DEVELOPMENT OF RICE FALSE SMUT (*USTILAGINOIDEA VIRENS*) SYMPTOMS UNDER THE ENVIRONMENTAL CONDITIONS OF BANGLADESH

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ABSTRACT

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Visible symptoms of rice false smut (RFSm) disease, types and time of appearance, and their seasonal distribution, provide an opportunity of fundamental understanding the disease cycle, and formulating first-step management interventions. Although rice false smut is not a new disease, its symptomology is not well investigated in Bangladesh. The present investigation was an attempt to find out symptom development of the disease under Bangladesh environment with their distribution within and between the seasons, and their time of appearance and development on host. This Multi-season and multi-year research were conducted in the Central Experimental Farm of Bangladesh Rice Research Institute, Gazipur, through intensive field-by-field monitoring. The RFSm disease produced symptoms on infected rice panicles through development of two distinct types of smut balls - orange and olivaceous greenish-black. The two ball-types were not the transformation of one another. The earliest symptom appeared around one week after panicle emergence as 'radiating white-belly' within infected spikelets; from this stage, it took 12 days to become fully developed smut balls. Appearance of the two ball-types was time-specific and was specifically influenced by temperature. Smut balls were recorded on the panicles of the regenerated tillers (known as 'ratoons') in the harvested hills, recorded as the first report in Bangladesh. Various insects were attracted to young, slightly sweetish, smut balls. The results provide the first step of understanding the disease symptoms which will aid in studying the disease cycle of rice false smut under the environmental conditions of Bangladesh.

Key words: Rice false smut, symptom, orange and olivaceous greenish-black smut ball, white smut ball, ratoon infection, insect's attraction, *Ustilaginoidea virens*, *Villosiclava virens*

INTRODUCTION

Like many other countries of the globe, rice false smut (RFSm) is increasingly becoming a disease of concern in Bangladesh. With gradual increasing incidence of the disease reported from farmers' fields since early 2010s, RFSm is now considered as an emerging disease in Bangladesh (Dhaka Tribune 2013; Nessa *et al*. 2015). The adoption of 'BRRI dhan49', a promising genotype for 'T. Aman' season in the country, is being seriously hindered by this disease (Kabir *et al*. 2015).

Rice false smut is a fungal disease (anamorph: *Ustilaginoidea virens* (Cooke) Takah.; teleomorph: *Villosiclava virens* (Nakata) E. Tanaka & C. Tanaka) was first reported in 1878 based on a specimen from Tirunelveli, Tamil Nadu, India by Cooke (Cooke 1878 cited by Tanaka *et al*. 2008). Tanaka and his associates (2008) elaborately described the nomenclature of the anamorph and teleomorph stages of the fungus. In brief, the original disease specimen was wrongly considered as 'a rice smut' and Cooke (1878) named the causal fungus as *Ustilago virens*. Eight years later, based on specimens collected in Japan, Patouillard (1887) independently named the fungus as *Tilletia oryzae*. Brefeld (1895) reported that *Tilletia oryzae* was an ascomycete which belonged to the fungi imperfecti, and not to the basidiomycete family *Ustilagineae* and proposed a new anamorphic genus *Ustilaginoidea* based on the characteristics of the conidial stage on rice and on *Setaria crus-ardeae*; Brefeld (1895) named the two species *U. oryzae* and *U. setariae*, respectively. Takahashi (1896) discovered that *Ustilaginoidea oryzae* was identical to *Ustilago virens*, and thus combined the scientific name as *Ustilaginoidea virens*, which is the current nomenclature of anamorphic stage of the fungus. According to scientific classification, the fungus is an

ascomycete, not a basidiomycete ('true smut'), hence received its common name as 'false smut'.

Rice false smut is an inflorescence disease. The symptom of RFSm disease only appears when rice crop is in flowering stage. Nessa *et al*. (2016) observed rice false smut balls predominantly formed on the lower half of the infected panicles. The fungus attacks individual grains. Upon infected, the grain turns into a large velvety mass (also known as 'pseudomorph'), which fully encloses the floral parts. This pseudomorph is commonly called as 'smut ball' (Guo *et al*. 2012) or 'spore ball' (Hashioka *et al*. 1951). Smut balls are initially silvery-white in colour, which turns yellow or orange and finally acquire dark green or almost black colour (Quintana *et al.* 2016). Because of the greenness in colour, smut balls are also termed as 'green balls' (Tanaka *et al*. 2008; Jecmen and TeBeest 2013). The balls are also known as 'pseudosclerotia' (Hedge *et al*. 2000; Tanaka *et al*. 2008) because of their physical hardness.

Diseases, caused by plant pathogens, express with a range of symptoms. Clear understanding on disease-specific symptoms, their types and time of appearance, their seasonal distribution, and relationship with climate is the first step for designing and formulating management interventions. Although RFSm is an ancient disease, its symptomology is not yet well investigated in Bangladesh.

With the above background, the present piece of research was undertaken to identify the symptoms ofrice false smut disease in Bangladesh, to study their distribution within and between the seasons, and their time of appearance and development on host and underlying weather influence.

MATERIALS AND METHODS

This multi-season and multi-year study were conducted in the 'West Byde' and 'East Byde' sections of the experimental farm of the Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh, located at 23°59′ N latitude, 90°24′ E longitude. Intensive field-by-field monitoring was carried out to record the symptom of smut balls during flowering to maturity period of 'Boro' (April to May of 2015, 2016 and 2017), 'Aus' (August to September of 2015, 2016 and 2017) and 'T. Aman' (October to December of 2014, 2015 and 2016) rice seasons. Monitoring included observation and recording of appearance of early symptom and types of smut balls, smut ball development steps and durations, presence of smut balls on ratoons, and visits of insects on smut balls.

Types of fresh smut balls were collected during monitoring. Each collected smut ball was longitudinally cut into two halves by sharp blade. The longitudinal sections of smut balls were photographed and internal structure was studied.

The temperature influence was measured on the time of appearance of specific symptoms, for which daily weather data were accessed from the Plant Physiology Division of BRRI.

RESULTS AND DISCUSSION

The symptom of rice false smut disease

The typical symptom of rice false smut disease appeared as popped roundish transformation of individual infected spikelets of rice panicles - the 'spore balls', often called 'smut balls'. Two types of such balls by colour were recorded: orange (O) (Fig.1a), and olivaceous greenish-black (OGB) (Fig. 1b). In addition, in a surprise discovery, two white smut balls (WSm) were identified on 28 November 2018) in a field in the West Byde section of BRRI headquarter research station (Fig 2). The WSm is believed to be *Ustilaginoidea albicans*, not *Ustilaginoidea virens*, as described by Honkura *et al*. (1991), Wang and Bai (1997) and Jecmen and TeBeest (2013). The symptomology of the white smut balls has not been further described in this paper.

Between the two types RFSm symptoms, olivaceous greenish-black balls, in general, were larger than the orange ones. The early symptom of RFSm was clearly visualized in between lemma and pelea around one week after emergence of the infected panicle (Fig. 3). However, through intense observation, radiating white-belly within infected spikelets was identified as the preliminary stage of the early symptom (Fig. 3 [inset]).

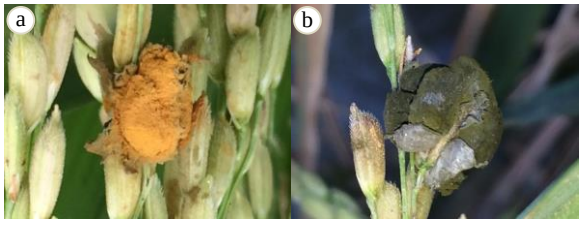

Figure 1. Identified two types of smut balls of rice false smut disease, (a) orange (O) and (b) olivaceous greenish-black (OGB).

Figure 2. White smut balls (WSm) found in West Byde, BRRI experimental farm in Gazipur on 28 Nov 2018 having similar appearance to those described as *Ustilaginoidea albicans* by Honkura *et al*. 1991; Wang and Bai 1997; Jecmen and TeBeest 2013.

Figure 3**.** Visualized early symptom of rice false smut disease (arrow heads) on an infected panicle around one week after panicle emergence. Inset shows 'radiating white-belly' symptom inside the infected spikelet (yellow arrow) and healthy spikelet (white arrow).

Development of smut balls

The full-size smut ball formation took about 12 days after initiation of the symptom (Figs. 4 and 5). The infected spikelet first turned whitish, and then erupted through lemma and pelea. The balls were covered by a membrane. The membrane contained both spores and mycelia.

Orange smut balls

Young orange smut balls were flattened, smooth, white, creamy white or light yellow in colour (Fig. 4a). The membrane covering the smut balls ruptured and orange chlamydospores exposed (Fig. 4b). The chlamydospores remained loosely attached at the surface of the balls (Fig. 4c). The orange chlamydospores subsequently turned into rusty, burnt orange or dark brown in colour over time (Fig. 4f).

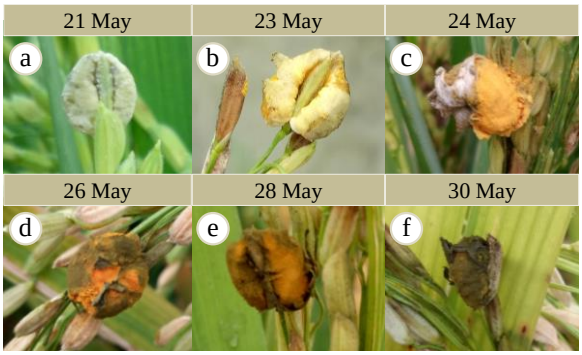

Figure 4. Steps of orange smut ball development in nature captured during 21 to 30 May 2017; letters a-f denote for the dates of observation (shown above corresponding photos). The ball on the first shown symptom (a) was about five day-olds (considering 'radiating white-belly' symptom as day-one).

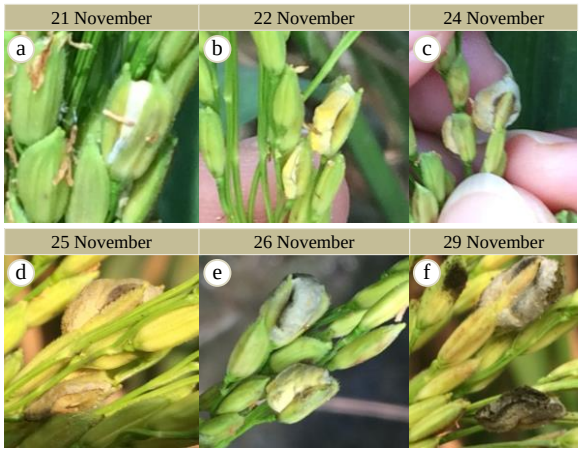

Figure 5**.** Steps of olivaceous greenish-black smut ball development in nature captured during 21 to 29 November 2014 in the study area; letters a-f denote for the dates of observation (shown above corresponding photos). The ball on the first shown symptom (a) was about four day-olds (considering radiating white-belly symptom as day-one).

Olivaceous greenish-black smut balls

Young olivaceous greenish-black smut balls were flattened, smooth, pale white, light yellow or light to dark ash in colour (Fig. 5a-c). The membrane covering the balls ruptured and dark-olive chlamydospores

exposed (Fig. 5d-e). The chlamydospores remained loosely attached at the surface of the smut balls (Fig. 5f). The dark-olive chlamydospores subsequently turned into dark green or sometimes black in colour over time (Fig. 5f).

In a separate set of observations, the first appearance of 'creamy white' smuts balls were recorded mostly between 10 and 12 days after panicle emergence. However, in few circumstances the appearance of such balls delayed up to 20 days after panicle emergence (Fig. 6).

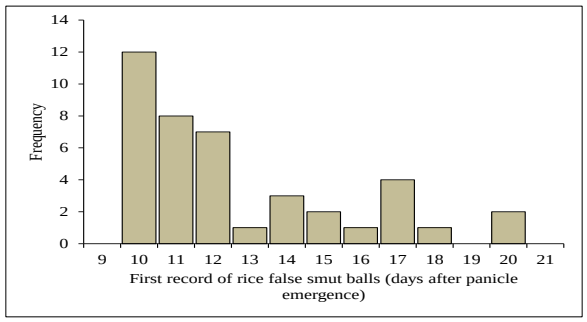

Figure 6. Frequency distribution of the first record 'creamy white' smut balls in 41 fields during 'T. Aman' season of 2014 in the study site.

Internal structure of orange and olivaceous greenish-black smut balls

In orange smut ball, the internal structure showed three distinct layers: the innermost layer was white, followed by a yellowing layer and the outer layer was orange in colour (Fig. 7a).

In olivaceous greenish-black smut ball, the internal structure showed four distinct layers: the innermost layer was white, followed by a pale-yellow layer, then a layer of orange-yellow and the outer most layers were olivaceous greenish-black in colour (Fig. 7b).

The internal structure of over-aged orange smut ball showed only two layers: white innermost layer and burnt dark brown outer layer (Fig. 8a).

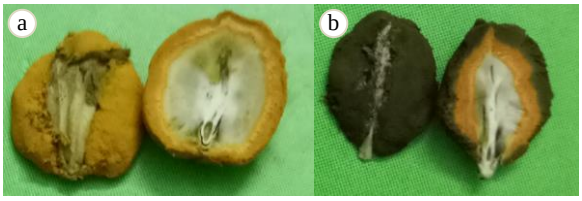

Figure 7. Internal structure of orange (a) and olivaceous greenish-black (b) smut ball of rice false smut disease.

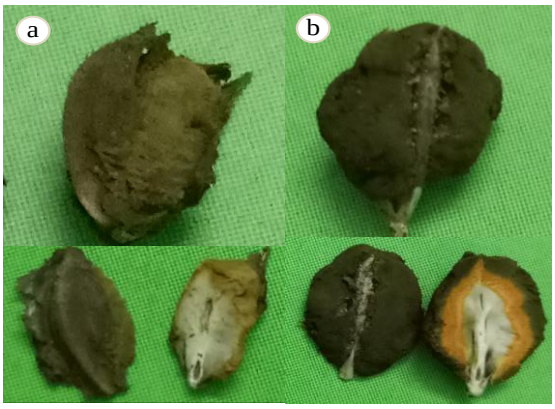

Figure 8. Internal structure of over-aged orange (a) and olivaceous greenish-black (b) smut ball of rice false smut disease.

Appearing time of orange and olivaceous greenish-black smut balls

The appearing time of orange and olivaceous greenish-black balls was also different in rice false smut disease. Orange balls appeared in all three rice growing seasons – 'Boro', 'Aus' and early 'T. Aman' (during October and early November). On the other hand, olivaceous greenish-black balls rarely observed in early 'T. Aman', and they were totally absent in 'Boro' or 'Aus' season. It only appeared in late (mid-November onwards) 'T. Aman' crops. Exceptionally though, in the second week of November 2014, few orange balls transformed into blackish colour (Fig. 9), but olivaceous greenish-black balls were never transformed into orange balls.

Temperature threshold for olivaceous greenish-black formation and turning orange smut balls into blackish colour

The first appearance of greenish-black ball formation occurred around 10 November, 24 October and 20 November in 2014, in 2015 and 2016, respectively. During the same time (10 November, 2014), some orange smut balls also transformed into blackish colour. The figure below (Fig. 10) indicates, that were the times when the difference of day-night temperature started to rise above 11C (calculated as 10-day moving average). We have termed this regime as 'threshold temperature' for formation of olivaceous greenish-black ball, and switching of the colour of orange ball to blackish.

Presence of smut balls on ratoons

In addition to main crops, in a surprising observation, the RFSm was recorded on 'ratoons' (re-generated tillers) in the harvested hills (Fig. 11).

Figure 9. Transformation of orange smut balls into blackish-coloured smut balls of rice false smut disease on two separate samples gathered from experimental site during 11 – 26 November, 2014.

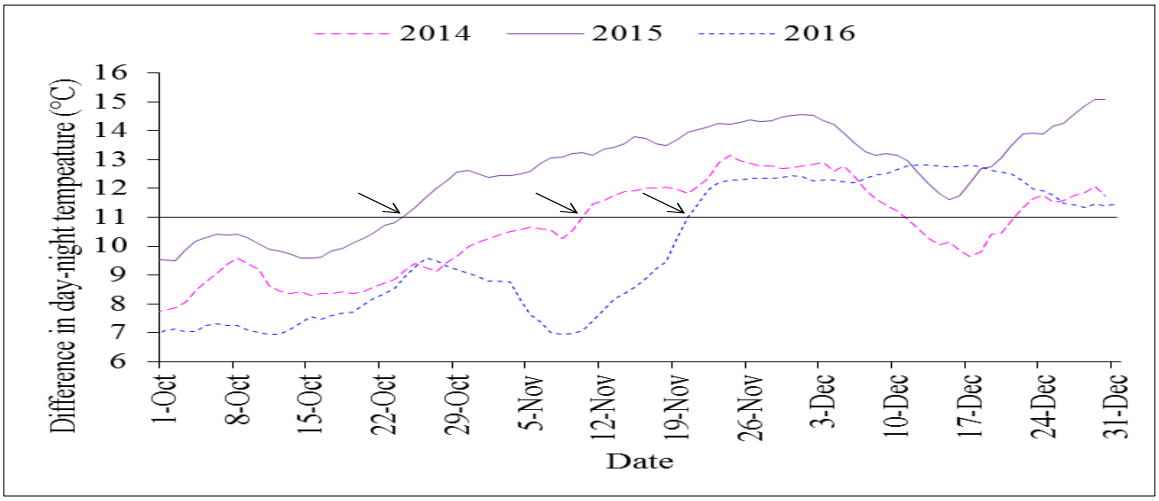

Figure 10. Difference in day-night temperatures, presented as 10-day moving average, during maturity period of T. Aman rice (October to December) in the study area in three years. The arrows show the start date of temperature difference above 11C in respective years.

Figure 11. Symptom as smut balls (circle) of rice false smut disease in the ratoons. Rectangles indicate the points of originating ratoons from where the tillers of the main crop were harvested.

Smut balls attracted by insects

Smut balls were found attracted by various insects, such as ant, fly and larvae of insects. Among those, ants were very much attracted to young smut balls (Fig. 12). Young smut balls (similar to Fig. 4(b) and 5(d)) when tasted by tongue found slightly sweetish.

Figure 12. Various insects found visiting on rice false smut balls in the experimental site.

Two types of smut balls by borne – orange (O) and olivaceous greenish-black (OGB) - were identified in the present study. Ikegami (1961) reported a similar divergence of smut ball appearance on infected The appearance of orange and 'OGB' type smut ball was time-specific. For example, the orange smut balls appeared in all three rice growing seasons, while the 'OGB' were only evident during late ''T. Aman'' season with lowering atmospheric temperature when the difference of day-night temperature started to rise above 11C (termed as 'threshold temperature'). This threshold also switches the orange ball to blackish colour. Ikegami (1961) also observed that smut balls formed under conditions of 25C showed the highest percentage of yellow balls, whereas high occurrence of greenish balls observed under temperatures lower than 25C. There were two pathways of colour formation in smut balls, according to Ikegami (1961): one, appearance of the colour at the time just after formation of the balls (according to inner alterations), and the other, colour changing after occurrence induced by environment (light and temperature).

Rani (2014) observed yellow balls having three layers (thick yellowing brown in the outermost, light yellow in the middle and white in the centre), whereas green ones had two layers (dark green in the outermost and yellow and white in the centre). This study also identified orange smut balls having three distinct layers of internal structure; but over-aged orange smut ball showed only two layers: white innermost layer and burnt dark brown outer layer. On the other hand, the internal structure of olivaceous greenish-black smut ball showed four distinct layers, chronologically olivaceous greenish-black, orange-yellow, pale-yellow and white from outer to centre.

panicles in Japan. He classified smut balls by colour as yellow, yellowish-green, greenish-yellow and green. Some literature term yellow balls as orange or orange-yellow (Rani 2014) and green as greenish–black or black (Rani 2014; Quintana *et al*. 2016) and presented dark balls (green or greenish-black) as the advance stage (by colour change over time) of light-coloured (yellow or orange balls). Most of the literature reveals that the smut balls show up as orange by rupturing a silvery-white membrane and exposing orange chlamydospores, which initially become yellow-orange and subsequently turning green, olive-green, and then greenish black at maturity (Lee and Gunnell 1992; Kim and Park 2007; Abbas *et al*. 2014; Jecmen 2014). Present investigation shows that 'OGB' smut ball was not the advance stage of orange smut ball, rather 'OGB' smut ball borne as 'OGB' smut ball. Usually, orange smut ball develops into burnt-orange or dark-brown over time. Ikegami (1961) also found similar result that the colour was essentially age-dependent, the aged the balls were, the darker was the colour.

Literature report existence of 'white' smut balls, which was first reported in Japan (Honkura *et al*. 1991), and subsequently in China (Wang and Bai 1997) and in the USA (Jecmen and TeBeest 2013). This study also identified couple of such type smut balls for the first time in Bangladesh, and likely the first report in South Asia. The white smut balls have been described as *Ustilaginoidea albicans*, not *U. virens* (Honkura *et al*. 1991; Wang and Bai 1997; Jecmen and TeBeest 2013).

Early symptom visualised in this study under natural field condition in around one week after panicle emergence (PE) as 'radiating white-belly' within infected spikelet. In artificial inoculation, Fan *et al*. (2015) recorded such symptoms around 10 days after PE (when the inoculation was done about a week before PE). The full-size smut ball formation observed in the study in about 12 days after initiation of the symptom, indicating the process completed in 19 days after PE. In artificial inoculation, Fan *et al*. (2015) recorded full size balls around 21 days after PE. Abbas *et al*. (2014) report that smut balls emerge about 20 days after the initial infection of spikelets of the rice panicle during flowering.

The present study recorded smut balls on the panicles of the regenerated tillers (otherwise known as ratoons) in the harvested hills. Recently, Rashmi *et al*. (2016) have reported existence of smut balls on ratoons in Kerala, India. The epidemiology of RFSm with respect to point of entry of the pathogen into plant is still on debate. A series of recent papers being successful in causing artificial inoculation at booting stage before

heading (Ashizawa *et a*l. 2012) led to the opinion that spores (ascospores or chlamydospores) enter into the booting sheath along with water flowing on the top leaves. Guo *et al*. (2012) view the hypothesis that water flowing brings the primary infection source into the sheath is consistent with the observation that the disease is much more severe when rice heading stage is located in rainy and high humidity days. In the present study, there was no rainfall recorded during mid-November 2014 to early-January 2015 in the ratoon infection period, and most of the symptom producing ratoons either lacked or had degenerated flag leaves (Nessa *et al*. 2018). Furthermore, false smut balls predominantly form on the lower half of the infected rice panicles (Nessa *et al*. 2016). Under these circumstances, the hypothesis of "water flowing brings the primary infection source into the sheath along with water flowing on the top leaves" is probably not correct. Various insects were found scavenging on smut balls; the smut balls appeared to be sweetish and not poisonous to those insects.

The results of the present investigation clearly reveal chronological development of rice false smut symptoms with the variations of rice growing season, temperature and other environmental conditions of Bangladesh. Orange and olivaceous greenish-black coloured smut balls are the typical symptoms of rice false smut disease. The smut balls visibly appear around one week after panicle emergence, which took 12 days to become fully developed. Olivaceous greenish-black coloured smut balls are not the transformation of orange smut balls and their internal structure is also different.

Temperature influences the appearance of the two ball-types - orange smut ball appears when the difference of day-night temperature remains below 11C, while the difference becomes above 11C the olivaceous greenish-black smut ball appears. The 11C, the 'threshold temperature', switches the orange ball to blackish colour.

From the identification of two distinct types of smut balls, research question arises whether the spores produced by the ball-types have equal virulence, and they are different by strain. As the ball-types are not the transformation of one another and their appearance is time-specific in relation to temperature, what individual role they play in inoculum build-up to carry the disease over the next season. Given the symptoms appear as early as one week after panicle emergence, the effectiveness of post-heading fungicide application may need to re-think as infection has already taken place. Position of the false smut balls on the lower half of the infected rice panicles (Nessa *et al*. 2016) and ratoon infection with lacked or degenerated flag leaves condition is completely reverse proposition of the hypothesis "water flowing brings the primary infection source into the sheath along with water flowing on the top leaves" as there was no rainfall recorded during the ratoon infection period. Finally, insects' attraction to smut balls and its slightly sweetish taste provides a sense that the balls are sweetish and non-poisonous to them.

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