

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.712.116>

An Eco-friendly Approach for the Management of Collar Rot of Brinjal Caused by *Sclerotium rolfsii* SACC.

Ruthy Tabing* and Shashi Tiwari

Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences, Naini, Allahabad – 211007, (U.P.) India

*Corresponding author

ABSTRACT

Brinjal (*Solanum melongena* L.) is one of the most important vegetable cultivated all over India. This crop is infected by many diseases and the most important disease reported in this crop causing yield loss about 90-100% is collar rot caused by *Sclerotium rolfsii* Sacc. Biological control of soil borne plant pathogen is gaining much importance due to its eco-friendly, economic and being harmless to non target organism in nature. Seven organic inputs namely *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis*, Neem oil, Eucalyptus oil, Mustard cake and Cow urine were evaluated against *Sclerotium rolfsii*. The *in vitro* evaluation revealed highest average mycelium growth inhibition with cow urine (@ 5%) concentration shows maximum inhibition (100%) followed by *Trichoderma viride* (72.08%) and Eucalyptus oil (57.11%). In the present study soil treatment (@ 2g/plot) and seedling root dip treatment (@ 100g/lit) with *Trichoderma viride* shows the lowest plant disease incidence (7.41%) significantly highest germination per cent recorded (95.89%) and highest plant height (72.63 cm). The soil treatment (@ 200g/plot) with soil amendment mustard cake has significant effect on seed germination (71.91%). Thus it is concluded that brinjal collar rot can be effectively managed by soil treatment and seedling root dip treatment with bio-agent *viz.* *Trichoderma viride*, *Pseudomonas* and *Bacillus*, botanicals *viz.* neem oil and eucalyptus oil, cow urine and soil amendment with mustard cake.

Keywords

Sclerotium rolfsii,
Trichoderma viride,
Pseudomonas,
Bacillus, Cow
 urine, Mustard
 cake, Brinjal, Collar
 rot

Article Info

Accepted:
 10 November 2018
 Available Online:
 10 December 2018

Introduction

Brinjal (*Solanum melongena* L.) is one of the important commercial vegetable crops grown in all parts of India and adapted to a wide range of climatic conditions. It is planted in three seasons, first in *Kharif* (June-Sep), second in *Rabi* (Nov-Feb) and third in the month of March. The solanaceous vegetable crop is covering an area of 704.96 ha with a

total production of 12,994.77 tonnes and productivity of 18.43 tonnes/ha. The leading brinjal producing countries in the order of importance are China, India, Japan, Italy and Spain. In India the major brinjal producing states in India are Andhra Pradesh, Maharashtra, Karnataka, Orissa, Madhya Pradesh and West Bengal. The fruit yield is about 16413 kg/ha and 200000 kg/ha in India and Andhra Pradesh, respectively. The fruits

contain approximately, 92.0 per cent protein, 0.3 per cent fats and some minerals. Dark purple brinjal has more vitamin C than those with white skin. Bitterness in brinjal is due to presence of glycoalkaloids. It is highly productive and usually finds its place as the poor man's crop. The green leaves are the main source of the supply of anti-scorbic vitamin-C and an excellent remedy for people suffering from liver complaints and diabetics (Chala, 1993).

More than ten diseases have been reported on brinjal from India (Rangaswami, 1972). The total cultivated area is about 530.30 ha, with productivity of 8703.80 t in India. The total cultivated area in Uttar Pradesh is about 3.13 ha with productivity of about 33.98 t. The fruit yield is about 16413kg/ha and in India (CMIE, 2007). The pathogen *S. rolfsii* has been reported to inflict the fruit yield losses up to 90-100% (Haque *et al.*, 2001; Jadon, 2009). Among the soil borne fungal diseases, collar rot caused by *Sclerotium rolfsii* Sacc has become one of the constraints resulting in yield losses upto 16-30 per cent (Singh and Dhancholia, 1991). The affected plant shows invasion of the fungus, in the form of a girdle in the collar region, just above the soil line. The girdling progresses upwards, along with the white mycelium. Later on cream to chocolate coloured sclerotia will be formed. Wilting occurs within 3-5 days and the entire plant dries up with the abolition of the green canopy. The dried leaves remain intact with the stem with poor root growth.

S. rolfsii is a soil borne facultative pathogen, belongs to the sub-division Deuteromycotina, which has wide host range of more than 500 species of cultivated and wild plants in tropical and sub tropical regions (Aycock 1966). The pathogen is polyphagous non target fungus attacking many crops in India (Prasad *et al.*, 1988) and throughout the world. The sclerotia can survive in the soil from a

few months to several years. After germination, *S. rolfsii* hyphae produce oxalic acid and pectinolytic and cellulolytic enzymes which kill and disintegrate host tissues, starting a new infection (Le. 2011).

Materials and Methods

Preparation of media

PDA: for *Trichoderma viride* and *Sclerotium rolfsii*

The potatoes were peeled and cut into small pieces and boiled in 500 ml of distilled water till they become soft. The extract obtained was filtered through muslin cloth and all the liquid was squeezed in beaker. 20 g of Agar was added bit by bit to the rest of 500 ml. Hot water to dissolve. Then 20 g of dextrose was added. Volume of broth was made up to 1000 ml by adding more distilled water. Then 200 ml of this solution was dispensed to each of five conical flasks and sterilized at 121°C at 15 lbs pressure /square inch for 15 minutes in an autoclave.

Nutrient agar: for *Bacillus subtilis*

King's B media: for *Pseudomonas fluorescens*

Isolation and purification of pathogen

The pathogen *Sclerotium rolfsii* was isolated from the infected brinjal plants showing typical symptoms of the disease. The part of collar or stem region showing typical symptoms of the disease was cut into small pieces. These pieces were surface sterilized with 0.1 % mercuric chloride solution for 30 seconds. These were then washed thoroughly in sterile water thrice to remove traces of mercuric chloride and then transferred aseptically to sterilized potato dextrose agar (PDA) plates. They were incubated at 27±1°C

and checked on 24 hour basis for the growth of the fungus. Later, the bit of fungal growth was transferred to PDA slants. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions (Rangaswamy, 1972).

Identification of pathogen

For identification of the pathogen was confirmed by observing the morphological features of colony and spore characteristics.

Maintenance of *Sclerotium rolfsii*

After obtaining the pure culture of *Sclerotium rolfsii*, the pathogen was transferred to agar slants of containing potato dextrose agar medium. After growing the culture of *Sclerotium rolfsii* the slants were kept at low temperature in refrigerator. After every 20-25 days the pathogen was transferred to fresh slants for maintenance of pathogen culture.

Mass multiplication

Mass cultures of *Sclerotium rolfsii* were prepared using wheat grains in 1000 ml conical flasks. wheat grains were soaked overnight in two per cent sucrose solution and air dry the remaining excess moisture then autoclaved, to which five discs (5 mm diameter) from three days old culture of test pathogen was added and thoroughly shaken. These inoculated conical flasks were incubated for 2 weeks at $28 \pm 2^\circ\text{C}$. The inoculated flasks were shaken periodically to allow the uniform growth and maximum utilization of substrate by the pathogen (Fig. 2).

Incubation of pathogen

The mass culture of *S. rolfsii* multiplied on wheat grains be directly incubated in the soil @ $100\text{g}/\text{m}^2$ of plot two weeks before

transplanting. The inoculation was done in all the plots with 3 replications. The mass culture well mixed with the soil. The pathogen *S. rolfsii* inoculated in seed bed soil was allowed to multiply with proper soil moisture.

Soil treatment

The seven treatments (Bio control-agents) *Trichoderma viride* @ 2g per plot. *Pseudomonas fluorescens* @ 2g per plot. *Bacillus subtilis* @ 2g per plot (Patil *et al.*, 2004) and (Botanicals) neem oil @ 5% concentration per plot, eucalyptus oil @ 5% concentration per plot, (Ramazeni *et al.*, 2002) mustard cake @ 200g per plot and Cow urine @ 5% per plot (Jandaik *et al.*, 2015) were applied in the soil at 6-7cm depth after one to two week of pathogen inoculation. The untreated plots will be left as control plot.

Seedling root dip treatment

The seedling roots were dipped into different treatments, *Trichoderma viride* @ 100g per liter. *Pseudomonas fluorescens* @ 100g per liter. *Bacillus subtilis* @ 100g per liter. (Patil *et al.*, 2004) Neem oil @ 50ml/1000ml of water. Eucalyptus oil @ 50ml/1000ml of water. Mustard cake @ 50ml/1000ml of water. Cow urine @ 50ml/1000ml of water, (Jandaik *et al.*, 2015) for 30 seconds and dried in shade for 15-20mins Prior to planting.

Results and Discussion

Pathogenicity test

Pathogenicity test was conducted for testing the infectivity of *S. rolfsii* isolated from infected plant on brinjal by artificial inoculation of the test fungus. The first symptoms appeared after 15 days of inoculation. Affected seedlings develop severe rot and become wilted and could easily be pulled out. The part of collar or stem region showing typical symptoms of the disease

were taken for re-isolation of the pathogen (Fig. 1). Then this re-isolated culture of pathogen compared with the original isolated culture. On comparison, re-isolated culture of pathogen was found to be identical with the previously isolated culture of pathogen, which confirms the Koch' postulates and pathogenic ability of isolated pathogen.

In vitro evaluation of different treatments against radial mycelium growth of *Sclerotium rolfsii*

Efficacy of different treatments on the radial mycelium growth of *Sclerotium rolfsii* is shown in (Table 1). Bio-agent and botanicals have profound effects on reduction of radial mycelium growth of the fungus. All the tested treatments significantly reduced radial mycelium growth of the fungus. Radial mycelium growth for all the tested treatments ranged from 0.0 to 9.0 cm recorded 5 days after inoculation. It was found that cow urine has significant effect in inhibiting the radial mycelium growth of *Sclerotium rolfsii*. Radial mycelium growth was not observed in cow urine (0.0 cm). Therefore cow urine has the highest inhibition percentage of (100 %). The lowest radial mycelium growth (0.67, 1.31, 1.67 and 2.20 cm) of *Sclerotium rolfsii* was recorded in case of *Trichoderma viride* at 2 day, 3 days, 4 days and 5 days after

inoculation. Then followed by eucalyptus oil (0.52, 1.07, 2.05, and 3.87 cm), *Bacillus subtilis* (1.45, 1.87, 2.05 and 4.27 cm), *Pseudomonas flourescens* (2.20, 2.72, 3.33 and 4.37 cm), neem oil (1.08, 4.90, 7.32 and 8.32 cm) and mustard cake (2.35, 6.42, 8.48 and 8.68 cm). The highest radial growth is observed in untreated Control (9.0 cm) (Fig. 3-7).

Evaluation of different treatments on plant disease incidence under field condition

The data recorded on plant disease incidence of collar rot of brinjal was done on 30, 60 and 90 days after transplantation. It was observed that plant disease incidence was significantly reduced in treated plots compared to untreated control plots (Table 2).

The lowest plant disease incidence (3.70, 7.41, 7.41) was recorded in *Trichoderma*. followed by *Pseudomonas* (7.41, 7.41, 11.11) of disease incidence, *Bacillus* (11.11, 14.81, 14.81) of disease incidence, cow urine (14.81, 14.81, 22.22) of disease incidence, neem oil (14.81, 22.22, 25.92) of disease incidence, eucalyptus oil (14.81, 25.92, 29.63) of disease incidence, mustard cake (29.63, 33.33, 40.74) of disease incidence were observed and the highest plant disease incidence (33.33, 44.44, 55.56) was observed in control plot (Fig. 8).



Fig.1 Formation of sclerotia around collar region of brinjal



Fig.2 Mass multiplication of *Sclerotium rolfsii* on wheat grains

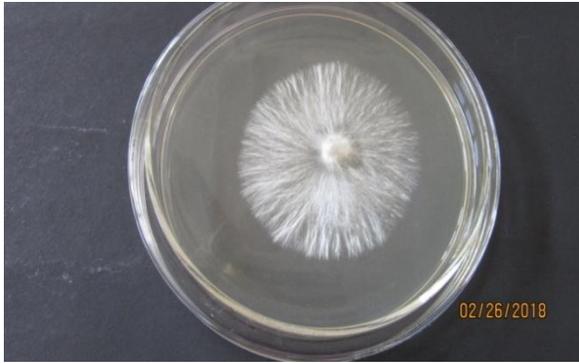


Fig.3 Mycelial growth of *Sclerotium rolfsii* on 3rd day



Fig.4 Sclerotia formation on 30 days after inoculation

Fig.5 *In vitro* evaluation of different botanicals on mycelia growth of *S. rolfsii*

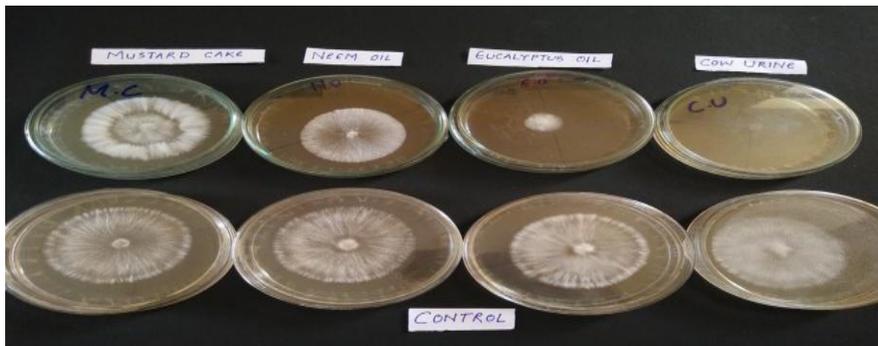


Fig.6 *In vitro* evaluation of different bio-agents on mycelium growth of *S. rolfsii*



Fig.7 *In vitro* evaluation of different treatments on the mycelial growth of *S. rolfsii* was recorded on 48, 72, 96 and 120 hours after inoculation (HAI)

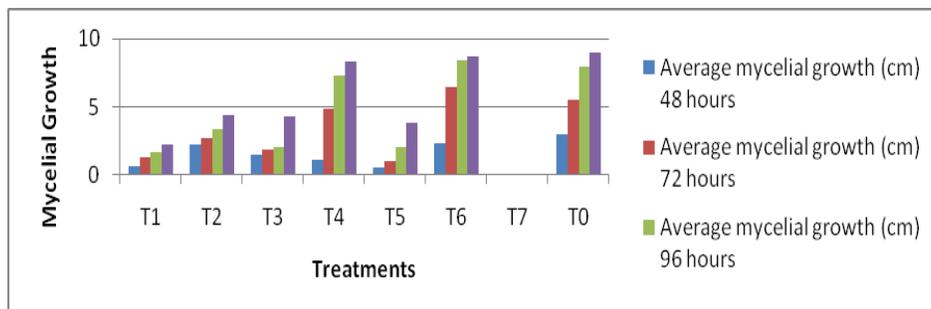


Fig.8 Evaluation of different treatments on plant disease incidence under field condition

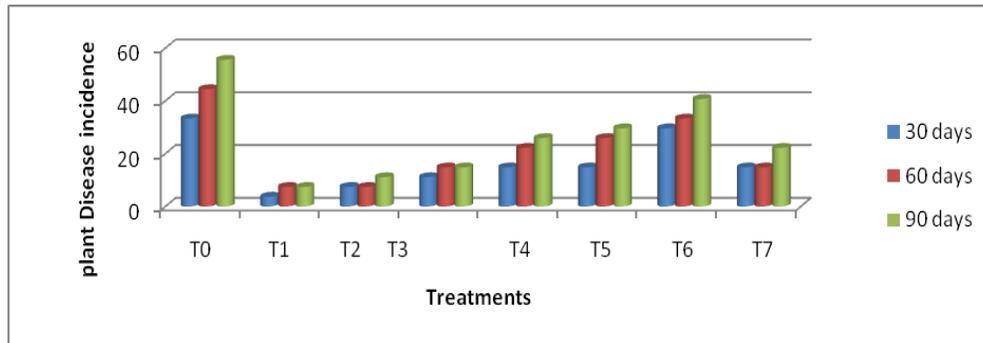


Table.1 *In vitro* evaluation of different treatments on the mycelial growth of *S. rolfsii* was recorded on 48, 72, 96 and 120 hours after inoculation (HAI)

Treatment	Average mycelial growth (cm)				Inhibition %
	48 hours	72 hours	96 hours	120 hours	
T ₀ -control	2.97	5.52	7.98	8.97	---
T ₁ - <i>Trichoderma viride</i>	0.67	1.32	1.67	2.20	72.08
T ₂ - <i>Pseudomonas fluorescens</i>	2.20	2.72	3.33	4.37	44.67
T ₃ - <i>Bacillus subtilis</i>	1.45	1.87	2.05	4.27	45.93
T ₄ -Neem oil	1.08	4.90	7.32	8.32	7.66
T ₅ -Eucalyptus oil	0.52	1.07	2.05	3.87	57.11
T ₆ -Mustard cake	2.35	6.42	8.45	8.68	3.55
T ₇ -Cowurine	0.00	0.00	0.00	0.00	100
S. Em	0.172	0.343	0.569	0.622	
CD (5%)	0.516	1.027	1.705	1.864	

Table.2 Evaluation of different treatments on plant disease incidence under field condition

Treatments	Plant Disease Incidence		
	30 days	60 days	90 days
T ₀ -control	33.33	44.44	55.56
T ₁ - <i>Trichoderma viride</i>	3.70	7.41	7.41
T ₂ - <i>Pseudomonas fluorescens</i>	7.41	7.41	11.11
T ₃ - <i>Bacillus subtilis</i>	11.11	14.81	14.81
T ₄ -Neem oil	14.81	22.22	25.92
T ₅ -Eucalyptus oil	14.81	25.92	29.63
T ₆ -Mustard cake	29.63	33.33	40.74
T ₇ -Cowurine	14.81	14.81	22.22
S.Em	4.84	6.99	7.82
CD (5%)	10.92	19.63	23.25

Different treatments have different effect on the mycelium growth. Minimum radial mycelium growth was observed in *Trichoderma viride* (2.2 cm) and cow urine (0.00). The above findings confirm the findings of Patil and Raut (2008) reported that the *Trichoderma viride* inhibited the mycelial growth of *Sclerotium rolfsii*.

Basak and Lee (2005) studied the efficacy and *in vitro* activities of cow urine and dung for controlling wilt caused by *F. oxysporum* f.sp. *cucumerinum* of cucumber. Cow urine showed 100 per cent inhibition of wilt pathogen. Joseph and sankarganesh (2011) studied the antifungal activity of panchagavya and cow urine against soil borne pathogen.

It was observed that plant disease incidence was significantly reduced in treated plots compared to untreated control plots. The lowest plant disease incidence (3.70, 7.41, 7.41) was recorded in *Trichoderma*. Franken *et al.*, (2002) observed that *Trichoderma* spp. Colonize plant roots prior to stimulation of plant growth and provide protection against invasion of infectious foreign organisms.

The summary and conclusion of the study are as follows:

Among all the treatments, cow urine was significantly effective in inhibiting the mycelium growth (0.0 cm), with highest disease inhibition percentage (100%). *Trichoderma viride* was found to be effective in reducing the growth of mycelium (2.2cm), with disease inhibition percentage of (77.81%). The highest mycelium growth was observed in control plate (9cm).

Under field condition, the disease incidence of collar rot of brinjal was recorded in 30, 60 and 90 DAT. The highest disease incidence (33.33, 44.44 and 55.56) was recorded in control plot. Followed by Mustard cake

(29.63, 33.33 and 40.74). The lowest disease incidence (3.70, 7.41 and 7.41) was recorded in plots treated with *Trichoderma viride*.

It can be concluded from the above findings that, since the fungus *Sclerotium rolfsii* is a soil borne pathogen, soil treatments with bioagents and botanicals have significant effect in controlling the collar rot in brinjal. *Trichoderma viride* has significant effect in reducing the plant disease incidence in field condition. Cow urine was found highly effective in inhibiting (100%) of mycelium growth in lab condition. The root dip method application helps the plant to resist the pathogen attack after transplantation. Usage of chemical methods leads to environment, soil and water pollution. Continuous usage of chemical makes the pathogen gain resistant to it. Hence, it can be concluded that eco-friendly approach for the management of collar rot is more advisable in today's world.

References

- 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.
- C. M. I. E. Agriculture.2007. Centre for Monitoring Indian Economy Pvt. Ltd. Andheri(East), Mumbai.
- Chala, M. L. 1993.Improvement of brinjal. In Advances in Horticulture (ed.) by Chadha K L, Malhotra Publishing House, New Delhi pp.105.
- Fery, R. L. and Dukes, S. D. 2002. Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: yield-loss estimates and sources of resistance. *Crop Protection*.21: 403-408.
- Franken, P.G., Khun and V. Gianianazzi-pearson. 2002. Development of molecular biology of arbuscular mycorrhizal fungi. pp. 325-348. *Molecular biology of fungal*

- development*. (Ed): H.D. Osiewacz. Marcel Dekker, New York.
- Gupta, S.K., Sharma, A., Shyam, K.R. and Sharma, J.C. 2002. Role of soil temperature and moisture on the development of crown rot (*Sclerotium rolfsii*) of French bean. *Plant Diseases Research*. 17: 366-368.
- Haque, M.M., Siddique, M.B., Meah, M.B. and Islam, M.N. 2001. Control of foot rot of brinjal through chemicals and organic soil amendments. *Journal of Biological Sciences*. 1:946-948.
- Jandaik, S., Thakur, P. and Kumar, V. 2015. Efficacy of Cow Urine as Plant Growth Enhancer and Antifungal Agent. *Advances in Agriculture* Volume 2015, Article ID 620368.
- Le, C.N. 2011. Diversity and biological control of *Sclerotium rolfsii*, causal agent of stem rot of groundnut. 152pp.
- Lewis, J. A. and Papavizas, G. C. 1991. Biocontrol of plant disease, the approach for tomorrow. *Crop protection*, 10: 95-105.
- Patil, P.R., Raut B.T., Shinde V.B. and Ingole M.N. 2004. Role of antagonists in inhibition of *Rhizoctonia bataticola* and *sclerotium rolfsii* causing seed/root/stem rot of sunflower. *Annals of plant Physiology* 18 (2): 195-197.
- Prasad, B. K., Thakur, S. P., Sinha, and Narayana, N. 1988. Influence of temperature on the soft rot of tomato fruit due to *Sclerotium rolfsii*. *Indian Phytopathology*, 44:256.
- Ramezani, H., Singh, H.P., Batish, D.R., Kohli, R. K. and Dargan, J. S. 2002. Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronellal. *New Zealand Plant Protection*. 55, 57–62.
- Rangaswami, G. 1972. Diseases of crop plants in India. Prentice hall of India Pvt. Ltd., New Delhi, 520pp.
- Singh, A. and Singh, H. B. 2004. Control of collar rot in mint (*Mentha* spp) caused by *Sclerotium rolfsii* using biological means. *Current Science*. 87 (3): 362-366.
- Singh, and Dhancholia, S. 1991. A noteworthy disease of brinjal caused by *Sclerotium rolfsii* Sacc. *Himachal Pradesh Journal of Agricultural Research*. 17 (1&2): 119-120.
- Singh, M. and Shukla, T. N. 1984. Control of collar rot of brinjal caused by *Sclerotium rolfsii*. *Indian Journal of Mycology and Plant Pathology* 14 (5):81-83.

How to cite this article:

Ruthy Tabing and Shashi Tiwari. 2018. An Eco-friendly Approach for the Management of Collar Rot of Brinjal Caused by *Sclerotium rolfsii* SACC. *Int.J.Curr.Microbiol.App.Sci*. 7(12): 929-936. doi: <https://doi.org/10.20546/ijcmas.2018.712.116>