

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 are 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.

Fungicides such as carbendazim was 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 (Alvindia and Natsuaki, 2008). 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 effectiveness of biocontrol agents in the management of soil-borne diseases had been reported in several crops (Meena *et al.*, 2002; Meena *et al.*, 2014). Biopesticides are cheaper, ecofriendly and do not pose 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 experiments 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 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

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

The plant height was recorded on 30 DAS. The leaf yield per hectare was recorded for each treatment and the data were statistically analyzed.

Induction of systemic resistance in *S. nigrum*

Solanum seeds were sown in earthen 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 32nd 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% H₂O₂. 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. It was found to be on par with 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 which 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*.

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 *et al.*, 2001). 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).

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.

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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 <i>T. asperellum</i> @ 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 <i>T. asperellum</i> @ 4 g/kg seed	20.6 (16.2)	22.5 (17.3)	21.6	31.4
T ₃ - Seed treatment with <i>T. asperellum</i> @ 4 g/kg seed + Soil application of <i>T. asperellum</i> @ 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 <i>T. asperellum</i> @ 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 <i>T. asperellum</i> @ 4 g/kg seed	52.7	47.4	50.1	16.7	13.7	15.2
T ₃ - Seed treatment with <i>T. asperellum</i> @ 4 g/kg seed + Soil application of <i>T. asperellum</i> @ 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 <i>R. bataticola</i>			
		1	3	5	7
1.	<i>T. asperellum</i> + <i>R. bataticola</i>	0.78	0.83	0.86	0.81
2.	<i>T. asperellum</i>	0.64	0.71	0.78	0.72
3.	<i>R. bataticola</i>	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 <i>R. bataticola</i>			
		1	3	5	7
1.	<i>T. asperellum</i> + <i>R. bataticola</i>	0.26	0.48	0.73	0.58
2.	<i>T. asperellum</i>	0.18	0.31	0.54	0.46
3.	<i>R. bataticola</i>	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