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Studies on morphological and cultural characteristics of *Ustilaginoidea virens* (Cke.) Tak.

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Abstract
The hyphae of *Ustilaginoidea virens* were septate, tightly woven and 2.5-3.0 µm in width. Initially, colony was hyaline later on turned orange, greyish and brown to black in colour. Conidia were sub globose, ovoid, hyaline, unicellular and measured 5.0 x 3.0µm. Chlamydospores formed on pseudo morph were initially smooth spherical and orange yellow coloured. At maturity, they became dark brown coloured, echinulate with rough surface and 5 to 6 µm in diameter. Maximum vegetative growth of *Ustilaginoidea virens* was recorded on potato dextrose agar (90mm) followed by glucose peptone agar medium (81.50 mm) of colony diameter. Oat meal agar medium supported maximum sporulation of the pathogen followed by rice meal agar medium.

Keywords: *Ustilaginoidea virens,* sporulation, growth and morphology

Introduction
Konkan is a major rice growing region of Maharashtra, with about 4.12 lakh hectares of land under rice cultivation. The total annual production is about 9.82 lakh tones, with average productivity of 2.38 tonnes ha1 (Anonymous, 2010-11). False smut has long been considered a minor disease (Ou, 1985; Dodan *et al*., 1996) [6, 1]. However, in recent years, it has become a serious problem because the disease affects the grain development and causes direct economic losses in the yield possibly due to high input cultivation for high-yield, increased use of hybrid varieties, and climate change. The climatic conditions which are favourable for the growth of the fungus are similar to those for good growth of the rice crop. In India, the disease has been reported to occur in moderate to severe form since 2000 onwards. Rice based cropping system is the tradition of *Konkan* region. Rice is the sole crop cultivated in *kharif* season. False smut is not a major disease under normal climatic conditions but it causes diminishing effect on the yield whenever the climate is favourable and the variety is susceptible. Field observations of last few years have indicated that occurrence false smut in the region is increasing on various traditional and newly introduced hybrids and high yielding varieties. Earlier the disease was considered as farmer's friendly diseases and locally known as 'Lakshmi disease' because its occurrence was always found to be associated with bumper yields. The reason behind this is that the climatic conditions which are favourable for the growth of the fungus are similar to those for good growth of the rice crop.

Materials and Methods
Morphological studies
Morphological studies were carried out by using potato dextrose agar as a basal medium. The slide culture technique was used to study the morphological characters of the test fungus. Five mm disc of solidified PDA in Petri plates was transferred with the help of incinerated cork borer to the centre of each sterilized micro slide. The disc was then inoculated with fungal inoculum and covered with sterilized coverslip. Such micro slides were placed on a pair of glass rods in a sterilized Petri plate lined with moist blotting paper. Plates were kept for incubation at room temperature (27 ± l O_C). Slides were observed after 48 hrs. Of incubation under microscope. Fungal growth on the micro slide was stained immediately with lacto phenol-cotton blue, covered with cover slip and was observed under compound microscope for morphological study such as shape and size of both hyphae and conidia.

Effect of different media on the growth

Cultural characteristics were studied on different media (Synthetic and Semi-synthetic) were analar in grade and sterilized by autoclaving at 1.054 kg/cm^{2}pressure for 15 minutes. The medium (20ml) was then poured aseptically in sterile petri dishes and allowed to solidify. Seven day old culture discs (diameter 5mm) were transferred aseptically on plates. These plates were incubated at room temperature (27±1Oc) for eight days. The observations were taken for colony type, colony colour, colour changes of substratum and colony diameter was recorded at an interval of 24 hours for seven days.

Effect of different media on sporulation

Sporulation was determined by making homogenous suspension of mycelial mat and counting number of spores per microscopic field.

The degree of sporulation was graded as follows,

i) Nil No sporulation
ii) Poor 1-3 spores
iii) Fair 4-6 spores
iv) Good 7-10spores
v) Excellent 11 and above spores
(Mehta, 1989).

Table 1: Media used for cultural studies.

Tr. No.	Synthetic media
T1	Glucose peptone agar medium.
T2	Asthana & Hawker's agar medium.
T3	Richard's agar medium.
T4	Coon's medium.
	Semi-synthetic media.
T5	Oat meal agar medium.
T6	Potato dextrose agar (PDA) medium.
T7	Rice meal agar medium.

Results and Discussion

Morphological studies

Full mycelial growth on PDA was recorded within 7 days with average growth rate of 13 mm within 24 hrs. After 48 hour and 96 hours, the colony characters were found changed circular radiating towards the periphery and colour changing from orange to greyish brown in the centre with hyaline cottony margin. Later on, the whole colony turned dull brown to black. The mycelium was hyaline initially, but later on turned to orange, greyish and finally dull brown to black. At this stage the septation was clearly visible. Hyphae were septate, tightly woven and measured 2.5-3 µm in width. Conidia were formed after germination of Chlamydospores. Chlamydospores at the time of germination gave rise to short, septate germ tube which beared conidia in clusters. The germination of Chlamydospores was observed after 24 hrs. and the germination was 15 to 20 per cent. Conidia formed in culture were hyaline, sub globose, ovoid and single celled with average size 5×3 µm Plate III. Chlamydospores formed on naturally infected glumes were observed under microscope. Chlamydospores were of two types on the basis of their developmental stages. In early stage, Chlamydospores were orange to yellow and spherical with smooth surface. Later on (at maturity), they turned dark brown, echinulate and with rough surface measuring from 5 to 6 µm Plate II. These results were closely conformity with Singh (1998) [11] and Rush *et al.*, (2000) [8] who stated that globose, yellowish green, velvety spore balls that were 2 to 5cm in diameter and covered by a thin orange membrane.

Effect of different media on the growth

The data revealed from table 2 & Plate I, that potato dextrose agar medium was the most suitable medium on which *Ustilaginoidea virens* attained 90 mm radial mycelial growth within 7 days. Similar findings were observed by Fu *et al*., (2013) [2] who recorded good growth of *U. virens* on potato sucrose agar and potato dextrose agar medium.

Effect of different media on sporulation

Excellent sporulation was obtained on Oat meal agar medium while, poor sporulation was recorded in Asthana and Hawker's and Richard's media. In the remaining media, sporulation was good. Initially, sclerotia were hyaline and smooth, but later on changed to orange and finally to greyish green, black in colour. At maturity, the mycelium exhibited red salmon coloured pigmentation this was closely conformity with Singh, (1998) [11] who stated that higher sporulation of *U. virens* was noted on Oat meal agar medium.

Summary and Conclusion

The mycelium of U. virens is having septate mycelium. Initially, colony was hyaline, later changed orange, greyish and brown to black in colour. The hyphae were septate, tightly woven and measured 2.5-3.0 µm in width. Conidia formed in culture were sub globose, ovoid in shape. The conidia were hyaline, single celled and measured 5.0 × 3.0 µm. Chlamydospores formed on pseudo morph were initially smooth spherical and orange yellow in colour. At maturity they became dark brown in colour, echinulate with rough surface and measured 5 to 6 µm in diameter. The Chlamydospores germinated to produce short septate germ tube with cluster of conidia at the tip. Cross section of pseudo morph revealed central white compact tissue surrounded by three sporiferous layers *viz.*, yellow, orange and black coloured, smooth young spores were observed in orange coloured layer. In the third layer black coloured spore mass was observed. Potato dextrose agar medium was found to be most suitable medium for growth of U. virens followed by Glucose peptone agar medium. Among the synthetic media, best growth was obtained on Glucose peptone agar medium and poor growth on Richards's medium. Oat meal agar medium supported for maximum sporulation of the pathogen followed by Rice meal agar medium. Among the synthetic media, best sporulation was obtained on Coon's medium and poor sporulation was on Richard's medium.

Table 2: Effect of different media on the vegetative growth and sporulation of *U. virens.*

Sr. No.	Medium	Average colony diameter (mm)	Growth character	Sporulation
1.	Asthana and Hawker's medium	78.00	Concentric rings, smooth growth, medium turned black at centre.	poor
2.	Richards medium	64.70	Cottony growth, white in colour, no change in colour of the medium.	poor
3.	Rice meal agar medium	81.30	Concentric rings formed no change colour of the medium.	Good
4.	Coon's medium	75.30	Concentric rings, Mycelial growth, whitish black in colour, medium turned black to brown in colour.	Good
5.	Glucose peptone medium	81.50	Profuse white growth, no change colour of the medium.	Good
6.	Oat meal agar medium	79.00	Concentric rings, fluffy growth, medium turned black to brown in colour.	Excellent
7.	Potato dextrose medium	90.00	Concentric rings, Profuse fluffy growth, medium turned brown in colour	Good
	S.E. =	0.87	C.D. at (1%) =	3.65
	Mean =	I 78.57	II 78.86	III 78.21

Plate I: Effect of different media on growth and sporulation of *U. virens* (Cke.) Tak.

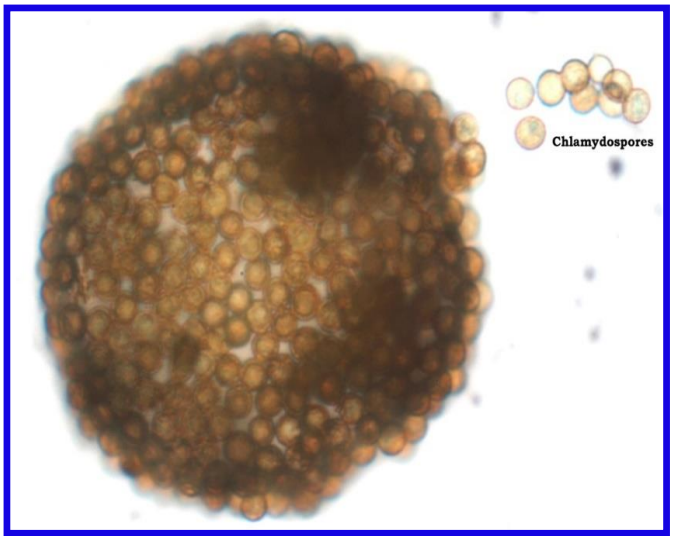

Plate II: Chlamydospores in sporeball.

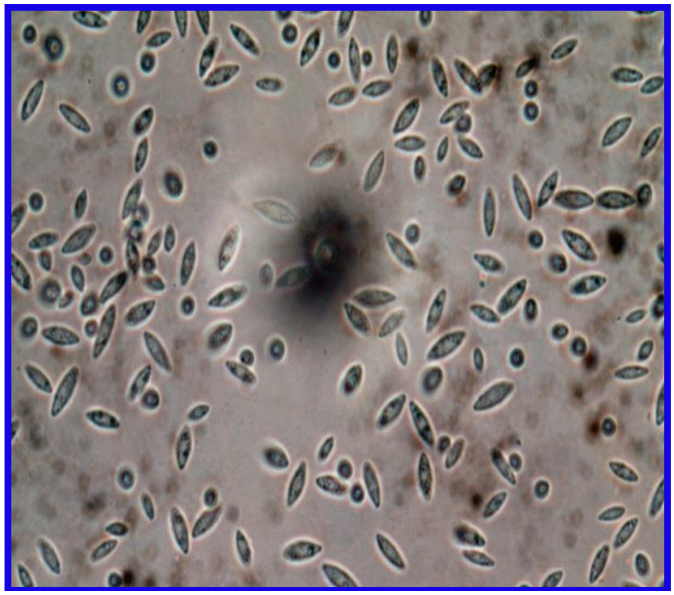

Plate III: Conidia of *U. virens* (Cke.) Tak. in culture.

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