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THE IMPACT OF TEMPERATURE AND NUTRIENT MEDIA ON THE GROWTH OF *EPICOCCUM NIGRUM*

Aleksandar VEMIĆ[1], Sanja JOVANOVIĆ[1], Katarina MLADENOVIĆ[1], Danilo FURTULA[1], Jelena BOŽOVIĆ[1], Radojica PIŽURICA[1]*

Abstract: *Fungus Epicoccum nigrum is one of the most important potential biocontrol agents of plant pathogens. To optimise the application methods, the study investigated the influence of temperature and nutrient media on Epicoccum nigrum mycelium growth. The optimal temperature for Epicoccum nigrum growth ranged from 17 to 25ºC. At 9 ºC, Epicoccum nigrum showed a delayed beginning of the growth compared to the other temperatures. The lowest mycelial growth was recorded on Sabouraud maltose agar (SMA), while cultures on malt extract agar (MEA), cornmeal agar (CMA) and potato dextrose agar (PDA) showed the same growth. The colour of cultures varied depending on temperature and nutrient media. The application of Epicoccum nigrum in biotechnical purposes was discussed.*

Keywords: growth conditions, strain, cultivation, biological control

UTICAJ TEMPERATURE I HRANLJIVE PODLOGE NA RAZVOJ *EPICOCCUM NIGRUM*

Sažetak: *Gljiva Epicoccum nigrum je jedan od najvažnijih potencijalnih agenasa biokontrole parazita biljaka. U cilju unapređenja metoda korišćenja, izvršeno je ispitivanje uticaja temperature i hranljive podloge na rast micelije Epicoccum nigrum. Optimalna temperatura za rast Epicoccum nigrum je bila u rasponu 17-25 ºC. Na temperaturi 9 ºC Epicoccum nigrum je pokazao kasniji početak rasta u odnosu na ostale temperature. Najmanji porast micelije je zabeležen na sabouraud maltoznom agaru (SMA) hranljivoj podlozi, dok su kulture na malc ekstrakt agar (MEA), kukurzna kaša agar (CMA) i krompir dektroza agar (PDA) hranljivim podlogama pokazale isti rast. Boja kultura je varirala u zavisnosti od temperature i hranljive podloge. Primena Epicoccum nigrum u biotehničke svrhe je diskutovana.*

Ključne reči: uslovi rasta, soj, gajenje, biološka kontrola

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1. INTRODUCTION

The genus *Epicoccum* was first described in 1815 by Link (Kirk et al., 2008). Species within this genus are ubiquitous ascomycetes, some of which are plant pathogens, while others serve as biocontrol agents against plant diseases (Taguiam et al., 2021). Fungi of the *Epicoccum* genus produce a wide range of metabolites that are not only valuable for biological control but also hold significant medical importance (Braga et al., 2018; Harwoko et al., 2021). Among them, *Epicoccum nigrum* Link is particularly notable, as it causes damage to various plant species while also acting as a biocontrol agent against other pathogens. Notably, *Epicoccum nigrum* exhibits high genetic variability (Fávaro et al., 2011; Li et al., 2022), with differences among strains often reflected in the morphological diversity of various isolates (Arenal et al., 2002).

Given these characteristics, it is essential to investigate different strains of this fungus to identify potential similarities and differences in their development. Temperature is one of the most critical factors influencing fungal growth. Studying the impact of temperature on the growth of *Epicoccum nigrum* can provide insights into its adaptation to various environmental conditions and help determine optimum conditions for its proliferation. Furthermore, combining these findings with data on the influence of nutrient media on mycelial growth enhances our understanding of the cultivation characteristics of this species. These results have practical applications in the cultivation and preservation of *Epicoccum nigrum* cultures, ultimately contributing to the improved production of its bioactive metabolites.

2. MATERIALS AND METHODS

2.1 Effect of Temperature on Culture Growth

The *Epicoccum nigrum* isolate, strain Z1MNE, was obtained from the mycological collection of the Institute of Forestry, Belgrade. Mycelial fragments measuring 6 × 6 mm were placed in the center of Petri dishes containing 3% malt extract agar (MEA; Biolab, Hungary; Torlak, Serbia) as the nutrient medium. The Petri dishes were incubated at four different temperatures: 9°C, 17°C, 25°C, and 30°C. The experiment was concluded after seven days, when the first culture had filled the Petri dish. Each temperature included 8–12 replicates. The culture diameter was measured in two perpendicular directions from the center of the Petri dish. Cultures exhibiting minimal growth were returned for further observation to allow for precise measurement.

2.2 Effect of Nutrient Medium on Culture Growth

An *Epicoccum nigrum* isolate identical to the one used in the temperature study was employed in this analysis. Four types of nutrient media were selected: malt extract agar (MEA; Lab M, UK), cornmeal agar (CMA; Himedia, India), Sabouraud maltose agar (SMA; Torlak, Serbia), and potato dextrose agar (PDA; Lab M, UK). Each medium was tested with 10 replicates. The experiment followed the same procedure as the temperature study.

2.3 Statistical Methods

The dimensions of the cultures in the experiments were tested for normality, homogeneity of variance, and normality of residuals. Since the conditions for parametric tests and models were not met, nonparametric tests were applied.

The Kruskal-Wallis test was used to test differences in culture dimensions across different temperatures and nutrient media. Dunn's post hoc test was conducted to compare individual culture pairs within the tested temperature and nutrient medium conditions.

All statistical analyses were performed using SPSS 27 (IBM Corp.) and Microsoft Office Excel 2021 (Microsoft Corp.).

3. RESULTS AND DISCUSSION

A statistically significant difference in *Epicoccum nigrum* mycelial growth was observed across different temperatures ($H = 37.344$; $p < 0.001$). No mycelial growth was recorded at 9°C and 30°C within the seven-day experimental period (Figure 1).

The highest growth rate was observed at 17°C and 25°C, which were identified as the optimum temperatures for *Epicoccum nigrum* growth (Figure 1). At 9°C, substantial mycelial growth was noted after 21 days, with an average colony diameter of 46.25 mm, i.e., 2.2 mm per day.

The colonies were flattened, compact, and slightly concentric. At 17°C and 25°C, the mycelium exhibited a yellow colouration with distinct zonation, whereas at 9°C, it developed a red colour, similar to that observed on the other tested nutrient media (Figure 1).

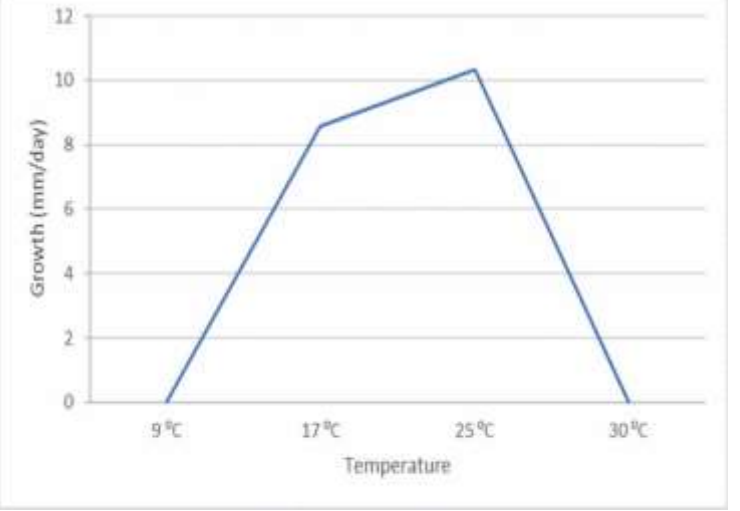

Figure 1. *Growth of Epicoccum nigrum Cultures at Different Temperatures*

A statistically significant difference was observed in the colony diameter of *Epicoccum nigrum* depending on the nutrient medium ($H = 18.859$, $p < 0.001$). The slowest growth was recorded on SMA, while growth on MEA, CMA, and PDA was comparable (Table 1).

On MEA nutrient medium, the cultures had the same characteristics as those observed in the temperature experiment—compact, flat, and zoned, with a yellow colour and multicoloured margins (Figure 1). On CMA, the colonies were compact and zoned, displaying a reddish-brown center with lighter edges (Figure 1). The cultures on SMA were also compact and zoned but appeared slightly darker than those on CMA (Figure 1). On PDA nutrient medium, the cultures were darker than on CMA and SMA, while similarly compact and flat (Figure 1).

Table 1. *Colony Diameter of Epicoccum nigrum on Tested Nutrient Media*

Nutrient Medium	Number of Replicates	Mean (mm)	Std. deviation (mm)
MEA	10	81.60a	1.81
CMA	10	83.80a	2.25
SMA	10	78.00b	3.21
PDA	10	82.20a	1.42

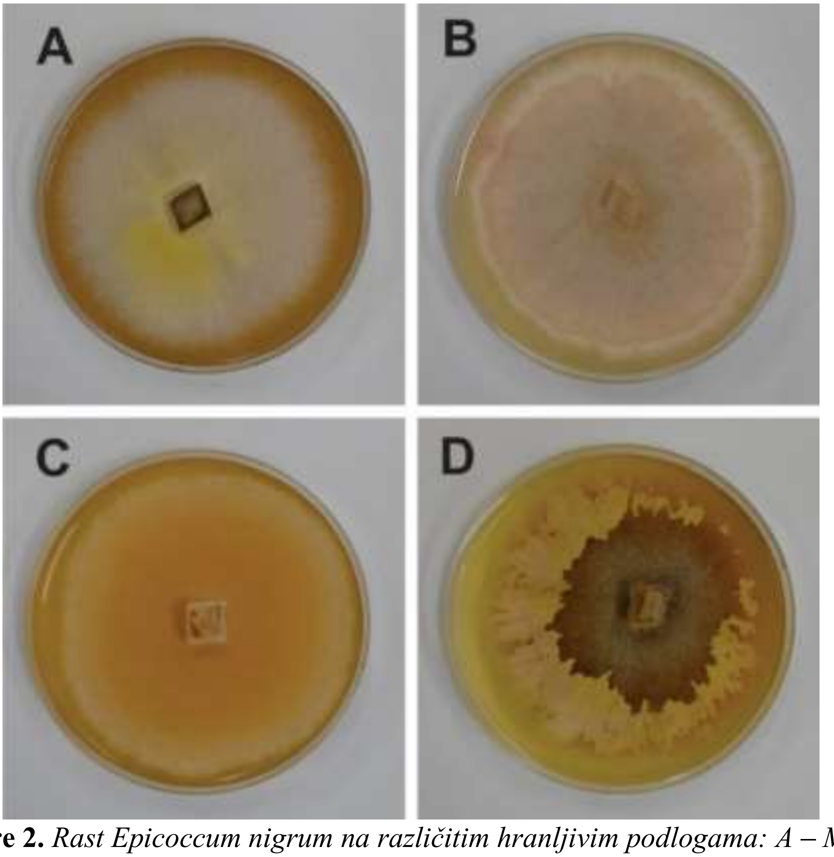

Figure 2. *Rast Epicoccum nigrum na različitim hranljivim podlogama: A – MEA, B – CMA, C – SMA, D – PDA*

Figure 2. *Growth of Epicoccum nigrum on Different Nutrient Media: A – MEA, B – CMA, C – SMA, D – PDA*

The following section presents the impact of *Epicoccum nigrum* on the suppression of specific plant pathogens in biotechnology, with a particular focus on forestry (Table 2).

Table 2. *Application of Epicoccum nigrum in the Suppression of Plant Pathogens*

Harmful organism	Type of damage	Reference
Aleternaria solani	Black spot Downy mildew	Abdel-Hafez et al. (2016)
Aspergillus flavus	Mould	Baldzhieva et al. (2024)
Botrytis cinerea	Gray mould	Christova & Slavov (2021) Baldzhieva et al. (2024)
Colletotrichum acutatum	Anthracnose	Angilè et al. (2025)
Diaporthe eres	Dieback, necrosis	Vemić et al. (2024a)
Fusarium graminearum	Damping-off, necrosis	Li et al. (2022) Nzabanita et al. (2022)
Fusarium sambucinum	Damping-off, necrosis	Vemić et al. (2024b)
Fusarium solani	Damping-off, necrosis	Ali et al. (2024)
Hymenoscyphus fraxineus	Tip dieback, necrosis	Kosawang et al. (2017)
Neonectria ditissima	Canker wounds	Papp-Rupar et al. (2023)
Phytophthora cinamomi	Root rot, dieback, ink disease	García-Latorre et al. (2022)
Pythium debaryanum	Root rot, damping-off	Hashem & Ali (2004)
Pythium ultimatum	Root rot, damping-off	Hashem & Ali (2004)
Pseudomonas savastanoi pv. *savastanoi (Psv)*	Tumors	Berardo et al. (2018)
Vericilum dahliae	Wilting	Angilè et al. (2025)
Sclerotinia sclerotiorum	White mould	Baldzhieva et al. (2024)

Epicoccum nigrum has extensive applications in various biotechnology-related fields. In short, polyketides, polyketide hybrids, and diketopiperazines are secondary metabolites of *Epicoccum nigrum* (Braga et al., 2018). Certain compounds, such as kinase and epicoccone, act as antioxidants (El Amrani et al., 2014). Other compounds, such as flavipin and its derivatives, exhibit antimicrobial, antialgal, and antinematodal properties (Braga et al., 2018). Additionally, some compounds, primarily pigments, have industrial applications (Mapari 2010). When analysing the metabolites of *Epicoccum nigrum*, differences were found in the genomes of isolates obtained from different substrates (Oliveira et al., 2017; Rutledge & Challis 2015). Therefore, it is essential to investigate the ecological characteristics of different isolates.

The obtained results are consistent with the study by Kaur et al. (2019), which identifies PDA as the most suitable nutrient medium for the growth of

Epicoccum nigrum. The nutrient medium can influence the pigment production of *Epicoccum nigrum* (Kaur et al., 2019). The yellow pigments include flavipin, 3-methoxyepicoccone, 7-methoxy-4-oxo-chroman-5-carboxylic acid methyl ester, epicoccalone, epicoccolides A-B, acetosellin, and β-carotene (Bamford et al., 1961; El Amrani et al., 2014; Foppen & Gribanovski-Sassu 1968; Kemami Wangun et al., 2008; Lee et al., 2007; Talontsi et al., 2013). The red pigments include epipyridone, epicoccarines A-B, 2-methyl-3-nonyl prodiginine, rhodoxanthin, and quinizarin (Dzoyem et al., 2017; Gribanovski-Sassu & Foppen 1967; Kemami Wangun & Hertweck 2007; Perveen et al., 2017).

Thus, this research facilitates the utilisation of the tested *Epicoccum nigrum* strain, which demonstrated the secretion of yellow and red pigments as well as antagonistic activity against the pathogens *Diaporthe eres* and *Fusarium sambucinum* (Vemić et al., 2024a, b).

4. CONCLUSIONS

This study examined the effect of temperature and nutrient media on the growth of *Epicoccum nigrum* cultures. The results of this study can be summarised as follows:

- There was a statistically significant difference in the growth rate of *Epicoccum nigrum* cultures exposed to different temperatures.
- The highest growth of *Epicoccum nigrum* cultures was recorded at 17°C and 25°C.
- At 9°C, culture growth started later and was slower compared to 17°C and 25°C.
- The mycelium was compact and flat at all temperatures. At optimal growth temperatures, the mycelium was yellow with zones of different coloUrs, while at 9°C, it was red.
- No mycelial growth was observed at 30°C.
- There was a statistically significant difference in the dimensions of cultures grown on MEA, CMA, SMA, and PDA nutrient media.
- The smallest growth of *Epicoccum nigrum* cultures was observed on SMA nutrient medium.
- No differences in culture growth were observed on MEA, CMA, and PDA media.
- The culture colour varied significantly depending on the type of nutrient medium. On MEA, the cultures were yellow with zones of different colours. Cultures grown on CMA and SMA were red with lighter edges, with darker colouration observed on CMA medium. PDA medium resulted in the darkest cultures, which were reddish-brown with lighter edges.

The obtained results can be applied to improve the cultivation and utilisation of *Epicoccum nigrum* cultures. Furthermore, the findings from this study contribute to the easier identification of specific *Epicoccum nigrum* strains and enable more accurate predictions of its spread in response to global changes.

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Aleksandar VEMIĆ, Sanja JOVANOVIĆ, Katarina MLADENOVIĆ, Danilo FURTULA, Jelena BOŽOVIĆ, Radojica PIŽURICA

Summary

The need for biological plant disease control necessitates an understanding of the bioecological characteristics of various fungal species and their strains. This study examined the effects of temperature and nutrient media on the growth of a tested *Epicoccum nigrum* strain.

Variability in the growth rate of the *Epicoccum nigrum* strain was observed depending on temperature and nutrient media after seven days from the beginning of the experiment. The optimum temperature range for culture growth was 17–25 °C, while its growth slowed at 9 °C. No growth was recorded at 30 °C. Malt extract agar (MEA), cornmeal agar (CMA), and potato dextrose agar (PDA) nutrient media had the same effect on culture growth, whereas growth was slower on Sabouraud maltose agar (SMA).

Mycelial colouration varied depending on temperature and nutrient media. *Epicoccum nigrum* cultures exhibited a red colour at 9 °C, while at higher temperatures, the mycelium appeared yellow with multi-coloured zones towards the edges of Petri dishes. On MEA, the culture colour remained consistent with that observed in the examination of the temperature effect. In contrast, cultures grown on CMA and SMA exhibited a red centre with lighter edges, with the CMA cultures appearing slightly darker than those on SMA. On PDA, the mycelium displayed a reddish-brown colour with lighter edges, appearing darker than those on CMA and SMA.

Finally, a literature review is provided on the potential application of *Epicoccum nigrum* for controlling some of the most significant plant pathogens, with a particular focus on forestry.

UTICAJ TEMPERATURE I HRANLJIVE PODLOGE NA RAZVOJ *EPICOCCUM NIGRUM*

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Rezime

Potreba za biološkim merama zaštite protiv biljnih bolesti uslovljava poznavanje bioekoloških karakteristika različitih vrsta gljiva i njihovih sojeva. Ova studija je ispitivala uticaj temperature i hranjive podloge na rast kultura testiranog soja *Epicoccum nigrum*.

Utvrđena je varijabilnost brzine rasta testiranog soja *Epicoccum nigrum* u zavisnosti od temperature i hranljive podloge posle 7 dana od početka ogleda. Temperaturni raspon 17-

25 ºC je bio optimalan za rast kultura, dok je na 9 ºC dolazilo do usporenog rasta kultura *Epicoccum nigrum*. Na temperaturi 30 ºC nije zabeležen rast. Hranljive podloge malc ekstrakt agar (MEA), kukuruzna kaša agar (CMA) i krompir dektroza agar (PDA) su pokazale isti efekat na rast kultura, a koji je bio sporiji na sabouraud maltoznom agaru (SMA).

Boja micelije se razlikovala u zavisnosti od temperature i hranljive podloge. Kulture *Epicoccum nigrum* su imale crvenu boju na temperaturi 9 ºC dok je na višim temperaturama micelija bila žuta, sa šarenim zonama prema ivicama petri šolja. Na MEA hranljivoj podlozi, boja kultura je bila ista kao prilikom ispitivanja uticaja temperature. Sa druge strane, boja kultura na CMA i SMA hranljivoj podlozi se odlikovala crvenim centrom sa svetlijim ivicama. Kulture su bile nešto tamnije na CMA hranljivoj podlozi u odnosu na SMA hranljivu podlogu. Na PDA hranljivoj podlozi, micelija je bila crveno braon boje sa svetlijim ivicama, odnosno tamnija u odnosu na CMA i SMA hranljivu podlogu.

Na kraju, naveden je literaturni prikaz mogućnosti primene *Epicoccum nigrum* za suzbijanje nekih od najznačajnijih patogena biljaka, sa posebnim osvrtom na oblast šumarstva.

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