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Allelopathic Potential of *Oxalis pes-caprae* Tissues and Root Exudates as a Tool for Integrated Weed Management

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Abstract: Laboratory and greenhouse pot experiments were conducted to evaluate the potential usefulness of *Oxalis pes-caprae* L. as allelopathic ground-cover species for integrated weed management. The effects of the extracts of several tissues and the exudates from living roots of *O. pes-caprae* plants were tested for their allelopathic activity on several plants. Duckweed fresh weight was significantly inhibited from phytotoxic activity of petiole tissues (and secondly leaves and stems) of *O. pes-caprae*. For all the kinds of tissues duckweed fresh weight was reduced with increasing extract concentrations. Besides, root exudates of Bermuda buttercup caused 62, 58 and 42% inhibition of the dry biomass production of tomato, oat and lettuce plants, respectively, confirming the remarkable allelopathic activity of this weed.

Key words: Bermuda buttercup, duckweed, tomato, lettuce, oat, weed control

INTRODUCTION

Bermuda buttercup (*Oxalis pes-caprae* L.) is a perennial small herb, member of Oxalidaceae family. It was introduced from South Africa in 19th century and now is one of the most noxious and invasive weeds of Mediterranean and other regions (Vilà *et al*., 2006; Castro *et al*., 2007; Vilà and Gimeno, 2007a, b). In Greece it is one of the most dominant weed in the olive grooves and vineyards (Damanakis and Markaki, 1990), while we can often find it in fallow land, too. This high competitive ability has to be investigated and further exploited, as long as *O. pes-caprae* has detrimental impacts by causing soil enrichment and stabilization of semi-stable areas and orchards and suppressing other weeds (Paspatis, 1987).

Now a days, allelopathy is not only considered as a common ability acquired by the plant kingdom through the course of evolution (Putnam and Tang, 1986), but is among the predominant forces in the development of plant communities and spatial patterns therein (Travlos and Paspatis, 2008).

However, a few studies reported to date have assessed the allelopathic potential and the secondary metabolites of *Oxalis pes-caprae* and other relative species (Campos *et al*., 2006; DellaGreca *et al*., 2007; Fossen *et al*., 2007), even if there is considerable evidence that a number of weed species may impose interference through allelopathic mechanisms (Paspatis, 1987; Shiraishi *et al*., 2002; Sala *et al*., 2007). The purpose of this study was to detect and evaluate the potential allelopathic activity of several Bermuda buttercup tissues on duckweed fresh weight and of root exudates on growth of tomato, lettuce and oat.

MATERIALS AND METHODS

The plant material was collected from a natural population established in Benaki Phytopathological Institute, during 2007. Entire plants and several plant parts of Bermuda buttercup (leaves, stems and petioles) were cut into small pieces, stored for a week at 0°C in the dark and extracted successively with deionised water at varying concentrations (0, 1, 2, 3, 4, 5, 7 and 10 mL 100 mL^{-1}).

The phytotoxicity of plant extracts was quantified with a bioassay using as test plant a species of duckweed (*Spirodella polyrhiza* L.) and measuring the decrease of its fresh weight. A small quantity of duckweed (0.30 g) was placed in 10 cm diametric plastic pots with 40 mL of a modified Hoagland's solution (of 0.25 strength) and 40 mL of each extract of *O. pes-caprae* plants, leaves, stems or petioles. The composition of the above mentioned solution was: 1 mL of KNO_3 1 M, 5 mL of KH_2PO_4 1 M, 5 mL of $Ca(NO_3)_2.4H_2O$ 1 M, 2 mL of $MgSO_4.7H_2O$ 1 M, 1 mL of FeEDTA 1 M and 1 mL of a solution with 2.86 g L^{-1} H_3BO_3, 1.81 g L^{-1} $MnCl_2.4H_2O$,

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0.22 g L^{-1} $ZnSO_4.H_2O$, 0.08 g L^{-1} $CuSO_4.5H_2O$ and 0.02 H_2MoO_4 (Hoagland and Arnon, 1950). The pots were placed in a growth chamber (GRW 1000T CMP, E. Chrisagis, Greece). Day/night length, air temperature and RH were 14/10 h, 25/20°C and 50/60%, respectively. Lighting was provided by four high pressure sodium lamps (Vialox NAV-T 400 4Y, Osram, Germany). The fresh weight of duckweed was measured after one week and used as an index of allelochemical activity of *O. pes-caprae* extracts.

Moreover, the phytotoxicity of *O. pes-caprae* root exudates was also quantified by means of bioassays using tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*) and oat (*Avena sativa*) plants and measuring their growth. Five bulbs of bermuda buttercup were placed in Grodan cubes (as a growing media) and wrapped with transparent polyethylene (PE) sheets, 0.05 mm thick (Plastica of Crete S.A., Greece). There was a daily irrigation with 200 mL of water, while once a week 200 mL of the above-mentioned modified Hoagland's solution (of full strength) was also added. The exudates from living roots were collected in a vial below the cubes and these exudates were added in corresponding pots (Grodan cubes) seeded with six tomato, lettuce and oat seeds, respectively. Control solutions were exudates of empty pots irrigated as above. Plants were collected 45 days after planting and the average fresh and dry weight per plant (60°C for 2 days) was determined.

The experimental design of all the conducted experiments was a randomized block with four replicates for each treatment and control. The doses needed to inhibit oat radicle growth to 50% of control radicle growth (hereafter called the I_{50} values) were determined from dose-response bioassays (Travlos *et al*., 2007a). For the analyses of the results from all the measurements SPSS software was used (SPSS, 1997). The differences among treatments were tested with Least Significant Difference (LSD) test, at p = 0.05.

RESULTS

There was a negative response of duckweed growth to the Bermuda buttercup extracts, while it was more intense with increasing extract concentrations (Table 1). In Table 2, there are shown the inhibitory effects of a range of aqueous extracts of leaves, stems and petioles of *O. pes-caprae* on duckweed growth. In general, there was a strong inhibition response of the leaves, stems and petiole samples in terms of the I_{50} estimates (2.7, 2.8 and 2.5 mg mL^{-1}). It is noticeable that stem and petiole extracts were adequate to cause a total inhibition of growth of duckweed (100%), even at the relatively low concentration of 5 mg 100 mL^{-1} (Table 2).

Table 1: Response of duckweed fresh weight (FW) to the extracts of blended *Oxalis pes-caprae* plants

Concentration[a] ($mL\ 100\ mL^{-1}$)	Duckweed FW (mg)	Inhibition or promotion[b] (%)
Control	540a[c]	0
1	530a	+2
2	610a	-15
3	370b	-31
4	70c	-87
5	0d	-100
7	0d	-100
10	0d	-100
	I_{50} = 3.32 mL 100 mL^{-1}	

[a]The I_{50} value represents the concentration of the allelopathic components to cause 50% inhibition of duckweed fresh weight as determined by probit analysis. [b] Inhibition or promotion compared with control are indicated with a minus or plus symbol, respectively. [c]Means followed by different letter(s) are significantly different at $p<0.05$, by the least significant difference test

Table 2: Response of duckweed to the extracts of *Oxalis pes-caprae* leaves, stems and petioles[a]

Concentration[b] ($mg\ 100\ mL^{-1}$)	Duckweed FW (mg) Leaves	Stems	Petioles
Control	900 (0)	900 (0)	900 (0)
0.32	1000 (+11)	870 (-3)	1000 (+11)
0.63	920 (+2)	1050 (+17)	940 (+4)
1.25	960 (+6)	1090 (+21)	820 (-9)
2.5	820 (-9)	620 (-31)	420 (-53)
5	90 (-90)	0 (-100)	0 (-100)
10	0 (-100)	0 (-100)	0 (-100)
	I_{50} = 2.7 mg mL^{-1}	I_{50} = 2.8 mg mL^{-1}	I_{50} = 2.5 mg mL^{-1}

[a]Numbers in parentheses indicate percent inhibition or promotion compared with control (minus or plus symbol, respectively). [b]The I_{50} value represent the concentration of the allelopathic components to cause 50% inhibition of duckweed fresh weight as determined by probit analysis

Table 3: Response of tomato, lettuce and oat fresh and dry weight (FW and DW, respectively) to the extracts of *Oxalis pes-caprae* living roots

Plant	Parameter	Treatment Control	Root exudates	Growth inhibition (%)
Tomato	FW (mg)	1400a[a]	280b	80
	DW (mg)	130a	50b	62
Lettuce	FW (mg)	1080a	710b	34
	DW (mg)	240a	140b	42
Oat	FW (mg)	510a	230b	55
	DW (mg)	120a	50b	58

[a]Means followed by different letter(s) in the same row are significantly different at $p<0.05$, by the least significant difference test

The fresh and dry weight accumulation of tomato, oat and lettuce was significantly inhibited by Bermuda buttercup root exudates by 34 to 80% (Table 3). The allelopathic effects of *O. pes-caprae* root exudates varied among three plant species. Indeed, root exudates showed a higher phytotoxic effect on tomato and oat, than on lettuce growth.

DISCUSSION

Present results further support and extend the biological activity of extracts from *Oxalis* species and their allelopathic effects on other plant species (Paspatis, 1987; Shiraishi *et al*., 2002; DellaGreca *et al*., 2007). These findings are in accordance with those of Travlos *et al.* (2007a) and Travlos and Paspatis (2008), whose studies showed that quantities of allelochemicals within plants vary with plant tissue. Besides, even if the extract of petioles was slightly more effective than leaves and stems, for all the tissues duckweed fresh weight was reduced with increasing extract concentrations (Table 2). Indeed, it is well known that the magnitude of phytotoxic activity is dependent upon the concentration and chemical stability of the active compounds (Travlos *et al*., 2007b; Travlos and Paspatis, 2008). We have also to note that *Spirodella polyrhiza* is among the most widely used plant indicators in allelopathic studies, since the bioassay is sensitive and reliable especially at the first steps of a screening procedure (Travlos *et al*., 2007a; Travlos and Paspatis, 2008). Moreover, duckweed species are highly sensitive to chemicals that inhibit the function of Photosystem II and their response by chlorosis is readily measurable through the drastic decrease in their fresh weight (Travlos and Paspatis, 2008). Similarly, tomato, lettuce and oat were included in this study since they germinate evenly, resulting in a uniform and rapid plant growth that enables qualification of biological response in plants. Furthermore, such hydroponic cultures of growth as the one used in our study are commonly used for assays of allelopathic activity (Asao *et al*., 2003; Travlos and Paspatis, 2008).

Moreover, the present study clearly highlights the significant inhibition of growth of tomato, oat and lettuce by means of the exudates from living roots of Bermuda buttercup plants. Shiraishi *et al.* (2005) had also observed allelopathic effects of root exudates from relative species (likewise *O. deppei*) on lettuce growth. However, among the three tested plants, lettuce was less inhibited, possibly because it is also known to contain water-soluble allelopathic substances (Chon *et al*., 2005). Therefore, Bermuda butternut root exudates showed a higher phytotoxic effect on tomato and oat, than on lettuce growth (34-42% inhibition).

CONCLUSION

Since growth of several plant species is very susceptible to extracts and living root exudates, it seems that one of the main reasons of the wide distribution and dominance of *O. pes-caprae* is certainly due to allelopathic potential of the species. Our hypothesis is further supported by the recent study of Sala *et al.* (2007), who showed that *O. pes-caprae* is a relatively poor competitor. Under the view of integrated weed management and development of more environmentally feasible methods of weed control, the indicated allelopathic activity of plants like *O. pes-caprae* could be exploited and accomplished with future studies focusing on the identification and isolation of the responsive allelochemicals.

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