# Suppression of the zearalenone production, sporulation, and pigmentation in *Fusarium culmorum* by 6-demethylmevinolin, an inhibitor of the aflatoxin B1 biosynthesis

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## INTRODUCTION

*Fusarium* fungi are common for many world regions. They infect many economically important crops and actively reproduced on the grain and other products during storage. *Fusarium* fungi produce some dangerous mycotoxins including zearalenone (ZEN), which pose a serious threat for humans and animals.

ZEN is one of the most economically important and widespread toxins. Being produced via the polyketide biosynthetic pathway, it represents a macrocyclic lactone of β-resorcylic acid (Fig. 1). It is able to impair functioning of a reproductive and endocrine systems of the organism, as well as to suppress the immunity.

ZEN possesses high thermal and UV resistance and can be degraded only by strong alkalis or oxidants havin very limited use for the treatment of food and feed products. Therefore, non-toxic natural compounds able to inhibit the ZEN biosynthesis may be of great demand in the food and agricultural industries.

Earlier we showed that 6-demethylmevinolin (6-DMM, **Fig. 1**) produced by *Penicillium citrinum* is able to almost completely suppress biosynthesis of aflatoxin B1, another polyketide mycotoxin, even at low concentrations, which do not suppress the growth of its producer [1]. The purpose of this study was the evaluation of the 6-DMM ability to inhibit the ZEN biosynthesis in one of its producing species, *F. culmorum*.

## MATERIALS AND METHODS

Lactone form of 6-DMM was obtained using an overproducing *Penicillum citrinum* 18–12 strain developed by a multi-step induced mutagenesis. The strain cultivation and 6-DMM isolation and purification were carried out as described earlier [1].

Submerged culture of a toxigenic *Fusarium culmorum* strain M-01-55/3 was grown in 250-mL flasks with 50 mL of a liquid Myro medium. 6-DMM diluted in a minimal volume of ethanol was added to the sterilized nutrient medium in three different concentrations (20, 50, and 100 μg/mL) after its autoclaving and prior inoculation with the conidial suspension of the fungus. Inoculated flasks were incubated on a shaker for 8 days at 25ºC and 250 rpm. All concentration variants were arranged in three replications.

ZEN production by the fungus was determined by RP-HPLC.The effect of 6-DMM on the mycelium pigmentation in a toxin-producing fungus was assessed by the strain cultivation on both agarized and liquid media. The effect of 6-DMM on the spore formation process was estimated by a microscopic examination of fungal colonies grown on agarized medium supplemented with 6-DMM.

The statistical treatment of data was carried out using a Statistica 6.0 software package. The significance of differences ($p < 0.05$) of the means between the experimental and control values were determined using a *t*-test for the independent variables.

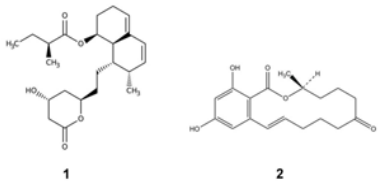


**FIGURE 1. Structural formules of (1) 6-demethylmevinolin and (2) zearalenone.**

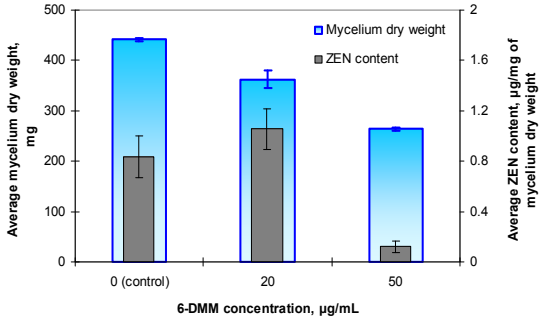


**FIGURE 2. Effect of 6-DMM on the mycelium growth an ZEN production in *F. culmorum* M-01-55/3.**

## RESULTS

The results of the evaluation of a 6-DMM effect on the mycelium growth and ZEN production in F. culmorum M-01-55/3 are shown in **Fig. 2**. As the concentration of 6-DMM increases, a fungal growth suppression remains significantly lower than the inhibition of ZEN production. Thus, 6-DMM inhibits the toxin biosynthesis more specifically than the growth of a fungal mycelium. 6-DMM (20 μg/mL) did not influence on the ZEN biosynthesis, though reduced the biomass of a producer by 26% comparing to the control. At 50 μg/mL of 6-DMM, ZEN production by the M-01-55/3 strain was inhibited by 93.0±1.1% on average, though the growth of a producer was suppressed only by 40%.

The 6-DMM presence (8 μg/mL) in the agarized nutrient medium resulted in a clearly visible discoloration of the mycelium (**Fig. 3**). This discoloration was also observed at higher 6-DMM concentrations. In the case of the strain cultivation in a liquid medium, a small discoloration was observed at a 6-DMM concentration of 50 μg/mL, while a complete discoloration was registered at a concentration of 100 μg/mL (**Fig. 4**).

No any presence of spores was observed in colonies grown on the agarized medium containing 20 μg/mL of 6-DMM or higher, whereas an abundant spore formation was observed in the control.

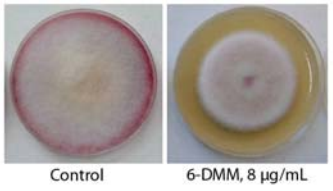


**FIGURE 3. Effect of 6-ДММ on the mycelium pigmentation in a *Fusarium culmorum* M-01-55/3 cultivated on agarized medium.**

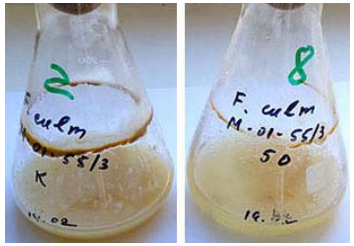

**FIGURE 4. 6-DMM-induced discoloration of the *F. culmorum* M-01-55/3 mycelium in a liquid culture. Left: control (no 6-DMM); right: 6-DMM (50 μg/mL).**

## CONCLUSIONS

**6-DMM, able to inhibit the early stages of the biosynthesis of polyketides including aflatoxin B1, was shown to be able to successfully inhibit the biosynthesis of ZEN, as well as to suppress sporulation and biosynthesis of pigments in *F. culmorum*.**

**The analysis of available publications and patents did not show any information relating to the above-described properties of the studied compound. Therefore, the obtained results are new and evidence a rather wide range of the 6-DMM activity in relation to the biosynthesis of different types of mycotoxins that makes this compound to be promising for the practical use to prevent mycotoxin accumulation in food and feed products.**

## ACKNOWLEDGMENTS

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## REFERENCES

1.Dzhavakhiya V.G., Voinova T.M., Popletaeva S.B., et al. *Toxins* 8, 313 (2016).