Antimicrobial Activity of Catechol and Pyrogallol as Allelochemicals

Ismail Kocaçalışkan[a,*], Ismet Talan[b], and Irfan Terzi[c]

[a] Department of Biology, Faculty of Arts and Sciences, Dumlupınar University, Kütahya, Turkey. Fax: (+90) 2742652056. E-mail: ismailkc@dumlupinar.edu.tr
[b] Institute of Science and Technology, Dumlupınar University, Kütahya, Turkey
[c] Department of Biology, Faculty of Education, Dumlupınar University, Kütahya, Turkey

* Author for correspondence and reprint requests

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Catechol and pyrogallol are allelochemicals which belong to phenolic compounds synthesized in plants. Their antimicrobial activities were investigated on three bacteria (*Pseudomonas putida, Pseudomonas pyocyanea, Corynebacterium xerosis*) and two fungi (*Fusarium oxysporum, Penicillium italicum*) phytopathogenic species as test organisms using the disc diffusion method. Both catechol and pyrogallol were found to have antibacterial effects on all the bacteria used in the study at 5 and 10 mm concentrations. Catechol has also been found to have an antifungal effect on the fungi used in the study, whereas no antifungal effects of pyrogallol were observed. The most sensitive species among the bacteria was *P. putida* which was inhibited by the allelochemicals even at 1 mm concentration.

Key words: Allelochemical, Antimicrobial Activity, Catechol, Pyrogallol

Introduction

Synthetic chemicals commonly used to control plant diseases not only pollute the environment but they are also harmful to human health. Thus there has developed a world-wide interest in searching for antimicrobial activity of natural plant compounds. Especially allelochemicals have been emphasized recently, because natural compounds have a short half-life since they are biodegradable, and therefore are considered environmentally and toxicologically safer than synthetic compounds (Inderjit *et al.*, 1999; Vyvyan, 2002; Macias *et al.*, 2004).

Allelochemicals which have a special emphasis as natural compounds are major agents of allelopathy in nature. The chemical interactions that occur among living organisms including plants, insects and microorganisms are called allelopathy, and the organic compounds involved in allelopathy are called allelochemicals. Sometimes an allelochemical produced by one organism is harmful to another and beneficial to a third organism; but they are generally toxic and cause stress and even dead (Rice, 1979; Rizvi and Rizvi, 1992; Kocaçalışkan and Terzi, 2001).

Phenolic allelochemicals are believed to function as defensive agents against invading microbes and as signal molecules in plant interactions with pathogens (Inderjit *et al.*, 1999). For example, the allelochemicals catechol and pyrogallol belonging to phenolic compounds are synthesized by the shikimate pathway in plants. Catechol has been isolated by Kuiters and Sarink (1986) from leaf and needle litter of several deciduous (*e.g.*, beech, birch, oak, hazelnut, maple, willow, poplar) and coniferous trees (*e.g.*, spruce-fir, Douglas-fir, larch), and there is also an indication that it is synthesized abundantly in onions and released by their outer layer cells (Farkas and Kiraly, 1962). On the other hand, pyrogallol is an important degradation product of plant debris/litter. In addition, both catechol and pyrogallol are the most common polyphenols in soils, and they are often precursors in synthesis of humic-like polymers (Inderjit *et al.*, 1999).

Catechol has been shown to have antifungal effects on *Colletotrichum circinans* fungus (Farkas and Kiraly, 1962). However, we have not encountered any reports about neither antibacterial effect of catechol nor antibacterial and antifungal effects of pyrogallol on phytopatohgenic bacteria and fungi. Therefore, we studied the antibacterial and antifungal effects of catechol and pyrogallol against three bacteria and two fungi species, *Pseudomonas putida, Pseudomonas pyocyanea, Corynebacterium xerosis, Fusarium oxysporum, Penicillium italicum*, which are common plant pathogens in soil (Leloğlu, 1973; Rizvi and Rizvi, 1992; Öner, 2002).

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Materials and Methods

For testing antimicrobial activity of catechol and pyrogallol, three bacteria (*Pseudomonas putida, Pseudomonas pyocyanea, Corynebacterium xerosis*) and two fungi (*Fusarium oxysporum, Penicillium italicum*) were chosen as test organisms. All the organisms used in the study, which are phytopathogenic soil microorganisms, were obtained from Microbiology Laboratory of Science Faculty of Uludağ University, Bursa, Turkey.

The bacteria were cultured on sterilized agar medium in Petri plates (prepared as 3.6% nutrient agar, pH 7). The inoculation was carried out by a sterile swab. Fungi were cultured on sterilized agar medium in Petri plates (prepared in 3:6:1 ratios as agar, malt extract and peptone, pH 6), and similarly inoculated by a sterile swab.

Solutions of catechol and pyrogallol allelochemicals were prepared as 1, 5 and 10 mM concentrations. Disc diffusion method was used to apply the allelochemicals. 0.1 ml of each allelochemical solution was deposited on a sterile paper disc (6.4 mm in diameter) and the discs were dried in an incubator at 50 °C. Then the discs were placed on the surface of Petri plates containing bacteria or fungi. Each plate contained two discs, one containing the allelochemical and the other containing 30 μg amicacin antibiotic (supplied by the microbiology laboratory of the State Hospital, Sakarya Turkey) for comparison. The plates of bacteria were incubated at 37 °C for 24 h; the plates of fungi were incubated at 27 °C for 5 d. After incubation periods, antimicrobial activities of the allelochemicals were determined by measuring the diameter of inhibition zones (DIZ) around each disc by an inhibition zone reader and recorded in mm. The experiments were repeated three times. Microorganisms were assumed as sensitive to an allelochemical if the DIZ was larger than 17 mm, less sensitive if the DIZ was between 14–17 mm, and resistant if the DIZ was smaller than 14 mm (Çetin, 1973).

Results and Discussion

In this study, catechol was found to have both antibacterial and antifungal activities whereas pyrogallol had only antibacterial but no antifungal activity (Table I). As seen in Table I, generally, both allelochemicals were not effective at 1 mM concentration with the exception of *P. putida*. However, the allelochemicals were found as effective as amicacin antibiotic against the bacteria at 10 mM. In some cases, 5 mM concentration of the allelochemicals was nearly as effective as 10 mM. The most sensitive species among the bacteria was *P. putida* which was affected by the allelochemicals even at 1 mM concentration. However, the most resistant bacterial species was *P. pyocyanea* whose DIZ was zero at 1 mM of both allelochemicals. On the other hand, the second bacterial species in sensitivity to the allelochemicals was *C. xerosis.*

The largest inhibition zone was recorded for *P. putida* with 31.3 mm DIZ by the activity of 10 mM catechol. Antifungal activity of catechol was also observed. That is, both *F. oxysporum* and *P. italicum* were found sensitive to catechol at 5 and 10 mM concentrations. However, the fungal species were found resistant to pyrogallol even at 10 mM

Table I. Antimicrobial activities of catechol and pyrogallol allelochemicals against three bacteria and two fungi species determined by the disc diffusion method. Values in the table are diameter of inhibition zones, DIZ (mm), as mean of three replicates.

Treatments		Bacteria			Fungi	
		Pseudomonas putida	*Pseudomonas pyocyanea*	*Corynebacterium xerosis*	*Fusarium oxysporum*	*Penicillium italicum*
Catechol	1 mM	16.0⁺ ± 1.0	0.0⁻ ± 0.0	11.6⁻ ± 1.5	0.0⁻ ± 0.0	0.0⁻ ± 0.0
	5 mM	27.3* ± 1.2	15.3⁺ ± 0.6	20.0* ± 1.0	17.3* ± 1.2	22.5* ± 1.5
	10 mM	31.3* ± 0.6	20.6* ± 2.9	21.6* ± 1.5	29.8* ± 1.0	26.5* ± 1.5
Pyrogallol	1 mM	15.3⁺ ± 2.1	0.0⁻ ± 0.0	9.6⁻ ± 1.2	0.0⁻ ± 0.0	0.0⁻ ± 0.0
	5 mM	22.3* ± 2.1	15.5⁺ ± 1.5	15.6⁺ ± 2.1	7.8⁻ ± 0.8	6.8⁻ ± 0.3
	10 mM	26.0* ± 0.0	20.0* ± 1.0	27.3* ± 0.6	12.6⁻ ± 0.6	11.0⁻ ± 1.7
Amicacin	30 μg	30.0* ± 1.0	26.3* ± 0.6	25.3* ± 0.6	ND	ND

* Sensitive (DIZ above 17 mm). ⁺ Less sensitive (DIZ between 14–17 mm). ⁻ Resistant (DIZ below 14 mm) (Çetin, 1973). ND, not determined. ±, Standard deviation from the mean.

concentration. The largest inhibition zone in *F. oxysporum* fungi was found with 29.8 mm DIZ by the activity of 10 mM catechol. At 10 mM catechol, the inhibition zone obtained for *P. italicum* was 26.5 mm. Inhibition zones of both fungi were zero at 1 mM concentration of catechol and pyrogallol.

Takahama (1997) reported that dopa was synthesized abundantly in broad bean leaves as a phenolic allelochemical. We have tested the activity of dopa against the microorganisms used in our present study; however, no significant inhibitory activity was observed at the concentration of 10 mM (data not shown).

Bacteria of *P. putida* and *P. pyocyanea* are Gram-negative but *C. xerosis* is Gram-positive (Leloğlu, 1973). This indicates that the allelochemicals used in the study have inhibitory effects on both Gram-positive and Gram-negative bacteria.

All the bacteria and fungi tested in this study are phytopathogenic microorganisms. For example, *Pseudomonas* causes withering disease in tomato plants, and *Corynebacterium* causes ring spot disease in several plants such as tomato and potato. *Fusarium oxysporum* causes wilt disease in several plants such as cotton, melon, water melon and cucumber. *Penicillium italicum* causes blue mold rot disease in citrus plants (Leloğlu, 1973; Rizvi and Rizvi, 1992; Öner, 2002).

Allelochemicals may be preferred in controlling plant diseases mainly from point of environmental protection, because they are friendly to the environment since their degradability is easy as compared to synthetic chemicals in nature. For example, catechol has been shown to be polymerized rapidly in soil by enzymatic reaction or autooxidation (Martin *et al.*, 1979). Also, it has been found that 30% of catechol was degraded to CO_2 after six months in soil under laboratory conditions (Cheng *et al.*, 1983).

So far, some allelochemicals have been shown to be effective in inhibiting growth of microorganisms. For example, juglone which is produced by walnut trees was found active against a broad spectrum of microorganisms, including bacteria and fungi (Krajci and Lynch, 1978; Dawson and Seymour, 1983; Clark *et al.*, 1990). Rhein which is the most active compound of rhubarb roots was found to have strong antibacterial capacity (Didry *et al.*, 1994). Allelochemicals are, generally, tested against clinically important microorganisms; however, their effects on phytopathogenic microorganisms are not investigated in detail.

As conclusion, the results indicate that catechol may be used in controlling both bacterial and fungal diseases but pyrogallol may be effective against only bacterial diseases. Therefore, it is suggested that catechol and pyrogallol may be important components in disease management caused by bacterial or fungal species used in this study and reduce the dependence on synthetic chemicals.

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