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Cultural Variability, Viability and Longevity of Teliospores of *Sorosporium paspali-thunbergii* in Kodo Millet

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A B S T R A C T

The teliospores of *Sorosporium paspali-thunbergii* were observed in spore balls of 17-34 x 42-57 µ. The individual teliospores are globose, sub-globose, angular in shape and yellowish brown to dark brown in colour. The size of teliospore ranged 6-13 x 4-19 µ. Maximum teliospore germination was observed in 2 % glucose solution over 1 % glucose solution and tap water. Maximum radial growth was observed in Potato Dextrose Agar medium followed by Oat Meal Agar medium, Corn Meal Agar medium and Czapek's dox Agar medium. Potato Dextrose Agar medium produced white mycelial colony, submerged and regular fast growth and no pigmentation. White to yellow colony, Sub merged, regular moderate growth and no pigmentation were observed in Oat meal agar medium. Light pink pigmentation, white to light pink mycelial colony and cottony, regular moderate growth were observed in Czapek's dox Agar medium. In Corn meal agar medium was recorded off white colour mycelial colony, cottony, regular moderate growth and no pigmentation. Storage medium and storage period were also observed to influence the viability of teliospores. Highest germination was recorded in teliospores stored in paper bags at 4^0 C. Reduction in longevity of teliospores ranged from 24.6 to 44.6 % in different storage medium over a period of 30 to 105 days.

Introduction

Kodo millet (*Paspalum scrobiculatum* L.) is a small grain cereal indigenous to India (De wet *et al*., 1983) belonging to family Poaceae (Gamineae) and is extensively cultivated in tribal and hilly areas without any or low input by tribal and poor farmers for their consumption. The area of the kodo millet under cultivation is declining, but kodo millet still contributes to the regional food security of the dry and marginal lands, where major *kharif* crops fail to yield. The crops is cultivated is about 224 thousands hectares in India with productivity of 312 kg $kgha^{-1}$ (Anon, 2011). Among the biotic stresses particularly diseases, head smut caused by *Sorosporium paspli-thunbergii* (Henn.) Ito (*Sorosporium paspali thunbergii (*Henn.) Vanky) is an important fungal disease and is

endemic in all the states of the country. The pathogen is externally seed borne in nature however infection may also take place through soil borne inoculum that can reduce production in terms of grain yield. Head smut was first report from Queensland, Australia (McAlpine, 1910). Later on, the disease was reported in India by Butler (1918) and China by Teng (1947). In Madhya Pradesh, the disease was reported first time from Dindori and Jabalpur by Mishra *et al*., 1976. Viswanath (1992) reported 30 to 40 % loss in grain yield, whereas Jain and Yadava (1997) estimated a loss of 13.15 to 32.98 % at the disease incidence of 13.15 to 40.15 % in different varieties of kodo millet. The symptom appears in the crop at flowering stage and affected panicles are completely transformed into a long sorus which contains black powdery spore masses. Management of the disease through growing resistant varieties is the cheapest and best way, though few fungicides were found very effective *in Vitro* and *in Vivo*. Few released, pre-released and landraces of kodo millet resistant to head smut were reported.

Materials and Methods

To study the morphological characteristics, teliospores of the *Sorosporium paspali thunbergii* were collected from the freshly harvested smut sorus and measured the colour, size and shape of spore balls and teliospores under compound microscope in the different magnification (x) of 10 x 40.

For cultural characteristics studied, teliospores were collected from the freshly harvested smut samples. In this study we used four different culture media to know the better mycelial growth measured through the radial growth in mm. Sterilized teliospores of *Sorosporium paspali thunbergii* were placed at the centre of 85 mm petriplates containing 15 ml. test media. All the plates were incubated at 28^0 C and measured the radial growth. The radial growth was measured in mm 72, 120 and 168 hours after incubation. The culture characters of the pathogen (Colony colour, growth pattern etc.) were recorded by visual observation. Pigmentation was also recorded by colour production from the back side of the culture plate.

Different culture media used in this study were given in Table 1.

For spore morphology and measurement studied, teliospores of the *Sorosporium paspali thunbergii* were collected from the freshly harvested smut sorus and measured the size using ocular micrometer and shape of spore balls and teliospores under compound microscope in the magnification (x) of 15 x 40.

For spore germination studies, teliospores stored along with host were used for germination studies in different nutrients. The aqueous suspension of teliospores was prepared in tap water, 1 % glucose and 2 % glucose solution. Then a drop of the suspension was placed on the cavity slides in 4 replications. The cavity slides were kept in moist chamber prepared in petri plates and incubated at room temperature for 24 hours. Germination of teliospores was recorded under compound microscope and percent germination was calculated using following

Formula:

$$\text{Percent germination} = \frac{\text{Number of spore germinated}}{\text{Total no. of spores observed}} \times 100$$

For the teliospore longevity study of the head smut pathogen stored in different storage medium viz. paper bags and polythene bags at

4^0C in the refrigerator and at room temperature was studied at 15 days interval in cavity slides in 4 replications. The teliospore germination was recorded and germination percentage was calculated.

Formula:

$$\text{Percent germination} = \frac{\text{Number of spore germinated}}{\text{Total no. of spores observed}} \times 100$$

Results and Discussion

The results indicate on spore ball, size, colour and measurement of teliospores are presented in Table 1. Teliospores of *Sorosporium paspali-thunbergii* remains in loose ball like masses and disintegrated into individual spores in the water with little pressure. Size of spore ball ranged 17-34 x 42-57 µ and individual teliospores were globose, sub-globose and angular in shape, measured 6-13 x 4-19 µ. Teliospores were yellowish brown to dark brown in colour with thick smooth wall (Plate 1).

The results on germination of teliospores were taken from smut sorus enclosed in boot leaf with host and germination as studied in tap water, 1% glucose solution and 2% glucose solution. The results of teliospore germination are presented in table 2. Significant differences in teliospore germination were observed in various treatments. Maximum mean teliospore germination was recorded in 2% glucose solution (58.0%) followed by 1% glucose solution (53.4%) and tape water (50.3%). Maximum teliospore germination was recorded in 2% glucose solution over 1% glucose and tape water as also reported by Ahmed (1991), Nemade (2012) and Nemade *at el.* (2015b). Zundel (1953), Ahmad (1991), Shivas (2010), Nemade (2012) and Nemade *at el.* (2015b) also reported similar type of observations, which confirms the present findings.

The results on different cultural characteristics of *Sorosporium paspli-thunbergii* were studied in four culture media are presented in Table 3. The colony colour, growth pattern and pigmentation were found diverse in all culture media. *Sorosporium paspli-thunbergii* produced submerged, regular fast growing white colony without pigmentation in Potato Dextrose Agar (PDA). In Oat Meal Agar (OMA) media, fungus produced white to light yellow colony, submerged, regular moderate growing and without pigmentation. White to light pink colony, cottony, regular moderate growth with light pink pigmentation were recorded in Czapek's dox Agar medium whereas off white colony, cottony, regular moderate growth without pigmentation was found in Corn Meal Agar (CMA) (Plate 2 and 3).

The radial growth of *Sorosporium paspli-thunbergii* was recorded in four culture media at different time intervals i.e. 72, 120 and 168 hrs after incubation (Table 4). Significant differences in radial growth recorded in different culture media. Radial growth of the fungus ranged 35.3 to 42.7 mm, 48.6 to 62.0 mm and 68.7 to 89.3 mm after 72, 120 and 168 hours of incubation, respectively. Maximum mycelial growth of fungus was recorded in Potato Dextrose Agar (89.3 mm) followed by Oat Meal Agar (88.0 mm), Corn Meal Agar (88.0 mm) and Czapek's dox Agar (68.7 mm) after incubation of 168 hrs. The radial growth of *Sorosporium paspli-thunbergii* in Potato Dextrose Agar, Oat Meal Agar and Corn Meal Agar medium was at par. Ahmed (1991) and Nemade (2012) also reported maximum radial growth in Potato Dextrose Agar medium followed by followed by Czapak's Ager medium.

The results on viability and longevity, germination of teliospores were studied in at different storage medium *i.e.* paper bag, polythen bags and different time of intervals. Data of teliospore germination in two storage

medium and six storage periods were presented in Table 5 and Fig. 6. All the treatments were found to be significant. Teliospore germination was recorded 61.5 % after 30 days of storage in paper bags at 4^0 C, which gradually decreased and only 46.4 % germination was recorded after 105 days of storage. Similarly, 59.8 % teliospore germination was recorded after 30 days of storage and after 105 days of storage only 40.7 % germination was noted. In polythene bag storage at 4^0 C, spore germination was 47.1 % and 33.5 % after 30 and 105 days of storage, respectively. Teliospore stored in polythene bags at room temperature, germination percentage recorded 55.6 % after 30 days and 30.8 % after 105 days of storage.

Table.1 Different culture media used in this study

(A) Potato Dextrose Agar (PDA) medium

Composition	Quantities
Potato (peeled and sliced)	200 g
Dextrose	20 g
Agar-agar	20 g
Distilled water	1000 ml

(B) Czapek Agar (CA) Medium

Composition	Quantities
Sucrose	30 g
NaNO3	3 g
K2HPO4·H2O	1 g
MgSO4·7H2O	0.5 g
KCl	0.5 g
FeSO4	0.01 g
Agar	15 g
H2O(Distilled water)	1000 ml

(C) Oatmeal Agar (OMA) Medium

Composition	Quantities
Oatmeal	30 g
Agar-agar	15 g
Distilled water	1000 ml

(D) Corn Meal Agar (CMA) Medium

Composition	Quantities
Corn meal	200 g
Agar-agar	15 g
Distilled water	1000 ml

Table.1 Characteristics of *Sorosporium paspali-thunbergii*

S. No.	Characteristics	Colour and measurements
1	Size of spore bolls *	17-34 x 42-57 µ
2	Size of teliospores**	6-13 x 4-19 µ
3	Shape of teliospores	Globose, sub-globose and angular
4	Colour of teliospores	Yellowish brown to dark brown

*Mean of 30 spore bolls **Mean of 30 teliospores

Table.2 Effect of nutrients on teliospore germination of *Sorosporium paspali-thunbergii*

S. No.	Treatments	Germination (%)
T_1	Glucose solution (1%)	54.2(47.41)*
T_2	Glucose solution (2%)	60.5(51.06)
T_3	Tap water	50.3(45.17)
	Mean	55.0 (47.87)
	SEm	1.33
	CD (5%)	3.70
	CV(%)	4.83

* Figures are arc sin transformed values

Table.3 Cultural characteristics of *Sorosporium paspli-thunbergii* of kodo millet on different culture medium

S. No.	Medium	Colony colour	Growth pattern	Pigmentation
1	Potato Dextrose Agar	White	Sub merged, regular fast	No pigmentation
2	Oat Meal Agar	White to light yellow	Sub merged, regular moderate	No pigmentation
3	Czapek's dox Agar	White to light pink	Cottony, regular moderate	Light pink
4	Corn Meal Agar	Off white	Cottony, regular moderate	No pigmentation

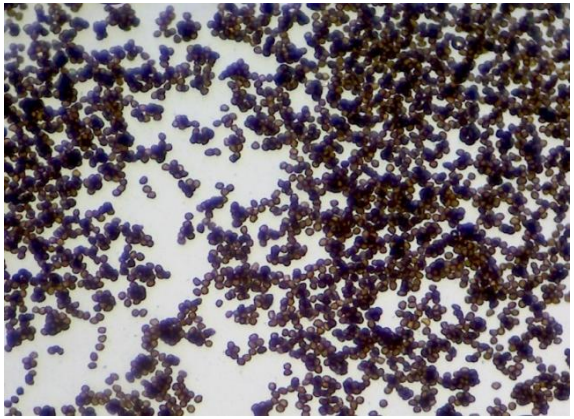
Table.4 Radial growth of *Sorosporium paspli-thunbergii* on different culture media

S. No.	Medium	Colony diameter (mm)		
		72 hrs	120 hrs	168 hrs
1	Potato Dextrose Agar	42.7	62.0	89.3
2	Oat Meal Agar	35.3	57.3	88.0
3	Czapek's dox Agar	39.3	48.6	68.7
4	Corn Meal Agar	38.7	57.3	88.0
	CD(P=0.05)	2.171	2.667	2.437

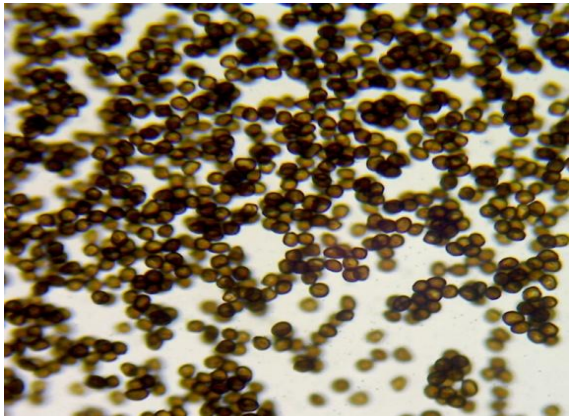
Table.4.6 Effect of storage medium and period on teliospore germination of *Sorosporium paspali-thunbergii*

S. No	Storage at different medium	Storage at different time intervals (days)							% reduction in teliospore germination
		30	45	60	75	90	105	Mean	
1	Teliospores in paper bags at 4°C	61.5 (51.65)*	59.5 (50.48)	56.8 (48.91)	54.7 (47.70)	50.5 (45.29)	46.4 (42.88)	54.9 (47.81)	24.6
2	Teliospores in paper bags at room temp.	59.8 (50.65)	56.5 (48.73)	54.9 (47.81)	51.6 (45.92)	46.5 (42.99)	40.7 (39.64)	51.7 (45.97)	31.9
3	Teliospores in polythene bags at 4°C	47.1 (43.34)	44.3 (41.73)	42.5 (40.69)	40.4 (39.47)	37.4 (37.70)	33.5 (35.37)	40.9 (39.76)	28.9
4	Teliospores in polythene bags at room temp.	55.6 (48.22)	46.4 (42.94)	43.5 (41.27)	38.2 (38.17)	34.7 (36.09)	30.8 (33.71)	41.5 (40.11)	44.6
	Mean	56.0 (48.45)	51.7 (45.97)	49.4 (44.66)	46.2 (42.82)	42.3 (40.57)	37.9 (38.00)	47.2 (43.39)	
	SEm±	0.301	0.392	0.386	0.334	0.330	0.343		
	CD (5%)	0.98	1.29	1.30	1.08	1.06	1.13		

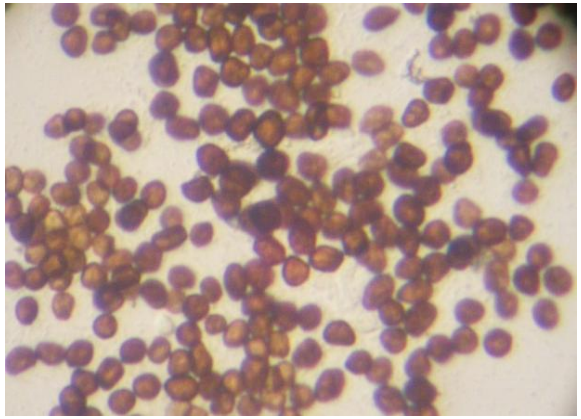
* Figures in parentheses are arc sin transformed values



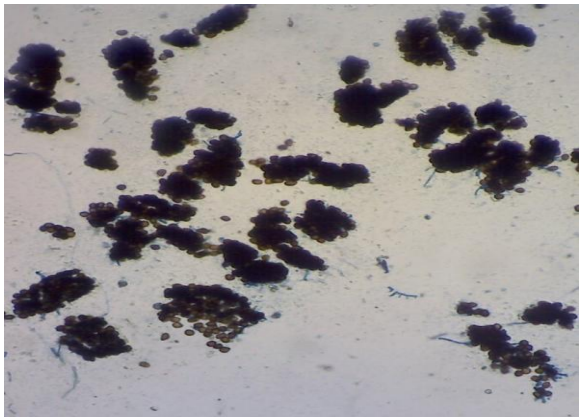
Non teliospore germination at 100 X magnification



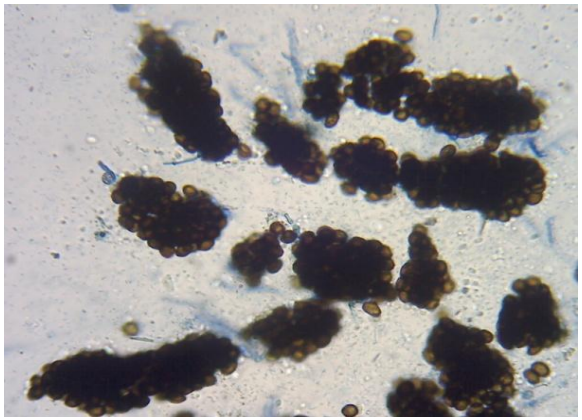
Nonteliospore germination at 200 X magnification



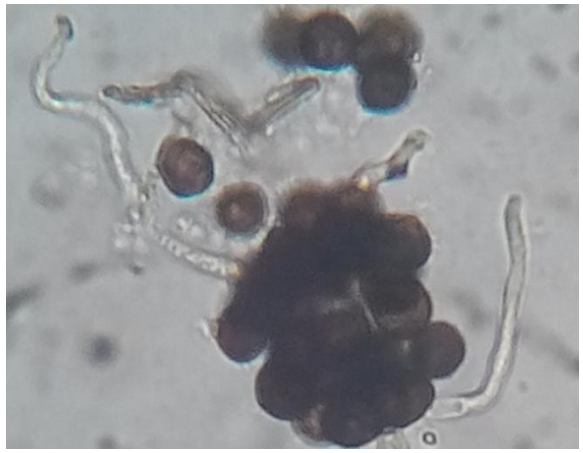
Non teliospore germination at 400 x magnification



Teliospore germination at 100 x magnification

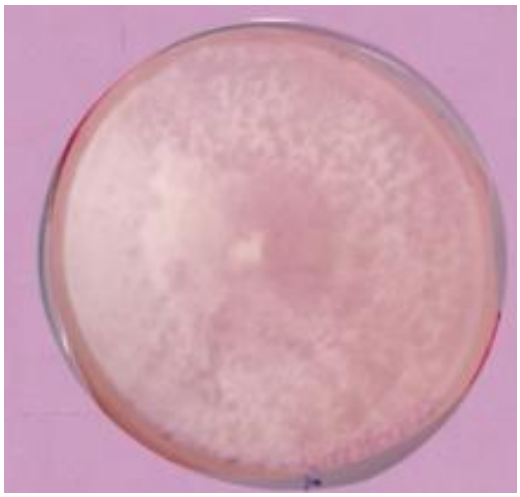


Teliospore germination at 200 X magnification



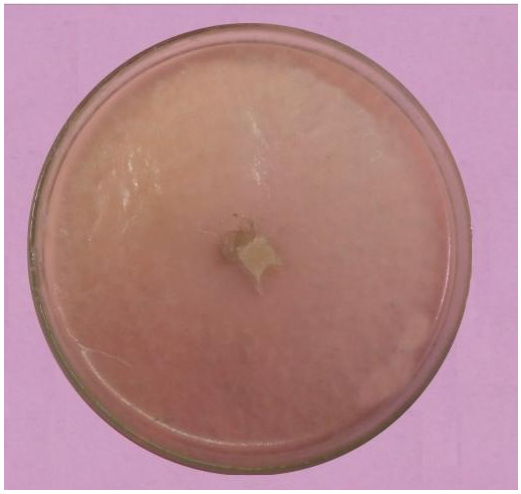
Teliospore germination at 400 x magnification

Plate 1.Non germinated and germinated teliospores of *Sorosporiumpaspalithunbergii*of kodo millet.

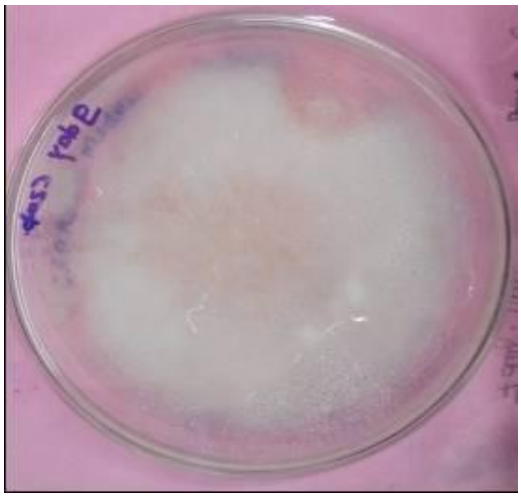


B- Potato Dextrose Agar

A- Oat Meal Agar



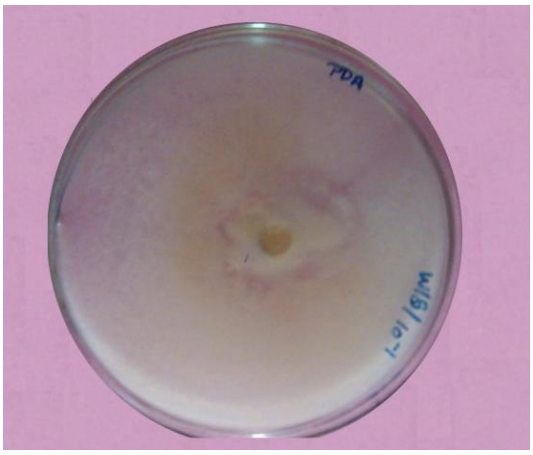
C - Corn Meal agar



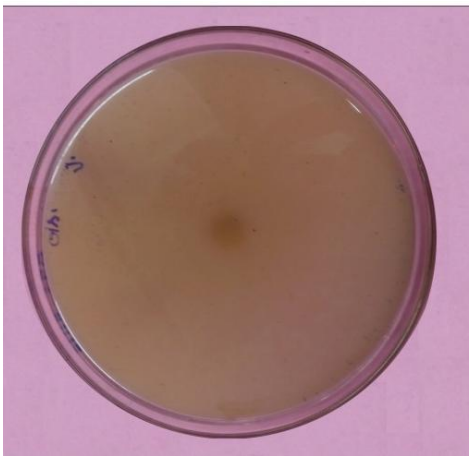
D- Czapek Dox agar

A- Front view of culture plates

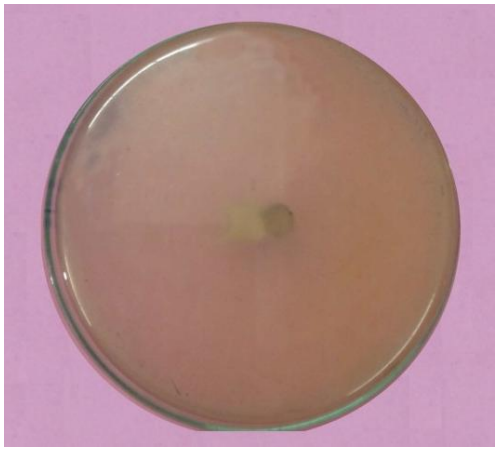
Plate.2 Radial growth of *Sorosporium paspali thunbergii* in different medium



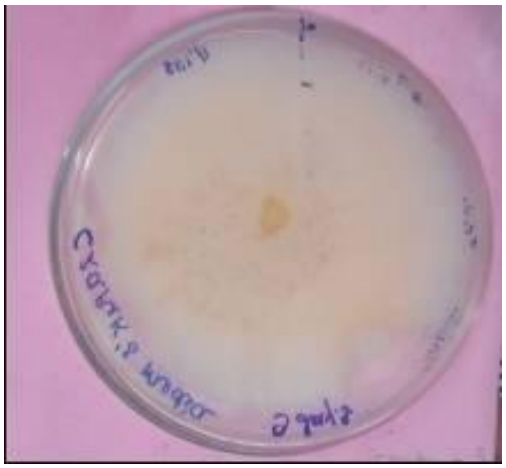
A- Potato Dextrose agar



B - Oat Meal Agar



C- Corn Meal Agar



D- Cazpek Dox Agar

A- Back view of culture plates

Plate.3 Radial growth of *Sorosporium paspali thunbergii* in different medium

Mean percent germination varied from 40.9 to 54.9 % in different storage medium was highest in teliospores stored in paper bags at 4°C (54.9 %) over a period of 30 to 105 days, where as lowest was recorded in teliospores stored in polythene bags at 4°C (40.9 %) over a period of 30 to 105 days. Maximum percent reduction in teliospore germination was recorded in teliospore stored in polythene bags at room temperature (44.6 %) over a period of 30 to 105 days, where as minimum percent reduction was recorded in teliospore stored in paper bags at 4°C(24.6 %) over a period of 30 to 105 days. Teliospore longevity of *Sorosporium paspali thunbergii* has been reported seven months by Ahmed (1991) that confirms the reduction of teliospores germination with storage period. Nemade (2012) also reported similar type of findings.

In conclusions, teliospores remains in spore balls, measured 17-34 x 42-57 µ and individual teliospores are globose to sub-globose, angular and yellowish brown to dark

brown in colour. Size of teliospore varied from 6-13 x 4-19 µ. Maximum radial growth of *Sorossporium pappali thunbergii* was in Potato Dextrose Agar medium after incubation of 168. White mycelial colony, submerged and regular fast growth and no pigmentation produced by the PDA medium. Viability of teliospores was maximum in 2 % glucose solution. Maximum viability was found in teliospores stored in paper bags at 4° C. Longevity of teliospores reduced significantly from 24.6 to 44.6 % with storage period from 30 to 105 days.

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