Occurrence, Virulence, Inoculum Density and Plant Age of *Sclerotium rolfsii* Sacc. Causing Collar Rot of Peppermint

Arjunan Muthukumar* and Arjunan Venkatesh
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India

Abstract
Roving survey was conducted during 2011-2012 around Coimbatore, Dindigul, Erode, Hosur, Krishnagiri, Namakkal, Salem and Theni districts to assess the incidence of collar rot of peppermint. The maximum incidence of 32.33% was recorded in Therkupalayam village of Coimbatore District. The pathogen was isolated from infected samples and 8 *Sclerotium rolfsii* isolates were maintained. The level of pathogenicity of these isolates revealed that *S. rolfsii* (I) from Therkupalayam in Coimbatore District was the most virulent and caused higher levels of collar rot incidence (93.66%). An inoculum load of *S. rolfsii* at 5% to 1 kg of soil registered the maximum incidence of 92.66% collar rot of peppermint. Significantly higher per cent of wilting of 92.66% was recorded in plants at 20 days after emergence and it was found significantly more susceptible compared to rest of the treatments.

Keywords: Peppermint; *Sclerotium rolfsii*; Survey; Pathogenicity; Inoculum levels

Introduction
*Mentha piperita* L. is an important aromatic perennial herb grown throughout the world, belonging to the family Lamiaceae. It 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]. *Mentha* is cultivated in Himalaya-hills, Haryana, Uttar Pradesh, Punjab and Bihar. Of these, Uttar Pradesh is the largest producing state in the country contributing 80-90% of the total production followed by Punjab, Haryana, Bihar and Himachal Pradesh. Peppermint oil is used to cure indigestion, headaches, colic, gingivitis, irritable bowel syndrome, spasms and rheumatism. It is also used in the manufacture of mint sweets and chocolates. In India, peppermint is grown throughout the year [2] and it is affected by several fungal diseases caused by *Rhizoctonia solani* [3], *Verticillium dahliae* [4], *Collectotrichum coccodes* [5], *R. solani* [6] and *Sclerotium rolfsii* [7]. Of these, collar rot caused by *Sclerotium rolfsii* is a major constraint in the peppermint cultivation in Tamil Nadu. It is a soil borne plant pathogen which causes considerable damage to the crop and the disease intensity in the field ranged from 5 to 20% [7]. The objectives of the present study are i) to survey on the incidence of collar rot of peppermint from Tamil Nadu, ii) to assess the virulence of *S. rolfsii* isolates iii) to know the susceptible stage of crop to collar rot of peppermint.

Materials and Methods
Survey on the incidence of collar rot of mint in different districts of Tamil Nadu
Surveys were carried out in important mint growing tracts *viz.*, Coimbatore, Dindigul, Erode, Hosur, Krishnagiri, Namakkal, Salem and Theni districts of Tamil Nadu to assess the severity of collar rot incidence. At each place, three fields were selected. Five plots (1 m²) were selected randomly in each field and 100 plants in each plot were selected randomly. Observations were recorded and per cent disease incidence was calculated using the following formula.

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\text{Per cent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100
\]

Isolation and maintenance of pathogen
The collar rot symptoms were collected from major mint growing tracts of Tamil Nadu pertaining to districts such as Coimbatore, Dindigul, Erode, Hosur, Krishnagiri, Namakkal, Salem and Theni. The infected plant materials brought back from the field were washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface sterilized in 0.5% sodium hypochlorite solution for 5 min. and rinsed in three changes of sterile distilled water. The segments were separately dried in between sheets of sterile filter paper and placed (3 pieces per plate) on fresh potato dextrose agar (PDA) medium [8] impregnated with streptomycin, and incubated for seven days at 28 ± 2°C.

A total of eight isolates (I1 to I8) causing collar rot was isolated from infected plant samples collected from different tracts of Tamil Nadu. The fungal growth on 5th day, which arose through the sclerotial bodies were i) to survey on the incidence of collar rot of peppermint from Tamil Nadu, ii) to assess the virulence of *S. rolfsii* isolates ii) to know the susceptible stage of crop to collar rot of peppermint.

Mass multiplication of *S. rolfsii* isolates
A total of eight isolates were multiplied in sand corn-meal medium [12]. Two hundred grams of sand corn meal mixture was taken in 500 mL conical flasks and mixed with 30% of distilled water and they were sterilized at 15 lb pressure for 20 min. The pure culture of each isolate of *S. rolfsii* was inoculated to these flasks under aseptic conditions and incubated at 28 ± 2°C for 20 days. These flask were shaken periodically

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*Corresponding author: Arjunan Muthukumar, Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India, Tel: 09443129959; E-mail: muthu78ap@yahoo.co.in

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Effect of different levels of inoculum of \textit{S. rolfsii} (I$_1$) on the incidence of collar rot of mint

The soil, sand and farmyard manure were sieved by passing through 2 mm mesh and sterilized separately and then mixed in 1:1:1 per cent proportion, respectively. After mixing, it is filled in 15×30 cm surface sterilized earthen pots. \textit{S. rolfsii} culture grown on sand-corn meal medium for 20 days was mixed to each pot so as to give different inoculum levels viz., 0, 1, 2, 3, 4 and 5%. The pots filled with sterilized soil without inoculum served as control (un-inoculated). Each treatment was replicated three times. Apparently healthy cuttings of mint (8/pot) were planted in pots. Water was added to the pots at regular intervals to maintain the soil moisture. The observation on the incidence of collar rot was recorded.

Identification of susceptible stage of the crop to collar rot of mint

To know the susceptible stage of the crop, an experiment was conducted under glasshouse condition. Five stages of the mint crop were taken for their susceptible reaction with the collar rot causal pathogen \textit{S. rolfsii}. These stages of crop were maintained in the earthen pots of 15×30 cm diameter filled with sterilized soil. After raising all the stages, the inoculum was added at 5% level. After 20 days, number of plants showing wilting symptom due to \textit{S. rolfsii} was recorded (Table 1).

Results

Survey of collar rot incidence of mint in Tamil Nadu

Roving survey was conducted during 2011-2012 around Coimbatore, Dindigul, Erode, Hosur, Krishnagiri, Namakkal, Salem and Theni districts to assess the incidence of collar rot of mint and the data are presented in the Table 2. The symptoms in the field were recognized by wilting and drying of the entire plant. Upon close observations on such affected plant revealed the presence of white cottony mycelium at the collar region, dark brown coloured mustard like sclerotial bodies were found adhering on the mycelial growth of the affected stem and at collar region (Plates 4 and 5).

| Table 1: Treatments of \textit{Sclerotium rolfsii} at different stages. |
|-----------------|---------------|----------------|
| \textbf{Treatments} | \textbf{Stage} | \textbf{Days after emergence} |
| T$_1$ | 1$^a$ | Zero stage |
| T$_2$ | 2$^b$ | 20 days old crop |
| T$_3$ | 3$^c$ | 40 days old crop |
| T$_4$ | 4$^d$ | 60 days old crop |
| T$_5$ | 5$^e$ | 80 days old |
| T$_6$ | 6$^f$ | 100 days old crop |

*Values in each column followed by the same letter are not significantly different according to the DMRT method (P=0.05).

Table 2: Survey of collar rot of mint in Tamil Nadu.
ranged from 14.00 to 32.33%. The maximum incidence of 32.33% was recorded in Therkupalayam village of Coimbatore District. This was followed by Attupalam village of Theni district (30.33%). Whereas, the least incidence of 14.00% was recorded in Pochampalli village of Krishnagiri district.

Pathogenicity of *S. rolfsii* isolates on mint

The data presented in Table 3 revealed that the level of pathogenicity varied between the isolates. Among the 8 isolates of *S. rolfsii*, the isolate-I1 (Therkupalayam) recorded the maximum disease incidence of 93.66%. It was followed by the isolate-I8 and I3 collected from Attupalam and Alangudi which were found to differ significantly from isolate-I1. The isolate-I4 (Pochampalli) recorded the least incidence of collar rot recording 56.66%. The isolate-I1 was found to be more virulent when compared to other isolates tested in the present study. Hence, the isolate-I1 alone was taken for the subsequent experiments.

Effect of different inoculum levels on the incidence of collar rot of mint

Among the various inoculum levels tested, 5% inoculum load of *S. rolfsii* to 1 kg of soil registered the maximum disease incidence of 92.66% collar rot which was followed by the 4% inoculum load of *S. rolfsii*. Ultimately the results emphasized that the disease incidence increased with the increase in the inoculum load up to 5% beyond which the disease incidence was found to decline (Table 4).
Tamil Nadu ranged from 14.00 to 33.00%. The maximum incidence of 32.33% was recorded in Therkupalayam village of Coimbatore district. This was followed by Attupalam village of Theni district (30.33%). However, the disease incidence was relatively less in Pochampalli village of Krishnagiri district (14.00%). The higher incidence of collar rot in Therkupalayam village may be due to continuous cropping and presence of pathogen over long period, because continuous cultivation of any crop over the season and years will build up inoculum level to such an extent as observed by various workers [14-16].

Pathogenicity test was carried out under pot culture conditions in glass house by inoculating with pathogenic culture of S. rolfsii. Among the isolates tested, S. rolfsii isolated (I1) from Therkupalayam area pertaining to Coimbatore district was found to be highly virulent in causing collar rot compared to other isolates investigated in the present study. This was followed by isolate (I5) from Attupalam area pertaining to Theni district. Earlies studies proved the pathogenicity of S. rolfsii on cardamom in pot culture studies by inoculating 25 days old sclerotial cultures which was grown on sand corn meal medium and observed the symptoms a week after inoculation [17]. Similarly, variation in the virulence of the isolates of S. rolfsii causing collar rots of chilli, potato and soybean has been reported by several workers [16,18,19]. Present study also corroborate with the findings of above researchers explaining that the isolate of S. rolfsii (I1) is highly virulent in causing collar rot disease.

In the present study, it was observed that S. rolfsii can infect all the stages of the mint crop, when inoculum was added to all the stages. But the susceptible stage of the crop can be identified based on the stage at which the crop shows the maximum symptoms of collar rot caused by S. rolfsii. It was found that, after 20 days of inoculation (T1, 20 days after emergence stage) mint crop showed maximum mortality (92.66%) and it was identified as the most susceptible stage. Similarly, chilli seedlings were found most susceptible to the attack of S. rolfsii during first thirty days of the growth and the per cent infection of the plant reduced with ageing [20]. Barley seedlings were found most susceptible to the attack of S. rolfsii during first fifteen days of the growth and the per cent infection of the plant reduced with ageing [21]. The most susceptible growth stage of groundnut S. rolfsii infection was at 15 days old plant and the least mortality in 105 days old plants [3]. The above results lend support to the present findings.

Among the inoculum levels tested, 5% inoculum load of S. rolfsii registered the maximum incidence of collar rot, which was followed by 4% inoculum level. Similarly, Sclerotium wilt of potato where the incidence increased with the increase in concentration of inoculum upto 4% [22]. Higher inoculum density resulted in highest disease incidence, which may be due to the fact that higher inoculum always ensures the certainty of the infection. Similar results have been reported by several workers [23-25].

References