# CHARACTERIZATION OF ANTAGONISM OF FUNGI *EPICOCCUM NIGRUM* AND *DIAPORTHE ERES*

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## Abstract

Fungus *Diaporthe eres* represents a pathogen that occurs on a large number of hosts in forestry. In order to create strategies for reduction of damages and use of ecologically justified measures of protection of this insufficiently studied complex of species, *in vitro* research of antagonistic activity of *Epicoccum nigrum* was carried out. The study of antagonism of *Epicoccum nigrum* and *Diaporthe eres* included dual cultures method. The measurement of diameter of cultures, determining of the appearance of the mycelium and the way of cultures' reaction was carried out after 14 days. The results showed that the growth of *Diaporthe eres* mycelia in cultures with *Epicoccum nigrum* was statistically significantly smaller compared to control cultures. The cultures of *Diaporthe eres* were inhibited, whereby there was no overgrowth of mycelia of *Diaporthe eres* with mycelia of *Epiccocum nigrum*, which indicates that the antagonism was primarily reflected in the prevention of growth. The growth of *Epicoccum nigrum* was also statistically significantly smaller compared to control cultures, as well as *Diaporthe eres,* which indicates that the antagonism was not absolutely pronounced, and that for more pronounced inhibition of *Diaporthe eres* a larger quantity of mycelia is needed. Part of the cultures of *Epicoccum nigrum* showed forming of red pigment during reaction with the cultures of *Diaporthe eres*, indicating that there could be further research of its part in the antagonism process. On the other hand, clear forming of the pigment of *Diaporthe eres* was not recorded in dual cultures compared to control cultures. The obtained results enable the development of practical application of *Epicoccum nigrum*.

**Keywords:** *biological protection, dual cultures, interaction, antagonism.*

## Introduction

Genus *Diaporthe* represents species of fungi that appear in different ecological conditions in forestry, as plant pathogens and saprophytes (Gomes *et al*., 2013; Horst, 2013). The taxonomic situation of this genus is very complex and the need for defining species is necessary due to creating adequate strategies of introduction of plants and phytosanitary protection measures (Crous and Groenewald, 2005; Wingfield *et al*., 2012). Especially since a certain number of members of this genus and related imperfect forms of fungi can occur on various hosts, or have wide geographical distribution. (Udayanga *et al*., 2011; Gomes *et al*., 2013). A complex of species *Diaporthe eres* is of great importance and present on many different hosts and also morphologically variable (Castlebury *et al*., 2002; Udayanga *et al*., 2014).
Genus *Epiccocum* consists of a large number of species, out of which *E. nigrum* (*E. purpurascens*), *E. layuense*, *E. dendrobii*, *E. mezzettii* and *E. minitans* represent the biocontrol agents of a large number of plant pathogens, whereby the species *Epicoccum nigrum* especially stands out (Taguiam *et al*., 2021). The mechanism of action of antagonism of *Epicoccum nigrum* to pathogenic organisms is necessary to research due to the need of creation of strategies for wide application of this species. The base for understanding the

antagonism between *Epicoccum nigrum* and pathogenic fungi are certainly the processes of growth of their mycelia. These researches are especially significant when studying the possibilities of protection against different complexes of species in order to obtain the data as detailed as possible on their reaction to antagonistic agents. The obtained results will enable at the same time better knowledge on antagonistic characteristics of certain strains of the species *Epicoccum nigrum* and reactions of certain members of the complex of species *Diaporthe eres* to their application.

In accordance with the above-mentioned, the objective of this research was to examine the influence of the used strain of *Epicoccum nigrum* to the development of the cultures of *Diaporthe eres*. The tested null hypotheses were the following: i) The used strain of *Epicoccum nigrum* cannot have effect on growth arrest of *Diaporthe eres*; ii) The mode of antagonism of the used strain of *Epicoccum nigrum* to *Diaporthe eres* cannot be characterized.

## Materials and Methods

The experiment of in vitro effects of *Epicoccum nigrum* on *Diaprthe eres* in dual cultures was placed on 3% malt extract agar nutrient medium (MEA; 30 g/l malt Biolab, Hungary; 20 g/l agar Torlak, Serbia), on the temperature of 25ºC in December 2023. The used isolates of *Diaporthe eres* and *Epicoccum nigrum* were taken from mycological collection of the Institute of Forestry in Belgrade and the Faculty of Forestry of the University of Belgrade.

The total of 15 dual cultures of *Epicoccum nigrum* and *Diaporthe eres* were set up. The control group for *Epicoccum nigrum* and *Diaporthe eres* contained 5 cultures of each fungus. Parts of mycelia 5x5 mm of *Diaporthe eres* and *Epicoccum nigrum* were placed by sterilised scalpel in petri dishes on the same distance of 2.5 cm from the edges of petri dishes. One part of mycelia of *Diaporthe eres* and *Epicoccum nigrum* were placed per each petri dish. The control groups contained pure cultures of *Epicoccum nigrum* and *Diaporthe eres*. The experiment was completed after 14 days when all the cultures showed evident growth.

The measurement of the diameter of cultures was carried out in two transverse directions. In antagonistic cultures measurement of the growth was carried out on the places of the largest growth of mycelia. In control cultures, the measurement of the diameter was carried out from the centre of petri dish.

The testing of the normality of the arrangement and homogeneity of variance of culture dimensions was performed by Kolmogorov-Smirnov test, with Lilliefors correction and Levene’s test. Kruskal Wallis test with Dunn’s post hoc test was used for comparing the dimensions of cultures of *Diaporthe eres* exposed to antagonism of *Epicoccum nigrum* and control cultures. The descriptive statistics was used for presenting the dimensions of cultures in the experiment. All statistical analyses were carried out using the software package SPSS 27 (IBM Corp.) and Microsoft Office Excel 2021 (Microsoft Corp.).

## Results and Discussion

Kruskal Wallis test showed statistically significant difference in the diameter of the cultures ($H = 34.899$; $p < 0.001$, Figure 1). Also, different pairs of cultures showed statistically significant difference in the development (Figure 1). The cultures of *Diaporthe eres* exposed to the effect of *Epicoccum nigrum* were significantly smaller compared to their control group (Figure 1, Figure 2). On the other hand, antagonistic cultures of *Epicoccum nigrum* had the smallest dimensions, as a consequence of preventing the growth of mycelia of *Diaporthe eres* (Figure 1, Figure 2).

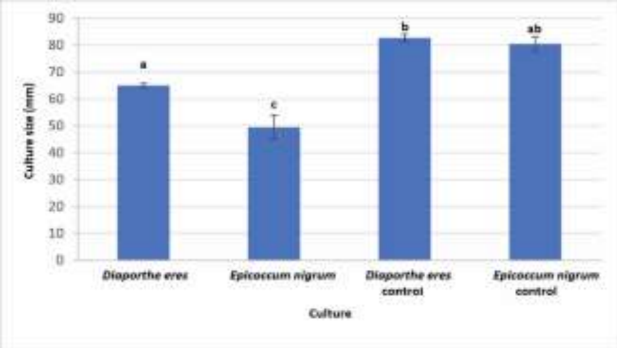


Figure 1 Dimensions of cultures of *Diaporthe eres* and *Epicoccum nigrum*
Note: Statistically significant differences were marked by different letters ($p < 0.05$)

All cultures had a distinctive appearance, in accordance with the characteristics of their isolates (Figure 2). Dual cultures of the tested fungi did not show forming of specific reaction zones between mycelia of *Diaporthe eres* and *Epicoccum nigrum* (Figure 2). However, certain colonies of *Epicoccum nigrum* in dual cultures showed pronounced secretion of red pigments which is possibly associated with metabolic enzymes, i.e. antagonistic activity (Figure 2). The secretion of black pigment in *Diaporthe eres* was recorded in all cultures, including inoculum places, so it is not considered the exclusive product of antagonism (Figure 2). In dual cultures overgrowth of mycelia of *Diaporthe eres* with mycelia of *Epicoccum nigrum* was not recorded, but the antagonism was reflected in occupation of space for growth (Figure 2).

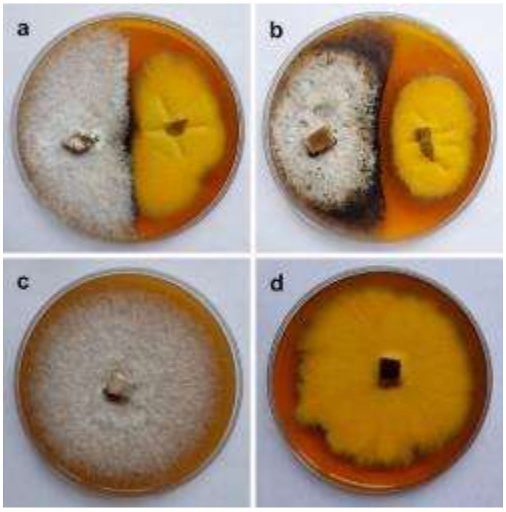


Figure 2 Pure cultures of *Diaporthe eres* and *Epicoccum nigrum*: a-b – antagonism (*Diaporthe eres* on the left, *Epicoccum nigrum* on the right), c – *Diaporthe eres* control, d – *Epicoccum nigrum* control

This study showed the way of growth of *Diaporthe eres* in case when it is exposed to interaction with *Epicoccum nigrum*. The obtained results enable development and improvement of strategies of application of *Epicoccum nigrum* as an agent of biological protection against *Diaporthe eres*. That is, the first null hypothesis was rejected and alternative hypothesis was accepted that the used strain of *Epicoccum nigrum* can have an effect on growth arrest of *Diaporthe eres*. Also, the results of this study are in line with the previous research that showed inhibitory potential of *Epicoccum nigrum* towards *Diaporthe* spp. (Abramczyk *et al*., 2020).

The performed experiment showed that blocking of the growth of *Diaporthe eres* was primary mechanism of antagonism of *Epicoccum nigrum*. In the cultures of *Diaporthe eres* exposed to *Epococcum nigrum* overgrowth of mycelia was not recorded as in the antagonism towards *Rhizoctonia solani* (Lahli and Hijri, 2010), nor degradation of mycelia as in antagonism towards *Phytophthora infestans* (Li *et al*., 2013). In this way, second null hypothesis was rejected and alternative hypothesis was accepted that the way of antagonism of the used strain of *Epicoccum nigrum* to *Diaporthe eres* can be characterised.
However, the occurrence of red pigment was noted which was present to a lesser extent in pure malt extract agar cultures, indicating the potential role of metabolites as factors that participate in antagonism or affects the inhibition of development independently. There are different methods of stimulation of natural pigments of *Epicoccum nigrum*, using potato dextrose agar and liquid nutrient media (Mapari *et al*., 2008; Kaur *et al*., 2019). Above all, forming of pigments of *Epicoccum nigrum* while interacting with *Diaporthe eres*, indicates bioecological characteristics of *Epicoccum nigrum* that can be significant for selection of antagonistic strains. The used isolates of *Diaporthe eres* and *Epicoccum nigrum* had the same growth rate, which gives a possibility of estimate of antagonistic potential of *Epicoccum nigrum*. It is noticeable that during the inhibition of *Diaporthe eres*, the colony of *Epicoccum nigrum* is also significantly smaller. This indicates that the antagonism of the tested strain of *Epicoccum nigrum* is not too pronounced and that a larger quantity or number of applications in necessary for more successful application against this pathogen. Similar examples are found while studying the antagonism of *Epicoccum nigrum* against *Botrytis cinerea*, where certain strains of *Epicoccum nigrum* did not succeed to limit the growth of *Botrytis cinerea* (Ogórek and Plaskowska, 2011), while other strains showed satisfactory effect (Christova and Slavov, 2021).
Finally, the need of studying the interaction of *Epicoccum nigrum* with certain fungi, is the first step in defining the way of reaction of plant host to *Epicoccum nigrum* aimed at successful protection from the said pathogen. From the obtained results, we conclude that the antagonism of *Epicoccum nigrum* towards *Diaporthe eres* is significantly reflected in this possibility to colonise the plant and reduce the space for growth of this pathogen. The experiences from agriculture showed that it stimulates the growth and biomass, as well as that it was re-isolated most frequently from the surface of the plant (Favaro *et al*., 2012; Ogórek *et al*., 2020). However, it is necessary to take in consideration that certain strains of *Epicoccum nigrum* are highly pathogenic towards certain woody species in forestry, which requires a detailed knowledge of pathogenicity of this fungus and to some extent limitations in application. In any case, in the future, it is necessary to develop methods by which *Epicoccum nigrum* would be more applied in the field and its positive effects would be clearer on different species of trees and shrubs, especially the ones that relate to antagonism towards *Diaporthe eres*.

## Conclusion

Based on the obtained results from in vitro study on the effect of *Epicoccum nigrum* to the growth of *Diaprthe eres*, the conclusions that arise from them can be presented in the following way:

- There was statistically significant inhibition of growth of mycelia of *Diaporthe eres* in cultures that were exposed to *Epicoccum nigrum* compared to pure cultures of *Diaporthe eres*.
- Mycelia of *Epicoccum nigrum* showed red pigment in contact with mycelia of *Diaporthe eres*. In this way, it is possible that during antagonism metabolites and mycotoxins are excreted that affect the development of pathogen. In any case, the occurrence of red pigment is noted in antagonistic strains of *Epicoccum nigrum*, i.e. efficient in inhibition of

*Diaporthe eres*, and to a certain extent it can serve as a starting point for selection of these strains of fungi and it is necessary to be further studied.

- Antagonistic colonies of *Epiccocum nigrum* had smaller dimensions compared to the colonies of *Diaporthe eres*, which indicates that antagonism was not very pronounced, i.e. that in the case of more pathogenic strains of complex of species *Diaporthe eres* it is necessary to use larger quantity of *Epicoccum nigrum* or larger number of treatments.
- Antagonism of *Epicoccum nigrum* in relation to *Diaporthe eres* was reflected in preventing of the growth of mycelia, whereby there was no overgrowth or disintegration of mycelia. This phenomenon leads to the conclusion that the antagonism of *Epicoccum nigrum* is characterised by occupying the space for growth of *Diaporthe eres*. Therefore, strategies are necessary which will enable better development of *Epicoccum nigrum* as a positive reaction of the host plant aimed at creating conditions for application of antagonism.

The obtained results enable development of the method for practical application of *Epicoccum nigrum* in protection of woody species in forestry from the complex of species *Diaporthe eres*.

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