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Evaluation of different inoculation technique against false smut of rice caused by *Ustilaginoidea virens* for creating epiphytotic conditions

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Abstract
Rice false smut caused by *Ustilaginoidea virens* (Cke.) Tak. is a devastating disease all around the world including India. In India, this diseases causes an economic yield losses i.e., 0.5 to 49 per cent depending on the host variety. Among various inoculation methods adopted at different stages employing on Damini rice variety which showed that spraying of conidial suspension (2×10^5 spores ml^{-1}) of *U. virens* at booting and late booting stage was ideal for infection with maximum infected tillers (41.54% and 37.82%) and smut balls (7.45 and 6.78) respectively.

Keywords: Rice, false smut, *Ustilaginoidea virens*, inoculation, epiphytotic

Introduction
Rice (*Oryza sativa* L.) is a crucial staple food crop, supplying more than half of the world's population. Rice does not show its full production potential due to several biotic and abiotic stresses. Among biotic factors, the major factor is disease caused by fungi, bacteria, viruses and others. The most important diseases of rice are blast, false smut, sheath blight, stem rot and bacterial blight. Rice False smut is major one which is now an emerging devastating threat to rice production (Ladhalakshmi *et al*., 2012 and Bashyal *et al*., 2020) [9, 2]. False smut of rice is due to the fungus *Ustilaginoidea virens* (Cooke) Takahashi (Teleomorph *Villosiclava virens*) (Tanaka *et al*., 2008) [14]. For the first time it was reported from Tirunelveli in Tamil Nadu state (Cooke, 1878) [4].
The pathogen replaces all or part of the kernel with an abundant mass of yellow-dark green spores and culminates with velvety smut ball formation. The sori of *U. virens* erupt through the palea and lemma (Brooks *et al*., 2010) [3]. The loss in grain yield is due to a reduction in test weight of grains, chaffiness and sterility of spikelet neighbouring the smut balls (Ladhalakshmi *et al*., 2012) [9]. In Uttar Pradesh, an incidence up to 5-85 percent was recorded (Singh *et al*., 2014) [13]. Currently, the illness has reached the position of worrying malady, principally due to extensive cultivation of high-fertilizer-responsive cultivars and hybrids, changes in meteorological conditions, and greater application of nitrogenous fertilizers (Rani *et al*., 2015) [11].
False smut not only reduces yield but also lowers rice grain quality, compromising food security and economic stability in rice-dependent nations. This research endeavors to evaluate different inoculation techniques to induce epiphytotic conditions conducive to false smut development. The significance of this study lies in its potential to provide insights into disease management strategies.

Materials and Methods
The experiments were conducted in the Net house of Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, U.P. during 2020-21.

Standardization of inoculation techniques for creating epiphytotic conditions of false smut of rice

Five different inoculation techniques were used to create epiphytotic conditions using rice *cv.* Damini. The experiment was conducted under net house conditions.

Conidial suspension (2 x 10^5 ml^{-1}) made from a culture is used for this study. The detail of method of inoculation techniques were given in table 1.

Soil inoculation with smut balls

The smut balls (10-15) were placed in the soil below 2-3 cm. Later, 21 day old seedlings (Damini) were transplanted and watered regularly.

Table 1: Different method of inoculation technique.

S. No.	Inoculation methods	Crop stage
1.	Soil inoculation with smutted balls	-
2.	Seedling treatment with smut balls suspension	-
3.	Spray inoculation	Early booting
		Late booting
4.	Syringe inoculation	Early booting
		Late booting
5.	Dusting	Early booting
		Late booting

Seedling treatment with smut balls suspension

The 21 days old seedling roots were treated with the smut balls spores suspension i.e. 2 ml and transplanted in the pot and watered regularly.

Spray inoculation

The conidial suspension (2 x 10^5 ml^{-1}) made from fresh culture was used to spray with hand sprayer in two crop growth stages i.e., early booting and late booting until the head and glumes were wetted until suspension dropped down. Then, pots were kept in moist chamber for 48 hrs. Then transferred to normal room temperature.

Syringe inoculation

Conidial suspension (2 x 10^5 ml^{-1}) made from fresh culture was injected into the leaf sheath of rice plant at two crop growth stages i.e., early booting and late booting until it overflowed. Then, pots were kept in moist chamber for 48 hrs.

Dusting inoculation

Fresh false smut infected panicle was used for this method. The infected panicle was directly dusted into the plant at two crop growth stages i.e., early booting and late booting until it overflowed. Then, pots were kept in moist chamber for 48 hrs.

Results and Discussion

Effective inoculation technique for creating disease in plants is pre-requisite for development of resistant variety. Five different inoculation methods *viz.*, I_1 Soil inoculation with smutted balls, I_2 Seedling treatment with smut balls suspension, Spray inoculation (I_3 Early booting and I_4 Late booting stage), Syringe inoculation (I_5 Early booting and I_6 Late booting stage), Dusting (I_7 Early booting and I_8 Late booting stage) on a susceptible variety Damini with isolate Uv1 (Ayodhya) (Table 2). The smut symptoms were observed after 12-13 days after inoculation. The results obtained were presented in Table 2. Maximum infected panicles were found in I_3 (41.54%) followed by I_4 (37.82%), I_5 (28.45%), I_6 (20.14%). Minimum infected panicles were found in I_7 (12.17%) and I_8 (08.05%). No infected panicles were found in I_1 and I_2. The maximum number of smut balls per panicle was found in I_3 (7.45) followed by I_4 (6.78).

Table 2: Evaluation of different inoculation techniques for creating artificially epiphytotic conditions of false smut disease cv. Damini.

Inoculation method		Crop stage	Infected Panicles	Smut balls per panicle
Soil inoculation with smutted balls	I_1	-	0.00 (0.28)	0.00 (0.28)
Seedling treatment with smut balls	I_2	-	0.00 (0.28)	0.00 (0.28)
Spray inoculation	I_3	Early booting	41.54 (40.12)	7.45 (15.83)
	I_4	Late booting	37.82 (37.94)	6.78 (15.09)
Syringe inoculation	I_5	Early booting	28.45 (32.23)	3.17 (10.25)
	I_6	Late booting	20.14 (26.66)	2.53 (9.14)
Dusting	I_7	Early booting	12.17 (20.41)	0.96 (5.60)
	I_8	Late booting	08.05 (16.46)	0.68 (4.71)
CD (P=0.05)			0.88	0.58
CV			2.30	4.38

The findings were also corroborated with the findings of Singh (2009) [12], Pannu *et al.* (2010) [10] and Kaur *et al.* (2018) [7] that the disease incidence and disease severity was maximum in case of spray inoculated followed by syringe inoculation and dropper inoculation method. Wang *et al.* (2008) [15] also studied the inoculation methods against *Ustilaginoidea albicans*, and observed that inoculations by spraying and injection method resulted in maximum disease development.

In contrast to this, Ashizawa *et al*., 2011 [1]; Haiyong, 2012 [5]; Haiyong *et al*., 2015 [6]; Kumar *et al*., 2016 [8] found that the injecting the conidial suspension at booting stage produces maximum number of infected tillers as compared with the spray inoculation. Number of smut balls varied from 0.03 to 50.00 in field and from 0.10 to 25.00 in greenhouse conditions on 18 different cultivars. No infected panicles were found in I_1 soil inoculation with smutted balls and I_2 seedling treatment with smut balls suspension.

The similar result was also reported by Kumar *et al*., 2016 [8].

Conclusion

In conclusion, this study assessed various inoculation methods for inducing false smut disease in the Damini variety, emphasizing the importance of effective disease creation for developing resistant crops. Among the techniques examined, spray inoculation during early booting emerged as the most effective, resulting in the highest disease incidence and severity. These findings align with previous research, highlighting the efficacy of spray

inoculation in disease development. However, contrasting results from other studies suggest the need for tailored inoculation approaches based on specific plant-pathogen interactions. Notably, soil inoculation and seedling treatment showed minimal infection, consistent with prior observations. This research provides valuable insights into optimizing inoculation techniques, aiding future studies on disease resistance mechanisms and the breeding of resilient crop varieties.

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