Response of Some Vegetable Legume Plants to Foliar Application of Some Antioxidants

Wael. M. Abd El-Hakim

Vegetable Res. Dept., Hort., Res. Inst., ARC, Giza, Egypt
faissalfadel@yahoo.com

Abstract: In this investigation, two field experiments were carried out at the experimental farm of Mallawy Agricultural Research Station, Minia, Egypt, during the two successive fall and winter seasons of 2012/2013 and 2013/2014**,** respectively. *Phaseolus vulgaris* cv. Nebraska and *Pisum sativum* cv. Master B*, Vicia faba* cv. Nubaria-1 (formely Giza Blanca) were used to study the influence of three antioxidants organic acids namely, Salicylic acid (SA), Vitamin E and Acetyl salicylic acid (ASA) on some yield characters, chemical constituents and antioxidative activities of total phenolic compounds (TPCs). Five concentrations i.e., 0.1, 0.5, 1.0, 2.0 and 4.0 mM were used from each antioxidant substance. Some physical properties and approximate analysis (dry matter DM, crude protein CP; crude fiber CF, crude lipids CL and ash content AC) were studied. The results indicate that the investigated legumes are rich and good sources of CP, CF and AC. Number of dry pods per plant, 100-seed weight and dry seeds yield of the three studied crops were significantly affected by using the antioxidant treatments. The best compound that gave, in average, the highest values was acetyl salicylic acid (ASA) at 1 m*M* concentration, on the other hand at 0.1 and 0.5 m*M* concentrations the highest value was obtained by using Vitamin E.. The nitrite concentration (NO_2^{-1} mg/kg) in the three legumes ranged from 4.20 to 6.5 and did not reach toxic limit level (i.e. 10 mg/kg). The highest level of nitrite was recorded in Master B and the lowest one in Nebraska. These results meaning that the consumption of these vegetable legume seeds is save. All seed samples contain less than 200 mg NO_3^{-1} ion/kg and the highest concentration (155 mg/kg NO_3^{1-}ion) was recorded in extracts of Master B and the lowest one in Nubaria 1. Concentrations of SA in legume seed samples were higher in all treatments compared with the untreated samples (control) and the uptake of SA differs according to the given doses. Changes in SA levels causing by spraying treatment was also studied and showed sharp increases in SA contents. The concentrations of TPCs and total flavonoids (TFs) in the crude extracts of whole seeds of the studied legumes were assayed and the results indicate that seeds of Nubaria-1 (dark coat seeds) contain higher levels of TPCs (8.3 mg/g) than those determined in seeds of Nebraska (7.4 mg/g) and much higher than Master B (6.5 mg/g) whereas, TFs concentration was the highest in extracts of Nebraska.

[Wael. M. Abd El-Hakim. **Response of Some Vegetable Legume Plants to Foliar Application of Some Antioxidants.** *J Am Sci* 2014;10(12s):1-12]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 1

Keywords: Legumes, antioxidants, yield, physical and chemical constituents.

1. Introduction

Dry beans (kidney bean, faba bean) are an integral part of diets in a significant portion of the world population, but the potential benefits of consuming beans from a "health benefits" point of view have largely been overlooked. The importance of the vegetable legumes (common bean, pea and broad bean) is due to its high protein content which very needed for human nutrition (Aggarwal *et al*., 2004).

Consumption of dry beans has been linked to reduced risk of cardiovascular disease, diabetes mellitus, obesity, cancer and diseases of digestive tract (Bazzano *et al.,* 2001, Geil and Anderson, 1994), heart disease (Anderson *et al.,* 1984) and colon cancer (Bazzano *et al.,* 2001). These potential health benefits of beans have been attributed to presence of secondary metabolites such as phenolic compounds that possess antioxidant properties (Lazze *et al.,* 2003; Azevedo *et al.,* 2003).

The common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes, consumed worldwide as pods of snap beans or dry seeds (Takeoka *et al.,* 2003). Common bean (*P. vulgaris* L.) is a traditional food in human diet, low in fat and rich in proteins, vitamins, complex carbohydrates and minerals (Abd El-Naem *et al.,* 2006).

The majority of the antioxidant capacity of fruits or vegetables may be from some compounds such as falvonoids, isofalvonoids, flavones, anthocyanins, catechins, vitamin C, E or β carotene (Kahkonen *et al.,* 1999).

Foliar application of vitamin E at 0.1 ml/l and 0.3 ml/l significantly improved vegetative growth and yield of bean plants compared to control plants especially at the higher concentrations (El-Tohamy and El-Greadly, 2007).

Acetylsalicylic acid (aspirin; 2-acetoxybenzoic acid) has been used for >100 years for pain (Paterson *et al*., 2006). It is one of the basic preparations used in the therapy of cardiovascular diseases, leads to irreversible reduction of platelet aggregation (Stejskal *et al*., 2001).

Salicylic acid is widely present in plants and functions as a hormonal mediator of the systemic acquired resistance response. Thus, it is present in a large scale of fruits, vegetables, herbs and spices of dietary relevance. The recognized effect of consuming fruits and vegetables on lowering risk of colon cancer may be partly attributable to salicylates in plant-based foods (Paterson *et al*., 2006).

Using antioxidants such as L-ascorbic acid, (L-AA) and salicylic acid (SA) are suggested for improving yield as well as quality of the seeds of vegetable legumes (Moustafa, 1999). Application of antioxidants, namely, salicylic acid, acetyl salicylic acid and L-ascorbic acid instead of using synthetic auxins for stimulating growth and productivity of various legume crops are considered important tasks for pomologists. These compounds, as non-enzymatic materials, have beneficial effect on catching the free radicals or the reactive oxygen species (ROS), namely, singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radicals and ozone produced during photosynthesis and respiration processes. Leaving these free radicals without chelating or catching leads to lipids oxidation, loss of plasma membrane permeability and death of cells within plant tissues. They also have an auxinic action **(**Rao *et al*. 1997).

Many publications on *P.vulgaris* have focused on antinutritional aspects of seed coat polyphenols such as condensed tannins (Elias *et al.,* 1995). However, it has been reported that polyphenols have anti-carcinogenic and antioxidant properties (Gamez *et al.,* 1998). According to (Hagerman *et al*., 1998), condensed and hydrolyzable tannins of relatively high molecular weight have also shown to be effective antioxidants with greater activity than simple phenols. It is generally believed that antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases (Cardador-Martinez *et al.,* 2002).

Polyphenols are reducing agents, and together with other dietary reducing compounds, such as vitamin C, vitamin E and carotenoids, they protect the body's tissues against oxidative stress. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases and inflammation.

Phenolic compounds are plant secondary metabolites that are biosynthesized through the shikimic acid pathway (Herrmann 1995; Taiz and Zeigler 1998). Most common classes of plant phenolics are having antioxidant properties include those derived from the products of the phenylpropanoid-acetate pathway. Flavonoids are the most abundant polyphenols in our diets. They can be divided into several classes according to the degree of oxidation of the oxygen heterocycle: flavones, flavonols, isoflavones, anthocyanins, flavanols, proanthocyanidins and flavanones.

We aimed too in this investigation to determine total phenolic compounds (TPCs), total flavonoids (TFs) in seeds of three common legumes, and study the effect of spraying treatment by three different antioxidants (SA, Vitamin E and ASA) on phenolic compounds (TPCs).

2. Material And Methods

Two field experiments were carried out at the experimental of Mallawy Agricultural Research Station, Minia, Egypt, during the two successive fall and winter seasons of 2012/2013 and 2013/2014, respectively. *Phaseolus vulgaris* cv. Nebraska, *Pisum sativum* cv. Master B and *Vicia faba* cv. Nubaria-1 were used to study the influence of three antioxidant organic acids namely, salicylic acid (SA), Vitamin E and acetyl salicylic acid (ASA) on dry seed yield, chemical constituents,.Five concentrations i.e., 0.1 m M, 0.5 mM, 1.0m M, 2 mM and 4.0 mM used prepared from each antioxidant substances. These concentrations were used as spraying treatment for the resultant plants. Control group was also conducted.

The seeds were rinsed and sown at September, 3, in the first season, (2012) and 6 at the second season, (2013) for common bean, at 14th and 17th November 2012/2013 and 2013/2014 for pea and faba bean, respectively.

Table 1: Physical and chemical analyses of the experimental soil.

Soil constituent	**Value**	**Soil constituent**	**Value**
Texture grade	Clay loam	Organic matter	1.41%
Sand	8.63%	$CaCO_3$	2.13%
Silt	58.57%	Available N	48.36 ppm
Clay	32.80%	P	11.15 ppm
pH (1.2.5 soil suspension)	8.12	K	76.15 ppm
E.C. (dslm, 1:5 soil water extract)	1.17	B	0.33 ppm

E.C. = Electrical conductivity

Fifteen treatments (3 chemical substances at five concentrations with treatment (foliar spraying) as well as control were arranged in a randomized completely randomized design with three replicates for each crop.

Seeds were sown at one side of the row at 10, 15, 20 cm apart for common bean, pea and broad bean, respectively. Each row was 4.5 m long and 0.60 m width. Each experimented plot was (10.5 m^2) and contained three ridges. Growing plants were thinned to two plants per a hill, after two weeks from sowing date. Plants were sprayed with distilled water or specific antioxidant as foliar application three times after 30, 45, 60 days from planting date..

Soil analysis was carried out according to (Wilde *et al*., 1985) and the averages of the obtained data are shown in Table (1).

Yield and its components

At harvest time, twenty plants from each plot were taken randomly to determine the average of:

(**1**) Number of dry pods/plant.

(**2**) Weight average of 100 seeds (g)

(**3**) Dry seeds yield/plant.

Chemical composition of dry seeds yield

1- Seed Samples.

Dry seeds were samples were collected from each crop and preparing for analysis.

2- Flour preparation.

The seeds were ground into meal using a mortar and coffee grinder and kept in a refrigerator at 4°C until analysis according to AOAC, (2005).

3- Preparation of dry defatted meal.

The flour samples were defatted with ice-cold acetone in a blinder. The defatted matter (acetone powder) was air dried and stored in plastic bags at 4°C until using (AOAC, 2005).

Proximate analysis:

Chemical composition of legume samples (Moisture content, total ash content, crude protein (% N x 6.25), total crude lipids, crude fibers) were determined according AOAC, (2005). All determinations were performed in triplicates and the means were calculated.

Determination of total carbohydrates (TCs):

The total carbohydrates were calculated by difference as follows:

TCs =100–(%Total lipids + %Crude fiber + %Crude ash + %Total proteins)

Extraction and determination of nitrite and nitrate: -

The nitrite and nitrate were extracted from finally powdered meal of all samples by 1% K_2SO_4 solution and determined spectrophotometrically as described by (Saad, 1991).

Determination of salicylic acid (SA):

To quantify SA, the ethyl acetate was used (5mM) for SA extraction then the extract was concentrated (1:3) under vacuum. Chromatographic examination (CE) for concentrated extract was made to distinguish either free-SA or glucosylated-SA are present. After CE; only one spot was obtained, i.e. endogenous free SA concentration was determined by adding 5 ml of 2 *M* $FeCl_3$ and 3 ml of water to 1 ml of concentrated extract (Meyer *et al.,* 1992). The absorbance of the purple iron-SA complex, which developed in the aqueous phase, was measured at 527 nm and compared with a standard curve of SA dissolved in ethyl acetate.

Extraction of total flavonoids (TFs):-

Defatted meal sample seeds (30 g) were extracted in a Soxhlet extractor with 100 ml ethanol for 1 hour and the extract filtered according to the method described by (Beninger *et al.,* 1997).

Determination of total flavonoids (TFs): -

A known volume of extract was placed 10 ml volumetric flasks. Distilled water was added (make 5 ml) and 0.3 ml $NaNO_2$ (1:20) was added then mixed. 3 ml $AlCl_3$ (1:10) were added 5 min later then after 6 min, 2 ml 1*M* NaOH was added the total volume was completed to 10 ml with distilled water. The solution were mixed well again and the absorbance was measured against a blank at 510 nm (Beninger *et al.,* 1997).

Total phenolic compounds (TPCs):-

Half gram of powdered field bean flour was refluxed with 50 ml methanol containing 1% HCl for 4 hr. The obtained extract was used to estimate the amount of phenolic compounds as tannic acid equivalent according to the Folin-Denis procedure (Forrest and Bendall, 1969).

Statistical analysis.

All data of each season were subjected to statistical analysis according to the procedure outlined by (Steel and Torrie, 1981). A combined analysis for the two seasons was applied after testing homogeneity of error variances according to Bart lett's test. The differences among the means of all treatments of all studied characters for each crop were compared by using Duncan's rang test as described by (Gomez and Gomez 1984).

3. Results and Discussion

Physical properties for studied legume seeds are given in Table (2). These results show that 100-seed weight of Nubaria-1 is the highest weight and the half of this weight is recorded for Nebraska seeds. Percentages of 100-seed coat weights compose 10-17% from the whole legume seeds.

The illustrated data in Table (3) indicated that no significant between the three antioxidants substances. The best concentration when spraying resultant plants by salicylic acid, Vitamin E and acetyl salicylic acid was1.0 mM.

Table 3: Some physical properties of the studied legumes

Constituent	Legume seeds		
	Phaseolus vulgaris var. Nebraska	*Pisum sativum* var. Master B	*Vicia faba* var. Nubaria-1
100-seed weight	50.94 g	16.10 g	115.70 g
100-seed coats wt	8.15 g	1.5 g	18.6 g
seed coats color	White	Light green	Brown

Table 4: Effect of the spraying treatment by some antioxidants on number of dry pods/plant of the three crops.

		Phaseolus vulgaris (cv. Nebraska)		*Pasium sativum* (cv.Master B)		*Vicia faba* (cv.Nubaria 1)	
Antioxidants	SA	14.58	a	14.48	a	13.63	a
	V.E	15.02	a	14.12	a	14.47	a
	ASA	15.00	a	14.42	a	13.55	a
Concentration	0.0	14.33	c	13.20	d	13.10	cd
	0.1	14.77	c	14.50	c	14.07	c
	0.5	16.20	b	15.83	b	15.13	b
	1.0	17.80	a	17.70	a	17.17	a
	2.0	14.20	c	13.03	d	12.93	d
	4.0	11.90	d	11.77	e	10.90	e
Salicylic acid (SA)	0.0	15.00	d-f	13.20	f-k	13.10	cd
	0.1	14.60		14.10	d-i	13.80	cd
	0.5	16.10	c-e	15.60	c-e	14.60	bc
	1.0	17.80	ab	17.70	ab	17.10	a
	2.0	12.50	gh	12.80	g-k	12.50	de
	4.0	11.60	h	12.00	jk	11.00	e
Vitamin (E)	0.0	14.50	ef	14.20	d-h	14.00	cd
	0.1	14.90	d-f	14.90	d-f	14.50	bc
	0.5	16.40	b-d	16.00	b-d	16.00	ab
	1.0	17.50	a-c	16.90	a-c	16.80	a
	2.0	14.60	ef	12.70	g-k	14.00	cd
	4.0	11.60	h	11.20	k	10.70	e
Acetyl salicylic acid (ASA)	0.0	13.50	fg	12.20	h-k	12.20	de
	0.1	14.80	d-f	14.50	d-g	13.90	cd
	0.5	16.10	c-e	15.90	b-d	14.80	bc
	1.0	18.10	a	18.5	a	17.60	a
	2.0	15.50	de	13.60	e-j	12.30	de
	4.0	12.50	gh	12.10	i-k	11.00	e
Grand mean		**14.9**		**14.3**		**13.8**	
$LSD_{0.5}$%for antioxidants		0.52		0.48		1.56	
$LSD_{0.5}$%for concentrations		0.88		1.03		1.01	
$LSD_{0.5}$% for interaction		1.53		1.78		1.75	

In pea, the treated plants with Vitamin E at level 4.0 mM produced lower pods number compared to all treatments. In general, SA increased in the number of pods/plant of three crops, increased the number of pods/plant and that was observed with increasing SA concentrations until 1.0 m*M*. But, the higher concentrations of SA caused reduction in the number of pods/plant. The results suggest the importance of moderate concentrations of SA (1.0 m*M*) to improve number of pods/plant.

These effects of SA at low concentrations may be due to inhibiting of phosphate uptake and potassium absorption (Glass, 1973 and 1974) and reduction of K absorption **(**Harper and Balke, 1981). The improve happened by moderate concentrations (1.0 m*M*) may be due to that SA increases flower longevity via inhibition of ethylene production (Lesilie and Romani, 1986),but the highest value was obtained by using acetyl salicylic acid (ASA) of the legumes under study at 1 m*M*, the lowest value (10.7) at 4.0 m*M* concentration of (*Vicia faba* cv Nubaria 1) with vitamin E.

El-Tohamy and El-Greadly, 2007 found that foliar application of vitamin E at 0.1 ml/l and 0.3 ml/l significantly improved vegetative growth and yield of bean plants compared to control plants especially at the higher concentrations.

Salicylic acid (SA) belongs to a divers group of plant phenolics that play an essential role in the regulation of plant growth, development and interaction with other organisms (Harborne, 1980). According to the role which has been played by SA in plants, it was called a plant hormone (Raskin, 1992).

Radiolabel ling studies showed that salicylic acid was an essential component in the signal transduction pathway leading to systemic acquired resistance (SAR), which is synthesized from phenylalanine and benzoic acid in cucumber (*Cucumis sativus* L.) plant inoculated with pathogens (Neuwly *et al*., 1995).

Results in Table (5) showed that antioxidants were increased weight 100seed for the three crops generally, but the high value was obtained in common bean by used ASA at 1 mM (59.0g), the highest value at (0.1 and 0.5 m*M*) when used Vitamin E of the three crops, on the other hand at 4m*M* gave the lowest value (36.20,10.00,87.00 g) when used SA for (common bean, pea, and broad bean) compared with control (52.00, 16.50,116.0 g) respectively.

Spraying plants with salicylic acid (SA) improved most growth characters i.e maize plants (Hussein *et al*., 2007a) onion plants (Amin *et al*., 2007).

Table (5): Effect of the spraying treatment by some antioxidants on the weight of 100 seed (g) in common bean, pea and broad bean.

		Phaseolus vulgaris (v. Nebraska)		*Pasium sativum* (v.Master B)		*Vicia faba* (v.Nubaria 1)	
Antioxidants	SA	50.03	b	15.83	b	118.0	b
	V.E	52.42	a	16.76	a	129.5	a
	ASA	49.07	c	15.67	b	118.2	b
Concentration	0.0	50.94	d	16.10	d	115.7	e
	0.1	53.17	c	16.87	c	121.3	d
	0.5	54.43	b	17.60	b	129.7	b
	1.0	56.53	a	18.60	a	141.7	a
	2.0	48.17	e	15.96	d	126.0	c
	4.0	39.80	f	11.40	e	97.00	f
Salicylic acid (SA)	0.0	52.00	fg	16.50	e-g	116.0	k
	0.1	52.00	fg	16.40	e-g	119.0	J
	0.5	53.30	d-f	17.00	de	127.0	g
	1.0	57.80	ab	16.07	gh	151.0	a
	2.0	42.00	j	16.00	gh	116.0	k
	4.0	36.20	l	10.00	k	87.00	o
Vitamin (E)	0.0	51.00	gh	16.20	f-h	115.0	k
	0.1	55.00	cd	17.30	d	124.0	h
	0.5	56.00	bc	18.30	b	133.0	e
	1.0	56.20	bc	18.10	bc	141.0	c
	2.0	44.70	i	15.80	gh	111.0	l
	4.0	40.20	k	11.70	j	98.00	n
Acetyl salicylic acid (ASA)	0.0	49.83	h	15.60	h	116.0	k
	0.1	52.50	e-g	16.90	d-f	121.0	I
	0.5	54.00	de	17.5	cd	129.0	f
	1.0	59.00	a	20.20	a	148.0	b
	2.0	54.40	c-e	17.50	cd	136.0	d
	4.0	43.00	ij	12.50	i	106.0	m
Grand mean		**50.507**		**16.086**		**121.89**	
$LSD_{0.5}$%for antioxidants		0.737		0.476		1.069	
$LSD_{0.5}$%for concentrations		1.019		0.403		0.812	
$LSD_{0.5}$% for interaction		1.765		0.698		1.406	

Table (6) revealed that the highest seed yield /plant was obtained, when the seeds and resultant plants had been treated by acetyl salicylic acid (ASA) at level 1.0 mM (56.2g) in common bean and 129.1g in broad bean). But in pea there is no significant effect for salicylic acid, Vitamin E and acetylsalicylic acid treatments. The highest seed yield/plant of pea was obtained when the plants treated with acetyl salicylic acid 1mM (39.0g).On the other hand, treatment of plants by Vitamin E at level 4.0 m*M* gave the lower value (12.60 g) SA and ASA. Decreasing of seed yield may be due to the SA treatment at high level stimulated ethylene production (Liang – Wusheng *et al*., 1997).

Spraying plants with á-tocopherol at the rate of 200 ppm improved growth characters of cowpea plants, although improvement was only significant for plant height and stem dry weight (Hussein *et al*., 2007b).

Similar inhibition of potato Micro – propagation and micro- tuberization were occurred by adding 4 m*M* salicylic acid to modified MS medium (Gad El – Hak *et al*., 2002). Salicylic acid treatment improved yield and its componend of onion plants (Amin *et al*. 2007), mung bean (Singh and Kaur, 1980), and tomato fruits (Moustafa, 1999).

Chemical analysis

approximate analysis for studied legume seeds are given in Table (7). These data indicate that studied legumes are rich and good sources of dietary fibers (<4.53 - 6.50%) and ash content. Similar results were reported by (Rehman *et al.,* 2001).

Table 6: Effect of the spraying treatment by some antioxidants on dry seeds yield/plant (g) of the three crops.

		Phaseolus vulgaris (v. Nebraska)		*Pasium sativum* (v.Master B)		*Vicia faba* (v.Nubaria 1)	
Antioxidants	SA	33.65	b	24.10	a	80.00	b
	V.E	36.87	a	23.88	a	82.63	a
	ASA	34.13	b	23.21	a	76.37	c
Concentration	0.0	28.20	d	18.40	d	62.00	d
	0.1	39.47	c	25.33	c	81.97	c
	0.5	46.60	b	33.27	b	99.00	b
	1.0	52.73	a	37.60	a	121.40	a
	2.0	23.93	e	14.67	e	63.07	d
	4.0	18.37	f	13.10	f	50.53	e
Salicylic acid (SA)	0.0	28.20	f	18.40	f	62.00	jk
	0.1	37.90	e	24.23	e	78.90	h
	0.5	44.50	c	31.70	d	95.20	f
	1.0	51.00	b	37.00	ab	126.10	b
	2.0	23.50	g	14.60	hi	60.00	k
	4.0	16.80	i	13.20	i	49.00	m
Vitamin (E)	0.0	29.20	f	19.40	f	62.80	jk
	0.1	42.00	d	26.10	e	84.00	g
	0.5	49.10	b	35.10	bc	102.90	d
	1.0	51.00	b	36.80	ab	109.10	c
	2.0	25.00	g	13.50	i	66.00	l
	4.0	19.70	h	12.60	i	52.00	l
Acetyl salicylic acid (ASA)	0.0	27.20	f	17.40	fg	61.20	jk
	0.1	38.50	e	25.67	e	83.0	g
	0.5	46.20	c	33.00	cd	98.9	e
	1.0	56.20	a	39.00	a	129.10	a
	2.0	23.30	g	15.90	gh	63.20	J
	4.0	18.60	hi	13.50	i	50.60	lm
Grand mean		**34.88**		**23.73**		**79.67**	
$LSD_{0.5}$%for antioxidants		0.94		1.28		1.63	
$LSD_{0.5}$%for concentration		1.15		1.30		1.56	
$LSD_{0.5}$%for interaction		1.99		2.26		2.70	

Table 7: Some chemical composition of the studied legumes .

Constituent	Legume seeds		
	Phaseolus vulgaris var. Nebraska	*Pisum sativum* var. Master B	*Vicia faba* var. Nubaria-1
Dry matter %	91.6±2.24	90.0±2.2	88.1±1.9
Total ash content	3.90±0.08	3.65±0.09	2.75±0.07
Crude fiber %	4.53±0.18	5.25±0.3	6.50±0.82
Crude lipids %	4.46±0.08	3.95±0.09	1.52±0.03
Crude protein %	29.50±1.62	23.5±1.2	25.7±1.30
Total carbohydrates*	**57.61**	**63.65**	**63.53**

*TCs= 100–(Total lipids + Crude fiber + Crude ash + Total proteins)

The highest level of crude protein (29.50%) was recorded in seeds of *Phaseolus vulgaris* cv. Nebraska followed by *Vicia faba* cv. Nubaria-1 (25.7%) then *Pisum sativum* cv. Master B (23.5%). Results recorded here are in a good agreement with those reported by several investigators (Ibuki *et al.,* 1986; Ali 1996 and Salah-El-Din 2002).

Nitrite (NO_2^{1-}mg/kg) and Nitrate NO_3^- mg/kg concentrations

Results given in Fig.(1) show that the nitrite concentration in the three legumes ranged from 4.20 to 6.5 mg/kg and did not reach toxic limit level. The highest level of nitrite was recorded in Master B(6.5 mg/kg) and the lowest one is in Nebraska.(4.2 mg/kg) (MAFF, 1987) showed that NO_2^{-1} concentration must not exceed the toxic limit in drinking water for human (10 NO_2^{-1} mg/kg).

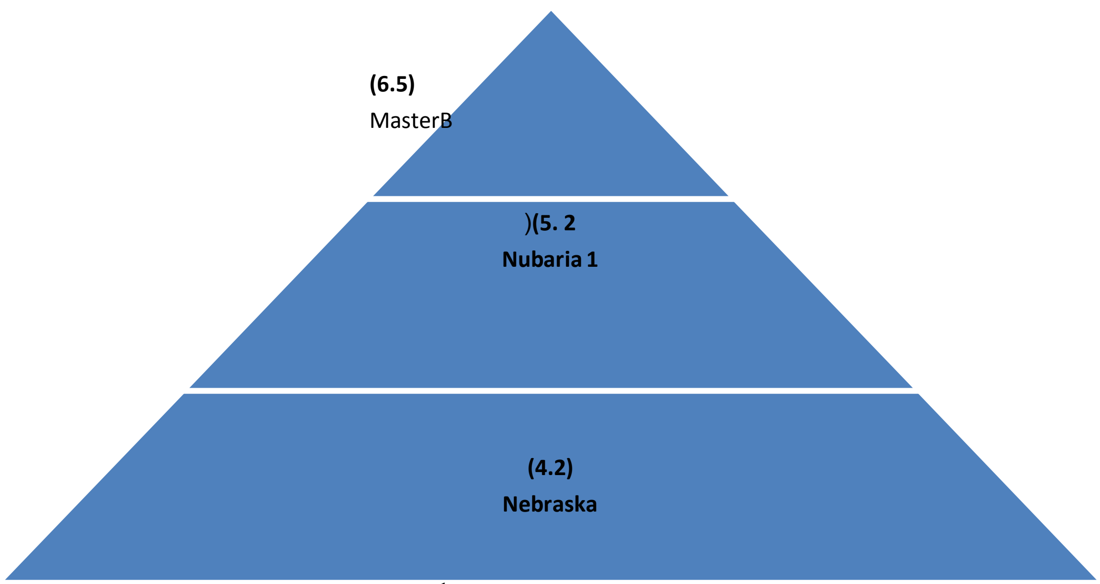

Fig. (1): Nitrite (NO_2^{1-}mg/kg) concentrations in legume samples

These results mean that the consumption of legume seeds is save. Similar results on different kidney bean genotypes were reported by (Abd El-Naem *et al.,* 2007).

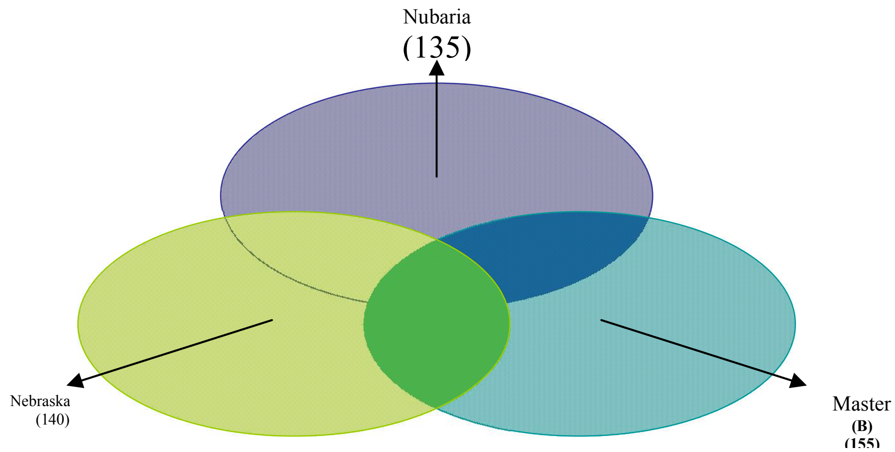

Fig. (2): Nitrate (NO_3^- ion/kg) concentrations in legume samples.

(Fig. 2) showed that, all legumes seeds contain less than 200 mg NO_3^- ion/kg and the highest concentration (155 mg/kg NO_3^{1-}ion) was recorded in extracts of Master B and the lowest one in Nubaria 1(135mg NO_3^- ion/kg.). The nitrite content of most fresh market vegetables is low and usually of the order of 1-2 mg/kg (Corre and Breimer, 1979). Whereas broad beans, peas, cauliflowers and kidney bean generally contain less than 200 mg/kg (MAFF, 1987). Thus, nitrate contents of group 1 are mainly less than 200 mg NO_3^- ion/kg; this group includes peas and other crops.

Salicylic acid (SA) content:-

The concentrations of SA in legume seeds were spectrophotometrically assayed in both control and all treated ones. Results presented in Fig. (5) indicated that SA concentration was recorded to be 140, 160 and 130 µg/ml extract in the untreated samples of Nebraska, Master B and Nubaria 1 respectively. Levels of SA were higher in all treatments compared with the control and the uptake of SA differs according to the given doses.

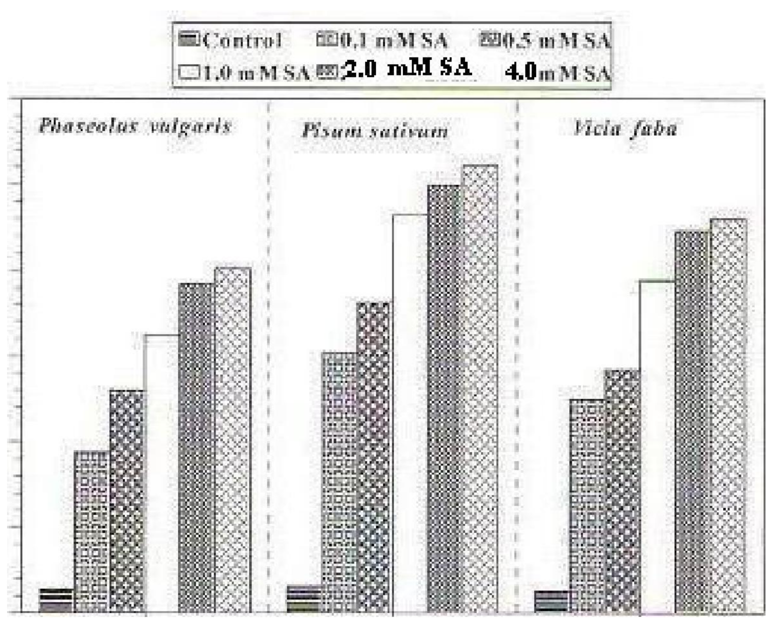

Fig. (3): Levels of SA in legume samples

Effect of spraying treatment with SA on SA as antioxidant in samples:-

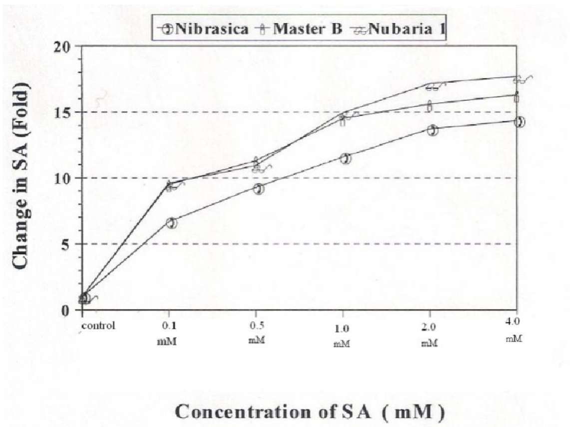

Fig. (4): Effect of spraying treatment with SA concentrations on SA content.

Changes in SA levels causing by treatment was studied and the results are given in Fig. (4). Results show sharp increases in SA contents. Treatment with lower concentrations (0.1 mM SA) led to increases in SA with 6.67-; 9.5- and 9.6-fold for Nibrasica; Master B and Nubaria 1, respectively, whereas, higher concentrations (4.0 mM) result in 14.35-; 16.31- and 17.69-fold for Nibrasicak; Master B and Nubaria-1, respectively.

SA is a phenol, ubiquitous in plants generating a significant impact on plant growth and development, photosynthesis, transpiration, ion uptake and transport. It also induces specific changes in leaf anatomy and chloroplast structure. SA is recognized as an endogenous signal, mediating in plant defence against pathogens.

It is clear that the application of SA on plants led to the accumulation of SA and consequently increased its endogenous level (Dat *et al.,* 1998b). These observations suggest that SA could be involved in heat acclimation and that its action may be linked to oxidative stress (Dat *et al.,* 1998a and Dat *et al.* (1998b) explored the possible involvement of SA in heat-stress physiology using the mustard seedling system characterized by (Dat *et al.* 1998a) in which exogenous SA can induce a period of thermotolerance similar to that of conventional heat acclimation. If endogenous SA has a function during heat acclimation, changes in SA levels would be expected.

Many studies have shown that responses to infection are mediated by endogenous SA (Mur *et al.,* 1997). Ozone and UV light also induce SA accumulation (Sharma *et al.,* 1996) as does high-light which induced H_2O_2 accumulation in catalase-deficient transgenic tobacco (Chamnongpol *et al.,* 1998).

TPCs and TFs in legume seeds:

The concentrations of TPCs and TFs in the crude extracts of whole seeds of studied legumes were spectrophotometerically assayed and the results are given in Fig. (5a). Results indicate that seeds of *Vicia faba* var. Nubaria-1 contain higher levels of TPCs (8.3 mg/g) than those determined in seeds of *P. vulgaris* var. Nebraska (7.4 mg/g) and much higher than *P. sativum* var. Master B (6.5 mg/g). These results indicate that the levels of TFs in three legume samples varied considerably from 1.5 to 2.1 mg/g of the whole seeds of *P. sativum* var. Master B and *P. vulgaris* var. Nebraska respectively. Our results are in good agreement with those recorded by (Fawzy, 1998). Condensed and hydrolyzable tannins of relatively high molecular weight have also been shown to be effective antioxidants with greater activity than simple phenolics (Hagerman *et al*., 1998).

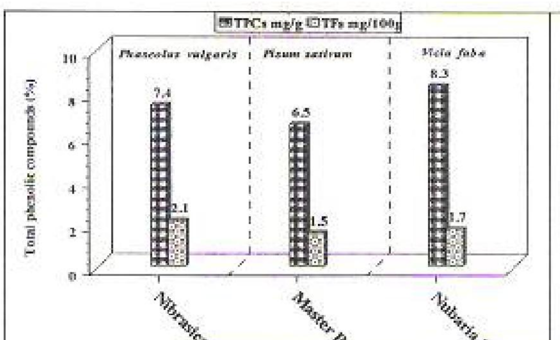

Fig. (5a): TPCs and TFs in legume seeds.

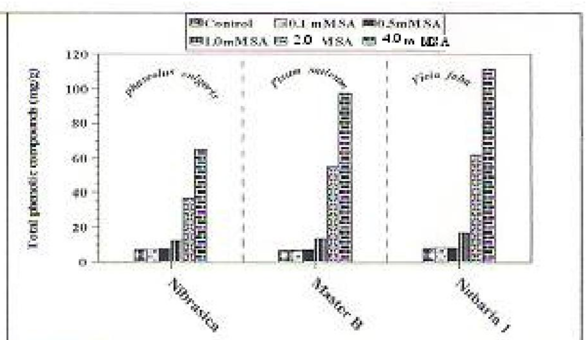

Fig.(5b): Effect of SA (spraying) on TPCs of the legume samples.

Results in Fig. (5b) show the effect of SA as antioxidant on TPCs content in Nebraska, Master B and Nubaria-1. It is noted that the endogenous phenolic content in Nebraska seeds treated by 4.0 mM SA, increased to be 8.78-fold of that recorded in control (7.4 mg/g). On the other hand, the phenol content in Master B and Nubaria-1 increased to reach the maximum values 98 and 112 mg/g respectively as percentage of the corresponding values of the control seeds. With further increase of SA concentration, the antioxidants as phenols contents was increased. There are differences in the content of condensed tannins of legumes seeds depending on the color of seed coats. The white (Nebraska) varieties of legumes contain usually lower concentrations of tannins than those with red, black or bronze seed coats. These results are in good agreement with those reported by (Troszynska *et al.,* 2006).

Conclusions

Antioxidants function by scavenging free radicals via donation of an electron or ahydrogen atom, or by deactivation of prooxidant metal ions and singlet oxygen (Shahidi, 2002), (Morello *et al*, 2002) stated that the primary role of antioxidants is to prevent degredation induced by free radical reactions. They noted that antioxidants function by hydrogen abstraction and metal ion assisted electron transfer. The antioxidant donates hydrogen atoms to the free radicals, thus inhibiting the propagation of the autocatalytic chain reaction.

Importance of phenolic content may be discussed from two points of view. First, evaluating the negative effects of consumed phenols and second, estimating their positive contribution to health. For example, polyphenols from dry beans may act as antioxidants to inhibit the formation of damaging free radicals that result from the natural degradation of foods (Namiki, 1990). Flavonoids obtained commercially (Sichel *et al*., 1998) and isolated from plant species (Gamez *et al*., 1998) are known to be effective free radical scavengers. There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidants activity of these compounds (Keli *et al*.,1996).

At the lowest concentrations (0.1 and 0.5 m*M*) of vitamin E gave the higher value than SA and ASA due to the high molecular weight of vitamin E.

Recommendation

From the previous results of this investigation, it could be recommend that spraying *vulgaris* cv. Nebraska and *Pisum sativum* cv. Master B*, Vicia faba* cv. Nubaria-1 (formely Giza Blanca) plants with (ASA) at 1.0 m*M* because it gave the highest value,and used the lowest concentrations (0.1 and 0.5 m*M*) of vitamin E because it gave the higher value than SA and ASA.

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12/11/2014