Journal of Microbiology, Biotechnology and Food Sciences

Ismael et al. 2012 : 1 (February Special issue) 931-941

REGULAR ARTICLE

INFLUENCE OF VARIETY, LOCALITY AND SOIL CONTAMINATION ON TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF FABABEAN GRAINS

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ABSTRACT

Polyphenolic compounds are secondary metabolites present in many plant species, including legumes. Their content depends on various factors, such as cultivar, pedo-climatic and cultivation conditions. The influence of cultivar, locality and soil contamination on the total polyphenols and antioxidant activity of fababean was studied. Fababean cultivars were cultivated under different climatic conditions in Iraq and Slovak Republic. The value of polyphenol content was in the range 1583.54 - 3374.91 $mg.kg^{-1}$ and their antioxidant activity from 16.62 - 38.40 % of DPPH inhibition. The locality and variety were significant factors in explaining differences between the total polyphenols values in fababean. In fababean (cv. Saturn, Zobor) grown under model conditions of vegetation pot trial using the soil (Čakajovce locality, Slovakia) with graduated loading by Zn, the influence of soil contamination on TP content and AOA was not confirmed, only the correlation between cumulated Zn content and TAC in cv. Zobor (at the highest contamination) was strongly negative (r= -0,99).

Keywords: fababean, polyphenols, antioxidant activity, zinc

INTRODUCTION

Legumes are excellent source of protein, dietary fibre, starch, micronutrients and bioactive compounds with low level of fat. Pulses have shown numerous health benefits, e.g.

lower glycaemic index for people with diabetes, increased satiation and cancer prevention as well as protection against cardiovascular diseases due to their dietary fibre content **(Chillo *et al.*, 2008)**. Legumes are a rich source of polyphenols, which have high antioxidant activities **(Cardador-Martinez *et al.*, 2002; Troszynska *et al.*, 2002)**. Recently there has been great interest in evaluation of antioxidant activity of phenolic compounds in leguminous seeds. Antioxidant activity has been reported for extracts of legumes such as pea; white, green, red and navy beans; beach pea; lentils; everlasting pea; Jack bean; azuki bean; and cowpea **(Lopez-Amoros *et al.*, 2006; Rocha-Guzman *et al.*, 2007)**. However, in recent years, there has been an increasing interest in other legumes such as fababean.

The common names of fababean (*Vicia faba* L.) are broad bean, faba bean, horsebean, windsorbean, tickbeans. Dry beans are widely known for their fibre, mineral and protein contents; however, its nutraceutical value is yet to gain as much attention in the prevention of chronic diseases **(Dinelli *et al.,* 2006)**. Despite its importance, few studies have been conducted to analyse the application of micronutrients to chickpea or bean, e.g. Zn. Zinc is one of the most important trace elements essential to human, and zinc deficiency is common in most of the legume growing areas of the world. Next, the presence of antinutrient, e.g. polyphenols seems to be one of the reasons why zinc is a limiting nutrient in many diets of people dependent on beans as a staple food. On the other hand, polyphenols presence in food is connected with antioxidant effect on human health. The current indicators for zinc deficiency, such as plasma or hair zinc concentrations, have poor sensitivity and specificity and do not change with marginal zinc deficiency **(Gibson *et al.*, 2008)**. The consequences of zinc deficiency in adults have been understudied despite the recognition of symptoms of Zinc deficiency for decades. Moreover, a considerable body of evidence suggests that Zinc deficiency may increase the risk of some chronic diseases including cancer **(Leone *et al.*, 2006)**. Polyphenols are most commonly distributed in plant kingdom and phenolic compounds play many roles in higher plants. They may combine with proteins either reversibly by hydrogen bonding or irreversibly by oxidation. When phenols become oxidized they form compound called quinones. Being an important group of secondary metabolites, phenolics may act as modulators of plant development by regulating indoleacetic acid catabolism **(Arnaldos *et al.*, 2001)**.

The type of cultivar, growth location, and soil contamination **(Lachman *et al.*, 2006; Musilová *et al.*, 2010)** can influence the content of polyphenols, too. Therefore the comparing the contents of polyphenols in the seeds of fababean grown in the different localities (Slovakia, Iraq) were the objective of our study. Next, in model conditions of vegetation pot experiment, we investigated the effect of accumulation of selected micro nutrient content (zinc) on total polyphenols and antioxidant activity assessed in faba bean grown in soil with increasing rates of the zinc contamination.

MATERIAL AND METHODS

Material

Fababean (*Vicia faba* L.) samples at full ripeness were obtained from local market in Slovakia (cv. Saturn, cv. Zobor from Zelseed firm), and two varieties (marked as R2, R3) were provided from Erbil Research in Iraq.

Extraction

For 12 hours extraction, dry milled material (5 g) was used and continuously extracted by a Twisselmann extractor with methanol (80%, v/v).

Total polyphenols (TP)

Total polyphenols were determined by the method of **Lachman *et al.* (2003)** and expressed in mg eq. gallic acid per kg dry matter. The total polyphenol content was estimated using Folin-Ciocalteau assay (Merck, Germany) on the spectrophotometer Shimadzu 710 (Japan).

Antioxidant activity (AOA)

The free radical scavenging activity of the extracts was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method of **Brand-Williams *et al.* (1995)**.

Soil

The contents of available nutrients in the solution were determined by Mehlich III method. Contents of risky heavy metals were determined in different soil extracts *(aqua* *regia*; NH_4NO_3, c=1 $mol.dm^{-3}$; HNO_3, c=2 $mol.dm^{-3}$). Atomic absorption spectrometry analysis was finally used.

It was difficult to compare the influence of locality (Slovakia, Iraq) by data which vary in using extraction solvent, conditions and legislative. For this reason, in this work soils were evaluated according to recent legislative norm valid in Slovakia (Law No. 220/2004). By this norm, the limit values of risky elements are considered to be critical values of agricultural soil in relationship to the plant and are also harmonized with EU limits.

- In Iraq there are mountains over 3.000 meters above sea level along the border with Iran and Turkey to the remnants of sea-level marshes in the southeast. Much of the land is desert or wasteland. Average annual temperatures range from higher than 48°C in July and August, below freezing in January. Annual average rainfall is 100 and 180 mm. Our area of interest was the mountainous region of northern Iraq, which receives appreciably more precipitation than the central or southern desert region. The exact Iraqi locations of the cultivation areas were: Aqra (36°44'42"N, 36°56'42"E), Erbil Ankawa (36°14'6"N, 44°02'42"E), Erbil (36°16'12"N, 43°50'E), Alton kupri (35°45'18"N, 44°09'18"E). Characteristics of soils of the four Iraqi locations are presented by Tables 1-3.

Table 1 Soil reaction, nutrients content ($mg.kg^{-1}$) and humus content (%) in soils from Iraq

Locality	pH (KCl)	Ca	Mg	K	P	N	hummus
Aqra	7.69	10856.0	314.0	302.5	88.9	609.0	3.41
Erbil Ankawa	7.64	10558.0	328.0	290.5	23.4	609.0	2.44
Erbil Research	7.67	10872.0	328.0	307.5	23.7	504.0	1.46
Alton kupri	7.60	10808.0	296.0	256.5	26.4	546.0	3.41

Table 2 Heavy metals content ($mg.kg^{-1}$) in soils from Iraq (soil extract by *aqua regia*)

Locality	Zn	Cu	Co	Ni	Cr	Pb	Cd
Aqra	64.4	25.8	24.2	125.2	56.0	20.2	1.22
Erbil Ankawa	61.6	25.2	24.2	122.6	54.8	19.0	1.28
Erbil Research	63.2	25.6	23.2	122.4	51.8	20.8	1.28
Alton kupri	61.0	26.0	22.2	122.4	52.2	21.0	1.34
limit value*	150	60	15	50	70	70	0.7

*Law 220/2004

Table 3 Heavy metals content (mg.kg^{-1}) in soils from Iraq (soil extract by NH_4NO_3, c=1 mol.dm^{-3})

Locality	Zn	Cu	Co	Ni	Cr	Pb	Cd
Aqra	0.070	0.065	0.080	0.125	0.005	0.115	0.014
Erbil Ankawa	0.080	0.075	0.090	0.095	0.030	0.125	0.014
Erbil Research	0.070	0.080	0.100	0.135	0.025	0.195	0.021
Alton kupri	0.070	0.095	0.085	0.115	0.020	0.140	0.018
critical value*	2	1	-	1.5	-	0.1	0.1

*Law 220/2004

Soils, from which the fababean samples were taken, had alkali soil reaction and they presented medium and high humus concentrations levels. The values of total risk elements contents (Table 2) in soil extract by *aqua regia* were under the concentrations of defined limit value with the exception of Cd and Ni, because its total contents were exceeded on all sites (in average 41.6% and 60.1% respectively). For assessing of the hygienic status of soils, the mobile and available forms of risk elements are the most important. In soil samples the releasable risk elements contents were also determined in the solution of NH_4NO_3 (c = 1 mol.dm^{-3}). All of determined values were lower than critical value (Table 3), only the maximal available soil content of mobile Pb forms was exceeded. In soils with alkali soil reaction these forms are less mobile (soil reaction is one of the factors influencing risk elements toxicity to plants: the most of them become more available to plants as pH decreased).

Under the model conditions of pot trial the rate of polyphenols accumulation in fababean grains depending on the extent of soil contamination by zinc that was applied to the soil in the form of solutions of its soluble salts was observed.

Characteristics of soil collected from site Čakajovce (Nitra upland with annual rainfall 600-700 mm, and annual temperature 8-9 ^{o}C, Slovak Republic) are given in Table 4.

Table 4 Nutrients and heavy metals content ($mg.kg^{-1}$), hummus content (%) and soil reaction in the soil from Čakajovce locality

Nutrients	N	K	Ca	Mg	P	hummus	pH KCl
	1225.0	291.0	5210.0	380.0	90.8	1.4	7.2
Heavy metals	Zn	Cu	Cr	Cd	Pb	Co	Ni
Aqua regia	48.5	19.8	27.4	0.72	18.3	13.0	29.2
limit value*	***150***	***60***	***70***	***0.7***	***70***	***15***	***50***
HNO_3 ($c = 2\ mol.dm^{-3}$)	8.7	8.4	1.8	0.29	9.6	5.5	7.3
reference value A_1*	***40***	***20***	***10***	***0.3***	***30***	***-***	***10***
NH_4NO_3 ($c=1 mol.dm^{-3}$)	0.025	0.085	0.06	0.026	0.11	0.14	0.16
critical value*	***2***	***1***	***-***	***0.1***	***0.1***	***-***	***1.5***

*Law 220/2004

**Decision of the Ministry of Agriculture SR No. 531/1994-540

The soil was characterized by high content of potassium and phosphorus, very high content of magnesium, and neutral soil reaction suitable for the legume cultivation. This is the uncontaminated soil, only Cd content is slightly higher than the limit value. But the reference value A_1 (intended for potentially mobilized forms extracted by in HNO_3 solution) and critical value (intended for potentially mobilized forms extracted in NH_4NO_3 solution) were not exceeded.

In model conditions of vegetation pot experiment, we used only Slovak varieties (cv. Zobor and Saturn) in 4 variants with four replications. Six kilograms of soil was weighted into plastic bowl-shaped pots with average 20 cm and height of 25 cm with foraminate bottom. Loading of soil by zinc was realised on the basis of hygienic limits of Decision of the

Ministry of Agriculture SR No. 531/1994-540. Zinc was applied in the form of $ZnSO_4.7H_2O$:

1st variant: 0 mg $Zn.kg^{-1}$ soil

2nd variant: 40 mg $Zn.kg^{-1}$ soil (reference value A_1 in HNO_3 solution)

3rd variant: 250 mg $Zn.kg^{-1}$ soil

4th variant: 500 mg $Zn.kg^{-1}$ soil (indicative value determining contamination of soils in HNO_3 solution)

RESULTS AND DISCUSSION

Influence of variety and locality

Four fababean cultivars were cultivated in fields of regions with different environmental growth conditions. According to the obtained results, the polyphenols content (TP) in the tested cultivars was significantly different and was influenced by locality. Polyphenols content in fababean cultivars in relation to locality decreased as following: R3> R2 (Ankawa)> Saturn> R2 (Aqra)> Zobor> R2 (Alton kupri).

Table 5 Total polyphenols content (mg eq. gallic acid.kg^{-1} DM) and antioxidant activity (% of DPPH inhibition)

cultivar (locality)	TP	AOA
R2 (Aqra)	1898.71±26.20 a	16.62±0.81 a
R2 (Erbil, Ankawa)	2702.44±37.95 b	24.54±0.64 b,c,d
R2 (Alton kupri)	1583.54±13.79 a,b,c,e,f	21.66±2.12 d
R3 (Erbil Research)	3374.91±13.38 c	18.24±0.39 b,c,d
Saturn (Slovakia)	1905.38±9.37 a,e	16.94±0.56e
Zobor (Slovakia)	1741.60±14.50d,f	38.40.±0.22 f

Data are expressed as means of six replications ± standard deviation. Values in the same column with the different letters present significant differences $p < 0.05$ using t-test for independent samples.

Our results suggest that more severe climatic conditions have caused a slight increase in the total content of polyphenolic substances. For growing fababean warm and dry areas appears as the best. Therefore the R2 seeds from Alton kupri locality have shown the lowest TP content (this cultivar is grown in area suitable for growing fababean), while in the same R2 variety grown in mountain area (Aqra) with higher rainfall, was recorded more higher TP amount. **Lachman *et al.* (2006)** investigated the effect of weather conditions on the TP contents of potatoes and their results also showed that an upland cooler site with higher rainfall provide the seeds with a higher content of TP. On the other hand, the highest TP value of cv. R2 or R3 from Erbil locality with medium rainfall also indicates more influence on TP content. This statement can be also attributed to the soil type of this area (loam and clayey red colour soil with lower hummus content-Table 1). **Berger *et al.* (1999)** and **Nikolopoulou *et al.* (2007)** identified that TP content has been negatively correlated with soil clay content for legume grown in the Mediterranean environment, thus not show our results.

Antioxidant capacity was in relation to genotype, ranging from 16.62 to 24.54 % of DPPH inhibition, but unaffected by location in varieties grown in Iraq.

The conditions for growing fababean in the Slovakia are not good particularly because it is difficult to reach the required amount of seeds, also due to temperatures in the vegetation, which will satisfy the temperature requirements crops. The values of TP content of Slovak cultivars corresponds to results of cv. R2 from Iraq with similar climate condition.

About the impact site is not enough literature demonstrable knowledge and scientists attaching greater importance to other factors: the influence of variety, and stress factors.

Influence of zinc addition in the soil

Zinc deficiency is recognized as one of the most common and widespread micronutrient deficiencies in most agricultural zones. In our model conditions, we verified the ability of fababean cv. Saturn and Zobor to accumulate zinc in relation to the total content of polyphenolic substances and antioxidant activity. Intentional addition of gradual doses of Zn into soil was reflected in its content in monitored grain. Increasing levels of Zn in the soil were reflected in increasing Zn content in fababean seeds, which were decomposed with using HNO_3 by microwave digestion. Zinc content exceeded the value determined by the Food Codex SR (< 50 $mg.kg^{-1}$) only in D variant at the indicative value determining contamination of soils. Zinc bioavailability was also studied by **Khan *et al.* (1998)** and **Ramírez-Cardenas *et al.* (2010)**.

Table 6 Zinc and polyphenols content ($mg.kg^{-1}$), antioxidant activity (% inhibition of DPPH) in cv. Saturn seeds after zinc application in soil

Variant / Zn application	Zn	TP	AOA
A: 0 mg Zn^{2+} (control)	30.60	1905.38±9.37 a	16.94±0.56 a
B: 40 mg Zn^{2+}	19.85	1954.18±15.25 a,c	19.76±0.62 b
C: 250 mg Zn^{2+}	39.95	2179.33±11.90 c,d	27.99±0.92 c
D: 500 mg Zn^{2+}	50.50	2414.57±17.46 d	13.08±0.74 d

Table 7 Zinc and polyphenols content ($mg.kg^{-1}$), antioxidant activity (% inhibition of DPPH) in cv. Zobor seeds after zinc application in soil

Variant / Zn application	Zn	TP	AOA
A: 0 mg Zn^{2+} (control)	20.85	1741.60±14.50a	38.45±0.22 a
B: 40 mg Zn^{2+}	10.35	2552.40± 18.3b,c,d	40.82±0.21 b
C: 250 mg Zn^{2+}	49.60	2646.85±13.99 c	35.47±0.87 c,a
D: 500 mg Zn^{2+}	55.55	2361.08±5.47 d	38.34±0.89 a,b,c

In tables 6-7, data are expressed as mean ± standard deviation. Values in the same column with the different letters present significant differences $p < 0.05$ using t-test for dependent samples.

The reaction on the stress caused by action of heavy metals is the production of various metabolites that might have diverse effects on the antioxidant system in plants **(Bystrická *et al.*, 2011)**. In our experiment the intentional application of zinc to the soil in the relation to production of total polyphenols was studied. As it could be seen from the Tables 6-7, the results of TP showed that there are significant differences among variants of experiment. We can state that increasing levels of Zn in the soil were reflected in increasing TP content in fababean grains in A, B, C, D variants, with exception D variant of cv. Zobor (the content of TP decreased). Testing of all values, between TP content and the cumulative amount of zinc in fababean has not been confirmed statistical significance. Correlation coefficients are very low. But in the case values of D variant of cv. Zobor was determined a strong dependence (r = - 0.99) between the accumulated Zn amount and TP content. **Musilová *et al.* (2010)** did not also confirmed Cd and Zn the influence of soil contamination on TP content in potato tubers.

When evaluating AOA, the correlation between the content of accumulated zinc and antioxidant activity was not confirmed. The highest total antioxidant capacity was observed in B and C variant for Zobor, Saturn fababean seeds, respectively. For cv. Saturn, although TP content and AOA tendency increased with the increasing of amount of Zn application in soil, the value 13.08 % of AOA in D variant was the lowest among the both cultivars. For cv. Zobor, the value 38.34 % of AOA in D variant was comparable with control (38.45 %), which may be related with TP content decreasing in this variety. We can conclude, in our experiment intentional addition of heavy metals had no clear impact on the antioxidant activity. There are also many disunited opinions on the relationship of heavy metals and the antioxidant activity. **Michalák (2006)**, **Kachout *et al.* (2009)** determined the increase of AOA after heavy metals treatment. According to **Hegedűs *et al.* (2001)** the heavy metals can cause decrease in the antioxidant activity.

CONCLUSION

Legumes are considered to be a promising crop in the view of human nutrition, therefore it is important to pay attention to entry of polyphenolic compounds to food chain. Four fababean cultivars were cultivated under different climatic conditions in Iraq (cv. R2, R3) and Slovak Republic (cv. Zobor. Saturn). The polyphenols content (TP) in the tested cultivars was significantly different and was influenced by locality. The antioxidant capacity was in relation to genotype, but unaffected by location in varieties grown in Iraq. We can also conclude that the TP content in fababean seeds may increase after Zn application, but concurrently results of analysis indicated that polyphenols formation and amount (as response to stress) is probably genetically determined or is conditioned of cultivation way **(Reddivari *et al.*, 2007)**.

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