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Research Note

∝-Difluoromethylornithine Inhibits the Growth of Fungus *Macrophomina phaseoli*

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Abstract: ∝-Difluoromethylornithine (DFMO), a specific enzyme activated inhibitor of ornithine decarboxylase (ODC), significantly inhibited the mycelial growth of the fungus *Macrophomina phaseoli* (Tassi) Goidanich. Putrescine (Put), when added to the nutrient medium at a concentration of 0.25 mM, decreased the inhibitory effect of DFMO. These results suggest that polyamines (PAs) are essential for the growth of fungi and that DFMO is applicable to the alleviation or prevention of crop losses due to phytopathogenic fungi.

Key Words: *Macrophomina phaseoli*, polyamines, fungi growth, ∝-Difluoromethylornithine

∝-Difluorometilornitinin *Macrophomina phaseoli* Mantarının Büyümesine Ket Vurması

Özet: Ornitin dekarboksilaz (ODC) enziminin spesifik inhibitörü ∝-difluorometilornitinin (DFMO) 0,1, 1,0 ve 2,0 mM konsantrasyonlarda *Macrophomina phaseoli* (Tassi) Goidanich mantarında misel büyümesini kayda değer bir şekilde inhibe ettiği saptandı. Besleyici ortama 0,25 mM konsantrasyonda putresin (Put) ilave edildiğinde ise DFMO nun ket vurucu etkisinin azaldığı belirlendi. Bu bulgular sonucunda poliaminlerin mantar büyümesinde esas işleve sahip olduğu ve fitopatojenik mantarların neden olduğu ürün kaybının önlenmesi amacıyla DFMO nun kullanabilirliği ortaya çıkmış olmaktadır.

Anahtar Sözcükler: Macrophomina phaseoli, poliaminler, mantar büyümesi, ∝-Difluorometilornitin

Introduction

It has been established that polyamines (PAs) are essential for the growth and development of bacteria, fungi (Tabor and Tabor, 1985) higher plants (Palavan and Galston, 1982; Slocum et al., 1984) and mammals (Metcalf et al., 1978). A diamine putrescine (Put), the precursor of PAs, arises either directly by decarboxylation of L-ornithine in a reaction catalyzed by ornithine decarboxylase (ODC) and/or by decarboxylation of L-arginine by arginine decarboxylase (ADC), leading to the formation of Put through agmatine and N-carbamoylputrescine intermediates. In most fungi and mammals (Tabor and Tabor, 1985), the first pathway is the only route for Put synthesis. Difluoromethylarginine (DFMA) and difluoromethylornithine (DFMO), which specifically and irreversibly block ADC and ODC (Metcalf et al., 1978; Kallio et al., 1981) respectively, have been used to specify the initial route of Put biosynthesis in many organisms. Since the PAs spermidine (Spd) and spermine (Spm) are formed from Put, effective blockage of Put formation can deprive the organisms of these PAs. These observations revealed the possibility that specific inhibition of fungal ODC with DFMO might inhibit spore germination and the spread of the infection, without significantly affecting PA biosynthesis in the host plant tissues which can use the uninhibited ADC pathway. Thus Rajam and Galston (1985) have reported that 0.5 M DFMO supplied to cultures of phytopathogenic fungi on synthetic medium resulted in a marked inhibition of growth and that such inhibition was preventable by Put or Spd. Later, Birecka et al. (1986) confirmed this observation in *Helminthosporium maydis* (Nisikado and Miyake), showing that Put but not cadaverine fully prevented the inhibitory effect of DFMO. Subsequently, Rajam et al. (1985) applied DFMO spray to bean leaves infected with uredospores of *Uromyces phaseoli* (Pers.) G. Winter, which led to partial or complete protection against infection, depending on the inhibitor concentration and timing.

Recently, Walters and Robins (1994) described new Put analogues (E-BED and E-TED) that were effective in preventing fungal infection. For sometime we have tested the DFMO effects on the growth of 10 different phytopathogenic fungi (*Phytophora infestans* (Mont.) de Bary., *Rhizoctonia solani* Kühn, *Botrytis cinerea* Per.: Fr., *Macrophomina phaseoli* (Tassi) Goidanich, *Fusarium oxysporum* Schl.: Fr., *Drechslera sorokiniana* (Sacc.) Subramanian and P.C.Jain) which are important for Turkish agriculture. The ODC inhibitor was effective in the case of *Botrytis cinerea*, *Drechslera sorokiniana* and *Macrophomina phaseoli* (Palavan-Ünsal et al., 1994). Since the latter fungus had not been investigated in detail previously it became the focus of the experiments reported here.

Materials and Methods

Cultures of *Macrophomina phaseoli* were kindly provided by Ege University, Agricultural Faculty, Department of Plant Protection. These cultures were maintained in 2% potato dextrose agar (PDA) medium. Inhibitor and Put solutions were added to the sterile media to final concentrations of 0.1, 1.0 and 2.0 mM for DFMO and 0.25 mM for Put. The media were dispensed aseptically into mm diameter sterile glass petri dishes (20 ml per dish) and allowed to solidify. Control petri dishes contained only culture medium and sterile distilled water with neither inhibitor nor Put. Each petri dish was inoculated at its centre with a plug of mycellium 7 mm in diameter. The replicates used for each concentration were incubated at 24°C in the dark and colony diameters in cm were measured at 48, 72, and 96 hours after inoculation along two diameters at right angles to one another.

Results and Discussion

Figure 1 shows the effect of DFMO on the mycelial growth of *Macrophomina phaseoli*. DFMO at concentrations of 0.1, 1.0 and 2.0 mM inhibited the growth of this fungus significantly. The inhibitory effect of DFMO was more pronounced at concentrations of 1.0 and 2.0 mM. These inhibition ratios were higher during the first 48 and 72 hours compared to 96 hours after inoculation. Concentrations of 1.0 and 2.0 mM DFMO inhibited the mycelial growth 68 and 86% respectively compared to the control conditions 48 h after inoculation. These inhibition percentages were decreased gradually towards 96 h after inoculation. Inhibition ratios of 1.0 and 2.0 mM DFMO were 42 and 56% respectively compared to the control condition 96 h after inoculation.

A diamine Put concentration of 0.25 mM did not stimulate the mycelial growth significantly in *Macrophomina phaseoli* (Figure 2) at all tested inoculation times (48, 72 and 96 h). Similarly, Birecka et al. (1986) reported that Put alone at 0.25 mM had no significant effect on fungal growth in *Helminthosporium maydis*, but 0.25 mM Put applied with 0.1 mM DFMO have an inhibitory effect on mycelial growth 48 h after inoculation. The inhibitory effect of DFMO (0.1 mM) on mycelial growth increased when applied together with Put, but we did not obtain the same results 96 h after inoculation. On the other hand, the inhibitory effect of 1.0 mM DFMO with 0.25 mM Put on mycelial growth increased compared with 48 h after inoculation and this stimulation was determined at 96 h after inoculation also. We established the same profile for 0.25 mM Put plus 2.0 mM DFMO application on mycelial growth. In summary, we observed reversion effects of Put on DFMO

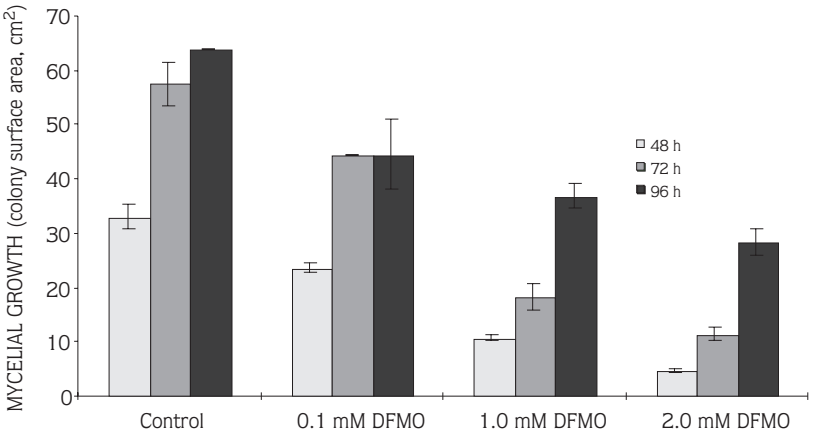

Figure 1. The effect of ∝-Difluoromethylornithine on growth of *Macrophomina phaseoli*. Control: Distilled water. Values are average of 6 replicates.

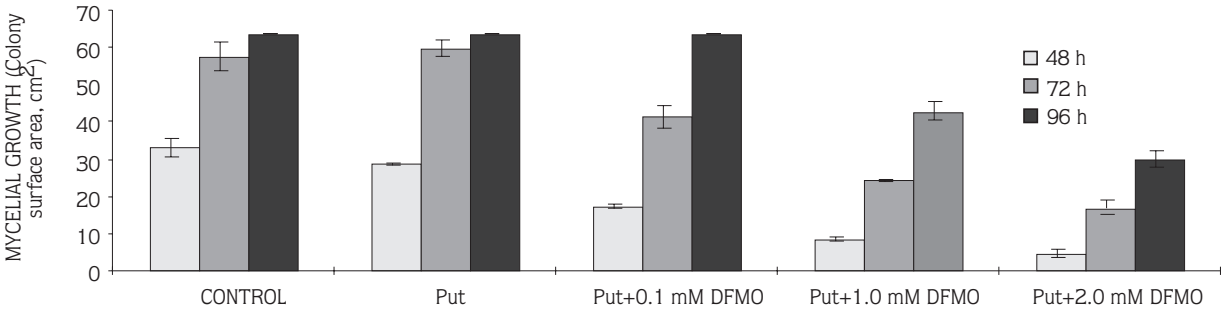

Figure 2. The effect of putrescine (Put: 0.25 mM) and ∝-Difluoromethylornithine on growth of *Macrophomina phaseoli*. Control: Distilled water. Values are average of 6 replicates.

inhibition in mycelial growth only after 96 h after inoculation and not before.

Some reports on the inhibitory effects of DFMO on different fungi (Rajam and Galston, 1985; Rajam et al., 1985; Palavan-Ünsal et al., 1994) showed significant differences between genera in their sensitivity to this inhibitor. These genus-dependent differences in sensitivity to DFMO may be due to the differences between genera. In the application of Put differences may also be caused by the different sensitivity in *Macrophomina phaseoli*.

The present findings show that specific and irreversible inhibitors of the PA biosynthetic enzyme ODC can inhibit the mycelial growth and that such inhibition might be decreased by exogenously applied PAs. This indicates that the mechanism of inhibition is due to the prevention of PA biosynthesis in spores.

According to Rajam and Galston (1985), DFMO sprays were effective in growth inhibition of *Uromyces phaseoli* also. The effective DFMO concentration (0.5 mM) produced no abnormal symptom on the host plants and were also able to protect the unsprayed parts of the plant.

These results suggest that DFMO and other inhibitors of enzymes in the PA biosynthetic pathway may prove to be applicable to the alleviation or prevention of crop losses due to phytopathogenic fungi.

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