Antifungal activity of anthocyanins from purple field corn cob against *Botrytis cinerea* and *Fusarium* species

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SUMMARY

Purple corn is a pigmented variety of Zea mays L. The color is a result of anthocyanins in the epidermal cells of the plant. This preliminary study aimed to examine the inhibitory effect of crude anthocyanin extracts from purple field corn cob to growth of plant pathogenic Botrytis cinerea and two Fusarium species. Our results clearly indicated the antifungal effect of purple corn extract toward mycelial growth of tested plant pathogen fungi.

Keywords: anthocianin, purple corn, antifungal activity, *Botrytis cinerea*

INTRODUCTION

Flavonoids are known to be one of the largest classes of naturally-occurring polyphenolic compounds. This class of plant secondary metabolites is largely responsible for the colors of many fruits and flowers. More than 4,000 flavonoid compounds have been characterized and classified according to their chemical structure (Murray, 1996). They have been reported to possess a variety of biological activities including antioxidant, antibacterial, antifungal, and antiviral activities (Bylka et al. 2004; Özçelik et al. 2008). Anthocyanins are a class of flavonoid compounds responsible for the bright attractive orange, red, purple, and blue colors of most fruits and vegetables.

There are various kinds of corn in the world, and they have various colors such as white, yellow, red, purple, brown, green and blue. The color of purple corn is due to anthocyanin. Purple corn color has been using for coloring beverages, jellies, candies and so on in Japan (Aoki et al. 2002). Recently, anthocyanins have been reported to have various biological activities, such as antioxidant, anti-mutagenic, and anticancer activities (Koide et al., 1997; Gabrielska et al, 1999; Yoshimoto et al., 1999).

This study was designed to examine the preliminary evidence of crude anthocyanin containing extracts from purple field corn cob to inhibit the growth of plant pathogenic *Botrytis cinerea* and two *Fusarium* species (*F. culmorum* and *F. equiseti*).

MATERIALS AND METHODS

Anthocyanin containing purple corncob extract was prepared from 10 g of purple corncob powder added to a 100 ml of aqueous ethanol and stored for 24h at room temperature. The extract was filtered and then concentrated in a rotary evaporator under reduced pressure. The resulting alcohol free syrup was stored at 4 °C until use in antifungal tests.

F. equiseti and *F. culmorum* isolated from the surface of stored grains were selected and cultured on potato dextrose agar (PDA) and incubated at 25 ºC. In the test a 10 mm mycelial plug was taken from the edge of a 3-day-old colony and placed on the center of PDA plates and make several hole around center in which filled with various concentrations of extract.

Mycelial inhibition tests of anthocyanin was performed with two *B. cinerea* strains isolated from infected plants in Hungary and the two *Fusarium* species were inoculated to PDA and incubated at 25 ºC for 3 days. PDA amended with evaporated extract from corncob was used in the inhibition test. For the controls distilled water was added to the medium. A 10 mm mycelial plug was taken from the edge of a 3-day-old fungal colony, and placed on the center of PDA plates amended with 7.5% ethanol free crude extracts and for control plates. Two replicates of each concentration were used for all isolates. Inoculated plates were incubated at 25 ºC for 2 days in dark, and the mycelial growth was recorded.

Inhibition was calculated with the following equation:

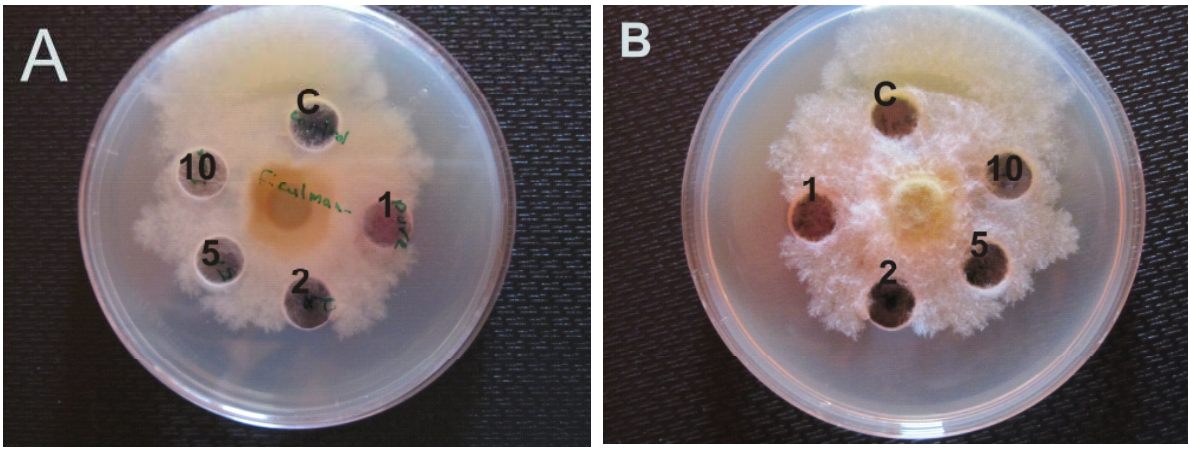
$$inhibition\ (\%) = 100 - \left(\frac{D_a\,(mm)}{D_c\,(mm)} \times 100 \right)$$

, where $D_{a:}$ is average fungal colony diameter on media with anthocyanins, and $D_{c:}$ is average fungal colony diameter on control media (without anthocyanins).

RESULTS AND DISCUSSION

Two *Fusarium* species (*F. equiseti* and *F. culmorum*) were examined in the agar diffusion tests of anthocyanins, where hole in PDA plates were filled with non diluted crude extract, moreover 2, 5 and 10 times diluted extracts and water as control. The result (*Figure 1*) showed that both crude extract of anthocyanins from corncob and diluted ones inhibited the mycelia growth of both *Fusarium* species.

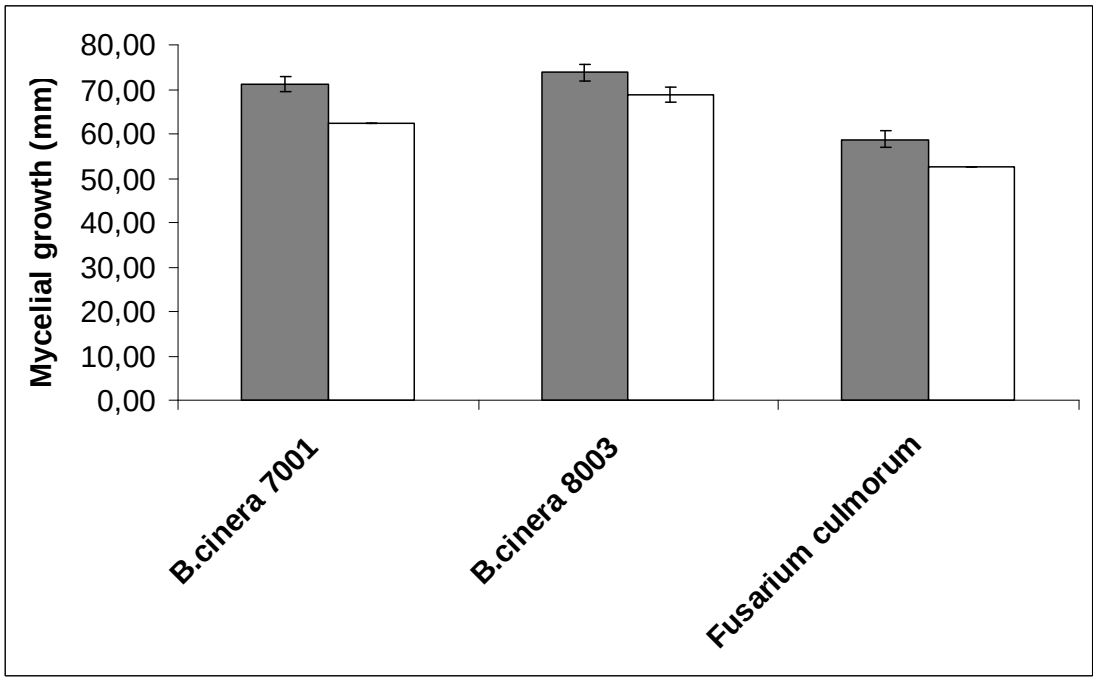
Figure 1: **Detection of antifungal activity of purple field corn cob with agar diffusion method.**



A: *Fusarium equiseti*, B: *Fusarium culmorum*. C: control, 1: non diluted extract, 2: two times diluted extract, 5: five times diluted extract, 10: ten times diluted extract.

Mycelial growth inhibition of purple corn extract was tested on two *B. cinerea* strains and *F. culmorum*. The growth was detected as mycelia diameter 2 days after inoculation (*Figure 2*). Results clearly indicate the inhibition of anthocyanin containing purple corn extract to mycelial growth in all cases.

Figure 2: **Mycelial growth of different plant pathogen fungi in the presence and absence of purple corn extract.**



Grey column: control, white column: with purple corn extract. Bars indicate standard deviation.

Table 1

Growth of different fungi on potato dextrose agar in the presence and in the absence (control) of anthocyanin extract of purple corn

Fungal strains	Inhibition (%)
Botrytis cinerea (strain 7001)	12.3
Botrytis cinerea (strain 8003)	6.8
Fusarium culmorum	10.6

Calculating the inhibition of the 7,5% ethanol free crude extracts on the complex medium, a moderate (7% – 12%) inhibition of purple corn extract could be detected for the growth of *Botrytis cinerea* and *Fusarium culmorum*.

CONCLUSIONS

Purple corn is a pigmented variety of *Zea mays* L. In Peru and Bolivia, a traditional drink, to which beneficial effects for health are attributed, is prepared cooking the corn with spices. In the plant, the anthocyanins appear in epidermal cells, where it is believed to have a protective function against UV-B radiation (Escribano-Bailón et al., 2004). Hammerschmidt and Nicholson (1977) showed the importance of anthocyanins in the resistance of the maize to anthrachnose. Our results also supported the antifungal effect of purple corn extract toward different plant pathogen fungi. Further studies necessary to test the possibilities of application the extract in plant protection.

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