APTEFF, 40, 1-220 (2009)
DOI: 10.2298/APT0940009D
UDC: 664.5:66.061.3:582.28
BIBLID: 1450-7188 (2009) 40, 9-16
Original scientific paper

ANTIMICROBIAL ACTIVITY OF CARAWAY, GARLIC AND OREGANO EXTRACTS AGAINST FILAMENTOUS MOULDS

Gordana R. Dimić, Sunčica D. Kocić-Tanackov, Dušanka J. Pejin, Jelena D. Pejin, Ilija J. Tanackov and Danijela Tuco

Inhibitory effect of caraway, garlic and oregano extracts (0.07, 0.1, 0.5, 1 and 2%), against four moulds species was investigated. The caraway extract had the strongest inhibitory effect by inhibiting the germination of Emericella nidulans, Penicillium commune and P. implicatum at the concentration of 0.1% and Aspergillus tamarii at the concentration of 0.5% during 7 days of incubation at 25°C. The extract of garlic only partially inhibited the growth of A. tamarii and P. commune. However, it inhibited completely the growth of P. implicatum and E. nidulans at the doses of 0.5 and 1%. Oregano partially inhibited all mould species, significantly reducing the growth of colonies, especially of E. nidulans (93.3%).

KEY WORDS: Spice extracts, antifungal activity

INTRODUCTION

Moulds highly prevail in nature and frequently contaminate human food. Some of them produce secondary metabolites such as aflatoxins, ochratoxin A, stergmatocystine, which are cytotoxic and carcinogenic, and as such present a potential health hazard for humans (1). Medium moisture food (0.75-0.90 a_w), low moisture food (< 0.75 a_w) and sour food are especially susceptible to the presence of moulds. The development of moulds on food can be expected in cases when inappropriate sanitary practice is applied in production plants. Moulds occur more frequently than other microorganisms on food products during storage and distribution as a consequence of inadequate conditions.

Essential oils extracted from spices and other herbs, as well as their biologically active components, have been intensively investigated for their potential role in the protection of food from microorganisms, especially the foodstuffs with short shelf-life, such as bread, bakery products, cakes, salads, fresh fruits and vegetable, fish, etc. which are the most susceptible to microbial spoilage. Being natural antimicrobial agents, their usage

Dr. Gordana R. Dimić, Assoc. Prof., gordanad@tf.uns.ac.rs, Sunčica D. Kocić-Tanackov, M.Sc., Assist., Dr. Dušanka J. Pejin, Prof., Dr. Jelena D. Pejin, Assist., Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia; Dr. Ilija J. Tanackov, Assoc. Prof., Faculty of Technical Science, University of Novi Sad, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia; Danijela Tuco, B.Sc. Etol JVE d.o.o., Bulevar Vojvode Stepe 40, 21000 Novi Sad, Serbia

can minimize the application of synthetic preservatives and additives, preserving simultaneously food freshness and sensory quality. Preserving properties of spices and their extracts have been recognized long ago; their residues have been found on old Egyptian mummies (2) and there are evidence of their usage as antiseptic agents (3).

Studies have shown that some spices like vanilla do not possess antifungal activity (4) whereas some of them have a stimulating effect (5, 6, 7, 8).

This study was aimed at investigating the antifungal potential of caraway, garlic, and oregano extracts against some food-borne fungi.

EXPERIMENTAL

Materials

Commercially available food grade ethanol extracts of caraway, garlic, and oregano were provided from Etol, Celje, Slovenia. Test cultures for antifungal investigations, *Aspergillus tamarii, Emericella nidulans, Penicillium commune* and *P. implicatum* were taken from the culture collection of the Laboratory for Food Microbiology, Faculty of Technology in Novi Sad, isolated from food. The cultures were maintained on potato-dextrose agar (PDA) slants at 4º C.

Preparation of inoculum

Prior to the experiment, moulds were cultured on PDA slants for 10 days until fully sporulated. Spores were taken by adding 10 ml of medium which contained 0.5% Tween 80 and 0.5% agar in sterile distilled water (4), scraped with sterile loop and aseptically transferred into sterile test tubes. Spore suspension obtained in this way was adjusted to final concentration of 2×10^6 spores/ml using the hemocytometer, and used for further work.

Antifungal test

The inhibition of mould growth was determined by performing daily measurements of the radial growth of colonies cultured on PDA medium which contained spice extracts (each plate separately) in the following concentrations 0.07, 0.1, 0.5, 1 i 2% (v/v). For test moulds, PDA plates without any added material were made and used as control plates. The solid plates were inoculated with spore suspension containing 1µl (10^3 spores/ml) in the centre of the medium and were incubated for 7 days at 25^{o}C. Diameter of the growth was determined by averaging the radial growrth of the colony in two orthogonal directions. Each test was run in triplicate.

RESULTS AND DISCUSSION

Inhibitory concentrations for caraway, garlic and oregano extracts against *A. tamarii, E. nidulans, P. commune* and *P. implicatum* are presented in Table 1.

APTEFF, 40, 1-220 (2009) UDC: 664.5:66.061.3:582.28
DOI: 10.2298/APT0940009D BIBLID: 1450-7188 (2009) 40, 9-16
Original scientific paper

Table 1. The inhibitory activities of spice extracts against moulds

Extract	Conc. (%)	Inhibition colony growth (%)			
		A. tamarii	*E. nidulans*	*P. commune*	*P. implicatum*
Caraway	0.07	12.7	20.0	14.8	11.1
	0.1	22.2	33.3	37.0	77.8
	0.5	47.6	100	100	100
	1	100	100	100	100
	2	100	100	100	100
Garlic	0.07	1.6	22.2	11.1	11.1
	0.1	14.3	31.1	18.5	33.3
	0.5	25.4	73.3	26.0	100
	1	30.1	100	33.3	100
	2	33.3	100	59.2	100
Oregano	0.07	12.7	11.1	14.8	5.5
	0.1	17.5	15.5	18.5	11.1
	0.5	30.1	31.1	22.2	16.7
	1	47.6	48.9	29.6	55.5
	2	79.4	93.3	74.1	66.7

As can be seen from data presented, the caraway extract exhibited the strongest inhibitory activity that was particularly expressed against *E. nidulans* and both *Penicillium* species (*P. commune* and *P. implicatum*) which did not grow at extract doses over 0.1%. At this level, the growth of *P. implicatum* was markedly reduced (77.8%). The level over 0.5% was needed to completely inhibit *A. tamarii*.

The garlic extract at 0.5 and 1% concentrations inhibited only partially the growth of *A. tamarii* and *P. commune* and completely the growth of *P. implicatum* and *E. nidulans*. If compared to *A. tamarii*, stronger antifungal activity against *P. commune* was observed in all applied concentrations. The level of growth reduction in the presence of garlic extract for *A. tamarii* ranged from 1.6 to 33.3% and for *P. commune* between 11.1 to 59.2%.

Although none of the tested species was completely inhibited by the oregano extract, high concentrations were found to significantly inhibit the growth of colonies. The 2% extract inhibited almost completely (93.3%) the growth of *E. nidulans*. Against *A. tamarii* and *P. commune*, the same extract concentration exhibited approximately 70% inhibition rate (79.4 and 74.1%, respectively), whereas *P. implicatum* was found to be the least sensitive species.

The effect of caraway, oregano and garlic extracts on the germination and growth rate of moulds is presented in Figures 1-3.

At the concentrations 0.1 and 0.5%, the caraway extract delayed the beginning of germination of *A. tamarii* by two and three days, respectively, as compared to the control. The appearance of the growth of *E. nidulans* and *P. commune* was not under the influence by the increased extract concentration; however, the differences in their growth rate were noticed during the next days. At 0.1% concentration, colonies of *P. implicatum* became visible only on the sixth day after the inoculation of agar plates.

UDC: 664.5:66.061.3:582.28
BIBLID: 1450-7188 (2009) 40, 9-16
Original scientific paper

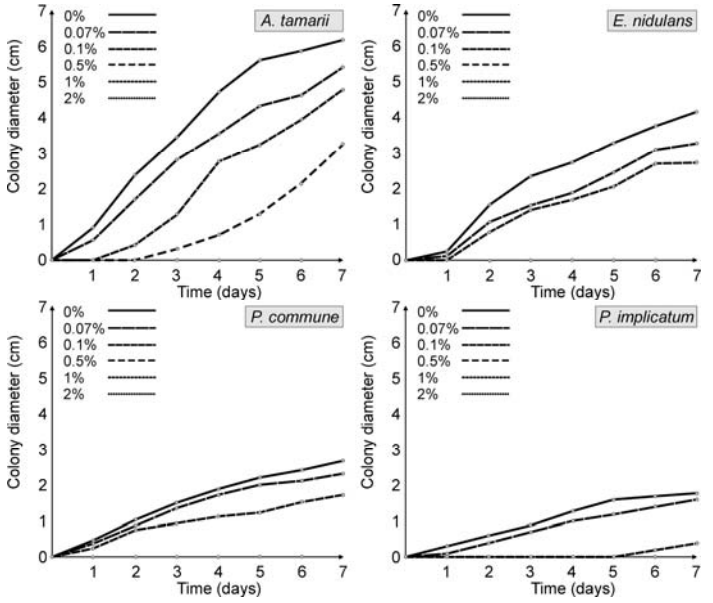

Fig. 1. Effect of caraway extract on the growth of moulds

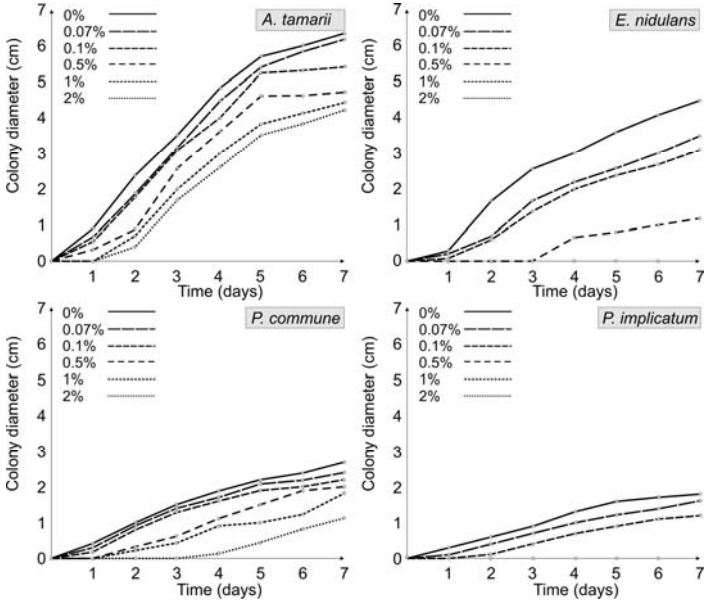

Fig. 2. Effect of garlic extract on the growth of moulds

The growth rate decline with increase of garlic extract contents in the agar medium was especially pronounced in the case of *P. implicatum* and *E. nidulans*, pointing to the greater sensitivity of these spesies (Fig. 2). Higher concentrations did not significantly influence the appearance of *A. tamarii*, whereas *P. commune* did not grow in the presence of 2% garlic extract until the fourth day.

At lower levels of oregano extract (Fig. 3), the begining of germination was delayed by two days only in the case of *P. implicatum*, at the concentration of 0.5%. The 1 and 2% concentrations delayed the growth of *P. implicatum* by two and four days and *E. nidulans* by two and five days. The growth of *A. tamarii* and *P. commune* was delayed only at the concentracion of 2%, for three days. Stronger inhibitory effect on the growth rate of *E. nidulans* was noticed at the concentrations over 0.1% for *A. tamarii* and *P. implicatum* over 0.5% and at *P. commune* over 1%.

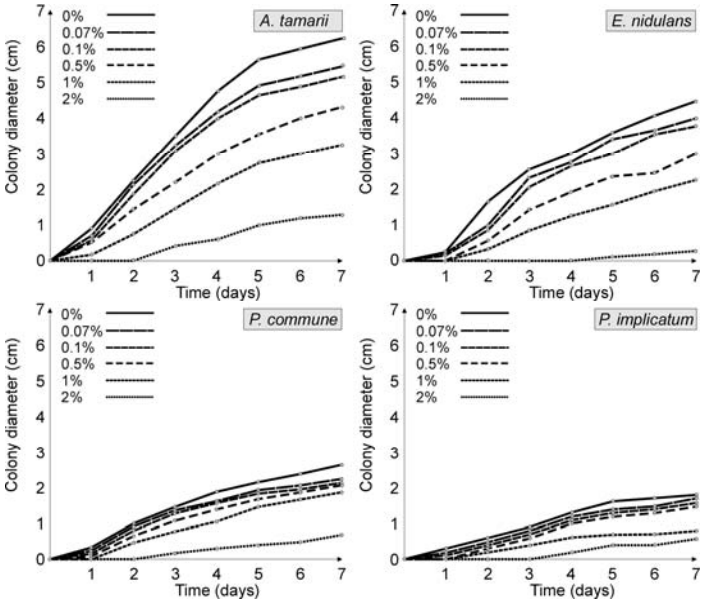

Fig. 3. Effect of oregano extract on the growth of moulds

The increasing concentrations of caraway, garlic and oregano extracts caused the absence or delay in germination of tested fungi, showing various inhibitory effects on the growth rate reduction. Caraway was more efficient at lower concentrations, compared to garlic and oregano. Moreover, it was the only extract to inhibit the growth of three species (out of the four tested) during the whole period of incubation (7 days) at 25°C. Previous studies also reported strong inhibitory effect of caraway on *Penicillium* species. Studies have shown that *P. aurantiogriseum*, *P. corylophilum, P. commune* and *P. griseofulvum* were completely inhibited at 1% dose (8, 9).

Antifungal properties of the tested extracts are due to their major constitutive components, carvacrol (from caraway and oregano), limonene (from caraway), thymol (from

oregano) and sulfur compounds (from garlic) (10, 11). Phenol compounds such as carvacrol, thymol, eugenol, vanillin, geraniol and cinnamaldehyd are known antimicrobial agents (11-17). Phenolic OH-group is very reactive and easily forms hydrogen bonds with active sites in enzymes (6). According to Soliman and Badea (18), caraway showed inhibitory effect on *Aspergillus flavus* and *A. parasiticus* at 2000 ppm dose and on *A. ochraceus* and *Fusarium moniliforme* at 3000 ppm. Nielsen and Rios (4) showed that esential oils of mustard, garlic and clove are effective in preventing the growth of moulds usually present in bread. Garlic show antifungal activity against certain *Aspergillus, Penicillium* and *Fusarium* species (19, 20). Guyenot et al. (3) investigated the protective effect of essential oils of 16 spices against xerophilic fungi from genera *Eurotium, Aspergillus* and *Penicillium*, common spoilage organisms in bakery products. It was also reported that oregano extract is capable to completely inhibit *Aspergillus parasiticus* at the level of 2% in agar medium (6), which is in compliance with our results. Essential oils of cinnamon and oregano proved to be very effective in growth inhibition of *Fusarium proliferatum* (21).

CONCLUSION

This study proved that the tested spice extracts can be potentially protective agents against filamentous fungi, frequent contaminants of food. Caraway extract exhibited high efficacy already at 0.5% dose. Garlic was the most effective against *E. nidulans* and *P. implicatum*. Oregano exhibited strong inhibitory effect although was unable to completely inhibit the fungal growth.

REFERENCES

1. van Egmond, P.H.: Mycotoxins: detection, reference materials and regulation, in Introduction to food- and airborne fungi. Eds. R.A. Samsin, E.S. Hoekstra and J.C. Frisvad, Ponson & Looyen, Wageningen, Utrech, Netherlands (2005) pp.332-338.
2. Silliker, J.H., R.P. Elliott, A.C. Bairol-Barker, F.L. Bryan, J.H.B. Christian, D.S. Clark, J.C. Olson and Jr.T.A. Roberts: Microbial Ecology of Foods. Vol.II, Academic Press, New York, London (1980).
3. Guynot, E.M., J. A. Ramos, L. Seto, V. Purroy, V. Sanchis and S. Marin: Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. J. Appl. Microbiol. **94** (2003) 893-899.
4. Nielsen, V.P. and R. Rios: Inhibition of fungal growth on bread, by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. Int. J. of Food Microbiology **60** (2000) 219-229.
5. Mabrouk, S.S. and M.A.N. El-Shayeb: Inhibition of aflatoxin formation by some spices. Z. Lebensm. Unters. Forsch. **171** (1980) 344-347.
6. Özcan, M.: Inhibitory effect of spices extracts on the growth of *Aspergillus parasiticus* NRRL2999 strain. Z. Lebensm. Unters Forsch A **207** (1998) 253-255.
7. Boyraz, N. and M. Özcan: Antifungal effect of some spice hydrosols. Fitoterapia **76** (2005) 661-665.

8. Dimić, G. and S. Kocić-Tanackov: Antifungal activity of spices extracts on the growth of *Penicillium commune* and *Penicillium griseofulvum*, 6th Congress of Medical Microbiology, Beograd, 11-14 June 2008, Book of Abstracts, pp. 335-336.
9. Dimić, G., S. Kocić-Tanackov and D. Karalić: Inhibitory Effect of Spice Extracts on Growth of *Penicillium aurantiogriseum* and *Penicillium corylophilum*, 54th International Meat Industry Conference, Vrnjačka Banja, 18-20 June 2007, Book of Abstracts, p. 89.
10. Baratta, M.T., H.J.D. Dorman, S.G. Deas, D.M. Biondi and G. Ruberto: Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosmary, oregano and coriander essential oils. J. Essent. Oil Res. **10,** 6 (1998) 618-627.
11. Ceylon, E. and C.Y. D. Fung: Antimicrobial activity of spices. J. Rapid. Meth. Autom. Microbiol. **12** (2004) 1-55.
12. Bullerman, L.B., F.Y. Lien and S.A. Seir: Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J. of Food Science **42** 4 (1977) 11007-1116.
13. Hitokoto, H., S. Morozumi, T. Wauke, S. Sakai and H. Kurata: Inhibitory effects of spice on growth and toxin production of toxigenic fungi. Appl. Environ. Microbiol. **39** 4 (1980) 818-822.
14. Moleyar, A.V. and P. Narasimham: Antifungal activity of some essential oil components. Food Microbiol. **3** (1986) 331-336.
15. Mahmound, A.L.E.: Antifungal action and antiaflatoxigenic properties of some essential oil constituents. Lett. Appl. Microbiol. **19** (1994) 110-113.
16. Matamoros-Leon, B., A. Argaiz and A. Lopez-Malo: Individual and combined effects of vanillin and potassium sorbate on *Penicillium digitatum, Penicillium glabrum*, and *Penicillium italicum* growth. J. Food Protect. **62** 5 (1999) 540-542.
17. Moriera, M.R., A.G. Ponce, C.E. del Valle and S.I. Rouza: Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT **38** (2005) 565-579.
18. Soliman,K.M. and R.I. Badeaa: Effect of oil extrated from some medicinal plants on different mycotoxigenic fungi. Food Chem. Toxicol. **40** (2002) 1669-1675.
19. Mei-chin, Y. and T. Shih-ming: Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. Int. J. Food Microbiol. **49** (1999) 49-56.
20. Benkeblia, N.: Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). LWT **37** (2004) 263-268.
21. Velluty, A., V. Sanchis, A.J. Ramos, J. Egido and S. Marin: Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum*. Int. J. Food Microbiol **89** (2003) 145-154.

APTEFF, 40, 1-220 (2009)
DOI: 10.2298/APT0940009D
UDC: 664.5:66.061.3:582.28
BIBLID: 1450-7188 (2009) 40, 9-16
Original scientific paper

АНТИМИКРОБНА АКТИВНОСТ ЕКСТРАКАТА КИМА, БЕЛОГ ЛУКА И ОРИГАНА НА ФИЛАМЕНТОЗНЕ ПЛЕСНИ

Гордана Р. Димић, Сунчица Д. Коцић-Танацков, Душанка Ј. Пејин, Јелена Д. Пејин, Илија Ј. Танацков и Данијела Туцо

Инхибиторна активност екстраката кима, белог лука и оригана (0,07, 0,1, 0,5, 1 и 2%) је испитивана против четири врсте плесни. Екстракт кима је имао најјачи инхибиторни ефекат спречавајући герминацију *Emericella nidulans*, *Penicillium commune* и *P. implicatum* при концентрацији од 0,1% и *Aspergillus tamarii* при концентрацији од 0,5% током седам дана инкубирања на 25°C. Екстракт белог лука је само парцијално инхибирао раст *A. tamarii* и *P. commune* и потпуно *P. implicatum* и *E. nidulans* при концентрацијама 0,5 и 1%. Оригано је парцијално инхибирао све четири врсте плесни, али је раст колонија био значајно смањен, нарочито код *E. nidulans* (93,3%).

Received 24 July 2009
Accepted 14 September 2009