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SHORT COMMUNICATION

Management of *Sclerotium rolfsii* causing basal rot of *Piper longum* through organic approaches

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Abstract

Sclerotium rolfsii Sacc causes serious yield loss of *Pippali* (*Piper longum* Linn.). Catkin, the fruit of *Pippali* is directly used in medicinal industry so, use of chemical fungicides is not encourages for the cultivation of *Pippali*. The present study was carried out to bring orgnaic management strategy of the crop. Six native isolates of fungal and bacterial antagonists were tested against *S. rolfsii* and found *T. harzianum* was found as the best agents. In in vitro studies, sclerotial production was found drastically reduced when exposed to biocontrol agents. Result on field experiment showed that soil application of mustard oil cake (MOC) @ 1 kg/2X2 m^2 plot fortified with *T. harzianum* @ 5 ml/m^2 found effective in managing the basal rot with disease reduction upto 10.65% with increased plant growth parameters. This was followed by soil application of *T. harzianum* @ 5 ml/m^2 with disease incidence of 14.98%.

Keywords *Piper longum* · Biological management · *Sclerotium rolfsii* · Basal stem rot · Organic amendment

Pippali, *Piper longum* Linn. is an under-shrub with erect and slender branches belonging to the family piperaceace. *Pippali* commonly known as Indian long pipper, pipli or pippali, a flowering plant which grows throughout the year (Dorman and Deans 2000). Leaves are simple, alternate, stipulate and petiolate or nearly sessile. Flowering is nearly throughout the year; inflorescence is spike; fruit greyish green or darker grey berries. It is believed to be originated from North East India especially in hotter parts of India ranging from central Himalayas to Assam (Oommen et al. 2000). *Pippali* is normally cultivated for its medicinal property which are being largely exploited in the ayurvedic industry for different diseases in humans. Diseases like respiratory tract, cough, bronchitis, asthma, as counter irritant, analgesic can be cured with *Pippali*. The main ingredient or constituent of the *Pippali* is the piperine found in the catkin (fruit). In pharmacological studies the piperine is used as antibacterial (Reddy et al. 2001), antiallergic activity (Chatterjee 1999), anti-tumour activity (Bai and Xu 2000), intestinal disorders (Ghoshal et al. 1996) etc. Different biotic and abiotic stresses are found to be associated with cultivation of the crop, which not only caused reduction in the yield but also causes economic loss in the ayurvedic industry. Out of the different biotic stresses, basal stem rot caused by *Sclerotium rolfsii* is the most devastating ones causing yield loss upto 70.0–90.0%. Considering the importance of the diseases and its application in the medicinal industry the present experiment was conducted with an aim to develop an effective organic disease management practices for basal stem rot of *Pippali* and to determine the effect organic practice on various plant growth parameter and yield.

Six biocontrol agents including 4 fungal viz., *Trichoderma harzianum, T. asperellum, T. pseudokoningii* and *Beauveria bassiana*, and two bacterial biocontrol agents viz., *Bacillus subtilis* and *Pseudomonas fluorescens* were selected for the in vitro efficacy against *S. rolfsii*. The cultures of biocontrol agents were collected from culture bank of Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam. The efficacy test was conducted during 2017–18 by dual culture method (Vincent 1947) with three replication. Observation on mycelial growth inhibition and sclerotial production was recorded when full growth was recorded in control plate. The data on mycelial growth inhibition and sclerotia production was converted to per

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cent inhibition over control. The best biocontrol agent was selected for field efficacy of bioformulation prepared with the isolate.

The experiment was carried out at Experimental Garden for Horticulture Orchard, Department of Horticulture, Assam Agricultural University, Jorhat, Assam during 2017–18 and 2018–19 with the seven-treatment combination each of which was replicated for thrice. The treatment combinations were, T_1: soil application of mustard oil cake (MOC) @ 1 kg/plot, T_2: soil application of *Org-Trichojal* (a liquid bioformulation of *T. harzianum*) @ 5 ml/m^2, T_3: soil application of MOC @1 kg/plot fortified with *Org-Trichojal* (40 ml of *Org-Trichojal* was mixed with 1000 g of MOC), T_4: soil application of neem oil cake (NOC) @ 1 kg/plot, T_5: soil application of NOC @1 kg/plot fortified with *Org-Trichojal* (40 ml *Org-Trichojal* was mixed 1000 g of NOC), T_6: soil application of carbendazim @0.3%, T_7: absolute control. The whole experiment was conducted in plot size of (plot size: 2×2 m^2) for all the treatments and replications.

The *T. harzianum* based bio-formulation *Org-Trichojal* was collected from the Nanolab, Department of Plant Pathology, AAU, Jorhat.

Org-Trichojal enriched organic amendment of mustard oil cake (MOC) and neem oil cake (NOC) was prepared by mixing 40 ml of *Org-Trichojal* in 1000 g of MOC and NOC. A heap was made with this mixture and kept in shade and covered with gunny bags for 7 days. Water was sprinkled over the heap twice in a day for maintaining the 40.0% moisture. At an interval of three days heap of MOC and NOC were mixed thoroughly and incubated. After 10 days the mixture was re-mixed with fresh MOC and NOC at 1:10 ratio and repeated the above steps. After 10 days of incubation the MOC and NOC will be enriched with inoculam of *Org-Trichojal* and ready for its field application. cfu count of the organism was calculated twice once at before starting the experiment and another at final harvest by serial dilution technique in Trichoderma selective medium (TSM).

Fresh elite planting materials of *Pippali* (JPL-19) of Bokakaht district of Assam, India was collected from the germplasms maintained at AICRP on MAP and Betelvine. The yield of this elite germplasm was 533.34 kg/ha on dry basis. *Piperine* content was found to be 5.54 as compared to national variety Viswam with *Piperine* content of 5.15 released from Kerala.

The pathogen causing basal stem rot of *Pippalii* was isolated from the freshly infected *Pippali* plants showing typical symptom of basal stem rot. The pathogen was purified by hyphal tip culture method and maintained by periodic transfer in fresh PDA slant and storing in refrigeration at 4 °C. For field inoculation, the pathogen was mass cultured in 4% maize meal medium (MSM) for 15 days and inoculated the experiments plot @ 0.02% seven days prior to the planting.

The field experiment was laid out in randomized block design with a plot size of 2×2 m. Field was prepared by 2–3 times ploughing followed by harrowing. After ploughing farm yard manure was applied in each plot as basal dose.

To study the field efficacy of different treatment combination, fortified and non fortified organic amendment (MOC and NOC) with *Org-Trichojal* was added @ 1.0 kg/plot. The application was repeated twice at 6 month intervals. Control plots were applied with well rotten FYM as basal dose and twice at 6 month intervals. Each treatment was replicated thrice.

Observations on per cent disease incidence, per cent disease reduction over control, and plant growth parameters, catkin per plant, yield per plot and population dynamics of *T. harzianum* were recorded for each treatment and the best disease management module was assessed.

Study on in vitro efficacy of biocontrol agents against *S. rolfsii* by dual culture method showed that out of all the tested biocontrol agents, *T. harzianum* was found best in inhibiting the mycelial growth of *S. rolfsii* with per cent mycelial growth inhibition of 72.17% (Table 1). This was followed by *T. asperellum, P. fluorescens, T. pseudokoningii*

Table 1 In vitro efficacy of biocontrol agents on mycelial growth and sclerotial production of *Sclerotium rolfsii* causing basal rot of *Pippali*

Treatment	Mycelial growth (mm)	Mycelial growth inhibition over control (%)	No of sclerotia produced	Reduction of sclerotia production over control
Control (*S. rolfsii* alone)	90.00	–	106.0	–
T. harzianum	25.05	72.17	25.0	76.42
T. asperellum	28.15	68.72	32.0	69.81
T. pseudokoningii	38.65	57.06	52.0	50.94
B. bassiana	41.21	54.21	48.0	54.72
P. fluorescens	28.32	68.64	40.0	62.26
B. subtilis	45.23	49.74	54.0	49.16
SEm	0.68	–	1.11	–
CD ($p=0.05$)	2.12	–	3.76	–
CV (%)	2.46	–	3.45	

and *B. bassiana* with inhibition percent of 68.72%, 68.64%, 38.65% and 41.21% respectively. Mycelial growth recorded for *T. asperellum* (28.15 mm) and *P. fluorescens* (28.32 mm) was statistically at par with each other.

While studying the sclerotial production of *S. rolfsii* in presence of the tested biocontrol agents, it was found that the pathogen produces comparatively less sclerotia in presence of the biocontrol agents as compared to control. Lowest sclerotia production was recorded in plates treated with *T. harzianum* with reduction of 76.42% over control (Table 1).

This was followed by *T. asperellum* and *P. fluorescence* with reduction of 69.81% and 61.26% respectively. Microscopic observation on the mechanism of growth suppression showed that the biocontrol agents causes coiling and lysis of the mycelial of the pathogen. Besides, conidial accumulation was found more on the surface pathogens mycelium. Microscopic study also showed that the lysis was more for bacterial biocontrol agents than the fungal biocontrol agents.

The mycelial growth inhibition recorded in the present study might be due to the action of biocontrol agents that compete for nutrient, food and space with the pathogen and due to the faster multiplication rate of biocontrol agents like *Trichoderma* spp. Besides, due to action like coiling, lysis and conidial accumulation etc. suppresses the growth of pathogen as observed in the present study. The result of present study is in conformity with our earlier study where we have found that species of *Trichoderma* like *T. harzianum*, *T. viride* and *T. koningii* were effective in suppression of mycelial growth of *S. rolfsii* (Dutta and Das 2002), *Rhizoctonia solani* (Dutta and Das 1999), *Sclerotinia sclerotiorum* (Dutta et al. 2008) causing disease in tomato, soybean and French bean, respectively. Though in earlier study lysis or disintegration mycelium was reported as mechanism of growth suppression by fungal biocontrol agents like *Trichoderma* spp against soil borne plant pathogen like *R. solani* (Dutta et al. 2013) but in the present study lysis as mode of action was found in case of bacterial biocontrol agents (*P. fluorescence* and *B. subtilis*) but not for the fungal biocontrol agents. Lower sclerotial production observed may be due to action of metabolites of biocontrol agents that first ceases the growth of the pathogen and kill before reaching the maturity enough to form sclerotia.

Result presented in Table 2 showed that basal stem rot incidence was lowest (7.23%) when carbendazim was applied as soil application. This was followed by soil application of Mustard oil cake @1 kg/plot fortified with *T. harzianum* and soil application of *T. harzianum* with disease incidence of 10.65% and 14.98% respectively (Table 2). Plant growth parameter like plant height (158 cm), internode length (13.80 cm), catkin no/plot (6.93) and yield per plot (108.1 g) was found highest in plot where soil application of Mustard oil cake @1 kg/plot fortified with *T. harzianum* was done (Fig. 1). While studying the population dynamics of *T. harzianum* it was recorded that after 90 days of application of treatments population dynamics of *T. harzianum* found to increased from 4.87×10^3 to 8.46×10^5 cfu/g of soil in plots where soil application of Mustard oil cake @1 kg/plot fortified with *T. harzianum* was done. This was followed by soil application of *T. harzianum* (Table 2). No population of *T. harzianum* was observed in plots treated with carbendazim @ 0.3%. Application of mustard oil cake @ 2.0 percent enhances the growth of *Trichoderma* species whereas germination of sclerotia of *S. rolfsii* are inhibited at the same concentration (Desai et al. 2003). Similarly, Dutta and Das (2002) found that application of FYM culture of *T. harzianum* resulted in minimum disease incidence and enhanced dry mass of root and shoot and yield during the experimentation in management of collar rot of tomato caused by *S. rolfsii.* Similarly, in an another study it was found that soil application of *T. harzianum* enriched FYM could effectively reduce the infection of *R. solani* and enhances plant growth and yield attributing parameters of soybean. Increased in the yield might be due to the reduction of diseases as well as release of some growth promoting substances by *T. harzianum*. Workers like Altomare et al. (1999) reported that *T. harzianum* has the ability to produced Zn, Mn^{4+}, Fe^{3+}, and Cu^{2+} and increased iron availability enhancing iron uptake, thus helping in increasing the yield of potato. The solubilization and chelating abilities of *T. harzianum* helps in increasing yield of *Pippali* plants in the present study is supported by Harman et al. (2004), Wilson et al. (2008), Hermosa et al. (2012) and Bhuiyan and Sen (2013).

Since *Pippali* is medicinal plants and is highly utilized for curing different diseases of humans so it is not advisable to used or minimal used of chemicals like fungicide for management of different biotic stresses. Therefore, from the above experiment it can concluded that organic management of basal stem rot of *Pippali* is the need of the hour. Hence from this findings it will boost the production of *Pippali* and ultimately to the end users successfully for cultivation and in ayurvedic industry.

Table 2 Field trial on management of basal stem rot of long pepper, caused by *Sclerotium rolfsii*

Treatment	Initial population of *T. harzianum* ($\times 10^3$ cfu/g of soil)	Per cent disease incidence	% disease reduction over control	Population of *T. harzianum* (90 days after application) ($\times 10^5$ cfu/g of soil)	Internode length (cm)	Plant height (cm)	Catkin/plant (no.)	Yield/plot (g)
T_1: soil application of mustard oil cake (MOC) @ 1 kg/plot	3.45	21.32[a]	66.40	3.33	10.07	111.40	5.07	79.91
T_2: soil application of *T. harzianum*	3.71	14.98	76.39	6.47	10.27	106.87	6.80	70.41
T_3: soil application of MOC@1 kg/plot fortified with *T. harzianum*	4.87	10.65	83.22	8.46	13.80	158.00	6.93	108.18
T_4: soil application of neem oil cake (NOC) @ 1 kg/plot	1.54	18.76	70.43	0.28	10.00	127.00	4.67	54.5
T_5: soil application of NOC@1 kg/plot fortified with *T. harzianum*	1.43	17.65	72.18	0.15	10.27	119.73	4.83	54.37
T_6: soil application of carbendazim @0.3%	1.05	7.23	88.61	0.00	11.47	113.73	7.73	76.28
T_7: control (*S. rolfsii* alone)	1.53	63.45	–	0.05	7.50	72.67	4.50	16.12
SEm	0.11	0.95	–	0.41	0.87	1.82	0.92	1.56
CD ($p=0.05$)	0.26	2.73	–	1.23	2.56	5.46	2.87	4.87
CV (%)	4.78	0.85	–	14.17	12.11	2.46	18.13	3.56

[a]Data are pooled from the experimental result of 2017–18 and 2018–19

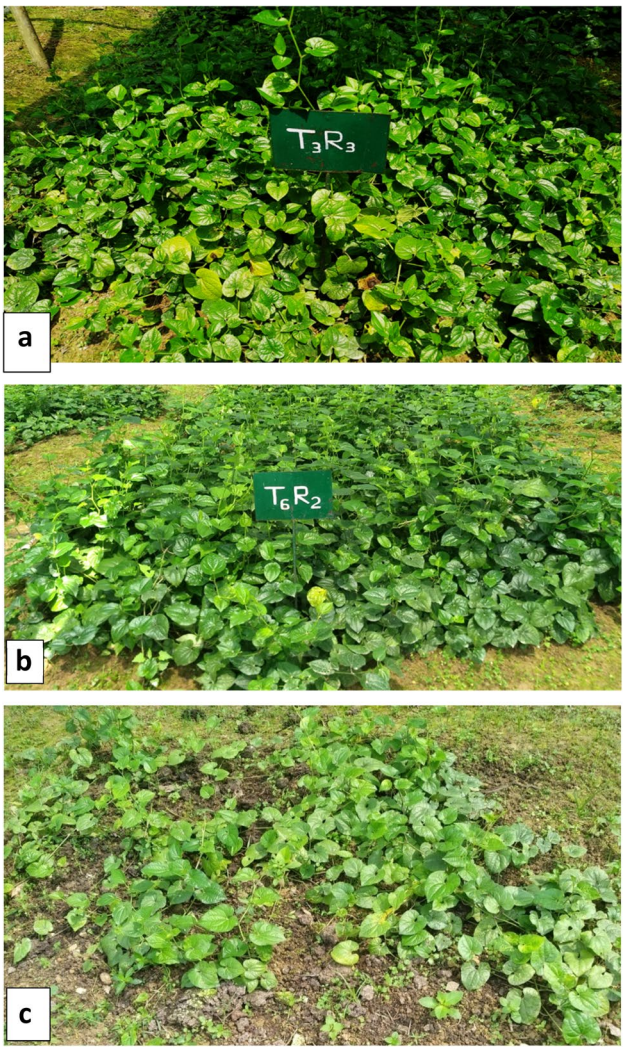

Fig. 1 Field efficacy of *T. harzianum* based bioformulation for the management of basal stem rot of *Pippali*. **a** Soil application of Mustard oil cake @1 kg fortified with *Trichoderma harzianum*+*S. rolfsii*. **b** Soil application of carbendazim @0.3%+*S. rolfsii*. **c** Control (*S. rolfsii* alone)

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