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Pathogenicity test of *Sclerotium rolfsii* isolates causing foot and root rot disease of betelvine (*Piper betle* L.)

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ABSTRACT

The experiment was conducted under *in-vivo* condition in a betelvine baroj at Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh. Nineteen isolates of *Sclerotium rolfsii* collected from different regions of Bangladesh designated as isolate-1 to isolate-19. Soil inoculated with *S. rolfsii* exhibited mycelial growth on the soil surface and around the base of the betelvine plant within 2-4 days after inoculation. Only 2 days after inoculation were required to manifest cottony colony on soil surface near root zone of inoculated betelvine plants by the isolate-3, 5, 7, 9 and 12. The first disease symptoms were observed within 6 to 16 days after inoculation where minimum days were required by the isolate-9 and maximum by the isolate-2 and 14. The highest lesion length (6.50 cm) was produced by the isolate-9 and isolate-13. All the isolates were found to be pathogenic in some cases, disease delayed due to their degree of pathogenicity. The isolate-9 showed highest disease incidence of 100% which was superior as compared to all other isolates at 15 days after inoculation. The isolate-19 showed least disease incidence of 66.66% even at 30 days after inoculation. Among the isolates, the most pathogenic one was isolate-9 collected from Kaligonj upazilla of Jhenaidah.

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Introduction

Betelvine (*Piper betle* L.) belongs to the family Piperaceae and is a perennial creeper grown for its leaves. It is an important commercial crop in India, Bangladesh and Srilanka and also most profitable amongst all cultivated crops. Diseases are the important factors limiting the productivity of betel leaf. Among the diseases foot and root rot caused by *Sclerotium rolfsii* is the major constraints for cultivation of the betelvine crop (Goswami et al., 2002). The fungus *S. rolfsii* is a facultative parasite and can maintain continuity of generation under adverse situation by the formation of resting structure called sclerotia. In India, stem rot caused by *S. rolfsii* is a major problem in most of states accounting for 10-11 percent yield loss (Prasad et al., 2012). In severely infected field, loss ranges from 10 to 25 percent and sometimes, it reaches up to 80 per cent (Mehan and McDonald, 1990).

The susceptibility of potential host plants varied depending on the isolates of *S. rolfsii* (Farr et al., 1989). There are several reports where various isolates of *S. rolfsii* have shown significant variations not only in their morphology but also in their pathological behavior (Sarama et al., 2002). However, pathogenicity of *S. rolfsii* in betelvine has not been well-researched especially in semi-arid tropics. This information is very useful for an effective control of this pathogen for better yield of betel leaf. Under the above facts the present piece of research was undertaken to test pathogenicity of different isolates of *S. rolfsii* causing foot and root rot disease of betelvine.

Materials and methods

Pathogenicity of 19 isolates of *S. rolfsii* isolated from foot and root rot infected stems of betelvine was tested following tissue planting method (Tuite, 1969; Mian,1995) in the year 2015 under *in-vivo* condition in a betelvine baroj. The boroj was constructed in the experimental field of the Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh. Each isolate was considered as a treatment. For each treatment three plants were used where one plant was grown in an earthen pot. The experiment was laid out in a randomized complete block design (RCBD) with 4 replications. The data of this experiment were statistically analyzed by using computer package program (Statistix 10). Duncan's multiple range test (DMRT) was performed for comparison of means of various treatments (Gomez and Gomez, 1983).

Growing Betelvine Plants

Potting medium was prepared by mixing soil, sand and well decomposed cow-dung in the proportion of 2:1:1 and were sterilized by formaldehyde. Formalin solution (4%) @ 200 ml/cft soil were mixed with the soil heap and the soil was covered with a polythene sheet for 48 hr for sterilization (Dashgupta, 1988). After 7 days, surface sterilized earthen pots were filled with the sterilized soil. Apparently healthy betelvine stem of variety Misti pan collected from Rajshahi were used to prepare cuttings. Forty centimeters long cutting having five nodes were prepared and grown in 14 inches diameter earthen pot containing potting medium at one plant per pot. The pots were placed inside a boraj and allowed to grow providing necessary care and management practices. One to two internodes below the bud point was dipped into the soil and kept touching with soil. Betelvine plants were fasten with bamboo sticks in baroj.

Inoculum preparation and inoculation with the S. rolfsii

The isolates of *S. rolfsii* were multiplied on barley grains (Gupta and Kolte, 1982). The barley grains were pre-soaked in 2% sucrose solution overnight, drained off excess solution and boiled in fresh water for 30 minutes and drained off again. These were transferred into 250 ml conical flasks @ 80 g and autoclaved at 121.6°C temperature, under 1.1 kg/cm^2 pressure for 20 minutes. The conical flasks were allowed to cool at room temperature and were inoculated with 5 mm discs of 4 days old culture of *Sclerotium rolfsii* isolates grown on PDA. Seven discs per flask were added and flasks were incubated for three weeks at 25±2°C. Similarly, ninteen isolates were separately cultured for inoculation of the betelvine plants.

After six months of plantation each isolates of causal pathogen (*S. rolfsii*) were inoculated separately. The plants were prepared for inoculation by removing top soil within 5 cm of the stem to a depth of 2 cm. A table spoon (5 g) of inoculum was placed in direct contact of entire circumference of the exposed stem. Finally, the inoculum was lightly covered with top soil for infection. The symptomology was recorded to test the pathogenicity of the causal pathogen.

Data Collection

The plant showing wilting and rotting symptoms at collar region were considered as infected plant and the plant without these symptoms were considered as healthy. The data were recorded until 30 days after inoculation on (1). days required for visible growth of mycelia on soil surface near base of the plant, (2). days required for appearance of visible disease symptom, (3). lesion length (cm) and (4). incidence of disease of foot and root rot of betelvine in pots were recorded base on total number of plants checked. The data on disease incidence were converted into arcsine values before statistical analysis.

Results and Discussion

Pathogenicity of the isolates of *S. rolfsii* causing foot and root rot disease of betelvine was tested in earthen pots, placed in betelvine baroj (orchard). The pathogenicity was determined based on mycelial growth on soil surface, early appearance of disease symptoms, lesion length and disease incidence.

Days Required for Appearance of Mycelium Growth On Soil Surface

Soil inoculated with *S. rolfsii* isolates exhibited mycelial growth on the soil surface and around the base of the plant within 2-4 days after inoculation. Only 2 days after inoculation (DAI) were required to manifest cottony colony on soil surface near root zone of inoculated betelvine plants in case of isolate-3, 5, 7, 9 and 12. The isolate-1, 2, 4, 6, 10, 11, 13, 15, 17 and 18 required 3 days and others needed 4 days to develop cottony colony on soil surface (Table 1).

Days Required for Appearance of 1st Symptom of Plants

Variable days were recorded on the appearance of first symptom of foot and root rot disease on betelvine. The first disease symptoms were observed within 6 to 16 DAI among different isolates of *S. rolfsii*, where the minimum days were required by isolate - 9 and the maximum by isolate-2 and 14 (Table 1).

Lesion Length

Lesion length ranged from 1.25 to 6.50 cm due to inoculation with different isolates of *S. rolfsii* at the base of betelvine plants. The largest lesion (6.50 cm) was observed in case of isolate-9 and Isolate-13. The smallest lesion (1.25 cm) was recorded in case of isolate-2 followed by isolate-14, 19 and 5 (Table 1). In severely infected plants, soft watery rotting symptoms and brown lesions advanced above the soil level appeared at the collar region. On the lesions, white mycelial growth having white and brown sclerotia depending on the maturity was observed (Figure 1).

Disease Incidence

The disease did not appear in uninoculated control plants at all stages of data collection. Disease incidence was 100% recorded from plants inoculated with isolate-8 and 9 at 15 DAI; with isolate-8, 9,11,12 and 15 at 20 DAI; with isolate-3, 4, 7, 8, 9,11, 12, 13, 15, and 16 at 25 DAI; and isolate-3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16 and 18 at 30 DAI (Table 2).

Table 1. Effect of various isolates of *Sclerotium rolfsii* on days to appear mycelium on soil, days to appear disease symptom and lesion length on stem of betelvine plant in pot culture.

Isolates	Days to appear mycelium on soil at DAI	Days to appear disease symptom at DAI	Lesion length on stem at 20 DAI*
Isolate-1 (BGPBSr-1)	3	12	$2.00^{fgh\alpha}$
Isolate-2 (BGPBSr-2)	3	16	1.25^{h}
Isolate-3 (BGPBSr-3)	2	10	3.25^{de}
Isolate-4 (BGPBSr-4)	3	12	2.25^{efgh}
Isolate-5 (BGPBSr-5)	2	10	1.75^{gh}
Isolate-6 (BGPBSr-6)	3	6	5.25^{bc}
Isolate-7 (BGPBSr-7)	2	8	4.50^{c}
Isolate-8 (JKPBSr-1)	3	7	5.25^{bc}
Isolate-9 (JKPBSr-2)	2	6	6.50^{a}
Isolate-10 (JKPBSr-3)	3	8	3.00^{ef}
Isolate-11 (KMPBSr-1)	3	8	4.25^{cd}
Isolate-12 (KMPBSr-2)	2	7	6.00^{ab}
Isolate-13 (KMPBSr-3)	3	7	6.50^{a}
Isolate-14 (RMPBSr-1)	4	16	1.50^{gh}
Isolate-15 (RMPBSr-2)	3	7	4.50^{c}
Isolate-16 (RMPBSr-3)	4	7	5.00^{bc}
Isolate-17 (CSPBSr-1)	3	12	2.50^{efg}
Isolate-18 (CSPBSr-2)	3	8	3.25^{de}
Isolate-19 (CSPBSr-3)	4	14	1.50^{gh}
Control (No isolates)	-	-	-

$^{\alpha}$Values within the same column with a common letter(s) do not differ significantly (P = 0.01), Note: *DAI = Days after inoculation; BG = Barisal-Gouronadi, PB = *Piper betle*, Sr = *Sclerotium rolfsii*, CS = Chittagong-Sitakunda, JK = Jhenaidah-Kaligonj, KM = Kushtia-Mirpur and RM = Rajshahi-Mohanpur

Table 2. Incidence of foot and root rot due to inoculation with various isolates of *Sclerotium rolfsii* at 10-30 days after inoculation

Isolates	Disease incidence (%) at days after inoculation (DAI)				
	10	15	20	25	30
Isolate-1 (BGPBSr-1)	$0.00^{g\alpha}(0.083)^{\beta}$	$41.66^{fg}(38.71)$	$41.66^{cd}(38.71)$	$66.66^{de}(52.79)$	$83.33^{bc}(69.81)$
Isolate-2 (BGPBSr-2)	$0.00^{g}(0.083)$	$0.00^{h}(0.083)$	$33.3^{d}(34.02)$	$66.66^{de}(52.79)$	$83.33^{bc}(69.81)$
Isolate-3 (BGPBSr-3)	$41.66^{ef}(38.71)$	$83.33^{bc}(69.81)$	$91.7^{a}(78.32)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-4 (BGPBSr-4)	$0.00^{g}(1.59)$	$58.33^{ef}(48.10)$	$75.0^{b}(61.31)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-5 (BGPBSr-5)	33.3f(34.02)	$33.33^{g}(34.02)$	$41.7^{cd}(38.71)$	$83.33^{bc}(69.81)$	$100.0^{a}(86.82)$
Isolate-6 (BGPBSr-6)	$50.00^{def}(43.41)$	$66.66^{de}(52.79)$	$66.7^{bc}(52.79)$	$75.00^{cd}(61.31)$	$100.0^{a}(86.82)$
Isolate-7 (BGPBSr-7)	$66.70^{cde}(52.79)$	$75.0^{cd}(61.31)$	$91.7^{a}(78.32)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-8 (JKPBSr-1)	$75.0^{abc}(65.12)$	$100.0^{a}(86.82)$	$100.0^{a}(86.8)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-9 (JKPBSr-2)	$91.7^{ab}(78.32)$	$100.0^{a}(86.82)$	$100..0^{a}(86.82)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-10 (JKPBSr-3)	$33.30^{f}(34.02)$	$66.66^{de}(52.79)$	$75.0^{b}(61.31)$	$58.33^{ab}(78.32)$	$100.0^{a}(86.82)$
Isolate-11 (KMPBSr-1)	$66.7^{cde}(52.79)$	$91.67^{ab}(78.32)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-12 (KMPBSr-2)	$66.7^{bcd}(56.61)$	$83.33^{bc}(69.81)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-13 (KMPBSr-3)	$83.3^{ab}(69.81)$	$91.67^{ab}(78.32)$	$91.7^{a}(78.32)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-14 (RMPBSr-1)	$0.00^{g}(0.083)$	$0.00^{h}(0.083)$	$33.3^{d}(34.02)$	$58.3^{e}(48.10)$	$75.0^{cd}(61.31)$
Isolate-15 (RMPBSr-2)	$83.30^{a}(69.81)$	$100.00^{a}(86.82)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-16 (RMPBSr-3)	$66.7^{cde}(52.79)$	$66.66^{de}(52.79)$	$91.7^{a}(78.32)$	$100.0^{a}(86.82)$	$100.0^{a}(86.82)$
Isolate-17 (CSPBSr-1)	$0.00^{g}(0.083)$	$33.33^{g}(34.02)$	$66.7^{bc}(52.79)$	$83.33^{bc}(69.81)$	$91.67^{ab}(78.32)$
Isolate-18 (CSPBSr-2)	$33.3^{f}(34.02)$	$33.33^{g}(34.02)$	$50.0^{cd}(43.41)$	$66.66^{de}(52.79)$	$100.0^{a}(86.82)$
Isolate-19 (CSPBSr-3)	$0.00^{g}(0.083))$	$33.33^{g}(34.02)$	$50.0^{cd}(43.41)$	$66.66^{de}(52.79)$	$66.66^{d}(52.79)$
Control (No isolates)	$0.00^{g}(0.083)$	$0.00^{h}(0.083)$	$0.0^{e}(0.083)$	$0.00^{f}(0.083)$	$0.00^{e}(0.083)$

$^{\alpha}$Values within the same column with a common letter(s) do not differ significantly (P = 0.01), $^{\beta}$Values with parentheses are arc-sign transformed values.

The highest disease incidence of 91.7% was observed when the plants were inoculated with the Isolate-9 followed by Isolate-15 (83.3%) and isolate-13 (83.3%) at 10 days after inoculation. No disease incidence was observed when plants were inoculated with Isolate-1, 2, 4, 14, 17 and 19 after 10 days of inoculation (Table 2). The untreated control pot did not show any symptom upto crop maturity.

Pathogenicity of the isolates of *S. rolfsii* isolated from foot and root rot infected betelvine plants collected from different areas was tested in earthen pots, which were placed in betelvine baroj. Out of 19, fourteen isolates caused 100% disease incidence in inoculated plants. Most of the isolates tested were pathogenic but some of them delayed disease development. Based on minimum days required for the appearance of mycelium growth on soil surface and for appearance of 1st symptom of plants and maximum lesion length and disease incidence, the isolate-9 (JKPBSr-2) collected from Kaligonj upazilla was noted as the most virulent.

Figure 1. Air dried colonized barley grains used as inoculum

(A); Barley grains colonized with Sclerotium rolfsii in conical flasks (B); preparation of betelvine plant in plot and pot soil for the inoculation (C); mycelium of S. rolfsii appeared on the soil surface (D); infected plant (E); Sclerotia of S. rolfsii developed on the soil surface around the stem base of betelvine plant (F, G).

The findings of pathogenicity tests were in agreement with the findings of other researchers. Sommat *et al*. (1982) made an investigation on the pathogenicity of *S. rolfsii* and found that the pathogen could infect its host (cotton) severely. Siddaramaiah (1988) proved the pathogenicity of *Sclerotium rolfsii* on cardamon 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.

Conclusion

The findings clearly indicated that all the19 isolates of *S. rolfsii* were found to be pathogenic causing foot and root rot disease of betelvine and the isolates were sharply varied in terms of degree of pathogenicity. The disease might be destructive to the crop at or before harvesting stage. The isolate-9 (JKPBSr-2) collected from Kaligonj upazilla of Zhenaidah district was noted as the most virulent.

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Conflict of Interest

The authors declare no conflict of interest.

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