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Research Article

Morphological Variation Amongst *Gleosporium ampelophagum* Causing Anthracnose of Grapes from Maharashtra in Pictorial Form

Chavan SS[1], VC Khilare[2]

[1]Department of Botany. S.G.R.G. Shinde Mahavidyalaya, Paranda
[2]Botany Research Centre, Vasantrao Naik Mahavidyalaya, Chh. Sambhajinagar.
Email: sschavan.09@gmail.com

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Abstract

Anthracnose of grapes, commonly known as bird's-eye spot, is an economically important disease in grape-growing regions of Maharashtra and is caused by the fungal pathogen *Gloeosporium ampelophagum*. The present study was undertaken to investigate the morphological variability among different isolates of *G. ampelophagum* collected from anthracnose-infected grape vineyards across various agro-climatic regions of Maharashtra. Diseased samples showing typical anthracnose symptoms were collected and the pathogen was isolated and maintained on CZA mediun . Considerable variation was observed among isolates with respect to colony characteristics such as color, texture, growth rate, zonation and margin. The formation of acervuli varied in size, distribution and presence or absence of setae. Microscopic examination revealed significant differences in conidial shape and size, although all conidia were hyaline and aseptate. Pictorial documentation of cultural characteristics, acervuli, setae and conidia was carried out using stereo-microscope and compound microscope to support the observed morphological variations. The study highlights the existence of considerable morphological diversity within *G. ampelophagum* populations in Maharashtra, which may have implications for disease epidemiology, pathogenic variability and management strategies of grape anthracnose.

INTRODUCTION

Grape (*Vitis vinifera* L.) is grown from temperate to warm regions; however, hot and dry climate is ideal. Indian grapes come in varied characteristics and are successfully grown in country. Modern pack-house facility with automatic forced air system for pre-cooling is available in all the commercial production areas. Traceability system is maintained for the product tracking. Extensive Residue Monitoring plan for monitoring the pesticide residues in grapes is implemented for consumer safety. More than 20 varieties are under cultivation in India. Currently, Thompson Seedless is the ruling grape variety occupying 55% of the area with its clones. Major grape-growing states are Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, and the north-western region covering Punjab, Haryana, western Uttar Pradesh, Rajasthan and Madhya Pradesh. Maharashtra ranks first in terms of production accounting for more than 75% of total production and highest productivity in the country. Grape is one of the important fruit covering an area of 60.2 thousand hectares occupying 1.30% of the total area. The total production of grapes in India is 1546 thousand MT. During the year 2008-09, total export was 1,31,364 MT worth Rs 545.33 crores. But increase in MRL (minimum residue level) of chlormequat chloride (growth hormone) 2800 containers were rejected from Nashik district amongst 3400. Export was declined suddenly and it was remained only at 46,752 MT worth Rs. 162.46 crores. The major export destinations in 2010-11 were European Union, Bangladesh, UAE and Germany. In addition to its importance as a fruit crop, it is valued for products such as winery and currant industry which

increases profitability. It is affected by various fungal diseases. Amongst them anthracnose is important one caused by *G. ampelophagum*. It infects on all succulent parts of the plant, including stems, leaves, petioles, tendrils, young shoots, and berries. Symptoms on young, succulent shoots first appear as numerous small, circular, and reddish spots. Dark reddish-brown to violet-black margins eventually surround the lesions. Lesions may coalesce, causing a blighting or killing of the shoot. Anthracnose on petioles appears similar to that on the shoots. Leaf spots are often numerous, the necrotic center of the lesion often drops out, creating a shot-hole appearance. Young leaves are more susceptible to infection than older leaves. On berries, small, reddish circular spots may become slightly sunken (Plate-I). Anthracnose is very severe in rainy season and results in total crop loss and also decreases the quality of berries if proper management strategies are not undertaken. Different fungicides like carbendazim, chlorothalonil, difenoconazole, iprodione, kitazin, mancozeb, propiconazole and ziram are recommended for the management of this pathogen (NRCG, 2007). However application programme of carbendazim, difenoconazole and propiconazole may influence the development of resistance in the pathogen.

MATERIALS AND METHODS

Isolation of *Gloeosporium ampelophagum*

The infected samples of grape anthracnose collected from different orchards known as 'grape belt' of Maharashtra like Pune, Sangli, Nashik and Solapur during crop seasons. Isolation of pathogen was done by inoculating the samples on Rose Bengal agar medium and the cultures were further purified and maintained on Czapek-Dox agar medium at 27± 1° C.

Morphological variation amongst various isolates of *G. ampelophagum*:

Isolates of *G. ampelophagum* were analyzed for their colony morphology, colour variation in colony, acervuli, size of conidia, and exudation. Various colony characters were studied with its exudates and conidial size by using Olympus CH 20i microscope equipped with Magnus Live camera and Magnus Pro and Analytics image capture software.

Culture media used:

Rose Bengal agar medium: Peptone-5 g, K_2HPO_4-1 g, $MgSO_4$-0.5 g, Glucose-10 g, Rose Bengal-0.001 g, Agar agar-20 g, Distilled water-1000ml

Czapek Dox agar medium: Sucrose-30 g, $NaNO_3$-2.0 g, KH_2PO_4-2.0g, $MgSO_4$-0.5 g, KCl -0.5 g, $FeSO_4$-0.1 g, Agar agar-20 g, Distilled water-1000ml

Anthracnose or Birds eye disease on grapes:

Causal organism: ***Gleosporium ampelophagum***

Symptoms:

1. Infection may occur on all succulent plant parts but is most common on young shoots, fruits, leaves and stem.
2. Shoot lesion appear as reddish spots that become elongated and sunken with gray centres and a dark reddish raised border.
3. Leaf spots are often numerous and resembles those on fruit. These spots develop in similar manner and eventually become circular with gray centers and red borders. The center often drops out leaving a shot hole appearance.
4) Young leaves are more susceptible than older leaves and are malformed when veins become infected.
5) The disease mainly occurs in rainy season.

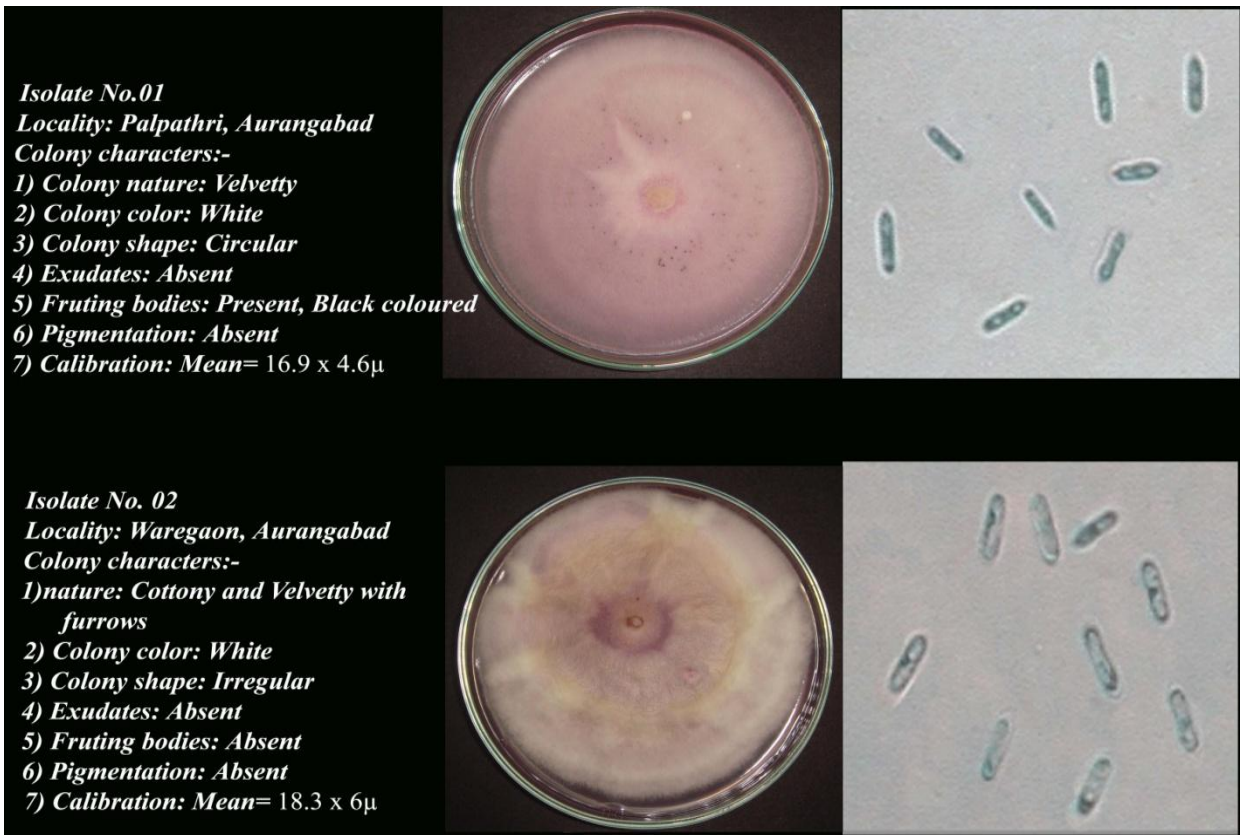
Symptoms of anthracnose disease on grapes crop:

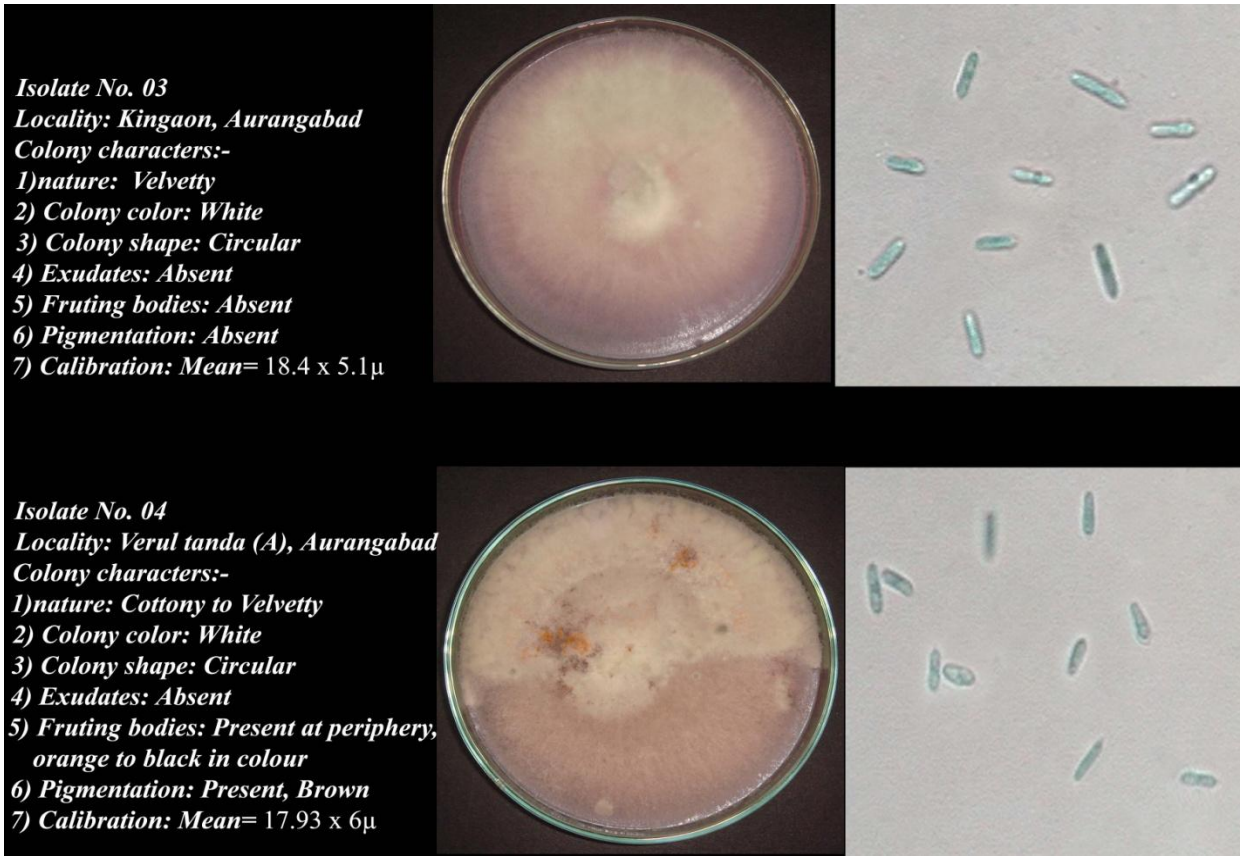
Infected fruit

Infected stem

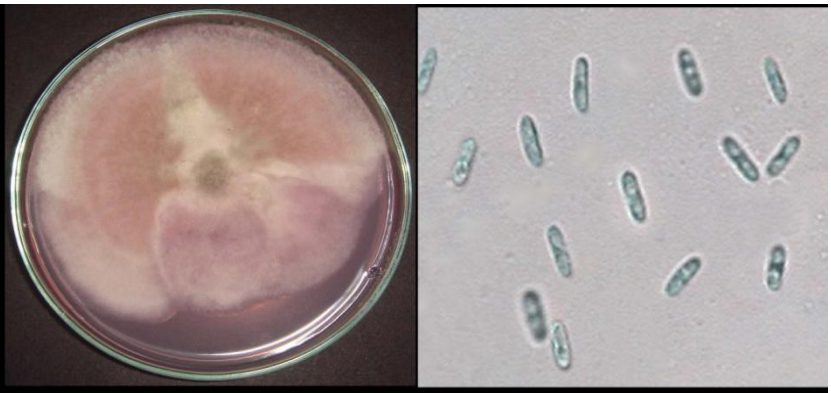
Infected leaves

Morphological variation amongst *Gleosporium ampelophagum* in pictorial form .

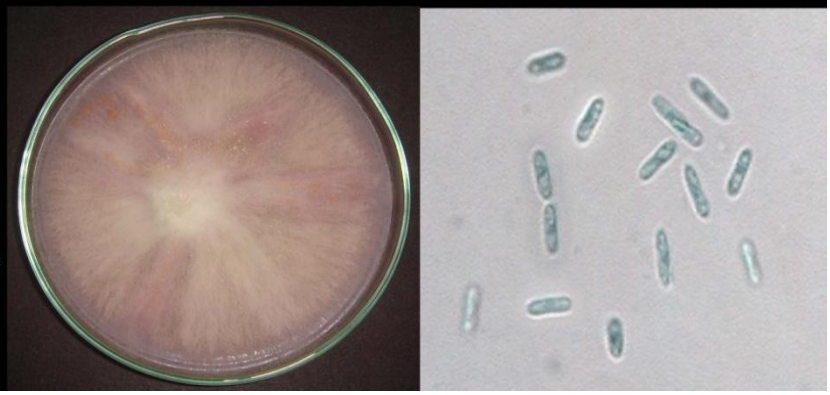

Isolate No.01
Locality: Palpathri, Aurangabad
Colony characters:-
1) Colony nature: Velvetty
2) Colony color: White
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Present, Black coloured
6) Pigmentation: Absent
7) Calibration: Mean= 16.9 x 4.6µ
Isolate No. 02
Locality: Waregaon, Aurangabad
Colony characters:-
1)nature: Cottony and Velvetty with furrows
2) Colony color: White
3) Colony shape: Irregular
4) Exudates: Absent
5) Fruting bodies: Absent
6) Pigmentation: Absent
7) Calibration: Mean= 18.3 x 6µ


Isolate No. 03
Locality: Kingaon, Aurangabad
Colony characters:-
1)nature: Velvetty
2) Colony color: White
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Absent
6) Pigmentation: Absent
7) Calibration: Mean= 18.4 x 5.1µ
Isolate No. 04
Locality: Verul tanda (A), Aurangabad
Colony characters:-
1)nature: Cottony to Velvetty
2) Colony color: White
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Present at periphery, orange to black in colour
6) Pigmentation: Present, Brown
7) Calibration: Mean= 17.93 x 6µ

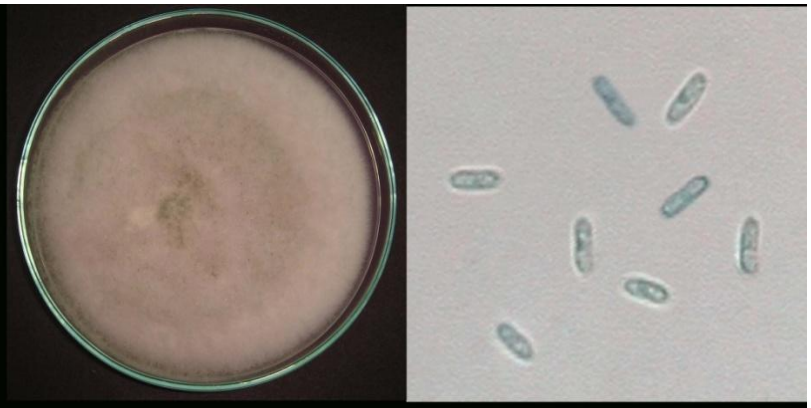
Isolate No. 05
Locality: Gopalwadi, Aurangabad
Colony characters:-
1)Nature: Cottony
2) Colony color: Snow white
3) Colony shape: irregular
4) Exudates: Absent
5) Fruting bodies: Absent
6) Pigmentation: Absent
7) Calibration: Mean= 18.7 x 7.9µ



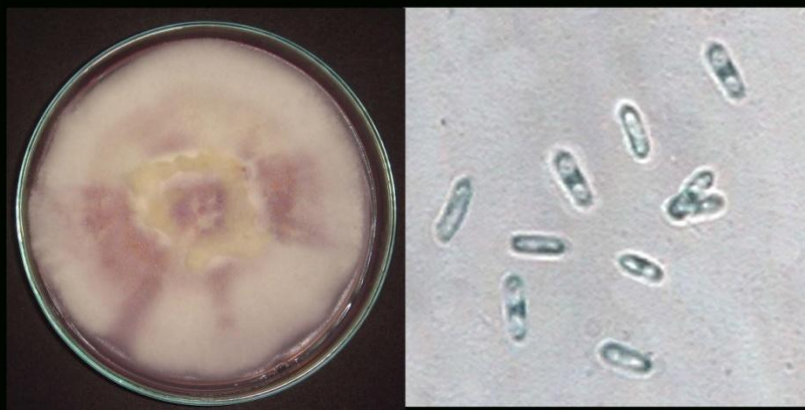
Isolate No. 06
Locality: Irle, Solapur
Colony characters:-
1)nature: Velvetty, growth in hairy pattern
2) Colony color: White
3) Colony shape: Circular
4) Exudates: Present
5) Fruting bodies: Present, Orange in colour, growth in furrowed pattern
6) Pigmentation: Absent
7) Calibration: Mean= 17.1 x 5.5µ

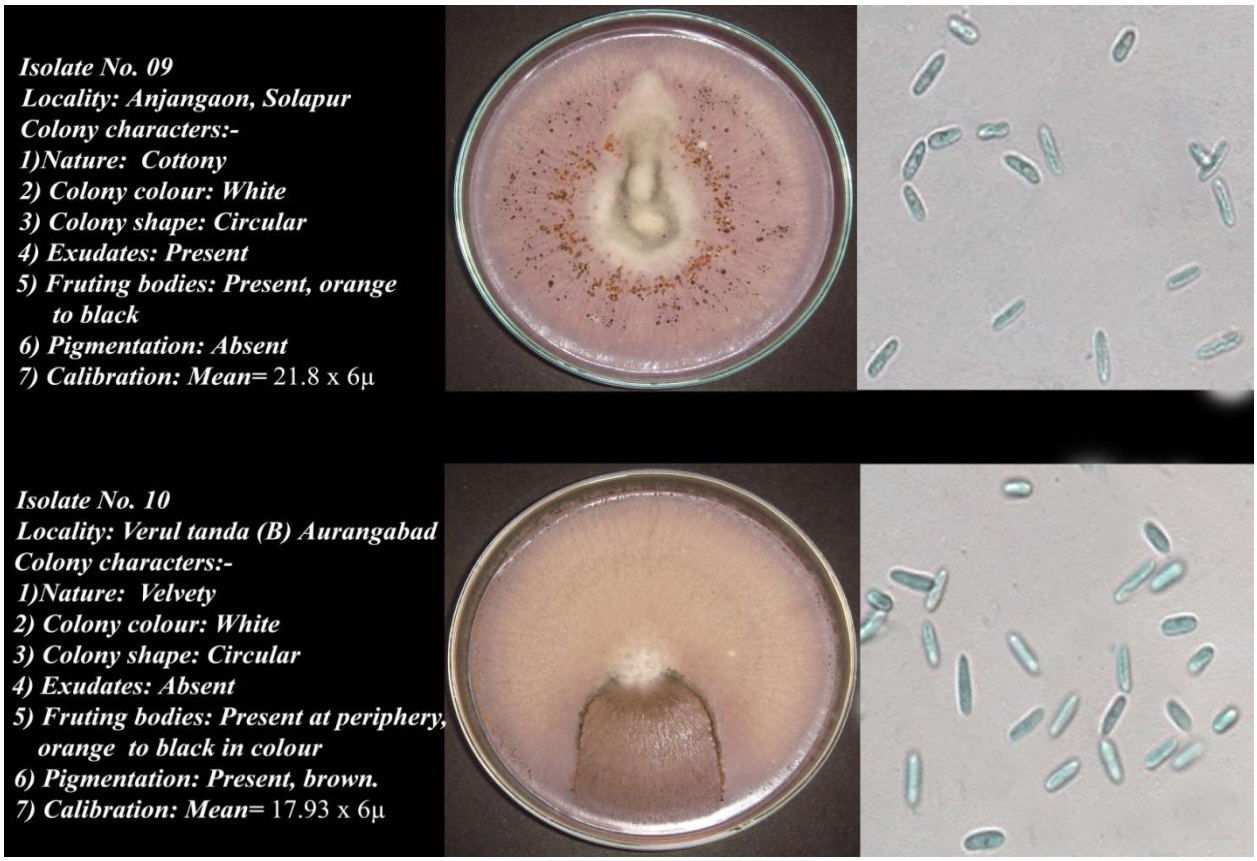


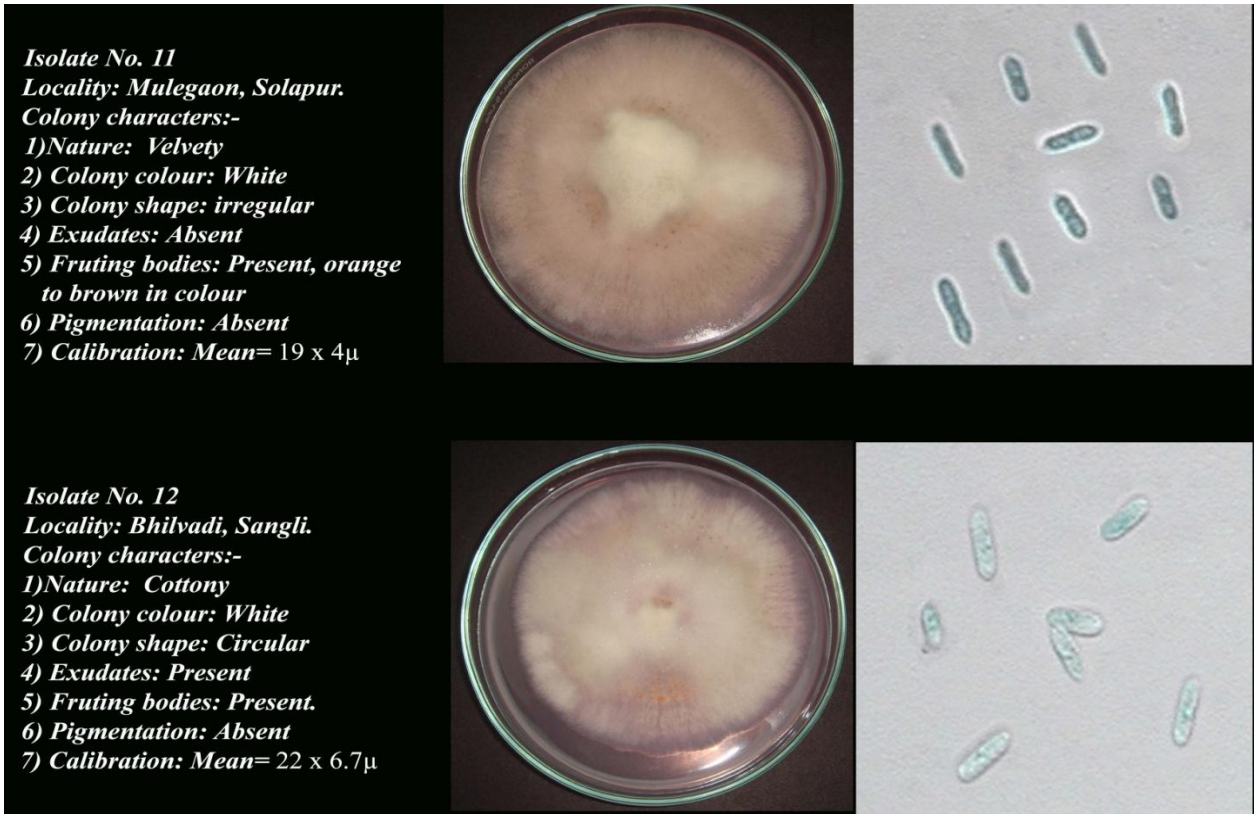
Isolate No. 07
Locality: Pattri, Aurangabad
Colony characters:-
1)Nature: Cottony
2) Colony colour: White
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Present, Black
6) Pigmentation: Absent
7) Calibration: Mean= 17.4 x 5µ

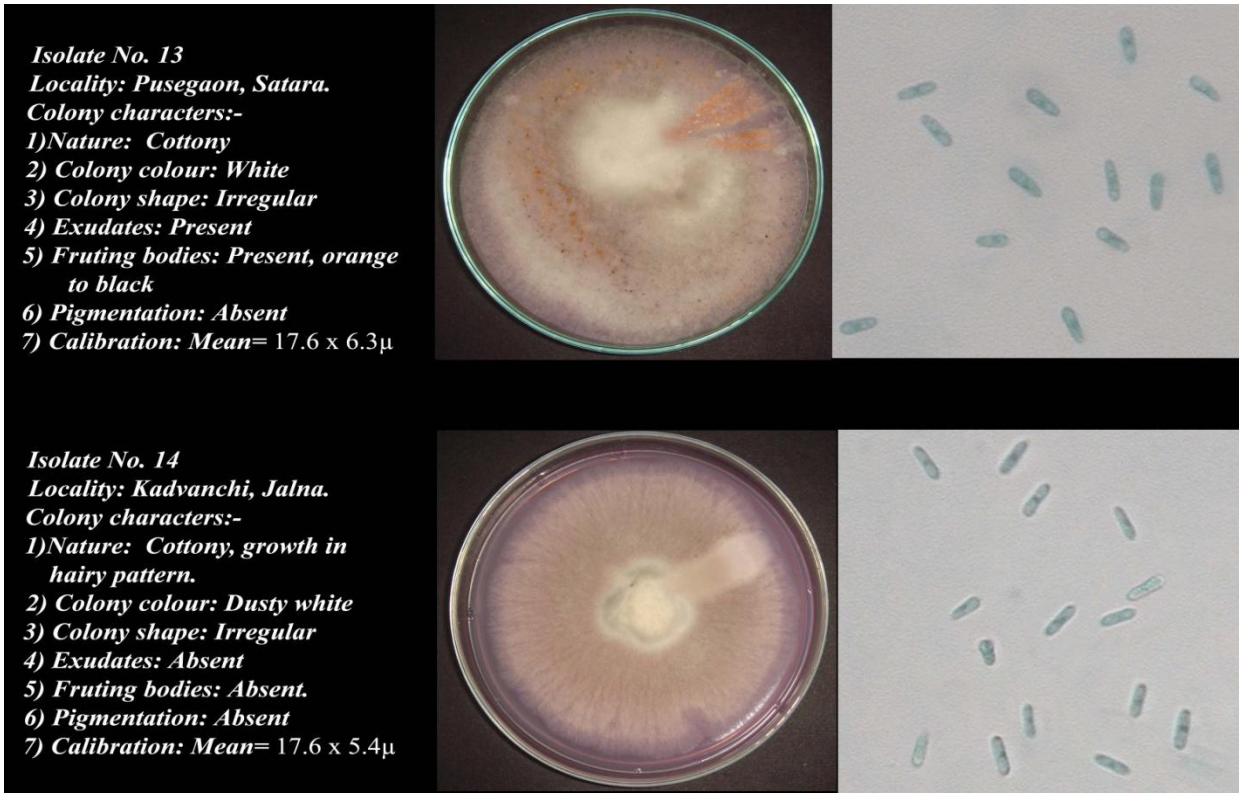


Isolate No. 08
Locality: Waluj, Solapur
Colony characters:-
1)Nature: Cottony and Velvety, Growth in clumps
2) Colony color: Snow white
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Present, orange in colour
6) Pigmentation: Absent
7) Calibration: Mean= 16.8 x 6.1µ



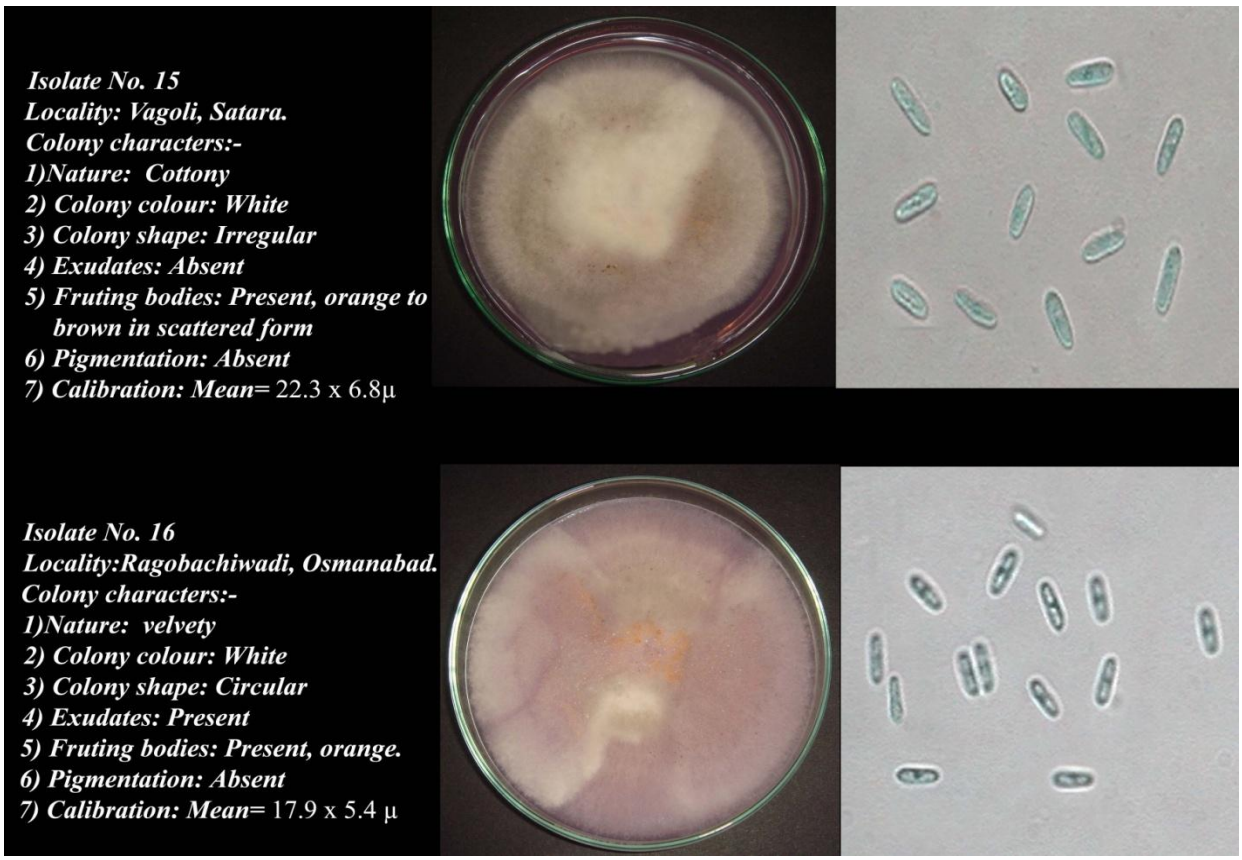

Isolate No. 09
Locality: Anjangaon, Solapur
Colony characters:-
1)Nature: Cottony
2) Colony colour: White
3) Colony shape: Circular
4) Exudates: Present
5) Fruting bodies: Present, orange to black
6) Pigmentation: Absent
7) Calibration: Mean= 21.8 x 6µ
Isolate No. 10
Locality: Verul tanda (B) Aurangabad
Colony characters:-
1)Nature: Velvety
2) Colony colour: White
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Present at periphery, orange to black in colour
6) Pigmentation: Present, brown.
7) Calibration: Mean= 17.93 x 6µ


Isolate No. 11
Locality: Mulegaon, Solapur.
Colony characters:-
1)Nature: Velvety
2) Colony colour: White
3) Colony shape: irregular
4) Exudates: Absent
5) Fruting bodies: Present, orange to brown in colour
6) Pigmentation: Absent
7) Calibration: Mean= 19 x 4µ
Isolate No. 12
Locality: Bhilvadi, Sangli.
Colony characters:-
1)Nature: Cottony
2) Colony colour: White
3) Colony shape: Circular
4) Exudates: Present
5) Fruting bodies: Present.
6) Pigmentation: Absent
7) Calibration: Mean= 22 x 6.7µ



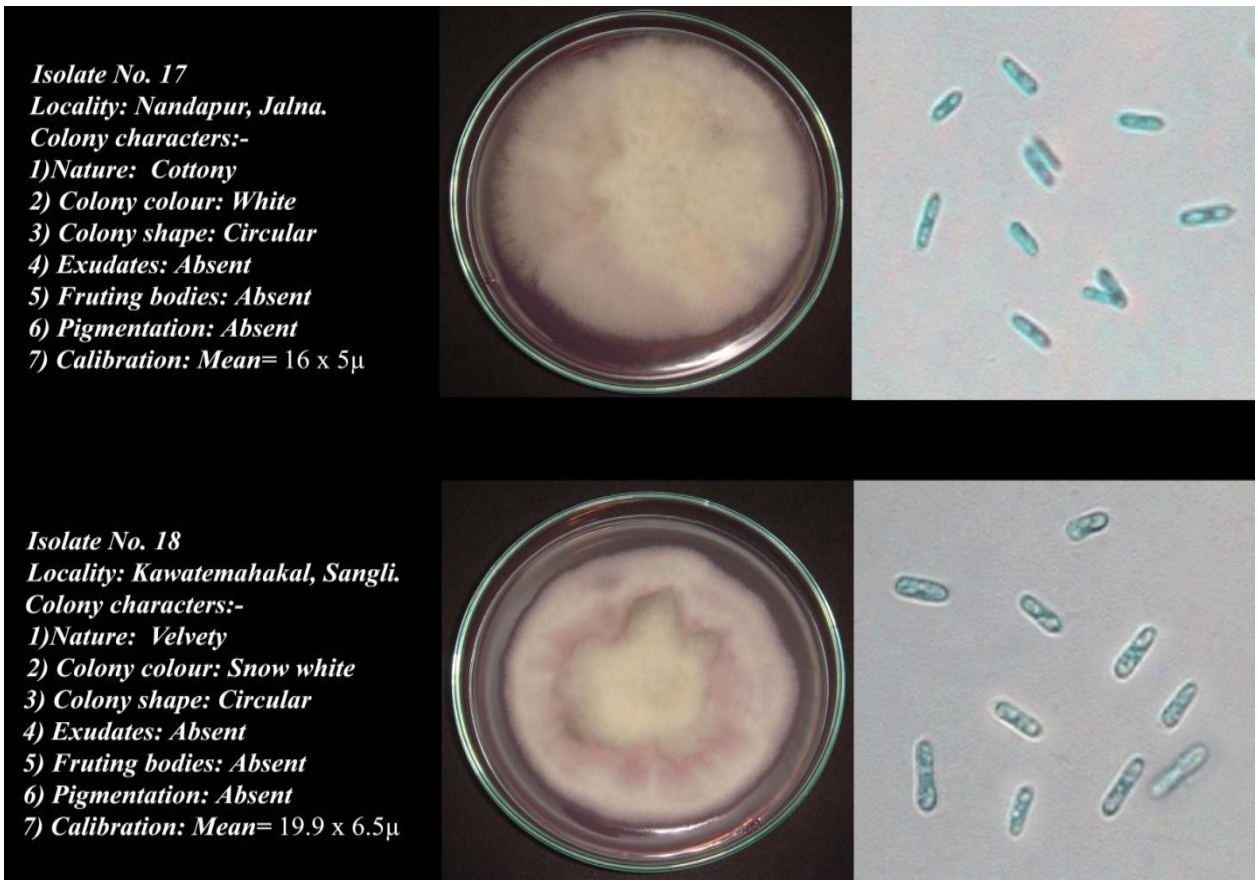
Isolate No. 13
Locality: Pusegaon, Satara.
Colony characters:-
1)Nature: Cottony
2) Colony colour: White
3) Colony shape: Irregular
4) Exudates: Present
5) Fruting bodies: Present, orange to black
6) Pigmentation: Absent
7) Calibration: Mean= 17.6 x 6.3µ

Isolate No. 14
Locality: Kadvanchi, Jalna.
Colony characters:-
1)Nature: Cottony, growth in hairy pattern.
2) Colony colour: Dusty white
3) Colony shape: Irregular
4) Exudates: Absent
5) Fruting bodies: Absent.
6) Pigmentation: Absent
7) Calibration: Mean= 17.6 x 5.4µ

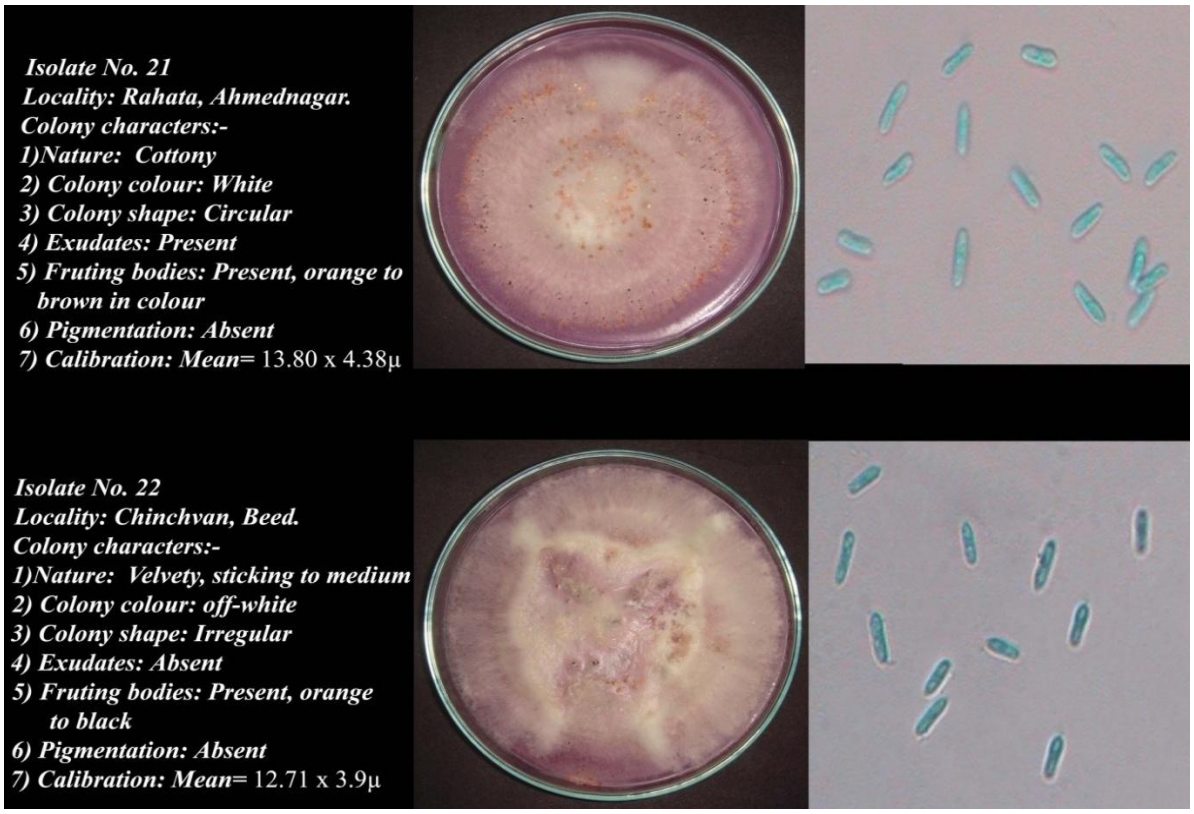


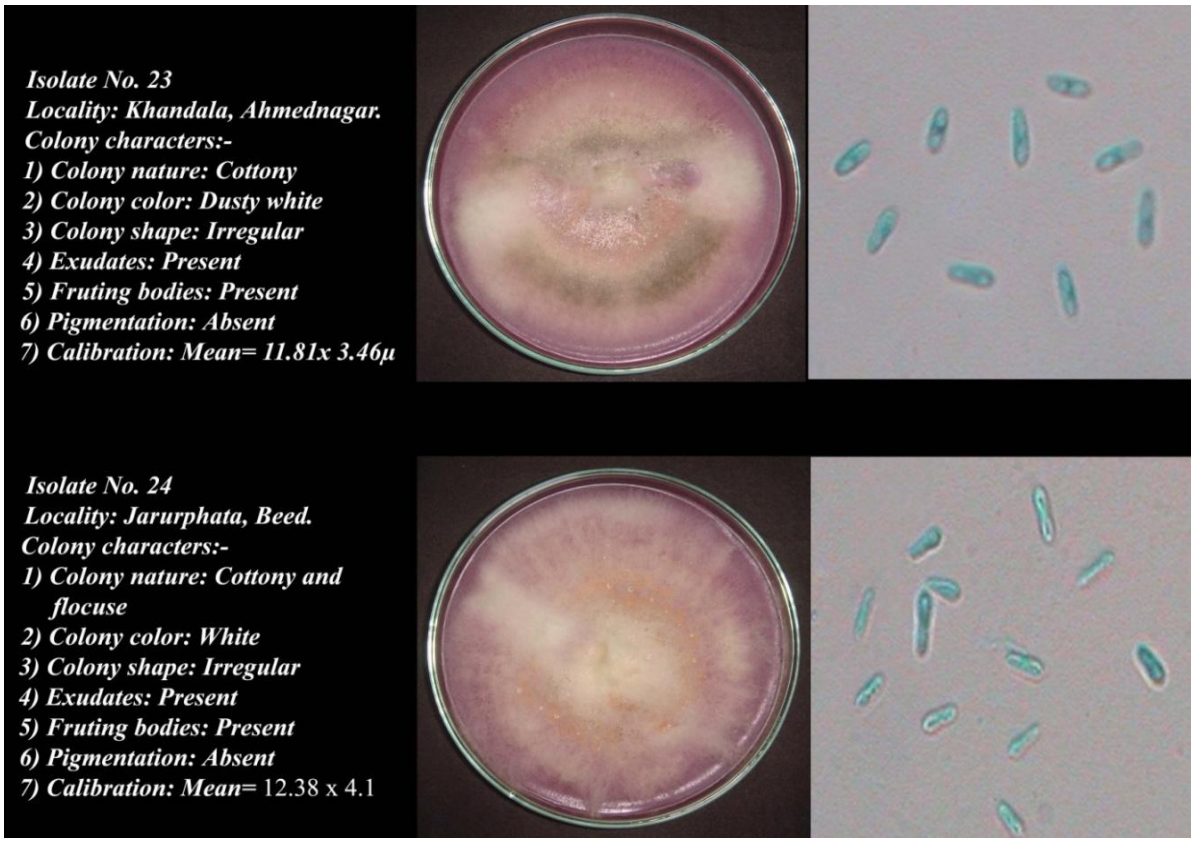
Isolate No. 15
Locality: Vagoli, Satara.
Colony characters:-
1)Nature: Cottony
2) Colony colour: White
3) Colony shape: Irregular
4) Exudates: Absent
5) Fruting bodies: Present, orange to brown in scattered form
6) Pigmentation: Absent
7) Calibration: Mean= 22.3 x 6.8µ

Isolate No. 16
Locality:Ragobachiwadi, Osmanabad.
Colony characters:-
1)Nature: velvety
2) Colony colour: White
3) Colony shape: Circular
4) Exudates: Present
5) Fruting bodies: Present, orange.
6) Pigmentation: Absent
7) Calibration: Mean= 17.9 x 5.4 µ


Isolate No. 17
Locality: Nandapur, Jalna.
Colony characters:-
1)Nature: Cottony
2) Colony colour: White
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Absent
6) Pigmentation: Absent
7) Calibration: Mean= 16 x 5µ
Isolate No. 18
Locality: Kawatemahakal, Sangli.
Colony characters:-
1)Nature: Velvety
2) Colony colour: Snow white
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Absent
6) Pigmentation: Absent
7) Calibration: Mean= 19.9 x 6.5µ


Isolate No. 19
Locality: Mohawadi, Nashik
Colony characters:-
1)Nature: Cottony.
2) Colony colour: whitish.
3) Colony shape: Irregular.
4) Exudates: Present.
5) Fruting bodies: Absent.
6) Pigmentation: Absent.
7) Calibration: Mean= 17 x 6.2 µ
Isolate No. 20
Locality: Shindri, Solapur
Colony characters:-
1)Nature: Cottony, growth in hairy pattern
2) Colony colour: White
3) Colony shape: Circular
4) Exudates: Absent
5) Fruting bodies: Present in centre, orange
6) Pigmentation: Absent
7) Calibration: Mean= 12.7 x 3.7µ


Isolate No. 21
Locality: Rahata, Ahmednagar.
Colony characters:-
1)Nature: Cottony
2) Colony colour: White
3) Colony shape: Circular
4) Exudates: Present
5) Fruting bodies: Present, orange to brown in colour
6) Pigmentation: Absent
7) Calibration: Mean= 13.80 x 4.38µ
Isolate No. 22
Locality: Chinchvan, Beed.
Colony characters:-
1)Nature: Velvety, sticking to medium
2) Colony colour: off-white
3) Colony shape: Irregular
4) Exudates: Absent
5) Fruting bodies: Present, orange to black
6) Pigmentation: Absent
7) Calibration: Mean= 12.71 x 3.9µ


Isolate No. 23
Locality: Khandala, Ahmednagar.
Colony characters:-
1) Colony nature: Cottony
2) Colony color: Dusty white
3) Colony shape: Irregular
4) Exudates: Present
5) Fruting bodies: Present
6) Pigmentation: Absent
7) Calibration: Mean= 11.81x 3.46µ
Isolate No. 24
Locality: Jarurphata, Beed.
Colony characters:-
1) Colony nature: Cottony and flocuse
2) Colony color: White
3) Colony shape: Irregular
4) Exudates: Present
5) Fruting bodies: Present
6) Pigmentation: Absent
7) Calibration: Mean= 12.38 x 4.1

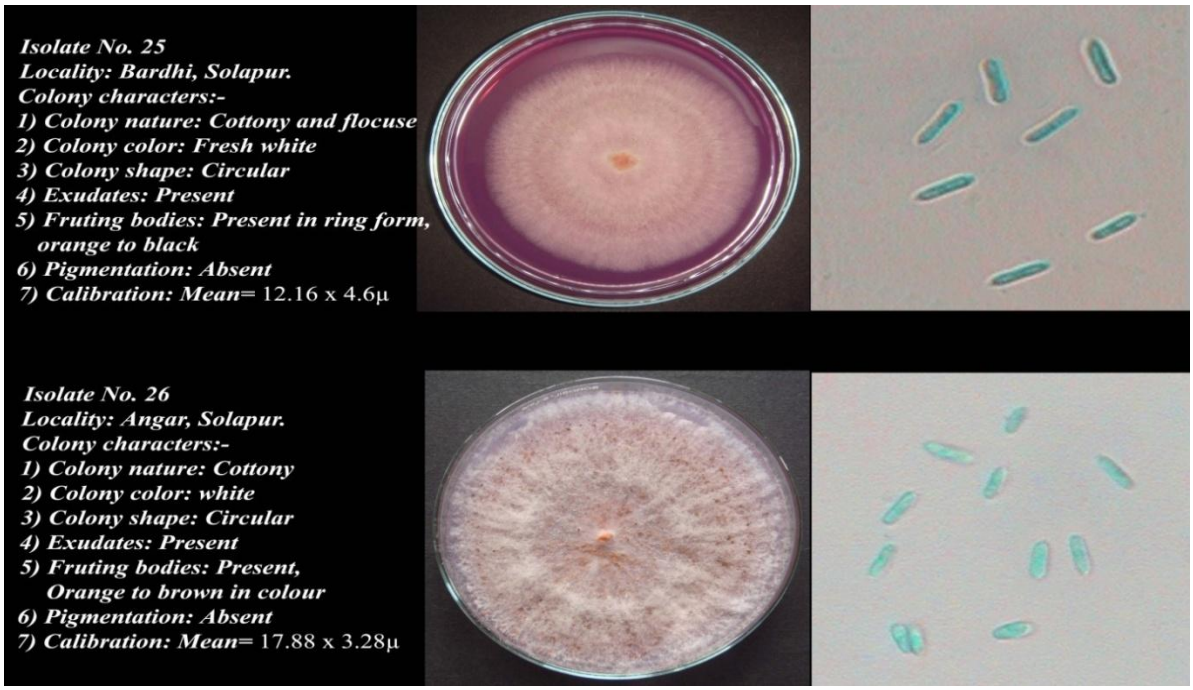


Table 1: Study of colony characters of different isolate of *Gloeosporium ampelophagum.*

Sr. No	Isolate No.	Colony Nature	Colony Shape	Colony Colour	Exudates	Pigmentation	Spore Calibration
	GA-01	Velvetty	Circular	White	Absent	Absent	16.7 x 4.6µ
2.	GA-02	Cottony and velvetty	Irregular	White	Absent	Absent	18.3x 6 µ
3.	GA-03	Veletty	Circular	White	Absent	Absent	18.4x 5.1 µ
4.	GA-04	Cottony and velvetty	Circular	White	Absent	Present	17.9 x 6 µ
5.	GA-05	Cottony	Irregular	Snowy white	Absent	Absent	18.7x 7.9 µ
6.	GA-06	Velvetty growth with hairy pattern	Circular	White	Present	Absent	17.1 x 5.5 µ
7.	GA-07	Cottony	Circular	White	Absent	Absent	17.4 x 50 µ
8.	GA-08	Cottony and velvety with clumps	Circular	Snow White	Absent	Absent	16.8 x 6.1 µ
9.	GA-09	Cottony	Circular	White	Present	Absent	21.8 x 60 µ
10.	GA-10	Velvetty	Circular	White	Absent	Present	17.9 x 60 µ
11.	GA-11	Velvetty	Irregular	White	Absent	Absent	19 x 40 µ
12.	GA-12	Cottony	Circular	White	Present	Present	22 x 6.7 µ
13.	GA-13	Cottony	Irregular	White	Present	Absent	17.6 x 6.3 µ
14.	GA-14	Cottony growth with hairy pattern	Irregular	Dusty white	Absent	Absent	17.6 x 5.4 µ
15.	GA-15	Cottony	Irregular	White	Absent	Absent	22.3 x 6.8 µ
16.	GA-16	Velvetty	Circular	White	Present	Absent	17.9 x 5.4 µ
17.	GA-17	Cottony	Circular	White	Absent	Absent	16 x 50 µ
18.	GA-18	Velvetty	Circular	Snow White	Absent	Absent	19.9 x 40 µ
19.	GA-19	Cottony	Irregular	Whitish	Present	Absent	17 x 6.2 µ
20.	GA-20	Cottony growth with hairy	Circular	White	Absent	Absent	12.7 x 3.7 µ

		pattern					
21.	GA-21	Cottony	Circular	White	Present	Absent	13.80 x 4.3 µ
22.	GA-22	Velvetty sticking to medium	Irregular	Off white	Present	Present	12.71 x 3.9 µ
23.	GA-23	Cottony	Irregular	Dusty white	Present	Absent	11.81 x 3.6 µ
24.	GA-24	Cottony and flocuse	Irregular	White	Present	Absent	12.38 x 4.1 µ
25.	GA-25	Cottony and flocuse	Circular	Fresh white	Present	Absent	12.16 x 4.6 µ
26.	GA-26	Cottony	Circular	White	Present	Present	17.88 x 3.2 µ

The present investigation focused on the study of morphological variability among different isolates of *Gloeosporium ampelophagum*, the causal organism of anthracnose of grapes, collected from major grape-growing regions of Maharashtra. Diseased grape samples showing typical anthracnose symptoms were collected from various agro-climatic zones, and the pathogen was successfully isolated and maintained on Czapek's Dox Agar (CZA) medium. The isolates exhibited considerable variation in cultural characteristics such as colony colour, texture, growth pattern, zonation and colony margin. Variations were also observed in the formation of acervuli with respect to their size, distribution, and presence or absence of setae. Microscopic examination revealed differences in the shape and size of conidia among isolates, although all conidia were hyaline and aseptate. Pictorial documentation of cultural and microscopic features was carried out using stereo and compound microscopes to support these observations. The results clearly indicate the presence of significant morphological diversity among *G. ampelophagum* isolates from various spots of Maharashtra.

The study clearly demonstrates that *Gloeosporium ampelophagum* populations associated with grape anthracnose in Maharashtra exhibit substantial morphological variability in both cultural and microscopic characteristics. Differences in colony morphology, acervuli formation, setae development, and conidial dimensions suggest the existence of diverse pathogen populations across different agro-climatic regions. This morphological diversity may influence the pathogenic behaviour, adaptability and epidemiology of the disease under varying environmental conditions. Understanding such variability is essential for accurate pathogen identification and for developing effective and region-specific disease management strategies. The findings of the present study provide a useful baseline for further investigations involving pathogenic variability, molecular characterization and integrated management of grape anthracnose.

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