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The effect of the natural extracts of garlic or *Eucalyptus* on the growth, yield and some chemical constituents in quinoa plants

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Abstract

Background: Quinoa seeds contain highly nutritive compounds, so it acquired increasing interest during the last years

Materials and methods: A field experiment was conducted at the experimental station of Agricultural Production and Research Station, National Research Centre, El Nubaria Province, El Behaira Governorate, Egypt, during two successive winter seasons (2016/2017 and 2017/2018). In this experiment, quinoa plants were sprayed with clove extract of garlic or leaf extract of *Eucalyptus globulus* with concentrations 5, 10 and 15% in addition to the untreated control treatment. Quinoa plants were sprayed twice 86 and 93 DAS (days after sowing).

Results: The results of vegetative growth stages indicated increases in quinoa growth due to the treatments with different concentrations of the two mentioned extracts. The results also revealed increases in photosynthetic pigments constituents (chlorophyll a, chlorophyll b and carotenoids) of quinoa leaves. The results also indicated increases in yield and oil content as well as different studied metabolic activity.

Conclusion: We can conclude from these results that spraying quinoa plants with natural clove extract of garlic or leaf extract of *Eucalyptus* for increasing growth and consequently yield.

Keywords: Garlic, *Eucalyptus*, Quinoa, Growth, Yield, Biochemical constituents

Background

Quinoa (*Chenopodium quinoa* Willd.) is an annual dicotyledonous herbaceous crop of the *Amaranthaceae* family and local to the Andean region in South Africa (Fuentes et al. 2012). Quinoa is a great wheat-free alternative to starchy grains. Increasing yield for an ever-growing world population has currently become a topic of great concern with regard to food security. Especially in Africa, agricultural productivity has not been able to cope with population growth, leading to increase in annual imports and food insecurity (Elewa et al. 2017).

Quinoa seeds contain highly nutritive compounds, so it acquired increasing interest during the last years (Nowak et al. 2016). Quinoa contains all ten essential amino acids, and its protein content ranges from 12.9 to 16.5% (Saturni et al. 2010 and Vega-Gálvez et al. 2010). In addition, its high content of the two essential amino acids lysine and methionine are deficient in many grains and legumes (Gesinski and Nowak 2011; Sánchez-Chino et al. 2015). Quinoa seeds have high biological protein value (73%) that is higher than rice, wheat and corn (56, 49 and 36% respectively) as have been reported by Vega-Gálvez et al. (2010) and Gesinski and Nowak (2011). It contains also high concentration of unsaturated fatty acids. The total lipid content of quinoa is 14.5%, with about 70–89.4% unsaturated fatty acids and contains many vitamins, such as thiamine, folic acid, vitamin C, vitamin B_6 and vitamin E (Vega-Gálvez et al. 2010). In the same trend, it contains mineral as calcium, magnesium and potassium in

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sufficient quantities and in bioavailable forms necessary for maintaining a balanced human diet (González Martín et al. 2014 and Nascimento et al. 2014) and large antioxidant capacity (Vega-Gálvez et al. 2010; Tang et al. 2016 and González Martín et al. 2014).

Plants produce metabolites which are inhibitors or stimulators depending on their concentrations. Some of these compounds are produced at certain concentrations that are phytotoxic to receiving organisms or stimulators (Rice 1984) and subsequently alter the growth or physiological functions of receiving species. These metabolites are extracted from different allelopathic plants such as *Eucalyptus globulus*.

The *Eucalyptus* species possess high allelopathic activity (Sasikumar et al. 2001 and Singh et al. 2005). Chromatographic analysis showed the presence of coumaric, gallic, gentisic, hydroxybenzoic, syringic and vanillic acids in *Eucalyptus globulus* leaf extract (Sasikumar et al. 2001 and El-Rokiek et al. 2011).

The garlic extract is the sap of garlic bulb *Allium sativum* (L.), which belongs to the family *Liliaceae*. It is distinguished by containing a high amount of amino acids, which contain sulphur element, such as cysteine and methionine (Synge 1971). As well, garlic contains the following materials: volatile oil, allicin, alliin, sugar, iodine and vitamins (Al Mayahi and Fayadh 2015). As mentioned by Abou Hussein et al. (1975a, 1975b), the garlic extract has many effects due to its hormonal (auxin-like) nature, which has an important role in lateral extension and elongation of cells. Concerning garlic acid effect, Helmy (1992) reported that application of fresh garlic clove extract solution either in ethyl alcohol or tap water to summer squash cv. Eskandarani plants at 250 mg DW/plant gave the best results in increasing the number of flowers. Ahmed et al. (2005) found that a greater increase in the number of pods of pea (cv. Meteor) was obtained with post-inoculation treatment with garlic extract at 10 g/l. Treatment of *Majorana hortensis* and *Salvia officinalis* by garlic extract at the concentration of 50 or 100% increased fresh, and dry weights, photosynthetic pigments of chlorophylls a and b and total soluble carbohydrates content in the first and second cut as well as the total oil content (Mady 2009).

Objective

This work aimed to increase quinoa yield by using low concentrations of garlic cloves and *Eucalyptus globulus* leaf extracts.

Material and methods

Allium sativum (garlic) plants were allowed for complete dryness in shadow, then the cloves were separated, cut and ground. Water extracts at concentrations of 15, 30, 45 and 60% were prepared.

A field experiment was carried out at the experimental station of Agricultural Production and Research Station, National Research Centre, El Nubaria Province, El Behaira Governorate, Egypt, at two successive winter seasons (2016/2017 and 2017/2018). Quinoa (*Chenopod quinoa*) cultivar (cv. Quinoa 1) was obtained from the Egyptian Ministry of Agriculture. The soil was ploughed twice, ridged and divided into plots. The area of each plot was 10.5 m^2 (15 rows; 3.5-m long and 20 cm apart between rows). Quinoa grains were cultivated during the first week of December (7 December 2016 and 2017). The plants were sprayed on both surfaces of the leaves with different concentrations of garlic clove water extract or *Eucalyptus globulus* water leaf extract at concentrations 0, 5, 10 and 15% twice at 86 and 93 days after sowing. The experimental design in this study was a randomized complete block design with four replicates. Samples of ten plants were taken at random from each plot of the four replications for determination of vegetative growth parameters (plant height, number of branches/plant, plant fresh and dry weight) and photosynthetic pigments.

At harvest, the weight of seeds/plant as well as the weight of 1000 seeds were taken. In addition, some chemicals in the analysis were determined in the yielded seeds such as carbohydrates%, oil %, some element content such as nitrogen, phosphorus and potassium, total phenolic contents, and flavonoids contents as well as antioxidant activity.

Measurements

Photosynthetic pigments

Total chlorophyll a and b and carotenoid contents in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue was ground in a mortar and pestles using 80% acetone. The optical density (OD) of the solution was recorded at 662 and 645 nm (for chlorophylls a and b, respectively) and 470 nm (for carotenoids) using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in milligrams per gram fresh weight.

Total carbohydrate

Determination of total carbohydrates was carried out according to Herbert et al. (1971). A known mass (0.2–0.5 g) of dried tissue was placed in the test tube, and then 10 ml of sulphuric acid (1 N) was added. The tube was sealed and placed overnight in an oven at 100 °C. The solution was then filtered into a measuring flask (100 ml) and completed to the mark with distilled water. The total sugars were determined colourimetrically according to the method of Dubois et al. (1956) as follows: an aliquot of 1 ml of sugar solution was transferred into a test tube and treated with 1 ml of 5% aqueous phenol solution followed by 5.0 ml of concentrated sulphuric acid. The tubes were thoroughly shaken for 10 min then placed in a water bath at 23–30 °C for 20 min. The optical density of the developed colour was measured at 490 nm using Shimadzu spectrophotometer model UV 1201.

Oil determination

The oil of quinoa seeds was extracted according to Kates and Eberhardt (1957); the powdered seeds are shaken overnight with isopropanol to chloroform (1:1). The solvent was evaporated under reduced pressure of the CO_2 atmosphere. The lipid residue is taken up in chloroform to methanol (2:1 *v*/*v*) and given a Folch wash; the dissolved total oils were purified by washing with 1% aqueous saline solution. The aqueous phases were washed with chloroform that was combined with the pure oil solution. Chloroform was evaporated and the total pure oil was weighed.

Element contents

Nitrogen, phosphorus and potassium contents were determined according to the official and modified methods of analysis (A.O.A.C. 1984).

Total phenolic contents

Total phenolic contents were extracted from dry seeds. A known weight was taken and extracted with 85% cold methanol (*v*/*v*) three times at 0°C. The combined extracts were collected and made up to a known volume with cold methanol. Then, 0.5 ml of the extraction was added to 0.5 ml Folin, shaken and allowed to stand for 3 min. Then, 1 ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60 min. The optical density was determined at a wavelength of 725 nm using a spectrophotometer as described by Danil and George (1972).

Free radical scavenging activity (DPPH%)

The free radical scavenging activity by different plant extracts was done according to the method reported by Gyamfi et al. (2002). Fifty microlitres of the plant extract in methanol, yielding 100 μg/ml respectively in each reaction, was mixed with 1 ml of 0.1 mM diphenyl-2-picrylhydrazyl (DPPH) in methanol solution and 450 μl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 μl) only was used as control of the experiment. After 30 min of incubation at room temperature, the reduction of the DPPH free radical was measured reading the absorbance at 517 nm. L-ascorbic acid and BHT were used as controls. The percent inhibition was calculated from the following equation:

$$\%\text{inhibition} = [\text{absorbance of control} \\ \text{-absorbance of test sample} \\ /\text{absorbance of control}] \times 100$$

Flavonoids contents

The flavonoid content of the crude extract was determined by the aluminium chloride colourimetric method (Chang et al. 2002). In brief, 50 μL of crude extract (1 mg/mL ethanol) was made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution was added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as milligram Rutin equivalent per gram dry weight.

Statistical analysis

Statistical analysis was statistically analyzed at 5% probability according to Snedecor and Cochran (1980).

Results

The results in Table 1 indicate that the vegetative growth characters shoot length, number of branches and fresh and dry weight of quinoa plant significantly increased at all concentrations of garlic clove extract as well as *Eucalyptus* leaf extract. The increase in these parameters reached maximum significant value using 15% of garlic clove extract or 10% of *Eucalyptus* leaf extract in comparison to the untreated control.

Data reveal that spraying quinoa plants with different concentrations of garlic clove extract or *Eucalyptus* leaf extract caused significant increases in the contents of chlorophyll a, chlorophyll b and carotenoids in quinoa leaves over untreated control. The remarkable significant increase was measured at 15% garlic clove extract or *Eucalyptus* leaf extract (Table 2).

Spraying garlic clove extract and *Eucalyptus* leaf extract at different concentrations showed significant increases in both plant height and number of branches/plant as well as dry weight/plant at the end of the season (Table 3). In addition, seed yield/plant and weight of 1000 seeds of quinoa plants revealed significant responses in comparison to their corresponding controls. The highest significant increase in seed yield/plant exceeded 100% of the untreated plant (121.6%) with the treatment of garlic extract at 15%. The weight of 1000 seeds realized corresponding results (58.8%) over an

Table 1 Effect of natural extracts of garlic and *Eucalyptus* on the vegetative growth of quinoa

Treatments	Concentration (%)	Shoot length	No. of branches	Fresh wt. (g)	Dry wt. (g)
Control	0	26.52	4.67	5.52	1.03
Garlic clove extract	5	32.52	5.33	6.45	1.85
	10	39.55	5.33	6.95	1.54
	15	36.52	6.52	7.52	1.65
Eucalyptus leaf extract	5	34.52	5.32	6.41	2.14
	10	38.52	5.67	7.52	2.34
	15	35.24	6.24	7.68	2.45
LSD at 5%		0.69	0.73	0.13	0.07

untreated plant with this later treatment. The increase in growth and yield was accompanied with a significant increase in the contents of total carbohydrates over the contents in seeds of untreated plants.

Quantitative estimation of oil in quinoa seeds revealed a significant increase in oil content by the natural extract of garlic and *Eucalyptus* application over the untreated control. The maximum significant increase was found with the treatment of garlic clove extract at 15% (Table 4). Regarding nitrogen and protein, the treatments of garlic and *Eucalyptus* showed significant increases over the untreated seeds with an elevated level at 10% of garlic cloves. Similarly, phosphorus and potassium recorded high significant increase over their corresponding controls reaching to marked level using *Eucalyptus* extract at 10%. The antioxidant activity and flavonoids as well as phenolic compound contents indicated significant increases over their corresponding controls. The treatment with *Eucalyptus* extract at 10% resulted in the maximum levels of the later three constituents (Table 4).

Discussion

Garlic (*Allium sativum* L.) extract as well as *Eucalyptus globulus* are thought to be good allelochemicals resources (Wang et al. 2014; El-Rokiek et al. 2011) and consequently can efficiently realize good management in agricultural sustainable development.

The results in Table 1 revealed a significant increase in quinoa growth represented by plant height, number of branches and fresh and dry weight by using leaf extract of *Eucalyptus* and garlic clove extract. The results also indicate an increase in photosynthetic pigment contents. This growth improvement in quinoa plants can be established by the fact that garlic extracts contain various growth-promoting compounds such as starch and vitamins and organosulphur compounds such as allicin and diallyl disulphide, etc. (Puvaˇca et al. 2014; Martins et al. 2016). However, in the current findings, there seems to be a strong correlation between the morphological indices and the developmental aspects such as chlorophyll or carotenoid contents. Several documented results revealed an increase in plant growth by using garlic extract, for example, increase in the number of flowers in summer squash cv. Eskandarani (Helmy 1992). Similar results also on squash were also obtained by El-Desouky et al. (1998). In addition, garlic extract at 10 g/L caused a great increase in the number of pods of pea plants (Ahmed et al. 2005). Similar results were obtained by Mady (2009) on *Majorana hortensis* and *Salvia officinalis*. Likewise, Hanafy et al. (2012) reported that the highest values

Table 2 Effect of natural extracts of garlic and *Eucalyptus* on contents of chlorophyll a, chlorophyll b and carotenoids in quinoa leaves

Treatments	Concentration (%)	Chl. a (mg/g fresh wt.)	Chl. b (mg/g fresh wt.)	Carotenoids (mg/g fresh wt.)	Total pigments (mg/g fresh wt.)
Control	0	12.52	8.54	4.52	25.58
Garlic clove extract	5	13.52	8.96	5.15	27.63
	10	14.52	10.15	5.95	30.62
	15	15.42	10.95	6.12	32.49
Eucalyptus leaf extract	5	13.45	9.01	4.97	27.43
	10	14.47	10.15	5.32	29.94
	15	15.95	10.74	6.54	33.23
LSD at 5%		0.43	0.10	0.27	0.55

Table 3 Effect of natural extracts of garlic and *Eucalyptus* on yield and yield components of quinoa plants

Treatments	Concentration (%)	Plant height (cm)	No. branches/ plant	Dry wt. (g)/ plant	Wt. of seeds (g)	Wt. of 1000 seeds(g)	Carbohydrates (%)
Control	0	53.66	10.66	9.68	4.66	2.33	60.25
Garlic clove extract	5	45.66	21.66	10.28	5.81	2.69	62.52
	10	54.00	29.00	16.75	8.65	3.02	62.78
	15	64.00	30.66	20.01	10.33	3.70	63.52
Eucalyptus leaf extract	5	50.33	18.66	10.15	6.52	3.13	61.52
	10	54.33	26.00	17.52	8.74	2.63	63.54
	15	42.33	15.00	9.05	6.85	3.44	63.47
LSD at 5%		2.61	3.24	2.73	0.53	0.14	0.40

of plant height, stem diameter, dry weight of leaves/plant, leaf area, total carbohydrates and N contents in *Schefflera arboricola* were obtained with garlic extract. Moreover, Sikandar et al. (2018) garlic extract treatment increased the growth parameters of pepper plant.

The *Eucalyptus* species possess high allelopathic activity that might be inhibitors or stimulatory according to concentrations (Singh et al. 2005). These allelopathic extracts contained different phenolic acids which might be phytotoxic or promoters (Sasikumar et al. 2001). In the present work, the used concentrations were promoters for quinoa growth, therefore increasing growth parameters, photosynthetic pigments and consequently yield.

The allelochemical materials in allelopathic plants might be phenolic acids as have been reported by many workers (Chon et al. 2003; Hegazy and Farrag 2007). In addition, the analysis of *Eucalyptus globulus* leaf extract by El-Rokiek et al. (2014) revealed the presence of phenolic acids such as caffeic, ferulic, benzoic, chlorogenic, hydroxybenzoic and cinnamic acids. Phenolic compounds played a significant role in the regulation of plant metabolic processes and act as a substrate for many antioxidants enzymes (Khattab 2007). Sharma et al. (2015) suggest that phenolic acid, e.g. ferulic acid at low concentration, can be used for improving the performance of wheat under various environmental constraints. Also, it was found that some phenolic acids at low concentrations stimulated primary root length, number and length of secondary roots and dry weight of *Deschampsia flexuosa* and *Senecio sylvaticus* (Kuiters 1989). Salicylic acid as an example at low concentration is an endogenous growth regulator of phenolic nature and acts as a potential non-enzymatic antioxidant that participates in the regulation of many physiological processes in plants, such as stomatal closure, photosynthesis, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan et al. 2003; Arfan et al. 2007). These results coincided with our obtained current results (Tables 2, 3 and 4).

In addition, the increases in metabolic activity of quinoa might have resulted from a corresponding increase in photosynthetic pigments (Table 2) which in turn increased carbohydrate contents in addition to the increase in protein due to the increase in nitrogen content (Coruzzi and Last 2000).

Table 4 Effect of natural extracts of garlic and *Eucalyptus* on the different chemical constituents of quinoa seeds at the end of the season

Treatments	Concentration (%)	Percentage (%)						Milligrams per gram dry weight	
		Oil	Protein	N	P	K	DPPH	Flavonoids	Phenolics
Control	0	8.48	14.49	236.22	266.55	398.63	34.28	41.69	111.25
Garlic clove extract	5	9.02	15.42	248.95	286.45	421.35	43.12	45.91	123.32
	10	9.67	16.58	263.63	321.47	439.30	47.02	50.31	136.87
	15	9.69	15.99	258.62	304.29	416.05	44.26	47.62	127.67
Eucalyptus leaf extract	5	9.14	15.67	253.17	303.33	435.61	44.89	47.05	123.83
	10	9.89	17.17	274.74	336.01	451.13	50.20	55.44	141.90
	15	9.56	16.17	260.13	315.34	443.67	46.55	51.01	129.30
LSD at 5%		0.13	0.37	2.69	3.74	1.89	0.50	0.82	0.86

In general, the increase in plant growth was accompanied by increases in metabolic activity which represented by different chemical constituents (El-Rokiek et al. 2012).

Conclusion

The results of the current study suggested using clove extract of garlic or leaf extract of *Eucalyptus* for increasing quinoa growth and consequently yield.

Abbreviations

Chl.: Chlorophyll; cv: Cultivar; DPPH: Diphenyl-2-picryl-hydrazyl; K: Potassium; N: Nitrogen; No.: Number; P: Phosphorus; Wt.: Weight

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Authors' contributions

KGER, MSS, MGD and MEEA performed the laboratory analysis, wrote the paper, performed the data and coordinated the data collection. All authors read and approved the final manuscript.

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Availability of data and materials

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