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A Study on the Effect of Different Soil pH on *Sclerotinia sclerotiorum* Infection and Growth of *Mentha arvensis*

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Abstract: *Sclerotinia sclerotiorum* is a broad-host-range phytopathogenic fungus. It also effect plants of *Mentha arvensis,* source of menthol which has great demand in pharmaceutical, flavouring and cosmetic industries. The present study was carried out to determine the role of soil pH (4.5, 6.0, 7.5 and 9.0) on *S. sclerotiorum* infection and the growth, biochemical parameters and oil yield of *M. arvensis* and in pot condition. Results showed that soil pH had effected either positively or negatively the growth and oil yield of *M. arvensis*. The maximum percent reduction in shoot height (20.1 %), shoot dry weight (25.8 %), roots and suckers dry weight (27.2 %) and oil yield (9.37 %) was observed in plants grown in soil pH 4.5 and inoculated with *S. sclerotiorum.* The maximum roots/suckers *S. sclerotiorum* infection was 52 % at pH 4.5. Chlorophyll a, chlorophyll b, total chlorophyll, total phenol and total sugar content of the plants inoculated with *S. sclerotiorum* as well as uninoculated plants increased with the increase in soil pH. Results demonstrated that pH is an important factor influencing the disease development and also affecting plant growth, oil yield and biochemical parameters.

Key words: *Sclerotinia sclerotiorum, Mentha arvensis,* soil pH, plant growth, oil yield.

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is an internationally important plant pathogen that causes important diseases such 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 [15]. Sclerotinia has been considered to be very difficult pathogen to control. The pathogen has been reported to cause damage up to 100% in sunflower and beans [22] and up to 80.2 % infection to *M. arvensis* [11].

Mints (*Mentha* spp., family Lamiaceae) have been grown and utilised since ancient times and are believed to have originated in the Mediterranean basin and spread to rest of the world. Mints have been cultivated on a large scale in many tropical countries of the world. *Mentha arvensis* L. (Japanese mint) is a rich source of menthol. Menthol oil has great demand in pharmaceutical, flavoring and cosmetic industries. The cultivation of mint is affected by a number of diseases caused by insects, fungi, viruses and plant- pathogenic nematodes. The ability of fungi to adapt to a wide range of conditions makes them an important group of pathogens. Several species of the following genera *Puccinia, Rhizoctonia, Macrophomina, Sclerotium, Fusarium, Verticillium, Curvularia, Alternaria, Erysiphae, Pernospora, septoria* and *Sclerotinia* are responsible for the diseases of mint [6,11].

Soil pH is considered as an important factor which affects plant growth as well as occurrence and severity of plant diseases caused by some

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pathogens. Soil pH controls availability of nutrient and some important biological functions in the soil matrix. In many diseases, it has been suggested that pH effects the pathogen, whereas, in some, a weakening of the host induced by the soil acidity, may affect the incidence and severity of the disease [1,13,14,21,24]. It was reported that *S. sclerotiorum* can maintain maximal activity of cell wall-degrading enzymes such as polygalacturonase through the acid environment [3]. Oxalic acid has been suggested to be an essential pathogenicity determinant in *S. sclerotiorum* [13]. Chen, *et al*. [5] indicated that *S. sclerotiorum* can coordinate environmental signals, such as pH to trigger a signaling pathway mediated by Smk 1 to induce sclerotia formation and sclerotial development is fundamental to disease cycle of this pathogen.

Thus, study was carried out in pots to determine the effect of soil pH 4.5, 6.0, 7.5 and 9.0 on *S. sclerotiorum* infection and the growth, biochemical parameters, and oil yield of *M. arvensis.*

Materials and methods

Maintenance of test pathogen

The fungus, *S. sclerotiorum* isolated and identified from the *M. arvensis* was maintained and cultured on sterilized potato dextrose broth for inoculation purpose.

Preparation of soil

The inner surface of 30-cm-diameter clay pots was painted with black enamel paint. It was done so to avoid the leaching of minerals from the pots. Dry sand was sieved through 710 µm mesh size sieve to remove coarse particles, stones and gravels. The sand was treated with 20 % hydrochloric acid (500 ml/kg sand) for 24 h in plastic containers. After that containers were left for further 24 h in the running tap water and thereafter the sand was washed for 10-12 times by stirring thoroughly in tap water. The washed sand was dried on a cleaned concrete platform and was sterilized by autoclaving. The painted clay pots were filled with the dry sand.

Preparation of Hoagland's solution and maintenance of pH

Complete Hoagland's solution was prepared by the procedure described by Hoagland and Arnon [7]. Four pH levels (4.5, 6.0, 7.5 and 9.0) of Hoagland's solution were prepared by adding 1M NaOH or 20 % HCl.

Transplanting and inoculation

Five cm length of healthy suckers of *M. arvensis* was transplanted singly into 30-cm- diameter each prepared pots. Pots were kept on concrete platform for the establishment of plants. At 4th leaf stage sand was removed exposing suckers and roots of *M. arvensis* and predetermined amount 3g of *S. sclerotiorum* mycelium in aqueous suspension was poured over the exposed roots/suckers, afterwards suckers were covered gently with sterilized sand. Pots were irrigated every day with 100-300 ml full strength Hoagland's solution at specified pH levels. There were four replicates for each treatment. The experiment was laid out as a completely randomized block design.

Recording of data

One hundred days after inoculation the experiment was terminated. Plant growth was determined by measuring shoot height, fresh and dry weights in grams (g) of shoot and roots/suckers. The per cent infection was calculated by measuring the infected portion in relation to total length of roots and suckers pieces [4]. The essential oil content was determined by hydro-distillation of fresh herb using Clevenger apparatus [10].

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 total chlorophyll content was calculated by using the specific absorption coefficient provided by Arnon [2].

Total sugar content of the third leaf from the apex was estimated by using the anthrone reagent method [12]. 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 spectro-

photometer. Blank was also run in the same way. The soluble sugars were calculated from a standard curve developed using glucose.

To estimate the total phenol content, third leaf from the apex was collected [23]. 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 Folincio Calteu reagent (1:1 diluted) was added. After 3 min, 1 ml of 35 % Na_2CO_3 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 of gallic acid.

Statistical analysis

All the data were analysed by analysis of variance followed by Duncan Multiple Range Test at $P \leq 0.05$ using computer software SPSS.

Results

Data presented in Table 1 indicate that soil pH had a significant effect on the growth and oil yield of *M. arvensis,* both in presence and absence of pathogen. An inversely proportional relationship was observed between pH levels and plant growth of *S. sclerotiorum* inoculated as well as uninoculated plants. Relationship between pH levels and oil yield was directly proportional in plants inoculated with *S. sclerotiorum.*

Plants inoculated with S. *sclerotiorum* had maximum reduction of 20.1, 25.8, 27.2, and 9.37 % in shoot height, shoot dry weight, and roots/suckers dry weight respectively at pH 4.5 (Table 1). Analyses of data indicated that there was a non-significant ($P \leq 0.05$) difference between pH 6.0 and 7.5 in shoot height, fresh and dry weights of root/sucker, and between pH 4.5 and 6.0 in shoot fresh and dry weights of the plants inoculated with pathogen. Howeverr, the reduction in growth of inoculated plants was significant ($P \leq 0.05$) as compared to uninoculated plants, irrespective of pH levels. Figure 1 shows the effect of *S. sclerotiorum* on the oil yield and root /suckers infection at different soil pH, it clearly shows that the oil yield had increased with the increase in soil pH. No difference in oil yield was observed between pH 6.0 and 7.5 in plants inoculated with *S. sclerotiorum*. Also, the relationship between oil yield and root/sucker infection by *S. sclerotiorum* was observed inversely proportional. All pH levels had a significant ($P \leq 0.05$) effect on the disease development. Highest percent roots/suckers infection by the S. *sclerotiorum* w observed at pH 4.5 followed by 6.0, 7.5, and 9.0. The maximum roots/suckers infection by fungus was 52% at pH 4.5.

Biochemical data of *M. arvensis* indicated that

Table 1. Effect of different soil pH on the growth of *Mentha arvensis* inoculated with *Sclerotinia sclerotiorum* (3g/pot) in pot condition

Soil pH	Shoot height (cm)		Shoot fresh weight (g)		Roots and suckers fresh weight (g)		Shoot dry weight (g)		Roots and suckers dry weight (g)	
	UI	I	UI	I	UI	I	UI	I	UI	I
4.5	77.1^{d}	61.1^{a}	141.5^{d}	105.5^{a}	138.0^{d}	102.0^{a}	33.7^{e}	25.0^{a}	26.5^{d}	19.3^{a}
6.0	71.5^{e}	59.0^{b}	129.3^{e}	102.0^{b}	120.5^{e}	93.5^{b}	31.0^{f}	24.0^{b}	23.4^{e}	18.0^{b}
7.5	66.5^{f}	59.5^{b}	118.5^{f}	95.8^{b}	115.5^{f}	92.0^{b}	28.5^{g}	22.7^{c}	22.5^{f}	17.7^{b}
9.0	59.1^{g}	54.0^{c}	105.9^{g}	90.0^{c}	102.0^{g}	88.5^{c}	25.6^{h}	21.6^{d}	19.6^{g}	16.9^{c}

Each value is an average of four replicates.

Data followed by different letters in the column of each parameter are significantly different ($P \leq 0.01$) according to Duncan's multiple range test.

UI = Uninoculated control; I=Inoculated with *S. sclerotiorum*

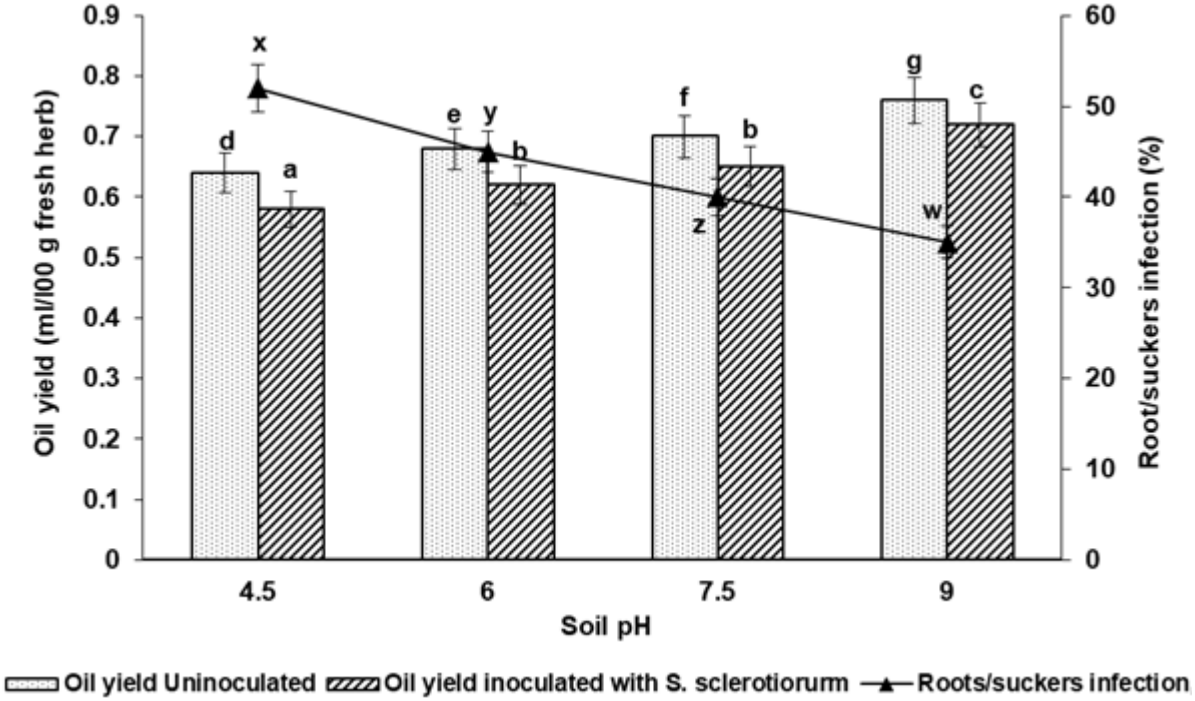

Figure 1. Effect of *Sclerotinia sclerotiorum* (3 g/pot) on disease infection of root/suckers and oil yield of *Mentha arvensis* at different soil pH in pot condition

Vertical bars show standard deviation of means of four replicates.Values with different letters at their top show significant difference ($P \leq 0.05$) as determined Duncan's multiple range test

the chlorophyll a, chlorophyll b, total chlorophyll, total phenol and total sugar content of the plants inoculated with *S. sclerotiorum* as well as uninoculated plants increased with the increase in pH (Table 2). Highest reduction in corresponding biochemical parameters was 14.0, 13.3, 13.17, 13.04 and 13.20 %, respectively, observed at pH 4.5 in plants inoculated with S. *sclerotiorum*. Analyses of data showed that the difference in chlorophyll a content of the leaves in the fungus free plants grown at pH 6.0 and 7.5 was non-significant ($P \leq 0.05$). The reduction in all biochemicals in inoculated plants was significant ($P \leq 0.05$) as compared with uninoculated plants irrespective of pH levels.

Discussion

Soil pH influence biotic factors as well as abiotic factors [14]. *S. sclerotiorum* is a broad-host-range phytopathogenic fungus, the acidic environment helps the fungus to grow and cause disease. Numerous pectinolytic, proteolytic, cellulytic, and other hydrolytic enzymes from *S. sclerotiorum* with acidic pH optima have been described [13]. Results depict that the plants inoculated with *S. sclerotiorum* had maximum reduction in plant growth parameters and oil yield at pH 4.5 followed by 6.0, 7.5 and 9.0 respectively. Similar results were observed on guar due to *S. rolfsii* [19] and on coriander due to *Protomyces macrosporus* [21]. A study demonstrated that the damage to the photosynthetic apparatus in the tobacco leaves induced by *S. sclerotiorum* was caused by the acidity of $H_2C_2O_4$ as well as by $C_2O_4^{2-}$ [25].

The roots/suckers infection due to *S. sclerotiorum* was found to be highest at pH 4.5 followed by pH 6.0, 7.5 and 9.0, respectively. Several other reports also indicated that *Sclerotinia* spp. could tolerate a wide range of pH, however this fungus is well adapted to an acidic substrate or environment [9,17]. Since the extracellular pectolytic and cellulolytic enzymes produced by *S. sclerotiorum* have optima pH below 5.0, therefore, the rapid disease development was observed at higher acidic pH [8,16]. Ambient pH conditions affect mul-

Table 2. Effect of different soil pH on the biochemical parameters of *Mentha arvensis* inoculated with *Sclerotinia sclerotiorum* (3g/pot) in pot condition

Soil pH	Chlorophyll a (mg/g fresh leaves)		Chlorophyll b (mg/g fresh leaves)		Total chlorophyll (mg/g fresh leaves)		Total phenol (mg/g fresh leaves)		Total sugar (mg/g fresh leaves)	
	UI	I	UI	I	UI	I	UI	I	UI	I
4.5	1.07^{d}	0.92^{a}	0.60^{e}	0.52^{a}	1.67^{e}	1.45^{a}	11.50^{e}	10.00^{a}	12.50^{e}	10.85^{a}
6.0	1.17^{e}	1.03^{b}	0.63^{f}	0.56^{b}	1.79^{f}	1.60^{b}	12.20^{f}	10.85^{b}	13.15^{f}	12.65^{b}
7.5	1.19^{e}	1.09^{b}	0.65^{g}	0.59^{c}	1.85^{g}	1.68^{c}	12.35^{g}	11.10^{c}	13.90^{g}	12.65^{c}
9.0	1.23^{f}	1.16^{c}	0.69^{h}	0.65^{d}	1.93^{h}	1.82^{d}	12.50^{h}	11.35^{d}	14.00^{h}	13.00^{d}

Each value is an average of four replicates
Data followed by different letters in the column of each parameter are significantly different ($P \leq 0.05$) according to Duncan's multiple range test
UI = Uninoculated control; I=Inoculated with *S. sclerotiorum*

tiple processes in *S. sclerotiorum* [24]. The sclerotial development was favoured by acidic ambient pH conditions but inhibited by neutral ambient pH, and transcripts encoding the endopolygalacturonase gene *g1* accumulated maximally under acidic culture conditions [13]. During plant infection, an acidic pH environment is necessary for the activities of many hydrolytic enzymes, including polygalacturonases which have been implicated as colonization and virulence factors in other plant infecting fungi [18,20].

The results demonstrated that pH is one of the important factors influencing disease development and affecting plant growth. On the basis of results it can be advised that for the cultivation of *M. arvensis,* the pH should be adjusted towards alkaline either temporarily or permanently by implementing appropriate means such as application of fertilizer or powder of limestone in their field. This will reduce the activity of pathogens and will increase the nutrient uptake by plants, thus resulting reduction in disease severity.

References

1. **Agrios, G.N. (2000).** Plant diseases caused by fungi. In: Plant Pathology. Ed. G.N. Agrios. Academic Press, London. pp. 645.
2. **Arnon, D.I. (1949).** Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris.* Plant Physiology. 24: 1-5.
3. **Bateman, D.F., Beer, S.V. (1965).** Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *sclerotium rolfsii*. Phytopathology. 5: 204-211.
4. **Biermann, B., Lindermann, R.C. (1981).** Quantifying vesicular–arbuscular mycorrhiza, proposed method towards standardization. New Phytology. 87: 63-67.
5. **Chen, C., Harel, A., Gorovoits, R., Yarden, O., Dickman, M.B. (2004).** MAPK regulation of sclerotial development in *Sclerotinia sclerotiorum* is linked with pH and cAMP sensing. Molecular Plant-Microbe Interactions. 17: 404-413.
6. **Haseeb, A., Shukla, P.K. (2001).** Nematode diseases of *Mentha* and their management. In: Role of Resistance in Intensive Agriculture. Eds. S. Nagarajan and D.P. Singh, Kalyani Publ., Ludhiana, India. pp. 60-77.
7. **Hoagland, D.R., Arnon, D.L. (1950).** The water culture method for growing plants without soil. California Agri. Exp. Stn. Cir. No. 347.
8. **Iliescu, H., Ionita, A., Jinga, V. (1988).** Ecological aspects of the fungi *Sclerotinia sclerotiorum* Lib. de Bary and S. *minor* Jagger, parasites on sunflower in Romania. Analele Institutului de

Cercetari Pentru Protectia Plantelor. 21: 29-44.
9. **Khare, K.B., Bompeix, G. Basuchaudary, K.C. (1981).** Factors affecting growth and sclerotial formation of *Sclerotinia minor in vitro.* Cryptogamie. Mycologie. 2: 289-297.
10. **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.* Nematology Mediterranean. 35: 75-79.
11. **Perveen, K., Haseeb, A., Shukla, P.K. (2010).** Effect of *Sclerotinia sclerotiorum* on the disease development, growth, oil yield and biochemical changes in plants of *Mentha arvensis*. Saudi Journal of Biological Sciences. 17: 291-294.
12. **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.
13. **Rollins, J.A., Martin, B.D. (2001).** pH Signaling in *Sclerotinia sclerotiorum*: Identification of a *pacC/RIM1* Homolog. Applied and Environmental Microbiology. 67: 75-81.
14. **Rousk, J., Brookes, P.C., Baath, E. (2009).** Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology. 75: 1589-1596.
15. **Saharan, G.S., Mehta, N. (2008).** Economic importance. In: Sclerotinia diseases of crop plants: Biology, Ecology and Disease Management. Springer, India. pp. 41-45.
16. **Sharma, R.C., Sharma, S.L., Sharma, I. (1984).** Protease activity of *Sclerotinia sclerotiorum* causing stalk rot of cauliflower. Indian Phytopathology. 37: 531-534.
17. **Shi, J.R., Wang, Y.Z. Shen, S.W., Chen, H.G., Jian, A.G. (1999).** Effect of pH on growth of *Sclerotinia sclerotiorum* in rape. Jiangsu Agricultural Sciences. 3: 46-49.
18. **Shieh, M., Brown, R.L., Whitehead, M.P., Carey, J.W., Cotty, P.J., Cleveland, T.E., Dean R.A.(1997).** Molecular genetic evidence for the involvement of a specific polygalacturonase, P2c, in the invasion and spread of *Aspergillus flavus* in cotton bolls. Applied and Environmental Microbiology. 63: 3548-3552.
19. **Singh, R.P., Gandhi, S.K. (1991).** Effect of soil pH and temperature on seedling mortality of guar caused by *Sclerotium rolfsii* and its fungicidal control. Indian Phytopathology. 44: 360-365.
20. **ten Have, A., Mulder, W., Visser, J., van Kan, J.A.L. (1998).** The endopolygalacturonase gene Bcpg1 is required for full virulence of *Botrytis cinerea*. Molecular Plant-Microbe Interactions. 11: 1009-1016.
21. **Tripathi, A.K., Pandya, R.K., Tiwari, R.J., Tripathi, M.L. (2002).** Response of stem gall disease *(Protomyces macrosporus*) and yield of coriander *(Coriandrum sativum)* to soil pH. Indian Journal of Agricultural Sciences. 72: 49-50.
22. **Tu, J.C. (1989).** Management of white mold of white beans in Ontario. Plant Disease. 73: 281-285.
23. **Vermerris, W., Nicholson, R. (2006).** Isolation and identification of phenolic compounds: a practical guide. In: Phenolic Compound Biochemistry. Springer, The Netherlands. pp. 151-196.
24. **Vylkova, S. (2017).** Environmental pH modulation by pathogenic fungi as a strategy to conquer the host. PLoS Pathogens. 13: e1006149.
25. **Yang, C., Zhang, Z., Gao, H., Liu, M., Fan, X. (2014).** Mechanisms by which the infection of *Sclerotinia sclerotiorum* (Lib.) de Bary affects the photosynthetic performance in tobacco leaves. BMC Plant Biology. 14: 240.