Efficacy of plant extracts against stored products fungi

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Summary The fungistatic activity of six aqueous extracts of plants were tested against *Aspergillus candidus*, *Aspergillus niger*, *Penicillium* sp. and *Fusarium culmorum*. The plants were, chamomile (*Anthemis nobilis* L.), cinnamon (*Cinnamomum verum* J. Presl.), French lavender (*Lavandula stoechas* L.), garlic (*Allium sativum* L.), malva (*Malva sylvestris* L.) and peppermint (*Mentha piperita* L.). The more concentrated extracts of chamomile and malva inhibited totally the growth of the tested fungi with malva being the most effective one.

Key words Plant extracts, Fungistatic activity, Natural pesticides

Worldwide, post harvest losses have been estimated at 50% and much of this is due to fungal and bacterial infections [22]. In developing countries, post harvest losses are often severe due to the lack of adequate handling and refrigerated controlled atmosphere storage facilities [10].

Fungi in stored products are responsible for the change of appearance, taste and flavour of the products, reduction of nutritional value, allergies, and production of mycotoxins.

Currently, synthetic fungicides are the primary means of controlling post harvest diseases [9,22]. However, growing concerns over the presence of chemical residues in the food chain, the development of fungicide-resistant strains of post harvest pathogens, and the revocation of registration of some of the more effective fungicides, have generated an interest in the development of safer alternatives to synthetic fungicides that are both effective and economically feasible [10].

Plant extracts are generally assumed to be more acceptable and less hazardous than synthetic compounds and could be alternative antifungal treatments [14].

Many reports are available on using plants to study the effects on fungal growth and mycotoxin production: thyme, sage, origano, coriander [20], clove [13,17], cinnamon, rosemary, lavender [6], cumin, pepper [5], garlic, onion [1,11], basil, saffron, marjoram and anise [13] are examples.

In this work, the fungistatic activity of six aqueous extracts has been evaluated, by analysing their effect on fungi growth.

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Material and Methods

Dried leaves of chamomile (*Anthemis nobilis* L.), cinnamon (*Cinnamomum verum* J. Presl.), French lavender (*Lavandula stoechas* L.), malva (*Malva sylvestris* L.), peppermint (*Mentha piperita* L.) (*Lavandula stoechas* L.) and garlic bulbs (*Allium sativum* L.) were macerated in 100 ml of sterile distilled water (sdw) (Table 1). The mixtures were strained through cheesecloth so that aqueous extracts were obtained. The cinnamon powder was diluted in 100 ml sdw, and put in an orbital incubator (160 rpm), for 48 h. The extracts were submitted to two centrifugations (8600 g), for 20 and 30 min. After this, the extracts were filtered through a 0.45mm filter under sterile conditions. Different concentrations of these extracts (Table 1) were added to potato dextrose agar (PDA), and the mixtures were poured into sterile Petri dishes (20 ml). After solidification, three plates of each extract were inoculated by placing 0.5 cm diameter discs of individual fungus in the centre of each plate. The discs were cut from the margins of actively growing colonies of *Aspergillus candidus* (Link), *Aspergillus niger* (van Tieghem), *Penicillium* sp. (Link), and *Fusarium culmorum* (W. G. Smith) Sacc isolated from cereals collected in warehouses, in S. Tomé e Príncipe islands, Africa. Plates of PDA without aqueous extracts were the controls. All cultures were incubated 28 °C, for eight days, at the end of which, fungal colonies diameters were measured. Results were analysed using ANOVA – 1 factor ($p<0.05$).

Results

Results of the effect of PDA media supplemented with different aqueous extracts on colonies diameters are presented in table 2. For chamomile and malva, the most effective extracts were the 0.92 g/ml and 0.60 g/ml respectively, by inhibiting totally the growth of all the tested fungi. The cinnamon extract with 0.60 g/ml was the best by inhibiting the growth of *A. niger*, *F. culmorum* and *Penicillium* sp. and reducing the growth of *A. candidus*. In relation to French lavender, the 0.88 g/ml extract was the most effective one, reducing the growth of *A. candidus*, *A. niger*, *Penicillium* sp. and *F. culmorum*. In the case of

Table 1. Quantities of the plant used and concentrations of their respective extracts added to PDA media.

Plants	Weight (g)	Concentrations (g/ml)
Chamomile	23	0.46, 0.69 and 0.92
Cinnamon	20	0.40 and 0.60
French lavender	44	0.79 and 0.88
Garlic	500	9 and 10
Malva	30	0.30 and 0.60
Peppermint	50	1 and 1.50

Table 2. Effect of PDA media supplemented with different aqueous extract concentrations.

Plants	Aqueous extracts (g/ml)	*A. candidus* (cm)	F / Fcrit	*A. niger* (cm)	F / Fcrit	*Penicillium* sp. (cm)	F / Fcrit	*F. culmorum* (cm)	F /Fcrit
Chamomile	0.46	3.90	488.4 / 4.066	7.86	9524.5 / 4.066	2.23	558.6 / 4.066	4.10	978.0 / 4.066
	0.69	3.43		7.53		1.86		0.50	
	0.92	0.50		0.50		0.50		0.50	
	control	6.66		7.90		3.36		7.63	
Cinnamon	0.40	4.43	368.1 / 5.143	8	16653.0 / 5.143	2.33	601.4 / 5.143	0.50	4124.7 / 5.143
	0.60	1.26		0.50		0.56		0.50	
	control	6.66		7.90		3.36		7.63	
French lavender	0.79	2.66	488.8 / 5.143	6.76	211.2 / 5.143	1.26	567.1 / 5.143	1.13	1715.6 / 5.143
	0.88	2.23		5.06		1		0.63	
	control	6.66		7.90		3.36		7.63	
Garlic	9.00	3.10	220.5 / 5.143	2.23	1304.7 / 5.143	1.16	552.4 / 5.143	0.66	1398.5 / 5.143
	10	0.50		0.50		0.50		2.66	
	control	6.66		7.90		3.36		7.63	
Malva	0.30	4.00	759.6 / 5.143	7.33	11449.8 / 5.143	1.03	1255 / 5.143	2.26	358.3 / 5.143
	0.60	0.50		0.50		0.50		0.50	
	control	6.66		7.90		3.36		7.63	
Peppermint	1	4.46	273.9 / 5.143	7.93	136.2 / 5.143	1.70	157.1 / 5.143	1.06	3031.9 / 5.143
	1.50	2.30		5.93		0.96		0.50	
	control	6.66		7.90		3.36		7.63	

F / Fcrit - Fvalue compared with correspondent critical value (Snedecor's F distribution). All F values are significant ($p<0.05$).

Table 3. Inhibition effect of the plant extracts tested.

Plants	Concentration (g/ml)	*A. candidus*	*A. niger*	*Penicillium* sp.	*F. culmorum*
Chamomile	0.46	-	-	+	++
	0.69	++	+	++	+++
	0.92	+++	+++	+++	+++
Cinnamon	0.40	++	++	++	++
	0.60	++	+++	+++	+++
French lavender	0.79	++	+	++	++
	0.88	++	+	++	++
Garlic	9.00	++	++	++	++
	10.00	++	+++	+++	+++
Malva	0.30	+	+	++	++
	0.60	+++	+++	+++	+++
Peppermint	1.00	+	-	++	++
	1.50	++	+	++	+++

(+++) 100% inhibition of fungi growth; (++) above 50% inhibition of fungi growth; (+) below 50% inhibition of fungi growth; (-) 0% inhibition of fungi growth.

garlic, the 10.00 g/ml extract inhibited totally the growth of *A. niger*, *F. culmorum* and *Penicillium* sp., and reduced the growth of *A. candidus*. It was the most effective. For peppermint, the 1.50 g/ml extract was the most effective, reducing the growth of *A. candidus*, *A. niger* and *Penicillium* sp. and inhibiting totally the growth of *F. culmorum*. The quantitative results presented in table 2 were transformed to qualitative results, which are presented in table 3.

Discussion

The search for new compounds to control fungi in stored products is a promising area of research. Natural compounds produced by the secondary metabolism of plants are potentially an important source of new types of fungicides. The knowledge about natural antifungal compounds from plants is scarce and practically non-existent for the control of fungi in stored products. In this work, all

fungi tested are mycotoxins producers. Among the six species of plants tested for their potential use for control of stored products fungi, chamomile and malva had the highest in vitro antimycotic activities (Tables 2 and 3). The chamomile and malva more concentrated extracts inhibited totally the growth of the four tested fungi, but malva was the most effective, since it gave the same result with a lower concentration. Literature references about this issue were not found. For the other plants, the level of inhibition varied according to the plant species and the fungus tested, as reported by [2,3,19]. *A. niger*, *Penicillium* sp. and *F. culmorum* showed a high susceptibility in relation to the highest extracts concentrations of the cinnamon and garlic. In the case of French lavender and peppermint *A. candidus* had more susceptibility than *A. niger* (Tables 2 and 3). There are different ways to prevent and suppress the fungi tested using synthetic fungicides [15], organic acids [8,12,18] and short synthetic antimicrobial peptides [16], but these compounds are only permitted in feedstuffs. Because of this it is important to select new substances which are easily decomposable, not environmental pollutants and with no residual or phytotoxic properties [4,5,21].

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