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Control of collar rot in mint (*Mentha* spp.) caused by *Sclerotium rolfsii* using biological means

Anand Singh and Harikesh Bahadur Singh*

National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India

Eight isolates of different species of *Trichoderma* and two isolates of *Gliocladium virens* were tested *in vitro* for their antagonistic activity against *Sclerotium rolfsii*, the cause of collar rot in mint. The most effective isolates of *T. harzianum* and *T. virens* were selected for disease-control studies in pots under greenhouse conditions. Inoculation of *Mentha arvensis*, *M. citrata*, *M. piperita* and *M. spicata* with the selected isolates of *T. harzianum* resulted in disease control ranging from 66.67 to 100%. Reduction in disease was accompanied with significant increase in herb and oil yield.

MENTHA (family Labiatae) is an important essential-oil-yielding herb grown throughout the world. Commercial cultivation is done to obtain its oil, which has different chemical constituents of economic importance, viz. menthol, menthone, methyl acetate, terpenes, etc. These constituents are used in medicinal preparation, toothpaste, mouthwash, perfumery, cosmetics and as flavouring agents. The menthol mint crop is extensively cultivated in India and about 70% of the international annual requirement is met from crops raised in the central region of the Indo-Gangetic plains[1]. A survey conducted by Central Institute of Medicinal and Aromatic Plants, Field Station (CIMAP, FS), Pantnagar, India revealed that the crop is severely affected by collar rot and wilt disease. The disease is caused by *Sclerotium rolfsii* Sacc which causes considerable damage to the crop. Disease intensity in the field ranged from 5 to 20%. Though collar rot and wilt disease of *Mentha* were reported way back in 1933 by Goto[2] from Japan, no further studies were undertaken on this disease. The first attempt to control the disease was by Pandotra and Ganguly[3] using chemical means. *S. rolfsii* is a pathogen of several crops and is not easy to control by conventional means[4,5].

In the past few years, management of diseases using biological antagonists has been increasing continuously. This is influenced by the idea that they may be potential alternatives to the use of chemicals for managing the plant diseases caused by soil-borne pathogens[6–8]. The present investigation is an attempt to control the collar rot disease of mints caused by *S. rolfsii* using fungal antagonists.

The collar rot pathogen, *S. rolfsii* has a wide host range. The collar rot of menthol mint *(Mentha arvensis)* was

*For correspondence. (e-mail: hbs1@rediffmail.com)

observed on mint plantations under natural conditions in the *tarai* regions of UP, India. In the survey, four species of mint, viz. *M. arvensis*, *M. spicata*, *M. piperita* and *M. citrata* grown commercially at CIMAP, FS were attacked by the collar rot pathogen. The *M. piperita* and *M. citrata* are new hosts of this pathogen[9]. Plants of all the four species showing symptoms of the disease were collected and the pathogen was isolated from both the sclerotia and the mycelium on potato dextrose agar (PDA) plates aseptically. Mass culture of the pathogen was prepared on sand–maize (3 : 1) medium. The medium was filled in Erlenmeyer flasks and sterilized at 15 p.s.i. for 1 h. The sclerotia of the pathogen were aseptically transferred in the flasks and incubated at 25 ± 2°C for three weeks. The flasks were completely filled with the mycelia and sclerotia of the pathogen, after three weeks.

Three fungal antagonists, viz. two strains of *Trichoderma harzianum* (IMI No. 359879 and ITCC No. 1065.95) and *Trichoderma virens* (ITCC No. 1066.95) (*T. virens* is the widely accepted synonym of *Gliocladium virens*)[10] were isolated from field soil of CIMAP, FS and other strains were obtained from Institute of Microbial Technology, Chandigarh, India. The antagonistic activity of these organisms was tested *in vitro* and the most effective ones were used in pots for disease management. Their activity *in vitro* was tested against the pathogen on PDA plates following the technique of Morton and Stroube[11]. From the zone of inhibition slides were prepared and observed under the microscope for hyphal interactions. In another test, the viability of the sclerotia of the pathogen was tested after subjecting them to attack by the different isolates of antagonists. Five similar-sized sclerotia of the pathogen were placed in a row on PDA plates. At the opposite end, bits of the antagonistic organisms were placed and the plates were incubated at 25 ± 2°C. After 10 days the sclerotia of the pathogen were taken out from these plates, surface-sterilized, re-inoculated on fresh PDA plates and incubated at 25 ± 2°C. The same process was repeated after different intervals. Observations on the sclerotial germination were recorded. The activity of biocontrol agents was also tested on liquid medium. Twenty sclerotia of similar size and shape from a 7-day-old culture plate were inoculated in 250-ml Erlenmeyer flasks containing the culture filtrate of different strains of biocontrol agents. Ten flasks for each treatment were maintained. The sclerotia were also inoculated in sterile distilled water to serve as control. Ten sclerotia were removed from each flask and plated onto fresh PDA plates after surface-sterilization with 0.2% $HgCl_2$. The process was repeated till no sclerotia remained viable in the treatment flasks.

Soil–compost mixture (50 : 50) was prepared and fumigated with 10% formalin. The mixture was covered with a plastic sheet for 24 h and then filled in earthen pots (24 cm diameter). For each species of *Mentha*, fifteen pots were prepared for experiments with both the antagonists. The mass culture of both the antagonists was prepared on sand–maize (3 : 1 ratio) medium in Erlenmeyer flasks by inoculating the mycelia of the antagonists on the medium and incubating at 25 ± 2° for three weeks. Two of the more effective antagonists, viz. *T. harzianum* (IMI No. 359879) and *T. virens* (TV, ITCC No. 1066.95) were selected for the pot experiments. Their mass cultures were also produced on sand–maize (3 : 1) medium. The pathogen was added to the sterilized soil–compost mixture in pots @ 20 g. The culture of *T. harzianum* and *T. virens* was added to these pots @ 20, 40, 60, 80, 100 g to give the ratios of 1 : 1, 1 : 2, 1 : 3, 1 : 4 and 1 : 5 of *S. rolfsii* (SR). For each species of mint, three pots were filled with 20 g of SR only to serve as control.

Suckers of *M. arvensis* and runners of *M. citrata*, *M. piperita* and *M. spicata* were sown in pots. The pots were kept in the open to provide natural conditions for the growth of plants as well as the fungi. Observations on the number of tillers, number of leaves and plant height were recorded after 110 days of sowing. Per cent disease control (PDC) was calculated by the following formula:

$$\text{PDC} = \frac{(\%\,\text{disease in check}) - (\%\,\text{disease in treatment})}{(\%\,\text{disease in check})} \times 100.$$

After 120 days the plants were harvested, weighed and oil was extracted from them. The oil was extracted from fresh leaves by Clevenger apparatus. Statistical analysis was done using the method described by Panse and Sukhatme[12] and simple correlation (r) was calculated according to Singh and Choudhary[13].

In the dual culture, the growth of the pathogen was inhibited completely as soon as its mycelium came in contact with the fungal antagonists. Thereafter, it ceased to grow. The colony of the pathogen was completely overrun by different antagonistic fungi and its mycelia were lysed. Attempts to isolate the pathogen from these plates resulted only in the isolation of the respective antagonists. The zone of inhibition, when observed under a microscope, showed coiling of the mycelium of the antagonistic organism around that of the pathogen. The results of the viability tests are given in Figure 1, which show that *T. virens* (ITCC No. 1066.95) was the most effective and *T. viride* (MTCC No. 800) was the least effective antagonistic organism among the fungi tested. Most of the antagonistic strains tested suppressed the sclerotial germination and completely killed the sclerotia within 20 days; only *T. viride* (MTCC No. 800) did so after 25 days. After 30 days, no sclerotia were found viable in the treatment assay flasks; however 100% sclerotia remained viable in control flasks.

The results of the pot experiments are given in Table 1. In pots with *T. harzianum* (TH, IMI No. 359879) and *T. virens* (TV, ITCC No. 1066.95), the growth of the pathogen was completely covered by that of fungal antagonists. It was observed that higher the rate of growth of antagonist, lower the rate of growth of collar rot pathogen,

SR. The minimum mycelial growth of the pathogen was observed in 1 : 4 ratio of SR + TH and SR + TV, whereas the pathogen grew quite well in 1 : 1 ratio of pathogen and both the antagonists. The disease control in *M. arvensis* by *T. harzianum* was 76.90% in 1 : 1 ratio and 100% in 1 : 4 and 1 : 5 ratios of SR + TH. The application of different rates of TH resulted in 69 to 76.90% disease control in *M. citrata*. Similarly, in *M. spicata* the disease control varied from 83.33 to 100% and from 66.67 to 100% in *M. piperita* by the application of different ratios of TH (Figure 2).

The application of different ratios of *T. virens* also showed variable degrees of disease control in different species of *Mentha*. It varied from 66.67 to 100% in *M. arvensis*, from 68.75 to 100% in *M. citrata*, 66.67 to 100% in *M. piperita* and 68 to 100% in *M. spicata*. The oil yield also increased by the application of different ratios of both the antagonists. The increase in oil yield was significantly more in the case of application of *T. virens* and the increase was dose-dependent.

Table 2 indicates that both herb yield and oil yield of different species of *Mentha* are negatively correlated with per cent disease incidence. However, the herb and oil yield were positively correlated with each other as well as the quantity of inoculum of *T. harzianum* or *G. virens*. The inoculum ratio was also negatively correlated with per cent disease incidence, indicating that higher inoculum controlled the disease better. However, after the application of a definite quantity of inoculum of biocontrol agents, the effect of inoculum showed no decrease in the disease.

The results of *in vitro* studies in the present investigation are in conformity with those of earlier workers[14,15]. The effect of different *Trichoderma* species against SR of bean (*Phaseolus vulgaris*) was evaluated. It was found that all species were effective to different degrees in arresting the growth of the pathogen[16]. Similar findings were also recorded in the present study. The present investigation is also in agreement with the findings of Silveira *et al.*[17]. Application of *T. virens* was more effective in increasing the herb yield and oil yield than application of *T. harzianum* in all the species of *Mentha*. Similar observations were also recorded by Singh *et al.*[18,19], who reported 65–85% reduction in disease incidence of wilt and rot disease of mint caused by *Rhizoctonia solani* using *T. harzianum* and *G. virens* and that the latter was better as a disease-control and growth-promoting agent for the host crop. Various workers have also recorded similar observations in different crops[20–24].

Plants of *Mentha* species grown in pots amended with *T. harzianum* and *T. virens* showed vigorous growth and higher oil yield than those in the control pots. The increase in herbage and oil yield in the presence of fungal antagonists may be due to the suppression and/or elimination

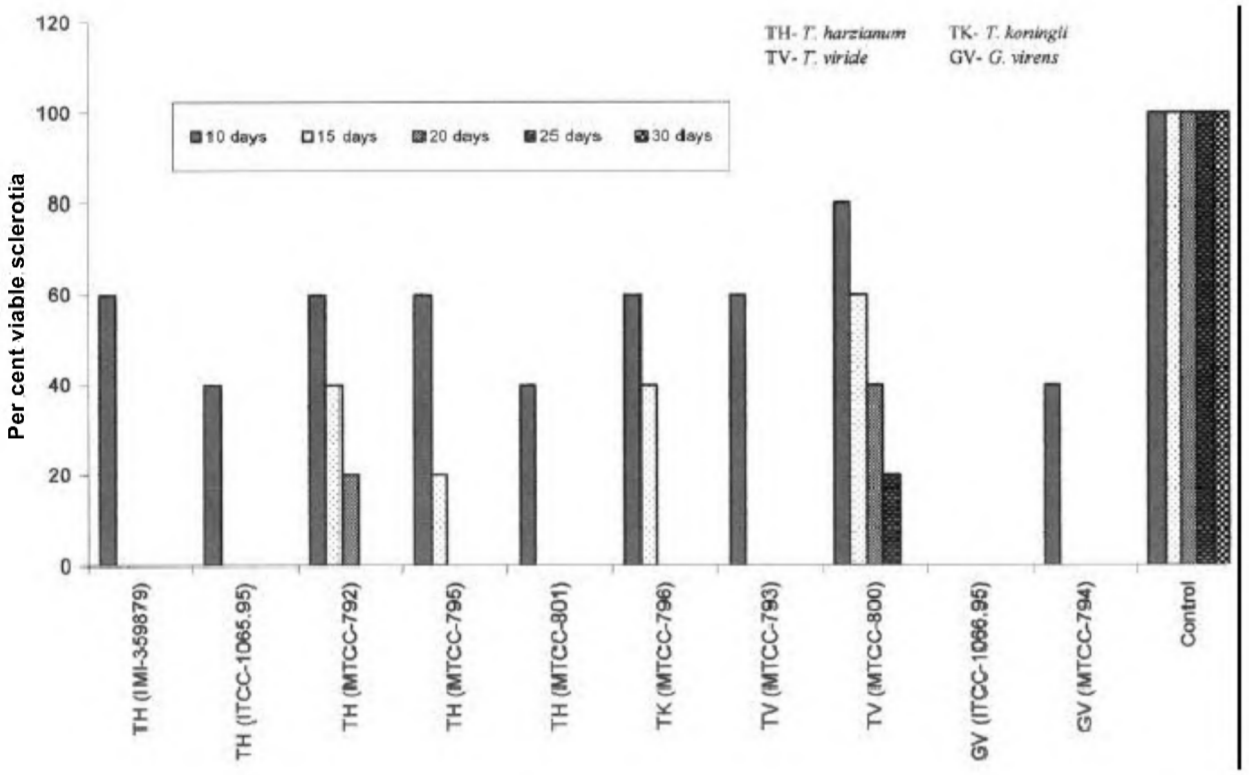

Figure 1. Effect of different fungal antagonists on the viability of sclerotia of *S. rolfsii* under *in vitro* conditions.

Table 1. Effect of *T. harzianum* (IMI No. 359879) and *T. virens* (ITCC No. 1066.95) on herb and oil yield and per cent disease control in different species of *Mentha*

Mentha spp.	Yield parameter	1 : 1	1 : 1	1 : 1	1 : 1	1 : 5	Control	CD at 5%
				T. harzianum				
M. arvensis	Herb yield (g)	98.0 ± 5.6	103.0 ± 19.4	104.7 ± 8.8	105.7 ± 17.4	117.7 ± 10.7	30.7 ± 0.67	28.5
	% oil yield	0.82	0.85	0.86	0.93	0.97	0.08	0.3
	% disease control*	76.9	84.4	92.2	100.0	100.0	–	34.7
M. citrata	Herb yield (g)	51.0 ± 0.6	62.3 ± 7.5	75.0 ± 12.5	80.7 ± 10.3	96.3 ± 6.0	22.3 ± 1.33	32.0
	% oil yield	0.98	1.23	1.21	1.28	1.64	0.40	0.4
	% disease control*	69.99	80.00	100.0	100.00	100.00	–	35.4
M. piperita	Herb yield (g)	73.3 ± 12.7	81.0 ± 4.9	100.0 ± 8.7	105.3 ± 2.9	120.3 ± 13.9	0.00#	38.8
	% oil yield	0.30	0.74	0.82	0.82	0.86	0.00	0.3
	% disease control*	73.91	73.91	86.96	100.00	100.00	–	33.9
M. spicata	Herb yield (g)	32.0 ± 1.33	34.7 ± 6.8	38.3 ± 4.4	40.7 ± 2.21	40.7 ± 3.23	20.9 ± 2.33	6.8
	% oil yield	0.24	0.31	0.49	0.49	0.51	0.20	0.3
	% disease control*	83.3	95.8	100.0	100.0	100.0	–	36.0
				T. virens				
M. arvensis	Herb yield (g)	80.0 ± 4.7	81.3 ± 4.9	90.7 ± 7.8	96.7 ±7.4	99.7 ± 2.33	30.7 ± 0.67	23.0
	% oil yield	0.86	0.88	1.00	1.03	1.22	0.08	0.4
	% disease control*	66.7	66.7	73.3	100.0	100.0	–	33.3
M. citrata	Herb yield (g)	91.7 ± 5.8	94.0 ± 6.3	107.3 ± 9.7	112.3 ± 7.6	120.3 ± 8.2	22.3 ± 1.33	31.7
	% oil yield	1.14	1.23	1.33	1.75	1.76	0.40	0.5
	% disease control*	68.8	68.8	100.0	100.0	100.00	–	35.4
M. piperita	Herb yield (g)	93.3 ± 2.5	93.7 ± 4.7	97.0 ± 3.9	112.3 ± 6.8	120.3 ± 9.1	0.00#	39.6
	% oil yield	0.62	0.75	0.85	0.86	0.96	0.00	0.3
	% disease control*	66.7	79.2	79.2	100.0	100.0	–	33.7
M. spicata	Herb yield (g)	37.7 ± 2.3	37.7 ± 3.7	41.7 ± 3.5	49.3 ± 5.7	49.3 ± 6.9	20.9 ± 2.33	8.6
	% oil yield	0.29	0.52	0.53	0.58	0.61	0.20	0.2
	% disease control*	68.0	92.0	100.0	100.0	100.0	–	35.9

*Percentage of control. #No of plants survived.

Figure 2. Effect of *T. harzianum* (IMI No. 359879) in different proportions (control, 1 : 1, 1 : 2, 1 : 3, 1 : 4 and 1 : 5 from left to right in each photograph) on different species of *Mentha* inoculated with collar rot pathogen. (Upper left): *M. arvensis*; (Upper right): *M. citrata*; (Lower left): *M. piperita*; (Lower right): *M. spicata*.

Table 2. Correlation coefficients (r) between oil yield, per cent disease incidence and inoculum ratio (SR + TH/TV) of different species of *Mentha* affected by collar rot disease

T. harzianum	Oil yield				Per cent disease incidence				Inoculum ratio			
	Ma	Mc	Mp	Ms	Ma	Mc	Mp	Ms	Ma	Mc	Mp	Ms
Herb yield	0.996	0.941	0.929	0.893	– 0.989	– 0.801	– 0.989	– 0.944	0.784	0.957	0.897	0.915
Oil yield	–	–	–	–	– 0.994	– 0.928	– 0.897	– 0.715	0.767	0.916	0.892	0.948
Per cent disease incidence	–	–	–	–	–	–	–	–	– 0.809	– 0.839	– 0.846	– 0.747
					T. virens							
Herb yield	0.987	0.952	0.982	0.906	– 0.987	– 0.987	– 0.988	– 0.897	0.853	0.834	0.812	0.934
Oil yield	–	–	–	–	– 0.972	– 0.948	– 0.986	– 0.838	0.868	0.933	0.859	0.930
Per cent disease incidence	–	–	–	–	–	–	–	–	– 0.886	– 0.859	– 0.865	– 0.747

*Ma, *M. arvensis*; Mc, *M. citrata*; Mp, *M. piperita*; Ms, *M. spicata*.

of the disease. SR cannot be easily controlled by the use of chemicals and large quantities of chemicals are required to check the germination of either sclerotia or mycelia. The efficacy of a particular compound also varies on different crops and the results are inconsistent from one growing season to another[25]. Thus, the two strains, viz. *T. harzianum* (IMI No. 359869) and *T. virens* (ITCC No. 1066.95) have the potential to control the collar rot disease of *Mentha* species and also increase the oil yield significantly which is otherwise drastically reduced in diseased plants.

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