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EVALUATION OF YIELD, CHEMICAL CONSTITUENTS AND ANTIOXIDATIVE ACTIVITIES OF PHENOLIC COMPOUNDS IN SOME VEGETABLE LEGUME TREATED WITH SOME ANTIOXIDANTS

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ABSTRACT

Two field experiments were carried out at Mallawy Agricultural Research Station, Minia, Egypt, experimental farm during the two successive fall and winter seasons of 2007/2008 and 2008/2009, respectively. *Phaseolus vulgaris* cultivar Nebraska and *Pisum sativum* cv. Master B, *Vicia faba* cv. Nubaria-1 (formely Giza Blanca) were used to study the influence of three antioxidant organic acids namely, salicylic acid (SA), acetyl salicylic acid (ASA) and L-ascorbic acid (L-AA) on some yield characters, chemical constituents and antioxidative activities of total phenolic compounds (TPCs). Five concentrations 0.1 m M, 0.5 mM, 1.0m M, 2.5 mM and 5.0 mM were prepared from each antioxidant substance. These concentrations were used for seeds soaking and foliar spray for the resultant plants. Some physical properties and approximate analysis (dry matter DM, crude protein CP; crude fiber CF, crude lipids CL and ash content AC) were determined. The results indicate that 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 were significantly affected by using antioxidant treatments. The best compound that gave, in average, the highest values was acetylsalicylic acid. Nitrite concentration (NO_2^{-1} mg/kg) ranged

from 4.50 to 6.7 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. This means that the consumption of these vegetable legume seeds is save. All seed samples contained less than 200 mg NO_3^{-1} ion/kg and the highest concentration (152 mg/kg NO_3^{1-}ion) was recorded in extracts of Master B and the lowest one in Nubaria 1.

Levels of L-AA are always high in the treated samples compared with the control. L-AA concentrations in extracts of Master B were higher (13.5 mg/100g) than those determined in Nubaria-1 (10 mg/100g) and Nibrasica. The application of L-AA in double treatment (soaking + spraying) led to a sharp increases in total concentration of L-AA being about 278% of its original amount in the untreated one of Nebraska; 249% of Nubaria-1 and 209% of Master B when 5 mM of L-AA was applied.

Concentrations of SA in legume seed samples were higher in all treatments compared with the untreated samples (control) and the uptake of SA differed according to the given doses. Changes in SA levels caused by double treatments (soaking + spraying) were 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 indicated 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 the extracts of Nebraska

Antioxidative activities (AOA) of crude extracts containing TPCs extracted from whole seeds of the three legumes were measured by ferric thiocyanate (FTC) method which measure the amount of peroxide formed during the initial stage of linoleic acid oxidation. The AOA of Nubaria-1 extracts which indicated that seed extracts were more effective in retarding the oxidation of linolic acid after more than 8 days of incubation than the corresponding extracts of Nebraska, and Master B seeds. In general, all vegetable bean extracts exhibited excellent antioxidative activities with about 90% inhibition of linoleic acid oxidation by the end of incubation period. These results indicated the importance of using antioxidants for increasing the antioxidative activities (AOA) of total phenolic compounds (TPCs) to limited of lipids oxidation (linoleic acid oxidation) in our study.

INTRODUCTION

Dry beans (kidney bean, faba bean) are an integral part of the diets for 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 (Aggarwal *et al.*, 2004 and Abd El-Hakim, 2006).

Consumption of dry beans has been linked to reduce 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 the presence of secondary metabolites such as phenolic compounds that possess antioxidant properties (Lazze *et al.,* 2003 and 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, rich in proteins, vitamins, complex carbohydrates and minerals (Abd El-Naem *et al.,* 2006).

Using antioxidants such as L-ascorbic acid, (L-AA) and salicylic acid (SA) are suggested for improving yield and 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, E and carotenoids, protect 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.

This investigation aimed to determine total phenolic compounds (TPCs), total flavonoids (TFs) in seeds of three common legumes and assay the AOA of TPCs extracted from different seeds. Studying the effect of soaking+spraying treatment by three different antioxidants (SA, ASA and L-AA) on the chemical components of seeds

MATERIALS AND METHODS

Two field experiments were carried out at Agricultural Research Station, Minia, Egypt, Mallawy experimental farm during the two successive fall and winter seasons of 2007/2008 and 2008/2009, 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), acetyl salicylic acid (ASA) and L-ascorbic acid (L-AA), on dry seed yield, chemical constituents, antioxidative potentials of total phenolic compounds. Five concentrations 0.1 m M, 0.5 mM, 1.0m M, 2.5 mM and 5.0 mM from each antioxidant substances. were used as seeds soak and /or foliar spray for the resultant plants. Control group was also conducted.

Seeds from common bean, pea and broad bean were soaked in distilled water or in the specific solutions of the antioxidants at 0.1, 0.5, 1.0, 2.5 and 5.0 mM for 30 min, 12 and 24 hours respectively. The seeds were rinsed and sown on September, 6, in the first season, (2008) and on 9 in the second season, (2009) for common bean, on 14^{th} and 17^{th} November 2007/2008 and 2008/2009 for pea and faba bean, respectively.

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

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. The common cultural practices known for each crop were followed. 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 seed (g) and 3] Weight of dry seeds yield/plant.

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

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

Soil constituent	Value	Soil constituent	Value
Texture grade	Clay loam	$CaCO_3$	2.08%
Sand	7.63%	Organic matter	1.35%
Silt	60.57%	Available N	45.13 ppm
Clay	31.80%	P	11.25 ppm
pH (1.2.5 soil suspension)	8.3	K	79.15 ppm
E.C. (dslm, 1:5 soil water extract)	1.14	B	0.3 ppm

E.C. = Electrical conductivity

Seed Samples.

Dry seeds samples were collected from each crop.

Flour preparation.

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).

Preparation of dry defatted meal.

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 use (AOAC, 2005).

Chemical composition

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

Extraction and determination of nitrite and nitrate: -

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 L-ascorbic:-

The indophenol method (2,6-dichlorophenol indophenol) described by (Mondy and Ponnampalam, (1986) was used for determination of ascorbic acid concentration in dry bean. L-AA was extracted using 1.25% oxalic acid solution.

Determination of salicylic acid (SA):

To quantify SA, the ethyl acetate (5mM) was used 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.

Determination of total carbohydrates (TCs):

Carbohydrates were calculated by difference:

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

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 in 10 ml volumetric flasks. Distilled water was added (make 5 ml) and 0.3 ml $NaNO_2$ (1:20) was added then mixed. $AlCl_3$ 3 ml (1:10) were added 5 min later then after 6 min, 2 ml 1M NaOH was added and the total volume was completed to 10 ml with distilled water. The solution was 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).

Antioxidative activity assay AOA: -

Antioxidative activity was carried out using the linoleic acid system (Osawa and Namiki, 1981). The degree of oxidation using ferric thiocyanate (FTC) was assayed according to the method described by Mitsuda *et al.,* (1966).

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).

RESULTS AND DISCUSSION

Physical properties and approximate analysis of the studied legume seeds are given in Table 2. Results show that 100-seed weight of Nubaria-1 was the highest weight and half of this weight was recorded for Nebraska seeds. Percentages of 100-seed coat weights compose 10-17% from the whole legume seeds. These data indicated that studied legumes are rich and good sources of dietary fibers (<4.0 - 6.5%) and ash content. Similar results were reported by Rehman *et al.,* (2001).

The highest level of crude protein (30.94%) was recorded in seeds of *Phaseolus vulgaris* cv. Nebraska followed by *Vicia faba* cv. Nubaria-1 (25.5%) then *Pisum sativum* cv. Master B (23.3%). Results recorded here are in good agreement with those reported by several investigators (Ibuki *et al.,* 1986; Ali 1996 and Salah-El-Din 2002, Abd El-Hakim, 2006).

Table 2 : Some physical properties and chemical composition of the studied legumes

Constituent	Legume seeds		
	Phaseolus vulgaris var. Nebraska	*Pisum sativum* var. Master B	*Vicia faba* var. Nubaria-1
100-seed weight	**55.0 g**	**15.9 g**	**110.5 g**
100-seed coats wt	**8.25 g**	**1.6 g**	**18.8 g**
seed coats color	**White**	**Light green**	**Brown**
Dry matter %	**90.6±2.24**	**91.0±2.2**	**89.1±1.9**
Total ash content	**4.10±0.08**	**3.65±0.09**	**2.75±0.07**
Crude fiber %	**4.33±0.18**	**5.25±0.3**	**6.50±0.82**
Crude lipids %	**3.86±0.08**	**3.95±0.09**	**1.52±0.03**
Crude protein %	**30.90±1.62**	**23.3±1.2**	**25.5±1.30**
Total carbohydrates*	**56.81**	**63.85**	**63.73**

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

The illustrated data in Table 3 indicate that no significant between the three antioxidants substances. The best concentration when soaking of seeds and spraying resultant plants by salicylic acid, acetyl salicylic acid and L-ascorbic acids was1.0 mM. In pea, the treated seeds and plants with salicylic acid at level and 5.0 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 mM. 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 mM) to improve number of pods/plant. These effects of SA at low concentrations may be due to inhibition of phosphate uptake and potassium absorption (Glass, 1973 and 1974) and reduction of K absorption (Harper and Balke, 1981). The improvement happened by moderate concentrations (1.0 mM) may be due to that SA increases flower longevity via inhibition of ethylene production (Lesilie and Romani, 1986).

Table 3 : Effect of soaking+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
	ASA	15.02 a	14.12 a	14.47 a
	AA	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
	2.5	14.20 c	13.03 d	12.93 d
	5.0	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	14.80 d-f	14.50 d-g	13.80 cd
	0.5	16.10 c-e	15.90 b-d	14.60 bc
	1.0	17.50 a-c	18.50 a	16.80 a
	2.5	12.50 gh	12.80 g-k	12.50 de
	5.0	11.60 h	12.00 jk	11.00 e
Acetyl salicylic acid (ASA)	0.0	14.50 ef	14.20 d-h	14.00 cd
	0.1	14.90 d-f	14.10 d-i	14.50 bc
	0.5	16.40 b-d	15.60 c-e	16.00 ab
	1.0	18.10 a	16.90 a-c	17.60 a
	2.5	14.60 ef	12.70 g-k	14.00 cd
	5.0	11.60 h	11.20 k	10.70 e
Ascorbic acids (AA)	0.0	13.50 fg	12.20 h-k	12.20 de
	0.1	14.60 ef	14.90 d-f	13.90 cd
	0.5	16.10 c-e	16.00 b-d	14.80 bc
	1.0	17.80 ab	17.70 ab	17.10 a
	2.5	15.50 de	13.60 e-j	12.30 de
	5.0	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

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).

Radiolabelling 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 4 show that ASA increased 100seed weight for the three crops more than SA and L-AA generally, but highest value was obtained in common bean used SA at 1 mM (59.0g) , At 5mM gave the lowest value (40.20,11.70,98.00 g) when used L-AA for common bean, pea, and broad bean compared with control (49.83, 15.60,116.0 g) respectively.

Spraying different 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 reveals that the highest seed yield /plant was obtained , when the seeds and resultant plants had been treated by acetyl salicylic acid 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, acetylsalicylic acid and ascorbic acids treatment. The highest seed yield/plant of pea was obtained when the seeds and the plants were treated with salicylic acid 1mM (39.0g). On the other hand, treatment of seeds and plants by salicylic acid at 5.0 mM level gave the lowest value (13.5 g). 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).

Table 4 : Effect of soaking+spraying treatment by some antioxidants on 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
	CA	52.42 a	16.76 a	129.5 a
	AA	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
	2.5	48.17 e	15.96 d	126.0 c
	5.0	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	55.00 cd	16.90 d-f	119.0 j
	0.5	56.00 bc	17.50 cd	129.0 f
	1.0	59.00 a	18.10 bc	141.0 c
	2.5	42.00 j	16.00 gh	116.0 k
	5.0	36.20 l	10.00 k	87.00 o
Acetyl salicylic acid (ASA)	0.0	51.00 gh	16.20 f-h	115.0 k
	0.1	52.50 e-g	17.30 d	124.0 h
	0.5	54.00 de	18.30 b	133.0 e
	1.0	56.20 bc	20.20 a	148.0 b
	2.5	57.80 ab	16.07 gh	151.0 a
	5.0	43.00 ij	12.50 i	106.0 m
Ascorbic acids (AA)	0.0	49.83 h	15.60 h	116.0 k
	0.1	52.00 fg	16.40 e-g	121.0 i
	0.5	53.30 d-f	17.00 de	127.0 g
	1.0	54.40 c-e	17.50 cd	136.0 d
	2.5	44.70 i	15.80 gh	111.0 l
	5.0	40.20 k	11.70 j	98.00 n
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 5 : Effect of soaking treatment by some antioxidants on weight 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
	ASA	36.87	a	23.88	a	82.63	a
	AA	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
	2.5	23.93	e	14.67	e	63.07	d
	5.0	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	26.10	e	84.00	g
	0.5	44.50	c	31.70	d	98.90	e
	1.0	51.00	b	39.00	a	126.10	b
	2.5	23.50	g	15.90	gh	60.00	k
	5.0	16.80	i	13.50	i	49.00	m
Acetyl salicylic acid (ASA)	0.0	29.20	f	19.40	f	62.80	jk
	0.1	42.00	d	25.67	e	83.00	g
	0.5	49.10	b	35.10	bc	102.90	d
	1.0	56.20	a	37.00	ab	129.10	a
	2.5	25.00	g	13.50	i	66.00	i
	5.0	19.70	h	12.60	i	52.00	l
Ascorbic acids (AA)	0.0	27.20	f	17.40	fg	61.20	jk
	0.1	38.50	e	24.23	e	78.90	h
	0.5	46.20	c	33.00	cd	95.20	f
	1.0	51.00	b	36.80	ab	109.10	c
	2.5	23.30	g	14.60	hi	63.20	j
	5.0	18.60	hi	13.20	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	

Similar inhibition of potato Micro – propagation and micro-tuberization were occurred by adding 5 mM 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).The results of yield characters in the crops under study are similar to those of Abd El-Hakim,(2006).

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

Results given in Fig. 1 show that nitrite concentration in the three legumes ranged from 4.50 to 6.7 and did not reach toxic limit level. The highest level of nitrite was recorded in Master B and the lowest one in Nebraska. 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).

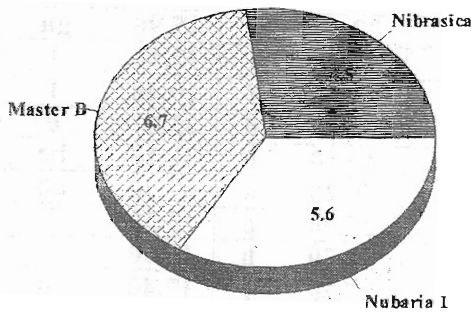

Fig. 1: Nitrite (NO_2^{1-}mg/kg) concentrations in legume samples

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

All legumes seeds contain less than 200 mg NO_3^- ion/kg and the highest concentration (152 mg/kg NO_3^{1-}ion) was recorded in extracts of Master B and the lowest one in Nubaria (1 Fig. 2). 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.

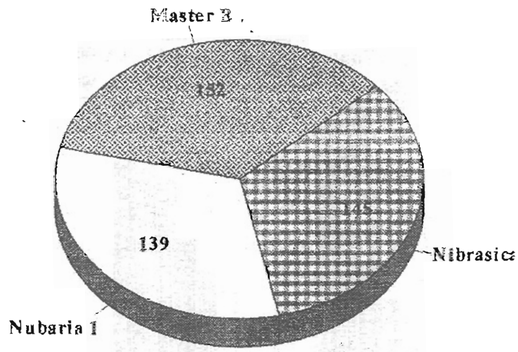

Fig. 2: Nitrate (NO_3^- ion/kg) concentrations in legume samples

L-Ascorbic acid (L-AA) Vitamin C content:-

L-AA was extracted from seed samples, and the results are given in Fig. 3. From Fig.4 the levels of L-AA were always high in the treated samples compared with the control. L-AA contents in extracts of *Pisum sativum* var. Master B were higher (13.5 mg/100g) than those determined in *Vicia faba* var. Nubaria 1(10 mg/100g) and *Phaseolus vulgaris* var. Nebraska, but these values are lower when compared with tomatoes or potatoes. These results are in good agreement with those obtained by Salah-El-Din, (2002).

Application significances of L-AA either soaking of seeds before plantation or spraying of plants could be discussed from two different views, first applied L-AA is accumulated in seeds and consequently increase the endogenous L-AA which protect the plants from pathogens and insects, second is L-AA plays an important role as ideal natural antioxidant in the lowering of damages causing by free radicals in protein and DNA molecules.

Effect of antioxidant on L-AA in the studied legumes:-

The application of L-AA in double treatment (Soaking + spraying) led to a sharp increase in whole concentration of L-AA (Fig. 4) being about 278% of its original amount in the untreated one of

Nebraska; 249% of Nubaria-1 and 209.11 Master B when 5 mM of L-AA was applied.

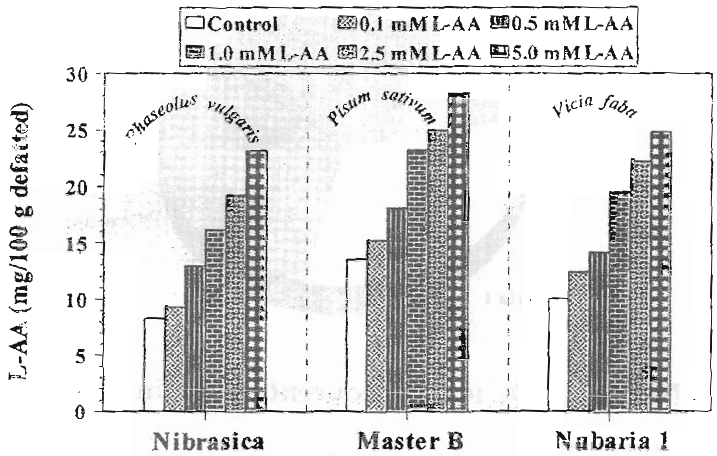

Fig. 3: Levels of L-AA in legume samples

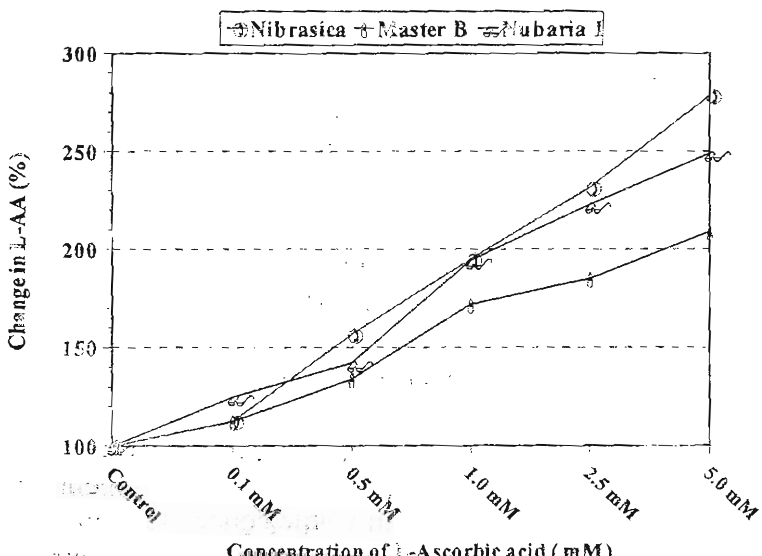

Fig. 4: Effect of antioxidant on L-AA in the legume samples

Generally, levels of L-AA were always high in the treated samples compared with the control and the uptake of L-AA differ according to the concentrations of applied L-AA. It is well documented that L-AA (vitamin C) biosynthesize in plants such as *Citrus* species, tomatoes, legume and many other plants with different rates, but what well happen when this natural antioxidant well be added as additional sources (spraying)?. It is expected that L-AA well be absorbed and accumulated in legume to play an important role as ideal natural antioxidant for lowering damages caused by free radicals in protein and DNA molecules. Considerable evidence from both recommended dietary alliance (RDA) may reduce the risk factors for chronic diseases such as, heart disease and cancer (Losnoczy *et al.*, 1996).

L-AA plays a key central role in detoxification of activated oxygen (Foyer *et al.*, 1991). It can react directly by reducing superoxide, hydrogen peroxide and hydroxyl radical or quenching singlet oxygen. Alternatively, it can react indirectly by regenerating α-tocopherol from α-chromanoxy radical or in the synthesis of zeaxanthin in the xanthophylls cycle.

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 recorded SA concentration was 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 differ according to the given doses.

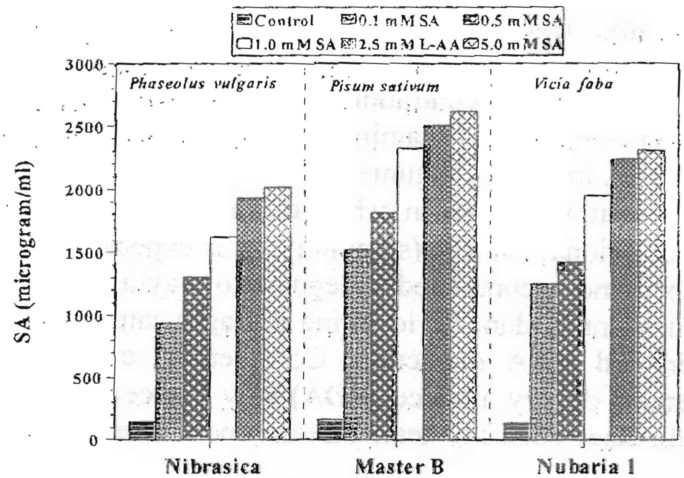

Fig. 5: Levels of SA in legume samples

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

Changes in SA levels caused by double treatments (soaking + spraying) were studied and the results are given in Fig. 6. Results show sharp increases in SA contents. Treatment with lower concentrations (0.1 mM SA) led to an increases in SA with 6.67-; 9.5- and 9.6-fold for Nibrasica; Master B and Nubaria 1, respectively, whereas, higher concentrations (5.0 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.

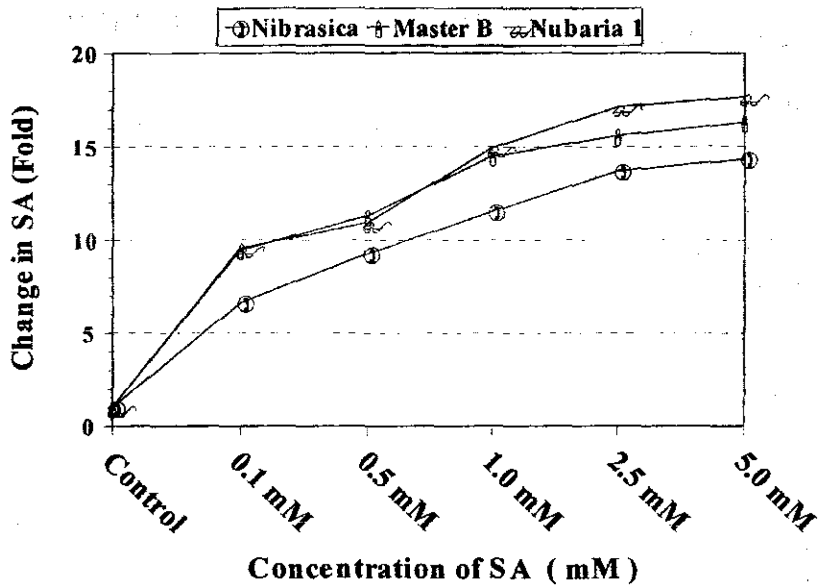

Fig. 6: Effect of (soaking + spraying) with SA on SA as antioxidant.

It is clear that 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). 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. 7a. Results indicated that seeds of *Vicia faba* var. Nubaria-1 contained 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 indicated 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. Nibrasica 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).

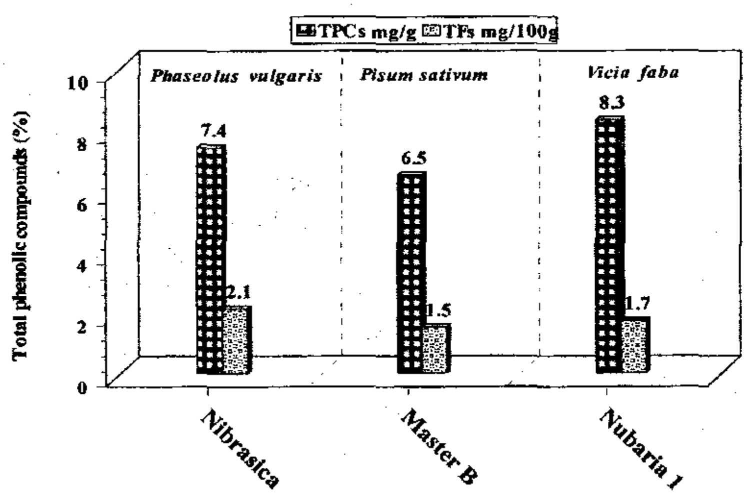

Fig. 7a: TPCs and TFs in legume seeds.

Treatment of legumes with antioxidant

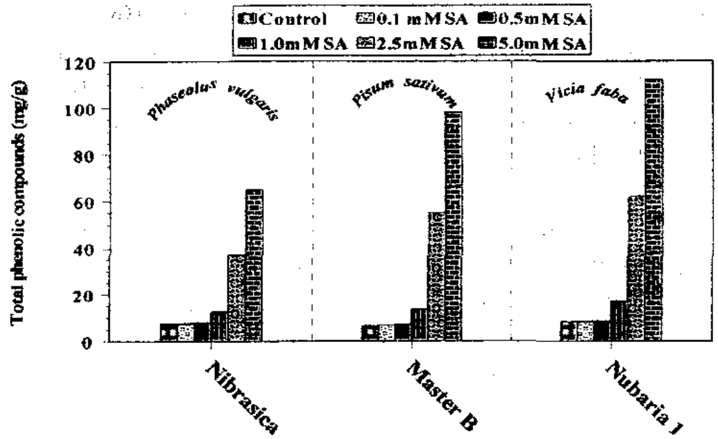

Fig.7b: Effect of SA (soaking+ spraying) on TPCs of the legume samples.

Results in Fig. 7b show the effect of SA as antioxidant on TPCs content in Nibrasica, Master B and Nubaria-1. It is noted that the endogenous phenolic content in Nibrasica seeds treated by 5.0 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 contained 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).

Antioxidative activities of TPCs extracted from dry bean

Antioxidative activities of TPCs-crude extracts from whole seeds of three legumes studied in this work were measured. The individual activity of extracts showed low absorbance values at 500 nm (Figs. 8) which indicate marked inhibition of oxidation of linoleic acid by all extracts. All of them were more effective than α-tocopherol, a common natural antioxidant.

The extracts of Nebraska, and Master B showed lower antioxidant activities (Fig. 8) than those recorded for Nubaria-1 extracts (Fig. 8) and this is consistent with the levels of phenolic compounds in these extracts (Fig. 8a). It could be concluded that the higher the phenolic content is the stronger in the antioxidative activity of the extract. The AOA of various extracts obtained from whole seeds with dark seed coat indicated that Nubaria-1 extracts were more effective in retarding the oxidation of linolic acid after more than 8 days of incubation than the corresponding extracts obtained from seeds with white seed coat. In general, the faba bean extracts exhibited excellent antioxidative activities with about 90% with more inhibition of linoleic acid oxidation by the end of incubation period while much lower inhibitory effects were observed by most of the whole seed extracts. Several studies were conducted with bean extracts in order to know its potential antioxidant activity. For example, in Tasuda, *et al.*, (1994), AOA was evaluated in pigments isolated from common beans. AOA was reported (Benninger and Hosfield, 2003) in extracts, condensed tannins and pure flavonoids from colored genotypes of common bean seed coats.

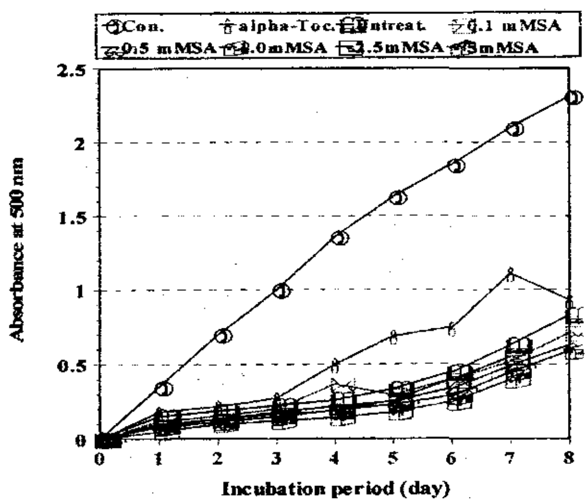

Fig 8a: Effect of SA on antioxidative activities of *V.faba Nubaria 1.*

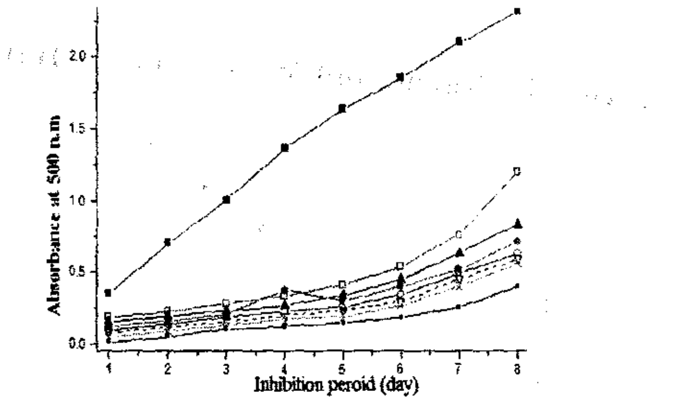

Fig 8b: Effect of ASA on antioxidative activities of *V.faba Nubaria 1.*.

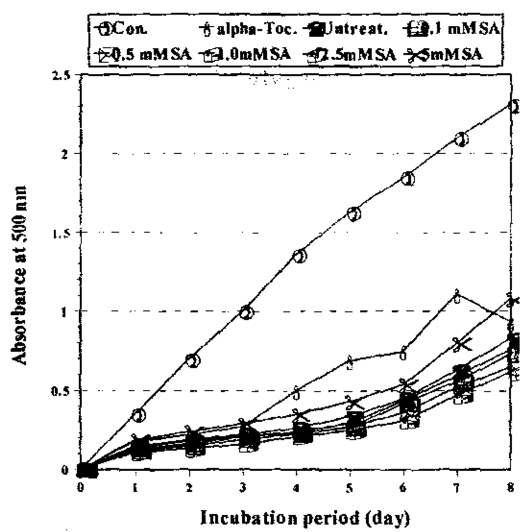

Fig 8c: Effect of L-AA on antioxidative activities of *V.faba Nubaria 1.*

Control —■— Alpha-tocopherol —□— Untreated —▲—
(0.1mM) —●—(0.5mM) —○— (1.0mM) - - ▽ - - (2.5mM) ---∗---
(5.0mM) —▪—

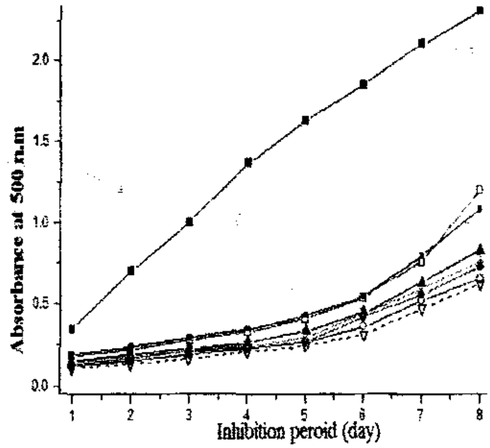

Fig 8d:Effect of SA on antioxidative activities of *P.sativum var.* Master B

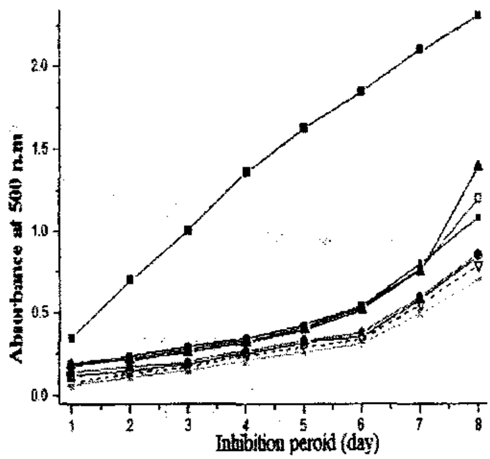

Fig 8e: Effect of ASA on antioxidative activities of *P.sativum var.* Master B.

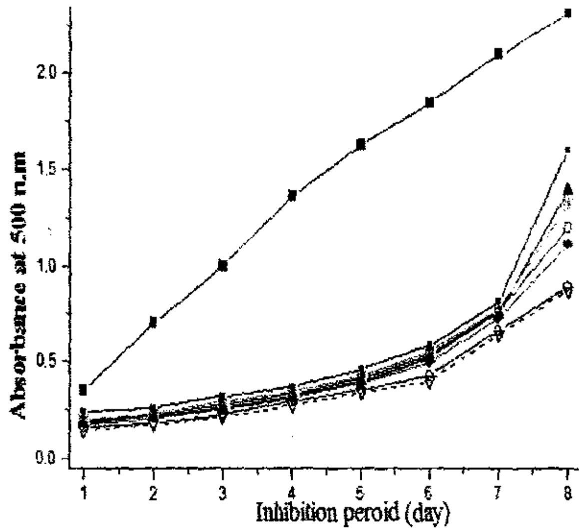

Fig 8f: Effect of L-AA on antioxidative activities of *P.sativum var.* Master B.

Control —■— Alpha-tocopherol —□— Untreated —▲—
(0.1mM) —●— (0.5mM) —○— (1.0mM) - - ▽ - - (2.5mM) —✳— (5.0mM) —•—

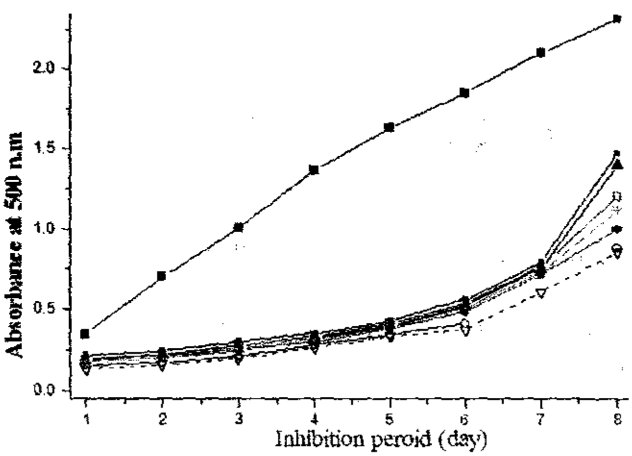

Fig 8g: Effect of SA on antioxidative activities of *P. vulgris var.* Nebraska

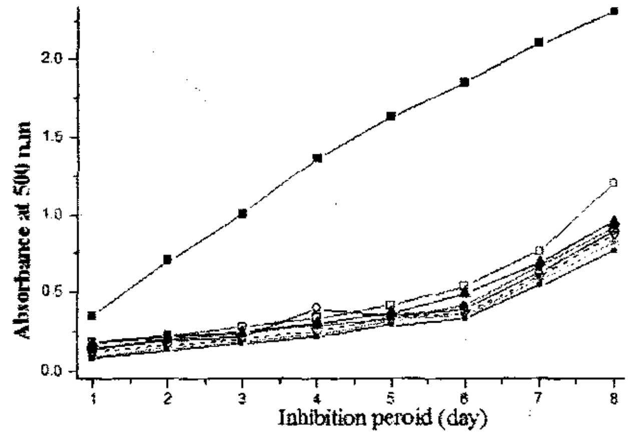

Fig 8h: Effect of ASA on antioxidative activities of *P. vulgris var.* Nebraska

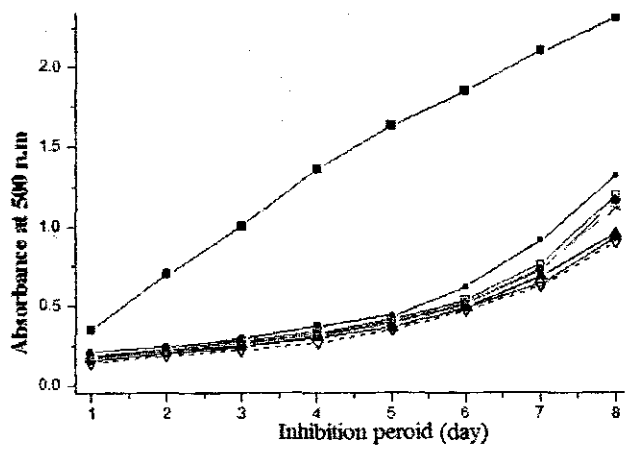

Fig 8j: Effect of L-AA on antioxidative activities of *P. vulgris var.* Nebraska

Control —■— Alpha-tocopherol —□— Untreated —▲—
(0.1mM) —◆— (0.5mM) —○— (1.0mM) - - ▽ - - (2.5mM) ---✶--- (5.0mM) —▪—

In foods, not only lipids but also DNA, proteins and carbohydrates can be damaged by reactive oxygen species (ROS). The ROS often encountered in biological systems include the radical's hydroxyl (OH.), superoxide (O^{-}_{2}.), and peroxyl (RO_2.) and non radicals, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). HOCl is a major active constituent of chlorine-based bleaches often used to disinfect equipment with which food will come into contact, (Morello *et al*, 2002)

The results presented in this work (Figs.8a, b, c, d, e, f, g, h and J) are consistent with those reported by other investigators who showed the antioxidative activity of phenolic compounds in the three crops under study. For example, Beninger and Hosfield, (2003) evaluated the abilities of tannin fraction extracts from the seed coats of ten *P. vulgaris* genotypes to prevent oil oxidation. These results suggest the possible value of the seed coat of kidney beans in the prevention of oxidative rancidity of food oils by the antioxidative effects of polyphenolic compounds present in the seed coats.

CONCLUSIONS

Antioxidants function by scavenging free radicals via donation of an electron or ahydrogen atom, or by deactivation of pro oxidant metal ions and singlet oxygen. Shahidi, (2002), and 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).

Finally, it could be concluded that, the methanolic-HCl extracts of TPCs extracted from whole dry bean exhibited antioxidant activity in linoleic acid. results indicated that importance of using antioxidants for increasing the antioxidative activities (AOA) of total phenolic compounds (TPCs) to limited of lipids oxidation (linoleic acid oxidation) in our study.

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التقييم المحصولى والمحتوى الكيميائي ونشاط مضادات الأكسدة للمركبات الفينولية فى بعض الخضر البقولية المعاملة ببعض مضادات الأكسدة

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أجريت هذه الدراسة خلال موسمين متتاليين في المزرعة البحثية بمحطة البحوث الزراعية بملوي وذلك خلال شتاء عامي ٢٠٠٨/٢٠٠٧ و ٢٠٠٩/٢٠٠٨ علي الفاصوليا صنف (نبراسكا) والبسلة صنف (ماستر بي) والفول الرومي صنف (نوبارية -١ (جيزة بلانكا) وذلك لدراسة تأثير ثلاثة من مضادات الاكسدة هم حمض السالسيليك وحمض الأسيتيل ساليسيلك وحمض الاسكوربيك علي المكونات المحصولية و الكيميائية ونشاط مضادات الأكسدة. تم تجهيز خمسة تركيزات هي ٠,١ ملليمولر، ٠,٥ ملليمولر، ١,٠ ملليمولر، ٢,٥ ملليمولر و ٥,٠ ملليمولر من كل مضاد أكسدة. ثم نقع البذور ورش النباتات الناتجة بهذه التركيزات هذا بالإضافة إلي نباتات غير معاملة (كنترول).

تم في هذه الدراسة تقدير بعض الصفات الفيزيائية للبذور وتحليلها كيميائياً (المادة الجافة، البروتين الخام، الألياف الخام ، الليبيدات الخام ومحتوي الرماد).

وتشير النتائج المتحصل عليها ان بذور الخضر البقولية تحت الدراسة مصادر جيدة وغنية للبروتين الخام والألياف والرماد.

وجد أن هناك تأثيرا معنويا باستخدام معاملات مضادات الأكسدة على عدد القرون الجافة/النبات ،وزن ال١٠٠بذرة ومحصول البذور الجافة للثلاث محاصيل تحت الدراسة.وكان حمض الأستيل ساليسيلك أفضل مركب حيث أعطى أعلى القيم.

تطرقت الدراسة إلي تقدير تركيزات النتريت (NO21-mg/kg) والتي تتراوح من ٤,٥ إلي ٦,٧ ولم تصل الي حد السمية (١٠ ملليجرام/كجم). وكان اعلي مستوي للنتريت في بذور البسلة صنف الماستر بي واقلها في بذور النبراسكا. وهذه النتائج تعني ان استهلاك هذه الخضر البقولية للتغذية عليها آمن. تحتوي بذور هذه العينات علي تركيزات من النترات اقل من ٢٠٠ ملليجرام/كجم وان اعلي تركيز (١٥٢ ملليجرام/كجم) سجل في مستخلصات البسلة صنف ماستر بي واقلها في النوبارية-١

مستويات حمض الاسكوربيك دائما عالية في العينات المعاملة مقارنة بالكنترول. تركيزات حمض الاسكوربيك في مستخلصات البسلة صنف ماستر بي اعلي (١٣,٥ ملليجرام/١٠٠جم) من تلك التي قدرت في مستخلصات الفول الرومي صنف نوبارية-١ (١٠ ملليجرام/١٠٠جم) ونبراسكا. إن استخدام حمض الاسكوربيك في المعاملة المزدوجة (النقع+الرش) أدت الي زيادات واضحة في تركيز حمض الاسكوربيك الكلي لتصل الي ٢٧٨% من كميتها الأصلية في البذور غير المعاملة للفاصوليا نبراسكا، ٢٤٩% للنوبارية-١ و ٢٠٩% للبسلة صنف ماستر بي عندما يستخدم تركيز ٥,٠ ملليمولر من الاسكوربيك.

تركيزات حمض السالسيليك في بذور العينات البقولية اعلي في كل المعاملات عند مقارنتها بالكنترول والجزء المأخوذ من حمض السالسيليك يختلف طبقا للجرعة. ولقد درست التغييرات في مستويات حمض السالسيليك نتيجة المعاملة المزدوجة (النقع+الرش) وتشير النتائج لحدوث زيادة واضحة في محتوي البذور من حمض السالسيليك.

لقد تطرقت الدراسة لتقدير تركيز المركبات الفينولية الكلية (TPCs) وتركيز الفلافونويدات الكلية (TFs) وتشير النتائج الي ان بذور الفول الرومي صنف نوبارية-١ (ذات القصرة الداكنة) تحتوي علي مستويات اعلي من الـ TPCs (٨,٣

ملليجرام/جرام) من تلك التي قدرت في في بذور الفاصوليا صنف نبراسكا (ذات القصرة البيضاء) (٧,٤ ملليجرام/جـرام) واعلـي بكثيـر مـن البـسلة صـنف ماسـتر بـي (٦,٥ملليجرام/جرام)، بينما تركيزات الفلافونويدات الكلية كانت الاعلي في مستخلـصات النبراسكا.

تم قياس نشاط المركبات الفينولية الكلية(TPCs) في المستخلـصات الخامـة المتحصله عليها من بذور البقوليات الثلاثة كنشاط لمضادات الأكسدة (AOA) بطريقة الثيوسيانات والتي تقيس كمية البيروكسيد المتكونة أثنـاء الأكـسدة الأوليـة لحمـض اللينوليك. أوضحت الدراسة أن (AOA) لمستخلصات الفول الرومي صنف نوبارية-١ كانت اكفأ في تأخير أكسدة اللينوليك بعد أكثر من ٨ ايام من التحضين من المستخلصات المناظرة المتحصل عليها من النبراسكا والماستر بي. وعموما فـان كـل مستخلـصات البقوليات تحت الدراسة أظهرت نشاط AOA ممتاز بنسبة ٩٠% في أكـسدة حمـض اللينوليك عند نهاية فترة التحضين . وهذه النتائج توضح أهميـة اسـتخدام مـضادات الأكسدة لزيادة نشاط مضادات الأكسدة للمركبات الفينولية الكليـة للحـد مـن أكـسدة الليبيدات (حمض اللينوليك) في دراستنا تلك