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RESEARCH PAPER

Effect of different temperatures, relative humidity, incubation period on conidial germination of *Erysiphe necator* causing powdery mildew of grapes

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Abstract: *In vitro* studies were conducted to assess the effect of different temperatures, relative humidity and incubation period on conidial germination of *Erysiphe necator* (powdery mildew) at the Department of Plant Pathology, College of Agriculture, Vijayapur during 2020-22. Physiological studies of *E. necator* revealed that incubation of powdery mildew fungal spore suspension at various intervals indicated fairly satisfactory conidial germination from 18 to 22 hrs and ultimately reached maximum at 24 hrs after incubation in distilled water. Among the different relative humidity (RH) levels used for conidial germination, maximum germination was observed at 80 per cent RH (82.67%). howver, relative humidity ranging from 80 to 85 per cent was found suitable for conidial germination of *E. necator*. Maximum conidial germination (83.69%) of *E. necator* was observed at 25 °C after 24 hrs and optimum temperature range for the conidial germination was 20-25 °C.

Key words: Grapes, Incubation period, Powdery mildew, Relative humidity, Temperature

Introduction

Grape (*Vitis vinifera* L.) is one of the most delicious, refreshing and nourishing sub-tropical fruit and its cultivation is one of the most remunerative farming enterprises in India. It is grown in a variety of soil. Berries are rich in minerals and vitamins, *viz.* A, B1, B2, C and K. Grape occupies the fifth position amongst fruit crops in India with a production of 2,920.09 thousand metric tonnes from an area of 138.91 thousand hectares. The area under grape is 1.23 per cent of the total area of fruit crops with the production of 2.8 per cent of total fruits produced in the country. About 80 per cent of the production comes from Maharashtra (2,286.44 thousand MT) followed by Karnataka (524.20 thousand MT and Tamil Nadu (58.93 thousand MT) (Anon., 2021). The production of grapevine is threatened by biotic (viruses, bacteria, fungi and insects) and abiotic stresses (*i.e.* drought, winter cold ect).

Powdery mildew (*Erysiphe necator*) is the most wide spread and destructive disease of grapevines worldwide. All green tissues of the grapevine are susceptible to powdery mildew infection. The disease appears as a whitish-grey powdery coating on the surface of leaves or on fruits the primary symptom appear as a chlorotic spot on the upper surface of the leaf that soon become whitish lesions, however, as the disease advances small black round structures (chasmothecia) begin to appear on the white powdery lesions. Whereas on shoots, appearance of brown/black diffuse patches are dominant and on dormant canes, these patches are reddish brown in colour. Severely infected leaves were prematurely dry and drop. Similarly,Infected berries were covered with the fungal structures, and turn to dark brown, shrivel and affected berries were split or may not ripen properly (Berkett and Cromwell, 2015). Like other fungal diseases,severity of the powdery mildew is also influenced by weather factors. Therefore, it is important to investigate influence of temperature, relative humidity and pH on disease severity, further, it is also help full in developing forewarning system to combat the disease. Considering these points in view, an attempt was made to study the effect of different temperatures, relative humidity and incubation period on conidial germination of *Erysiphe necator* causing powdery mildew of grapes.

Material and methods

Physiological studies related to *Erysiphe necator* (powdery mildew) of grapes

In vitro studies were conducted to assess the effect of different temperatures, relative humidity and incubation period on conidial germination of *E. necator*. Conidia were collected from powdery mildew infected grape leaves by using camel hair brush and used for following studies.

Effect of incubation period on conidial germination of *E. necator*

Conidial germination was studied up to 28 hrs incubation period at an interval of two 2 hrs. Spore suspensions were prepared in sterilized distilled water. Drop of spore suspension was placed on clean and sterilized cavity slides, three set of slides were kept for each observation and such slides were kept in moist chamber at room temperatureof 25± 1°C.Conidial germination was observed at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28 hrs. after incubation. Further, per cent germination was worked out by observing conidia in three microscopic fields under microscope at 10x magnification. Per cent conidial germination was calculated by using formula.

$$\text{Per cent conidia germination} = \frac{A}{B} \times 100$$

Where,

A - Number of conidia germinated

B - Number of conidia observed

Effect of relative humidity on conidial germination of *E. necator*

Effect of different levels of relative humidity (RH) on conidial germination was studied, using BOD incubator. The Petri dishes containing conidia suspension in cavity slides kept on BOD incubator for 24 hrs for each different relative humidity levels *viz*. 65, 70, 75, 80, 85, 90, 95 and 100 per cent, each treatment was replicated thrice. The per cent germination was worked out by number of spore germinated to total number of spores observed and expressed in percentage. The data obtained was analyzed statistically.

Effect of temperature levels on conidial germination of *E. necator*

Effect of different temperature levels on conidial germination was studied by taking spore mass in cavity slide. The cavity slides were kept in the moist chamber and were incubated at different temperatures *viz.* 5, 10, 15, 20, 25, 30, 35 and 40 ºC. Three replications were maintained for each treatment. Conidial germination was observed at 24 hrs. The per cent germination was worked out by number of spore germinated to total number of spores observed and expressed in percentage. The data obtained was analyzed statistically.

Observation on cleistothecial production

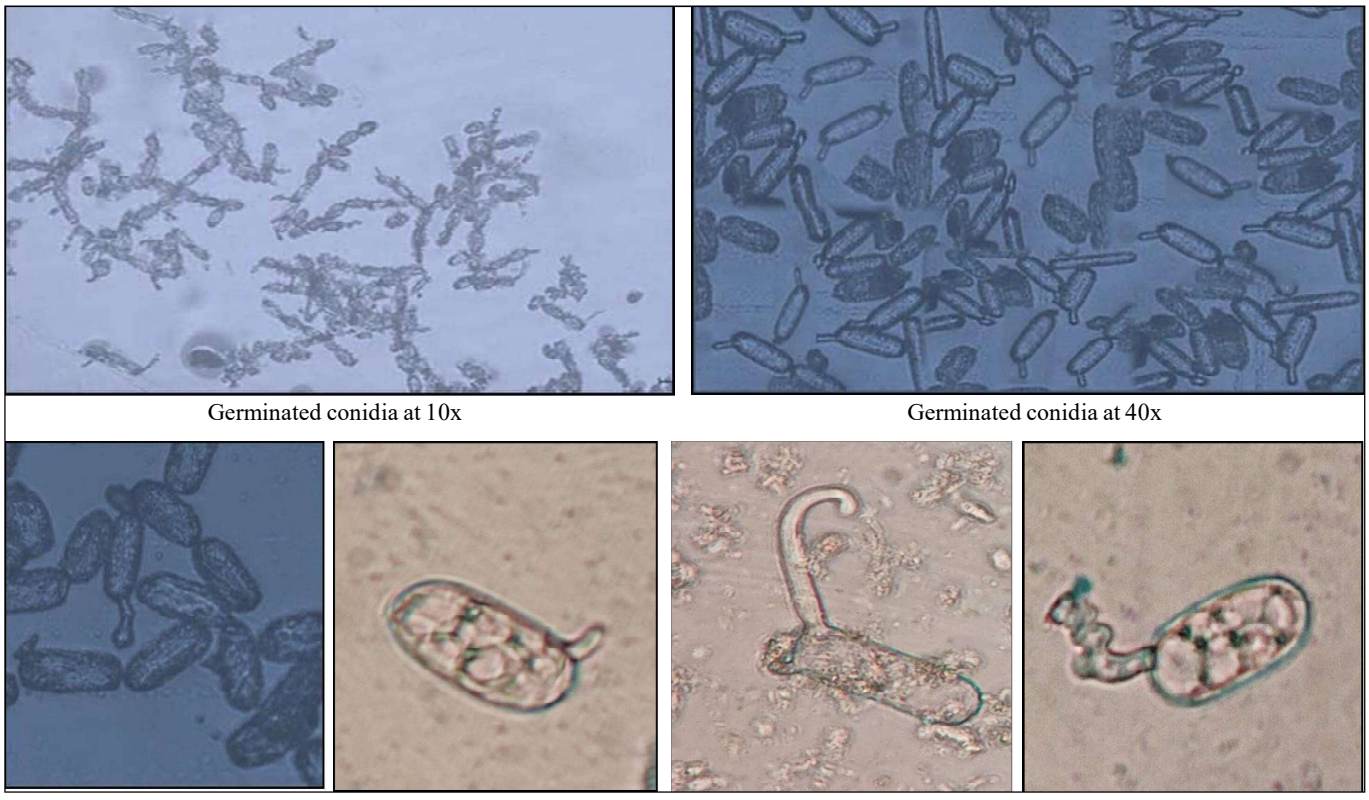
Cleistothecia is the sexual fruiting body of the powdery mildew in general Powdery mildew of grapes caused by *E. necator* in particular can survive from season to season either through dormant mycelium or sexual fruiting body (cleistothecia) or both. To understand the production of sexual stage studies were conducted at Department of Plant Pathology, Agriculture College, Vijayapur.

The fully infected powdery mildew leaves were collected from vineyard during October first week, 2021. The leaves were placed in well aerated thin cloth bag and incubated at room temperature 25±1°C. Later the leaves were observed at fortnightly intervals and observed under compound microscope at 40x magnification for the presence of cleistothecia. Accordingly, observation was recorded and tabulated

Results and discussion

Effect of incubation period on conidial germination of *E. necator*

The fungal conidial germination (Fig.1) depends upon incubation period, atmospheric temperature and relative humidity. Fungal conidial suspension of Powdery mildew was incubated at various intervals in distilled water (Table 1 and Fig. 2A) indicated that, satisfactory germination (26.67 to 31.00%) of conidia were observed from 18 to 24 hrs. however, conidial germination was declined at 26 hrs after incubation. Germination of infectious propagules is an important process in the life cycle of pathogen and disease development, which ultimately determines the host penetration and infection process. More and quick germination of spores also play a vital role in faster spread and development of the disease. In the present study,



Germinated conidia at 10x | Germinated conidia at 40x

Conidia with lobed appresoria | Conidia with germ tube and hooked appresoria

Fig. 1. Microphotograph showing conidial germination of *Erysiphe necator*

Table 1. Effect of incubation period for conidial germination of *E. necator*

Incubation period(hrs)	Conidial germination(%)
2	0.00 (0.00)*
4	1.93 (7.99)
6	6.92 (15.26)
8	7.26 (15.63)
10	11.33 (19.67)
12	15.66 (23.31)
14	18.33 (25.35)
16	20.00 (26.57)
18	22.27 (28.16)
20	25.67 (30.44)
22	26.67 (31.09)
24	31.00 (33.83)
26	19.51 (26.21)
28	13.11 (21.23)
S. Em. ±	0.608
C.D. @ 1%	1.771
S. Ed. ±	0.86
C.V. (%)	5.712

*Figures in the parenthesis indicate angular transformation

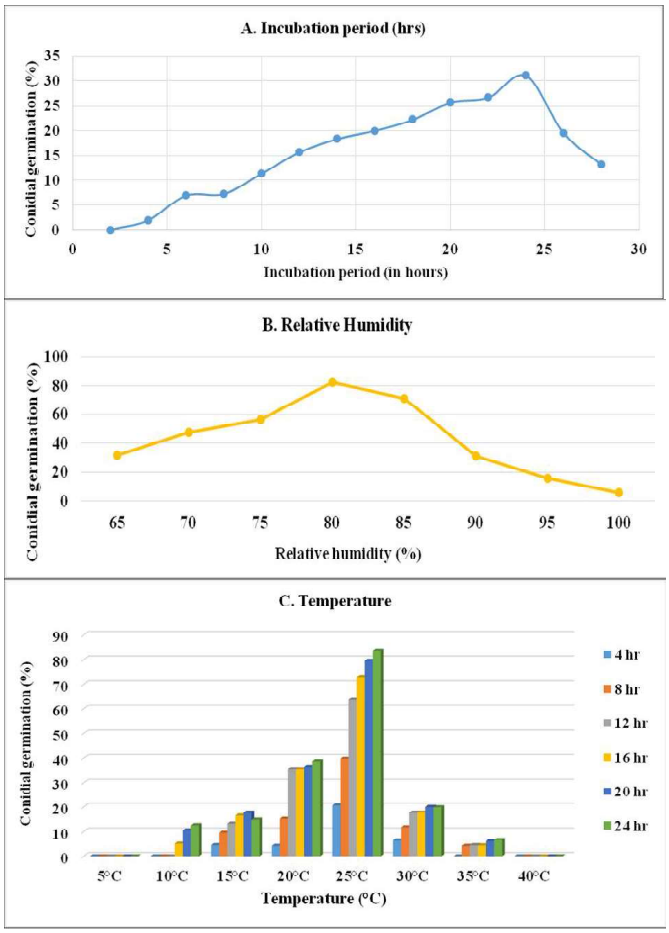


Fig. 2. Conidial germination of *E. necator* under different A) Incubation period (in hrs), B) Relative humidity (%) and C) Temperature (°C) conditions

maximum conidial germination (31.00%) in distilled water was observed at 24 hrs after incubation. These results are in evidence with the studies of Saharan and Saharan (1994) they reported maximum conidial germination of linseed powdery mildew was observed after 14 hrs of incubation. Similarly, Shivanna (2004) reported maximum conidial germination at 24 hrs after incubation.

Table 2. Effect of relative humidity on per cent conidial germination of *E. necator*

Relative humidity(%)	Conidial germination(%)
65	31.33 (34.04)*
70	47.33 (43.47)
75	56.33 (48.64)
80	82.67 (65.40)
85	71.00 (57.42)
90	31.00 (33.83)
95	15.80 (23.42)
100	5.67 (13.77)
S.Em. ±	1.014
C.D. @ 1%	3.067
S. Ed. ±	1.435
C.V. (%)	4.12

*Figures in the parenthesis indicate angular transformations

Effect of relative humidity on conidial germination of *E. necator*

Humidity is the another crucial factor for infection, reproduction and survival of powdery mildew fungus, considering this, varied range of realtive humidity were tested against conidial germination of *E. necator* (Table 2 and Fig. 2B). Among the eight different relative humidity (RH) levels tested, maximum conidial germination was observed at 80 per cent RH (82.67%) which was significantly superior over other RH levels. This was followed by 85 per cent RH (71.00%) and it gradually decreased to 31.00 per cent at 90 per cent RH whereas least conidial germination was observed at 95 per cent RH (15.80%) and 100 per cent RH (5.67%) at 25 °C for 24 hrs. Eventually Study revealed that,80 to 85 per cent relative humidity is required for optimum conidial germination of *E. necator*.. The findings are in agreement with Biju (2000), Carroll and Wilcox (2003), Shivanna (2004) and Sanjivareddi (2012).

Effect of temperature on conidial germination of *E. necator*

Temperature is one of the important factors for all metabolic activities of the fungus. Hence the effect of different temperature levels (Table 3 and Fig. 2C) germination of *E. necator* conidia was studied. The present study revealed that fungus showed variation in its spore germination at different temperatures. Howver, conidial germination was observed at temperature ranging from 10 to 35 °C whereas optimum temperature for maximum conidial germination (83.69%) was at 25 °C which was followed by 20 °C (38.62%). The least or no spore germination (0.00%) was observed at 5°C and 40 °C after 24 hrs of incubation. Similarly Aswathanarayana *et al.* (2003) reported that maximum conidial germination of *E. necator* was recorded at 20 °C temperature as well as at 80 per cent of relative humidity. Further more researches like Biju (2000), Carroll and Wilcox, (2003), Singh (2000) and Sanjivareddi (2012) were also reported the similar results. Sanjivareddi (2012) found highest germination of conidia at 25 °C and stated that 20 to 25 °C is optimum temperature range for conidial germination of *E. cichoracearum* f.sp. *helianthi*.

Table 3. Effect of temperature on per cent conidial germination of *E. necator*

Temperature(°C)	Per cent conidial germination (hours)					
	4	8	12	16	20	24
5	0(0.00)*	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
10	0(0.00)	00.00)	0(0.00)	5.14(13.05)	10.44(18.84)	12.47(20.62)
15	4.59(12.34)	9.5(17.88)	13.09(21.22)	16.52(23.96)	17.66(24.82)	14.92(22.73)
20	4.2911.87)	15.29(22.97)	35.49(35.23)	35.46(35.23)	36.42(37.09)	38.62(38.41)
25	20.53(26.92)	39.74(39.03)	63.62(52.87)	72.97(58.39)	79.53(63.08)	83.6966.16)
30	6.43(14.79)	11.7(19.97)	17.63(24.83)	17.67(24.88)	19.97(26.54)	19.82(26.35)
35	0(0.00)	4.3(11.92)	4.55(12.24)	4.48(12.01)	6.12(14.29)	6.59(14.81)
40	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Factors			S. Em ±	C.D. @ 1%	S. Ed. ±	C.V. (%)
Factor A(Temperature)			0.48	1.54	0.68	2.84
Factor B(Time period)			0.37	1.16	0.53	
Factor(A X B)			1.01	3.08	1.5	

*Figures in the parenthesis indicate angular transformations

Table 4. Production of cleistothecia of *Erysiphe necator* on grape during 2021-22

Days after incubation	Cleistothecial production
1st October 2021	NIL
15th October 2021	NIL
1st November 2021	NIL
15th November 2021	NIL
1st December2021	NIL
15th December 2021	NIL
1st January 2022	NIL
15th January 2022	NIL
1st February 2022	NIL
15th February 2022	NIL
1st March 2022	NIL
15th March 2022	NIL

Observations on cleistothecial production

The experiment results on cleistothecial production was (Table 4) revealed that, there was no cleistothecia production on fully infected leaves; however, observations were recorded periodically using microscope at 15 days' intervals, starting from 1st October, 2021 till the end of 15th March, 2022.

E. necator an obligate biotroph, can infect the leaves, berries, petioles and young buds. It requires a special mechanism to perpetuate through winter when vines are completely dormant. It has been identified that the pathogen perpetuates as cleistothecia (sexual stage) during winter in temperate countries. However, in tropical/subtropical regions of Karnataka; the production of cleistothecia is often speculative and has no role to play in pathogenesis. Hence, in the present experimentation to confirmthe production of cleistothecia was carried out in controlled conditions. Studies revealed that, the fortnightly observations for presence of the sexual stage since from 1st October 2021 to 15th March 2022 confirmed no cleistothecial production under conditions prevailing at study place (Vijayapur, Karnataka) which invariably recorded >30 °C in all the seasons. It clearly shows that the powdery mildew infection on vines carried from season to season may be through the dormant mycelium present under scale of buds but, not through cleistothecia.

However, Putto and Razdan (1987) and Thind *et al*. (1996) reported cleistothecial production under cool climate and green house conditions in Shalimar and Ludhiana, respectively. They described cleistothecia as black fruiting bodies, with coiled appendages. The diameter of cleistothecia varied from 75.0-112.5 x 92 µm and the asci 45-56 x 21-45 µm in size. Each ascus containing 4-6 ovate to ellipsoid ascospores measuring 12-21 x 7-10 µm. Cleistothecial production in South Africa was reported by Halleen and Holz (2001).

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