Int J Seed Spice, 14(2), December, 2024: 38-43 DOI: https://doi.org/10.56093/IJSS.v14i2.3

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Received: 18 January, 2025
Revision: 27 January, 2025
Accepted: 31 January, 2025

Citation
Nukum, K.N., Ghevariya, T.V., Der, H.N. and Radadiya, N.V. 2024. Impact of temperature and pH on the in vitro growth of *Pestalotia anacardii* causing grey leaf blight of Cashew. Int J Seed Spice.14 (2): 38-43

DOI
https://doi.org/10.56093/IJSS.v14i2.3

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Impact of temperature and pH on the in vitro growth of *Pestalotia anacardii* causing grey leaf blight of Cashew

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Abstract

Cashew is one of the significant fruit crops grown extensively in South Gujarat which was viewed as seriously impacted by grey leaf blight disease. In this experiment, exposure of microorganism to various Temperatures and pH to evaluate the mycelial development and colony diameter (mm) of *P. anacardii* was Carried out. For the temperature exposure plates were incubated at various temperatures *viz*.; average ambient temperature (27 ± 2°C), 20, 25, 30, 35 and 40°C with four replications. The outcomes uncover that there was essentially higher mycelial development of *P. anacardii* was seen at normal surrounding temperature for example 27 ± 2°C which was viewed as at standard with 30°C temperature followed 25°C temperature and 35°C temperature. Impact of eight pH levels viz., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 on mycelial development and No. of conidia/low power micro field (10x) of *P. anacardii* was observed. Dry mycelial development was seen at all the pH levels tried however it was most extreme at pH 5.0 (319.66 mg) which was at standard with pH 5.5 (307.33 mg), trailed by pH 6.0 (285.66 mg), pH 6.5 (153.66 mg). The sporulation was significantly superior at 5.0 pH (360.66 spores LPM^{-1}) followed by 5.5 pH (333.66 spores $litre^{-1}$ per minute (LPM) and 6.0 pH (309.33 spores LPM^{-1}). The pH studies indicated that the fungus could grow and sporulate under a wide range of pH from 5.0 to 8.0. However, pH ranging from 5.0 to 6.0 was ideal for growth and sporulation of the fungus.

Keywords: Cashew, *Pestalotia anacardii,*Temperature, pH, Grey Leaf Blight

Introduction

Cashew (*Anacardium occidentale* L.) is significant tropical tree crop. It belongs to the *Anacardiaceae* family, which incorporates about 60 genera and 400 species, among them mango (*Mangifera indica* L.) and pistachio (*Pistacia vera* L.) likewise included. Cashew trees are evergreen and can grow rapidly up to 20 m, however as a rule arrive at 8-12 m in level.Cashew developing states in India are

Maharashtra, Andhra Pradesh, Karnataka, Gujarat, Orissa and West Bengal. India has the distinction of being the world's biggest maker of cashew nuts (Anon., 2018). Cashews have a long history as a popular snack and a versatile ingredient in both sweet and savoury dishes. They are a staple in many cuisines, particularly Asian, where they enhance the flavour and texture of various meals. Cashew nut paste gives a rich, smooth, and creamy consistency to gravies of paneer *curry* and dum *aloo*. Beyond gravies, cashews also add a delightful crunch to various rice preparations. Grey leaf blight, caused by the fungal pathogen *Pestalotia anacardii*, poses a significant threat to cashew production, particularly in regions like South Gujarat where this crop is economically important. It causes heavy defoliation of leaves with decreased photosynthesis activity. The disease may kill smaller twigs ultimately affecting the setting of flowers and fruits resulting in huge economic loss. Khalequzamman et al. (2003)reported that the leaf spot disease of sapotafirst appeared as numerous small, reddish brown specks on the leaf lamina which gradually enlarged to form more or less circular spots measuring 1-3mm in diameter. Fully developed spots have greyish-centred lesions. Grey blight disease initially manifests as tiny yellow spots, each surrounded by a grey halo, on the leaflets. These spots progressively merge, forming irregular necrotic patches. As the disease advances, the center of these patches becomes a grayish-white, while the surrounding brown band darkens. Severe infections lead to complete blight, causing the leaf blade to dry out and shrivel (Sarkar (1960) reported that the growth of *Pestalotia* sp. was capable of developing at temperatures between 10 and 35 C and the ideal lies between 20-25 C. Understanding the environmental factors that influence the growth and development of this pathogen is crucial for developing effective disease management strategies. This study investigates the *in vitro* effects of temperature and pH on the growth of *P. anacardii*, aiming to identify the optimal conditions for its proliferation and, conversely, potential vulnerabilities that could be exploited for disease control.

Materials and Methods

Isolation, identification, maintenance of pathogen

The experiment was conducted at the Department of Plant Pathology, NAU, Navsari.The contaminated plant materials brought back from the field were washed and cut into 1mm leaf pieces including the advancing margins of infection. These leaf pieces were surface sterilized with 1:1000(w/v) Sodium hypochlorite solution for a minute followed by three resultingpieces of washing with sterilized distilled water and these pieces were then aseptically transferred to sterilized Petriplates.Containing 20 ml potato dextrose agar (PDA) medium (Ainsworth, 1961) impregnated with streptomycin and incubated for eight days at 27±2°C.The growthof the fungus developed after 48 hrs of incubation was sub-cultured to obtain pure culture by hyphal tip method that was further maintained by frequent sub-culturing. The isolate was used for further studies.The purified isolate was identified as *P. anacardii* in view of morphological and cultural characteristics (Ko,*et a*l.,2007).

Impact of different temperature on the growth of *P. anacardii*

The pathogen was grown study on PDA media and 20ml of potato dextrose agar was poured a 90mm sterile Petri plate. Such plates were inoculated with 5 mm mycelial disc obtained from the periphery of 7 days old culture of *P. anacardii* and incubated at various temperatures*viz.,*average ambient temperature (27 ± 2°C), 20, 25, 30, 35, and 40°C with four replications.Colony diameter was recorded day to day up to the complete coverage of plates with fungal growth.Count for sporulation was recorded from all four replications. Ten culture blocks of 5 mm diameter mycelial discs from 7 days old culture were suspended in 20 ml sterilized distilled water, homogenized and filtered through muslin cloth, a drop from such filtrate was examined under a microscope. The number of spores per microscopic field under low power magnification (10x) were recorded from three microscopic fields, selected randomly. The data thus obtained were statistically analysed.

Impact of various pH level on the growth of *P. anacardii*

The arrangement of variouspH viz., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were made and pH was adjusted by adding the suitable measure of HCl and NaOH in the PDA-broth medium.50 ml liquid PDA medium was poured into each 150 ml conical flask as per the treatment marked on it. Every treatment was replicated three times. After sterilizing at 121°C and 1.2 kg/cm^2

pressure for 20 minutes in the autoclave, these flasks were inoculated with a 5mm diameter disc of *P. anacardii* with the help of a sterilized cork-borer under aseptic conditions. Inoculated flasks were incubated at room temperature (27 ± 2°C) for 15 days. Mycelial mats were collected from three replications in each case previously weighted Whatman's filter paper no. 42 and dry in oven at 55°C for three sequential days until the consistent weight(Spore/litre per minute (LPM) was obtained.

Results and Discussion

The results of the present study reveal that temperature impactedsignificantly higher mycelial growth.Six different temperature ranges were tried to find suitable temperatures for the growth and sporulation of *P. anacardii*. The outcomes are presented in Table-1 and Figure-1.It is evident from the results that significantly higher mycelial growth (87.67 mm) of *P. anacardii* was observed at average ambient temperature *i.e.* 27 ± 2°C which was found to be at par with 30°C temperature (87.00 mm) followed by 25°C temperature (82.00 mm) and 35°C temperature (76.00 mm) while poor to moderate growth of *P. anacardii* was observed at 20°Ctemperatures (46.67 mm) followed by 40°C temperature (24.33 mm) (Table-1 and Figure-2).Tandon, 1961 recorded a significant difference among the temperature levels on mycelial growth and sporulation of *P. mangiferae* isolated from mango. Maximum mycelial mat was harvested at a temperature of 25°C followed by 30°C and sporulation was highest at 25°C.Das*et al*. 1976, also recorded similar results. According to Mishra and Chhotaray (1989),*P. mangiferae* could develop and sporulate best at temperatures ranging from 25-30^0 C. Data present in (Table-2 and Figure-3) revealed that dry mycelial weight was significantly pH 5.0 (319.66mg) which was at par with pH 5.5 (307.33 mg), followed by pH 6.0 (285.66 mg), pH 6.5 (153.66 mg), which was at par with pH 7.0 (109.66 mg). Significantly least growth of the fungus was recorded at pH 7.5 (57.66 mg) and pH 8.0 (47.00 mg). The sporulation was significantly superior at 5.0 pH (360.66 spores/LPM). The next best in order of merit were 5.5 pH (333.66 spores/LPM), 6.0 pH (309.33 spores/LPM), 6.5 pH (196.66 spores/LPM) followed by 7.0 pH (91.33 spores/LPM). The least sporulation was recorded at pH 7.5 (44.33 spores/LPM) and 8.0 pH (30.33 spores/LPM).Kyada, 2006, has noticed maximum mycelial growthwell at pH range 3.0 to 8.0 but pH 5.5 was ideal for growth and abundant sporulation in *P. guepinii*.In the present study, the pH from 5.0 to 6.0 were found to be most favourable for the radial growth and sporulation of the fungus.This study establishes that *P. anacardii* flourishes under warm temperatures (around 27-30°C) and slightly acidic conditions (pH 5.0-6.0). Understanding these optimal conditions is crucial for developing effective disease management strategies, such as adjusting cultural practices or timing fungicide applications to coincide with periods less favorable for pathogen growth. Further research may explore the combined effects of temperature and pH, as well as other environmental factors, to develop a more comprehensive understanding of grey leaf blight epidemiology

Table 1. Impact of Temperature on growth and sporulation of *P. anacardii*

Sr. No	Temperature(°C)	Average colony diameter (mm)	Sporulation (10x)
1	Average ambient temperature(27 ± 2°C)	9.39* (87.67)**	++++
2	20	6.67 (46.67)	+
3	25	9.08 (82.00)	+++
4	30	9.34 (86.67)	++++
5	35	8.75 (76.00)	++
6	40	4.98 (24.33)	+
	S.Em.±	0.05	
	C.D. at 5 %	0.17	
	C.V %	1.16	

*****Figures those outside parenthesis indicates SQR+0.5 transformed values
**Figures in parenthesis indicate original values

Sporulation category: No. of spores/low - power microscopic field(10x)

Degree of Sporulation	Sporulation category	No.of spores/low - power microscopic field(10x)
+	Poor	1-10 conidia/microfield
++	Moderate	11-25 conidia/microfield
+++	Good	26-40 conidia/microfield
++++	Excellent	>40 conidia/microfield

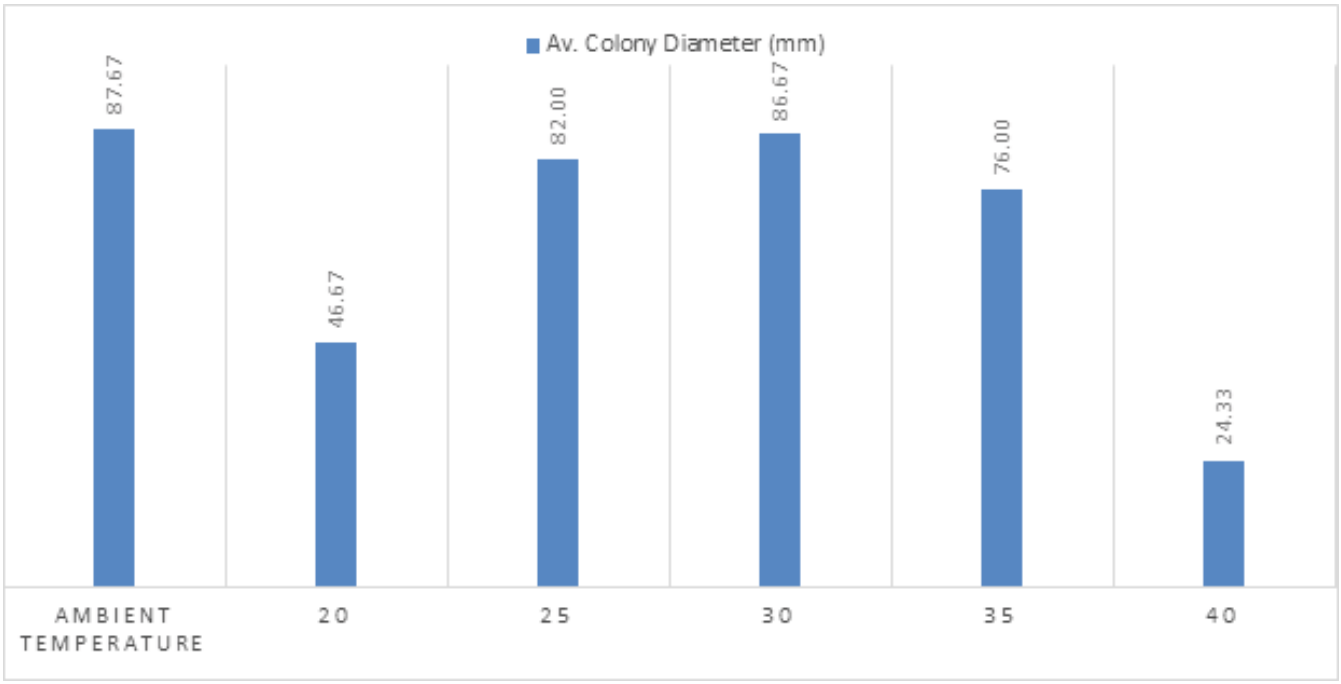

Figure 1. Impact of temperature on growth and sporulation of *P. anacardii*

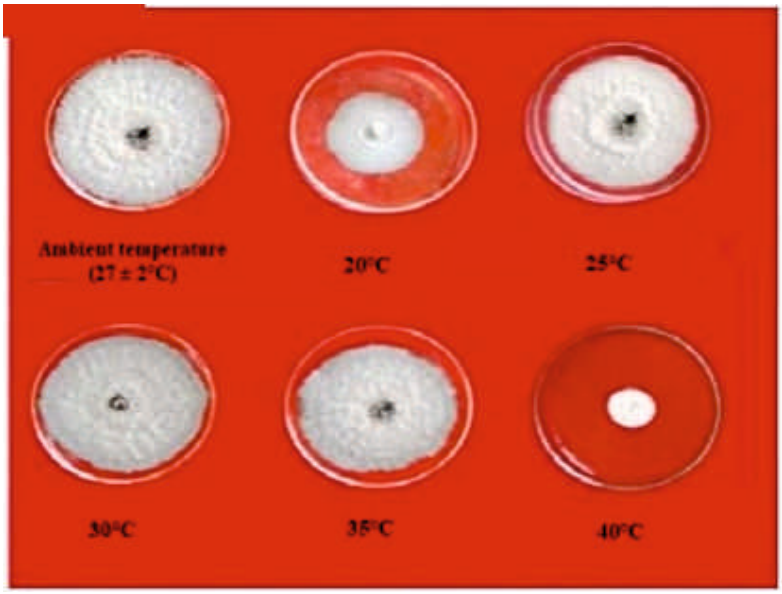

Figure 2. *In vitro* condition mycelium development of *P. anacardii* on PDA

Table 2. Impact of various pH levels on radial growth and sporulation of *P. anacardii*

Sr.No.	pH level	Liquid PDA medium (after 15 days)	
		Average dry weight of mycelium (mg)	No. of conidia/low power microfield (spores/LPM)
1	5.0	2.50* (319.66)**	2.55* (360.66)**
2	5.5	2.48 (307.33)	2.52 (333.66)
3	6.0	2.45 (285.66)	2.49 (309.33)
4	6.5	2.18 (153.66)	2.29 (196.66)
5	7.0	2.04 (109.66)	1.96 (91.33)
6	7.5	1.76 (57.66)	1.65 (44.33)
7	8.0	1.68 (47.00)	1.49 (30.33)
	S.Em±	0.004	0.004
	C.D at 5%	0.011	0.012
	C.V.%	0.30	0.32

*Figures those outside parenthesis indicates SQR+0.5 transformed values
**Figures in parenthesis indicate original values

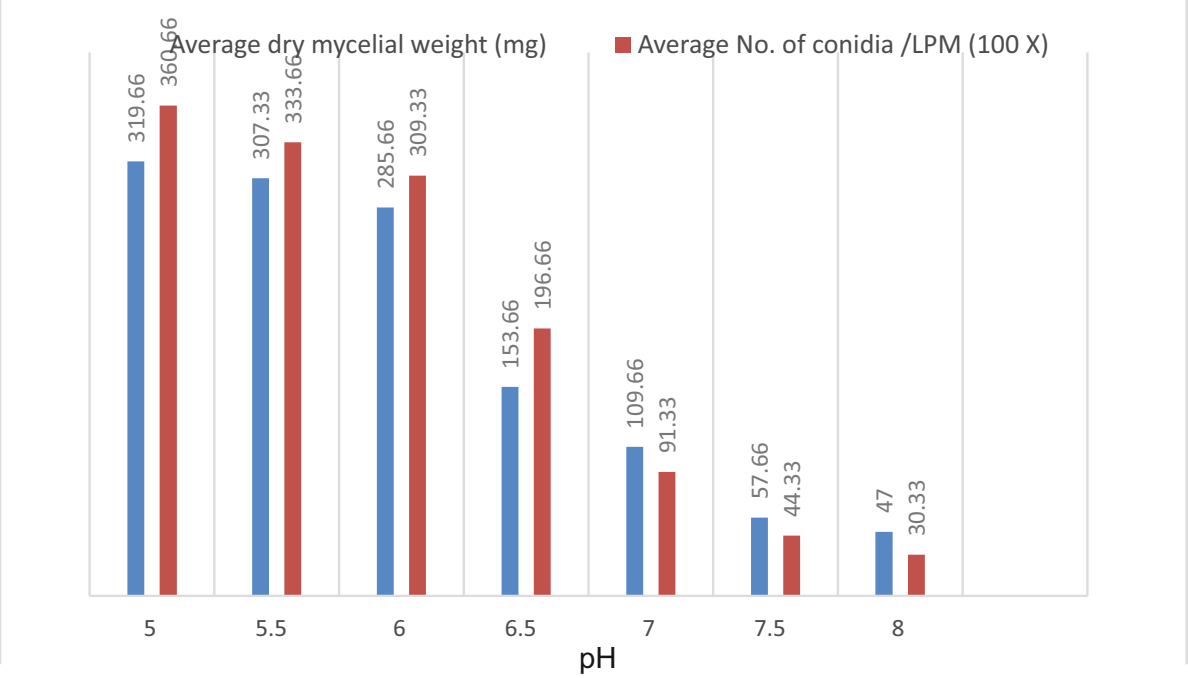

Figure 3. Impact of different temperature on growth and sporulation of *P. anacardii*

Conflict of interest

The authors declare that the research was conducted beyond any commercial or financial affairs that could be taken as a potential conflict of interest.

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