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Effect of *Sclerotinia sclerotiorum* on the disease development, growth, oil yield and biochemical changes in plants of *Mentha arvensis*

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Abstract Experiment was carried out to determine the effect of *Sclerotinia sclerotiorum* on the disease development, growth, oil yield and biochemical changes in the plants of *Mentha arvensis*. With the increase in initial inoculum levels of *S. sclerotiorum* a corresponding decrease in plant fresh and dry weights were recorded. The maximum reduction in the shoot-roots/suckers fresh weight and shoot-roots/suckers dry weights (39.8%, 43.6%, 40.3% and 42.9%), respectively, was observed at the highest initial inoculum level of 12 g fungal mycelium/5 kg soil as compared to uninoculated control. The infection of roots and suckers due to *S. sclerotiorum* increased with increasing initial inoculum levels. At the lowest initial inoculum (1.0 g mycelium/5 kg soil), infection was observed 18.0% and at the highest (12 g mycelium/5 kg soil), it was 80.2%. Significant ($P \leq 0.01$) reduction in oil yield, total chlorophyll, total phenol and total sugar content of *M. arvensis* plants was observed at the lowest inoculum level as compared to uninoculated control.

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1. Introduction

Mentha arvensis L. yielding essential oil on hydro-distillation from fresh herb is a rich source of menthol, a chemical, which is widely used in pharmaceutical, flavoring and cosmetic industries. Cultivation of mint crops has increased tremendously due to better economic returns of the oil and its components. Various plant pathogenic fungi are known to infect mints, causing severe damage to the crop.

The genus *Sclerotinia* belongs to Sclerotiniaceae; an important family of the class Ascomycotina. *Sclerotinia* spp. are polyphagous in nature, wide spread and destructive pathogens of vegetables, ornamentals, field crops, medicinal and aromatic

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plants. The distribution of various species of *Sclerotinia* is cosmopolitan but, they are most common in temperate regions. *Sclerotinia sclerotiorum* (Lib.) de Bary is an internationally important plant pathogen that causes important diseases known as white mold, Sclerotinia stem rot, wilt or stalk rot, or Sclerotinia head rot on a wide variety of broadleaf crops. This pathogen is known to infect about 500 species of plants (Saharan and Mehta, 2008). Sclerotinia has been considered to be a very difficult pathogen to control. The pathogen has been reported to cause damage up to 100% in sunflower and beans (Tu, 1989). However impact of this pathogen on *M. arvensis* has yet to be assessed. Keeping in view the importance of mentha crop and highly destructive nature of the sclerotinia rot present study was planned.

The aim of this study is to assess the damaging potential of *S. sclerotiorum* in relation with growth, oil yield, and biochemical changes in plants so that the information obtained through this study can be utilized in the development of management strategies and disease forecasting system for *S. sclerotiorum*.

2. Materials and methods

2.1. Isolation of *S. sclerotiorum* from roots and suckers of *M. arvensis*

Apparently infected roots/suckers of *M. arvensis* plants were collected from the Mentha growing fields. From the samples fungi were isolated on potato dextrose agar (PDA) medium. On the basis of cultural characters and microscopic observations, *S. sclerotiorum* was identified. For further confirmation the culture was sent to Indian Type Culture Collection (ITCC), Plant Pathology Division, Indian Agricultural Research Institute (IARI), New Delhi, India. The fungus, *S. sclerotiorum* isolated and identified from the *M. arvensis* was maintained and cultured on sterilized potato dextrose broth for inoculation purpose.

2.2. Transplanting and inoculation

To examine the effect of different initial inoculum levels of *S. sclerotiorum*, five cm length of healthy suckers of *M. arvensis* were transplanted singly into 30-cm-diameter clay pots containing 5 kg steam sterilized soil and farm yard manure (5:1) mixture. Pots were kept on concrete platform for the establishment of plants. At 4th leaf stage soil was removed exposing suckers and roots of *M. arvensis* and predetermined amount (0, 1, 3, 6, 9, 12 g) of *S. sclerotiorum* mycelium in aqueous suspension was poured over the exposed roots/suckers, afterwards suckers were covered gently with sterilized soil. There were five replicates per treatment, and the pots were arranged in a completely randomized block design.

2.3. Recording of plant growth parameters

One hundred days after inoculation, plants were carefully uprooted from pots and roots/suckers were washed in running tap water to remove the adhering soil particles. Excess water was removed with blotting paper. Plant growth was determined by measuring fresh and dry weights in grams (g) of shoot and roots/suckers. The percent reduction in plant growth over uninoculated control was also calculated. The per-

cent infection was calculated by measuring the infected portion in relation to total length of roots and suckers pieces (Biermann and Lindermann, 1981).

2.4. Oil extraction

The essential oil content was determined by hydro-distillation of fresh herb using Clevenger apparatus (Perveen et al., 2007). All aerial parts, including leaves and stems, of each replicate were cut into 2–3 cm long pieces and placed, with 400 ml of water, in 1-litre capacity round bottom flasks of the Clevenger apparatus. Distillation was conducted at a 90 °C heating mantle temperature for 1 h and the amount of condensed essential oil was recorded on the scale in the apparatus. The percentage reduction in yield relative to the uninoculated control was calculated.

2.5. Estimation of total chlorophyll

For the estimation of chlorophyll content, fresh leaf (0.2 g) sample was homogenized in 80% acetone, and then measured the absorbance (*A*) at 645 and 633 nm on spectrophotometer (Spectronic 20D) and then total chlorophyll content was calculated by using the specific absorption coefficient provided by Arnon (1949).

2.6. Estimation of total sugar

Total sugar content of the third leaf from the apex was estimated by using the anthrone reagent method (Rajvaidya and Markandy, 2006). Fresh leaf (0.5%) samples were transferred to 10 ml boiling 80% ethanol solution. The solution was filtered and final volume was made up to 50 ml. In 1 ml of this filtrate, 5 ml of anthrone reagent was added, and then heated in boiling water bath for 15 min. The test tubes were incubated for 20 min at room temperature (25 °C). Optical density was read at 620 nm on spectrophotometer. Blank was also run in the same way. The soluble sugars were calculated from a standard curve developed using glucose.

2.7. Estimation of total phenol

To estimate the total phenol content, third leaf from the apex was collected (Vermerris and Nicholson, 2006). Fresh leaf (0.5 g) sample was extracted with 30 ml methanol and the sample evaporated to dryness. The residue was dissolved in 0.5 ml methanol and volume was made to 25 ml with distilled water. One ml of extract was diluted to 6 ml with distilled water and 0.5 ml Folinio Calteu reagent (1:1 diluted) was added. After 3 min, 1 ml of 35% Na₂CO₃ was added to the reaction mixture and final volume was made up to 10 ml. The tubes were kept in darkness for 30 min and afterwards OD was recorded at 600 nm on a spectrophotometer. The phenol content was calculated from a standard curve gallic acid.

2.8. Statistical analysis

The data were subjected to ANOVA and least significant difference (LSD) at probability of 0.05 and 0.01 was used to compare the treatments.

3. Results

As initial inoculum levels of *S. sclerotiorum* increased, there was a corresponding decrease in plant fresh and dry weights (Table 1). The maximum reduction in the shoot-roots/suckers fresh weight and shoot-roots/suckers dry weights (39.8%, 43.6%, 40.3% and 42.9%), respectively, was observed at the highest initial inoculum level of 12 g fungal mycelium/5 kg soil as compared to uninoculated control. Analyses of data indicated that effects of all the inoculum levels on all the test parameters were highly significant ($P \leq 0.01$).

The infection of roots and suckers due to *S. sclerotiorum* increased with increasing initial inoculum levels. At the lowest initial inoculum (1.0 g mycelium/5 kg soil), infection was observed 18.0% and at the highest (12 g mycelium/5 kg soil), it was 80.2%. Significant ($P \leq 0.05$) differences were observed in the extent of infection among all the corresponding inoculum levels.

Significant ($P \leq 0.01$) reduction in oil yield, total chlorophyll, total phenol and total sugar content of *M. arvensis* plants was observed at the lowest inoculum level as compared to uninoculated control. Analyses of data indicated that differences in all above mentioned test parameters were significant ($P \leq 0.01$) among all the initial inoculum levels. Maximum reduction in oil yield, total chlorophyll, total phenol and total sugar 28.9%, 31.4%, 34.8% and 31.6%, respectively, was observed at the highest inoculum level as compared to uninoculated control (Table 2).

4. Discussion

The increasing inoculum levels of *S. sclerotiorum* resulted in a gradual increase in extent of reduction in shoot and root and sucker fresh and dry weights, oil yield, chlorophyll, total sugar and total phenol content in leaves and root/sucker infection

Table 1 Effect of different initial inoculum levels of *Sclerotinia sclerotiorum* (g mycelium/5 kg soil) on disease development and plant growth of *Mentha arvensis*.^a

Initial inoculum levels	Plant fresh weight (g)			Plant dry weight (g)			Roots ^b and suckers infection
	Shoot	Roots and suckers	Total	Shoot	Roots and suckers	Total	
0.0	130.5	122.2	252.7	31.0	23.5	54.5	0.0
1.0	119.2	109.1	228.3	28.2	21.0	49.2	18.00
	(8.6)	(10.7)	(9.6)	(9.0)	(10.6)	(9.7)	
3.0	105.7	96.5	202.2	25.0	18.5	43.5	40.00
	(19.0)	(21.0)	(20.0)	(19.3)	(21.3)	(20.2)	
6.0	93.5	82.6	176.1	22.0	15.7	37.7	65.00
	(28.3)	(32.4)	(30.3)	(29.0)	(33.2)	(30.8)	
9.0	85.4	75.5	160.9	20.1	14.5	34.6	72.50
	(34.5)	(38.2)	(36.3)	(35.2)	(38.3)	(36.5)	
12.0	78.5	68.9	147.4	18.5	13.4	31.9	80.25
	(39.8)	(43.6)	(41.7)	(40.3)	(42.9)	(41.5)	
L.S.D _{-0.05}	3.8	4.0	5.2	1.1	1.0	2.7	2.8
L.S.D _{-0.01}	5.2	5.4	7.2	1.5	1.4	3.6	4.0

Figures in parentheses are percent reduction over uninoculated control.

^a Each value is an average of five replicates.

^b Percent roots and suckers infection by *S. sclerotiorum*.

Table 2 Effect of different initial inoculum levels of *Sclerotinia sclerotiorum* (g mycelium/5 kg soil) on oil yield and biochemical changes in plants of *Mentha arvensis*.^a

Initial inoculum levels	Oil yield (ml/100 g fresh herb)	Chlorophyll content (mg/g fresh leaves)	Total phenol (mg/g fresh leaves)	Total sugar (mg/g fresh leaves)
0.0	0.76	1.69	12.90	15.50
1.0	0.74	1.63	12.25	14.75
	(2.60) ^b	(3.55)	(5.04)	(4.84)
3.0	0.69	1.55	11.60	14.00
	(9.10)	(8.28)	(10.08)	(9.68)
6.0	0.65	1.43	10.50	12.75
	(14.47)	(15.38)	(18.60)	(17.74)
9.0	0.58	1.31	9.25	11.20
	(23.68)	(22.48)	(28.29)	(27.74)
12.0	0.54	1.16	8.40	10.60
	(28.94)	(31.36)	(34.88)	(31.61)
L.S.D _{-0.05}	0.02	0.03	0.19	0.22
L.S.D _{-0.01}	0.03	0.04	0.27	0.30

^a Each value is an average of five replicates.

^b Figures in parentheses are percent reduction over uninoculated control.

due to fungus. Grau (1988) also observed a linear relationship in yield and severity of stem rot on soybean due to *Sclerotinia* spp. Similarly, del Río et al. (2007) reported considerable loss in the yield of canola crop due to sclerotinia rot.

In the present study, the results showed a decrease in chlorophyll content in the leaves of *M. arvensis*, when inoculated with the increasing inoculum levels of *S. sclerotiorum*. This is in an agreement with the report of Tariq and Jefferies (1985) who showed that chloroplast degeneration in sclerotinia infection is associated with oxalic acid secretion by the fungus which causes rupturing of the chloroplast membrane. Similarly, Pinto et al. (2000) observed 50% reductions in total chlorophyll content in the endophyte-infected maize plants. The reduction in chlorophyll content might be due to the reduction in protein nitrogen content.

There was a decrease in total phenol of the leaves of *M. arvensis* with increase in initial inoculum of *S. sclerotiorum*. Kumar et al. (1998) also reported reduction in total phenol content in the seeds of sunflower infected with *S. sclerotiorum*. Similar observations were also found in wheat due to *Urocystis agropyri* (Sindhani et al., 1996). It might have occurred due to altered rates of synthetic activity because of infection by pathogen (Howlett, 2006).

Increased reduction of total sugar was also recorded in the leaves of *M. arvensis*, with increasing inoculum levels of the fungus. Kumar et al. (1998) also observed similar results in seeds of sunflower infected with *S. sclerotiorum*. The effect seemed to be due to reduction in chlorophyll content which may have lead to a decrease in the electron transport components and a consequent reduction in carbohydrate synthesis.

5. Conclusion

The aim of this study was to assess the damaging potential of *S. sclerotiorum* in relation with growth, oil yield, biochemical changes in plants and it has been found that all the test initial inoculum levels of *S. sclerotiorum* caused significant damage to *M. arvensis*. On the basis of studies, it is concluded that the prediction of losses expected from a particular inoculum level of *S. sclerotiorum* in soil can be utilized in developing management strategies.

References

- Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24, 1–5.
- Biermann, B., Lindermann, R.C., 1981. Quantifying vesicular-arbuscular mycorrhiza, proposed method towards standardization. *New Phytol.* 87, 63–67.
- del Río, L.E., Bradley, C.A., Henson, R.A., Endres, G.J., Hanson, B.K., McKay, K., Halvorson, M., Porter, P.M., Le Gare, D.G., Lamey, H.A., 2007. Impact of *Sclerotinia* stem rot on yield of canola. *Plant Dis.* 91, 191–194.
- Grau, C.R., 1988. *Sclerotinia* stem rot of soybean. In: Wyllie, T.D., Scott, D.H. (Eds.), *Soybean Diseases of the North Central Region*. APS Press, The American Phytopathol. Soc., pp. 56–66.
- Howlett, B.J., 2006. Secondary metabolite toxins and nutrition of plant pathogenic fungi. *Cur. Opin. Plant Biol.* 9, 371–375.
- Kumar, B., Chahal, S.S., Ahuja, K.L., 1998. Effect of *Sclerotinia* head rot on some bioconstituents of sunflower seed. *Indian Phytopath.* 51, 359–360.
- Perveen, K., Haseeb, A., Shukla, P.K., 2007. Efficacy of pesticides, neem seed powder and bio-control agents on *Meloidogyne incognita* and growth and oil yield of *Mentha arvensis*. *Nematol. Medit.* 35, 75–79.
- Pinto, L.S.R.C., Azevedo, J.L., Pereira, J.O., Vieira, M.L.C., Labate, C.A., 2000. Symptomless infection of banana and maize by endophytic fungi impairs photosynthetic efficiency. *New Phytologist* 147 (3), 609–615.
- Rajvaidya, N., Markandy, D.K., 2006. Estimation of total sugar in plant material by anthrone-reagent method. In: *Microbiology*. APH Publ., New Delhi, India, pp. 264–269.
- Saharan, G.S., Mehta, N., 2008. Economic importance. In: *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*. Springer, India, pp. 41–45.
- Sindhani, G.S., Parashar, R.D., Hooda, I., 1996. Relationship between biochemical parameters and flag smut resistance in wheat. *Indian J. Mycol. Plant Pathol.* 26, 291–293.
- Tariq, V.N., Jeffries, P., 1985. Change occurring in chloroplasts of *Phaseolus* following infection by *Sclerotinia*: a cytochemical study. *J. Cell Sci.* 75, 195–205.
- Tu, J.C., 1989. Management of white mold of white beans in Ontario. *Plant Dis.* 73, 281–285.
- Vermerris, W., Nicholson, R., 2006. Isolation and identification of phenolic compounds: a practical guide. In: *Phenolic Compound Biochemistry*. Springer, The Netherlands, pp. 151–196.