



Epidemiological Insights into *Erysiphe polygoni*-Induced Powdery Mildew of Blackgram under *In-vitro* Conditions

V. Vasanthi ^{a*}, P. Kishore Varma ^b, M. Suresh ^c,
N. Kamakshi ^d, Ch. Rani ^b, D. Ramesh ^a, J. Vasanthi ^a
and B. Pushparajyam ^b

^a Agricultural College, Bapatla, India.

^b RARS, Lam, Guntur, India.

^c ICAR-IIOR, Hyderabad, India.

^d ARS, Bapatla, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author VV authored experimental design, executed statistical analyses, data tabulation, interpretation and draft of the manuscript. Author PKV contributed to the conceptualization of experiments, ideation, and manuscript revisions. Author BP throughout the data collection phases. Authors JV helped with the experiment's execution and logistical support. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Powdery mildew, incited by *Erysiphe polygoni* D.C, is a prominent foliar disease of blackgram (*Vigna mungo* L.) that adversely impacts all developmental stages, culminating in premature defoliation and yield losses reaching up to 70%. The disease initiates as subtle, white powdery

*Corresponding author: Email: vanapallivasanthi1998@gmail.com;

flecks on the abaxial surface of lower leaves subsequently proliferating into dense mycelial colonies that envelop aerial plant parts, persisting until physiological maturity. Despite its economic significance, the epidemiology of this pathogen under varying environmental conditions remains inadequately explored. The present investigation aimed to dissect the influence of environmental factors on conidial germination of *E. polygoni* under controlled *in vitro* conditions.

Methodology: A spectrum of carbon sources, temperature regimes and relative humidity (RH) levels were evaluated under *invitro* conditions at RARS, Lam, Guntur during December, 2024.

Results: Among carbon substrates, 2.0% dextrose demonstrated the highest conidial germination rate (77.33%), markedly surpassing other treatments. This was trailed by glucose (72.67% at 1.5%) and sucrose (72.00% at 1.5% and 70.67% at 2.0%) whereas sterile water yielded the lowest germination (51.33%). Temperature analysis revealed optimal germination (64.67%) at 24°C with significant inhibition at lower extremes—15°C (37.67%) and 18°C (40.67%). Humidity trials underscored 80% RH as the most conducive, achieving 78.33% germination whereas 50% RH exhibited a suppressive effect with germination plummeting to 36.33%.

Conclusion: This study underscores the intricate interplay of environmental parameters in regulating the infective potential of *E. polygoni*.

Keywords: *Erysiphe polygoni*; premature defoliation; epidemiology; conidia; environment

1. INTRODUCTION

Blackgram, a nutritionally enriched pulse crop, is prized for its high phosphoric acid content and adaptability to diverse cropping systems (Nitin, 2023). is a nutrient-dense pulse with a rich nutritional profile, making it a valuable dietary component, especially for vegetarians and vegans. Per 100 g, it provides 24% protein, 64 IU vitamin A, 0.41 mg thiamine (B1), 0.37 mg riboflavin (B2), 2.0 mg niacin, 7.5 mg vitamin C, 206 mg choline, 144 µg biotin, 90 µg folic acid, 3.5 mg pantothenic acid (B5), and 0.19 mg vitamin K (Nitin, 2023). Additionally, it is rich in soluble and insoluble dietary fibers, promoting digestive health, preventing constipation, and reducing cholesterol levels, which support cardiovascular health. Beyond its nutritional benefits, black gram enhances soil fertility through symbiotic nitrogen fixation with *Rhizobium* in root nodules, acting as a natural biofertilizer. It also serves as nutritious dairy feed, green manure, and helps stabilize soil, reducing erosion (Swaminathan et al., 2023). However, its productivity is severely constrained by biotic stresses, particularly powdery mildew caused by *Erysiphe polygoni* DC, the most destructive foliar pathogen of this crop (Meena et al., 2015). The disease predominantly impacts aerial plant parts, impairing photosynthetic efficiency and inducing physiological disturbances, culminating in yield reductions ranging from 20–40% annually (Chennaveeresh and Kulkarni 2017). Powdery mildew is most prevalent during the flowering and pod formation stages (35–40 days post-sowing) in the *Rabi* season, thriving under moderate temperatures (20–25°C) and elevated

relative humidity (80–90%) (Mishra et al., 2017). The initial infection manifests as white, powdery patches on leaves and other photosynthetic tissues which expand into circular lesions leading to tissue desiccation, distortion and premature leaf loss. Advanced stages of infection accelerate plant senescence, significantly reducing the crop's yield potential. The ectophytic fungus forms specialized haustoria within epidermal cells and produces conidiophores on leaf surfaces which bear hyaline, single-celled conidia in chains (Seethapathy, 2016). Morphologically, primary conidia are lanceolate and apically pointed, while secondary conidia are ellipsoid to cylindrical with rounded or truncate apices. At maturity, cleistothecia develop, releasing asci that contain ascospores which disseminate and initiate subsequent infections (Seethapathy, 2016). The impact of powdery mildew on black gram cultivation is influenced by several factors, including the choice of cultivar, the plant's developmental stage and environmental conditions such as humidity and temperature. The disease's global significance is underscored by its widespread occurrence in major black gram-growing regions, particularly the Indian subcontinent and Southeast Asia where it poses a persistent threat to production (Butler, 1918). Analyzing the epidemiology of powdery mildew in the context of environmental parameters allows for the quantification of relationships between independent climatic factors and disease progression enabling the formulation of predictive autoregressive models. Investigating the pathogen's response to climate variables such as relative humidity and temperature fluctuations provides invaluable

insights into its epidemiological dynamics under future climate scenarios. These findings lay the groundwork for precise disease forecasting and the development of site-specific, adaptive management strategies aimed at minimizing crop losses.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Inoculum

The inoculum of *Erysiphe polygoni* was collected early in the morning from blackgram (*Vigna mungo*) plants exhibiting characteristic powdery mildew symptoms, with leaves showing distinct fungal colonies on the abaxial surface. These conidia were gently removed using a sterile cotton pad and a camel's hairbrush and immediately transferred into a freshly prepared buffer solution (pH 7.0) using double-distilled water to maintain their viability. The resulting suspension was thoroughly agitated to ensure uniform dispersion and then adjusted to a concentration of 5000 conidia/ml using a hemocytometer, ensuring a standardized inoculum for subsequent experiments. Finally, the conidial suspension was incubated under controlled conditions for 24 hours to promote optimal germination, thereby preparing it for artificial inoculation and further disease progression studies. (Kumawat et al., 2016).

2.2 Effect of Different Concentrations of Sugar on Conidial Germination

Conidial suspensions of *Erysiphe polygoni* were prepared in sterilized distilled water, while sucrose and dextrose solutions were formulated at concentrations of 0.5%, 1.0%, 1.5% and 2.0% by dissolving the requisite quantities of sugar in 100 ml of distilled water. Distilled water and tap water without sugar served as controls. Sterilized three-depression cavity slides were used with two drops of conidial suspension (5×10^3 conidia/mL) placed per cavity for each sugar concentration. Three replicates were maintained for each treatment. The inoculated slides were incubated at 24°C for 24 hours. Observations on conidial germination were recorded after 24 hours examining 100 conidia per replication. The percentage of conidial germination was calculated using the formula provided below.

$$PG = \frac{A}{B} * 100$$

Where,

PG = Per cent germination,

A = Number of conidia germinated,

B = Total number of conidia examined

2.3 Effect of Temperature on Conidial Germination

The influence of temperature on conidial germination was evaluated by placing a drop of conidial suspension on sterilized cavity slides which were supported on glass rods inside Petri dishes. To maintain optimal moisture levels both halves of the Petri dishes were lined with sterilized filter paper sprayed with sterile water. Cavity slides were utilized instead of flat slides to prevent desiccation of the droplets (Prakash and Saharan, 1999). The experimental protocol adhered to the standards outlined by the Committee on Standardization of Fungicide Tests of the American Phytopathological Society (1943). The conidial suspension, containing approximately 20 conidia per microscopic field under 10×10 magnification was incubated at five temperature levels: 15°C, 18°C, 21°C, 24°C, 27°C and 30°C using a digital B.O.D. incubator. Three replicates were maintained for each temperature treatment. After 24 hours of incubation, the slides were removed, and a drop of lactophenol was immediately applied to arrest further conidial germination. The per cent germination was recorded as mentioned in above 2.1.

2.4 Effect of Relative Humidity on Conidial Germination

The impact of relative humidity (RH) on the germination of *Erysiphe polygoni* conidia was evaluated using the previously described method for inoculum preparation. Six distinct RH levels—50%, 60%, 70%, 80%, 90% and 100%—were established within desiccators by varying the proportions of glycerol and sterilized double-distilled water following the method recommended by Marcoli and Peter, 2005. The desiccators were then incubated in an incubator at 24°C for 24 hours. The per cent germination was recorded as mentioned in above 2.1.

3. RESULTS AND DISCUSSION

3.1 Effect of Different Concentrations of Sugar on Conidial Germination

The study investigates the influence of varying sugar concentrations on the germination of

fungal conidia with a focus on dextrose and sucrose. The findings reveal a statistically significant impact of sugar solutions on conidial germination rates. The highest germination rate (77.33%) was observed in a 2% dextrose solution which demonstrated significant superiority over other media. This was followed by 1.5% dextrose and 1.5% sucrose solutions which recorded germination rates of 72.67% and 72.00% respectively. Control treatments, including tap water, sterile water and distilled water exhibited comparatively lower germination rates of 57.33%, 51.33% and 56.67%, respectively. Among the sugar solutions tested, the lowest germination rate (58.08%) was associated with 0.5% sucrose (Table 1 and Fig. 1). These findings underscore the critical role of carbon sources in fungal physiology, particularly the ability of higher sugar concentrations, such as 2% dextrose, to enhance conidial germination. Conversely, reduced sugar concentrations or water-based treatments result in significantly lower germination rates. This study provides valuable insights into fungal growth dynamics under varying nutritional conditions and highlights the potential implications of carbon source optimization in fungal biology. The findings were in accordance with Jyothi *et al.* 2014 who reported that maximum germination of conidia was observed at 2.0 per cent dextrose solution (75.50%) at 24h after incubation. Very poor germination was recorded in sterile water compared to tap water. Similarly, Ashwini *et al.*, 2021 reported that After 24 hours of incubation, maximum conidial germination (84.64%) was observed in 1.5 per cent glucose solution which differed significantly with remaining treatments followed by glucose

(79.15%) at 2 per cent concentration. Least conidial germination was noticed in distilled water (64.28%).

3.2 Effect of Temperature on Conidial Germination

The conidial germination of *Erysiphe polygoni* was significantly influenced by atmospheric temperature, as evident from the data presented in Table 2 and Fig. 2. The highest conidial germination rate (64.67%) was observed at 24°C, which differed significantly from the germination rates recorded at other temperature levels. This was followed by germination rates of 57.33% at 21°C and 49.33% at 27°C. The lowest germination rate (37.67%) was recorded at 15°C. Interestingly, 24°C was identified as the optimum temperature for maximal conidial germination of *Erysiphe polygoni*, as temperatures both below and above this threshold caused a significant reduction in germination. These findings align with the results reported by Kumawat *et al.* (2016), who observed maximum conidial germination at 24°C. Similarly, Ashwini *et al.* (2021) documented a peak germination rate of 58.12% at 25°C, which significantly differed from germination rates at other temperatures. Poor germination rates of 8.28% and 20.23% were recorded at lower temperatures of 5°C and 10°C, respectively. This study underscores the pivotal role of temperature as a key abiotic factor affecting the distribution, growth and reproductive capacity of *Erysiphe polygoni*. Understanding the temperature-dependent dynamics of conidial germination provides critical insights into fungal ecology and its potential management under varying climatic conditions.

Table 1. Optimization of sugar solution concentrations for conidial germination of *Erysiphe polygoni* DC

S.no	Treatment	Concentration (%)	Conidia Germination (%)
1	Sterile Water	-	51.33(45.79) ^f
2	Distilled Water	-	56.67(48.86) ^e
3	Tap water	-	57.33(49.24) ^e
4	Dextrose	0.5	60.00(50.79) ^{cde}
5	Dextrose	1	63.67(52.96) ^c
6	Dextrose	1.5	72.67(58.51) ^b
7	Dextrose	2	77.33(61.60) ^a
8	Sucrose	0.5	58.67(50.02) ^{de}
9	Sucrose	1	62.00(51.97) ^{cd}
10	Sucrose	1.5	72.00(58.08) ^b
11	Sucrose	2	70.67(57.24) ^b
12	SEm ±		0.87
13	CD (P≤ 0.05)		2.55
14	CV(%)		2.84

Figure in the parenthesis are Arc sin transformed values

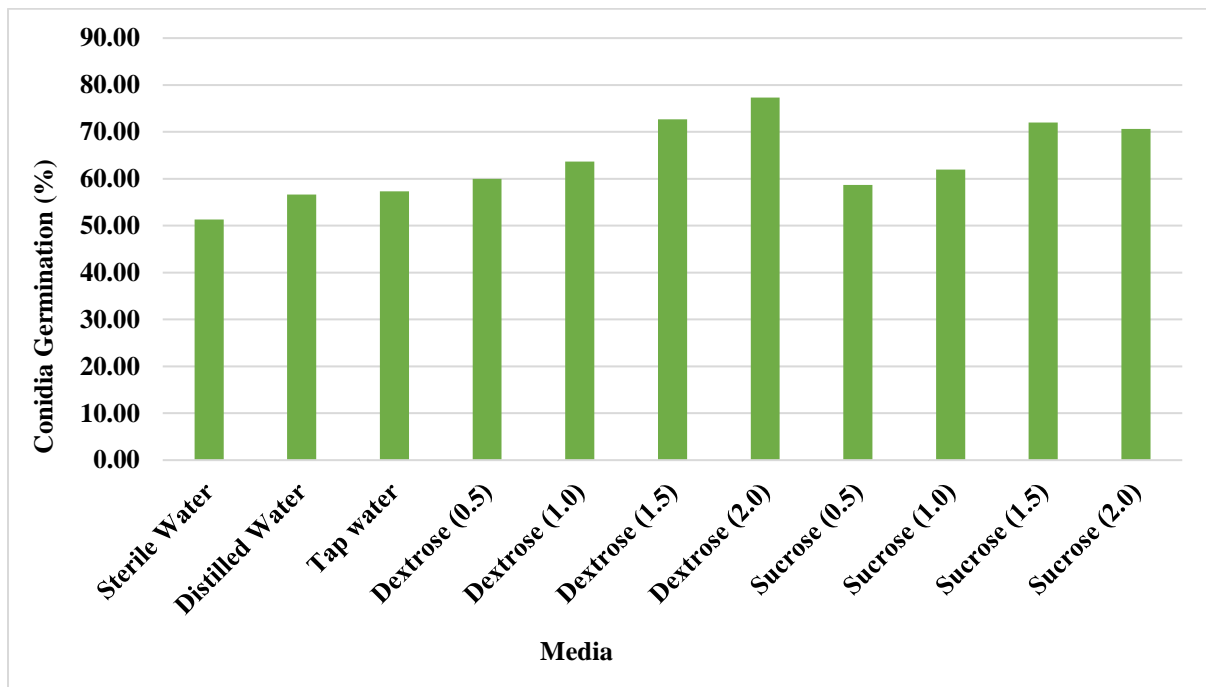


Fig. 1. Optimization of Sugar Solution Concentrations for Conidial Germination of *Erysiphe polygoni* DC

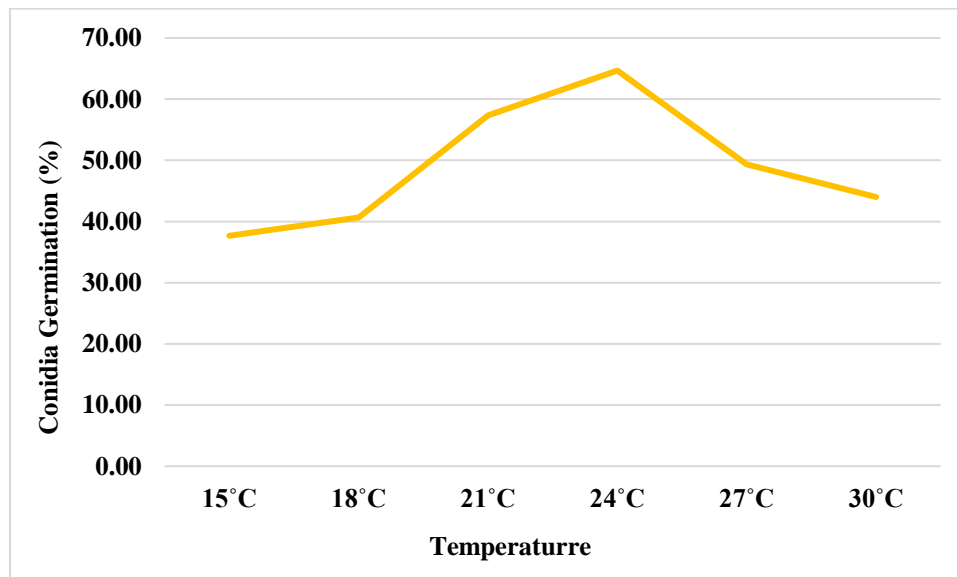


Fig. 2. Effect of temperature on conidial germination of *Erysiphe polygoni* DC

3.3 Effect of Relative Humidity on Conidial Germination

The present study investigated the impact of relative humidity (RH) on conidial germination under controlled conditions at 24°C. The results demonstrated that conidial germination was significantly influenced by RH levels. The maximum germination rate, 78.33% was

observed at 80% RH which was statistically superior to all other treatments. The second highest germination rate, 68.33% was recorded at 70% RH followed by 55.00% at 90% RH and 46.67% at 100% RH. These levels varied significantly among themselves. The lowest germination rate, 36.33% occurred at 50% RH (Table 3 and Fig. 3). These findings align with the observations of Jyothi *et al.* (2014), who reported

optimal conidial germination of 70.50% at 80% RH, and Vikas and Ratnoo (2011) who found that RH levels between 60% and 80% were conducive for conidial germination of *Erysiphe polygoni* on fenugreek. The study emphasizes the critical role of relative humidity in facilitating conidial germination which is essential for fungal

infection and disease progression. It highlights that 80% RH is optimal for maximum germination providing insights into how environmental conditions influence pathogen activity. This knowledge can be leveraged to develop targeted strategies for managing fungal diseases under varying climatic conditions.

Table 2. Effect of temperature on conidial germination of *Erysiphe polygoni* DC

Sno.	Temperature	Conidia Germination (%)
1	T1 (15°C)	37.67(37.88) ^e
2	T2 (18°C)	40.67(39.64) ^{de}
3	T3 (21°C)	57.33(49.24) ^b
4	T4 (24°C)	64.67(53.56) ^a
5	T5 (27°C)	49.33(44.64) ^c
6	T6 (30°C)	44.00(41.58) ^d
7	SEm ±	0.94
8	CD (P≤ 0.05)	2.77
9	CV(%)	3.70

Figure in the parenthesis are Arc sin transformed values

Table 3. Role of Relative Humidity in Regulating Conidial Germination of *Erysiphe polygoni* DC

Sno.	Relative Humidity (%)	Conidia germination (%)
1	50	36.33(37.09) ^e
2	60	40.67(39.64) ^{de}
3	70	68.33(55.78) ^b
4	80	78.33(62.29) ^a
5	90	55.00(47.89) ^c
6	100	46.67(43.11) ^d
7	SEm ±	1.39
8	CD (P≤ 0.05)	4.09
9	CV(%)	5.08

Figure in the parenthesis are Arc sin transformed values

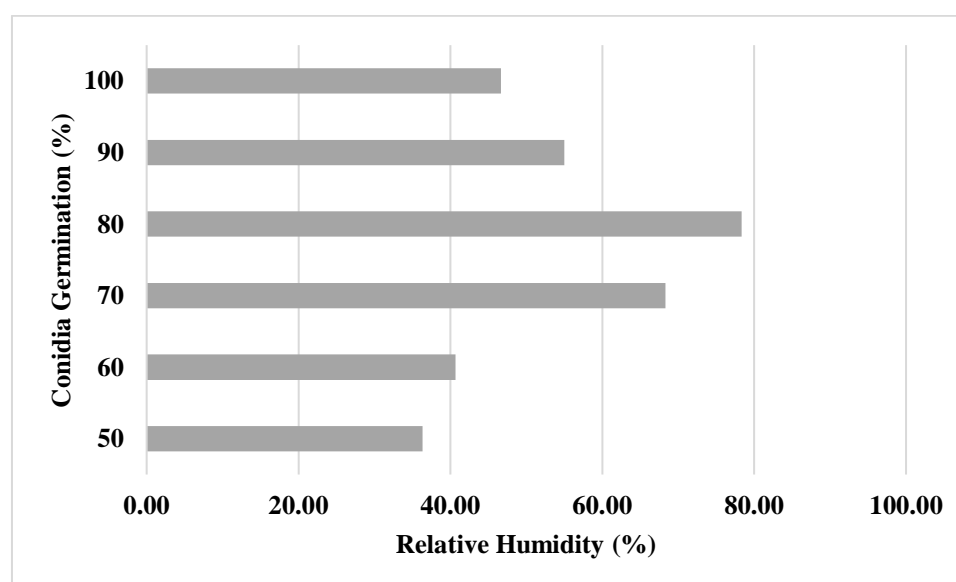


Fig. 3. Effect of relative humidity on conidial germination

4. CONCLUSION

The study elucidates the critical influence of nutritional and environmental factors on conidial germination. Carbon sources play a pivotal role with sugar concentrations significantly affecting fungal physiology. Temperature is identified as a key abiotic factor, regulating growth and reproduction within specific thresholds. Relative humidity further modulates germination dynamics, reflecting its importance in disease establishment and progression. These findings provide valuable insights into fungal ecology and offer potential applications in disease management. By understanding these interactions, strategies can be developed to mitigate fungal infections under varying climatic and environmental conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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