# Biological management of root rot disease in *Solanum nigrum*

**ABSTRACT**

The root rot disease of *Solanum nigrum* caused by *Rhizoctonia bataticola* is a serious fungal disease in Tamil Nadu. Yellowing of leaves, discolouration and rotting of roots and dark brown lesions on the stem are the prominent symptoms of root rot disease. The field experiments were conducted in the farmer's field at Kaalipalayam, Coimbatore district, Tamil Nadu during 2015-2016 and 2016-2017 to find out the effect of fungicides and biocontrol agents for the management of root rot disease of *S. nigrum.* The results revealed that soil application of *Trichoderma asperellum* at 2.5 kg/ha mixed with neem cake at 250 kg/ha followed by seed treatment with *T. asperellum* at 4 g/kg seed was effective in managing the root rot disease and increased the leaf yield.

*Keywords* **:** Biocontrol, Solanum, root rot, carbendazim

**INTRODUCTION**

*Solanum nigrum* L. (black nightshade) is an important vegetable cum medicinal plant belonging to the family Solanaceae. The leaves and fruits are used as vegetable. It contains alkaloids *viz.,* solamargine, solanigrine and solasonine. The plant is used for asthma, ulcer, dropsy, cough etc. It contains alkaloids *viz.,* solamargine, solanigrine and solasonine. In India, *S. nigrum* is used as hepatoprotective agent. The root rot disease of *S. nigrum* caused by *Rhizoctonia bataticola* is a serious fungal disease in Tamil Nadu state of India. Yellowing of leaves, discolouration and rotting of roots and dark brown lesions on the stem are the prominent symptoms of root rot disease. The presence of sclerotial bodies as small, black dot-like structures is seen in the stem portions. The pathogen survives in the soil for several years. The yield loss ranged up to 30 to 40 per cent due to this disease. The pathogen produces pycnidia which are black, flask-shaped structures with an ostiole. Numerous pycnidiospores are released from the pycnidium (Kaur *et al*., 2012).

Fungicides such as carbendazim were reported to control root rot disease in various crop plants. Management of disease through fungicides alone leads to cause soil residual problem and health hazards, besides involving higher input cost. Biological control through the use of antagonistic microorganisms has recently emerged as a viable disease management strategy. The main modes of action of the biocontrol agent include competition for nutrients and space, production of cell wall degrading enzymes, production of antifungal diffusible and volatile metabolites and mycoparasitism. The efficient use of rhizosphere microorganisms to control plant pathogens had been reported worldwide in different plants (Meena *et al*., 2002; Animisha *et al*., 2012; Meena *et al*., 2014; Meena and Rajamani, 2014; Khadse *et al*., 2015; Meena, 2016).

Induced Systemic Resistance (ISR) is an activated resistance process that is activated by biological or abiotic factors and is dependent on the physical or chemical barrier of the host plant, and its action is characterized by no direct killing or inhibition of the pathogen, but through the induction of plant disease resistance to disease prevention and control purposes (Bitas *et al*., 2013). Root colonisation by specific non-pathogenic microorganisms such as plant growth promoting rhizobacteria can induce a systemic increase in resistance. Biopesticides are cheaper, eco-friendly and do not pose a risk of the pathogen developing resistance. Hence, the present research is carried out to manage root rot disease of *S. nigrum* using the bioagent, *Trichoderma asperellum.*

## MATERIALS AND METHODS

### Isolation of pathogen

The pathogen *R. bataticola* was isolated from the infected roots of *S. nigrum* and maintained on potato dextrose agar (PDA) slants (Rangaswami, 1972).

### Field studies

Field eperiments were conducted in the farmers field at Kaalipalayam, Coimbatore District, Tamil Nadu on the management of root rot disease of *S. nigrum* during 2015-2016 and 2016-2017. The seeds of *S. nigrum* were treated with *Trichoderma asperellum* talc formulation at the rate of 4 g per kg of seed before sowing. Soil application of *T. asperellum* talc formulation at the rate of 2.5 kg/ha mixed with neem cake @ 250 kg/ha was done during the time of field preparation. The combination treatment of seed treatment and and soil application of bioagent, *T. asperellum* was also made. For comparison, the seeds were treated with fungicide carbendazim at the rate of 2 g per kg of seed and drenched with carbendazim at 1 g per litre on 30 days after sowing. The root rot disease incidence was assessed on 30 days after sowing by counting the number of infected plants and total number of plants.

Per cent disease incidence was calculated using the formula

$$PDI = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

The plant height was recorded on 30 days after sowing. The leaf yield per hectare was recorded for each treatment and the data were statistically analyzed by IRRISTAT.

### Induction of systemic resistance in *S. nigrum*

*Solanum* seeds were sown in earthern pots. The bioagent, *T. asperellum* talc powder formulation was applied to soil at the rate of 5 g per kg of soil at 30 days after sowing. The pathogen, *R. bataticola* was multiplied in sand-maize medium and challenge inoculated at the rate of 5 g per kg of soil on 32$^{nd}$ day after planting. Leaf samples were collected at 1, 3, 5 and 7 days after pathogen inoculation to assay the change in activities of defense related enzymes *viz*., peroxidase (PO) and polyphenol oxidase (PPO).

### Peroxidase activity (PO)

One g of leaf tissue was ground in 1 ml of 0.1M phosphate buffer (pH 7.0) in a pre-cooled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The

supernatant was used as enzyme source to assay the activities of PO and PPO. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.1 ml of enzyme extract and 0.5 ml of 1% $H_2O_2$. The change in absorbance of the reaction mixture was recorded at 420 nm in an UV-visible spectrophotometer at 30 sec interval for 3 min from zero second of incubation. The enzyme activity was expressed as change in absorbance of the reaction mixture/min/g of fresh tissue (Hammerschmidt *et al*., 1982).

**Polyphenol oxidase (PPO)**

The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer pH 6.5 and 0.1 ml of the enzyme extract. The reaction was initiated by the addition of 0.2 ml of 0.01M catechol. The enzyme activity was expressed as change in absorbance of the reaction mixture/min/g of fresh tissue (Mayer *et al*., 1965).

**Results and Discussion**

The results of the field experiments conducted on the management of root rot disease of *S. nigrum* showed that soil application of *T. asperellum* @ 2.5 kg/ha mixed with neem cake @ 250 kg/ha followed by seed treatment with *T. asperellum* at the rate of 4 g per kg seed was effective in managing the root rot disease which recorded the lowest disease incidence of 15.5 per cent. Seed treatment with carbendazim at 2 g per kg seed followed by drenching with carbendazim at 1 g per litre on 30 days after sowing recorded 16.9 per cent disease incidence. The highest root rot disease incidence (31.5 per cent) was observed in the control (Table 2). Saxena and Moly Saxena (1995) reported that seed coating and soil application of *Trichoderma viride* was effective in controlling dry root rot of chickpea.

The plant growth parameter *viz.,* plant height was found to be maximum (51.4 cm) in the soil application of *T. asperellum* @ 2.5 kg/ha mixed with neem cake @ 250 kg/ha followed by seed treatment with *T. asperellum* at 4 g per kg seed. Seed treatment alone with *T. asperellum* at 4 g per kg seed recorded the plant height of 50.1 cm. Carbendazim seed treatment recorded the plant height of 45.0 cm. The plant height was found to be minimum (42.7 cm) in the control (Table 2). Meena and Rajamani (2015) observed the plant growth promotion in glory lily by dipping the tubers in *P. fluorescens* talc formulation.

In addition to disease reduction, the leaf yield was also found to be increased in the effective treatment. The maximum green leaf yield of 18.9 t/ha was recorded in soil application of *T. asperellum* @ 2.5 kg/ha mixed with neem cake @ 250 kg/ha and seed treatment with *T. asperellum* at the rate of 4 g per kg seed. Seed treatment with carbendazim @ 2 g/kg seed followed by drenching with carbendazim @ 0.1% on 30 DAS recorded the leaf yield of 16.7 t/ha. The leaf yield of 14.1 t/ha was observed in seed treatment alone with carbendazim @ 2 g/kg seed. In control, the lowest yield of 13.4 t/ha was observed (Table 2). Meena (2016) reported the effectiveness of *P. fluorescens* in the management of root rot disease of *Coleus forskohlii*.

Induced systemic resistance (ISR) of plants against pathogens is a widespread phenomenon that has been intensively investigated with respect to the underlying signalling pathways as well as to its potential use in plant protection. Elicited by a local infection, plants respond with a salicylic-dependent signalling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance that is effective against fungi, bacteria and viruses. Changes in cell wall

composition, de novo production of pathogenesis-related proteins such as chitinases and glucanases, and synthesis of phytoalexins are associated with resistance, although further defensive compounds are likely to exist but remain to be identified (Heil and Bostock, 2002). Use of antagonistic organisms is often associated with the onset of defense mechanism by expression of various defense-related enzymes *viz.*, β-1,3-glucanase, chitinase, PAL, PO, PPO and accumulation of phenols (Meena and Rajamani, 2015). In the present study, enhanced activities of defense-related enzymes *viz*., peroxidase and polyphenol oxidase were observed in *Solanum* plants in response to the application of *T. asperellum* formulation against *R. bataticola* indicating the induction of systemic resistance.

Treatment with *T. asperellum* challenged with *R. bataticola* led to enhanced activity of peroxidase (0.86 changes in absorbance/minute/g of leaf tissue) upto fifth day and declined thereafter throughout the experimental period of seven days. Uninoculated control recorded less induction of peroxidase activity (0.64 changes in absorbance/minute/g) and showed decline from fifth day onwards (Table 3). Ramyabharathi *et al*., 2012 highlighted the induction of defense enzymes *viz*., chitinase and β-1,3-glucanase in tomato plants infected with *Fusarium oxysporum* f.sp. *lycopersici* by the biocontrol agent *Bacillus subtilis* EPCO16.

Probability of polyphenol oxidase oxidizing phenolics to highly toxic quinones might be involved in the terminal oxidation of diseased plant tissue and attributed for its role in disease resistance. In the present study, plants treated with *T. asperellum* challenged with *R. bataticola* showed maximum polyphenol oxidase activity (0.73 changes in absorbance/minute/g). It was followed by *R. bataticola* (0.63 changes in absorbance/minute/g) and *T. asperellum* (0.54 changes in absorbance/minute/g). Uninoculated control plants recorded less induction of β-1,3-glucanase (0.17 changes in absorbance/minute/g) (Table 4). The induction of various defense compounds against die-back and fruit rot in chillies by *P. fluorescens* was observed by Meena and Marimuthu (2007). Sivakumar (2017) highlighted the induced systemic resistance in tuberose by *Bacillus subtilis.* The role of *Trichoderma virens* in inducing systemic resistance was demonstrated by Lamdan *et al*., 2015.

**Acknowledgement**

The financial support rendered by ICAR – All India Co-ordinated Research Project on Medicinal and Aromatic Plants and Betelvine is gratefully acknowledged.

## Conclusion

The present study indicated the usefulness of *T. asperellum* formulation for the management of root rot disease in *S. nigrum*. Hence, this approach can be exploited as it is natural, safe, effective, persistent and durable alternative to chemical pesticides for controlling plant diseases.

**Ethical approval and consent: NA**

## References

Animisha S, Zacharia S, Jaiswal K, Pandey P. Integrated management of chickpea wilt incited by *Fusarium oxysporum* f.sp. *ciceris*. Int. J. Agric. Res. 2012; 41: 1-7.

Bitas V, Kim HS, Bennett JW, Kang S. Sniffing on microbes : diverse roles of microbial volatile organic compounds in plant health. MPMI. 2013; 26: 835-843.

Hammerschmidt R, Nuckles EM, Kuc J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum legenarium.* Physiol Plant Pathol. 1982; 20: 73-82.

Heil M, Bostock RM. Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann Bot. 2002; 89: 503-512.

Kaur S, Dhillon GS, Brar SK, Vallad GE, Chand R, Chauhan VB. Emerging phytopathogen *Macrophomina phaseolina* : biology, ecconomic importance and current diagnostic trends. Critical reviews in Microbiology. 2012; 38: 136-151.

Khadse RR, Giri GK, Raut SA, Bhoye BB. *In vitro* efficacy of fungicides and bioagents against wilt of pigeonpea caused by *Neocosmospora vasinfecta*. Sci. Int. 2015; 3: 82-84.

Lamdan N, Shalaby S, Ziv T, Kenerley CM, Horwitz BA. Secretome of *Trichoderma* interacting with maize roots : Role in induced systemic resistance. Mol Cell Proteomics. 2015; 14: 1054-1063.

Mayer AM, Harel E, Shaul RB. Assay of catechol oxidase a critical comparison of methods. Phytochemistry. 1965; 5: 783-789.

Meena B. Integrated disease management of root rot in *Coleus forskohlii.* World Journal of Pharmaceutical Research. 2016; 5: 1312-1317.

Meena B, Marimuthu T. Induction of systemic resistance against die-back and fruit rot of chillies by *Pseudomonas fluorescens.* In: Recent Trends in Horticultural Biotechnology, Keshvachandran *et al. (*Eds.), New India Publishing Agency, New Delhi; 2007.

Meena B, Rajamani K. Biological control of leaf blight disease in *Gloriosa superba* using *Pseudomonas fluorescens.* Journal of Pure and Applied Microbiology. 2015; 8: 2579-2586.

Meena B, Radhajeyalakshmi R, Marimuthu T, Vidhyasekaran P, Velazhahan R. Biological control of groundnut late leaf spot and rust by seed and foliar applications of a powder formulation of *Pseudomonas fluorescens.* Biocontrol Sci Technol. 2002; 12**:** 195-204.

Meena B, Ramjegathesh R, Ramyabharathi SA. Evaluation of bio-control agents and fungicides against the stem bleeding disease of coconut. Journal of Plantation Crops. 2014; 42: 1-5.

Ramyabharathi SA, Meena B, Raguchander T. Induction of chitinase and β-1,3-glucanase PR proteins in tomato through liquid formulated *Bacillus subtilis* EPCO16 against *Fusarium* wilt. Journal of Today's Biological Sciences: Research & Review. 2012; 1: 59-66.

Rangaswami G. Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd. New Delhi; 1972.

Saxena DR, Moly Saxena. Evaluation of antagonists against dry root rot pathogen of chickpea. Indian J. Mycol. Pl. Pathol. 1995; 25: 130-133.

Sivakumar SS. Standardization and development of IDM module for wilt-nematode complex disease in tuberose (*Polianthes tuberose* L.). M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore; 2017.

**Table 1. Effect of *Trichoderma asperellum* on the management of root rot disease of *S. nigrum***

| Treatments | Disease incidence | Mean | Disease |
|---|---|---|---|

| | (%) | | | reduction over control (%) |
|---|---|---|---|---|
| | Trial I (2015-2016) | Trial II (2016-2017) | | |
| $T_1$- Soil application of *T. asperellum* @ 2.5 kg/ha mixed with neem cake @ 250 kg/ha | 23.9 (17.6) | 27.3 (20.1) | 25.6 | 18.7 |
| $T_2$- Seed treatment with *T. asperellum* @ 4 g/kg seed | 20.6 (16.2) | 22.5 (17.3) | 21.6 | 31.4 |
| $T_3$- Seed treatment with *T. asperellum* @ 4 g/kg seed + Soil application of *T. asperellum* @ 2.5 kg/ha mixed with neem cake @ 250 kg/ha | 14.7 (13.4) | 16.3 (14.1) | 15.5 | 50.8 |
| $T_4$- Seed treatment with carbendazim @ 2 g/kg seed | 24.2 (18.3) | 27.1 (20.4) | 25.7 | 18.4 |
| $T_5$- Drenching with carbendazim @ 0.1% on 30 DAS | 17.1 (14.3) | 19.7 (15.3) | 18.4 | 41.6 |
| $T_6$- Seed treatment with carbendazim @ 2 g/kg seed + Drenching with carbendazim @ 0.1% on 30 DAS | 15.3 (14.1) | 18.4 (14.9) | 16.9 | 46.3 |
| $T_7$- Control | 29.4 (21.6) | 33.6 (22.4) | 31.5 | - |
| CD (P=0.05) | 2.4 | 2.7 | 2.6 | |

The values are mean of three replications

**Table 2. Effect of treatments on plant height and yield of *S. nigrum***

| Treatments | Plant height (cm) | | Mean | Leaf yield (t/ha) | | Mean |
|---|---|---|---|---|---|---|
| | Trial I (2015-2016) | Trial II (2016-2017) | | Trial I (2015-2016) | Trial II (2016-2017) | |
| $T_1$- Soil application of *T. asperellum* @ 2.5 kg/ha mixed with neem cake @ 250 kg/ha | 47.2 | 40.5 | 43.9 | 15.9 | 13.6 | 14.8 |
| $T_2$- Seed treatment with *T. asperellum* @ 4 g/kg seed | 52.7 | 47.4 | 50.1 | 16.7 | 13.7 | 15.2 |
| $T_3$- Seed treatment with *T. asperellum* @ 4 g/kg seed + Soil application of *T. asperellum* @ 2.5 kg/ha mixed with neem cake @ 250 kg/ha | 54.6 | 48.2 | 51.4 | 19.4 | 18.3 | 18.9 |
| $T_4$- Seed treatment with carbendazim @ 2 g/kg seed | 48.4 | 41.6 | 45.0 | 13.2 | 15.0 | 14.1 |
| $T_5$- Drenching with carbendazim @ 0.1% on 30 DAS | 49.1 | 43.7 | 46.4 | 15.3 | 16.9 | 16.1 |
| $T_6$- Seed treatment with carbendazim @ 2 g/kg seed + Drenching with carbendazim @ 0.1% on 30 DAS | 52.3 | 45.3 | 48.8 | 18.1 | 15.2 | 16.7 |
| $T_7$- Control | 46.9 | 38.4 | 42.7 | 12.8 | 13.9 | 13.4 |
| CD (P=0.05) | 2.4 | 3.1 | 2.8 | 2.1 | 1.8 | 1.9 |

The values are mean of three replications

**Table 3. Induction of peroxidase activity upon treatment with *T. asperellum* formulation**

| S.No. | Treatments | Peroxidase activity (Changes in absorbance/min/g) | | | |
|---|---|---|---|---|---|
| | | Days after inoculation with *R. bataticola* | | | |
| | | 1 | 3 | 5 | 7 |
| 1. | *T. asperellum* + *R. bataticola* | 0.78 | 0.83 | 0.86 | 0.81 |
| 2. | *T. asperellum* | 0.64 | 0.71 | 0.78 | 0.72 |
| 3. | *R. bataticola* | 0.72 | 0.79 | 0.81 | 0.76 |

| | | | | | |
|---|---|---|---|---|---|
| 4. | Untreated Control | 0.51 | 0.58 | 0.64 | 0.61 |

The values are mean of five replications

CD (P=0.05)
Treatments = 0.018
Days = 0.024
Treatments x Days = 0.051

**Table 4. Induction of polyphenol oxidase activity upon treatment with *T. asperellum* formulation**

| S.No. | Treatments | PPO activity (Changes in absorbance/min/g) | | | |
|---|---|---|---|---|---|
| | | Days after inoculation with *R. bataticola* | | | |
| | | 1 | 3 | 5 | 7 |
| 1. | *T. asperellum* + *R. bataticola* | 0.26 | 0.48 | 0.73 | 0.58 |
| 2. | *T. asperellum* | 0.18 | 0.31 | 0.54 | 0.46 |
| 3. | *R. bataticola* | 0.21 | 0.34 | 0.63 | 0.48 |
| 4. | Untreated Control | 0.14 | 0.19 | 0.17 | 0.15 |

The values are mean of five replications

CD (P=0.05)
Treatments = 0.024
Days = 0.038
Treatments x Days = 0.072