;11:306-373.
14. Dennis C, Webster J. Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. Transactions of the British Mycological Society 1971;57:25-39.
15. Dias MC. Phytotoxicity: An Overview of the Physiological Responses of Plants Exposed to Fungicides. Journal of Botany 2012;10.1155/2012/135479.
16. El-Katatny MH, Gudelj M, Robra KH, Elnaghy MA, Gubitz GM. Characterization of a chitinase and an endo-b-1,3- glucanase from *Trichoderma harzianum* Rifai T24 involved in control of the phytopathogen Sclerotium rolfsii. Applied Microbiology & Biotechnology 2001;56(1-2):137–143.
17. Franke MD, Brenneman TB, Stevenson KL, Padgett GB. Sensitivity of Isolates of *Sclerotium rolfsii* from Peanut in Georgia to Selected Fungicides. Plant Disease 1998;8(5):578-583.
18. Karthikeyan V, Sankaralingam A, Nakkeeran S. Biological control of groundnut stem rot caused by *Sclerotium rolfsii* (Sacc.). Archives of Phytopathology and Plant Protection 2006;39(3):239-246.
19. Ma Z, Felts D, Michailides TJ. Resistance to azoxystrobin in *Alternaria* isolates from pistachio in California. Pesticides Biochemistry & Physiology 2003;77(2):66-74.
20. Marcuzzo LL, Schuller A. Survival and viability of sclerotia from *Sclerotium rolfsii* on the soil. Summa Phytopathogica 2014;40(3):281-283.
21. Mishra RK, Jaiswal RK, Kumar D, Saabale PR, Singh A. Management of major diseases and insect pests of onion and garlic. Journal of Plant Breeding and Crop Science 2014;6(11):160-170.
22. Mordue JEM. *Sclerotium rolfsii*. CMI descriptions of pathogenic fungi and bacteria. Commonwealth Mycological Institute, Kew, Surrey, England, 1974, 410.
23. Mullen J. Southern blight, Southern stem blight, White mold. The Plant Health Instructor, 2001, 10.1094/PHI-I-2001-0104-01.
24. Nettles R, Watkins J, Ricks K, Boyer M, Licht M, Atwood LW *et al*. Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean. Applied Soil Ecology 2016;102:61-69.
25. Patel JK. Bioefficacy of *Trichoderma spp*. against *Sclerotium rolfsii* Sacc., an incitant of collar rot of chickpea *in vitro*. The Bioscan 2018;10(4):1745-1748.
26. Punja Z K. The biology, ecology, and control of *Sclerotium rolfsii*. Annual Review of Phytopathology 1985;23:97-127.
27. Punja ZK. *Sclerotium (Athelia) rolfsii*, a pathogen of many plant species. In G.S. Sindhu, Advances in plant pathology, Academic Press, 1988, 523-534.
28. Punja ZK, Damiani A. Comparative growth, morphology and physiology of three *Sclerotium* species. Mycologia 1996;88(5):694-706.
29. Punja ZK and Sun LJ. Genetic diversity among mycelial compatibility groups of *Sclerotium rolfsii* (teleomorph *Athelia rolfsii*) and *S. delphinii*. *Mycological Research* 2001;105(5):537-546.
30. Punja ZK, Huang ZS & Jenkins SF. 1985. Relationship of Mycelial Growth and Production of Oxalic Acid and Cell Wall Degrading Enzymes to Virulence in *Sclerotium rolfsii*. Canadian Journal of Plant Pathology 1985;7(2):109-117.
31. Raut I, Doni MB, Calin M, Oancea F, Vasilescu C, Sesan TE *et al.* Effect of Volatile and Non-Volatile Metabolites from *Trichoderma* spp. against Important Phytopathogens. Revista De Chime-Bucharest 2014;65(11):1285-1288.
32. Rolfs PH. Tomato blight. Some hints. Bulletin of Florida Agricultural Experimental Station 1892, 18.
33. Saccardo PA. *Sclerotium rolfsii*. Sylloge Fungorum XXII, Pavia, Italy 1913, 1500.
34. Senthilkumaran R, Savitha S, Sivakumar T. A study on diseases of *Vanilla planifolia* and their management through Biocontrol Agents. International Journal of Advanced Research in Biological Sciences 2010;1(8):192-204.
35. Saigan S, Seraji A, Moghaddam SAM. Identification and investigation on antagonistic effect of *Trichoderma* spp. on tea seedlings white foot and root rot (*Sclerotium rolfsii* Sacc.) *in vitro* condition. Pakistan Journal of Biological Science 2008;11(19):2346-2350.
36. Shem MY, Starr JL, Keller NP, Woodard KE, Lee TA. Distribution of isolates of *Sclerotium rolfsii* tolerant to pentachloronitrobenzene in Texas peanut fields. Plant Disease. 1998;82:103-106.
37. Shukla R, Pandey AK. Pathogenic diversity of Sclerotium rolfsii isolates, a potential biocontrol agent against *Parthenium hysterophorus* L. African Journal of Environmental Science and Technology 2008;2(5)-124-126.
38. Singh A, Singh D. Biocontrol of *Sclerotium rolfsii* Sacc. Causing Collar Rot of Brinjal. Journal of Biological Control 1994;8(2):105-110.
39. Tu CC, Kimbrough JW. 1978. Systematics and Phylogeny of Fungi in the *Rhizoctonia* Complex. Botanical Gazette 1978;139(4):454-466.

40. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature 1927;159:850
41. Yaqub F, Shahzad S. *In vitro* evaluation of microbial antagonists against *Sclerotium rolfsii*. Pakistan Journal of Botany 2005;37(4):1033-1036.
42. Yenis A, Lewis JA, Papavizas GC. Interactions between *Sclerotium rolfsii* and *Trichoderma* spp: Relationship between antagonism and disease control. Soil Biology & Biochemistry 1984;16(4):391-395.