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Allelopathic effects of orange (*Citrus sinensis* L.) peel essential oil

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RESUMO

(Efeitos alelopáticos do óleo essencial da casca da laranja (*Citrus sinensis* L.)). Plantas daninhas são o principal problema da agricultura no mundo, causando anualmente um prejuízo de U$95 bilhões. A principal forma de combate às plantas daninhas são os herbicidas sintéticos. Entretanto, o uso continuado desses produtos encarece a produção, reduz a qualidade dos alimentos, deixa resíduos tóxicos no ambiente e ameaça a saúde humana e animal. Existe assim, uma demanda por técnicas menos agressivas de controle. O uso de compostos alelopáticos de descartes de culturas para esse fim é uma alternativa. A laranja é uma das maiores culturas do mundo, e gera grandes quantidades de resíduos. Existem evidências da bioatividade no óleo essencial da casca da laranja. Nesse trabalho, nosso objetivo foi verificar a existência de propriedades alelopáticas desse óleo. Para isto, extraímos o óleo por arraste de vapor de água utilizando o aparelho de Clevenger, e colocamos os produtos volatilizáveis do óleo essencial em contato com plântulas de *Euphorbia heterophylla* L. e *Ipomoea grandifolia* (Dammer) O'Donell. O resultado foi inibição tanto quantitativa quanto qualitativa das plântulas

Palavras-chave: agricultura orgânica, alelopatia, controle de pragas, descartes de cultivos, Limoneno, plantas invasoras de culturas

ABSTRACT

(Allelopathic effects of orange (*Citrus sinensis* L.) peel essential oil). Crop weeds are the main problem in agriculture, causing a worldwide annual loss of about US$95 billion. The principal method for control is the use of synthetic herbicides. The continued use of these products increases crop costs, reduces crop quality, and leaves toxic residues in the environment, which are a threat to human and livestock health. Therefore, there is a demand for environmentally friendly methods of weed control. The use of allelopathic compounds from crop residues is an alternative. Orange is one of the biggest crops in the world, and its cultivation generates large amounts of residues. There is strong evidence of bioactivity in orange peel essential oil. Therefore, the objective in this work was to verify the allelopathic proprieties of this oil. We extracted the oil from the peels of recently discarded oranges using water vapor flow with a Clevenger extractor, and tested it against the growth of *Euphorbia heterophylla* L. and *Ipomoea grandifolia* (Dammer) O'Donell seedlings when placed in contact with the oil vapor. The results were both quantitative and qualitative in the inhibition of the seedlings.

Key words: Allelopathy, crop residues, Limonene, organic agriculture, weeds, weed controly

Introduction

Crop weeds are the main problem in agriculture, causing a worldwide an annual loss of about US$95 billion (FAO 2009). The principal method to control weeds is the use of synthetic herbicides (Hong et al. 2004). Although these products have brought unquestionable gains to agriculture, their use increases production cost, reduces crop quality (resulting in less healthy plants and more expensive food) and, contaminates the environment, which is a threat to human and livestock health (Silva et al. 2005). Furthermore, the continued use of herbicides has led to a selection of resistant weed biotypes, on which a commercial dose of herbicide has a limited effect (Gelmini et al. 2001). Therefore, there is a growing demand to replace synthetic herbicides with environmentally friendly weed control.

The possibility of weed control using allelochemicals is widely recognized (Rice 1995, Souza *et al.* 2002, Piccolo *et al.* 2007, Dayan *et al.* 2009). Allelochemicals are compounds from the secondary metabolism of plants, and present several advantages over herbicides, such as less toxicity, a shorter half

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life and higher water-solubility (Duke et al. 2000). Furthermore, because they have a lower specific action mechanism, they are less likely to select resistant biotypes (Reigosa *et al.* 2001, Durán-Serantes *et al.* 2002).

A main shortcoming of the commercial use of allelochemicals is their production at a large scale, because most donor plants are non-cultivated, poorly-studied species. One possible solution is to determine the allelochemical compound and then artificially synthesize it; however, this is far beyond the reality of most farmers. Furthermore, the synthetic versions of natural compounds are not accepted in organic agriculture in most countries. Another solution, which has been successfully tested, is the use of crop residues as allelochemical sources (Rizvi *et al.* 1999). Crop residues are likely to be used at a commercial scale because they are available in large amounts.

Brazil is one of the world's largest orange (*Citrus sinensis* L.) producers. Therefore, residues from this crop might be an enormous source of allelochemicals. The orange peel, frequently discarded after juice extraction, is rich in essential oil. Essential oil of several species is able to inhibit the germination and growth of other species (Duke et al. 2002), and the orange peel essential oil seems to be highly bioactive (Sharma & Tripathi 2006, Ali *et al.* 2007, Tsai 2008). In preliminary assays, we detected strong inhibitory effects of orange peel essential oil on the germination and initial growth of lettuce (*Lactuca sativa* L.).

The objective of this work was to determine the allelopathic proprieties of orange peel essential oil on two of the most common weeds in Brazil: *Euphorbia heterophylla* L. and *Ipomoea grandifolia* (Dammer) O'Donell. Due to its high bioactivity and to the preliminary assay results, we predicted that the orange peel essential oil would be able to inhibit these species.

Material and methods

Essential oil extraction

We extracted the essential oil using water vapor flow, with a Clevenger type extractor. We used the crumbled peels of 14 oranges recently rejected from a juice store and about two liters of distilled water. We kept the oil in a freezer until the experiment started.

Bioassays

For the bioassay, we used plastic boxes (8x13x5 cm) with a double layer of filter paper (80 gm^{-2}, 205 μm), 10 mL of distilled water, and 10 weed seedlings (pre-germinated for 24h in distilled water). Inside the box we also placed an 8 mL beaker with 0.7 mL of essential oil, so only the oil vapor reached the seedlings (Fig 1). In an incubator (28 °C, with 12 h/12 h light regime at 83.16 μmol $m^{-2}s^{-1}$), we allowed the seedlings to grow for five days. After that, we measured the root and shoot lengths of each seedling. For control we used the same procedure, but with an empty 8 mL beaker. We also performed a qualitative analysis of the seedlings, by searching for any malformation or injury.

Data analysis

The experimental design was entirely casualized. For both treatment and controls we assembled six replicates, and analyzed the data using the Mann-Whitney test ($p<0.05$).

Results

Seedlings size and mortality

The contact with the essential oil vapor reduced the length of both the roots and shoots (and therefore, the total size) of *E. heterophylla* seedlings. The number of dead plants was also higher for those in the presence of oil. *Ipomoea grandifolia* seedlings were more resistant. Only shoots were affected, and no seedlings died.

Malformations

Besides the quantitative reduction of seedlings size, the essential oil also led to malformations. *Euphorbia heterophylla* seedlings exposed to the oil vapor suffered cotyledon injuries, malformation of the secondary roots, and presented fragile shoots (Fig 2A). *Ipomoea grandifolia* seedlings also presented cotyledon and secondary root malformations (Fig 2B). The shoots of both species were red in the control and almost white in the treatment, showing pigmentation differences.

Discussion

The orange peel essential oil inhibited *E. heterophylla* seedlings. Besides reducing the average seedling size, the oil also led to several injuries and bad formations in both the roots and shoots. In several cases, injuries were severe enough to kill the seedling. *Ipomoea grandifolia* seedlings were less sensitive to the oil, and root size and total size of the seedling were not affected in this species. Nevertheless, the contact with the volatilized oil reduced shoot size, and led to morphological changes, particularly cotyledon malformations and a change in shoot color. Therefore, quantitative analysis alone may underestimate the inhibitory effects of the orange peel essential oil on this species.

In the end, there was still oil in all of the beakers. This suggests that essential oil vapor saturation inside the plastic box can be reached with less than 0.7 mL of oil. Therefore, it is reasonable to propose that similar inhibitory effects could be produced with a smaller amount of oil. Most allelopathic surveys with essential oils use emulsions of the oils in their assays. Furthermore, the target species varies (Alves *et al.* 2004; Liu *et al.* 2008) and thus, it is difficult to compare our results with others. Nevertheless, contact with the oil vapor inhibited *E. heterophylla* and *Ipomoea grandifolia*, which suggests that the orange peel essential oil has high inhibitory effects.

The bioactivity of the orange peel essential oil is well documented. Ali *et al.* (2007) detected anti-mitotic propri-

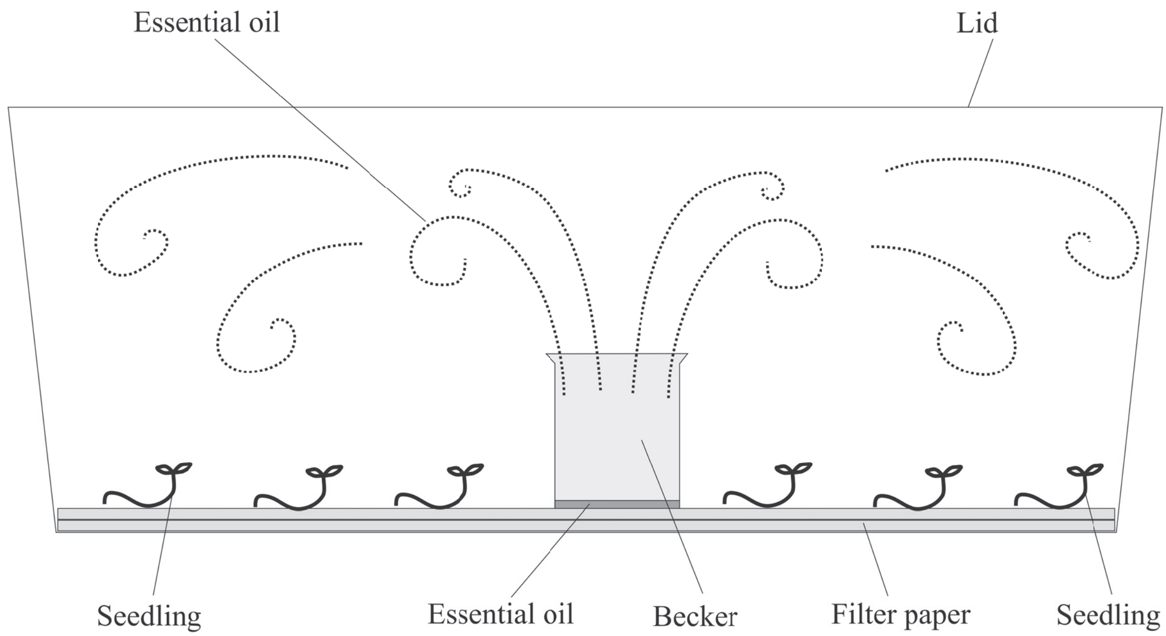

Figure 1. Initial growth bioassay of weeds.

Figure 2. *Euphorbia heterophylla* L. (A) and *Ipomoea grandifolia* (Dammer) O'Donell. (B) seedling growth without (main image) and with (detail) contact with orange (*Citrus sinensis* L.) peel essential oil.

Table 1. Average size (cm) of shoots (ST), roots (RT), total size, and mortality (M, in number of deaths) of *Euphorbia heterophylla* L. and *Ipomoea grandifolia* (Dammer) O'Donell. in contact with orange (*Citrus sinensis* L.) peel essential oil. Values followed by * differs significantly from the control by the Mann-Whitney test (p<0.05).

	Euphorbia heterophylla				*Ipomoea grandifolia*			
	ST	RT	Total	M	ST	RT	Total	M
Control	5.52	8.10	13.40	0.16	3.45	3.92	7.38	0
Treatment	1.75*	5.71*	2.79*	7.16*	2.67*	4.09	6.77	0

eties and Tsai (2008) showed that the oil can kill nematodes. Sharma &Tripathi (2006) documented fungicide proprieties. Our results corroborate this because we found strong inhibitory activities.

The main compound in the essential oil of citric fruits is limonene (Tsai 2008). In oranges, this monoterpene represents 84.2% of the essential oil in the peel. This high concentration has been suggested as the reason of the high bioactivity of orange peel essential oil (Tsai 2008). Limonene is also present in the essential oils of non-citric species (Agostini *et al.* 2005; Apel *et al.* 2005; Apel *et al.* 2006; Carvalho Filho *et al.* 2006; Silva *et al.* 2009), which are frequently associated with high inhibitory effects (Azirak & Karaman 2008; Silva *et al.* 2009; Souza Filho *et al.* 2009). To the best of our knowledge, there has been no allelopathic study that used isolated limonene, but it is reasonable to propose that this was the main compound responsible for the allelopathic effects found (because we used a purified version of the orange peel essential oil, one might choose to call this a phytotoxic effect).

The citric industry is one of the world's largest agroindustries, and the juice manufacturing process creates enormous amount of residues (Tsai 2008). Although further research is required, particularly in applicable studies (Inderjit & Weston 2000), the present work showed that it is possible to control weeds using an allelochemical from citric crop residues. This is an environmentally friendly weed control, and helps dispose residues produced by the citric industry.

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