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Influence of p^H on Growth and Sclerotia Formation of *Sclerotium rolfsii* Causal Agent of Foot Rot Disease of Betel Vine.

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Abstract: *An experiment was conducted to evaluate the effect of different pH levels (4.5, 4.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) on radial growth, and formation of sclerotia, weight of sclerotia of Sclerotium rolfsii on PDA (Potato Dextrose Agar) medium and fresh weight and dry weight of S. rolfsii on PDB (Potato Dextrose Broth) medium. Radial growth, fresh weight and dry weight, at different pH levels differed significantly at 1% level of significance but number of sclerotia formation and their weight did not differ. Optimum pH for radial growth, fresh weight and dry weight were found 5.0 to 6.0, 4.5 to 6.5 and 4.5 to 6.5, respectively.*
Key word*: pH, Sclerotium rolfsii, Betel Vine*

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I. Introduction

Betel vine (*Paper Betle* L.) is an important perennial dioecious creeper belonging to the family Piperaceae and probably a native to Malaysia. It is locally known as "Pan". Humid and moist shaded conditions are favorable for vine growth and also favor a variety of root and foliage diseases. The recurrent of the diseases leads to complete destruction and crop failure after a few years.

Sclerotium rolfsii Sacc. (Teleomorph: Corticium rolfsii Curzi) is a serious soil borne pathogenic fungus and harmful to many crops which are economically valuable in most of the tropical and sub-tropical region of the world (Aycock, 1966). It also causes diseases of other crops. It has a wide host range (Talukder, 1974) and it has been referred as an almost omnipathogenic organism (Talukder, 1974) *Sclerotium rolfsii* is known to be a ubiquitous pathogen that causes mild to extreme crop losses depending on environmental condition including temperature, moisture and amount of crop debris available (Aycock, 1966). The sclerotia can germinate mycelogenically forming mycelia which again form sclerotia depending upon environmental and nutrition status of the substratum (Aycock, 1966).

The disease caused by *Sclerotium rolfsii* commonly known as seedling blight, foot rot, collar rot and Southern blight of many crops (Talukder, 1974; Chowdhury, 1946; Ahmed, 1986). *Sclerotium rolfsii* initiate infection usually at the collar region of susceptible host (Punja and Grogan, 198). The mycelial growth and sclerotia production of this fungus is influenced by many factors including pH, temperature, individual nutrients and volatile compounds like ethanol and C/N ratio (Devi, et al., 1999; Prithiviraj, et al., 2000; Linderman, and Gilbert, 1973).

II. Materials and Methods

Stems of Chachi variety of betel vine showing typical symptoms of *Sclerotium rolfsii* was collected from Digholia Upazila of Khulna District. The fungus was isolated following standard procedures (Dhingra and Sinclair, 1985). Diseased stems were thoroughly washed under running tap water. The stems were cut into convenient size (about 1 cm) containing black lesion where healthy and diseased tissue remains together. The cut stem pieces were first thoroughly washed by sterilized distilled water and then transferred to 0.1% sodium hypochloride (NaOCl) solution and kept there for 5 minutes. Surface sterilized stem pieces were transferred on sterilized blotting paper to remove excess NaOCl solution. The cut pieces were then placed onto sterilized water agar in glass petriplates with the help of sterilized forceps and incubated in room temperature $(30\pm2)^0$C until mycelial formation. All the works were done in a laminar air flow cabinet. Fungal isolates was identified based on the characteristics of hyphae and sclerotia (Lilly and Barnett, 1951)

Pathogenicity test was done on excised stem of betel vine. Healthy stems of betel vine were cut into pieces (about 1 inch.) and surface sterilized with 70% ethanol for 10 seconds. Stem pieces were injured softly by flame sterilized pointed needles and then placed onto sterilized water agar. Advance hyphae were cut from 30

hours old pure cultures aseptically with the help of cork borer and then placed at three plates (both ends and centre) onto the stems (Dhingra and Sinclair, 1985)

Eight different pH levels namely 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were taken as treatments. Hundred (100) ml PDA was taken on 250 ml conical flask. Different pH were adjusted by adding either 0.1N HCl or 0.1N NaOH and measured by pH meter. Advance hyphae of 32 hours old culture grown on PDA at pH 6.0 were cut by flame sterilized 5 mm cork borer. The inoculated petriplates were kept in the growth chamber at a temperature of 26 ± 2^0C for 54 hours.

Radial growth of the isolates was measured by averaging the diameters taken for each colony and then these plates were kept there for 30 days for sclerotia formation.

After 30 days the sclerotia of each petriplates were separated by using camel hair brush and the number of sclerotia of each petriplates was counted manually. The weight of sclerotia of each plate was measured by using an electrical balance.

For the measurement of fresh weight and dry weight of the fungus *Sclerotium rolfsii* was grown on Potato Dextrose Broth (PDB). Hundred (100) ml PDB was taken in each 250 ml conical flask. The pH of the PDB was adjusted to 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 by adding either 0.1N HCl or 0.1N NaOH where needed. The PDB was inoculated by 5 mm mycelial block, one for each, taken from 32 hours culture grown on PDA. The inoculated conical flask was kept in the growth chamber at a temperature of 26 ± 2^0C for 7 days. After incubation the fungal mass in each conical flask was separated by filtration using Whatman paper no. 1. The filtrates were discarded and fungal mass on blotting paper was kept in the laboratory at room temperature for 40 hours for air drying. Then fresh weight was measured by using electrical balance. The fungal mass of all treatments were then oven dried at a temperature of 70^0C for 72 hours. Then the oven dried fungal mass were weighted to have dry weight.

III. Results and Discussion

Samples with characteristics symptoms of foot rot of betel vine (dark lesion develop on the stem just below the soil level and upto 10 cm height, the leaves turn yellow, become flaccid and drop and ultimately the entire vine wilts and die) were collected from Digholia Upazila, Khulna (Plate-1). After isolation of the fungus from the collected sample the characters of sclerotia were observed both by naked eyes and under microscope. The color of the mycelia was light and branches are formed with acute angle. These characters indicated that the fungus was *Sclerotium rolfsii* (Lilly and Barnett, 1951. After inoculation, characteristics symptoms of the disease were produced on excised stems of betel vine (Plate-2) which were identical to the symptoms of the sample. So, it was confirmed that the *Sclerotium rolfsii* isolate was pathogenic.

Influence of pH on radial growth, sclerotia formation, fresh weight and dry weight of *Sclerotium rolfsii.*

Influence of pH on growth of *Sclerotium rolfsii* was significantly different at ($p<0.01$_) different pH level (Table-1). Among all the pH the radial growth was highest at pH 5.5 followed by at pH 5.0. Radial growth at pH 5.5 was statistically similar with radial growth at pH 5.0 and 6.0 but different from radial growth at other pH. Radial growth at pH 4.5, 6.0, 6.5, 7.5 and 8.0 was statistically similar. On the other hand radial growth at pH 6.0, 6.5, 7.0 and 8.0 was statistically similar and radial growth at pH 8.0 was statistically different from other pH i.e. 4.5, 5.0 and 7.0. The lowest radial growth was found at pH 7.5 which was statistically different from all other pH levels.

Table-1 Influence of pH on Radial growth of *Sclerotium rolfsii* on PDA medium

pH level	Radial growth (cm)
4.5	7.137 bc
5.0	7.775 ab
5.5	8.012 a
6.0	7.325 abc
6.5	7.113 bc
7.0	7.000 bc
7.5	5.700 d
8.0	6.863 c
Level of significace	**

Here,

**= Significant at 1% level of probability

*= Significant at 5% level of probability

Growth at pH 8.0 was significantly higher than at pH 7.5. The result is somewhat inconsistent. Mycelial growth of *Sclerotium rolfsii* was higher at pH range 4.7 (Dey, et al., 1992) and at 5.0 to 7.5. In this experiment the optimum pH range for radial growth may be 5.0-to 6.0. This range is somewhat narrow then the range found by the other authors. This little bit deference might be due to difference of isolate. The number and weight of sclerotia formed on PDA at different pH did not differ significantly. The apparently higher number of sclerotia were formed at pH 5.0(253) followed by at pH 6.0 (241) and at pH 4.5 (232) (Table-2). Apparently lower number of sclerotia were formed at pH 5.5 (111) followed by pH 7.0 and 8.0.

Table-2 Influence of pH on the number and weight of sclerotia of *Sclerotium rolfsii* on PDA medium.

pH level	Number of sclerotia	Weight of sclerotia (gm)
4.5	232	0.106
5.0	253	0.104
5.5	111	0.058
6.0	241	0.103
6.5	218	0.078
7.0	147	0.080
7.5	227	0.073
8.0	175	0.084
Level of significace	NS	NS

Here,

NS= Non significant

Apparently higher weight was measured at pH 4.5 (0.106 gm) followed by at pH 5.0 and at pH 6.0. Apparently lower weight was found at pH 5.5 (0.058 gm) followed by pH 7.5 (0.073 gm), 6.5 (0.078 gm) and pH 7.0 (0.080 gm)

Influence of pH on fresh weight and dry weight of the fungus *Sclerotium rolfsii* on PDB was significantly different ($p<0.01$) at different pH level. Highest fresh weight was at pH 5.5 followed by pH levels 4.5, 6.0 and 6.5, which were statistically with the value at pH 5.5 (Table-3). Fresh weight at ph 7.0 was significantly different from pH 7.5 and pH 5.5 but similar with all other pH levels. The lowest fresh weight was observed at pH 7.5. Dry weight of *Sclerotium rolfsii* was also highest at pH 5.5. It was statistically similar with the dry weight at pH 4.5, 5.0, 6.0 and 6.5 but different from others. The lowest dry weight was also observed at pH 7.5, which was significantly different from all other pH levels. Dry weight at pH 4.5, 5.0, 6.0, 6.5, 7.0 and 8.0 were found similar.

Table-3 Influence of pH on fresh weight and dry weight of sclerotia of *Sclerotium rolfsii* on PDB medium

pH level	Fresh weight 9gm)	Dry weight (gm)
4.5	1.557 ab	1.278 ab
5.0	1.460 b	1.170 ab
5.5	1.898 a	1.503 a
6.0	1.610 ab	1.303 ab
6.5	1.535 ab	1.260 ab
7.0	1.380 b	1.120 b
7.5	0.636 c	0.470 c
8.0	1.268	1.030 b
Level of significace	**	**

Here,

**= Significant at 1% level of probability

*= Significant at 5% level of probability

IV. Conclusion

Sclerotium rolfsii identified from the naturally infected samples of foot rot of betel vine. pH 5.5 was more effective for radial growth of *Sclerotium rolfsii* and less at pH 5.0. pH 5.0 was more effective for sclerotia formation of *Sclerotium rolfsii* and less at pH 5.5. pH 4.5 was more effective for weight of formed sclerotia of *Sclerotium rolfsii* and less at pH 5.5 Fresh weight and dry weight was found high at 5.5 pH level and low at pH 7.5. All the highest radial growth, fresh weight and dry weight were found at pH 5.5, which indicate that there is a consistency among the parameter studied.

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