EFFECT OF SPICE EXTRACTS ON THE GROWTH OF *PENICILLIUM* SPECIES

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ABSTRACT

The inhibitory effect of various concentrations (0, 0.1, 0.5, 1 and 2%) of caraway, garlic and oregano extracts on growth of four species of *Penicillium* were investigated. Among the spices used, caraway showed the strongest effect on all moulds. It completely inhibited growth of *P. aurantiogriseum* at the level of 0.5% extract and *P. commune, P. griseofulvum* and *P. corylophilum* at the level of 1%. The garlic extract completely inhibited the growth only of *P. aurantiogriseum*, but was ineffective against *P. commune* and *P. griseofulvum*. Oregano showed partial effect in inhibition of all four species, with significant growth reduction at concentration of 2%.

1. INTRODUCTION

Aromatic plants (either fresh or dried) have been widely used for flavouring of various foods. Essential oils and extracts obtained from natural aromatic plants have been gaining increasing attention in food industry due to their tremendous potential in the prevention of microbial spoilage of food and as an alternative to synthetic preservatives. It has been confirmed that caraway, cloves, mustard, coriander, thymus, and cinnamon are effective antimicrobial agents which inhibit fungal growth (Karapiran, 1985; Soliman and Badeaa, 2002; Suhr and Nielsen, 2003; Velluty et al., 2003). Moreover, many studies revealed that some spices do not exert antifungal activity, e.g., vanila (Nielsen and Rios, 2000) whereas some have stimulative effect (Mabrouk and El-Shayeb, 1980; Ozcan, 1998; Boyraz and Ozcan, 2005).

Essential oils are complex and consist of many components. Their active constituents such as eugenol, thymol, cinnamaldehyde, cariofilen, geraniol, sulphuric and other compounds have been subjected to extensive research (Bullerman et al., 1977; Hitokoto et al., 1980; Moleyar and Narasimham, 1986; Mahmound, 1994; Mansour et al., 1996; Matamoros-Leon et al., 1999; Moriera et al., 2005). Guynot et al. (2003) reported about the preventive activity of essential oils against fungi through contact with volatiles at various water activity levels. Authors found that five out of total 16 essential oils completely inhibited the growth of all tested fungi over a wide range of water activities (0.80-0.90 aw). Somewhat earlier, Nielsen and Rios (2000) demonstrated that mustard essential oil and its major constituent allyl isothiocyanate (AITC) can be successfully used to prevent fungal growth. Fungi are responsible for the development of offensive odour and production of cytotoxic, carcinogenic and allergenic compounds. They prevail more frequently than other microorganisms on medium or low humid, acidulous food which has been inadequately stored.

The objective of this work was to examine the inhibitory potential of extracts of caraway, garlic and oregano against four fungal species from genus *Penicillium* which have been frequently encountered in food and recognized as food spoilage agents. *P. aurantiogriseum, P. griseofulvum* and *P. commune* are known producers of many toxic

metabolites (Frisvad and Thrane, 2004) whereas *P.corylophilum* is the most xerophilic of all *Penicillium* species (Pitt and Hocking, 1985) but its toxigenic activity has not been affirmed (Singh et al., 1991).

2. MATERIALS AND METHODS

2.1. Materials

Extracts of caraway, garlic and oregano used in this study were commercially available products procured from ETOL Celje Comp., Slovenia. Test microorganisms *P. commune, P. griseofulvum, P. aurantiogriseum* and *P. corylophilum* were maintained on Potato dextrose agar (PDA) slants at 4°C. For the experiment, the microorganisms were cultivated on the same medium for 10 days at 25°C.

2.2. Antifungal assessment

Determination of antifungal activity was conducted on PDA medium. The test medium was poured into 250 ml Erlenmeyer flasks and autoclaved at 121°C for 15 min. After sterilization, spice extracts were separately added to the flasks to obtain concentrations: 0, 0.1, 0.5, 1 and 2%. The culture medium was then poured into sterile Petri plates (9 cm diameter) in equal volumes (12 ml per plate).
Suspension of conidial spores was prepared in a medium that contained 0.5% Tween 80 and 0.5% agar in distilled water (Nielsen and Rios, 2000). The concentration of suspension was adjusted to 10^6 spores/ml using a haemocytometer. For each tested extract and concentration, triplicate plates were centrally inoculated by spreading 1 µl of spore suspension (10^3 spores/ml), including a control samples, too.
The growth inhibition was evaluated by daily measurement of radial growth of colonies during the days of incubation at 25°C.

3. RESULTS AND DISCUSSION

Inhibitory effect of all spice extracts against the tested *Penicillium* species are displayed in Table 1. The extract of caraway completely inhibited the growth of *P. aurantiogrisum* at 0.5% dose. At the same dose, the growth of *P. griseofulvum* was almost completely inhibited (91.3%) whereas significant inhibitory effects were observed with *P. commune* and *P. coryphilum* (50.0 and 73.1%, respectively). At 1% dose, the growth of all tested microorganisms was inhibited.
The extract of garlic was totally inefficient against *P.commune*, it even exhibited a growth stimulating effect within the tested doses. Also, the highest doses applied (1 and 2%) did not show any activity against *P. griseofulvum* whereas *P. corylophilum* was partially inhibited (34.6 and 53.8%). Solely *P. aurantiogriseum* was completely inhibited by the garlic extract.

The lowest applied dose of oregano extract was found inefficient against *P. griseofulvum, P. aurantiogriseum* and *P. corylophilum* with weakly inhibitory effect against *P. commune*. The next dose (0.5%) was also actually inefficient against *P. commune*, but it reduced the growth of other tested species by 13.6% (*P. griseofulvum*), 14.8% (*P. aurantiogriseum*) and 7.7% (*P. corylophilum*). Although the oregano extract did not exhibit complete

inhibitory effect against any of the tested species, a 2% dose had strong activity which was maximal (85.2%) against *P. aurantiogriseum* and minimal (50%) against *P. commune*.

Table 1. Antifungal activity of spice extracts on the growth of moulds

Extract	Conc. (% v/v)	Inhibition colony growth (%)			
		P. commune	*P. griseofulvum*	*P. aurantiogriseum*	*P. corylophilum*
Caraway	0.1	10.7	19.6	33.3	19.2
	0.5	50	91.3	100	73.1
	1	100	100	100	100
	2	100	100	100	100
Garlic	0.1	0	0	7.4	15.4
	0.5	0*	0	25.9	23.1
	1	0*	2.3	100	34.6
	2	0*	2.3	100	53.8
Oregano	0.1	11.1	0	0	0
	0.5	13.0	13.6	14.8	7.7
	1	29.6	68.2	40.7	38.5
	2	50	83.6	85.2	84.6

0* -stimulation colony growth

The effects of caraway, garlic and oregano on germination and growth rate of fungi is presented in Figures 1, 2 and 3.

The growth rate of *P. aurantiogriseum* was more markedly reduced in the presence of caraway extract indicating higher sensitivity of the species. Low extract concentration (0.5%) delayed the colony growth of *P. commune* for 4 days, *P. corylophilum* for 6 days and *P. griseofulvum* for 7 days but it was found inefficient in the case of other species (Fig. 1).

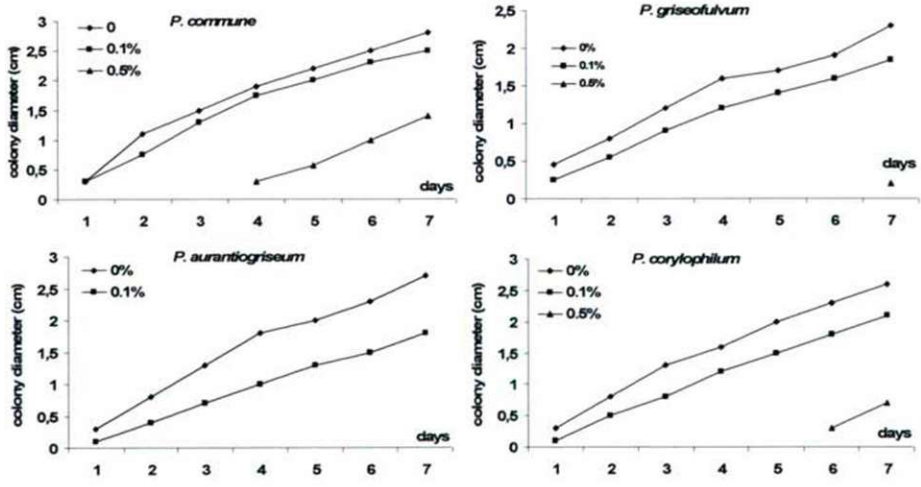

Fig. 1. Inhibition of Penicillium spp. by caraway extract

In all treatments with the garlic extract, the period of colony growth delay for *P. commune* and *P. griseofulvum* was virtually the same as for the control. Garlic favoured the growth of these fungal species. Moreover, this effect was intensified with increasing extract

concentration in the case of *P. commune*. At 0.5% concentration, *P. aurantiogriseum* and *P. corylophilum* were completely inhibited for 2 days. At higher concentrations, the growth of *P. corylophilum* was delayed for one more day (Fig. 2).

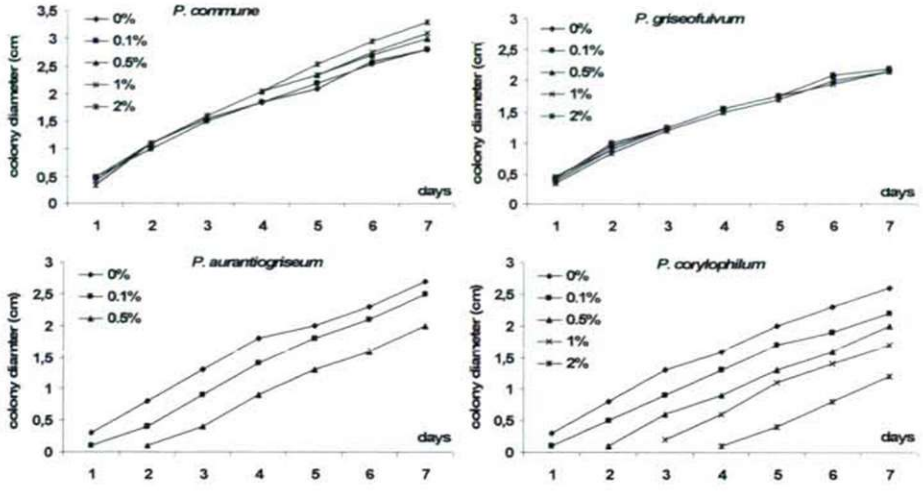

Fig. 2. Inhibition of Penicillium spp. by garlic extract

The treatments with oregano extracts revealed a growth delay for *P. aurantiogriseum* and *P. corylophilum* at concentrations over 0.5% for the same number of days. The colony growth continued with similar activity over the further experimental period. The inhibitory effect on the growth of *P. griseofulvum* at 2% concentration remained constant until the fifth day of the experiment after which it was gradually diminished (Fig. 3).

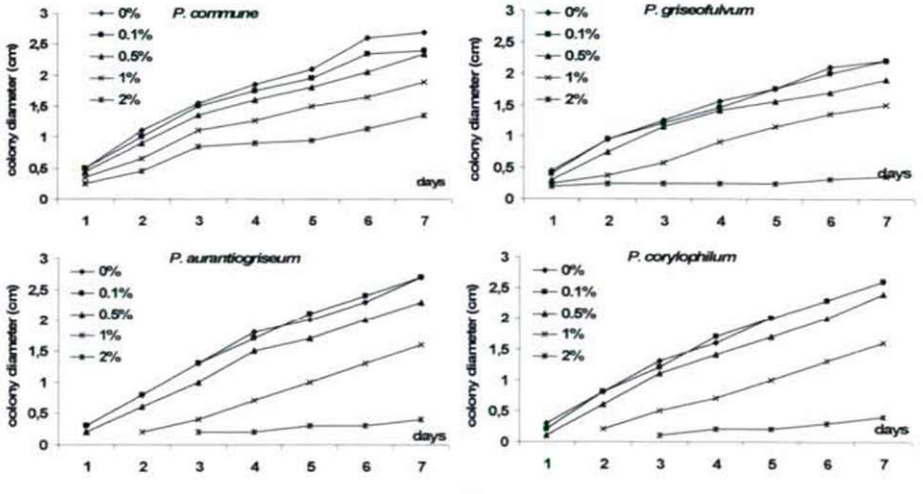

Fig. 3. Inhibition of Penicillium spp. by oregano extract

The experimental results demonstrated that solely the caraway extract had the ability to completely inhibit the growth of all tested *Penicillium* species. The garlic extract showed a

total inhibitory effect on *P. aurantiogriseum* and partial on *P. corylophilum* whereas it was ineffective against the other two species. The oregano extract failed to inhibit the germination of the tested fungal species; however, it exerted a strong antifungal activity at the highest concentration. The most sensitive species to the effect of spice extracts was *P. auratiogriseum*. The increasing concentrations of extracts caused an absence or growth delay of fungal species and showed various effects on the rate of fungal growth that ranged from inhibitory to stimulating.

The antifungal activity of the tested extracts is associated with their major constituents such as carvacrol (caraway, oregano), limonene (caraway) and sulphuric compounds (garlic) (Barrata et al., 1998; Ceylon and Fung, 2004).

It has been reported that essential oils of caraway, clove, garlic, onion and oregano might prevent the synthesis of sterigmatocystine, aflatoxins and ochratoxin A produced by some toxigenic fingi (Hitokoto et al., 1980; Hasan and Mahmoud, 1993; Basilico and Basilico, 1999). Benkeblia (2004) concluded that EO extract from garlic behaved different to that of red onion by showing strong inhibitory effect against *P. cyclopium* at lower doses (50 and 100 ml/l). Moreover, micromorphological changes in fungi have been documented after exposure to volatile essential oils of some spices (Arras and Usai, 2001; Rassoli et al., 2006).

3. CONCLUSION

The current results confirmed that the shelf-life of food can be ensured with natural antimicrobial agents such as spice extracts. Their application can contribute to lowering the use of synthetic preservatives commonly used to limit the fungal growth. *Penicillium* species are the most important contaminants of stored food commodities and present a considerable health risk.

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