

**BOLL ROT OF COTTON (*Gossypium hirsutum* L.) CAUSED BY  
*RHIZOPUS ORYZAE* WENT & PRINS. GEERL.-  
A NEW RECORD IN BANGLADESH**

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Cotton plant (*Gossypium hirsutum* L.) belonging to the family Malvaceae is an important fiber yielding plant throughout the world. During the period of January and February 2013, severe boll rot symptom was noticed on cotton varieties CB-1 to CB-11 those were grown in the Botanic Garden, University of Dhaka. *Rhizopus oryzae* Went & Prins. Geerl. was diagnosed as causal agent of boll rot of cotton. Among the cotton varieties examined CB 6 and CB 11 were found severely attacked by the fungus showing boll rot symptom. Disease severity of fruit recorded 0-6 scale. Prevalence of *R. oryzae* was 100%. Present investigation revealed that *R. oryzae* was pathogenic to cotton. The boll rot of cotton caused by *R. oryzae* is new record in Bangladesh.

Cotton plants suffer from more than 20 fungal diseases throughout the world (Wikipedia, 2013). In Bangladesh, the most common diseases of the crop are anthracnose, Alternaria leaf spot, Cercospora leaf spot, Rust, Sclerotium rot, Wilt, and Boll rot (Lutfunnessa and Shamsi, 2011). Among the diseases, boll rot is considered as the most destructive one. In the USA, at least 170 microorganisms are capable of causing cotton boll rot. The majority of the damage is attributed to the species of *Fusarium*, *Diplodia*, *Glomerella*, *Xanthomonas*, *Rhizopus* and *Alternaria* (Guthrie *et al.*, 1994). *Sclerotium rolfsii* is identified as one of the causes of boll rot in Bangladesh (Shamsi *et al.*, 2008). Considering importance of the disease, the present investigation was conducted to identify fungal pathogens associated with the disease under Bangladesh conditions.

During the period of January and February 2013, cotton varieties CB-1 to CB-11 those were grown in the Botanic Garden, University of Dhaka, exhibit a high level of boll rot infection.

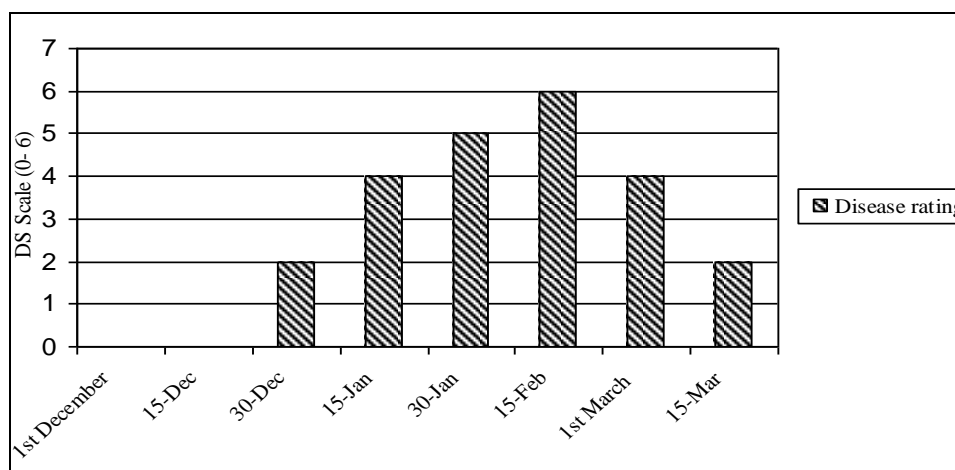
For visual estimation of severity of infected fruit, 0-6 point scale has been designed following 0 – 9 point scale used for rating of all foliar diseases studied (PDI=McKinney's Index, Ghosh *et al.*, 2009). No infection – 0, 1 = upto 10% fruit area infected, 2 = 10 – 20% fruit area infected, 3 = 20 – 30% fruit area infected, 4 = 30 – 40% fruit area infected, 5 = 40 – 50% fruit area infected, 6 = more than 50% fruit area infected.

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Cotton plants of varieties CB1 to CB11 having boll rot infected fruits were selected. Disease symptoms and DS observed on naturally infected boll were recorded (Fig. 1). Boll rot infected fruit samples of cotton were collected for further studies. The fungus associated with the diseased samples was isolated following tissue planting method (CAB, 1968). The samples were washed with fresh water, cut into small pieces (2 mm × 2mm) having 50% healthy tissues, surface sterilized with 10% chlorox for 3-5 minutes and rinsed with sterilized distilled water for three times. Surface sterilized inocula were placed in Petri dishes containing potato dextrose agar (PDA) at 3 inocula per plate. The inoculated plates were incubated at 25±1° C for 3 – 5 days. Fungus grew from the specimens was transferred to petridishes containing fresh PDA. After sporulation, temporary mount was prepared and observed under a compound microscope. Morphological characters of hyphae, sporangiophores, sporangia and sporangiospores of the fungus grew from the specimens were recorded. Pathogenicity of the fungus was also performed following detached fruit inoculation method (Shamsi *et al.*, 2010).

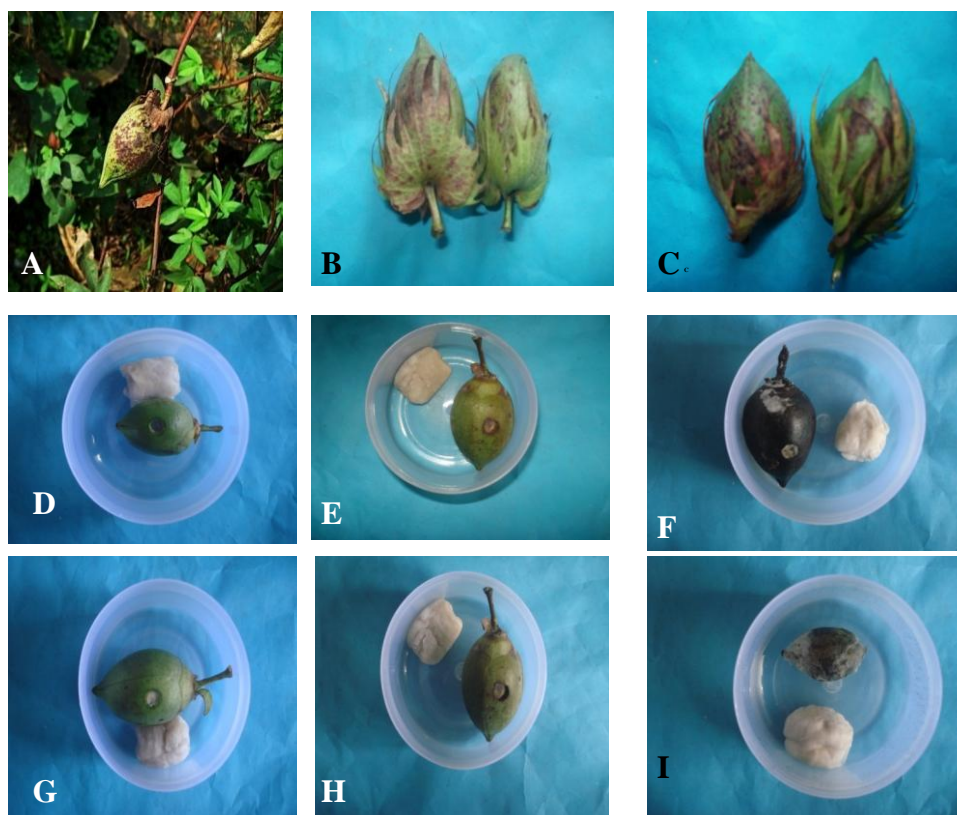
Boll rot disease of cotton is one of the notorious disease of cotton. In the present investigation, *Rhizopus oryzae* Went & Prins. Geerl. (= *R. arrhizus* Fisher) was first time isolated from boll rot infected cotton variety CB6 and CB11. Disease severity of boll rot was recorded at (0-6) DS scale during the months of December 2012 to March 2013. Highest severity of the boll rot disease was recorded as DS-6 in the second week of February followed by DS-5 in 30<sup>th</sup> January. Lowest DS-2 was recorded in 15<sup>th</sup> February and 15<sup>th</sup> March (Fig. 1).



**Fig. 1. Disease severity of cotton boll during 15 December 2012-15 March 2013.**

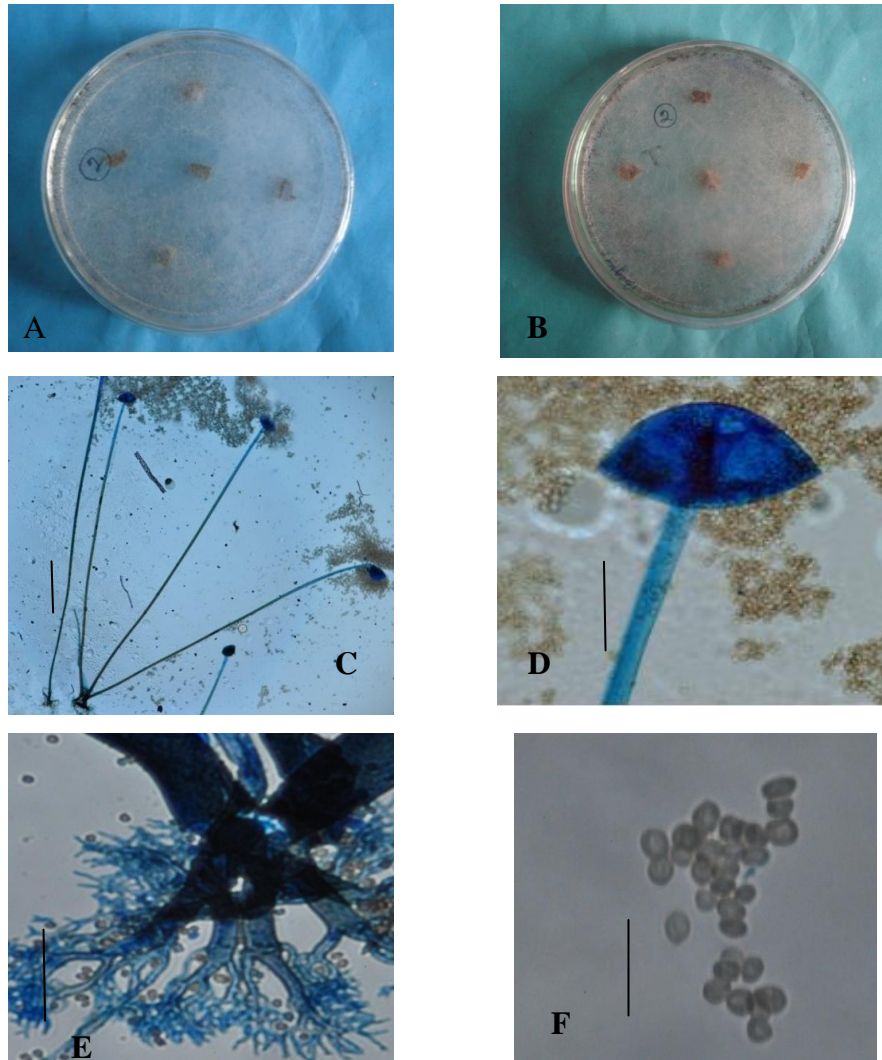
A zygomycetous fungal species was frequently associated with rotted boll of cotton with highest prevalence on CB-6 and CB-11. Comparing characters with appropriate Key books, the fungus was identified as *Rhizopus oryzae* Went &

Prins. Geerl. (Barnett and Hunter, 2000 and Schipper, 1984). Colonies of the fungus was white cottony, transparent at first becoming blackish-grey depending on the amount of sporulation. Very fast growing at 25<sup>0</sup> C temperature, fluffy, reaching 5-8 mm in height in PDA medium. Sporangiphore and sporangia developed within 72 hours of inoculation and within subsequent three hours inoculated plates were completely covered by the sporulating structures of the fungus. Sporangiphores are up to 1500 µm in length and 18 µm in width, smooth walled, non-septate, mostly unbranched, arising from stolons opposite to rhizoids usually in groups of 3 or more. Rhizoids are medium sized, local swellings and dichotomous branching present. Sporangia are globose, often with a flattened base, grayish black, powdery in appearance, up to 175 µm in diameter containing many spores. Columella are globose, subglobose or oval, up to 130 µm, soon collapse to an umbrella-like form after spore release. Sporangiospores are angular, subglobose, hyaline and aseptate (Fig. 2).



**Fig. 2.** Photograph showing : A. Naturally infected cotton boll caused by *R. oryzae*; B. Infected boll of CB- 6; C. Infected boll of CB-11; D. Uninoculated cotton boll of CB-6 (control) ; E. Inoculated cotton boll of CB- 6 after 3 days; F. Inoculated cotton boll of CB- 6 showing rot symptom after 7days of inoculation; G. Uninoculated cotton boll of CB- 11 (control); H. Inoculated cotton boll of CB- 11 after 3 days; I. Inoculated cotton boll of C-9 showing rot symptom with mycelial mat of *R. oryzae* after 7days of inoculation.

*Rhizopus oryzae* isolated from cotton boll rot was found to be pathogenic on the crop. After artificial inoculation the isolated fungus developed symptom similar to the symptom recorded from naturally infected boll (Fig. 3).



**Fig. 3.** Photograph showing *Rhizopus oryzae*: A. 3 days old colony of *Rhizopus oryzae*; B. 7 days old Colony of *R. oryzae*; C. Sporangium and sporangiophore; D. Sporangium with sporangiospores; E. Rhizoids and F. Sporangiospores, [Bars = 100  $\mu$ m (C), 50  $\mu$ m (D, E), 20  $\mu$ m (F)]

Boll rot exhibit a high level of complexity in its symptomology and pathogenicity. Present investigation estimated that 75% of total fruit loss due to boll rot symptom caused by *R. oryzae*.

Cotton boll is an excellent substrate for the growth of fungi. So far *Ascochyta gossypii*, *Colletotrichum gossypii*, *Fusarium* spp., *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl, *Botryosphaeria rhodina* (Cooke) Arx, *Phytophthora* spp. and *Rhizoctonia solani* have been reported from boll rot symptom. *Sclerotium rolfsii* was reported on stem and root of infected cotton. From Bangladesh, *Sclerotium rolfsii* was isolated from infected cotton boll of CB3 for the first time (Shamsi *et al.*, 2008). In the present study, *R. oryzae* was the exclusive fungal species isolated from infected inocula and it was found to be pathogenic on inoculated fruits.

The boll rot of cotton caused by *R. oryzae* is new record in Bangladesh and literature search does not show reports of its presence in other parts of the world where cotton is cultivated.

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