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STUDY THE EFFECT OF SOWING DATE AND WEATHER PARAMETERS ON FALSE SMUT (*Ustilaginoidea virens*) AND KERNEL SMUT (*Tilletia barclayana*) OF RICE (*Oryza sativa L.)*

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ABSTRACT
False smut caused by *Ustilaginoidea virens* and kernel smut caused by *Tilletia barclayana* fungal diseases of rice has become a serious production constraint in various rice-growing regions of Punjab. Disease incidence and severity varied significantly between climate change and varieties. In the field experiment during Kharif season 2018 observed that the maximum of the false smut incidence (27.49%) and severity (12.0%) recorded on variety Pusa44 followed by variety PR127 on late sowing.The relationship between the disease incidence and relative humidity (79.8%) and temperature (29.7°C) were significant. Rainfall did not influence the false smut but high rainfall (94mm) with temperature (34.1°C) and relative humidity (82.6%) favour the maximum kernel smut incidence (26.93%) and severity (47.18%) with variety PR127, followed by variety Pusa44 on early sowing. The maximum incidence of false smut was found when crop get flowered during October to November and kernel smut incidence was more in August to September.

KEYWORDS: rice-growing regions, false smut, Pusa44, Kharif season.

INTRODUCTION

India is the second largest producer as well as consumer of rice in the world. In India total area under cultivated of rice is 43.86 million with production 112.91 million tones and with productivity is about 3.62 tonnes per hectare during 2017-18 (Anonymous, 2019a). In Punjab it is the most widely grown Kharif crop and occupied 3.06 million hectares with a production of 19.9 million tonnes and its average yield was 6.52 tonnes per hectare during 2017-18 (Anonymous, 2019b). False smut or Green Smut or Pseudo smut of rice caused by the fungal pathogen, *Ustilaginoidea virens* (Cooke) Takahashi, was first reported from Tirunelveli in Tamil Nadu (Cooke 1878) and Kernel smut of rice is also known as grain smut (Biswas, 2001), is caused by the pathogen *Tilletia barclyana*, was recorded first time in Egypt in 1999 (Ismail, 2003). The typical symptoms of false smut appear at the maturity stage and in kernel smut symptom appear at grain formation stage of rice crop. In case of false smut individual grain is transformed into smutted balls which changed from orange to yellowish green at the maturity (Kumar 2012) but in kernel smut individual grain in the panicle transformed into black powder spores (Singh 1998). In false smut the Chlamydospores shape varied from elliptical, globose to irregularly round with prominent spines on the outer surface, initially in white colony that changed to yellow and finally became green with thick and double layer wall on media (Pilla *et al.*, 2017) and played an important role in the secondary infection of the host (Zhou *et al*., 2008).In kernel smut the spores are globose or elliptical, light to dark brown in young stage then transformed into black color at maturity (Pandey, 2015). On the germination the teliosporesgive rise to promycelium bearing the primary sporidia which are long, cylindrical to produce secondary sporodia (Allantoid and Filliform) are responsible for floral infection under favourable conditions (Chahal 2001; Elshafey, 2013).The growth characteristics on media of *T. barclayana* found white cottony colour and powdery, the colony was convex, deeply imbedded and incorporated within media.

Yield loss from the *Ustilaginoideavirens* and *Tilletia barclyana* was from 5 to 85% (Ladhalakkshmi *et al.*, 2012) and 5 to 15% (Priya *et al.*, 2018) respectively in different regions of India on different varieties (CGKB 2014; Sharma *et al.*, 1993). False smut and kernel smut causes quantitative and qualitative losses at 25 to 30ºC temperature (Salam *et al*., 2016). Relative humidity 70-80% and not much rainfall favourthe false smut but high relative humidity (RH > 85%) and rain showers at the time of ear emergence highly favour the kernel smut disease (RKMP, 2011).

The major incidence of rice false smut disease can be avoided by planting rice varieties in such a way that the crops do not flower during mid-October to mid-November (Nessa, 2017). By manipulating the time is easy way to controlling the incidence offalse smut (Ahonsi and Adeoti, 2003; Brooks *et al*., 2011). The incidence of kernel smut of rice is more in the early maturing varieties than medium and latematuring variety (Singh and Pavgi, 1970; Singh 1975). The present study to examine the effect of sowing time and weather parameters on incidence and severity of false smut and kernel smut and attempt has been made to identify the best sowing for rice to control the incidence of false smut and kernel smut of rice.

Material and methods

Collection of diseased samples

The field experiments were conducted at Research Farms of Guru Kashi University, Department of Plant Pathology, Talwandi Sabo, Bathinda, Punjab, during Kharif season 2018-19. The selected varieties *i.e.* PR111, PR122, PR114, PR127 and Pusa44 were sown at different sowing date at fortnight interval i.e. 10th May, 25thMay and 10thJune 2018. Split plot design was used and plot size was maintained 3x2.5m^2 and 15x20cm plant spacing was maintained. All cultural practices followed according to the package practices of PAU. Observations were recorded at the harvesting time as the number of panicle affected in the selected plants and number of florets affected per panicle among randomly selected plants. The disease severity and disease incidence was calculated as (Singh and Dube, 1978).

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased panicle}}{\text{Total number of inspected panicle}} \times 100$$

$$\text{Disease Severity (\%)} = \text{Percentinfected tillers x Percent smutted balls}$$

$$\text{Percent smutted balls} = \frac{\text{Number of smutted balls/ panicle}}{\text{Total number of grains/ panicle}} \times 100$$

The data recorded in all the experiments was statistically analyzed using EDA statistical programme, at Central Computer Laboratory, Guru Kashi University, Talwandi Saboand the differences among means were tested by using Least Significant Difference (LSD) values at 5% level of probability.

Isolation of culture:

The sterilized samples of false smutted balls with 1% sodium hypochlorite solution for 1 min followed by50 % ethanol, were dried by keeping them between filter paper and put the cut pieces of diseased samples on the YPDA media.. The samples were incubated in the BOD at 27 ±2°C for about two weeks to get the pure culture of fungus. Similarly, the sterilized teliospores of black smut transferred onto a 2% water agar. The plates were incubated at 25 ± 2°C for 15 days in the BOD for germination of teliospores.

Purification and maintenance of both cultures:

Hyphal tip method (Aboul-Nasr M B et al 2014)) used for purification of both cultures (False smut and black smut) under laminar flow conditionsandinoculated petriplatesincubated at the desired temperature in the BOD (Biological Oxygen Demand). As the fungus grow in the center the advancing edge of the mycelium havinghyphal tips well separated from each other. These hyphal tips transferred individually to separate agar slants in the test tubes using red hot inoculation needle. Thsesehyphal tips in the tubes developed into pure colony.

Identification and characterization of Pathogens:

False smut (*Ustilaginoidea virens*)

The characteristics of the fungal colony on media and the details of their morphology were recorded and culture was identified by using the slides and observes it under the microscope at 40 X.

Black smut (*Tilletia barclayana)*

Identification of *T. barclyana* isolates was carried out according to the morphological, microscopic characteristics and type of teliosporegermination in Plant Pathology Laboratory using the key given by Fischer and Holton (1957).

Weather Parameter data

The weather data collected from RRS (Bathinda) from August to November 2018, to know the effect of different sowing date of rice on incidence of false smut and kernel smut of rice.

RESULTS AND DISCUSSION

Effect of different sowing date on incidence and severity of Diseases

During Kharif, 2018-19, the results (Table1and Graph 1, 2) revealed that the disease incidence and severity percentage of both collected false smut (Figure 1) and kernel smut (Figure 2) in all three sowing dates varied with different varieties.

False smut

False smut disease incidence and severity (Table 1) increased steadily with delay in sowing. The late sowing of rice was recorded highest incidence 4.54, 9.63, 15.2, 6.60, 27.4 and severity 1.38, 4.60, 11.3, 5.85, 12.0 percentage at flowering stage during October to November as compare to early sowing with minimum disease incidence 0.99, 0.52, 1.12, 0.92, 5.49 and severity 0.13, 0.03, 0.20, 0.18, 1.25 percentage amongst three sowing dates. Nessa (2017) also conclude that the major incidence of rice false smut disease can be avoided by planting rice varieties in such a way that the crops do not flower during mid-October to mid-November. Narinder and Singh (1989) also observed that the rice plants transplanted early, found less incidence *i.e*. 47% and 48.2% when transplanted on 5 and 25 July, respectively.

Kernel smut

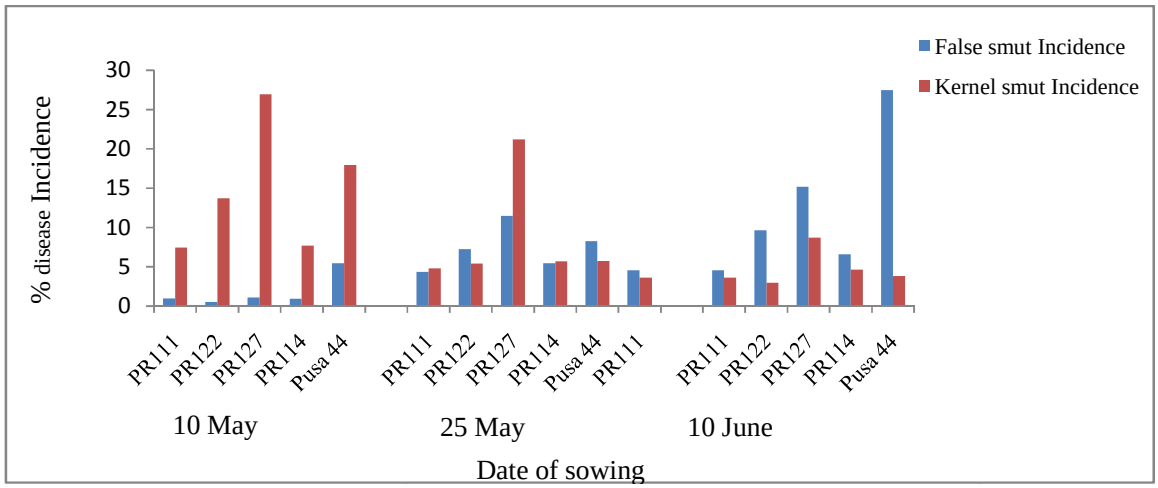
Kernel smut disease incidence and severity (Table 1) decreased steadily with delay in sowing. The early sowing of rice was recorded maximum disease incidence 7.46, 13.7, 26.9, 7.68, 17.9 and severity 12.12, 6.60, 47.2, 12.7, and 20.4 percentage at flowering stage during August to September as compare to late sowing with minimum disease incidence 3.62, 2.99, 8.72, 4.64, 3.83 and severity 0.21, 0.07, 0.64, 0.18, 0.25 percentage were recorded amongst three sowing dates. The pervious study showed that the short duration varieties and early sowing varieties suffer more than late maturing varieties (RKMP 2011; Anonymous 2016).

(a) development of mycelium (b) emerging of white mycelium (c) developing yellow Chlamydospores (d) Fully developed chlamydospores (e) changing color, yellow to green (f) fully developed green color smutted ball Figure 2: Kernel smut symptoms

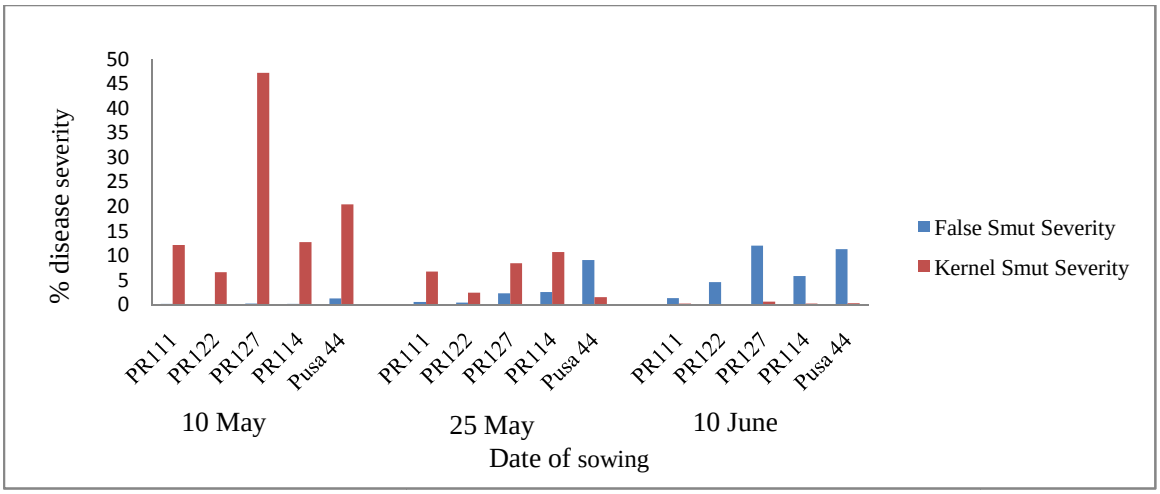
FIGURE 1: Development stages of false smut in the field

TABLE: 1 Disease Incidence (DI %) and disease severity (DS %) of false smut at Kernel smut at different sowing date

Sowing Date	10th May				25th May				10th June			
Varieties	False smut		Kernel smut		False smut		Kernel smut		False smut		Kernel smut	
	DI(%)	DS(%)	DI(%	DS(%)	DI(%)	DS(%)	DI(%)	DS(%)	DI(%	DS(%)	DI(%	DS(%)
PR111	0.99	0.13	7.46	12.1	4.37	0.60	4.84	6.71	4.54	1.38	3.62	0.21
PR122	0.52	0.03	13.7	6.60	7.25	0.47	5.40	2.46	9.63	4.60	2.99	0.07
PR127	1.12	0.20	26.9	47.2	11.5	2.33	21.2	8.42	15.2	11.3	8.72	0.64
PR114	0.92	0.18	7.68	12.7	5.46	2.60	5.71	10.7	6.60	5.85	4.64	0.18
PUSA44	5.49	1.25	17.9	20.4	8.26	9.11	5.73	1.51	27.4	12.0	3.83	0.25
Mean	1.80	0.36	14.7	19.8	7.69	3.03	8.57	5.96	12.6	7.03	4.76	0.27



GRAPH 1: Disease Incidence of False and Kernel smut of rice at different sowing date



GRAPH 2: Disease Severity of False and Kernel smut of rice at different sowing date

Identification and characterization of Pathogens: False smut

After 7-14 days of incubation the chlamydospores of *Ustilaginoidea virens* germinate to produced milky white colony with fluffy mycelium with flat or slightly convex surface, compact and leathery mycelium (Plate-FS1) which appeared orange or yellowish (Plate-FS2) and the mycelium show continued its growth on the PDYA slant (Plate-FS3). Sharma and Joshi (1975); Baite *et al.* (2014) studied the growth and sporulation of *Ustilaginoidea virens.* It produce creamy white colony with fluffy, compact and leathery mycelium, almost round, later on they became orange yellow and finally olive green and powdery.

Kernel smut

In the *Tilletiabarclyana*, the teliospores germinated into restricted colonies after cultured on the water agar (**Plate-KS1)**. These were then be cultured directly on solid media (PDYA) **(Plate-KS2)**. After 14 days of incubation at 19 °C with a 12 h light cycle typically produce white- smooth leathery surface and raised colony deeply imbedded on PDYA slant (**Plate-KS3**). Similar observation of culture colonies of *Tilletia barclyana* recorded in pervious study by Chahal *et al* 2001 and Elshafey, 2013.

FS1 **FS2** **FS3** **KS1** **KS2** **KS3**

PLATE - 1 (Morphological study) FS1- growth after 14th day on PDYA; FS2-Orange and white color; FS3-Colony of False smut on PDYA slants; KS1-Primary Sporidia growth on agar medium; KS2-growth of KS on PDYA; KS3-Colony of Kernel smut on PDYA

Microscopicstudyof spores and mycelium:

False smut

The chlamydospores (from the outer region of the smut ball) were round to elliptical, thick and double walled. They appear yellow color in center and their thick wall appears black**. (Slide-F1)** and there germination was observed under compound microscope at 10X(Slide-F2)after inoculation on PDYA, mycelium was slender, branched, septate hyphae bear conidia (Slide-F3). Similar microscopic study recorded in pervious study (Ladhalak shmi *et al.,* 2012 and Rani, 2014).

F1 **F2** **F3** **K1** **K2** **K3** **K4**

Slide-1 (Microscopic Study): False smut; F1-Chlamydospores; F2-Mycelium bearing conidia; Kernel smut; K1-Teliospores;K2-Germination of teliospores;K3-Filliform(Secondary) sporidia;K4-Allantoid sporidia

Kernel smut:-

The spore (teliospore/ Teleutospore) of *Tilletiabarclyana* appear light brown and while few were brown to black in color and round in shape (slide F3) under compound microscope. Similarly Singh (1998) observe the teleutospores are pulverulent, light brown to black, globose or sub-globose measuring 15-32 in diameter or 22.5-28.7- in size withor without an appendage/ apiculus. On germination, it was observed teleutospores give rise to promycelium (Slide K2), bear cluster of primary sporidia which germinate to secondary sporidia called Filliform (Slide K3) and Allantoid. (Slide K4). Chahal *et al.* (2001) observed germination of matureteliospores and found that the primary sporidia give rise to filliform and allantoidssporidia.

Effect of weather conditions on incidence of false smut and kernel smut of rice

Weather data (Table 2) revealed that there is significant relationship of the five most growing varieties (PR111, PR122, PR127, PR114 and Pusa44) in Punjab related to false smut incidence with

1) False smut

Weather data (Graph 3) revealed that there is significant relationship of the five most growing varieties (PR111, PR122, PR127, PR114 and PUSA44) in Punjab related to false smut incidence with low temperature, low relative humidity and low or no rainfall. The weather conditions with Max and Min temperature (29.7 and 14.3°C), Max and Min RH (79.8 and 39.6%) and no Rainfall for these five varieties favour the higher incidence 4.54, 9.63, 15.2, 6.60, 27.4%respectively of false smut in the late sowing

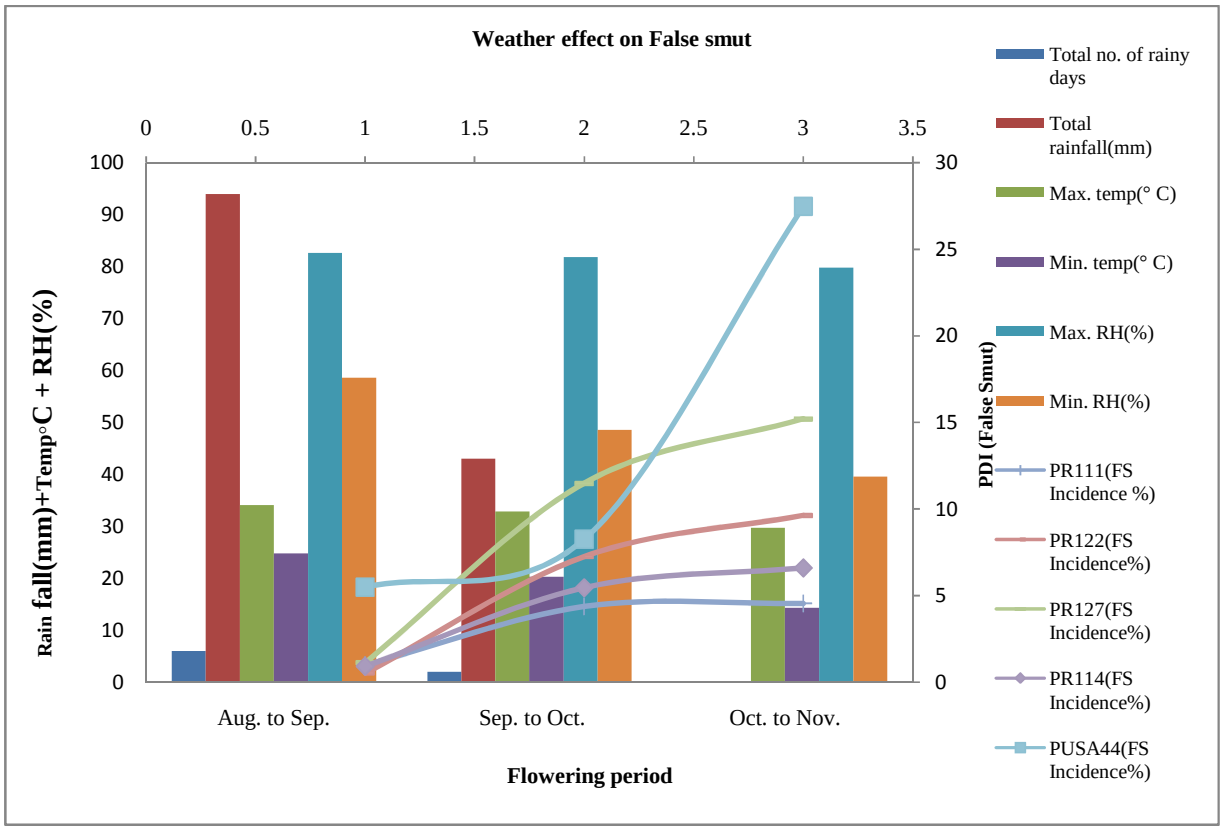
when the cop flowered on October to November observed as compare to early sowing when the crop get flowered during last week of August to mid-September with Max and Min temperature (34.1 and 24.8° C), Max and Min RH (82.6 and 58.6%), Rainfall (94mm) and number of rainfall (6D)with less incidence 0.99, 0.52, 1.12, 0.92, 5.49%respectively. The pervious study showed that the incidence of false smut is favored by high relative humidity 70 - 80%, warm weather temperature between 25 and 30ºc and not much effected by rainfall, late sowing (Salam *et al.*, 2016)**.**

2) Kernel smut

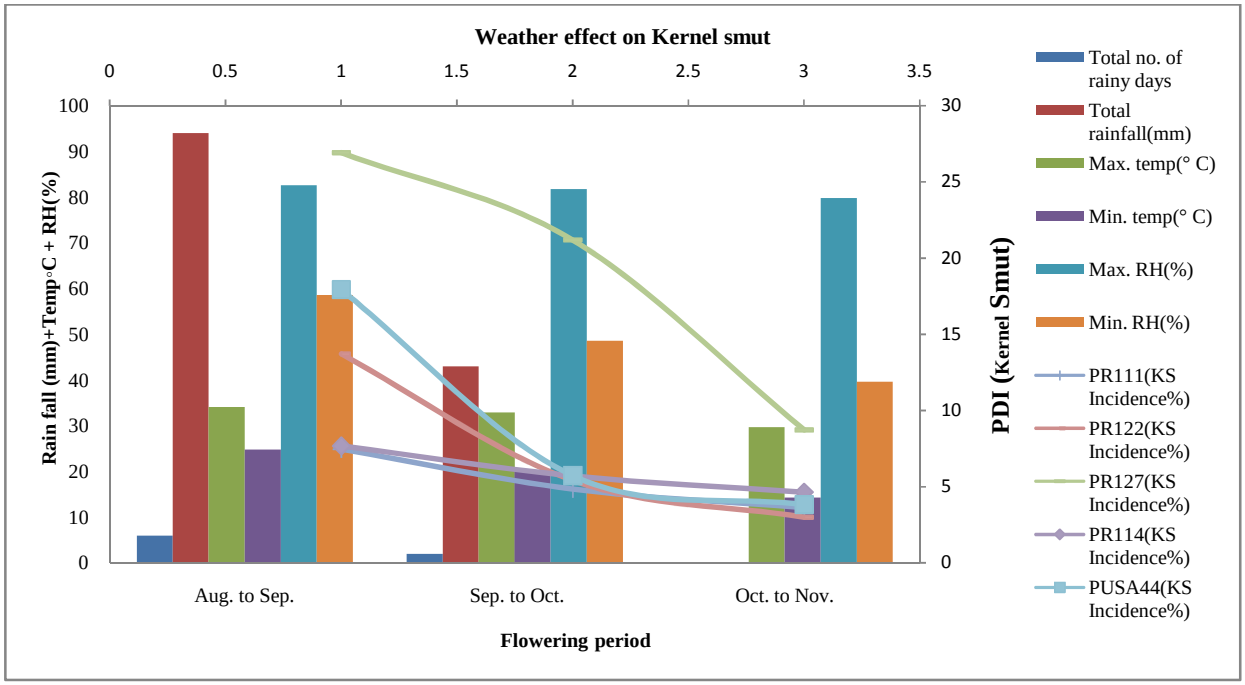
Weather data (Graph 4) revealed that there is significant relationship of the five most growing varieties (PR111, PR122, PR127, PR114 and Pusa44) in Punjab related to Kernel smut incidence with high temperature (Max. and Min.), High relative humidity (Max. and Min.) and high rainfall. The weather conditions with Max and Min temperature (34.1and 24.8°C), Max and Min RH (82.6 and 58.6%) with 94mm rainfall for these five varieties favour the higher incidence7.46, 13.7, 26.9, 7.68, 17.9% respectively of Kernel smut the early sowing when the cop flowered during last week of August to mid-September observed as compare to late sowing when the crop get flowered on October to November with Max and Min temperature (29.7°C and 14.3°C), Max and Min RH (79.8 and 39.6%) without Rainfall having minimum incidence 3.62, 2.99, 8.72, 4.64, 3.83% respectively. The temperature range of 24-33 °C coupled with high relative humidity (above 85%) and frequent light showers at the time of ear emergence are friendly for infection and development of kernel smut (Kaurav and Mathur, 1980; RKMP, 2011; Gupta *et al.*,2002).

TABLE: 2 Effect of weather conditions on *U. virens* and *T. barclayana*

Sowing time	Flowering time	No. of Rainy Days	Total Rainfall (mm)	Temperature (° C)		Relative Humidity (%)		Percent disease incidence									
				Maxtemp (°C)	Min temp (°C)	Max RH (%)	Min RH (%)	False Smut					Kernel Smut				
								PR 111	PR 122	PR 127	PR 114	PUSA 44	PR 111	PR 122	PR 127	PR 114	PUSA44
10th May	Aug. to Sep.	6	94	34.1	24.8	82.6	58.6	0.99	0.52	1.12	0.92	5.49	7.46	13.7	26.9	7.68	17.9
25th May	Sep. to Oct.	2	43	32.9	20.3	81.8	48.6	4.37	7.25	11.5	5.46	8.26	4.84	5.40	21.2	5.71	5.73
10th June	Oct. to Nov.	0	0	29.7	14.3	79.8	39.6	4.54	9.63	15.2	6.60	27.5	3.62	2.99	8.72	4.64	3.83



GRAPH: 3 Weather Effect on Incidence of false smut at different flowering period



GRAPH: 4 Weather Effect on Incidence of Kernel smut at different flowering period

CONCLUSION

During the Kharif season 2018-19, the third sowing (10 June) show maximum false smut incidence (4.54, 9.63, 15.21, 6.60, 27.49%)and severity (1.38, 4.60, 11.3.00, 5.85, 12.0%) and minimum kernel smut incidence (2.99, 3.62, 3.83, 4.64, 8.72%) and severity (0.07, 0.18, 0.21, 0.25, 0.64%) as comparison to first sowing (10 May) with minimum false smut incidence (0.99, 0.52, 1.12, 0.92, 5.49%)and severity (0.13, 0.03, 0.20, 0.18, 1.25%)but maximum kernel smut incidence (26.93, 17.93, 13.71, 7.68, 7.46%) and severity (47.18, 20.37, 12.70, 12.12, 6.60%) for five varieties PR111, PR122, PR127, PR114 and Pusa44 respectively. There is significant relationship of weather parameter on disease incidence and severity percentage. At their flowering time Kernel smut require high maximum (34.1˚**C**) and minimum (24.8˚**C**) temperature, high maximum (82.6%) and minimum (58.6%)relative humidity with maximum rainfall as compare to False smut with low temp (29.7˚C and 14.3°**C**), low RH (79.8 and 39.6%) with no rainfall.

The present study is more informative to farmer that they should be sown the rice varieties in such a way that crop do not flower in August to september in kernel smut prone areas and October to November in case of false smut prone areas.

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