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MORPHOLOGICAL CHARACTERIZATION AND BIOLOGICAL MANAGEMENT OF *GLOEOSPORIUM AMPELOPHAGUM* (PASS.) SACC CAUSING ANTHRACNOSE OF GRAPES IN INDIA

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ABSTRACT

Grape an important fruit crop, has been found to face a serious threat due to anthracnose disease. The disease on leaves appeared as small irregular dark brown lesions, which later developed greyish centre with dark brown margins that eventually dried and dropped resulting in peculiar shot hole appearance. On twigs (vines) the disease initially appeared as light brown circular lesion which on elongation became elliptical and developed sunken ashy grey centre. Coalescing of numerous such lesions culminated in canker formation. Berry symptoms were initiated as circular reddish brown lesions, which later coalesced, resulting in shrivelling and mummification of berries. The pathogen was isolated on potato dextrose agar medium and its pathogenicity was proved. The fungus produced circular, cottony colony with light green centre and creamish margins which later turned olive green with radial furrows. Acervuli and conidia were formed 10 days after incubation at 25±2 °C. Conidia (6.21 × 3.87µm) were oblong and hyaline to brownish in colour. Based on its colony characters, morphological characters and by comparing with authentic descriptions as well as through pathogenicity the pathogen causing anthracnose disease of grapes was identified as *Gloeosporium ampelophagum* (Pass.)Sacc**.** The fungus showed highest mycelial sporulation of 0.81×10^6/ml on oat meal agar with a pH 6.5 at 25-30 °C. Among the bio-agents screened, *Trichoderma harzianum* exhibited maximum inhibition in mycelial growth of 62.53 per cent that can be effectively used for the management of the disease.

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INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crop in temperate, tropical and subtropical regions of the world (Ghosh *et al.*, 2017). In India, grapes are grown in Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu as tropical fruit, in Uttar Pradesh, Punjab and Haryana as subtropical, and as temperate in Jammu and Kashmir, Himachal Pradesh occupying an area of 155 thousand hectares with an annual production of about 3358 thousand metric tons (FAO, 2021). In Jammu and Kashmir grapes are grown in Kupwara, Baramulla, Bandipora, Ganderbal, Budgam and Srinagar districts covering an area of 400 hectares with production of 1,600 metric tons (Ahmad *et al.*, 2021). Grape, fruiting berry of the deciduous woody vine can be eaten fresh as table fruit and can also be used for making wine, jam, juice, jelly, raisins, vinegar and grape seed oil. In India bulk of production (78%) is used as fresh/table grape followed by raisin (17.20%) (Rao *et al.*, 2014). Grape is a good source of potassium, sodium, calcium, phosphorus, iron and vitamins such as

vitamin-C, vitamin-A, vitamin-K, carotenes and B-complex (Bhagwat *et al.*, 2014).

Although the agro-climatic conditions of Kashmir valley are favourable for cultivation of the grapes, yet its productivity has been relatively low compared to other parts of the world owing to many biotic and abiotic factors. The production of grapes in Kashmir is suffering from qualitative and quantitative losses inflicted by the onslaught of several diseases (Ghuffar *et al.*, 2018). Grape cultivation has been vulnerable to many serious pests and diseases of which, fungal diseases are the most destructive and take a heavy toll on the crop at various stages of crop growth. Anthracnose or "bird's eye spot", as referred popularly, has been one of the major diseases on grapes that appears from the beginning of the crop growth and causes long lasting effect on the vine growth and reduced yield potential of the crop if proper management is not adopted in time (Mathukorn *et al.*, 2012).

Grape anthracnose disease is considered to be the major threat to successful grape cultivation worldwide. Likewise in India, anthracnose has become a potential threat to grape cultivation. In north India, it appears every year and reduces the quality and quantity of the crop apart from making vines weak (Thind and Nirmalijit, 2005). Though chemical fungicides have been extensively used worldwide to control various pathogens, their use has many attendant problems such as environmental pollution, deteorating human health, development of pathogen resistance to fungicide and phytotoxicity (Dubey *et al.*, 2008). To minimize these problems, researchers have sought to develop biological control agents for controlling diseases that might be more environment-friendly. Phytopathogens have been controlled by using plant extracts (Bashir *et al.*, 2020) and several antagonistic microorganisms, including *Bacillus* spp. (Ongena and Jacques, 2008; Hyder *et al.*, 2020; Bibi *et al.*, 2017), *Streptomyces* spp. (Bressan, 2003), *Psudomonas* spp. (Hubbard *et al.*, 1983; Shahzaman *et al.*, 2016; Shahzaman *et al.*, 2017), and *Trichoderma* spp. (Sivan and Chet, 1986; Shakoor *et al.*, 2015).

Since fungi are sensitive to nutritional and environmental factors and their growth and sporulation is greatly influenced by the composition of nutrient media, pH and temperature. So an attempt was made to quantify and compare vegetative mycelial growth and sporulation of causal pathogen on different media under different temperature and pH, besides validation of the isolated pathogen at both morphological and physiological levels. In order to develop eco-friendly strategies biological agents were evaluated under *in vitro* conditions for suitable management of anthracnose disease.

MATERIALS AND METHODS

Symptomatological studies

Detailed studies on the development of grape anthracnose symptoms were conducted on susceptible grape cultivar, Anab-e-shahi. Vines were selected randomly at experimental trial conducted at Badampora, Ganderbal which were kept unsprayed throughout growing season to study symptoms under natural epiphytotic conditions. Four branches bearing leaves and twigs were tagged from each vine. Observations were recorded with regard to the first appearance of disease symptoms and different stages of its development i.e size, shape and colour of the lesions. The diseased leaves, twigs and fruits exhibiting typical anthracnose symptoms collected from the orchards were further examined by teasing the diseased portion under compound microscope. The leaves and twigs were then kept in moist chamber at 28±2 °C for 36 hours to get the acervuli bulged. These bulged acervuli were observed under stereoscopic microscope and temporary mounts in cotton blue, lectophenol and distilled water were prepared. Acervuli and conidia were examined for their colour, shape and size.

Isolation of the pathogen

Pathogen was isolated from diseased samples exhibiting typical anthracnose symptoms using tissue segment method (Rangaswami, 1958) on potato dextrose agar (PDA), in sterilized Petriplates and incubated at 28±2 °C temperature in biological oxidation demand incubator (BOD) for seven days.

Secondly, the diseased samples were kept in moist chamber followed by incubation at 28±2 °C for 24 hours to get the swollen acervuli. The bulged acervuli were lifted and directly placed aseptically on potato dextrose agar medium and incubated at 28±2 °C for 3-4 days (Jamshidi and Salahi, 2012).

Morphological characteristics of the pathogen

For morphological studies, semi-permanent slides were prepared from 7 days old culture in lactophenol stained with cotton blue. The slides were examined under

compound microscope (100X) with respect to following characters of the causal organism:

Hyphae: Septation, colour and width
Acervuli: Shape, colour and size
Conidia: Shape, colour and size

Cultural characteristics of the pathogen

Artificially grown colonies were visually examined and observations for colony characters such as nature of growth of fungal colony and nature of pigmentation, if any, were recorded.

Identification of the pathogen

On the basis of morphological and cultural characteristics the pathogen was identified which was further proved by performing pathogenicity test and by comparing with authentic descriptions (Kore and Gurme, 1979; Jamadar and Sataraddi, 2011; Mathukorn *et al.*, 2012).

Pathogenicity test

Pathogenicity of the isolated pathogen was performed by detached leaf technique to confirm Koch's postulates. In detached leaf technique, one set of leaves was given injuries with the help of sterilized teasing needle while as another set kept uninjured. Inoculation were made by spraying spore suspension (1×10^5 spores per ml) from young culture of isolated pathogen on the adaxial surface of both injured and uninjured leaves with the help of atomizer. The injured and uninjured leaves of third set were sprayed with sterilized water and served as a check. These leaves were maintained for three weeks and were examined daily for symptom development. The symptoms were microscopically examined for presence of acervuli and conidia. The pathogenicity was confirmed after satisfying Koch's postulates.

Studying the effect of physiological factors on growth of fungal pathogen

Effect of different solid media on the radial mycelial growth and sporulation of causal pathogen

The radial mycelial growth and sporulation of the causal pathogen was studied on five different solid media *viz*., potato dextrose agar, Richard's agar, Czapek (Dox) agar, corn meal agar and oat meal agar.

Each of the media to be evaluated was poured separately in 250 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 15 lbs pressure per square inch for 20 minutes. Thirty ml of each test media was poured into sterilized Petri plates under aseptic conditions. Inoculations were made with uniform culture bits (3mm diameter) from actively growing fungus culture. Each treatment was replicated four times in completely randomized design (CRD). The inoculated Petri plates were incubated at 28±2 ^{o}C for 7 days. The radial mycelial growth in all the four replications from each medium was recorded and average of the four replications was taken as final observation.

The sporulation was studied, by thoroughly homogenizing a 3mm mycelial disc in 5ml of sterilized distilled water. The spore suspension thus obtained was used for counting the number of spores with the help of haemocytometer. Media supporting the best radial mycelial growth and sporulation of the test fungus was used as a basal medium for further studies.

Effect of different temperature regimes on the radial mycelial growth and sporulation of causal pathogen

To study the radial mycelial growth and sporulation of causal pathogen at different temperature regimes, the best media evaluated above was poured separately in 250 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 15 lbs pressure per square inch for 15 minutes. Thirty ml of the media was poured into sterilized Petri plates under aseptic conditions. Inoculations were made with uniform culture bits (3mm diameter) from actively growing fungus culture and were incubated at five different temperature regimes *viz.* (10, 15, 20, 25, and 30 °C) for 7 days to study the best suited temperature level. Each treatment was replicated four times in completely randomized design (CRD). The radial mycelial growth in all the four replications was recorded and average of the four replications was taken as final observation.

The sporulation was studied, by thoroughly homogenizing a 3mm mycelial disc in 5ml of sterilized distilled water. The spore suspension thus obtained was used for counting the number of spores with the help of haemocytometer.

Effect of different pH regimes on the radial mycelial growth and sporulation of causal pathogen

To study the radial mycelial growth and sporulation of

causal pathogen at different pH, best media of five different pH (5, 5.5, 6, 6.5, and 7) was prepared. The media pH was adjusted with 0.1 N sodium hydroxide (NaOH) or 0.1N hydrochloric acid (HCl) (Naik *et al.*, 1988) and was poured separately in 250 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 15 lbs pressure per square inch for 20 minutes.

Thirty ml of the media of different pH was poured into sterilized Petri plates under aseptic conditions. Inoculations were made with uniform culture bits (3 mm diameter) from actively growing fungus culture and were incubated at best temperature evaluated in previous experiment for 7 days to study the best suited pH level. Each treatment was replicated four times in completely randomized design (CRD). The radial mycelial growth in all the four replications was recorded and average of the four replications was taken as final observation.

The sporulation was studied, by thoroughly homogenizing a 3 mm mycelial disc in 5 ml of sterilized distilled water. The spore suspension thus obtained was used for counting the number of spores with the help of haemocytometer.

In vitro evaluation of biological agents against the pathogen

Antagonistic effect of the three bio-agents *viz*., *Trichoderma harzianum, Bacillus subtilis*, and *Pseudomonas fluorescens* available at bio-control lab, Division of Plant Pathology (SKUAST-K), Shalimar, were evaluated against the test pathogen. The antagonistic effect was bio-assayed by adopting dual culture method (Huang and Hoes, 1976).

The culture discs of 3mm diameter taken from 7 days old culture of test pathogen and bio-agent were cut with a sterilized cork borer and aseptically placed at two opposite ends of 90mm diameter Petri plates containing sterilized oat meal agar medium. The pathogen and respective test bio-agent bio-agent was allowed to grow up to 7-10 days. The experiment was laid out in complete randomized design (CRD) with four replications for each treatment. After ten days of incubation at 30℃ temperature, observations for relative radial mycelial growth of antagonists and pathogen were recorded. Per cent inhibition in radial mycelial growth of test pathogen was calculated by employing the following formula:

$$\% \text{ RMG inhibition} = \frac{\text{RMG in check} - \text{RMG in treatment}}{\text{RMG in check}} \times 100$$

RMG = Radial mycelial growth

RESULT AND DISCUSSION

Symptomatology

Symptoms of the anthracnose disease were studied under natural conditions of infection on leaves, twigs and berries of unsprayed vines. During the periodic observation of marked trees, the initial disease symptoms were noticed in the first week of May and reached its peak during August. Disease symptoms as presented in Table 1, initially appeared on leaves as small, dark brown slightly sunken circular to irregular spots ranging from 3-6mm with an average of 4.5mm in size in the 1st week of May. Later on lesion turned light brown with irregular margins and size ranged from 12-16 mm with an average of 13.50 mm in the last week of May. These lesions increase in size ranging from 17-19mm with an average of 17.50 mm in the first week of June. These lesions later on coalesce to form large necrotic patches ranging from 22-24 mm with an average of 22.10 mm in the 1st week of July (Figure 1a). Formation of irregular necrotic patches takes place ranging from 32-36mm with an average of 34.50mm in the lst week of July. Discoloration and downward curling of leaves was observed in the 1st week of August (Figure 1b). The infection extended to petioles, as elliptical brown and sunken lesions that ultimately results in defoliation in the 3rd week of August (Figure 1c).

Disease symptoms on shoots and tendrils as presented in Table 2, appeared as small isolated light brown spots and circular in outline ranging from 5-7 mm with an average of 5.6 mm in the 1st week of May. Later the lesions turned elliptical and slightly sunken ranging from 8-10mm with an average of 8.5 mm in the 3rd week of May. These spots later on increase in size ranging from 9.0-11mm with an average of 9.5 mm in the 1st week of June. The tissue at the border of these lesions becomes slightly raised, forming a dark coloured rim, while the central area turns to ashy gray in colour in the last week of June (Figure 1d). These lesions later on increase in size ranging from 15-18 mm with an average of 15.50 mm in the 1st week of July. Coalescing of lesions to form large necrotic patches was observed in last week of July attaining size of 30-35 mm with an average of

32.10 mm. Canker formation of the twigs takes place in the 1st week of August attaining size of 40-42mm with an average of 40.50mm. The lesions may be so numerous as to give rough, scarred appearance to shoots in the last week of August. In mid-summer, during wet weather, small pink spots may be observed in the depressed centre of the lesions, that contains the spore masses of the causal agent.

The disease on berries as presented in Table 3 start as small dark red spots ranging from 2-3mm with an average of 2.5mm in the 1st week of June. Later on lesion increase in size ranging from 7-10 mm with an average of 9.5 mm and colour changes to purple with greyish centre in the last week of June. These spots later on produces typical "bird's eye spot" having gray centre surrounded by reddish brown zone in the 1st week of July. These lesions later on increase in size ranging from 12-15 mm with an average of 12.50 mm in the last week of July. Coalescing of lesions to form large necrotic patches was observed in 1st week of August attaining size of 18-20 mm with an average of 18.5 mm and ultimately leads to the rotting of berries in the last week of August (Figure 1e).

The symptomatological studies of the disease observed were compared with the authentic description given by Thind and Nirmalijit (2005), Mathukorn *et al.* (2012) and Ellis (2012) with which these characters closely relate.

Colony characters

The fungus inoculated on Petriplates were critically observed for colony characters, growth behaviour on oat meal agar and are presented in Table 4. After 3 days of incubation at 25±2 °C, the colony appeared as circular and cottony with light green centre and creamish margins with groves inside measuring about 15.20-20.34 mm in diameter (Figure 2a) which after 7 days, turned circular and cottony, olive green in colour, surrounded by flat creamy margins measuring about 31.23-34.78mm in diameter (Figure 2b) and after 10 days, changed to floccose greyish white with black centre and development of radial furrows (Figure 2c) attained diameter of 76.10-79.23 mm.

Figure 1 a. Coalescing of lesions on leaves, b. Chlorosis and downward curling of leaf margins, c. Elliptical, brown and sunken lesions, d. Elliptical and sunken lesions with ashy grey centre, e. Shrivelled and rotten berries of grapes.

Table 1: Symptomatological development of Anthracnose disease of grapes on leaves.

	Diameter (mm)		Shape	Colour	Other characters
	`Range	Average			
April					
22	-		-	-	Disease free
May					
05	3-6mm	4.5mm	Circular to irregular	Dark brown	Slightly sunken spots
13	6-8mm	7.5 mm	-do-	-do-	-do-
21	10-12 mm	11.5 mm	-do-	-do-	-do-
29	12-16mm	13.50 mm	Irregular	Light brown	-do-
June					
06	17-19mm	17.50mm	Irregular	Light brown	Shot hole formation
14	19-21 mm	21.10 mm	-do-	-do-	-do-
22	21-22mm	21.50mm	-do-	-do-	-do-
30	22-24 mm	22.10 mm	-do-	-do-	-do-
July					
07	25-27mm	26.50mm	Mostly irregular	Light brown	Numerous Spots start coalescing
16	28-30mm	28.10mm	-do-	-do-	-do-
23	31-33 mm	31.50 mm	-do-	-do-	Formation of irregular necrotic patches
31	32-36 mm	34.50 mm	-do-	-do-	-do-
August					
08	32-36mm	32.1 mm	-do-	-do-	Chlorosis of highly infected leaves
16	36-37 mm	36.1 mm	-do-	-do-	-do-
24	37-38 mm	37.1mm	-do-	-do-	Petiole infection
31	38-39 mm	38.1mm	-do-	-do-	Defoliation

Figure 2. Morpho-cultural characters of *Gloeosporium ampelophagum*. a. 3 days old colony, b. 7 days old colony, c. 10 days old colony, d. Mycelium, e. Acervuli, f. Conidia of *G. ampelophagum.*

Table 2. Symptomatological development of Anthracnose disease of grapes on twigs.

	Diameter (mm)		Shape	Colour	Other characters
	Range	Average			
April 22		-	-	-	Disease free
May					
05	5-6mm	5.6mm	Circular	Light brown	Slightly sunken spots
13	6-7mm	6.5 mm	-do-	-do-	-do-
21	7-8 mm	7.5 mm	-do-	-do-	-do-
29	8-10mm	8.5mm	Elliptical	Light brown	-do-
June					
06	9-11mm	9.5mm	Elliptical	Light brown	-do-
14	10-13mm	11.50mm	-do-	-do-	-do-
22	13-14mm	13.50mm	-do-	-do-	-do-
30	14-15mm	14.50mm			Dark coloured rim, central area turns to ashy gray in colour
July					
07	15-18mm	15.50mm	Irregular	Light brown to Ashy grey	-do-
16	20-24mm	20.50mm	-do-	-do-	-do-
23	25--28mm	25.50mm	-do-	-do-	-do-
31	30-35mm	32.10mm	-do-	-do-	
August					
08	36-38 mm	37.50mm	Mostly irregular	Ashy grey	Canker formation
16	38-39 mm	38.50mm	-do-	-do-	-do-
24	39-40 mm	39.50mm	-do-	-do-	-do-
31	40-42mm	40.50mm	-do-		

Table 3: Symptomatological development of Anthracnose disease of grapes on berries.

	Diameter (mm)		Shape	Colour	Other characters
	Range	Average			
June					
15	2-3mm	2.5mm	Circular	Dark Red	Slightly sunken spots
13	4-6mm	4.5 mm	-do-	-do-	-do-
21	6-7 mm	6.5 mm	-do-	-do-	-do-
29	7-10mm	8.50 mm	-do-	Purple red	Greyish centre
July					
06	10-12mm		Oval	Grey centre with reddish brown zone	Bird's eye spot
14	12-13mm	12.50mm	-do-	-do-	-do-
22	12-14mm	12.50mm	-do-	-do-	-do-
30	12-15mm	13.50mm	-do-	-do-	-do-
August					
07	18-20mm	18.50mm	Irregular	Dark brown	Mummification of berries
16	18-20mm	18.50mm	-do-	-do-	-do-
23	18-20mm	18.50mm	-do-	-do-	-do-
31	18-20mm	18.50mm	-do-	-do-	-do-

Morphological characters

The morphological characters of the *Gloeosporium ampelophagum* (Pass.) Sacc were studied on culture under *in vitro* conditions and are presented in Table 4. Observations revealed that the pathogen produced branched, septate, smooth and brownish hyphae measuring 4.51-4.89 µm in width (Figure 2d). Acervuli developed in concentric rings after 10 days of incubation were round shaped, brown-black in colour (Figure 2e) measuring 74.21-78.01 µm in size. Conidia were single celled, hyaline to brownish, oblong conidia measuring 6.41-7.70 × 3.51-4.08 µm (Figure 2f).

The morphological characters of the pathogen observed in culture were compared with the authentic description given by Thind and Nirmalijit (2005) and Mathukorn *et al.* (2012) with which these characters closely relate.

Pathogenicity

Pathogenicity of isolated pathogen was performed on detached young healthy leaves of susceptible cultivar Anab-e-shahi. It was observed that typical symptoms of disease appeared after 5-6 and 11-15 days on injured and uninjured leaves respectively and there was no symptom development on control. Confirmation was done by re-isolating the pathogenic fungus from inoculated diseased leaves.

Identification of pathogen

Based on morphological, cultural characteristics, pathogenicity test, and by comparison with the authentic descriptions the fungus was identified as *Gleosporium ampelophagum* (Pass.)Sacc. (Jamadar and Sataraddi, 2011; Thind and Nirmalijit, 2005; Mathukorn *et al.*, 2012).

Physiological studies of causal pathogen

Effect of different media on the radial mycelial growth and sporulation of *Gloeosporium ampelophagum* (Pass.) Sacc

In order to ascertain the best solid media for the maximum mycelial growth and sporulation, the pathogen was grown on five different solid media viz., potato dextrose agar, Richard's agar, Czapek (Dox) agar, corn meal agar, and oat meal agar. The average radial mycelial growth (mm) and sporulation was recorded in each media after 10 days of incubation at 25±2 ^{o}C and the results obtained are presented in Table 5 and Figure 3.

Table 4: Morpho-cultural characteristics of *Gleosporium ampelophagum* (Pass.) Sacc. causing anthracnose disease of grapes (*Vitis vinifera* L.).

Thallus part		Shape and character	Colour	Size	Septation
Colony	3 days	Initially circular and cottony with groves inside	light green centre and creamish margins	15.20-20.34 mm	-
	7 days	Circular, cottony surrounded by flat white mass	Olive green	31.23-34.78mm	-
	10 days	Circular, floccose with radial furrows	Greyish white	76.10-79.23 mm	-
Hyphae	-	Branched and smooth	brownish	4.51-4.89 µm (Av: 4.72 µm width)	Septate
Acervuli	-	Round shaped	brown to black	74.21-78.01 µm (Av: 75.23 µm)	-
Conidia	-	Oblong	Hyaline to brownish	6.41-7.70 × 3.51-4.08 µm (Av: 6.21 × 3.87µm)	Aseptate

The data revealed that fungus can utilize number of media for its growth. The average maximum radial mycelial growth of 71.77 mm was recorded on oat meal agar which differs significantly from potato dextrose agar with radial mycelial growth of 66.60mm. Growth of 63.57mm was recorded on corn meal agar that was significantly different than on Czapek (Dox) agar (59.60mm). While as least radial mycelial growth of 54.16mm was observed on Richard's Agar.

The data further revealed that average conidial production of 5.18×10^{6} conidia per ml of water was recorded on oat meal agar followed by corn meal agar

(0.94×10^6 conidia per ml) of water. Average conidial production of 3.86 and 2.99 $\times10^6$ conidia per ml was recorded on potato dextrose agar and Czapek (Dox) agar respectively. Minimum conidial production of 2.08×10^6 conidia per ml of water was recorded on Richard's agar.

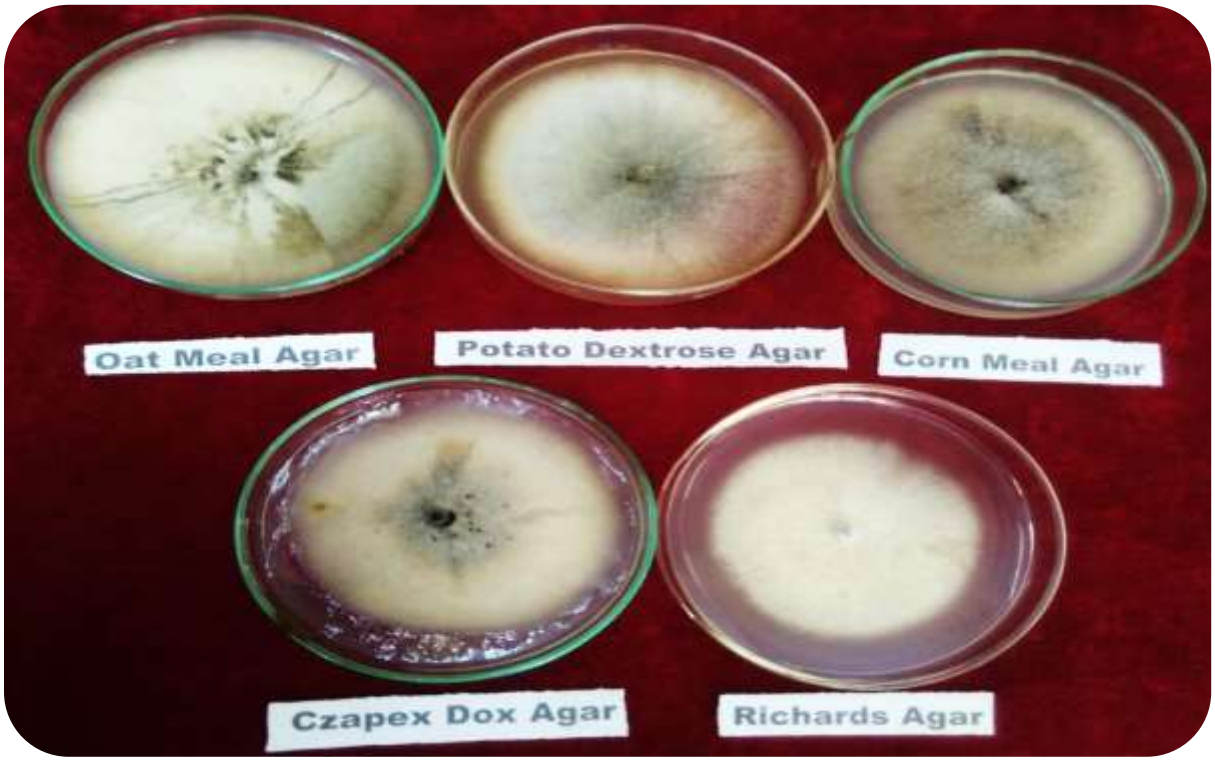

Figure 3. Effect of media on radial mycelial growth and sporulation of *Gleosporium ampelophagum.*

Table 5. Effect of different media on growth and sporulation of *Gleosporium ampelophagum* (Pass.)Sacc. at 25±2 ˚C after 7 days of incubation.

Media	Radial mycelial growth (mm)*	Sporulation (1×10^6/ml)
Oat Meal Agar	90.22 (71.77)[a]	0.81 (5.18)[a]
Potato Dextrose Agar	84.23 (66.60)[b]	0.45 (3.86)[c]
Corn Meal Agar	80.24 (63.57)[c]	0.58 (4.38)[b]
Czapek (Dox) Agar	74.42 (59.60)[d]	0.27 (2.99)[d]
Richard's Agar	65.70 (54.16)[e]	0.13 (2.08)[e]
CD ($p\leq0.05$)	1.90	0.25

* Figures in parenthesis are arc sin transformed values based on mean of four replications.

Effect of different temperature regimes on the radial mycelial growth and sporulation of *Gloeosporium ampelophagum* (Pass.) Sacc

In order to ascertain the best temperature for the maximum mycelial growth and sporulation, the pathogen was grown on best media obtained above and evaluated at five different temperature viz., 10, 15, 20, 25 and 30 °C. The average radial mycelial growth (mm) and sporulation was recorded at each temperature after 7 days of incubation and the results obtained are

presented in Table 6 and Figure 4. The radial mycelial growth increase with increase in temperature. The maximum average radial mycelial growth of 64.88 mm was recorded at 30 °C followed by 58.86mm at 25 °C and 40.79 mm at 20 °C. Minimum radial mycelial growth of 38.01 mm was recorded at 10 °C.

The data further revealed that average maximum conidial production of 5.95× 10^6 conidia per ml of water was recorded at 25 °C which was at par at 20 °C with conidial production of 5.31× 10^6 conidia per ml of water at 30°C. Conidial production of 5.24 × 10^6 per ml of water was recorded at 30 °C. Minimum conidial production of 2.24× 10^6 per ml of water was recorded at 10 °C.

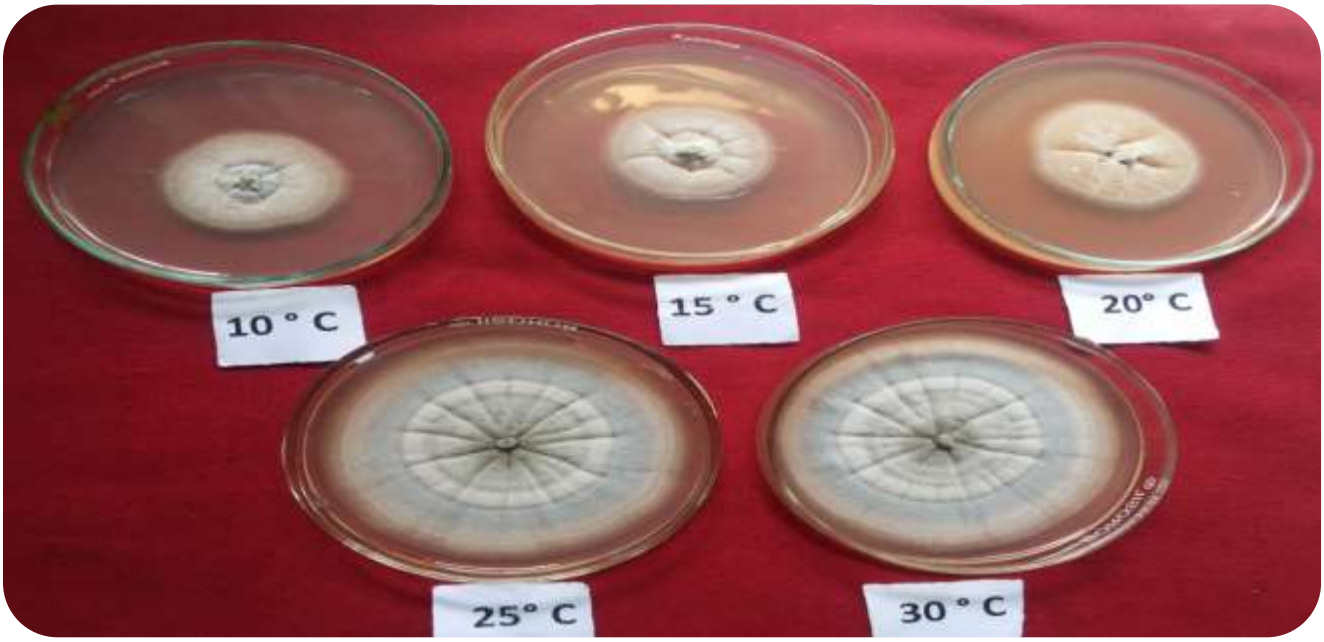

Figure 4. Effect of temperature on growth and sporulation of *Gleosporium ampelophagum* after 7 days of incubation.

Table 6. Effect of different temperature on growth and sporulation of *Gleosporium ampelophagum* (Pass.)Sacc. at 25±2 °C after 7 days of incubation.

Temperature	Radial mycelial growth (mm)*	Sporulation (×10^6/ml)
10	37.95 (38.01)^d	0.08 (2.24)^b
15	44.72 (38.61)^d	0.35 (2.95)^b
20	49.27 (40.79)^c	0.86 (5.31)^a
25	74.84 (58.86)^b	1.08 (5.95)^a
30	81.29 (64.88)^a	0.83 (5.24)^a
CD (p≤0.05)	1.63	1.80

* Figures in parenthesis are arc sin transformed values based on mean of four replications.

Effect of different pH regimes on the radial mycelial growth and sporulation of *Gloeosporium ampelophagum* (Pass.) Sacc

The data presented in Table 7 and Figure 5 revealed that acidic pH supports radial mycelial growth. The average maximum radial mycelial growth of 64.88 mm was recorded at 6.5 pH followed by 45.17 mm at 6.0 pH. There was significant difference in radial mycelial

growth at pH 5.5 and 7 showing radial mycelial growth of 41.12mm and 38.59 mm respectively. Minimum radial mycelial growth of 36.03 mm was recorded at pH of 5.0. The data further revealed that average maximum conidial production of 5.95× 10^6 conidia per ml of water was recorded at 6.5 pH followed by 5.24 × 10^6 conidia per ml of water at pH of 7.0 and 5.18 ×10^6 conidia per ml of water at pH of 6.0. Minimum conidial production of 3.39×10^6 conidia per ml of water was recorded at 5.0 pH. These results are in agreement to various researchers delineating that pathogen causing anthracnose of grapes exhibits maximum radial mycelial growth at pH 6.5 (Pandey *et al.*, 2011; Bhat *et al.*, 2019; Fayaz *et al.*, 2021a).

Figure 5. Effect of pH on the radial mycelial growth and sporulation of *Gleosporium ampelophagum.*

Table 7. Effect of different pH on growth and sporulation of *Gleosporium ampelophagum* (Pass.)Sacc. at 25±2 ˚C after 7 days of incubation.

pH	Radial mycelial growth (mm)*	Sporulation (×10^6/ml)
5.0	34.66 (36.03)^e	0.35 (3.39)^e
5.5	43.29 (41.12)^c	0.62 (4.51)^a
6.0	50.32 (45.17)^b	0.81 (5.18)^a
6.5	81.29 (64.88)^a	1.08 (5.95)^a
7.0	38.95 (38.59)^d	0.83 (5.24)^a
CD (p≤0.05)	1.63	1.80

*Figures in parenthesis are arc sin transformed values based on mean of four replications.

Growth of mycelium and sporulation are influenced by the medium, pH and temperature (Kumara and Rawal, 2010). The best medium for growth and conidial production of *G. ampelophagum* (Pass.) Sacc was oat meal agar where in maximum radial mycelial growth and conidial production was obtained, whereas the

minimum radial mycelial growth and conidial production was observed on Czapek (Dox) agar. These results are in close agreement with those of Kore and Gurme (1979) who reported maximum radial mycelial growth on potato dextrose agar followed by oat meal agar and maximum sporulation on oat meal agar than all other media tested, however, the slight variation may be attributed to isolate difference. The present investigation are further supported by the findings of Fayaz *et al.* (2021b) who reported that oat meal agar supported highest vegetative growth and sporulation of *Sphaceloma ampelinum* causing anthracnose of grapes. Further, the findings may be attributed to the fact that microorganisms differ in their nutritional requirements, and get affected by the deficiency of rapidly metabolisable nutrients (Cochrane, 1958; Fayaz *et al.*, 2021a). Media containing carbohydrates, lipids, proteins and elements are basic requirements of microorganisms as these nutrients provide energy for biosynthesis and cell maintenance (Hilton, 1999).

In vitro evaluation of bio-agents against *Gloeosporium ampelophagum* (Pass.) Sacc

Perusal of data presented in Table 8 revealed that all the three bio-agents significantly inhibited the radial mycelial growth of the test fungus compared to check. *Trichoderma harzianum* proved most effective bio-agent, providing maximum radial mycelial growth inhibition of 62.53 per cent. It was followed by *Bacillus subtilis* and *Pseudomonas fluorescens* which provided 45.49 and 41.29 per cent inhibition in radial mycelial growth respectively.

Table 8. *In vitro* efficacy of different bio-agents inhibiting the radial mycelial growth of *Gleosporium ampelophagum* (Pass.) Sacc.

Treatments	Average radial mycelial growth (mm)	Inhibition in radial mycelial growth (%)
Trichoderma harzianum	16.75	78.63 (62.53) [a]
Bacillus subtilis	43.5	50.88 (45.49) [b]
Pseudomonas fluorescens	50.18	43.57 (41.29) [c]
Check	84.39	
CD ($p \leq 0.05$)		3.2

The results are also in conformity with those of Jamadar and Lingaraju (2009), who revealed superiority of *T. harzianum* to *P. fluorescens* against *Elsinoe ampelina* causing grape anthracnose. The present results are in accordance with the results of Fayaz *et al.* (2021b) who reported *T. harzianum* efficient in inhibiting the radial mycelial growth of *Sphaceloma ampelinum* the causal agent of grape anthracnose The significant reduction in the growth of *G. ampelophagum* (Pass.) Sacc by antagonists may be due to competition for food, space, production of antibiotics or by lysis of hyphae (Sawant and Sawant, 2006).

CONCLUSION

The temperature of 25-30 ℃ and pH 6.0-6.5 are the favorable conditions that increases the capacity of pathogen to show maximum growth and sporulation on oat meal agar which might be due to best moisture retention, nutrient availability and activity of enzymes under these conditions. Application of synthetic fungicides has been the traditional strategy for the management of diseases. The increasing concern for health hazards and environmental pollution due to chemical use has necessitated the development of alternative strategies for the control of various diseases. Management of anthracnose of grapes by employing microbial agents has been demonstrated to be most suitable strategy to replace the chemicals which are either being banned or recommended for limited use.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.