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A Comparative Analysis of Culture Media for Optimizing the Mycelial Growth and Sporulation of *Stemphylium vesicarium* Cause of White Blotch of Onion

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Abstract: This study was conducted to identify a cheap and suitable culture medium for the mycelial growth and sporulation of *Stemphylium vesicarium* and to determine the cultural and morphological variability of this pathogen. A total of 24 isolates of *S. vesicarium* collected from eight different onion growing areas were characterized in terms of cultural and morphological aspects. Front colony colors were greenish brown to dirty white, deep grey to whitish, light grey to whitish, deep greenish white, light grey and dirty white to greenish. Reverse colony colors were brown, deep brown and light brown. Colony shapes were circular and irregular with umbonate, raised and flat type colony elevation. Colony textures were cottony, fluffy and velvety with entire, undulate and filiform type colony margin. Among the culture media, V-7 juice agar found to be the most suitable culture media for mycelial growth of *S. vesicarium.* The sporulation of the fungus was remarkably influenced by V-7 juice mixed with potato dextrose agar (PDA) media, this media exhibited the highest sporulation (87.76-169.0/mm^2) of *S. vesicarium*in comparison with other media. The minimum days (28 d to 31 d) for conidial production were observed on V-7 juice agar medium. The length of conidia varied from 14.6 µm to 30.6 µm. The maximum mean length of conidia was 29.97 µm found in isolate DSSA, while the minimum mean length 17.36 µm was found in isolate MSMM 02. The breadth of conidia ranged from 4.7 µm to 15.7 µm. The maximum mean breadth of conidia was 12.55 µm found in the isolate DSSA, while the minimum mean breadth 9.760 µm was found in the isolate CCKH 02. The horizontal septation varied from 1 to 3 and the longitudinal septation varied from 0 to 4.

Key words: Onion, white blotch, *S. vesicarium*, V-7 juice agar.

1. Introduction

White blotch of onion, commonly known as stemphylium blight which is caused by *Stemphylium vesicarium*, is an important disease throughout the world. As per literatures in Refs. [1-3], onion crop is affected by 66 diseases, and among them stemphylium blight is an important one. In Bangladesh, onion production is gradually decreasing due to this major disease problem. Onion seed production is also severely affected by this disease, because it causes the breaking of floral stalks [4]. About 80%-85% losses were caused by *S. vesicarium* affecting leaves and inflorescence stalks of onion [5]. As onion (*Allium cepa*) is one of the most important and familiar spices crop throughout the world, so attention must be given on white blotch disease. Out of 15 important vegetables and spice crops listed by FAO, onion stands the second in terms of annual world production [6]. Among the onion producing countries, China tops the list with 205.08 million tons onion production, followed by 133.72 million tons in India, 33.21 million tons in USA and 19.23 million tons in Iran, whereas the production of onion in Bangladesh is only 1,159,259 tons [7], which is very lower compared to other onion growing countries in the world. The onion production per unit area is gradually decreasing in Bangladesh due to diseases problem [8]. Stemphylium blight has become more widespread in the onion growing region during recent years.

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Isolation and characterization of causal organism of *Stemphylium* from infected onion plant or plant parts is rather difficult. To study the *Stemphylium* in lab condition is a problem, particularly because of its slow growth habit on routine laboratory artificial culture media, like potato dextrose agar (PDA). Sometimes, it grows as mycelium, but does not sporulate easily in the culture media, and without sporulation it is difficult to identify a pathogen. Hashemi et al. [9] also reported that *Stemphylium* sp. do not sporulate well on ordinary synthetic media. To overcome this problem, many researchers have tried to find out the suitable culture media for the mycelial growth and sporulation of *Stemphylium* sp.. Borges et al. [10] harvested 7×10^4 conidia/mL of *S. botryosum* in V-8 juice agar medium from alfalfa. Salter and Leath [11] also used V-8 juice agar and achieved 1×10^4 conidia/mL to 5×10^4 conidia/mL. In Bangladesh, V-8 juice agar medium is unavailable and somewhat costly. Thus, conducting research on *Stemphylium* sp. cost effective and easily available media is necessary. So the present study was undertaken to identify a cheap and suitable culture medium for the mycelial growth and sporulation of *S. vesicarium* and to determine the cultural and morphological variability of this pathogen.

2. Materials and Methods

2.1 Collection, Isolation, Purification and Preservation of S. vesicarium

S. vesicarium was isolated by tissue planting methods from diseased leaves of onion grown at different locations. Purification was carried out by transferring conidia of *S. vesicarium* into PDA medium and incubated at 25 ± 1 °C with maintaining alternating dark and light period. The pure cultures of *S. Vesicarium* were preserved in refrigerator at 4 °C for further use.

2.2 Cultural Variability Study of S. vesicarium Isolates

A total of 24 isolates of *S. vesicarium* were used for cultural variability. For this target, 5 mm diameter mycelial discs of 7-day-old culture were transferred to the centre of different culture media—PDA, V-7 juice agar and combined PDA + V-7 juice agar (Table 1) and incubated at 25 ± 1 °C. Radial mycelial growth was measured after 3 d of incubation at 25 ± 1 °C. Three replications were maintained for each isolate in a completely randomized design. The radial mycelial growth was recorded from the 3rd days to 15th days after inoculation. Growth per day was calculated by Eq. (1):

$$\text{Growth rate (cm/d)} = (\text{growth on a day} - \text{growth on previous day})/2 \quad (1)$$

Sporulation time and conidia production were measured and recorded by using pure culture of *S. vesicarium* grown on PDA, V-7 juice agar and combined PDA + V-7 juice agar media. The conidia produced per unit surface area were measured using haemacytometer and digital microscope. The measurement was according to Chauhan and Pandey [12] as Eq. (2):

$$\text{No. of conidia/mm}^2 = \frac{\text{No. of conidia per mL suspension}}{\text{Total surface area of suspension}} \quad (2)$$

Finally, front and reverse colony color, shape, elevation, margin and texture of 24 isolates of *S. vesicarium* colony were recorded on PDA for cultural variability.

2.3 Morphological Variability Study of S. vesicarium Isolates

Morphological variability of 24 isolates of *S. vesicarium* in the terms of conidial shape, color, size and septation were observed and recorded on PDA medium. The 30-days-old, 40-days-old and 90-days-old pure culture was used for morphological variability.

2.4 Experimental Design

The experiment was laid out in a completely randomized design with three replications.

Table 1 Composition of different culture media for 1 L.

Name of media	Composition	
	Ingredient	Amount
PDA media	Potato slice	200 g
	Dextrose	20 g
	Agar	17 g
	Distilled water	1,000 mL
V-7 juice agar media (vegetables juice agar)	Tomato	174 mL
	Carrot	8 mL
	Celery	4 mL
	Spinach	4 mL
	Beet	4 mL
	Lettuce	4 mL
	Onion	2 mL
	Agar	15 g
	$CaCO_3$	2 g
	Distilled water	800 mL
Combined PDA + V-7 juice agar media	Components of PDA medium	200 mL
	Components of V-7 juice agar media	800 mL

2.5 Statistical Analysis

The data were analyzed by statistical software, MSTAT-C computer package program. The data were subjected to an analysis of variance, and least significant difference (LSD) were used to separate means and compared with Dunkan's multiple range test (DMRT), where *F* values indicated significantly differences at 5% level of probability.

3. Results

3.1 Cultural Variability of S. vesicarium Isolates

3.1.1 Mycelial Growth of *S. vesicarium* on Different Culture Media

The effect of different culture media, i.e., PDA, V-7 juice agar and combined PDA + V-7 juice agar, had a significant role on per-day radial mycelial growth of *S. vesicarium* (Table 2 and Fig. 1). The fungus grew well on V-7 juice agar medium and the maximum growth was recorded as 0.610-0.650 cm/d. The minimum radial mycelial growth (0.423-0.483 cm/d) was recorded on PDA medium, preceded by combined PDA + V-7 juice agar medium (0.470-0.503 cm/d).

3.1.2 Conidium Production of *S. vesicarium* on Different Culture Media

Marked variations were observed on conidium production of *S. vesicarium* on PDA, V-7 juice agar and combined PDA + V-7 juice agar culture media. The highest number (87.76-169.0/mm^2) of conidia was recorded on combined PDA+V-7 juice agar media, and the lowest number (20.73-36.67/mm^2) of conidia was found on PDA media, preceded by V-7 juice agar (55.01 to 92.12/mm^2) as clear shown in Fig. 2.

3.1.3 Sporulation Time of *S. vesicarium* on Different Culture Media

The effect of PDA, V-7 juice agar and combined PDA + V-7 juice agar culture media on sporulation time of *S. vesicarium* is presented in Fig. 3. The earliest (28.33-31.00 d) conidia production was observed on V-7 juice agar in respect of all isolates. On PDA, comparatively more days (86.33-90.00 d) were required for conidia production.

3.1.4 Characteristics of Colony Color, Shape, Elevation, Margin and Texture on PDA

The isolates of *S. vesicarium* exhibited variations in colony characters, like color, shape, elevation, margin and texture. Front colony colors were greenish brown to dirty white, deep grey to whitish, light grey to whitish, deep greenish white, light grey and dirty white to greenish, whereas reverse colony colors were deep brown, brown and light brown. Circular and irregular colony shapes were found in *S. vesicarium*, respectively. Colony elevation was umbonate, raised

Table 2 Daily radial mycelial growths of 24 isolates of *S. vesicarium* on different culture media in different location.

Isolates	Location (district/sub-district)	Mean colony diameter of radial mycelia growth/day (cm)*		
		On PDA agar	On V-7 juice agar	On combined PDA + V-7 agar
DSSA	Sher-e-Bangla Nagar, Dhaka	0.460	0.616	0.473
DSTR 01	Savar, Savar	0.480	0.630	0.483
DSTR 02		0.480	0.626	0.470
MMBH	BAU, Mymensingh Sadar	0.433	0.640	0.493
MTBB 01	Trishal, Trishal	0.476	0.633	0.490
MTBB 02		0.463	0.620	0.500
RBHR 01	Rajshahi, Bagmara	0.466	0.650	0.486
RBHR 02		0.453	0.626	0.480
RBHR 03		0.443	0.640	0.496
GJBS	BARI, Joydebpur	0.440	0.640	0.496
GGBB 01	Gazipur Sadar	0.430	0.620	0.486
GGBB 02		0.453	0.626	0.480
CCKH 01	Chandina, Comilla	0.423	0.640	0.496
CCKH 02		0.483	0.616	0.496
CCKH 03		0.460	0.616	0.490
JJLL 01	Jamalpur Sadar	0.466	0.636	0.486
JJLL 02		0.446	0.630	0.476
JJLL 03		0.470	0.633	0.490
MSMM 01	Manikganj, Shibalaya	0.460	0.620	0.503
MSMM 02		0.470	0.633	0.486
MSMM 03		0.463	0.640	0.486
FFKU 01	Faridpur, Faridpur Sadar	0.456	0.623	0.480
FFKU 02		0.476	0.626	0.500
FFKU 03		0.453	0.610	0.483
LSD (0.05)		0.0519	0.0519	0.0519
CV		6.40%	2.72%	2.84%

*Means of three observations for each isolate.

and flat type. Entire, undulate and filiform margins were found in *S. vesicarium* colony. Colony texture was cottony, fluffy and velvety. These results are presented in Table 3.

3.2 Morphological Variability of S. vesicarium Isolates

3.2.1 Conidial Shape, Color and Size of *S. vesicarium* on PDA

Distinct variations were observed in conidial shape, color and size of 24 isolates of *S. vesicarium* on PDA. Conidial shape was ovoid, oblong and ovoid to oblong type, which were light brown, brown and deep brown in color. The length of conidia varied from 14.6 µm to 30.6 µm. The maximum mean length (29.97 µm) was recorded in DSSA isolate, and the minimum mean length (17.36 µm) in isolate MSMM 02. The breadth of conidia ranged from 4.7 µm to 16.24 µm. The highest mean breadth (12.55 µm) was recorded in isolate DSSA and the minimum mean breadth (9.760 µm) was in CCKH 02 isolate. These results are presented in Table 4.

3.2.2 Septation of *S. vesicarium* Conidia on PDA

Distinct variations were observed in horizontal and longitudinal conidial septation of 24 isolates of *S. vesicarium*. The maximum mean number of horizontal septation was recorded in DSTR 01 (2.500) isolate and the minimum (1.600) was in isolates of MTBB 01,

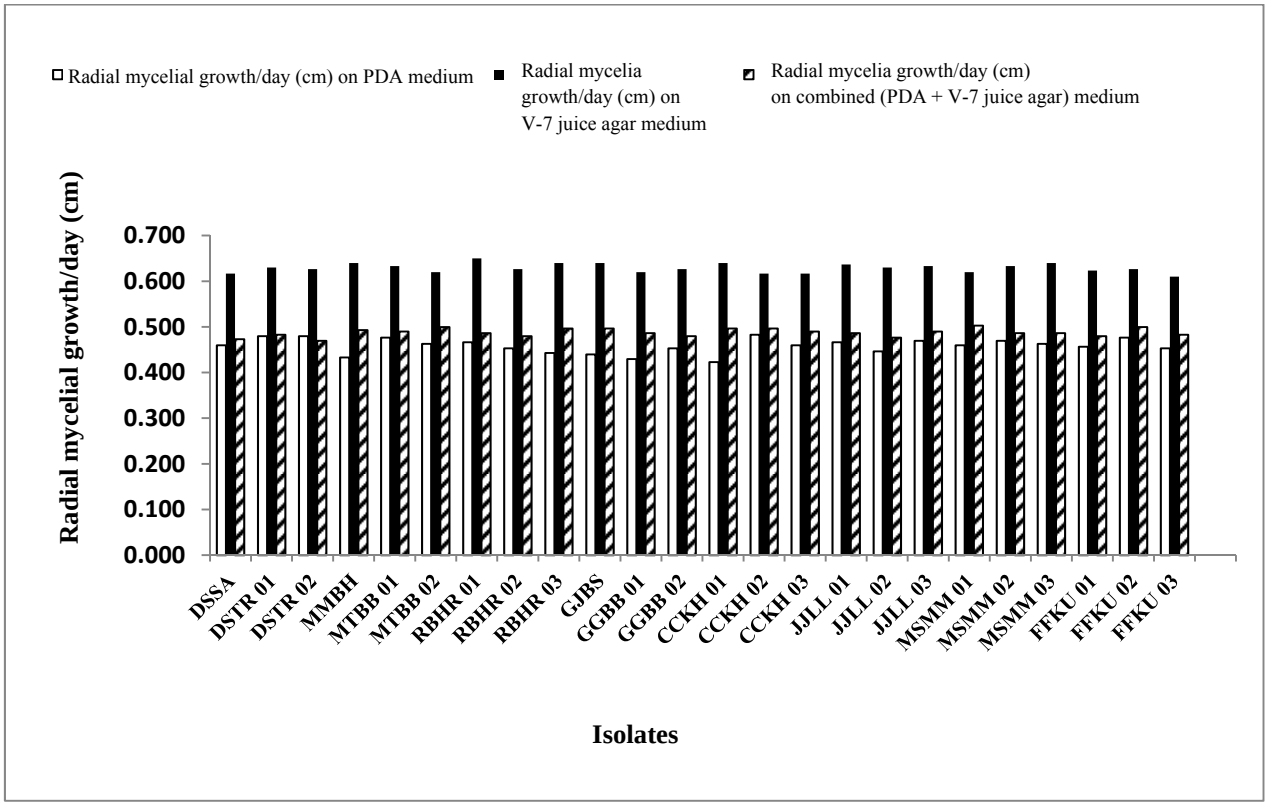

Fig. 1 Radial mycelial growth of *S. vesicarium* on PDA, V-7 juice agar and combined PDA + V-7 juice agar media.

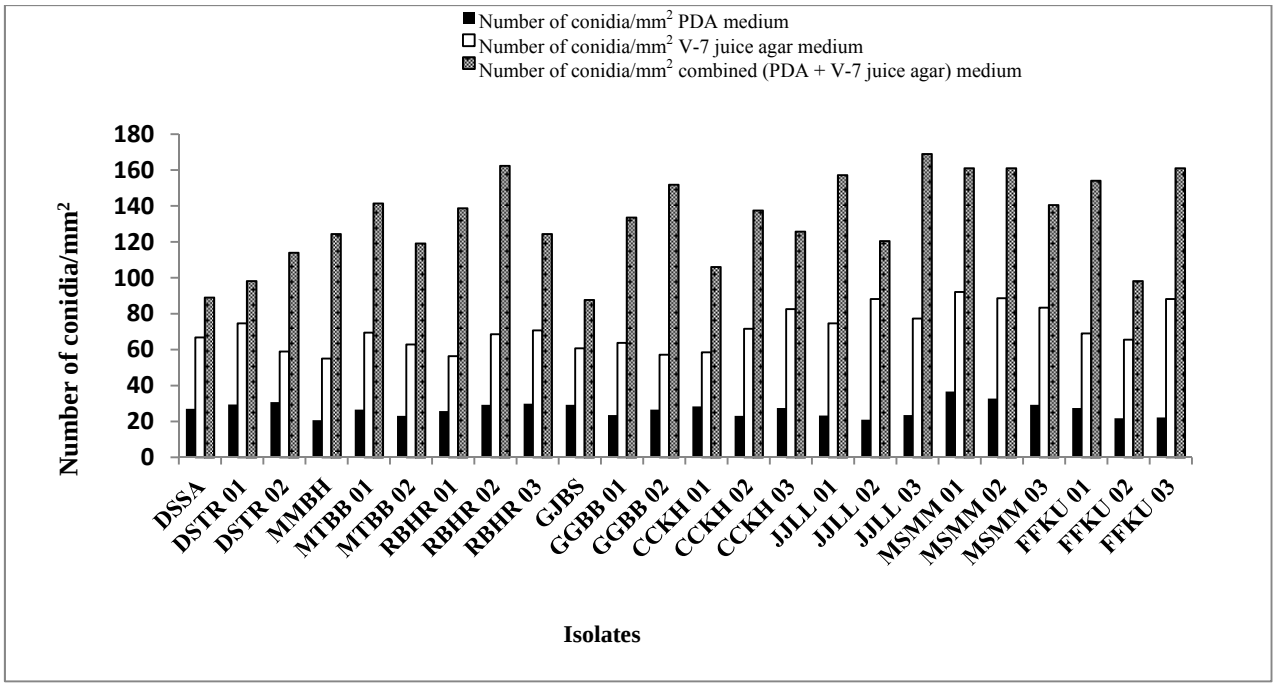

Fig. 2 Conidia production of 24 isolates of *S. vesicarium* on PDA, V-7 juice agar and combined PDA + V-7 juice agar media.

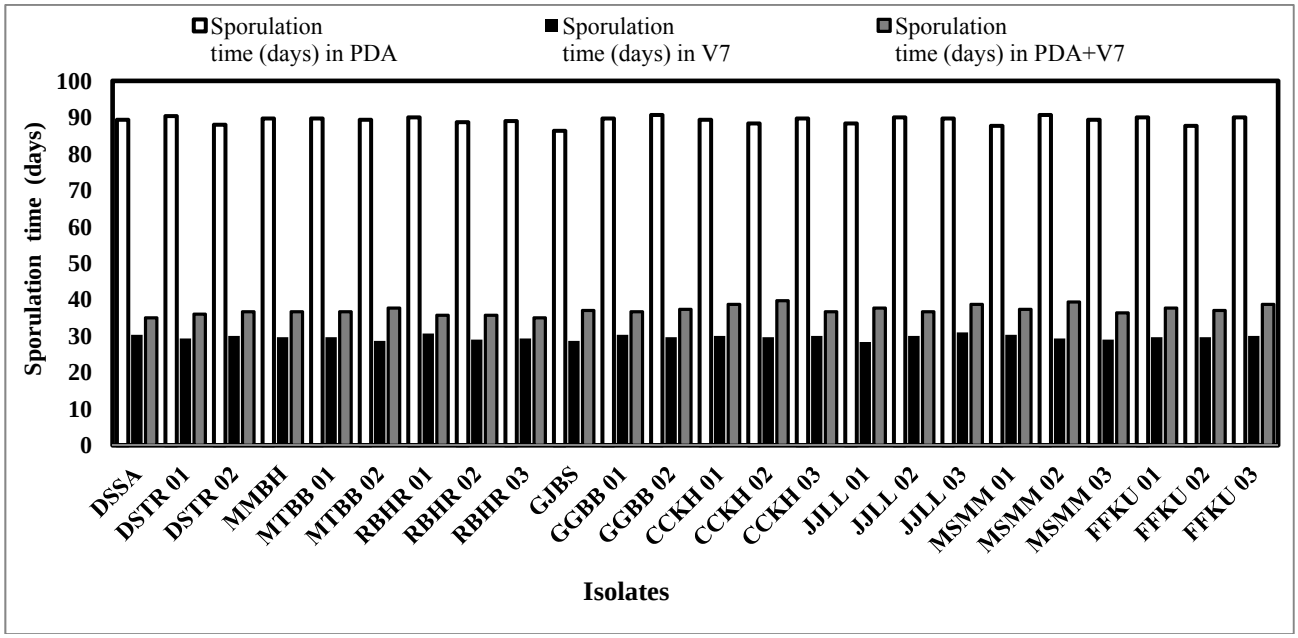

Fig. 3 Sporulation time (days) of 24 isolates of *S. vesicarium* on PDA, V-7 juice agar and combined PDA + V-7 juice agar media.

Table 3 Front and reverse colony color, shape, elevation, margin and texture of 24 isolates of *S. vesicarium* on PDA.

Isolates	Cultural variability on PDA					
	Front colony color	Reverse colony color	Colony shape	Colony elevation	Colony margin	Colony texture
DSSA	Greenish brown to dirty white	Deep brown	Irregular	Raised	Undulate	Cottony
DSTR 01	Deep greenish white	Deep brown	Irregular	Umbonate	Entire	Cottony
DSTR 02	Greenish brown to dirty white	Deep brown	Circular	Raised	Entire	Cottony
MMBH	Light grey	Light brown	Circular	Raised	Entire	Cottony
MTBB 01	Deep grey to whitish	Deep brown	Irregular	Raised	Filiform	Fluffy
MTBB 02	Deep grey to whitish	Deep brown	Circular	Raised	Entire	Fluffy
RBHR 01	Dirty white to greenish	Brown	Circular	Flat	Entire	Velvety
RBHR 02	Deep grey to whitish	Brown	Circular	Raised	Filiform	Fluffy
RBHR 03	Greenish brown to dirty white	Light brown	Irregular	Raised	Undulate	Cottony
GJBS	Deep grey to whitish	Brown	Circular	Umbonate	Entire	Fluffy
GGBB 01	Greenish brown to dirty white	Light brown	Circular	Umbonate	Entire	Cottony
GGBB 02	Greenish brown to dirty white	Light brown	Circular	Raised	Entire	Cottony
CCKH 01	Greenish brown to dirty white	Light brown	Circular	Raised	Entire	Velvety
CCKH 02	Deep grey to whitish	Brown	Circular	Umbonate	Filiform	Fluffy
CCKH 03	Deep grey to whitish	Brown	Irregular	Umbonate	Undulate	Fluffy
JJLL 01	Deep grey to whitish	Brown	Circular	Raised	Undulate	Fluffy
JJLL 02	Deep grey to whitish	Brown	Irregular	Umbonate	Filiform	Fluffy
JJLL 03	Deep grey to whitish	Brown	Circular	Raised	Filiform	Fluffy
MSMM 01	Deep grey to whitish	Brown	Irregular	Raised	Entire	Fluffy
MSMM 02	Deep grey to whitish	Brown	Irregular	Raised	Undulate	Fluffy
MSMM 03	Deep grey to whitish	Deep brown	Circular	Raised	Filiform	Fluffy
FFKU 01	Deep grey to whitish	Deep brown	Circular	Raised	Filiform	Fluffy
FFKU 02	Light grey to whitish	Light brown	Circular	Raised	Entire	Cottony
FFKU 03	Deep grey to whitish	Deep brown	Irregular	Umbonate	Filiform	Fluffy

Table 4 Shape, color and size of conidia of 24 isolates of *S. vesicarium* on PDA.

Isolates	Shape	Color	Conidial size			
			Conidial length[1] (μm)		Conidial breadth[1](μm)	
			Range	Mean	Range	Mean
DSSA	Ovoid	Deep brown	29.5-30.6	29.97	4.7-14.5	12.55
DSTR 01	Oblong	Light brown	20.4-27.8	25.13	7.7-15.1	9.80
DSTR 02	Oblong	Light brown	23.5-26.2	24.43	6.0-15.3	11.89
MMBH	Ovoid to oblong	Light brown	19.0-25.3	23.13	6.9-14.5	11.10
MTBB 01	Ovoid	Deep brown	20.0-27.6	24.87	8.2-12.1	10.15
MTBB 02	Ovoid	Deep brown	23.7-25.2	24.60	9.6-14.7	11.36
RBHR 01	Ovoid	Brown	19.8-27.9	23.86	9.4-14.4	11.86
RBHR 02	Oblong	Brown	22.4-23.1	22.67	8.1-13.8	10.81
RBHR 03	Ovoid to oblong	Light brown	21.2-24.9	22.60	9.3-13.7	10.89
GJBS	Ovoid to oblong	Deep brown	16.9-22.7	20.74	8.1-12.1	11.12
GGBB 01	Oblong	Light brown	17.6-25.7	21.27	9.0-14.6	10.98
GGBB 02	Oblong	Light brown	24.9-28.1	26.80	8.2-14.7	11.73
CCKH 01	Oblong	Brown	15.0-19.1	17.50	8.2-15.7	11.29
CCKH 02	Oblong	Brown	20.2-27.7	25.42	6.7-16.2	9.76
CCKH 03	Oblong	Light brown	20.8-28.4	25.37	7.7-14.2	10.34
JJLL 01	Ovoid	Deep brown	19.9-26.4	24.02	7.9-14.9	10.54
JJLL 02	Oblong	Brown	18.3-23.2	20.73	8.7-13.9	11.01
JJLL 03	Ovoid	Light brown	16.3-21.4	19.47	7.3-13.3	10.95
MSMM 01	Ovoid to oblong	Deep brown	15.5-22.3	18.09	7.3-12.9	9.82
MSMM 02	Ovoid to oblong	Brown	14.6-20.5	17.36	7.3-14.4	10.23
MSMM 03	Oblong	Brown	19.0-25.3	23.32	9.2-12.8	10.13
FFKU 01	Oblong	Light brown	19.7-24.6	19.98	9.6-13.5	9.86
FFKU 02	Ovoid	Brown	16.9-25.2	21.87	7.9-14.3	10.52
FFKU 03	Ovoid	Deep brown	17.2-24.9	24.52	8.0-13.9	11.23
LSD (0.05)				0.563		0.395
CV				1.50%		2.23%

[1]Means of 10 observations for each isolate.

RBHR 01, GGBB 01 and MSMM 01. The maximum mean number of longitudinal septation (2.500) was recorded in MMBH isolate and the minimum (1.400) in GGBB 02 isolate.

4. Discussion

In this study, three different culture media were used to identify which would be suitable in the terms of facilitating radial mycelial growth. Results showed that among the three selected culture media, V-7 juice agar gave the best performance, while the lowest radial mycelial growth was observed on PDA medium. Results revealed that presence of seven different vegetables juice in culture medium favored radial mycelial growth of *S. vesicarium.* But in the terms of sporulation capacity, combined PDA + V-7 juice agar medium exhibited the highest performance (87.76-169.0 conidia/mm^2) in comparison to other media used. Kumar [13] also identified a suitable culture medium for the sporulation of *S. botryosum* and found that the fungus produce the highest number of conidia (84.7 ± 6.0 × 10^4 conidia/mL) on V8 juice potato dextrose agar (V8-P) medium. All the isolates showed variation in respect of sporulation time on different media. The sporulation time of *S. vesicarium* on PDA varied from 86.33 d to 90.67 d with the maximum sporulation time in isolate GGBB 02 and MSMM 02, respectively, and the minimum in GJBS isolate. On V-7 juice agar, the sporulation time for all isolates varied from 28.33 d to 31.0 d with the

maximum sporulation time in isolate JJLL 03 and the minimum in JJLL 01. On combined PDA + V-7 juice agar, the sporulation time varied from 35.0 d to 39.67 d with the maximum sporulation time in isolate CCKH 02 and the minimum in DSSA and RBHR 03 isolates, respectively. The results of the present investigation showed that *S. vesicarium* produces conidia on V-7 juice agar earlier than on PDA and combined PDA + V-7 juice agar medium.

Colonies of *S. vesicarium* showed deep grey to whitish, greenish brown to dirty white, light grey to whitish, deep greenish white, light grey and dirty white to greenish color from frontal view on PDA. The results are in agreement with Salter and Leath [11], who found that the colony color of *Stemphylium solani* isolated from pepper was gray in color and gray to light brown growth found in case of *Stemphylium lycopersici* isolated from tomato plants on PDA. Hosen et al. [14] observed greenish brown and dirty white color colony of *Stemphylium botryosum* isolated from lentil plants. Whereas, reverse colony colors were deep brown, brown and light brown. Circular and irregular shaped colonies were found in all the isolates with entire, undulate and filiform margin on PDA. The results are in agreement with Hosen [15], who found that the colony margin of *Stemphylium botryosum* isolated from lentil plants was entire and regular. Umbonate, raised and flat type colony elevation were found among all the isolates with cottony, fluffy and velvety texture on PDA. Arzanlou et al. [16] found flat type colony elevation of *S. vesicarium* grown on potato carrot agar (PCA) media. The results of textural growth of *S. vesicarium* are in agreement with Mehta [17], who found that the mycelium of *Stemphylium solani* isolated from cotton was velvety, cottony or immersed. Similarly, Hosen et al. [14] also observed velvety, effuse and fluffy type colony texture of *Stemphylium botryosum* isolated from lentil plants. Ovoid, oblong and ovoid to oblong shaped conidia of *S. vesicarium* were observed under digital microscope in this research work. The present findings agreed with the report of Ellis [18] and Koike et al. [19], who found oblong to ovoid shaped conidia of *Stemphylium*, and reported that the conidia of *Stemphylium botryosum* were broadly ovoid. Oblong, ovoid or broadly ellipsoidal shaped conidia of *Stemphylium luffae*, *Stemphylium lycii* and *Stemphylium cucumis* was also found by Pei et al. [20]. Remarkable variations were also observed in conidial color of 24 isolates of *S. vesicarium* on PDA, where deep brown, brown and light brown color conidia were observed. The current findings were well supported by some reported works [17, 19, 21], which worked on *Stemphylium solani* and *Stemphylium lycopersici* and observed tan to light brown colored conidia. Koike et al. [19] and Zheng et al. [21] found brown colored conidia of *Stemphylium botryosum* and *Stemphylium solani*.

Marked variations were observed in conidial length and breadth of *S. vesicarium* on PDA. The length of conidia varied from 14.6 μm to 30.6 μm, and the breadth of conidia ranged from 4.7 μm to 15.7 μm. The present findings agreed with the report of Arzanlou et al. [16] and Ellis [18], who measured 25-40 μm × 13-21 μm and 20-24 (-30) μm × 12-15 μm conidial length and breadth of *S. vesicarium*. Hosen et al. [14, 15], Koike et al. [19] and Simmons [22] also observed 33-35 μm × 24-26 μm, 17-28 μm × 13-19 μm, 10-25 μm × 5-15 μm and 12.35-23.45 μm × 10.5-15 μm onidia of *Stemphylium botryosum*. In this research, the horizontal septation of *S. vesicarium* conidia varied from 1 to 3, and the longitudinal septation varied from 0 to 4. These findings collaborate with the reports in other Refs. [11, 16, 18] which found that *S. vesicarium* conidia have 1-3 transverse, 1-4 longitudinal or oblique septa and 1-5 transverse and several longitudinal septa. Pei et al. [20] noted 1-3 dark colored transverse septa and 2-7 longitudinal or oblique septa in *Stemphylium solani*. They also found 3-5 transverse septa and 2-5 longitudinal or oblique septa in *Stemphylium luffae* [20].

In this research work, only three types of cultural media were used, but for the establishment of suitable

cultural medium for the growth and sporulation of *S. vesicarium*, more research work should be performed.

5. Conclusions

From the present research, it is concluded that all isolates of *S. vesicarium* showed variations in the terms of cultural and morphological aspects. Different culture media had profound effect on radial mycelial growth of *S. vesicarium.* V-7 juice agar medium appeared to be the best for supporting the maximum mycelial radial growth of this fungal pathogen. Combined PDA + V-7 juice agar appeared to be the best for the sporulation of *S. vesicarium*.

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