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Sclerotia of rice false smut disease in Bangladesh

Bodrun Nessa[1,2*], Moin Us Salam[3], Md Abul Kashem[4]

[1]Department of Plant Pathology and Seed Science, Sylhet Agricultural University, Sylhet 3100, Bangladesh
[2]Bangladesh Rice Research Institute (BRRI), Gazipur 1701, Bangladesh
[3]Freelance Consultant (Agricultural Research, Development and Modelling), 115/3 East Bhurulia, Joydebpur, Gazipur 1700, Bangladesh
[4]Department of Soil Science, Sylhet Agricultural University, Sylhet 3100, Bangladesh

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alihossain.ppath@bau.edu.bd

**Corresponding Author*
Bodrun Nessa
runu.brri@yahoo.com

ABSTRACT

It is perceived that sclerotia of the pathogen, in addition to chlamydospores, play an important role in the epidemiology of rice false smut disease. The propagule has been identified only in five rice growing countries of the world, and never recorded in Bangladesh. This study was undertaken to identify the sclerotia, quantify their concentration on smut balls and estimate the time of its formation. Intensive field-by-field monitoring was conducted during 2014, 2015 and 2016 at experimental farm of the Bangladesh Rice Research Institute, Gazipur. Sclerotia of rice false smut pathogen was first recorded on the 14th December 2014. Sclerotia were observed only on olivaceous greenish-black smut balls, but not on orange smut ball. During the three years study, on average, 30.61 ± 9.79% (± is 95%confidence interval) olivaceous greenish-black smut balls borne sclerotia, the average number being 1.63 ± 0.09 per sclerotia-bearing ball. The 'threshold temperature' for sclerotia formation was estimated as above 11°C difference in day-night temperature. Consistence presence and high concentration of sclerotia in smut balls signify its role in the epidemiology of the disease in the agroecology of Bangladesh.

Keywords: Sclerotia, olivaceous greenish-black smut balls, rice false smut, *Ustilaginoidea virens*, *Villosiclava virens*

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1 Introduction

Rice false smut (RFSm) (anamorph: *Ustilaginoidea virens* (Cooke) Takah.; teleomorph: *Villosiclava virens* (Nakata) E. Tanaka & C. Tanaka) is an important disease of rice in almost all the rice growing countries of the globe (Nessa et al., 2015a,b). The disease has the potential to infect the crop year-round across the three rice growing seasons in Bangladesh, 'Aus' (mid-March to mid-July), 'T. Aman' (mid-July to mid-November) and 'Boro' (mid-November to mid-March) (Nessa et al., 2018). However, in terms of adversity, RFSm is the disease of 'T. Aman' rice in the country (Nessa et al., 2018) along with part of India (Shetty and Shetty, 1985). With increasing incidence being reported from farmers' fields since early 2010s, RFSm is now an emerging disease in Bangladesh (Khatun et al., 2021).

Although rice false smut is an ancient disease, its epidemiology is not well understood especially under the changing environments, and particularly under Bangladesh condition (Nessa, 2017). The typical symptom of the disease appears on grains ball like colonies (composed of chlamydospores) called 'smut balls' (Guo et al., 2012). Three types of smut balls - orange, olivaceous greenish-black and white - has been clearly described by Nessa et al. (2021). The pathogen can produce both chlamydospores and sclerotia as fruiting bodies (Nessa, 2017). Production of sclerotia on the surface of smut ball (Sakurai, 1934; Fan et al., 2016; Ikegami, 1963; Singh and Dube, 1976) is also a part of disease symptom. Sclerotia can survive in soil as long as 11 months (Singh et al., 1985) and act as the primary inoculum (Sakurai, 1934; Fan et al., 2016; Singh and Dubey, 1984; Milao, 1992; Wang et al., 1998).

Though the disease was first reported in 1878, sclerotia were first identified half a century later in early 1930s in Japan (Sakurai, 1934; Fan et al., 2016). Till to date, this propagating structure has been reported only in five countries namely Japan (Sakurai, 1934; Fan et al., 2016), Myanmar (Seth, 1945), Korea (In et al., 1984), India (Singh and Dubey, 1984) and China (Wang et al., 1998). To best of our knowledge, sclerotia of rice false smut pathogen have not been recorded in Bangladesh before this study. It is, therefore, unknown whether sclerotia has any role in the epidemiology of RFSm in the country.

With the above background, this study was attempted on (i) identifying the sclerotia of rice false smut disease pathogen, (ii) quantifying concentration of sclerotia on smut balls and (iii) estimating the time of sclerotia formation.

2 Materials and Methods

The study was conducted in the experimental farm of the Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh, located at 23°59′ N latitude, 90°24′ E longitude, which facilitated wide scope for this research. Intensive field-by-field monitoring, during the 50% maturity to harvesting stage of crops, was carried out to search sclerotia on smut balls during November to December of 2014, 2015 and 2016 on T. Aman rice. Sclerotia were searched and confirmed following the physical features described by Ou (1972) and Tanaka et al. (2008). The identified sclerotia were photographed in nature. Seven, 14 and 8 fields during 2014, 2015 and 2015, respectively, were inspected where rice variety 'BRRI dhan49' flowered in late autumn for sampling. BRRI dhan49 was selected as the variety was widely suspected as susceptible to the disease. Altogether 791, 486 and 994 false smut infected panicles were randomly picked up in 2014, 2015 and 2016, respectively (Table 1).

The percentage sclerotia-bearing balls was calculated as:
(Number of sclerotia-bearing smut balls / total number of smut balls) *100.

The number of sclerotia per sclerotia-bearing smut balls was calculated as:
(Number of sclerotia / number of sclerotia-bearing balls) * 100

Results were expressed year-wise by averaging the sampled fields and 95% confidence interval was also calculated. For calculating the potential number of sclerotia-bearing smut balls per infected panicle, the total number of smut balls and the number of sclerotia-bearing smut balls in the infected panicle were counted separately for each of the sampled panicles collected in 2015. Their relationship was presented in graph as: (i) number of sclerotia-bearing smut balls to total number of smut balls in an infected panicle, and (ii) fraction of sclerotia-bearing smut balls (number of sclerotia-bearing smut balls / total number of smut balls in an infected panicle) to total number of smut balls in an infected panicle. The upper bounds of the data in the graphs were drawn in straight lines following the principle presented by French and Schultz (1984).

Table 1. Sampling location and sample size (number of infected panicles) for collecting data on sclerotia formation on smut balls in 'T. Aman' rice of 2014, 2015 and 2016 in the study site

Location†	No. of infected panicles collected		
	2014	2015	2016
1	90	27	116
2	175	24	189
3	62	27	181
4	205	46	107
5	199	133	152
6	30	28	119
7	30	53	64
8	–	20	26
9	–	26	–
10	–	14	–
11	–	21	–
12	–	28	–
13	–	26	–
14	–	13	–
Total	791	486	994

† Sampling location (field)

Using weather data from BRRI, the 10-day moving average of daily difference of day and night temperatures (Δdnt, daily maximum – daily minimum temperature in ºC) was calculated for each day during 1 October to 31 December. The statistics (Δdnt) were plotted in X:Y graph against the calculation period (1 October to 31 December). The date of the first appearance of sclerotia in 2014, 2015 and 2016 T. Aman crops was recorded through field observation. Following the principle of Milao (1992), the Δdnt that closely matched with the first appearance of sclerotia in 2014, 2015 and 2016 was designed as the likely date or the 'temperature threshold' of sclerotia appearance.

3 Results

3.1 Identification of sclerotia

After long waiting and rigorous search, sclerotia of rice false smut pathogen was identified on the 14th of December 2014 in one of the fields of study site (Fig. 1). The date was marked as the first report of the presence of true sclerotia, the resting stage of rice false

Figure 1. The first discovery of a sclerotium (arrow) on an olivaceous greenish-black smut ball of rice false smut disease on the 14th of December 2014 at the Bangladesh Rice Research Institute (BRRI) research station, Gazipur, Bangladesh

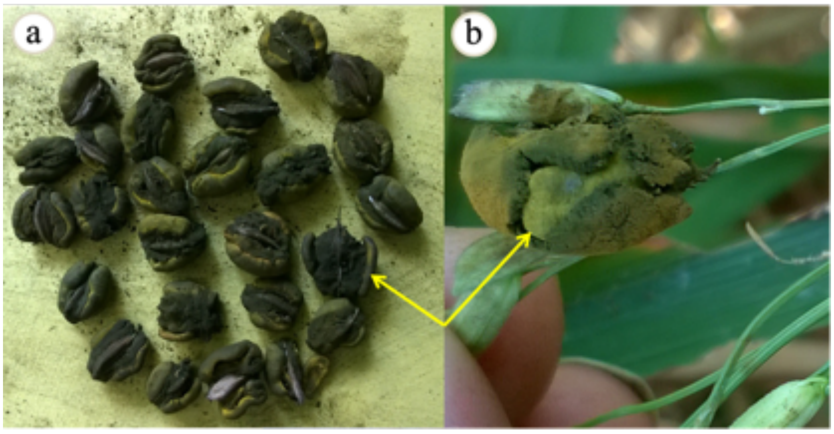

Figure 2. Sclerotia-bearing olivaceous greenish-black smut balls (a, b)

Figure 3. Sclerotia of various shapes and sizes collected from smut balls in the study area: (a) 2015 and (b) 2016

smut pathogen (*Villosiclava virens*), in the country.

Sclerotia were observed on olivaceous greenish-black smut balls, attached on their surfaces (Fig. 2a, b). However, sclerotia were not observed on orange smut balls.

The sclerotia were hard, dark-olive green to nearly black in colour, flat, irregular oblong, clavate, reniform, botuliform, horse-shoe shape to indefinite in shape (Fig. 3). The inner-side of sclerotia was concave and the outer-side was convex (Fig. 4).

Sclerotia were loosely attached to smut balls (Fig. 5). In some cases, two sclerotia joined together on the surface of a smut ball and remained hanging like a 'flying bird' (Fig. 6). Sclerotia detached in course of time upon slight physical manoeuvring (Fig. 7).

After detachment, sclerotia left yellowish orange hole on the surface of smut balls (Fig. 8). The holes were filled up over time (Fig. 9).

3.2 Transverse section of sclerotia

The transverse section of the sclerotia showed the outer tissue composed of compact cells, while the inner tissue appeared hyaline cells (Fig. 10).

3.3 Population of sclerotia

During 2014, 2015 and 2016 'T. Aman' season, 127, 4504 and 409 sclerotia were collected, respectively, from sampled infected panicles (Fig. 11).

Sclerotia were observed on infected panicles having smut balls, numbering 1 to 77; but not all those produced sclerotia. The highest number of 26 sclerotia-bearing smut balls was found on a panicle bearing 35 smut balls (Fig. 12a). On percentage basis, potentially all the smut balls up to 24 per infected panicle developed sclerotia; thereafter, it gradually declined and reached to 1% at 77 smut balls per infected panicle (Fig. 12b).

Across the studied field during three years, on average, 30.61 ± 9.79% (± is 95% confidence interval) olivaceous greenish-black smut balls borne sclerotia (Table 2). However, there was variation in the number of sclerotia-bearing smut balls between the three years. Significantly the highest number of sclerotia-bearing smut balls (51.94 ± 9.96%) recorded in 2015, compared to 9.72 ± 7.10% in 2014 and 8.74 ± 6.27% in 2016.

The average number of sclerotia per sclerotia-bearing ball was 1.63 ± 0.09 in the three years; this was significantly low (1.50 ± 0.14) in 2015 and high (1.80 ± 0.05) in 2014 (Table 2).

3.4 Formation of sclerotia

Sclerotia initiated below the outer surface of smut balls (Fig. 13a). As the size increased, the surface of smut balls started cracking (Fig. 13b) and eventually fully developed sclerotia exposed (Fig. 13c-d)).

3.5 Time of sclerotia appearance

Sclerotia were observed in the later part of crop maturity, early-November to mid-December, during the 'T. Aman' season of 2014, 2015 and 2016. The appearance of sclerotia was early in 2015 than 2014 or 2016. The pattern of this difference was related to the timing of high temperature difference between day and night. The 10-day moving average in the difference of day-night temperature started to rise above 11 °C from 24 October in 2015, 10 November in 2014 and 20 November in 2016 (Fig. 14). This marked the 'threshold temperature' of sclerotia appreance on smut balls.

4 Discussion

This study identified sclerotia on olivaceous greenish-black smut balls (but not on orange ones) in all three years of investigation during 2014 to 2016 in 'T. Aman' season. As far literature indicate, sclerotia have been reported only in five countries - Japan (Sakurai, 1934; Fan et al., 2016), Myanmar (Seth, 1945), Korea (In et al., 1984), India (Singh and Dubey, 1984) and China (Wang et al., 1998). The observed shapes and sizes of sclerotia in this study were comparable to what have been reported in literature (Singh and Dube, 1976; Tanaka et al., 2008; Fan et al., 2016).

In Japan, Hashioka et al. (1951) observed one to four sclerotia in nature on the smut balls. Rathaiah and Bhattacharyya (1993) report that sclerotia (usually 2 in number) formed on the surface of smut balls, never within. In this study, the average number of sclerotia per sclerotia-bearing smut ball was recorded as 1.63 ± 0.09, which was close to the number (usually 2) reported in the literature.

For three years observation, on average a quarter of the smut balls produced sclerotia. Rathaiah and Bhattacharyya (1993) reported from Assam in India, that less than 1% of the smut balls in a rainfed lowland rice crop (July-December) had sclerotia. On the other hand, Ikegami (1963) reported 23.3% sclerotia-bearing smut balls in his investigation in Japan; our record (30.61 ± 9.79) was higher than Ikegami's findings.

Variation in the production of sclerotia was observed between the years; the number was very high in 2015 compared to 2014 and 2016. Fan et al. (2016) also found such variation between years; for example, in their field observation 21.2% smut balls produced sclerotia in 2015 compared to only 1.8% in 2014. The

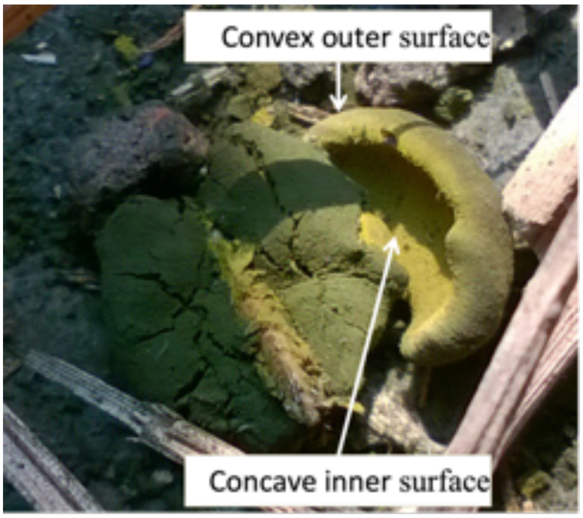

Figure 4. Typical shape (inner and outer side shown in arrows) of sclerotia of rice false smut disease observed in the study

Figure 5. Loosely attached sclerotia on rice false smut balls. A sclerotium just started detaching from (a), and resting on (b, c) smut balls. Two sclerotia separating from one smut ball (d)

Figure 6. Two sclerotia joined together and remained loosely attached on the outer surface of smut balls (a-d) creating a structure of 'flying bird'

Figure 7. Sclerotia detaching from smut balls – early (a) and advance stage (b) of the phenomenon

Figure 8. Yellowish orange hole on the surface of smut ball (a) after detachment of sclerotium (b)

Figure 9. The holes left on the surface of smut balls after detachment of sclerotia were being filled up (a-d)

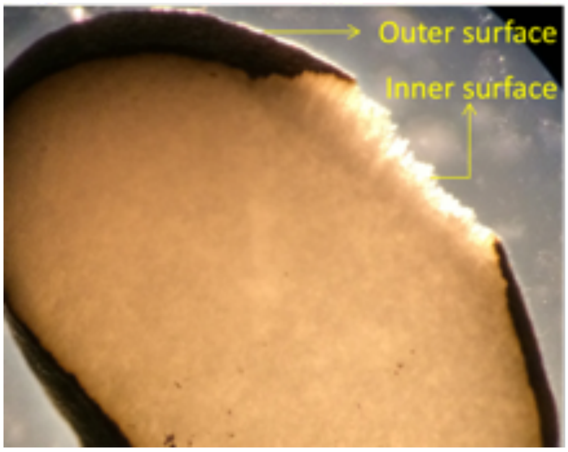

Figure 10. Transverse section of a sclerotium under 10× compound microscope

Table 2. Percentage of smut balls developed sclerotia and number of sclerotia per sclerotia-bearing smut ball in the 'T. Aman' rice of 2014, 2015 and 2016 in the study site

Year	Smut balls developed sclerotia (%)	Number of sclerotia per sclerotia-bearing smut ball
2014	9.72 ± 7.10	1.80 ± 0.05
2015	51.94 ± 9.96	1.50 ± 0.14
2016	8.74 ± 6.27	1.70 ± 0.13
Average	30.61 ± 9.79	1.63 ± 0.09

Values are mean ± standard error

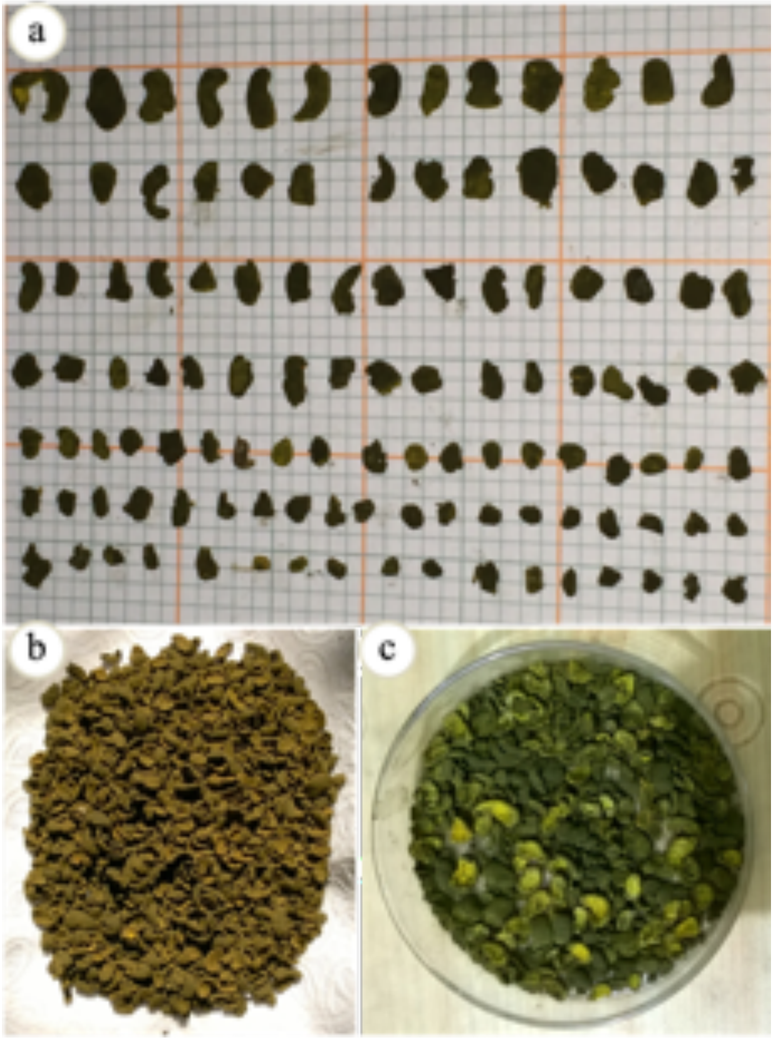

Figure 11. Sclerotia harvested from sampled smut balls during 'T. Aman' season of 2014(a), 2015(b) and 2016 (c)

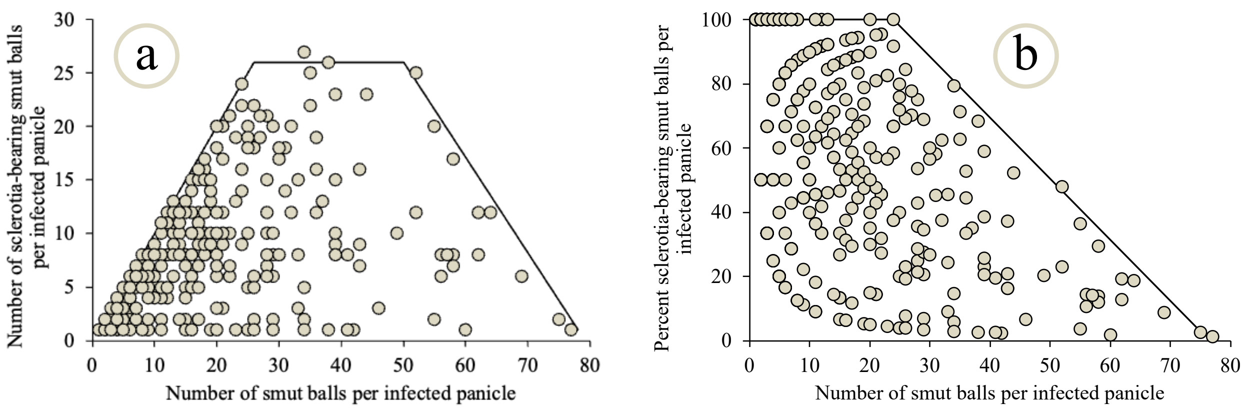

Figure 12. The relationship between number of sclerotia-bearing and total smut balls on rice panicles infected with false smut disease (a); and the fraction of sclerotia-bearing smut balls in relation to total balls on rice panicles infected with false smut disease (b)

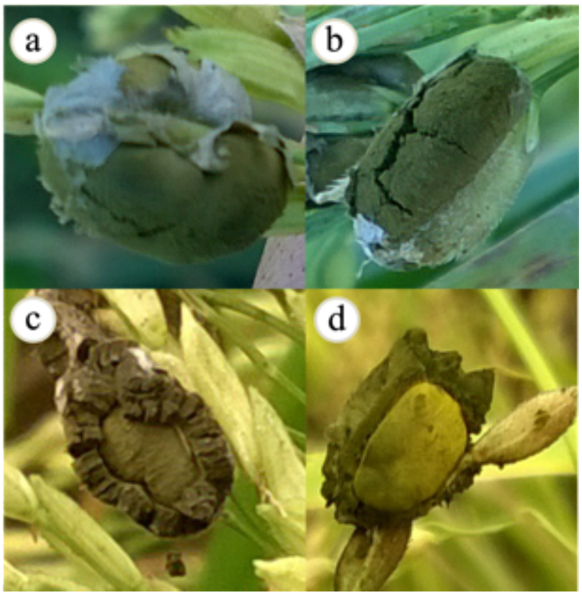

Figure 13. Development of sclerotia on smut balls. (a) sclerotium initiating below the outer surface of smut ball; (b) surface of sclerotium containing smut ball started cracking; (c) fully developed sclerotium exposing; (d) loosely attached sclerotium on surface of smut ball

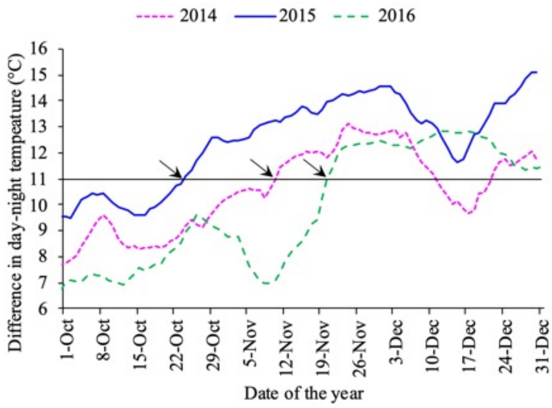

Figure 14. Difference in day-night temperatures, presented as 10-day moving average, during maturity period of T. Aman rice (October to December) in the study area in three years. The arrows show the start date of temperature difference above 11 °C in respective years, designated as the 'temperature threshold' for the appearance of sclerotia in smut balls

abundance of sclerotia in 2015 probably resulted from longer sclerotia-formation period, triggered by early occurrence of favourable temperature for sclerotia formation as evident in Fig. 14.

In our study, sclerotia were observed in both poorly and heavily infected (measured as number of smut balls per infected panicle) panicles; the maximum of 26 sclerotia-bearing smut balls were recorded on a panicle bearing 35 smut balls.

Literature indicates sclerotia form especially in late autumn, with relatively lower temperature, and when temperature differences between day and night is higher (Milao, 1992). In the present study, sclerotia were also observed in the later part of crop during late 'T. Aman' season (November-December of 2014, 2015 and 2016), when atmospheric temperature was low. Above 11 °C of difference in day-night temperatures explained the timing of appearance of sclerotia in the three years. So, time of the first appearance of olivaceous greenish-black ball and switching of the colour of orange ball to blackish (Nessa et al., 2021), and sclerotia appearance appears to be the same and related to the 'threshold temperature' (the difference of day-night temperature started to rise above 11 °C). Ikegami (1963) reported the reason of sclerotia appearance as more fluctuation of diurnal temperature in late autumn. Fan et al. (2016) observed that low temperature during rice filling is an importantly inducing factor for sclerotial formation in *V. virens*. Findings of Fan et al. (2016) further showed that 3-days of night temperature at 15 ºC were enough to induce sclerotial formation. Under Bangladesh condition, sclerotia do not form in other rice growing seasons (Aus and Boro) (Nessa et al., 2018) due to unsuitability of temperature requirements, which prevents the formation of olivaceous greenish-black smut ball; sclerotia form only on such type of smut ball.

5 Conclusion

This study recorded sclerotia – its various natural structures – in specific time corresponding to a 'temperature threshold', and on specific type of smut ball, which provides a clue on searching sclerotia of rice false smut disease where it has not discovered yet. The presence of large number of sclerotia in smut balls indicates the sclerotia are probably the primary source of inoculum in the ecology of the study area. These findings open a new insight of epidemiology of the fungus especially with respect to understanding its role in the disease cycle.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Fan Ll, li Yong M, yang Li D, jia Liu Y, hui Lai C, ming Chen H, min Cheng F, wei Hu D. 2016. Effect of temperature on the development of sclerotia in *Villosiclava virens*. Journal of Integrative Agriculture 15:2550–2555. doi: 10.1016/s2095-3119(16)61400-4.

French RJ, Schultz JE. 1984. Water use efficiency of wheat in a Mediterranean-type environment II: some limitations to efficiency. Australian Journal of Agricultural Research 35:765–775. doi: 10.1071/ar9840765.

Guo X, Li Y, Fan J, Li L, Huang F, Wang W. 2012. Progress in the study of false smut disease in rice. Journal of Agricultural Science and Technology 2:1211–1217.

Hashioka Y, Yoshino M, Yamamoto T. 1951. Physiology of the rice false smut, *Ustilaginoidea virens* (Cke.) Tak. Research Bulletin of Saitama Agricultural Experimental Station 2:1–20.

Ikegami H. 1963. Occurrence and development of sclerotia of the rice false smut fungus. Research Bulletin Faculty of Agriculture Gifu University 18:47–53.

In MS, Nou TH, Yu SH. 1984. Studies on the formation and germination of sclerotia of *Ustilaginoidea virens*. Research Reports of the Office of Rural Development [in Korean with an English summary] ORD 26:67–91.

Khatun MT, Nessa B, Salam MU, Kabir MS. 2021. Strategy for rice disease management in Bangladesh. Bangladesh Rice Journal 25:23–36. doi: 10.3329/brj.v25i1.55177.

Milao QM. 1992. Studies on infection route of rice false smut. Journal of Yunnan Agricultural University 7:40–42.

Nessa B. 2017. Rice False Smut Disease in Bangladesh: Epidemiology, Yield Loss and Management. PhD thesis, Department of Plant Pathology and Seed Science, Sylhet Agricultural University, Sylhet, Bangladesh.

Nessa B, Salam MU, Haque AHMM, Biswas JK, MacLeod WJ, Ali MA, Halder KP, Galloway J. 2015a. A simple yet robust model for estimating yield loss from rice false smut disease (*Ustilaginoidea virens*). American Journal of Agricultural and Biological Sciences 10:41–54. doi: 10.3844/ajabssp.2015.41.54.

Nessa B, Salam MU, Haque AHMM, Kashem MA, Kabir MS. 2018. Weather condition, seasonal variation and ball development pattern in relation to rice false smut disease in Bangladesh. Bangladesh Rice Journal 22:57–64. doi: 10.3329/brj.v22i1.41840.

Nessa B, Salam MU, Haque AM, Biswas JK, Kabir MS, MacLeod WJ, D'Antuono M, Barman HN, Latif MA, Galloway J. 2015b. Spatial pattern of natural spread of rice false smut (*Ustilaginoidea virens*) disease in fields. American Journal of Agricultural and Biological Sciences 10:63–73. doi: 10.3844/ajabssp.2015.63.73.

Nessa B, Salam MU, Kashem MA. 2021. Development of rice false smut (*Ustilaginoidea virens*) symptoms under the environmental conditions of Bangladesh. Journal of Plant Pathology 37:61–68.

Ou SH. 1972. Rice diseases Kew. Commonwealth Mycological Institute.

Rathaiah Y, Bhattacharyya A. 1993. Sclerotia of false smut (Fsm) of rice from Assam, India. International Rice Research Notes 18:48.

Sakurai M. 1934. On the causal fungus of rice false smut. Annals of the Phytopathological Society of Japan 3:70–71.

Seth LN. 1945. Studies on the false-smut disease of paddy caused by *Ustilaginoidea virens* (Cke.) Tak. Indian Journal of Agricultural Science 15:53–55.

Shetty SA, Shetty HS. 1985. A hitherto unrecorded collateral host of *Ustilaginoidea virens* (Cke) Tak. Current Science 54:646–647.

Singh RA, Dube KS. 1976. Occurrence of true sclerotia in claviceps oryzae-sativae the causal organism of false smut of rice. Current Science 45:772–773.

Singh RA, Dubey KS. 1984. Sclerotial germination and ascospore formation of *Claviceps oryzae-sativae* in India. Indian Phytopathology 37:168–170.

Singh RA, Dubey KS, Verma RK. 1985. Survival of *Claviceps oryzae sativae* - the incitant of false smut of rice. Indian Phytopathology 38:442–446.

Tanaka E, Ashizawa T, Sonoda R, Tanaka C. 2008. *Villosiclava virens* gen nov, comb nov, the teleomorph of *Ustilaginoidea virens*, the causal agent of rice false smut. Mycotaxon 106:491–501.

Wang S, Bai YJ, Zhou YL, Yao JM, Bai JK. 1998. The pathogen of false smut of rice. Acta Phytopathologica Sinica 28:19–24.

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