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# EVALUATION OF THE ALLELOPATHIC POTENTIAL OF QUINOA (*CHENOPODIUM QUINOA* WILLD.)

**Dimitrios J. Bilalis[1,*], Ilias S. Travlos[1], Anestis Karkanis[2], Maria Gournaki[1], Giannis Katsenios[1], Dimitra Hela[3] & Ioanna Kakabouki[3]**

[1]Laboratory of Agronomy, Faculty of Crop Science, Agricultural University of Athens, 75 Iera Odos str., 11855, Athens, Greece.

[2]Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Fytokou str., 38446, N. Ionia, Magnisia, Greece.

[3]University of Ioannina, Dep. of Business Administration of Food & Agricultural Products, 20 Seferi str., 30100 Agrinio, Greece.

[*]Corresponding author. E-mail: bilalisdimitrios@yahoo.gr

## ABSTRACT

**The allelopathic effects of three plant tissues of *Chenopodium quinoa* (leaves, roots and inflorescences) on oat growth were investigated using *in vivo* tests. Oat growth (fresh and dry weights of above and underground parts) was significantly inhibited from phytotoxic activity of inflorescence tissues, leaves and roots of *C. quinoa* in pot experiments. The inhibiting actions of aqueous crude extracts of the several plant parts were examined using three bioassay methods: (a) seed germination and radicle growth of oat, (b) fresh and dry weight of bean and (b) fresh weight of duckweed plants. All three *in vitro* tested species exhibited greater phytotoxic response from the inflorescence tissues, than from the other parts of quinoa, confirming the results of *in vivo* studies on the potential allelopathic activity of this promising crop.**

**Key words:** Quinoa; allelopathy, oat; bioassay, duckweed, bean.

## INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a highly nutritious plant traditionally grown in the Andean highlands of Bolivia, Peru and Ecuador. Recently it has acquired an additional interest as an export crop generating resources for the poor regions where it is usually grown (Hellin and Higman, 2003). It belongs to the group of crops known as pseudocereals (Cusack, 1984; Koziol, 1993) that includes other domesticated chenopods, amaranths and buckwheat. Its grain has a high-protein content with abundance of essential amino acids, and a wide range of vitamins, minerals and saponin (Bilalis et al., 2012; Repo-Carrasco et al., 2003). Recently, there has been growing interest in a number of countries (especially in Europe), initiating introduction and research work on quinoa (Galwey, 1992; Jacobsen, 1997).

Molisch (1937) coined the term allelopathy to refer to biochemical interactions between all types of plants including microorganisms. Nowadays, 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 (Rice, 1984; Travlos and Paspatis, 2008).

An early sowing would enable quinoa to have a head start over weeds as the plant can attain good growth during this period. This is more important, since there is an absence of any recommendation or use of herbicides to control weed populations in quinoa and generally hand weeding is done. A number of crop plants have been reported to acquire allelopathic potential that affects the growth of other species (Karkanis et al., 2010).

Weed control has a major impact on grain yield of quinoa. The crop has a relatively slow first growth, which could result to an insufficient weed competition (Bhargava et

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al., 2006). Therefore, an eventual allelopathic activity of species like that could offer some alternatives through of integration of several weed management practices and may help reduce herbicide dependency in agriculture. Unfortunately, very few studies reported to date have assessed *in vitro* the allelopathic potential of *C. quinoa*, even if there is considerable evidence that several *Chenopodium* species may impose interference through allelopathic mechanisms (Jiménez-Osornio et al., 1996; El-Khatib et al., 2004; Batish et al., 2006).

This study reports a preliminary investigation into the allelochemical characteristics of quinoa. The objective of this paper was to detect and evaluate the potential allelopathic activity: (a) of different quinoa tissues on oat seedling growth and (b) of plant extracts on oat radicle growth, bean seedling growth and duckweed fresh weight and estimate their dose-response to aqueous extracts of varying concentrations.

## MATERIAL AND METHODS

### Pot experiment

Plant material was collected at the reproductive stage of a quinoa crop established in Agricultural University of Athens (AUA), Greece (latitude 37$^o$ 59) N; longitude 23$^o$ 42′ E) in June 2011. The soil was clay loam (CL 0-25 cm; 34.1% clay, 28.8% silt and 37.1% sand), calcareous (15.9% $CaCO_3$), with a pH value of 7.29 and relatively moderate organic matter (determined according to Wakley and Black, 1934) and nitrogen content (1.47 and 13.4%, respectively), with sufficient levels of nitrate, moderate in available phosphorus and rich in available potassium and sodium (104.3, 9.95, 590 and 110 ppm, respectively). For the purposes of allelopathic experiments three tissue types were used: (i) inflorescences, (ii) leaves and stems, and (iii) roots. Bioassay experiments were used to determine the inhibitory potential of each of these tissues on oat above- and below- ground growth in pot experiments. Oat (*Avena sativa*) was included in this study since it has been used extensively in allelopathy research as the receiver plant to test compounds released by a donor plant. Oat seeds germinate evenly, resulting in a uniform and rapid plant growth that enables qualification of biological response in plants. In addition, oat biotest is considered as a sensitive and easily facilitated method (Rice, 1984). Eight seeds of oat were placed and grown in 10 cm diametric plastic pots containing 5 g freeze tissues per pot filled with perlite, in a growth chamber (GRW 1000T CMP, E. Chrisagis, Athens, Greece). The seeds were placed 2 cm deep in 16 test pots. The pots were watered daily with equal volumes of deionised water. All pots were watered to maintain adequate moisture. Day/night length, air temperature and RH were: 12/12 h, 26/17$^o$C and 50/70% respectively. Lighting was provided by four high pressure sodium lamps (Vialox NAV-T 400 4Y, Osram, Gmbh, Munich, Germany) The above- and below-ground growth of oat were measured after two weeks and used as an index of allelochemical activity. Seedlings were collected two weeks after planting and the average shoot fresh and dry weight per pot was determined. The experimental design was a randomised block with four replicates for each treatment and control.

### Plant extracts bioassay

The phytotoxicity of plant extracts was quantified with an *Avena sativa* seed bioassay. The several plant parts (leaves and stems, roots, inflorescences) were cut into small pieces, stored for a week at 0$^o$C in the dark and extracted successively with deionised water. Aqueous dilutions of the initial crude extracts were bioassayed on filter paper in plastic Petri plates. Ten oat seeds were placed onto two layers of 9-cm filter paper in Petri plates treated with 3 ml of test solution, covered, and incubated at 24$^o$ C in the dark.

The experimental design was a randomised block with four replicate Petri dishes for each treatment, while in the control solutions only deionised water was added. Inhibitory concentrations were calculated after 7 days and used as an index of allelochemical activity. An analysis was conducted according to Finney (1962). A 5-mm radicle length was considered germinated, while seeds that did

not germinate were considered to have a radicle length at 0 mm. Growth was quantified by measuring the radicle length of germinated oat and was also expressed as a percentage of radicle elongation in control dishes. The dose needed to inhibit oat radicle growth to 50% of control radicle growth (hereafter called the $I_{50}$ value) was determined from dose-response bioassays (Finney, 1978). All statistical analyses were conducted using the Statistica 9 software package (StatSoft, Inc. 2300 East 14th Street, Tulsa, OK 74104, USA).

Moreover, the phytotoxicity of *C. quinoa* extracts was also quantified by means of a bioassay using common bean plants (*Phaseolus vulgaris*) and measuring some of the main features of their first growth. *Phaseolus* species are also often included in similar studies (Jiménez-Osornio et al., 1996; Batish et al., 2006). Additionally, beans germinate evenly, resulting in a uniform and rapid plant growth that enables qualification of biological response in plants, while such hydroponic systems of growth like the selected one are commonly used for assays of allelopathic activity (Leather and Einhellig, 1985; Travlos and Paspatis, 2008). Two bean plants in the stage of two real leaves (shoot height of about 4-6 cm) were held with an aphrolex strip of 1cm width, in the top of plastic cups, with their roots inside 40 ml of a modified Hoagland's solution (of 0.25 strength) and 40 ml of quinoa extracts (or only deionised water in the case of control). The composition for 1 l of the above mentioned solution was: 1ml 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 $l^{-1}$ $MnCl_2$ $4H_2O$, 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 plants were placed in a growth chamber with the same conditions as in the case of the above-mentioned pot experiment. The experimental design was a randomised block with four replicate plastic bowls for each treatment. The above- and below-ground growth of beans were measured after 7 days and used as an index of allelochemical activity. There the same data analysis like in oat was conducted, and the $I_{50}$ value for bean root dry weight was also determined.

Furthermore, the same dilutions of the initial inflorescence extracts of quinoa were tested via another bioassay using as test plant a species of duckweed (*Spirodella polyrhiza* L.) and measuring the decrease of its fresh weight. This plant indicator has been used in several allelopathic studies, since the bioassay is sensitive and reliable especially at the first steps of a screening procedure. 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 (Leather and Einhellig, 1985; Economou et al., 2002).

## RESULTS AND DISCUSSION

### Pot experiment

The response of oat bioassay to the quinoa debris varied among the three types. The inflorescences showed a higher phytotoxic effect on the oat underground growth. The fresh and dry weight accumulation was significantly inhibited, more than the leaves and roots, when they were incorporated on the perlite surface (Table 1). Fresh and dry weights of underground oat parts were significantly inhibited from phytotoxins derived from quinoa inflorescences, even if inhibitory substances of intermediate strength were released from quinoa debris derived from leaves and roots, too. Our observations further support and extend the biological activity of compounds derived of *Chenopodium* species (Jiménez-Osornio et al., 1996; El-Khatib et al., 2004; Batish et al., 2006). These findings are in accordance with those of Putnam and Duke (1978), Rice (1984) and Travlos and Paspatis (2008), whose studies showed that quantities of allelochemicals within plants vary with plant tissue.

*Table 1*. Response of oat growth to perlite-incorporated plant tissues of *Chenopodium quinoa* debris

| Plant tissue | Above ground | | Underground | |
|---|---|---|---|---|
| | Fresh wt[a] (mg) | Dry wt (mg) | Fresh wt (mg) | Dry wt (mg) |
| Inflorescences | 132.15 (16.91)* | 8.63 (0.78)* | 215 (17.32)*[b] | 12.10 (1.38)* |
| Leaves and stems | 166.23 (11.18)* | 9.87 (1.29) | 302.5 (60.76)* | 13.96 (3.12)* |
| Roots | 182.44 (9.21) | 10.14 (1.23) | 312.13 (26.81) | 14.87 (1.41) |
| Control | 205.71 (22.03) | 11.80 (2.64) | 366.05 (23.86) | 16.79 (1.26) |

[a]Average of four replicates and in parathensis the corresponding standard deviation.
[b]Values are significantly different from those of respective control at the levels of *P<0.05 according to LSD test.

Allelochemicals may be synthesized and stored in other tissues and then transported into new leaves and inflorescence. Alternatively, it may indicate transport of allelochemicals from leaves to the inflorescences (Heisey, 1990). Furthermore, as the radicles of newly germinated seeds are very susceptible to phytotoxins, it is possible that one of the reasons of the wide distribution and dominance of *C. quinoa* is due to allelopathic potential of the species.

**Plants extract bioassays**

The tissue debris of quinoa was found to have an inhibitory effect on the oat growth bioassay and the crude plant extracts also demonstrated inhibition in oat seedling growth. In table 2, the inhibitory effects of a range of several aqueous extracts of leaves and stems, roots and inflorescences on oat growth is shown. There is a strong inhibition response of the inflorescence sample, as the $I_{50}$ estimate (26 mg $ml^{-1}$) was about five to six times lower than the average $I_{50}$ estimates of the leaf and root extracts, respectively. The strong inhibitory action of the sample of inflorescences was true even from the concentration of 10 mg $ml^{-1}$, as long as the oat radicle length was 45 % lower than the control.

Even if the inflorescence extract was significantly more effective than leaves and roots, for all the tissues radicle elongation 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 (Einhellig, 1986).

*Table 2*. Response of oat radicle elongation to the allelopathic components from *Chenopodium quinoa* crude extracts

| Roots | | Leaves and stems | | Inflorescences | |
|---|---|---|---|---|---|
| Concentration (mg $ml^{-1}$) | Radicle length (cm) | Concentration (mg $ml^{-1}$) | Radicle length (cm) | Concentration (mg $ml^{-1}$) | Radicle length (cm) |
| Control | 14.0 (0[a]) | Control | 13.5 (0) | Control | 13.7 (0) |
| **10** | **11.3 (19)** | **10** | **11.6 (14)** | **5** | **8.9 (35)** |
| 20 | 10.9 (22) | 20 | 10.2 (24) | 10 | 7.6 (45) |
| 40 | 8.9 (36) | 40 | 9.0 (33) | 20 | 7.2 (47) |
| 80 | 8.2 (41) | 80 | 7.7 (43) | 40 | 5.8 (58) |
| 160 | 6.9 (51) | 160 | 6.2 (54) | 80 | 4.4 (68) |
| 320 | 5.8 (59) | 320 | 4.9 (64) | 160 | 2.1 (85) |
| $I_{50}$[b] = 157 mg $ml^{-1}$ | | $I_{50}$ = 134 mg $ml^{-1}$ | | $I_{50}$ = 26 mg $ml^{-1}$ | |

[a]Numbers in parentheses indicate percent inhibition.
[b] The $I_{50}$ values represent the concentration of the allelopathic components to cause 50 % inhibition of radicle growth as determined by probit analysis.

The response of bean growth to quinoa extracts varied among the three types. The aqueous extracts of inflorescences showed a higher phytotoxic effect, especially on the bean underground growth. The fresh and dry weight accumulation was significantly inhibited in that case, more than the corresponding parameters of leaves and roots, when they were added to the growth medium of beans (Table 3).

*Table 3*. Response of common bean growth to *Chenopodium quinoa* extracts

| Plant tissue | Above ground | | Underground | |
|---|---|---|---|---|
| | Fresh wt (mg) | Dry wt (mg) | Fresh wt (mg) | Dry wt (mg) |
| Inflorescences | 1083 (91.33[a])*[b] | 77.76 (12.82)* | 265.6 (21.82)* | 69.14 (11.6)* |
| Leaves and stems | 1420 (122.61)* | 91.22 (11.73)* | 392.08 (17.6)* | 101.3 (19.17)* |
| Roots | 1690 (215.82)* | 93.5 (20.16)* | 446.1 (22.12)* | 124.52 (16.63) |
| Control | 2532.8 (214.38) | 190 (22.87) | 780 (82.75) | 175 (31.14) |

[a]Average of four replicates are followed by standard deviations in parenthesis.
[b]Values are significantly different from those of respective control at the levels of * P<0.05 according to LSD test.

Fresh and dry weights of underground bean parts were significantly inhibited from phytotoxins derived from quinoa inflorescences, even if inhibitory substances of intermediate strength were released from quinoa extracts of leaves and roots, too (Table 4). The differentiation of allocation of allelochemicals within the plants of quinoa is now well documented, while the high allelopathic activity of inflorescences is common among several weed species (Economou et al., 2002; Batish et al., 2006; Travlos and Paspatis, 2008).

*Table 4*. Response of duckweed fresh weight and common bean roots dry weight to the extracts of *Chenopodium quinoa* inflorescences

| Concentration (mg $ml^{-1}$) | Duckweed fresh weight (% inhibition) | Bean roots dry weight (% inhibition) |
|---|---|---|
| Control | 0[a] | 0 |
| 5 | 9 | 17) |
| 10 | 22 | 28 |
| 20 | 24 | 40 |
| 40 | 26 | 45 |
| 80 | 58 | 48 |
| 160 | 81 | 59 |
| 320 | 91 | 69 |
| $I_{50}$[a]=70 mg $ml^{-1}$ | | $I_{50}$=86 mg $ml^{-1}$ |

[a]The $I_{50}$ values represent the concentration of the allelopathic components to cause 50 % inhibition of duckweed fresh weight and the dry weight of bean roots as determined by probit analysis, respectively.

Moreover, the same dilutions of the initial inflorescence extracts of quinoa were tested via another bioassay using duckweed and measuring the decrease of its fresh weight. The inflorescence extracts caused a considerable inhibition response to both, bean and duckweed plants. It is also noticeable that only 320 mg $ml^{-1}$ of the most effective aqueous extract (from the inflorescences) could almost totally inhibit duckweed growth.

Nowadays, there is a certain need for the development and promotion of more environmentally feasible methods of pest control and especially weeds (Putnam and Duke, 1978; Travlos and Paspatis, 2008). Among such approaches as integrated weed management, the primary utility of allelopathy could be a solution, even if the positive impact of allelochemicals has only recently been seriously discussed. Under this view, the indicated allelopathic activity of noticeable plants like *C. quinoa* could be exploited and accomplished with future studies focusing on the identification and isolation of the allelochemicals. Besides, and despite the several limitations, allelochemicals should certainly serve as a model for future herbicides if environmental compatibility is a required feature.

## CONCLUSIONS

The allelopathic effects of three plant tissues of *Chenopodium quinoa* (leaves, roots

and inflorescences) on oat growth were investigated. Our results indicate that different parts of quinoa had different allelopathic activity. The tested species (oat, bean and duckweed) exhibited greater phytotoxic response from the inflorescence tissues, than from the other parts of quinoa (leaves and roots).

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